Biological Reference Data on CD(SD) IGS Rats - 1999

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PREFACE

The first edition of the “Biological Reference Data on CD(SD)IGS Rats-1998” was published in the end of the last year. This monumental accomplishment was made possible through the gracious contributions of both domestic and foreign members of the IGS Rat Study Group and the tireless efforts of Dr. Toshiaki Matsuzawa.

The study group members must have surprised at this book not only on appearance but on substantial content. This unique edition contained 43 reports totaling more than 260 pages of new information in easy to read format. The 1998 edition dealt primarily with general toxicology and reproductive data; while carcinogenicity data has been included in the 1999 edition. Differences in mortality, body weight, spontaneous tumor incidences, etc., between the IGS and the classical CD rat are discussed. One primary goal of the Carcinogenicity Subgroups of the IGS Rat Study Group is the collection and integration of neoplastic incidence data into existing databases. Likewise, the General and Reproduction Subgroups are planning similar activities. It is anticipated that this task will be completed by the year 2000.

A thorough understanding of the IGS rat as a model for safety evaluation coupled with standardization of breeding procedures would greatly facilitate the meaningful interpretation and evaluation of results and help to eliminate inherent confounding factors.

Final Thanks is given to Dr. Toshiaki Matsuzawa for his diligent work in preparation of this edition while he was serving with the Japan Pharmaceutical Manufactures Association, and acting as chairman of the Preclinical Evaluation Sub-Committee

In the fall of 1999
Hiroyuki Inoue, Ph.D. Chairman

PREFACE

The entire period from August through October of 1998 was devoted entirely to the preparation of the first edition of this book. Although two well known international companies graciously offered editorial assistance, the staff of the IGS Rat Study Group devoted their effects to editing in an attempt to minimize costs and save precious time. The first edition of the book was completed in December of 1998 and proved to be a most rewarding experience. A gala celebration, attended by several representatives from Western countries, was held at the Tokyo Pastoral on January 21, 1999. Although a great success, I was unable to attend this momentous occasion due to previous commitments.

Upon retirement from IGS Rat Study Group as vice-chairman, I recently accepted the position of chairman of the Preclinical Evaluation Subcommittee of Japan Pharmaceutical Manufacturers Association effective April, 1999, while maintaining my editorial responsibilities for the 1999 edition. Dr. Lee of Charles River, Inc. on his recent visit to Japan, informed me that this book was nicknamed "The Blue Book" as the authoritative source on the subject and that there will be numerous requests for free copies now and in the future.

There has been and will be a great deal of continued interest in the IGS rat. The integration of breeding practices among colonies, quality inspections along with confirmation, maintenance and assurance of phenotype will remain the primary goal of the IGS Rat Study Group. Consequently, the prompt pro-active involvement and contribution of IGS Rat Study Group will be essential to update and maintain this most useful document. Meticulous attention must be paid in the gathering and disseminating of this information with clear and accurate descriptions of observations and examinations while at the same time avoiding the incorporation of superfluous and artifactual findings which would only obscure the data base and result in erroneous decisions. As numerous artifactual results are often encountered in clinical pathology examinations, the first chapter of this book is devoted to clinical chemistry and urinalysis determinations in an attempt to explain and clarify the source of these findings.

The integration of "state-of-the-art" science, technology and historical data into SOP's and study protocols coupled with continued surveillance and updating of data bases is essential for the proper interpretation of pre-clinical safety evaluation.

In the fall of 1999
Toshiaki MATSUZAWA, Ph.D. Editor-in-chief

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We would like to acknowledge all those involved in the contribution of numerous papers and articles over the years and the secretaries and support staff assisting in this mammoth undertaking. Many thanks to Dr. R. Harling of HLS (U.K.) and Dr. J. L. Schardein of WIL (U.S.) for their valuable assistance and contributions. Numerous American and European individuals assisted from their branch and liaison offices in Japan. Special thanks to Mr. S. Mizuno of Covance, Mr. J. Hayakawa of HLS, Ms. M. Mochizuki of CRC Research, Institute and Mr. T. Nishizawa of Miki Sangyo.

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We wish to thank Ms. M. Kimishima of U-STAFF, Inc. for a copyright assistance and book number registration (ISBN), and to Mr. Y. Tsudome, Manager of Best Printing Inc., for printing and bookbinding.

Our deep-felt appreciation for the members and personnel from the companies concerned for past and continued support of the Study Group's activities.

Lastly we wish you, all concerned and readers, prosperity and health.

Toshiaki Matsuzawa, Ph. D. and Hiroyuki Inoue, Ph. D.

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CHAPTER 1

Introduction

Technical Factors Affecting Clinical Chemistry Values in Laboratory Rats

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ABSTRACT. Technical factors affecting clinical chemistry values in rats have become an ever increasingly important topic, but have been left unresolved. Basically, there are three reasons for this. First is the fact that the blood volume in rats is less than that of humans and adequate sampling induces stress in rats. Secondly, the use of human reagents for clinical chemistry in rats sometimes fails to provide the necessary specificity. Lastly, when statistically significant differences are evident in clinical parameters evaluated in toxicity studies, the lack of correlation with histological evaluation makes the findings meaningless in many cases. Two surveys were conducted on historical clinical data under the leadership of the members of the Japan Pharmaceutical Manufacturers Association [27, 30]. As a result, the overall variations and incidences of outlying data appear to decrease only in facilities that are concerned with artifactual occurrences. Many scientists and laboratories may be indifferent to these artifactual occurrences simply due to a general unawareness of the situation.

Clinical chemistry data of rats can vary according to sampling conditions and the measurement procedures. In this chapter, technical factors, e.g., fasting/feeding, sampling site, presence or absence of anticoagulants, differences between plasma and serum samples and storage, which are responsible for artifacts, are discussed. — Key words: Rat, Clinical Chemistry, Artifact

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INTRODUCTION

Charles River, Inc., a breeder of laboratory animals, noted that the demand and consequent production of CD(SD)IGS rats has increased dramatically, outweighing the demand for the classical CD strain of rats. Users of laboratory animals anticipate that a constant supply of CD(SD)IGS rats that are genetically stable will be continually and economically available. It is of paramount importance that these animals maintain the genetic stability and characteristics of the CD strain. Users would also expect the breeder to produce smaller animals with fewer gender differences and increased viability up to 80% at 2 years of age in the future. Based on data reported in the publication of IGS [31], body weight and food consumption were lower in CD(SD)IGS rats than in the CD(SD) strain at 31 weeks of age. Hematological data were comparable between these strains, but blood cholesterol and triglyceride levels tended to be lower in the CD(SD)IGS rats [32]. Although a constant supply of CD(SD)IGS rats is available due to a stable production system, if there are great inter-laboratory variations in evaluation techniques, genetic changes that may occur may not be apparent for the foreseeable future.

The Japan Pharmaceutical Manufacturers Association conducted two surveys on clinical chemistry data and published the results [25, 27, 30]. Repeated surveys revealed smaller variations and outlying data. However, clinical chemistry data continued to vary due to sampling conditions/analytical methods and factors such as age, strain, housing conditions, type of feed, water intake, anesthesia, sampling site, anticoagulants, etc. In addition to the results of these surveys, the results in our published reports are included for discussion. To minimize variations in clinical chemistry determinations due to artifacts, it is necessary for each laboratory to prepare a good historical control data base and update it periodically. In the previous edition, overall historical data was discussed [31]. This edition will focus on clinical chemistry values.

Abundant historical clinical chemistry data have been reported [5, 6, 14, 16, 44, 54]. The reader is referred to a paper by Riley [40] concerning artifacts affecting clinical chemistry values.

SOURCES OF VARIATIONS IN CLINICAL LABORATORY MEASUREMENTS

It is well known that clinical chemistry values vary with age [22, 37, 38, 46, 51, 53, 55], strain [23] and gender [25, 27]. Therefore, these factors are not discussed in this edition.

1. Type of feed, feeding and fasting

Feed is one factor that can affect clinical chemistry values. Feed containing protein between 20-29% is commonly used in the U.S.A. and Japan and 15-20% in the U.K. It is a questionable practice to provide feed of the same composition through all stages of development and ages (young, adult and old) from a nutritional and physiological viewpoint. Recently, feed with a lower protein content (18%) is commercially available in Japan. (See Appendix in the 1998 edition concerning nutritional ingredients of rat feed.) Although chronic nephropathy develops with age, at a higher incidence and at an earlier onset, greater variations in clinical chemistry values (decreased glucose, UN, GPT and ALP in SD rats [2]) are evident when animals are provided with high-protein diets. ALP activity decreases with decreased food consumption since the major blood ALP activities are derived from the small intestine/duodenum in rats.

Feeding and fasting conditions at the time of sampling are also influential. Therefore, these conditions should be controlled. As presented in Table 1, clinical chemistry values vary due to fasting [28]. It has been known that activated partial thromboplastin time (APTT) and prothrombin time (PT) are prolonged by fasting [49]. Triglyceride (Tg) levels under *ad libitum* feeding were approximately 2 times those under fasting, and the values in males tended to be greater than in females [27]. Although it has been reported that cholesterol levels under *ad libitum* feeding are higher than under fasting conditions [17, 33, 42, 45], these differences were negligible in the surveys conducted by JPMA. Free and ester-type cholesterol levels in plasma are reported to increase with age [36, 42]. Care should be taken with glucose evaluations since levels can be affected by sampling sites and intra-day variations associated with food consumption [3, 21, 46].

2. Animal handling and stress

It has been postulated that excitation or stress due to handling of animals at sampling or routine procedures may induce release of catecholamine or corticosteroids, resulting in effects on clinical chemistry values. It has been reported that glucose levels fluctuate due to excitation or fright when animals are restrained or not anesthetized at sampling [3]. Therefore, it is important to handle animals gently by a well-trained and experienced technical staff. Increases in GOT and CPK activities are often observed due to vigorous handling of animals and/or insufficient anesthesia [12, 46].

3. Blood sampling procedure

It is well known that blood chemistry determinations can be affected by sampling sites, methods of sampling and bleeding intervals. Blood samples should be collected in random order and should be appropriately treated (avoiding hemolysis, heparinization, suitable temperature, rapid and gentle centrifugation, and avoiding contamination with platelets and blood cells).

1) Bleeding intervals

Anemia occurs in animals when blood is taken over short intervals or if large amounts are collected at one time. This can result not only in changes in hematological parameters but also increases in plasma cholinesterase and GPT activities. Increased frequency of blood sampling may also result not only in fluctuations in hematocrit, RBC and reticulocyte values but also in GOT, GPT and ALP activities [43]. Sufficient consideration should be taken into account when sampling at numerous intervals to avoid transient anemia due to blood loss. The maximum sample volume that does not provide physiological fluctuations is said to be 10% of the entire volume [11]. The samples should be taken at the same time each day since some parameters, such as glucose, hormones and ALP in rats, exhibit intra-day variations [46]. Activated partial thromboplastin time (APTT) and prothrombin time (PT) have circadian rhythms (diurnal variations) and tend to be shortened in the day and prolonged in the night [7].

2) Hemolysis

Hemolysis affects coagulation and clinical chemistry parameters as well as hematological parameters [12]. AST, LDH, CPK and ALP activities and inorganic phosphorus, potassium and cholesterol levels increase by exudation from red blood cells. Generally, blood samples in which hemolysis is confirmed should not be used for examinations. Although, hemolysis is an extreme example, components also leak from blood cells when blood is allowed to stand, resulting in fluctuations of serum components.

Creatinine levels are sometimes apparently changed due to hemolysis, depth of anesthesia at sampling, and the effects of drugs administered as well as to measurement conditions [8, 19, 39]. Urinary creatinine levels also fluctuate due to handling of animals in sampling and feeding conditions [39].

3) Sampling site and anesthesia

It is important that sampling be conducted as quickly as possible in an attempt to minimize pain and stress to avoid effects on blood components. Sampling sites and anesthesia are very important factors in rats and mice [37, 47]. Carbon dioxide gas is sometimes used for anesthesia in Western countries [52].

4) Sampling site and sampling procedures

Sampling sites may vary between Western countries and Japan (Table 3). Sampling from the orbital sinus (retrobulbar venous plexus) is commonly employed in rats and mice in Western countries. However, this procedure sometimes induces artifacts and ocular damage if conducted improperly [11]. This procedure can also induce damage to surrounding tissues such as orbital tissue and Harderian glands with untrained staff [13, 34, 37, 47, 52]. Evaluations of clinical chemistry determinations with blood taken from the tail, jugular and sublingual veins have been made [1, 2].

Serum ALP, GOT, GPT and LDH activities and potassium levels were significantly lower in blood samples obtained from the posterior vena cava when compared to those from the orbital sinus [15]. This suggests that when the vessels and surrounding tissues are damaged, some parameters, particularly enzymatic activities, are affected by leakage of enzymes from the damaged vessels/tissues. Friedel *et al.* [10] reported that plasma LDH, GOT, GPT, CPK and ALP activities were significantly higher in blood samples obtained from the orbital sinus of rats when compared to those from the jugular vein. Puncture of the orbital sinus is widely used for repeated blood sampling from rats. It is well known that fluctuations of analytical values are greater by this method compared to those of sampling from the posterior vena cava. Values may vary according to the quality of sampling techniques. Therefore, skill, training and experience are essential for adequate sampling techniques. Comparisons of clinical chemistry values were made among the samples (heparinized plasma) taken from the posterior vena cava, orbital sinus and abdominal aorta. Although statistically significantly differences were found in some parameters such as GOT, GPT and aldolase activities, there were no parameters with biologically significant differences (Table 3), indicating the importance of sampling techniques (efficient and gentle handling, heparinization and rapid treatment of samples).

4. Treatment of samples after collection

Plasma enzyme levels can increase unless the plasma is separated from blood promptly after collection since platelets and red blood cells contain considerable LDH, CPK, LDH, GOT and/or potassium [5, 48]. Our experimental results are presented in Tables 4-7. As described in the section on “Hemolysis”, if blood samples are allowed to stand, serum GOT, LDH and CPK activities and potassium levels increase and sodium and glucose levels decrease. Therefore, prompt attention to samples is required. According to Matsumoto *et al.* [24], the serum LDH activities 3 hours after blood sampling compared to those 5 minutes after sampling increased as follows: approximately 1.1 times in humans, 7 times in dogs and 19 times in rats. The time from blood sampling until serum/plasma separation can drastically affect results in laboratory animals, particularly rodents. LDH, GOT [4, 9, 10] and CPK [35] activities increase while whole blood is allowed to stand at room temperature. These enzymes are considered to be released from platelets during the process of coagulation. The degree of the release from platelets is considerably different among animal species; slight in dogs and monkeys, but marked in rabbits and rodents [4, 9, 10]. Heparinized plasma is better for measurements since serum LDH and CPK activities are markedly increased.

5. Anticoagulants

Chelating agents such as EDTA affect ALP, LDH and calcium; therefore, heparin is suitable as an anticoagulant for the separation of plasma. However, heparin ammonium is not suitable for measurement of urea nitrogen levels by the urease-indophenol method since ammonium ions inhibit urease [12].

SEPARATION OF PLASMA/SERUM

CPK values are affected by the conditions of separation/storage of serum or plasma in rats [48]. Rapid increases in CPK activities are observed in serum after separation from whole blood which is allowed to stand at room temperature, while similar changes are not observed in plasma [15]. Therefore, it is necessary to consider which is more appropriate for examinations, serum or plasma, since normal values can vary between these samples [15, 27]. Platelets or red blood cells contain considerable CPK, LDH [41] and potassium; therefore, contamination with blood cells should be avoided at centrifugation. Refrigeration should also be avoided prior to and after centrifugation to prevent leakage of these enzymes or other components. In toxicity studies, both serum and plasma are used for blood chemistry determinations. It is recommended to use plasma since intracellular materials are markedly released from blood cells, particularly platelets in the process of coagulation in laboratory animals, especially rodents [18, 20, 26, 46].

Inter-species differences in potassium levels are presented in Table 8 and fluctuations in potassium levels after whole blood was allowed to stand are presented in Table 9. It is important when measuring potassium levels to use plasma and not to cool the samples.

DISPOSITION OF SAMPLES PRIOR TO EVALUATION

Clinical chemistry determinations usually require prompt analysis to correlate relationships with clinical signs. Therefore, samples should be measured immediately after collection. However, it is also necessary to understand that values can change after storage of samples. Potassium levels increase when potassium is released from blood cells into serum due to decreased active transport and passive diffusion. This phenomena is noted in both serum and plasma, but is more marked in serum. Active transport on and in red blood cell membranes is inhibited at 4℃, resulting in greater fluctuations in potassium levels than at room temperature [15]. Based on these facts, care should be taken in the handling of samples and data evaluation. Potassium levels and APTT values are presented in Tables 9 and 10, respectively. APTT was prolonged when stored under refrigeration [50] and inter-species differences were evident.

INCONSISTENCY OF VALUES DUE TO DIFFERENCES IN MEASUREMENT METHODS

There should be consistency in results between different laboratories, if the principles and methods (equipment, techniques, etc.) of detection are sufficient and if there is no interference from the components in the samples. However, it is often difficult to obtain consistent results even when the detection methods are the same. This was evident in results of control data in the surveys conducted by JPMA [30]. Therefore, historical control data should be stratified according to detection methods, equipment and sampling conditions at calculation. Taking into consideration that acquired values may vary due to buffers (types, concentrations and pH), substrates (types and concentrations), coenzymes (concentrations), temperature, etc., even when the same analytical principles are used, international harmonization of detailed procedures for clinical chemistry determinations is also required in the field of veterinary clinical chemistry and toxicity studies.

FUTURE PROSPECTS

Equipment for clinical chemistry determinations has been developed and even trace values are detectable. The precision control of the equipment is readily available [29]. However, interlaboratory differences continue to exist in sampling (blood and urine) and storage conditions as well as artifacts, resulting in greater variations from normal values. These variations could be easily improved in the near future.

Clinical chemistry values were used historically to support histopathological evaluations in toxicity studies in rats. However, we may have re-think our priorities since alterations in clinical laboratory values may be and often are the first early indicator of potential gross and microscopic lesions and are important indicators for extrapolation of risks to man. Therefore, constant diligence is required to generate accurate, precise, meaningful and reproducible results. I would like you, the reader, to consider the clinical chemistry data in IGS rats from the viewpoints as described above.

Table 1. Body weight, hematocrit and plasma chemistry values in male F344 rats

Parameters	Unit	Fasting time before blood sampling (hr)	
		0	16
Body weight	g	357±2	399±3**
Hematocrit	%	45±0	45±1
Alkaline phosphatase	KA	45.7±1.0	19.9±1.3**
Total protein	g/dl	6.7±0.1	6.4±0.1**
Glucose	mg/dl	168±3	153±6
Total cholesterol	mg/dl	62±1	49±2**
Triglycerides	mg/dl	116±15	63±6**
Urea nitrogen	g/dl	22±1	17±0**
Calcium	mEq/l	5.5±0.1	5.0±0.1**
Inorganic phosphorus	mg/dl	6.9±0.3	6.5±0.3
AST/GOT	IU/l	75±1	85±4**
ALT/GPT	IU/l	44±1	45±2

Values are means±S.E. of 10 animals. 20 weeks old.
Significantly different from the unfasted rats (0 h). **p<0.01
by Matsuzawa and Sakazume 1994. Comp. Haematol. Int. 4:152-156.

Table 2. Blood collection sites in the rats by JPMA survey

Bleeding Vessels	For Hematology %	For Blood Chemistry %
Cardiac	4	4
Aorta	47	65
Abdonimal	41	55
Carotid	4	6
Femoral	2	4
Vein	49	31
Jugular	13	5
Abdominal Vena Cava	12	13
Orbital sinus	8	8
Tail	7	0
Femoral	7	5
Others	2	0

by Matsuzawa 1992. Toxicologic Pathology 20: part 2. 528-533

Table 3. A comparison of three methods of bleeding sites: Plasma Chemistry

	Units	the inferior vena cava (IVC)	the abdominal aorta (AA)	the orbital venous plexus (OVP)
No. of animals used		50	50	50
GOT/AST	IU/l	67±1[a]	73±1**	70±1**
GPT/ALT	IU/l	34±0	37±1**	35±0
LDH	IU/l	150±11	181±23	166±13
CK	IU/l	143±3	155±5	162±6*
GGT	IU/l	1±0	1±0	1±0
Aldolase	IU/l	12.8±0.2	13.7±0.4	13.7±0.4
Amylase	IU/l	4462±34	4605±45*	4505±42
ALP	IU/l	463.6±3.3	457.2±3.5	470.4±4.6
T Bile acid	mg/dl	7.3±0.9	6.3±0.3	7.4±0.3*
Total protein	g/dl	6.0±0.0	6.0±0.0	6.0±0.0
Albumin	g/dl	2.6±0.0	2.6±0.0*	2.6±0.0*
Urea nitrogen	mg/dl	23±0	22±0	21±0**
Creatinine	mg/dl	0.5±0.0	0.5±0.0	0.5±0.0
Glucose	mg/dl	142±1.0	167±2.0*	139±2.0
T. Cholesterol	mg/dl	47±0	47±0	47±1
F. Cholesterol	mg/dl	9±0	8±0*	8±0
Triglycerides	mg/dl	54±2	54±2	54±2
Phospholipids	mg/dl	98±1	98±1	99±1
Calcium	mg/dl	5.0±0.1	4.9±0.1	5.0±0.1
I. phosphorus	mg/dl	6.7±0.1	6.4±0.1	6.3±0.1**
Sodium	mg/dl	143±0	142±0**	143±0
Potassium	mg/dl	3.6±0.1	3.7±0.1*	3.5±0.0
Chloride	mg/dl	103±0.0	104±0**	103±0

Significantly different from the value of inferior venena cava, *$p<0.05$, **$p<0.01$.
[a]: MEAN±SE, LDH: Lactic dehydrogenase, Creatine phosphokinase: CK, Gammaglutamyl transpetidase: GGT, Alkaline phosphatase: ALP
by Matsuzawa *et al.* 1994. Comp Haematol. Int. 4: 207-211.

Table 4. LDH and CK activity in plasma after freezing (n=10)

Platelet count (10^4/μl plasma)		LDH activity IU/l			CK activity IU/l		
		Not frozen	-20℃ 5 days	Increase ×	Not frozen	-20℃ 5 days	Increase ×
Ave	4.8	117.2	309.5	2.9	103.0	171.8	1.7
Min	3	71	199	1.8	84	141	1.4
Max	9	263	583	4.4	137	253	2.2

by Matsuzawa & Ishikawa 1993. Comp. Haematol. Int. 3: 214-219.

Table 5. Platelet count and LDH and CK activity in plasma

Platelet count (10^4/μl plasma)	LDH activity (IU/l)			CK activity (IU/l)		
	Not frozen	-20℃ 5 days	Increased activity	Not frozen	-20℃ 5 days	Increased activity
14	214	665	451	81	253	172
8	174	497	323	74	196	122
5	150	358	208	73	147	74
3	132	267	135	70	119	49

by Matsuzawa & Ishikawa 1993. Comp. Haematol. Int. 3: 214-219.

Table 6. GOT, GPT, LDH, CK and ALP activity in lysed platelets

No. of lysed platelets (10^4/μl)	GOT (IU/l)	GPT (IU/l)	LDH (IU/l)	CK (IU/l)	ALP (IU/l)
0.68	1	N	26	N	N
3.4	6	N	143	41	N
6.8	12	N	283	104	N
13.6	24	1	566	251	N
68	120	5	>2000	1285	0.4

N: no activity
by Matsuzawa & Ishikawa 1993. Comp. Haematol. Int. 3: 214-219.

Table 7. Bleeding site and CK activity (IU/l)

No. of rats	Orbital venous plexus (OVP)	Abdominal vena cava (AVC)
26	129±19	71±4

Mean±SE
by Matsuzawa & Ishikawa 1993. Comp. Haematol. Int. 3: 214-219.

Table 8. Serum and heparinized plasma potassium concentrations in four laboratory animals stored for 0 hour.

Sample	Potassium concentrations (mmol/l)			
	Dog	Monkey	Rabbit	Rat
Plasma	4.0±0.3	3.9±0.3	4.0±0.3	3.9±0.4
Serum	4.3±0.3*	4.7±0.5*	4.2±0.3*	4.8±0.5*

Mean±S.D. (n=10)
*Significantly different from the values for plasma, p<0.01
by Ito *et al*. 1998. Comp. Haematol. Int. 8: 77-81

Table 9. Dependence of serum and plasma potassium concentrations on time and temperature of whole blood storage: Rats

Sample	Temperature (℃)	Potassium concentrations (mmol/l)		
		0 h	2 h	5 h
Plasma	25	3.9±0.4	3.6±0.3[a]	3.7±0.4[b]
	4		4.2±0.4[a]	5.1±0.4[a]
Serum	25	4.8±0.5[a]	4.8±0.5	4.7±0.4
	4		5.3±0.5[c]	5.9±0.5[c]

Mean±S.D. (n=10).
[a]Significantly different from the values of plasma 0 hour, p<0.01
[b]Significantly different from the values of plasma 0 hour, p<0.05
[c]Significantly different from the values of serum 0 hour, p<0.01
by Ito *et al*. 1998. Comp. Haematol. Int. 8: 77-81

Table 10. Comparison of APTT time and stability of citrated plasma samples from rats, rabbits, dogs, monkeys and human stored at 4℃

Species		Time after storage (hour)					
		0(fresh)	1	2	4	8	24
Rat（F344）	♂	16.7±0.9	17.7±0.5	19.2±1.0**	20.2±1.2**	21.2±1.0**	27.3±1.8**
	♀	17.6±1.8	19.1±1.0	20.4±1.1**	23.8±2.2**	27.5±2.6**	34.5±2.6**
Rabbit（NZW）	♂	25.5±0.6	26.0±0.2	26.1±0.1	26.6±0.7	26.0±0.1	26.4±0.6
	♀	25.3±2.2	27.2±1.9	25.7±2.6	26.4±1.1	27.1±4.3	27.5±2.5
Dog（Beagle）	♂	12.0±0.5	12.0±0.8	12.0±0.7	12.3±0.4	12.3±0.2	12.2±0.3
	♀	12.3±0.9	12.1±0.9	12.2±0.9	12.5±0.7	12.6±0.8	12.7±0.9
Monkey（Cynom.）	♂	21.9±0.8	21.5±0.6	21.9±1.1	22.6±1.1	23.4±1.3	24.9±1.3**
	♀	20.6±1.9	20.6±1.9	20.6±1.8	21.2±2.0	22.5±2.1	24.7±0.7**
Human	♂	27.3±1.6	29.8±3.5	30.0±2.0	31.4±2.7*	32.2±4.0	31.2±2.9*

Mean±S.D. (n=6).
Significantly different from the 0 h values of each species, *:p<0.05, **:p<0.01
by Tabata *et al*. 1995. Comp. Haematol. Int. 5: 140-144.

REFERENCES

1. Angelov, O., Schroer, R. A. and Heft, S. *et al.* 1984. A comparison of two methods of bleeding rats: the venous plexus of the eye versus the vena sublingualis. J. Appl. Toxicol. 4: 258-260.
2. Apostolou, A., Saidt, L., and Brown, W. R. 1976. Effect of overnight fasting of young rats on water consumption, body weight, blood sampling, and blood composition. Lab. Anim. Sci. 26: 959-960.
3. Besch, E. L., and Chou, B. J. 1971. Physiological responses to blood collection methods in rats (36041). Pro. Soc. Exp. Biol. Med. 138: 1020-1021.
4. Breuer, Von J. und Stucky H. 1975. Enzymaktivitaeten im Serum and Plasma von Mensch, Hund und ratte sowie deren Veraenderungen beim Aufbewahren des Blutes. Z. Klin. Chem. Klin. Biochem. 13: 355-360.
5. Caisey, J. D. and King, D. J. 1980. Clinical chemical values for some common laboratory animals. Clin. Chemi. 26: 1877-1879.
6. Carakostas, M. C. and Banerjee, A. K. 1990. Interpreting rodent clinical laboratory data in safety assessment studies: Biological and analytical components of variation. Fund. Appl. Toxicol. 15: 744-753.
7. Cohen, M., Simmons, D.J. and Joist, J.H. 1978. Diural hemostatic changes in the rat. Thromb. Res. 12: 965-971.
8. Evans, G.O., 1986. The use of an enzymatic kit to measure plasma creatinine in the mouse and three other species. Comp. Biochem. Physiol. 85B: 193-195.
9. Friedel, R., and Mattenheimer, H. 1971. Freisetzung von Stoffwechselenzymen aus den Thrombocyten der Maus und des Meerschweinchens waehrend der Blutgerinnung. Z. Klin. Chem. Klin. Biochem. 9: 103-106.
10. Friedel, R., Trautschold, I. and Gaertner, K. 1975. EinfluB verschiedener Methoden zur Blutgewinnung auf Enzym Akitivitaeten im Serum Kleiner Laboratoriumstiere. Z. Klin. Chem. Klin. Biochem. 13: 499-505.
11. Gralla, E.J.1980. Chronic testing in animals: In vivo techniques, In: Introduction to biochemical toxicology, edited by Hodgson, E., Guthrie, F.E., Elsevier, New York: 385.
12. Hall, R.L. 1991. Clinical pathology of laboratory animals, in Animal models in toxicology editedby Gad, C. and Chengelis, C.P. 765-811. Marcel Dekker, Inc., New York.
13. Herck, H.V., Baumans, V. and Craats, N.R.V.D. 1992. Histological changes in the orbital regionof rats after orbital puncture. Lab. Anim. 26: 53-58.
14. Harris, E. K. 1974. Effects of intra- and interindividual variation on the appropriate use of normal ranges. Clin. Chem. 20: 1535-1542.
15. Ikeda, S. and Tsuda, S. 1985. In: Hematology & Clinical Chemistry, New Methods on Toxicity Studies, Shirasu and Hayama eds, Realiz, Tokyo, pp. 115-121.
16. Iwasawa, Y. 1984. Normal range of clinical pathology values in laboratory animals. Pharmacia (Jpn). Review No.13: 133-162.
17. Johnson, I.T., Livesey, G. and Gee, J. M. 1990. The biological effects and digestible energy value of a sugar-beet fibre preparation in the rat. Br. J. Nutr. 64: 187-199.
18. Ladenson, H. J., Tsai, B. L. M. and Michael, M. J. 1974. Serum versus heparinized plasma for eighteen common chemistry tests. Am. J. Clin. Pathol. 62: 545-552.
19. Letellier, G. and Desjarlais, F., 1985. Analytical interference of drugs in clinical chemistry: Ⅱ the interference of three cephalosporins with the determination of serum creatine concentration by the Jaffe reaction. Clin. Biochemi. 18, 352-356.
20. Lum, L. G. and Gambino, S. R. 1974. A comparison of serum versus heparinized plasma for routine chemistry tests. Am. J. Clin. Pathol. 61: 108-113.
21. Maejima, K., and Nagase, S. 1991. Effect of starvation and refeeding on the circadian rhythms of hematological and clinico-biochemical values, and water intake of rats. Exp. Anim. Jpn. 40: 389-393
22. Maejima, K. and Tamura, H. 1990. Hematology and clinical chemistry values of aged F344 rats. Lab. Anim. Technol. (Jpn). 25: 119 -129.
23. Masoro, E.J., Katz, M.S. and McMahan, C.A. 1989. Evidence for glycation hypothesis of aging from the food restricted model. J. Gerontol. (Bio Sci) 44: B20-22.
24. Matsumoto, K., Morino. T., Toriya, F., and Hayano, K. 1977. On the increase of rat and dog serum LDH activity due to stand the whole blood. Igaku no Ayumi Jpn. 102: 608-610.
25. Matsuzawa, T. 1992. Present status of animal clinical pathology examinations in the Japanese Pharmaceutical Manufacture Association. Toxicol. Pathol. 20: part 2 528-533.
26. Matsuzawa, T. and Ishikawa, A. 1993. The effect of preanalytical conditions on lactate dehydrogenase and creatine kinase activities in the rat. Comp Haematol Int 3: 214-219.
27. Matsuzawa, T., Nomura, T. and Unno, T. 1993. Clinical pathology reference ranges of laboratory animals. J.Vet. Med.Sci. 55: 351-362.
28. Matsuzawa, T. and Sakazume, M. 1994. Effect of fasting on haematology and clinical chemistryvalues in the rat and dog. Comp. Haematol. Int. 4:152-156.
29. Matsuzawa,T., Nomura, M., Yonezawa, H. and Unno, T.1995. Selection of appropriate parameters, use of a quality control concept, and suitable statistical analyses for clinical pathology examination of animals in toxicity studies; Results of a current survey by Japanese Pharmaceutical Manufacturers Association. Comp Haematol Int 5: 196-200.
30. Matsuzawa, T., Hayashi, Y. and Nomura, M. *et al.* 1997. A survey of the values of clinical chemistry parameters obtained for a common rat blood sample in ninety-eight Japanese laboratories. J. Toxicol. Sci. 22: 25-45.
31. Matsuzawa, T. and Inoue, H. 1998. ed by Matsuzawa and Inoue "Biological Referencce Data on CD(SD)IGS Rats - 1998". pp. 1-7. The Study Group of CD(SD)IGS, Yokohama.
32. Matsuzawa, T. and Inoue, H. ed. 1998. Biological Referencce Data on CD(SD)IGS Rats - 1998. The Study Group of CD(SD)IGS, Yokohama.
33. Mazur, A., Remesy, C. and Gueux, E. 1990. Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and on lipoprotein catabolism in rats. J. Nutr. 120. 1037-1045.
34. McGee, M. A., and Maronpot, R. R. 1979. Harderian gland dacryoadenitis in rats resulting from orbital bleeding, Lab. Anim. Sci. 29: 639-641.

35. Meltzer, H. Y. and Guschwan, A. 1972. Type I brain type creatine phosphokinase (CPK) activity in rat platelets. Life Sci. 11: 121-130.
36. Murawshki, U., Kriesten, K. and Egge, H. 1989. Age-related changes of lipid fractions and total fatty acids in the serum of female and male rats aged from 37 to 1200 days. Comp. Biochem. Physiol. 94: 525-536.
37. Neptun, D. A., Smith, C. N. and Irons, R. D. 1985. Effect of sampling site and collection method on variations in baseline clinical pathology parameters in Fischer-344 rats. 1. Clinical chemistry. Fund. Appl. Toxicol. 5: 1180-1185.
38. Onoue, M., Uchida, K. and Takahashi, T. 1978. Relationship between some biochemical measurements and histopathological changes in age-related kidney lesions of rats. Exp. Anim. Jpn. 27: 405-412.
39. Rikimaru, T., Oozeki, T. and Ichikawa, M. 1989. Comparisons of urinary creatine, skeletal muscle mass, and indices of muscle protein catabolism in rats fed *ad libitum*, with restricted food intake, and deprived of food. J. Nutr. Sci. Vitaminol. 35: 199-209.
40. Riley, J. H. 1992. Clinical pathology: Preanalytical variation in preclinical safety assessment studies-Effect on predictive value of analyze tests. Toxicol. Pathol. 20: Part 2, 490-500.
41. Rothwell, D. J., Jendrzejczak. B. and Becker, M. 1976. Lactate dehydrogenase activities in serum and plasma. Clin. Chem. 22: 1024-1026.
42. Ruggiero, F. M., Cafagna, F. and Gadaleta, M. N. 1990. Effect of aging and acetyl-L-carnitine on the lipid composition of rat plasma and erythrocytes. Biochem. Biophys. Res. Commun. 170: 621-626.
43. Sakai, T., Yoshida, S. and Fujimoto, R. 1989. Repeated phlebotomy-induced liver cell necrosis in rats. J. Toxicol. Pathol. Jpn. 2: 175-179.
44. Schwartz, E., Tornaben, J. and Boxill, G. C. 1973. The effects of food restriction on hematology, clinical chemistry and pathology in the albino rat. Toxicol. Appl. Pharmacol. 25: 515-524.
45. Shinnick, F. L., Ink, S. L. and Malett, J. A. 1990. Dose response to a dietary oat bran fraction in cholesterol-fed rat. J. Nutr. 120: 561-568.
46. Stevens, K. R. and Gallo, M. A. 1982. Practical considerations in the conduct of chronic toxicity studies, In: Principles and methods of toxicology, edited by Hayes A W, Ravan Press, New York, USA: 53-77.
47. Suber, R. L. and Kodell, R. L. 1985. The effect of three phlebotomy techniques on hematological and clinical chemical evaluation in Sprague-Dawley rats. Vet. Clin. Pathol. 14: 23-30.
48. Suzuki. T., Kondou, M. and Orita, S. 1983. Lactate dehydrogenese and creatine phoshokinase assay in rat plasma; Effect of contamination of platelets in 1500xG centrifuged plasma. J. Toxicol. Sci. Jpn. 4: 325
49. Suzuki, S. 1986. Specimen collection and influental factors in hematological examination. J. Toxicol. Sci. Jpn. 11: 401-403.
50. Tabata, H., Nakamura, S. and Matsuzawa, T. 1995. Some species differeces in the false prolongation of prothrombin times and activated partial thromboplastin times in toxicology. Comp Haematol Int 5: 140-144.
51. Unakami, S., Hirata, M. and Ichinohe, K. 1989. Separation and quantific action of serum alkaline phosphatase isozymes in the rat by affinity electrophoresis. Exp. Anim. Jpn. 38: 85- 88.
52. Walter, G. L. 1999. Effects of carbon dioxide inhalation on hematology, coagulation, and serum clinical chemistry values in rats. Toxicol. Pathol. 27: 217-225.
53. Waner, T., Nyska, A., and Chen, R. 1991. Population distribution profiles of the activities of blood alanine and aspartate aminotransferase in the normal F344 inbred rat by age and sex. Lab. Anim. Jpn. 25: 263-271.
54. Wolford, S. T., Schroer, R. A. and Gohs, F. X. 1986. Reference range data base for serum chemistry and hematology values in laboratory animals. J. Toxicol. Environ. Health. 18: 61-188.
55. Wolford, S. T., Schroer, R. A. and Gallo, P. P. 1987. Age-related changes in serum chemistry and hematology values in normal Sprague-Dawley rats. Fund. Appl. Toxicol. 8: 80-88.

The Importance of Urinalysis and Effects of Urine Collection Techniques in Toxicity Studies with Rats

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ABSTRUCT. The urinalysis parameters recommended in the JMHW guidelines for repeated dose toxicity studies, including urinary volume, pH, protein, glucose, ketone bodies, bilirubin, occult blood, specific gravity or osmotic pressure, electrolytes and microscopic examination of the sediment, with emphasis on rat urinalysis are reviewed in this paper. Fluctuations and individual variations of these parameters are greater than those encountered with blood determinations under the normal physiological conditions. Therefore, it is often difficult to detect minute changes in urinalysis in laboratory animals. Many investigators have concluded that overnight urine collection under food and water deprivation induces stress in animals and is not desirable from a humanitarian viewpoint. However, urine collection under *ad libitum* feeding and watering conditions cannot yield suitable data for the evaluation of toxicity. It is recommended that urine be collected from animals that were fasted overnight but which were supplemented with gastric intubations of water to minimize stress and contamination of samples; thereby urinary volume is more reflective of normal and adverse effects on kidney functions. Utilization of such a procedure can reduce the variations and false negative or positive results classically evident in urinalysis data in safety evaluation studies. — Key words: Rat, Urine collection and Urinalysis

CD(SD)IGS-1999: 9-22

INTRODUCTION

The following urinalysis parameters are recommended in the JMHW guidelines for repeated dose toxicity studies [66]: urinary volume, pH, protein, glucose, ketone bodies, bilirubin, occult blood, microscopic examination of the sediments, SG or osmolality and electrolytes. The Manual of the JMHW guidelines for toxicity studies of drugs [67] states, "Data on urinary volume and urinary constituents can contribute to the evaluation not only of renal function and the state of the urinary tract, but also of cardiac and endocrine function. An increase of normal constituents or the occurrence of abnormal constituents in the body fluids can be detected in urine with great sensitivity and rapidity inasmuch as urine is concentrated in kidneys and the urinary threshold for excretion of abnormal body fluid constituents is usually low. Techniques for urine collection, suitable for meaningful examinations, need to be developed since it can be extremely difficult to obtain adequate volumes of fresh urine samples from test animals as and when required". The lack of a more detailed discussion and explanation for the necessity of each parameter and species differences of this statement is somewhat unclear and leaves room for sounder approaches.

The recommendation of the expert panel of IHCPT for urinalysis determinations is more realistic [101, 102]. The major part of the recommendation is as follows: Uroanalytic tests are often inaccurate due to artifactual changes induced *in vitro* by suboptimal conditions in which timed urine samples are commonly collected from animals in metabolism cages [27, 76, 85, 97]. Optimally, contamination of samples with feed, drinking water, feces, vomit and blood should be minimized. In addition, the pooling of urine samples from animals is not recommended. Urinalyses are influenced by the conditions of collection, such as feeding, fasting, and sampling times. For these reasons, it is recommended that urine be collected in the same manner from concurrent control and treatment groups. Urinalysis should be conducted at least once during a study. It is optimal to conduct and interpret uroanalytic tests with other concurrent clinical laboratory tests. Urine sample quality can be maintained by collecting samples in a cooled container or one containing a suitable preservative. An overnight collection (approximately 16 hr) is recommended for routine analyses. It is recommended that the core tests should include an assessment of urine appearance (color and turbidity), volume, specific gravity or osmolality, pH, and either the quantitative or semi-quantitative determination of total protein and glucose. Microscopic examination of urine sediments and urinary minerals and electrolytes are not recommended in routine screening tests in non-clinical studies [101]. Additional, more sophisticated, tests with appropriate collection procedures may be necessary for evaluating test materials known or suspected of causing renal toxicity [14, 55, 90]. (Part concerning rats is cited.)

Urinary volume and constituents can differ according to the strain [5, 7, 16], gender [3] and age of the animals [1, 4, 9, 15, 28, 30, 69, 91, 100], the composition of feed and housing conditions [18]. Urinary volume is known to have intra-day variations associated with the activity of the animals [80]. Also urine volume is influenced by body weight and size, deit, temperature, and humidity.

Routine methods for qualitative and quantitative determinations of protein, glucose, ketone bodies, and birilubin/urobilinogen are readily available. The qualitative method employing a reagent strip test is commonly used in toxicity studies [21, 27, 77, 97]. Therefore, it is important to know the factors that can yield false positive or negative results and factors which can interfere with the reagent strip test. (See other references.) Fluctuations of urinary constituents and individual variations are greater than those of blood under normal physiological conditions. Therefore, it is often difficult to detect minute, meaningful changes in urinalysis determinations in animals. The effects of drugs on urinalysis should be considered in toxicity studies. Methods of urine collection, technical procedures and appropriate evaluations of urinalysis are reviewed in this paper.

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LITERATURE REVIEW

1. Urine collection and urinary volume

Urine is generally collected in toxicity studies to assess renal function. There are no major problems in urine collection for human urinalysis. Numerous references in the literature describe the effects and interactions of the endocrine system, antidiuretic hormone (ADH) and sympathetic nervous systems on urinary volume.

The causes of oliguria and auria are classified as follows: ⅰ) Pre-renal: decrease in circulating blood (due to hemorrhage, vomiting, diarrhea, edema, shock, decreased blood pressure, etc.), decreased cardiac output and constriction of renal blood vessels; ⅱ) Renal: disorders of glomeruli or renal tubules (due to toxic substances); ⅲ) Post-renal: obliteration of ureters and tumors. Pre-renal and renal oliguria and auria are classified on the basis of urinary osmolality and sodium levels. The osmolality is higher and the sodium level is lower in pre-renal diseases; while they are isotonic and nearly normal in renal diseases.

Conversely, the causes of polyuria are as follows: ⅰ) excessive water consumption, ⅱ) disorder of ADH secretion (central diabetes insipidus), ⅲ) hyporeactivity of urinary ductular cells to ADH (nephrogenic diabetes insipidus), ⅳ) increased osmolality of the urinary filtrate (Henle loop diuresis, hyperglycemia, urinary macromolecular substances, etc.). Urinary osmolality is low in cases ⅰ to ⅲ, ADH-reactive in case ⅱ and non-ADH-reactive in case ⅲ.

Diagnostic criteria of oliguria and polyuria in laboratory animals are not clear. There are variations due to physiological and urinary collection conditions. However, the urinary volume is not only an index of the renal function but also an important factor for quantification of urinary constituents. Therefore, it is important to minimize artifactual variations and to establish a historical database for conditions of urine collection in each laboratory. The urinary volume of rodents is far less than that of humans. Therefore, care must be taken to avoid evaporation of the samples. For analysis of electrolyte levels, it is necessary to dilute samples accurately and to measure within the calibration ranges of the instruments. Urine collection is one of the most difficult procedures to conduct in animal experiments. Therefore, sampling conditions must be taken into consideration for meaningful analysis.

Pooled urine is used to measure urinary volume and to conduct quantitative determinations. In toxicity studies, the urine is generally collected from animals in metabolism cages over a specified period (overnight, 16 to 24 hours). Some problems exist with this method, such as acclimation to metabolism cages and contamination of samples with feed, water, feces, sperm or hair. Results may vary due to conditions of sampling, i.e., presence or absence of an antiseptic, the duration of the collection, feeding and watering. Differences in these conditions should be considered not only for urinary volume but also for other parameters. Urinary volume in particular is markedly decreased due to water deprivation, resulting in fluctuations in electrolyte levels and specific gravity or osmolality. However, when water is provided *ad libitum*, contamination of the sample with water should be a concern. When 24-hour or overnight urine is pooled, evaporation of the urine should be avoided by using collection containers with a narrow mouth. Based on our experiments, approximately 5% of a urine sample may evaporate overnight if a wide-mouth container is used. In addition, it is sometimes necessary to preserve urine samples under light-shielded or refrigerated conditions depending on the parameter of examination. Appropriately designed apparatus for such conditions are commercially available. Although several types of metabolism cages are commonly in use [12, 13, 32, 99], the ideal apparatus has yet to be developed. In order to obtain precise and reliable data, appropriate sampling conditions and the use of well designed metabolism cages are essential. Fresh urine samples obtained by pressing the lower abdomen [33] or taken directly from the urinary bladder are occasionally employed.

In a survey conducted by JPMA [64], urine samples were routinely collected under conditions of feeding and watering; then under just fasting and final under food and water deprivation (Table 1). The most commonly used method is not always the appropriate one. The two most commonly used urinary collection periods are during the daytime for 6 hours and overnight for 16 to 24 hours (Table 2). Daytime urine samples were used mainly for qualitative tests such as pH, glucose, etc. Overnight samples were used for determinations of urine volume, electrolyte levels, etc.

In a survey conducted by JPMA [64, 65], there were considerable variations in historical data on rat urinary volume, most probably reflective of differences in urinary sampling conditions. Some data showed urinary volumes of 60-80 ml/animal/day. Contamination with tap water was suspected in these cases. It is inadvisable to use such data for meaningful evaluation of toxicity. Urinary data on SD rats in a CRO in the US was also investigated and marked variations in urinary volume were also evident. Urinary volume is decreased under conditions of fasting but watering since water consumption decreases during fasting [6, 60]. Fasting during urine collection is considered to be useful when investigating the urine-concentrating ability of the kidneys [101, 102]. However, such results may possibly reflect individual water consumption and not toxic effects on renal function.

Review of approximately 60 recent Japanese publications (1995-1999) revealed that urinalysis has been conducted in numerous repeated dose toxicity studies in rats. It is assumed that the JMHW guidelines were used as the sole criteria for these evaluations. Problems encountered included: only a few reports described the conditions of urine collection; contamination with tap water was suspected in several papers and the urinary volume did not reflect body weight and age in some instances. There is the apprehension that some studies were insufficient for the meaningful evaluations. Relatively well-designed papers are presented in Table 3, but concerns remain for aforementioned viewpoints. Conversely, urinalysis was not conducted or was conducted on only 3-4 parameters in repeated dose toxicity studies in some Western countries [8, 48, 55, 56], indicating the appropriate use of the intent of the guidelines for toxicity studies.

Many investigators have concluded that overnight urine collection under food and water deprivation induces stress in animals and is not desirable from a humanitarian viewpoint. However, urine collection under *ad libitum* feeding and watering conditions cannot yield suitable data for the evaluation of toxicity.

Therefore, it is important to seriously consider the purpose of urine collection. It is recommended that urine be collected from animals that were fasted overnight but which were supplemented with gastric intubations of water to minimize stress and contamination of samples; thereby urinary volume is more reflective of normal and adverse effects on kidney functions. Accurate water loading (per kg body weight) is achievable by this method. With the exception of toxicity studies of diuretics, it is preferable to administer 20-25 ml/kg of water via oral gavage immediately prior to initiation of urine collection under conditions of fasting and water-deprivation. For toxicity studies of diuretics, urine should be collected from animals provided with water via an automatic watering system *ad libitum* designed to avoid contamination of urine with water. Such systems are technically and economically available [25, 57]. This method has been in use by many Western CRO for 10 years or more.

1) Conditions of urine collection for determinations of urinary enzymes

a. Precautions during urine collections

At the time of sampling, gauze, glass wool or paper filters should be placed in metabolism cages to avoid contamination with feed and feces. To avoid contamination with water, it is required that the tip of a water bottle be set out of the cage, at a distance from which rats can drink readily (5-8 mm). Blood sampling should not be performed just prior to urine collection and animals should be observed for hemorrhagic changes and injuries of the limbs to avoid possible contamination.

b. Period of urine collection

Urine should be collected from food-fasted animals within 24 hours in a metabolism cage [82]. Sample collection time should be short when evaluating enzymes levels, particularly those derived from brush borders of the proximal convoluted tubules of the kidney, since peak urinary excretion occurs in approximately 6 hours and long collection times can decrease enzymatic activity.

2) Conditions of urine collection for examination of urinary sediment

Animals should be placed in clean metabolism cages and urine collected in a glass tube for 3 hours. Better packing of the sediment is achieved in glass as opposed to plastic. Care should be taken to avoid contamination with feces. If a minimum of 0.5 ml of urine cannot be collected by this method, urine should be forcedly voided by pressing on the lower abdomen.

Naturally or forcedly voided urine is collected from animals, but a sufficient urine volume cannot always be collected. Sufficient volume of urine from all animals is a prerequistic for urinalysis, so that fresh or 3-hour pooled urine is routinely collected from rats. Usually, 0.5-1 ml of urine can be collected within 3 hours from 80% or more of animals at the ages commonly used in toxicity studies. If naturally voided urine cannot be collected, animals should be held at the cervical region of the neck between the thumb and forefinger and placing the tail between the pinkie and bent backward by pulling the tail to enhance voiding. Care should be taken since this method provides physical stress to animals if technicians are not properly trained. Pressing the lower abdomen is also acceptable [33]. Animals should be deprived of feed and water once they are transferred to metabolism cages.

The method for collecting larger volumes of urine by loading with water immediately prior to transfer to the metabolism cage is described above. In such cases, it is advisable to collect more than 0.5-1 ml of urine to increase the precision of the measurements. Sixteen to 24-hour pooled samples are collected routinely in toxicity studies. The method of Addis is a highly quantitative one for examination of the urinary sediment.

2. Electrolytes (sodium, potassium and chloride)

For determinations of sodium and potassium levels, flamephotometry and ion-selective electrode methods are more commonly used than chemical determination methods, atomic spectrophotometry and enzymatic methods [20]. For the determination of chloride levels, coulometric titration and ion-selective electrode methods are commonly employed (chemical determination method and enzymatic method, less occasionally). Coexisting ions may be present during analyses [25]. The effects of Br^-, in particular, on Cl ionic electrodes should be of concern. Since ionic concentrations are measured by the coulometric titration and ion-selective electrode methods, electrolytes that combine with proteins are not detectable.

Urinary excretion of sodium, potassium and chloride are generally proportional to their respective dietary intake. Urinary electrolytes originate from the consumption of feed. When a large volume of physiological saline is used as a vehicle, the effects on urinary electrolytes should be considered. Electrolyte levels also fluctuate in response to the secretion of hormones participating glomerular filtration, resorption and excretion in the renal tubules and when water/electrolyte balances in blood vary. In rats, bacteria in the cecum participate in the absorption of electrolytes from the intestinal tract. In animals like rats, if the endobacterial flora is altered due to administration of antibiotics, absorption of sodium is inhibited and may result in decreased urinary sodium levels. As described previously, deprivation of water results in markedly decreased urinary volumes, which in turn can result in fluctuations in electrolyte levels and osmolality. Urinary electrolyte levels are generally higher in laboratory animals than in humans and samples from animals are usually diluted prior to analysis. Therefore, dilution should be conducted accurately and analysis should be conducted within the calibration ranges of the equipment. In studies of diuretics, electrolyte levels and specific gravity fluctuations are associated with increased urinary volume. Urinary electrolyte levels also vary according to intra-day variations, the strain and age of rats [7] as well drug administration [48].

3. Specific gravity and osmolality

In measuring specific gravity in animals, problems can be encountered using refractometers or reagent strips intended for human use since factors involved in specific gravity may differ between humans and animals. More reliable data may be obtained by measuring osmolality if sample volume is not an issue.

1) Specific gravity (SG)

Refractometers and reagent strips are routinely used to measure SG. Refractive rates are proportional to the concentrations of urinary solutes and correlate with the SG. Urinary refractom-

etry is readily measurable with a drop of urine. However, corrections may be necessary to correct for the presence of solutes such as glucose and protein.

The effects of the presence of the test substance and its metabolites should not be disregarded in toxicity studies. The principle of paper dipstick testing is to qualify the urinary positive ions (mainly Na^+) using reagent strips containing macromolecular electrolytes (methoxyethylen-maleic anhydride copolymer), buffer and pH indicators. This method has advantages in that corrections for the temperature and glucose are not necessary and can be used concomitantly with other qualitative tests. However, urea, the main determining component for the urinary SG, cannot be measured by this method since urea is not ionized. In addition, this method was developed for human urinalysis. Therefore, it is unclear how the urinary SG of animals, with different electrolyte compositions and pH, is affected. Of particular concern is the fact that the limit of sensitivity of SG in reagent strips is 1.030. However, the SG of rat and dog urine can occasionally exceed this limit and will not correlate with the refractive. Urine with a high pH exhibits a low SG. Coloration and turbidity of urine can be affected by the concentration of urine and must be interpreted with consideration of the urine SG.

2) Osmolality

Freezing and boiling methods can be used to measure osmolality. The former method is most commonly used. In this method, the freezing point depression of solutions are compared to pure water and calculated as follows: osmolality (mOsm/kg) = depression of freezing point (℃)/0.00186. Unlike specific gravity, corrections for temperature, for glucose and protein are not necessary for osmolality determinations.

SG and osmolality are indices of the urinary concentrating ability of the kidneys. In normal animals, urinary volume is inversely proportional to the SG or osmolality. However, this dose not hold true in morbid animals. Measurement of the SG is a common practice. However, the ability to concentrate urine is determined by molecular number (molar number) of solute particles in the urine. Consequently, osmolality measurements have replaced the SG as an index of the kidneys urinary concentrating ability. SG is particularly high when specimens contain protein, glucose, contrast media, etc. and values exceeding 1.050 cannot be measured using the common hydrometer. In such cases, it is necessary to measure osmolality. SG cannot be measured using dilutions of urine samples with high SGs; however, osmolality is measurable in diluted samples.

Osmolality of body fluids is regulated within very narrow ranges; whereas, urinary specific gravity and osmolality may widely vary with water consumption. Therefore, although urinary volume is essential to evaluate abnormalities based on the SG and osmolality, water consumption and their relationship to serum osmolality should also be considered.

4. pH

A pH meter (glass electrode) and reagent strips (uni- or multi-reagent strips) are commonly employed to measure urinary pH. A pH meter is utilized for more accurate measurements. In toxicity studies, multi-reagent strips are most commonly used. The multi-reagent strips contain mixed indicators of methyl red and bromthymol blue and approximate pH values range from 5 (orange) to 9 (blue). Urinary pH is generally higher in pooled than in fresh urine due to the generation of ammonia by bacterial action in the pooling process. Pooled urine samples can reach a pH of 8 or higher under non-aseptic conditions. Xylene, toluene, formalin and thymol are common antiseptics. They should be evaluated to determine potential effects on parameters being evaluated [19, 103]. Urine containing dextrose may decrease pH due to the oxidization capability of bacteria. Therefore, it is necessary to use fresh urine when measuring pH. When a multi-reagent strip is used, the strip should be promptly removed from the sample so as not to leave excessive urine on the strip which could decrease the pH due to elution of acidic buffer from the adjacent protein reagent portion of the strip.

In vivo blood pH is controlled within narrow ranges, due to the buffering capacity of hydrogen ions obtained from feed, water and normal metabolism. Acid-base balance is controlled by the buffering capacity of body fluids, ventilation of the lungs, bicarbonate ion (HCO_3) concentrations in the kidneys, excretion of neutralized acids, etc. Urinary pH is maintained through the intake of hydrogen ions and regulatory mechanisms in the kidneys. The presence of substances in the urine derived from test substances administered should be of concern to the investigation of toxicity studies. As urinary pH is increased following food consumption, it becomes somewhat difficult to evaluate toxic responses based on urinary pH alone.

Highly precise results cannot be anticipated with the use of simple reagent strips unless minimal cautions are followed. The most important aspect to consider when using paper strips is the proper storage and use. Reagent strips should be used as follows: store at room temperature (15-30℃) avoiding direct sunlight and high humidity; do not store under refrigeration once the seal is opened; take out only the necessary number of strips to be use to prevent degradation; avoid all contamination; do not use after the expiration date or if the strips are discolored. Fresh urine is preferable. If the test cannot be conducted immediately after sampling, the urine should be stored under refrigeration and allowed to come to room temperature before testing. It is better not to use: urine samples containing an antiseptic, samples which were stored frozen, supernatant fractions of urine and samples which were allowed to stand at room temperature for prolonged periods. Inter-experimenter differences can be a problem in gross observations using classic colorimetric tables; however, automatic analyzers for reagent strips have improved this problem. Maintenance and quality control of automatic analyzers are necessary; otherwise, even greater errors may occur.

5. Protein

Urinary protein is a sensitive indicator of renal toxicity and an important parameter in toxicity evaluations [5, 69, 90]. Various types of proteins are present in the urine, and albumin is most commonly determined. Recently, low molecular proteins such as β_2-microglobulin have been received attention as an indicator of drug-related renal disorders [2].

Tamm-Horsfall glycoprotein (TH glycoprotein) is secreted specifically from the distal convoluted tubule epithelium cells, and

its relationship with immunomodulating mechanisms has attracted much attention [51].

Reagent strips detect albumin adequately, but show low sensitivity to globulin [24]. Although reagent strips contain buffers, strong alkaline urine is tested after acidification with acetic acid. Other qualitative tests include the sulfosalicylic acid assay. This method is effective in distinguishing false positive results obtained with the reagent strip tests. As a quantitative test, a method of Coomassie brilliant blue G250 containing sodium lauryl sulfate (CBB-SDS method), a chromo-binding method, is widely used due to fewer differences among the protein types.

Proteinuria (albumiuria) is classified as aphysiological condition and a pathologic condition with serious consequences. Physiological proteinuria occurs transiently due to exercise, stress, etc. Orthostatic albuminuria is occasionally seen in humans, but has not been reported in animals. Morbid proteinuria is further classified as pre-renal, renal (gromerular, tubular) and post-renal based on etiological mechanisms. Pre-renal proteinuria, including hemoglobinuria caused by hemolytic anemia, myoglobulinuria caused by myositis, α_1-acid glycoproteinuria seen at inflammation, etc., contain relatively low molecular weight proteins which are concentrated prior to filtration in the gromeruli. Gromerular proteinuria is comprised of proteins exuded from the gromerular basement membrane due to gromerular disorders. As the disorder progresses, macromolecular proteins such as α_2-macroglobulin and IgM are also released. The presence of hematuria supports the diagnosis and prognosis of gromerular proteinuria. Low molecular proteins induced by tubular damage result in tubular proteinuria. Post-renal proteinuria develops in the urinary tract and genital organs and contains proteins derived from white blood cells and secretory exudates of the lymph vessels.

Examination of urinary sediments is thought to be important for the diagnosis of proteinuria.

Protein levels in dogs and rats are greater than those of humans [16]. Negative (−) to positive (+) protein results are commonly observed in dogs and rats. Elevations in urinary protein levels are a common finding in aged rats [4, 7, 28, 69, 100]. Gender and strain differences and effects of diet have been reported for urinary protein in rats [4, 16, 18, 35, 59, 69, 70, 78, 86, 98].

6. Low molecular proteins

Enzyme immunoassays for rat β_2-microglobulin has been established and a kit for β_2-microglobulin (EIA method) is commercially available. Urinary β_2-microglobulin levels were apparently increased as early as 4 days after dosing in rat models with induced renal disorders attained by administering cisplatin and aminoglycoside antibiotics, known inducers of disorders of the proximal convoluted tubules [98]. Urinary β_2-microglobulin in combination with urinary enzymes such as NAG is considered to be more accurate indicators of damage.

7. Phospholipids

Increased phospholipid levels are induced in rats by administering aminoglycoside antibiotics [44]. Therefore, measuring phospholipid levels is useful to assess the state of renal disorders induced by aminoglycoside antibiotics. There are two types of phospholipid determinations, total and fractional phospholipids. Thin-layer chromatography of 24-hour pooled urine samples from rats administered gentamicin at 10 mg/kg/day for 10 days reveals clearly elevated phospholipid fractions compared to control animals. Measurement of urinary phospholipids has the advantage that timing is not a consideration since there is no peak of excretion and excretion levels increase proportionally to dose levels and duration of administration, unlike enzyme determinations. The only disadvantage is one of complexity. Renal toxicity induced by aminoglycoside antibiotics can be readily detected by this method. Measurement of urinary phospholipids is recommended for comparisons of toxic changes at low dose levels due to its high sensitivity.

8. Glucose

In addition to glucose, lactose, fructose, pentose, galactose, etc. are excreted in the urine. Glucose is analyzed routinely and other sugars are rarely analyzed. The reagent strip tests using glucose oxidase-peroxidase reaction (GOD-POD reaction) is specific to glucose. Enzymatic methods using GOD-POD reaction is also major quantitative method of analysis.

In addition to glucosuria seen during hyperglycemia, renal glucosuria also occurs due to decreased resorption from renal tubules and altered gromerular filtration and transient glucosuria due to stress and fever. When glucosuria is evident, blood glucose levels should be determined to confirm whether the animal is hyperglycemia. Normal animal urine usually tests negative for glucose.

9. Ketone bodies

The presence of ketone bodies in the urine results from the catabolism of fatty acids resulting in the formation of substances such as; acetoacetic acid, β-hydroxy butyric acid and acetone. Acetoacetic acid and β-hydroxy butyric acid represent the most commonly found urinary ketone bodies.

A classic reagent strip using the nitroprusside reaction detect acetoacetic acid and acetone but not β-hydroxy butyric acid. Direct enzymatic methods are necessary for purposes of quantification of β-hydroxy butyric acid.

Ketone bodies are excreted in the urine when blood ketone body levels increase. The presence of urinary ketones in humans is indicative of diabetic ketoacidosis, hyperthyroidism, starvation, excessive vomiting and diarrhea, dehydration, fasting and fever. Evaluated urinary ketones is an index of a generalized deteriorated condition such as starvation, severe vomiting and diarrhea. Animal urine is generally negative for ketone bodies.

10. Bilirubin and urobilinogen

Bilirubin and urobilinogen are components of bile. The diagnostic importance of these substances is in the pathologic screening for diseases of the hepatobiliary system. However, bilirubin is regarded as a primary indicator since urobilinogen is difficult to evaluate due to the possibility of its false positive responses.

1) Bilirubin

A diazo reaction is used in both the reagent strips and quantitative analysis. Qualitative analysis for bilirubin includes an oxidation method.

The majority of urinary bilirubin is conjugated. The urine tests

positive for bilirubin when conjugated blirubin is increased in the blood due to disorders of liver function and obstruction of the bile ducts. Bilirubin is excreted in the urine before serum bilirubin levels increase. Therefore, detection of urinary bilirubin is useful in the early diagnosis of acute hepatitis. However, negative results for urinary bilirubin does not necessarily imply the absence of insult of the liver.

Concentrated dog urine under conditions of fasting may test positive for bilirubin. If positive results are obtained, it is necessary to test urinary SG. Bilurubin generally tests negative in the urine of animals.

2) Urobilinogen

There are 2 types of reagent strips using aldehyde and diazo reactions. The former occasionally exhibits false positive responses. As a quantitative test, the modified method of Watson using aldehyde reactions is more appropriate. Decreased urobilinogen cannot be detected with reagent strips.

The general diagnostic utility of urobilinogen determinations involve the detection of disorders of liver function, bile duct obstruction, hyper-biosynthesis of bilirubin (internal hemorrhage, increased hemocytocatheresis), decreased intestinal bacteria, retention of intestinal contents (constipation), etc.

11. Urinary enzymes

Known urinary enzymes in rats include: N-acetyl-β-D-glucosaminidase (NAG), alkaline phosphatase (ALP), glutathione-S-transeferase (GST), β-D-gralactosidase, alanine aminopeptidase (AAP), alanine aminotransferase (GPT), γ-glutamyl transpeptidase (γ-GTP), acid phosphatase (ACP), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH), asparate aminotransferase (GOT) and Na^+- and K^+-ATP-ase [14, 15, 22, 82, 83]. Human urine also contains similar enzymes with similar functions.

In toxicity studies in rats, ACP, ALP, γ-GPT, LDH, GPT, NAG, AAP, etc., were examined [17, 20, 23, 44, 61, 90, 104, 105].

1) Precautions on sample preparation

a. Handling and stability of urine samples

Urine samples should be handles in an ice bath or at approximately 4℃ to prevent deactivation of enzymes. ALP, AAP, γ-GTP and LDH may be deactivated by freezing and thawing; therefore, these parameters should be determined on the day of sampling or after storage at 4℃. AAP, ALP, γ -GTP and LDH are unstable under acidic conditions; while NAG is not stable under alkaline conditions. Both are stable under neutral conditions [46].

b. Removal of activating and inhibitory substances

Urinary sediment components, particularly cellular debris, possess enzymatic activity and therefore should be removed by centrifugation. Dialysis of urine samples is generally unnecessary. However, when the test article is excreted in the urine and inhibits the targeted enzyme activity, the inhibitory substances should be removed by dialysis, filtration with Sephadex or ultrafiltration or dilutions of urine samples.

Organomercurials inhibit enzymes containing sulfhydryl groups, such as NAG. ALP [31], LAP and LDH, which are distributed predominantly in the proximal convoluted tubules and cortex of the medulla, are inhibited in rats administered gentamicin or cephaloridine at high dose levels [84]. Bilirubin, hemoglobin, methylene blue and salicylic acid have been reported to be inhibitors of NAG [34]. Normal values of representative urinary enzyme activities in rats are presented in Table 4.

2) Expression of the results

Enzymatic levels are generally expressed as total amounts excreted over 24 hours. Spot sample can be corrected for activity utilizing creatinine clearance or activities. Creatinine levels are occasionally markedly decreased in cases of renal disorders, resulting in apparent increases in enzymatic activities. Therefore, when urinary enzymatic activities are corrected for creatinine levels, it is desirable to describe creatinine levels as well as enzymatic activities.

3) Factors influencing variations of urinary enzymes

a. Intra-day variations

NAG activities in dogs decrease rapidly after feeding. This is a function of urinary pH and activities decreased at a pH 8 or greater [45]. Therefore, when urine is collected in the evening to the following morning, feed should be provided in the morning. Rats are nocturnal and begin feeding at the start of the dark cycle. Therefore, urinary pH begins to increase with decreased NAG activity in 2-3hrs after lights out. The activity is lower in the urine collected during the night than during the day.

b. Gender differences

The NAG activity in rat urine is higher in males than in females when compared by the total amount of excretion/animal/day. However, there are no differences between males and females when the values are corrected for body weight or creatinine levels, indicating no essential gender differences. The AAP activity in male rats is higher than in females even after correction for body weight and creatinine levels.

c. Aging

Urinary NAG activity increase with age in rats. It has been reported that urinary enzymatic activities, except for ALP, in newborn rats (15-20 days of age) are not suitable for the evaluation of renal toxicity due to underdeveloped enzyme-synthesis pathways [15].

4) Importance of enzymatic activities for diagnosis of renal toxicity

(1) Correlation of enzymatic activities with urinalysis parameters and morphological changes

Generally, changes in low molecular proteins or excretion of enzymes in the urine appear earlier than those of other renal function tests in acute renal disorders. Urinary enzyme levels generally correlate with low molecular weight protein levels. However, both parameters do not always exhibit the same patterns since low molecular protein levels tend to indicate functional abnormalities such as disorders of resorption while fluctuations of enzyme levels reflect direct damage to cells. Urinary excretion of low molecular enzymes such as lysozyme is a good indicator of lowered resorptional capacity of the proximal convoluted tubules and correlates well with low molecular weight proteinuria [82].

There are other examples of correlation between the excretion patterns of urinary enzymes and insult to various areas of the kidneys, i.e., necrosis of the renal papilla and NAG/ALP ratio, disor-

ders of the distal convoluted tubules and LDH [26, 63, 92], etc.

Urinary enzymes are not necessarily good indicators in the evaluation of chronic renal toxicity based on the results of evaluations of biphenyl, carbon tetrachloride, mercury, lead, etc. in rats and humans [55]. The reasons for the lack of sensitivity of urinary enzymes in the evaluation of chronic renal toxicity are as follows: damaged cells during the acute phase are replaced with immature cells; immature cells have insufficient enzyme-synthesizing capabilities or develop tolerance to toxic drugs; enzymes become depleted in damaged cells; some enzyme activities normally increase with age (e.g., rat NAG activity) .

(2) Renal disorders induced by nephrotoxic substances and correlations with urinary enzymes

The relationship between renal disorders induced by nephrotoxins and associated urinary enzyme patterns are described in the literature. The following are representative examples:

a. Antibiotics

The target of aminoglycoside-type antibiotics such as gentamicin are lysosomes, as evident by a dose-dependent increase in the NAG activity [104]. In addition to the lysosome enzyme (NAG), analyses of γ-GTP, AAP, LDH activities and LDH isozymes are also useful.

b. Metals

With a few exceptions such as methyl mercury which inhibits lysosome function, mercuric chloride, chromic hydrochloride, uranyl acetate, etc., target the brush border surfaces of the proximal convoluted tubules resulting in increases in ALP, LDH, GOT, AAP, γ-GTP activities. However, decreases in these activities are commonly observed approximately 1 week after exposure due to cellular regeneration and/or the development of tolerance to these nephrotoxic substances [95].

c. Analgesics and anti-inflammatories

Large volumes of NAG are detected in humans with renal disorders induced by salicylic acid, phenacetin and aspirin. It is believed that useful animal models of human analgesic renal disorders are not available. Aspirin and phenacetin induce disorders of the renal tubules rather than the renal papilla in rats [79]. Brush border enzymes or LDH are good indicators of renal disorders induced by analgesics and anti-inflammatories in rats and NAG and LDH in humans.

5) Differences in urinary enzymes between humans and laboratory animals in the evaluation of renal toxicity

With the exception of some drugs such as analgesics, there are no appreciable differences in the types of enzymes excreted in the urine of humans and laboratory animals with renal disorders. The reasons for this are considered to be due to that there are no great differences in the localization of enzymes in the kidneys between humans and laboratory animals and that structure of the nephron (basic unit of the kidney) is comparable between the species.

Profiles of urinary excretion patterns of enzymes depend not only on types of nephrotoxic substances but also on dosing regimens such as dose levels, route and frequency of administration. Therefore, based on an understanding of the exposure profiles of the test article in humans, appropriate study designs with appropriate dose levels and observation intervals can be selected for animal studies.

12. Urinary sediment

When urine samples are centrifuged at 1500 rpm for 5 minutes, a sediment is formed. This sediment contains various components such as a cellular component (red blood cells, white blood cells, epithelial cells) derived from the urinary system (kidneys, ureters, urinary bladder), casts, precipitated salts, etc. Differences in the incidence and morphology of these components correlate with various disease states of the urinary system. Constituents of the sediment can vary widely in the early stages of onset of general nephritis, with little or no relationship to the progression of nephritis. Therefore, the severity of the disease is difficult to ascertain from examination of the sediment; however, subtle changes can be detected. Nevertheless, renal toxicity in animals can be identified by examination of the urinary sediment [21]. Effects of drugs on the urinary system through repeated administrations may be identified as nephrotoxic effects over time by examinations at appropriate intervals.

1) Components of urinary sediment

The urinary sediment can be examined non-stained or after Sternheimer-Malbin (SM) staining. The sediment from animals is generally examined after staining.

a. Red blood cells

Red blood cells in the sediment: average size, 6-8 μm; colorless to light yellow (non-staining) or colorless to light reddish purple (SM staining); disciform in most cases, confetti-shaped in hypertonic urine due to loss of water and sometimes swollen or ruptured in hypotonic urine (ghost cells). Care should be taken since red blood cells can be easily mistaken for other components (crystals, yeast, fat droplets, etc). Gromerular abnormalities and hemorrhage in the urinary system are suspected if whole red blood cells are observed in the sediment.

b. White blood cells

White blood cells in the sediment: average size, 10-12 μm; colorless to light yellow (non-staining) or pink to purple (SM staining); round. Fresh white blood cells are colorless to light blue even after SM-stained. Bacterial infections and inflammation of the urinary system are suspected, but normal urine also contains a small amount of white blood cells. White blood cells may be mixed in the urine of female animals (rats and mice) according to the stage of the estrous cycle (metestrus to diestrus, stages contain a large number of white blood cells).

c. Epithelial cells

Epithelial cells are classified by form and size as follows: squamous cells, round cells, spindle cells and small round cells (descending order of size). This classification is considered to be practical for toxicity studies, though there is another more detailed classification according to morphology and the volume ratio of the nucleus to cytoplasm (N/C ratio). Epithelial cells are purple red after SM-staining. Squamous cells are more reddish. A small amount of epithelial cells are present in normal urine. The incidence of round epithelial cells is increased in disorders of the renal tubules.

d. Casts

Tamm-Horsfall glycoprotein, secreted from the renal tubules, combines with protein derived from plasma and gells to form cylinders (hyaline casts) in the renal tubules. Hyaline casts contain-

ing other substances (cellular debris) are classified as red or white cell casts, epithelial, granular, waxy, bloody and fatty casts, etc. The urine is generally positive for protein when casts are present. Almost all casts dissolve in alkaline urine. Casts reflect abnormalities of the renal tubules and are indicative of urinary retention (cysts) and recanilization (voiding of cysts). Waxy casts are indicative of severe renal disorders.

e. Crystals

Various types of crystals can be observed in the urinary sediment such as; ammonium magnesium phosphate crystals (tube cap-shaped, rectangular, feather-shaped, readily apparent in alkaline urine), calcium oxalate crystals (regular octahedron, dumbbell-shaped, apparent in acidic or alkaline urine), amino acid crystals, etc. These same salts may be present in various shapes and sizes. A large amount of crystals are observed in normal urine. However, a specific salt may increase due to effects of the test article. Excreted test articles and/or their metabolic by-products may appear as specific crystals. Amino acid crystals are indicative of severe liver disorders.

f. Feed

Some dietary components may be present in the urine and resemble epithelial and white blood cells. Since it is difficult to distinguish them from normal constituents, care should be taken to avoid contamination of urine samples with animal feed.

g. Other

Sperm, bacteria, yeast and fatty droplets may be contained in the sediment.

2) Examinations of urinary sediment in rats

a. Treatment and storage of samples

The reagent strip test should be conducted immediately after sampling to obtain data on pH, occult blood, protein, etc., prior to examination of the sediment. The samples are then mixed with a drop of 10% formalin (approx. 25 μl) as an antiseptic and centrifuged at 1500 rpm for 5 minutes. The supernatant should be discarded (the glass tube is decanted to discard the supernatant and excess urine on the brim of the tube is wiped with tissue paper). The sediment should be stored in a hermetically sealed container under refrigeration (approx. 4℃) in the dark.

Although it is desirable to examine the sediment as early as possible to avoid changes in components of the sediment, microscopy of the sediment cannot always be conducted on the day of sampling due to logistics. It is best to conduct the examination within 2 or 3 days. The supernatant should be discarded prior to storage of the sediment; otherwise, interference may result from precipitated salts from the supernatant.

Some investigators believe that aspiration of the supernatant using an aspirator or pipette to leave a constant volume of sediment is more advisable than decantation. However, the decantation method is also useful to obtain a precise constant volume of sediment when it is conducted carefully according to the prescribed procedures.

A swing-typed centrifuge is desirable. It is believed that values of the sediment obtained using an angle-typed centrifuge are lower than those using a swing-type centrifuge since the sediment deposits at an angle to the bottom of centrifuge tube. Although centrifugation conditions have been set at 1500 rpm for 5 minutes, 500 G for 5 minutes is more desirable.

b. Preparation of the sediment specimens

The sediment sample (10 μl) and 10 μl of Sedi-stain solution (10-fold dilution with distilled water, Becton Dickinson) are stirred gently with a tip in circular motions on a glass slide, covered with a 18 × 18 mm cover glass and submitted for microscopic examination.

When a drop of a stock Sedi-stain solution is added to extremely small quantities of sediment, the specimen is deeply stained making cellular structures and components difficult to identify. Therefore, 10-fold dilutions are preferable. The sediment obtained by decanting can be re-examined using freshly stained specimens at a later time.

Before sampling of the sediment, the tube should be gently tapped against the palm of the hand to loosen the sediment and then shaken lightly. A mixer should not be used to avoid breakage of casts.

Sedi-stain solution is a Sternheimer-Malbin staining solutions commonly used for urinary sediment examinations of neutrophils, epithelial cells, casts, bacteria and other components that are readily and clearly stained with this solution.

c. Microscopy

Areas of microscopic fields vary according to the type of microscope. Therefore, it is advisable to use a standard microscope. Initially, the entire field is observed for appearance of cysts at a magnification of 10 × 10 (objective × ocular). If casts are observed, this field (18 × 18 mm) is regarded as one field and the grade of casts is determined. For red and white blood cells, epithelial cells and crystals, 10 fields are observed at 40 × 10 (objective × ocular) and the numbers per field are calculated to determine the grade according to standard criteria. Other components are observed at appropriate magnifications as appropriate. A magnification of 40 × 10 is commonly used for one field, but not limited to this.

d. Interpretation of the results

The presence of red blood cells and casts in the urine are generally considered to be abnormal. However, the presence of red blood cells is not necessarily abnormal since a small amount are present in the sediment of normal animals. A few white blood and epithelial cells are also routinely observed in untreated animals. Hyaline casts are occasionally observed in the sediment of normal humans. Based on the results of the studies of nephrototoxins in rats, hyaline casts are observed prior to appearance of epithelial, granular and waxy cysts. Therefore, the presence of hyaline casts may be an earlier indicator of the renal disorders.

3) Importance of urinary sediment examinations in the diagnosis of renal toxicity

Kanamycin has been reported to affect the convoluted portion of the renal tubules with increased epithelial cells and cysts in the sediment. The proximal and distal portions of the convoluted tubules are markedly dilatated in rats receiving puromycin aminonucleoside at 15 mg/kg for 2 weeks. Hyaline casts are evident in specimens lightly stained with eosin, but degenerative necrosis of the epithelial cells in the renal tubules is not observed [62].

4) Differences between laboratory animals and humans

Detailed, accurate and time consuming procedures to minimize stress to the animals are inevitable in collecting clean urine samples highly precise evaluations of the urine sediment. It is of utmost importance when conducting urinary sediment examinations to carefully consider the number of animals being evaluated, sampling methods, sample volumes and their potential effects on the outcome of the results to ensure the reliability and interpretations of results. If properly conducted, urinalysis can be useful adjuncts in the overall assessment of nephrotoxicity evaluations.

Since the urine volume from rats is very small, several factors should be considered before proceeding with examinations, including the selection of instruments, sample treatment and specimen preparation. For example, 1 ml glass tubes are preferable for collection since concentrated urine samples are small in volume. To stain extremely small quantities of sediment (approx. 30 μl), it is best to use 10-fold dilutions with distilled water with a commercially available staining solution (Sedi-stain), to mix with an equal volume (10 μ l) of sediment on a glass slide and not to use the staining solution according to routine methods, thereby avoiding deep, excessive staining.

As described previously, microscopic examination of the sediment cannot always be conducted on the day of sampling. Therefore, interpretations should take into consideration the fact that some alternations decreased activity of components, etc. may have occurred from the time of sampling. Based on the results of the studies of nephrototoxins in rats, hyaline casts are observed prior to appearance of epithelial, granular and waxy cysts. Therefore, the presence of hyaline casts may be an earlier indicator of the renal disorders.

Some urinary sediment findings induced by drugs are comparable between animals and humans and some are different. Findings may also vary not only due to species differences but also due to dose levels and time period. Care should be taken for extrapolation of the results to humans since urinary sediment findings may not necessarily reflect the severity of renal disorders.

Table 1. Feeding Condition and Urine Collection Period in Rats by JPMA survey

Tap Water	Diet	Day time	Over night
Give	Give	62 %	59 %
Give	Take	28	35
Take	Give	0	0
Take	Take	10	6

by Matsuzawa 1992.

Table 2. Main Parameters in Urinalysis and Urine Collection Period by JMPA survey

Parameters	Day time %	Over night %
Urinary volume	6	94
Qualitative tests*	68	32
Specific gravity	39	61
Osmolality	11	89
Na, K	8	92
Cl	4	96
Ca	13	87
Inorganic P	25	75
Color	46	54
Deposites	57	43

*: pH, glucose, ketones, bilirubin, protein, occult blood, urobilinogen Daytime: 6 hrs after dosing, Overnight: 16-24 hrs after dosing

Table 3. Volumes of rat urine collected overnight of repeated dose toxicity studies in published reports

Strain	Age	No. of	Body weight, mean±S.D, g		Urine volume, mean±S.D, ml		Food	Tap	Collection	References
	weeks	rats/sex	Male	Female	Male	Female		Water	time, hr	
Jcl:SD	31	12	761±63.0	402±53.3	21.9±14.54	14.4±3.70			21	96
Crj:CD	57	20	705±88.0	446±65.0	13.3±6.72	7.8±3.83	take	give	20	109
Slc:SD	10	10	291±17	182±12	7.92±2.22	6.44±2.77			24	50
	15	10	433±25	247±16	10.52±2.94	8.52±2.62				
Scl:SD	31	19-20	494±43	265±22	11.03±2.24	10.76±4.06			24	29
	40	10	625±56	322±33	12.90±3.11	13.26±3.08				
Scl:SD	9	10	337.0±19.16	213±9.69	14±6.4	10±4.8	take		16	52
Scl:SD	19	10	468.6±23.4	271.2±21.8	17.10±2.52	14.65±4.25	give	give	24	38
	24	10	533.7±51.3	278.9±29.4	17.32±1.95	14.35±4.82				
Slc:SD	10	12	317.6±19.9	198.4±12.3	114±2.5	10.7±2.7	give	give	20	72
	14	8	400.1±25.3	236.1±14.1	10.5±1.6	6.8±2.1				
Slc:SD	19	10	475.40±61.07	258.1±16.8	17.68±2.45	14.58±7.00	give		24	71
	24	10	497.90±48.67	277.2±36.17	12.64±2.25	11.63±4.87				
Jcl:SD	10	10	330.91±15.75	208.54±13.30	8.84±3.31	11.54±6.42	take	give	18	110
	14	10	426.37±46.74	251.30±13.30	15.75±8.44	8.43±8.38				
Crl:F344/BR	9	10	185.9±17.9	139.7±4.3	3.5±0.5	2.0±0.5	take	take	16	113
	13	5	229.6±14.6	149.1±6.5	8.5±2.5	7.0±2.0				
	31	20	359.5±21.7	183.3±11.3	5.1±1.5	4.5±3.0				
Jcl:SD	20	10	521.14±40.10	285.66±20.89	11.48±3.16	5.23±1.75	take	give	18	73
	33	10	608.04±43.80	335.28±27.56	11.50±3.49	8.22±4.81				
	37	9	628.00±58.43	348.07±27.82	21.99±15.41	11.08±8.89				

Body weight: at autopsy, Blank: No information or unclear information, take: deprived, give: provided

Table 3. Volumes of rat urine collected overnight of repeated dose toxicity studies in published reports (continued)

Strain	Age weeks	No. of rats /sex	Body weight, mean±S.D, g		Urine volume, mean±S.D, ml		Food	Tap Water	Collection time, hr	References
			Male	Female	Male	Female				
Jcl:SD	10	10	337.19±31.93	205.28±14.96	11.00±7.09	11.33±7.89	take	give		111
	14	10	451.54±41.83	247.73±17.83	10.00±2.65	5.34±2.15				
Jcl:Wistar	18	16	385.5±23.6	243.4±17.8	10.6±5.4	14.7±6.0		give	24	107
	24	6	402.7±28.2	253.8±13.9	10.2±4.0	17.0±11.8				
Scl:Wistar	58	30	489±25	280±20	3.9±1.5	5.9±1.9		give	16	108
	66	7	511±14	283±13	3.9±1.4	6.5±1.6				
Crj:CD	11	10	330±16	212±18	21±10	15±7			24	37
	33	10	612±62	304±36	29±5	23±9				
	37	10	658±57	329±29	30±11	21±10				
Crj:CD	32	20	605.6±64.9	309.6±42.8	17.26±6.12	9.96±2.58	take	give	22	40
	36	8	624.1±64.4	307.6±25.6	14.23±4.76	6.94±2.93				
OFA:SD	20	20	599.3±62.75	335.1±22.83	9.73±1.38	8.85±2.43				10
Slc:SD	18		437.7±33.2	236.1±12.9	10.58±2.44	11.07±3.69	give		21	49
	31		503.5±30.6	257.3±14.1	10.95±3.60	15.90±7.97				
Slc:Wistar	58	8	410.2±22.0	243.4±20.6	8.5±	7.0±	give		21	89
	62	8	410.0±22.9	233.1±19.0	9.8±	8.1±				
Scl:Wistar	19	16	310.6±15.1	175.8±8.2	8.0±	9.4±	give		21	89
	23	6	356.3±13.4	192.8±11.8	6.0±	7.3±				
OFA:SD	60	16	742.9±89.28	444.0±84.60	9.34±2.047	8.31±3.525			18	11
	68	8	779.6±79.40	482.3±62.35	11.91±2.084	8.49±1.518				

Body weight: at autopsy, Blank: No information or unclear information, take: deprived, give: provided

Table 3. Volumes of rat urine collected overnight of repeated dose toxicity studies in published reports (continued)

Strain	Age weeks	No. of rats /sex	Body weight, mean±S.D, g		Urine volume, mean±S.D, ml		Food	Tap Water	Collection time, hr	References
			Male	Female	Male	Female				
Crj:CD	19	12	490.1±48.4	288.5±24.6	18.4±8.9	9.0±3.9	give	give	24	93
	32	12	597.4±60.3	347.6±42.7	18.3±7.3	10.8±3.7				
	36	8	652.3±68.1	337.5±34.7	19.4±8.9	11.3±6.1				
Crj:CD	10	10	345.8±34.1	219.5±18.9	16.2±4.5	7.4±4.0	give	give	24	94
	14	6	431.9±49.3	255.3±18.3	17.7±7.0	11.9±3.0				
	10	16	266.3±29.8	183.8±15.1	9.9±2.4	8.5±2.4				
	14	6	369.7±26.3	239.2±25.2	18.0±3.7	11.7±4.7				
Crj:F344・Du	9	10	171.0±7.7	130.2±4.8	6.5±1.2	9.1±1.7			24	39
	13	6	249.0±11.0	168.7±3.8	7.4±1.6	6.6±2.1				
Crl:CD/BR	19	10	555±49	303±20	2.7±1.2	2.0±1.6			24	114
	23	10	583±50	337±24	3.2±1.6	2.5±0.7				
Slc:SD	9	5	324.8±24.0	214.6±12.1	14.9±2.3	11.3±2.3			24	87
	13	5	427.2±20.1	260.7±21.8	15.7±3.6	10.9±1.9				
Slc:SD	32	25	510.3±51.8	289.7±20.2	14.1±2.9	13.5±6.2			24	42
	40	5	571.4±78.0	307.7±28.0	15.6±3.2	11.5±3.8				
Slc:SD	9	10	341.6±18.4	211.4±12.5	16.5±2.3	13.1±4.7			24	43
Crj:CD	18	16	545.3±33.2	283.4±20.4	9.7±2.9	7.8±3.7			18	47
	22	6	587.5±28.1	297.9±19.2	12.4±4.0	9.0±4.1				
Jcl:SD	58	10	719±68.4	443±59.0	16.7±11.3	12.2±4.8	give		17	68
	62	8	731±94.6	462±49.3	15.4±6.0	29.2±10.0				
Crj:F344/Du	18	6	269±10	163±6	15.4±0.3	12.7±5.3			24	112

Body weight: at autopsy, Blank: No information or unclear information, take: deprived, give: provided

Table 4. Urinary enzyme activity in rats (Price et al, 1982)

Parameters[c]	Mean±S.D.
β-glucosidase	0.21±0.05a
β-galactosidase	2.7±0.9a
NAG	1±0.4a
ALP	1.1±0.62a
ACP	2.4±2.7a
LDH	199000±1000b

a: μ moles 4-methylumbelliferon/h/24hr urine
b: I.U./24hr urine
c: No.of rats used: 25

REFERENCES

1. Ali, B. H., Bashir, A. K. and Mugamer, I. T. El. Et al. 1996. Gentamicin nephrotoxicity in the rat: influence of age and diabetes mellitus. Human & Exptl Toxicol. 15: 51-55.
2. Allchin, J. P and Evans, G. O. 1986. A simple rapid method for the detection of rat urinary proteins by agarose electrophoresis and nigrosine staining. Lab. Anim. 20: 202-205.
3. Alt, J. M., 1980. The study of urinary protein excretion in male rats as compared with human proteinuria. Contr. Nephrol. 19: 79-87.
4. Alt, J. M., Hackbarth, H. and Deerberg, F. 1980. Proteinuria in rats in relation to age-dependent renal changes. Lab. Anim. 14: 95-101.
5. Alt, J. M., Maess, B. and Hackbarth, H. 1985. Species and strain differences in urinary protein excretion. Renal Physiol., Basel. 8: 301-309.
6. Apostolou, A., Saidt, L. and Brown, W. R. 1976. Effect of overnight fasting of young rats on water consumption, body weight, blood sampling, and blood composition. Lab. Anim. Sci. 26: 959-960.
7. Bellamy, D., Lamming, P. A. and Stevenson, A. 1970. Daily patterns of feeding and excretion in rats confined to metabolism cages: strain and age differences. Lab. Anim. 4: 215-225.
8. Bellringer, M. E., Smith, T. G. and Read, R. et al. 1995. β-Cyclodextrin: 52-week toxicity studies in the rat and dog. Food Chem. Toxicol. 33: 367-376.
9. Bengele, H, H., Mathias, R. S. and Perkins, J. H. et al. 1981. Urinary concentrating defect in the aged rat. Am. J. Physiol. 240: F147-F150
10. Bezancon , M. and Mizuno, H. 1998. A 6-month oral toxicity study of mizolastine in rats. Jpn. Pharmaol. Ther (Jpn). 26: suppl. 573-590.
11. Bezancon, M. and Tanaka, E. 1998. A 12-month oral toxicity study of mizolastine in rats with a recovery period of one month. Jpn. Pharmacol. Ther (Jpn). Suppl. 611-631.
12. Bla Blacck, W. D. and Claxton, M. J. 1979. A simple, reliable and inexpensive method for the collection of rat urine. Lab. Anim. Sci. 29: 25~254
13. Blass, E. M. 1972 An improved rat metabolism cage. Physiol. Behav. 9: 68l-683.
14. Bovee, K. C. 1986. Renal function and laboratory evaluation. Toxicol. Pathol. 14: 26-36.
15. Braunlich, H. and Fleck, C. 1981. Urinary enzyme excretion as an indicator of nephrotoxicity. Exp. Path. 20: 182-187.
16. Brdicko, R. 1984. Urinary proteins: interstrain differences in the laboratory rat. 15: 79-83.
17. Brown, D., Brown, J. and Whiting, P. H. 1996. Kidney and urine enzyme activity in experimental gentamicin nephrotoxicity in the Sprague-Dawley rat. Biochemical Sci. Transact. 24: 3175.
18. Camp, I. V., Ritskes-Hoitinga, J. and Lemmens, A. G. et al. 1990. Diet-induced nephrocalcenosis and urinary excretion of albumin in female rats. Lab. Anim. 24: 137-141.
19. Carolyn, S. L. 1975. A comparative study of two methods of urine preservation. Clin. Biochem. 8: 108-117.
20. Chahwala, S. B. and Harpur, E. S. 1983. Gentamicin-induced hypercalciuria in the rat. Acta Pharmacol. et toxicol. 53: 358-362.
21. Christenson, R. H., Tucker, J. A. and Allen, E. 1985. Results of dipstick tests, visual inspection, microscopic examination of urine sediment, and microbiological cultures of urine compared for simplifying urinalysis. Clin. Chem. 3: 448-450.
22. Dance, N., Price, R. G. and Robinson, D. et al. 1969. Galactosidase, β-glucosidase and N-acetyl-β-glucosaminidase in human kidney. Clin. Chim. Acta., 24: 189-197.
23. Davies, D. T. 1992. Enzymology in preclinical safety evaluation. Toxicol Pathol. 20 (3), Part 2, 501-505.
24. Doetsch, K. and Gadsden, R. H. 1973. Determination of total urinary protein, combining lowry sensitity and biuret specifity. Clin. Chem. 19: 1170-1178.
25. Durand-Cavagna, G., Owen, R. A. and Gordon, L.R. et al. 1992. Urothelial hyperplasia induced by carbonic anhydrase inhibitors (CAIs) in animals and its relationship to urinary Na and pH. Fund. Appl. Toxicol. 18: 137-143.
26. Ellis, B. G. and Price, R. G. 1975 Urinary enzyme excretion during renal papillary necrosis induced in rats with ethyleneimine. Chem. -Biol. Interact. 11: 473-482.
27. Evans, G.O. and Parsons, C. E. 1986. Potential errors in the measurement of total protein in male rat urine using test strips. Lab. Anim. 20: 27-31.
28. Everitt, A. V., Porter, B. D. and Wyndham, J. R. 1982. Effects of caloric intake and dietary composition on the development of protein, age-associated renal disease and longevity in the male rat, Gerontol. 28: 168-175.
29. Furukawa, S., Nishida, I. and Kikumori, M. et al. 1995. Twenty-six-week intravenous toxicity study of montirelin hydrate (NS-3) in rats followed by 9-week recovery test. J. Toxicol. Sci. (Jpn), 20: suppl. Ⅱ 191- 213.
30. Goldstein, R. S., Tarloff, J. B. and Hook, J. B. 1988. Age-related nephropathy in laboratory rats. FASEB J. 2: 2241-2251.
31. Guder, W. G., and Ross, B. D. 1984. Enzyme distribution along the nephron. Kidney Int. 26: 101-111.
32. Hansen, L., and HoIm, H. 1971. Apparatus for collecting feces and urine from rats in metabolic experiments. Lab. Anim. 5, 221-224.

33. Hayashi, S., and Sakaguchi, T. 1975. Capillary tube urinalysis for small animals. Lab. Anim. Sci. 25: 781-782.
34. Horak, E., Hopfer, S. M. and Sunderman Jr., F. W. 1981. Spectrophotometric assay for urinary N-acetyl-p-D-glucosaminidase activity. Clin. Chem., 27: 1180-1185.
35. Husman, B., Norstedt G. and Mode, A. et al. 1985. The mode of growth hormone administration is of major importance for the excretion of the major male rat urinay proteins. Molecular Cellular Endocrinol. 40: 205-210.
36. Ikegami, J., Sano, J. and Sadanaga, O. et al. 1995. Toxicity studies of KW-4679. - A 13-week repeated dose toxicity study in rats-Clin Report (Jpn) 29: 4749-4767.
37. Inoue, H., Mikami, S. and Kakamu, S. et al. 1996. Six months repeated dose toxicity study of percutaneously applied recombinant human basic fibroblast growth factor (KCB-1) in rats. -Followed by 4-week recovery test-. Clin Report (Jpn). 30: 1613-1645.
38. Ishibashi, S., Nakazawa, M. and Tawaratani, T. et al. 1996. A 13-week oral toxicity study of prulifloxacin (NM441) in rats followed by a 5-week recovery test. J. Toxicol. Sci. (Jpn), 21: suppl. Ⅰ. 89-111.
39. Ishii, S., Umemura, A. and Katayama, K. et al. 1995. Toxicity studies of GA-1080, a new TPN solution (5). - Four-week intravenous dose toxicity study of GA-1080 (L) with four-week recovery period in rats-. Jpn. Pharmacol. Ther (Jpn). 23: suppl. 2371-2401.
40. Ito, A., Yoshida, M. and Suzuki, K. et al. 1997. Six-month intravenous toxicity study of CR1505 in rats with and without one-month recovery study. Jpn. Pharmacol. Ther (Jpn) 25: 2597-2618.
41. Ito, F., Abuuchi, K. and Ohno, H. et l. 1998. A single and 2-week intravenous repeated dose toxicity studies of disodium mercaptoundecahydro-closo-dodeca-borate in rats. J. Toxicol. Sci. 23: 588-612.
42. Itoh, T., Deki, T. and Uchiyama, T. et al. 1995. Six-month intraperitoneal toxicity study of AVS in rats with two-month post-treatment recovery period. Jpn Pharmacol. Ther. (Jpn) 23: suppl. 69-102.
43. Itoh, T., Furuno, T. and Sugimoto, T. et al. 1995. One month intraperitoneal toxicity study of AVS in rats. Jpn Pharmacol. Ther. (Jpn) 23: suppl. 21-39.
44. Josepovitz, C., Levine, R. and Lane, B. et al. 1985. Contrasting efects of gentamicin and mercuric chloride on urinary excretion of enzymes and phospholipids in the rat. Lab. Invest. 52: 375-386.
45. Jung, K., Pergande, M. and Scfroder, K. 1982. Influence of pH on the activity of enzymes in urine at 37℃. Clin. Chem. 28: 1814.
46. Jung, K. and Sholz, D. 1980. An optimized assay of alanine aminopeptidase activity in urine. Clin. Chem. 26: 1251-1254.
47. Kashima, H., Aoyama, N. and Hamada, H. et al. 1996. Three-month oral toxicity study of azasetron hydrochloride in rats followed by 1-monh recovery test. Ohyou Yakuri (Jpn) 51: 263-285.
48. Kedderis, L. B., Bozigian, H. P. and Kleeman, J. M. et al. 1995. Toxicity of the protein kinase C inhibitor safingol administered alone and in combination with chemotherapeutic agents. Fund. Appl. Toxicol. 25: 201-217.
49. Kiguchi, M. and Shigeno, H. 1999. Ninety-day intravenous toxicity study of amrubicin hydrochloride (SM-5887) in rats followed by 90-day recovery study. Jpn. Pharmacol. Ther. (Jpn) 27: suppl. 91-110.
50. Kikumori, M., Nishida, I. and Kojima, M. 1995. Five-week intravenous toxicity study of montirelin hydrate (NS-3) in rats followed by 4-week recovery test. J. Toxicol. Sci. (Jpn), 20: suppl. Ⅱ 171- 189.
51. Kimura, K., Ogawa. S. and Watanabe, N. 1991. et al Immunohistochemical localization of physiologic urinary proteins in Wistar rats. Lab. Anim. Sci. 41: 466-470.
52. Kitagaki, T., Suzuki, T. and Koike, Y. et al. 1996. A 4-week repeated percutaneous dose toxicity study of calcipotriol (MC903) followed by a 4-week recovery test in rats. J. Toxicol. Sci. (Jpn), 21: suppl. Ⅱ 287-307.
53. Kitagaki, T., Suzuki, T. and Koike, Y. et al. 1996. A 26-week repeated percutaneous dose toxicity study of calcipotriol (MC903) followed by a 5-week recovery test in rats. J. Toxicol. Sci. (Jpn), 21: suppl. Ⅱ 325-343.
54. Kitagaki, T., Suzuki, T. and Koike, Y. et al. 1996. A 26-week repeated percutaneous dose toxicity study of calcipotriol (MC903) in rats. J. Toxicol. Sci. (Jpn), 21: suppl. Ⅱ 345-363.
55. Kluwe, W. M. 1981. Renal function tests as indicators of kidney injury in subacute toxicity studies. Tox. Appl. Pharmacol. 57: 414-424
56. Knight, E. V., Kimball, J. P. and Keenan, C. M. et al. 1996. Preclinical toxicity evaluation of tepoxalin, a dual inhibitor of cyclooxygenase and 5-lipoxygenase, in Sprague-Dawley rats and beagle dogs. Fund. Appl. Toxicol 33: 38-48.
57. Kobayashi, S., Clemmons, D. R. and Nogami, H. et al. 1995. Tubular hypertrophy due to work load induced by furosemide is associated with increases of IGF-1 and IGFBP-1. Kidney Int. 47: 818-828.
58. Koguchi, A., Kawai,Y. and Suetake, K. et al. 1997. A 4-wek and a 26-week repeated oral toxicity studies of betotastine besilate in rats. Ohyou Yakuri (Jpn) 53: 299-318.
59. Lawrence, G. M. and Brewer, D. B. 1981. Normal urinary protein composition in the female Wistar rat and its relationship to the proteinuria induced by intraperitoneal bovine albumin. Clin. Sci. 60: 693-702.
60. Lee, K. M., Reed, L. L., Bove, D. L. and Dill, J. A. 1998. Effects of water dilution, housing, and food on rat urine collected from the metabolism cage. Lab. Animal. Sci. 48: 520-525.
61. Mandal, A. K., Saklayen, M. G. and Taylor, C. A. 1991. An electron microscopy study of urinary sediment: Relationship between myeloid body excretion and gentamicin nephrotoxity in the rat. Renal Failure 13: 259-266.
62. Marek, K., and Wocka-Marek, T. 1994. Beta-N-acetyloglucosaminidase in urine as a sign of kidney damage in workers exposed to metallic mercury. Med. Pr. 5: 101-105.

63. Maruhn. D., Paar, D. D. and Hartmann, H. G. et al. 1980. Enzyme activities in urine in acute experimental renal damage. J. Clin. Chem. Clin. Biochem. 18: 686-687.
64. Matsuzawa, T. 1992. Present status of animal clinical pathology examinations in the Japanese Pharmaceutical Manufacturers Association. Toxicol. Pathol. 20. Part 3, 528-533.
65. Matsuzawa, T., Nomura, M. and Unno, T. 1993. Clinical pathology reference ranges of laboratory animals. J. Vet. Med. Sci. 55: 351-362.
66. Ministry of Health and Welfare in Japan, Notfication No. 24 of the Pharmaceutcal Affairs Bureau. 1989. Guidelines for toxicity studies of drug.
67. Ministry of Health and Welfare in Japan, Bureau. 1997. Explanation of guidelines for non-clinical studies of pharmaceuticals.
68. Nagai, A., Miyazaki, M. and Sanzen, T. et al. 1998. Twelve-month oral repeated dose toxicity study of osutidine (T-593), a novel antiulcer agent, in rats. Ohyou Yakuri (Jpn). 55: 149-168.
69. Neuhaus, O.W., and Flory, W. 1978. Age-dependent changes in the excretion of urinary proteins by the rat. Nephron. 22: 570-576.
70. Nikaido, H., Yamada, J. and Kondo, Y. 1992. Male urinary protein-1 (MUP-1) in the rat. J. Heredity 73: 119-122.
71. Nishiguchi, Y., Adachi, T. and Nakazawa, M. et al. 1997. A 13-week oral repeated dose toxicity study of (±)-4-diethylamino-1, 1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride monohydrate (NS-21), a novel drug for urinary frequency and incontinence, in rats followed by a 5-week recovery test. J. Toxicol. Sci (Jpn). 22: suppl. Ⅰ. 27-57.
72. Nishimura, N., Fukuda, K. and Yamazaki, S. et al. 1996. A 4-week oral toxicity study of prulifloxacin (NM441) in rats followed by a 4-week recovery test. J. Toxicol. Sci. (Jpn). 21: suppl. Ⅰ. 45-70.
73. Okamoto, M., Ide, M., Suzuki, H., Ishii, T. et al. 1998. Toxicity studies of sodium n-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetate tetrahydrate (ono-5046・Na) (5) 6-month repeated dose intravenous toxicity study in dogs with 1-month recovery test. J. Toxicol. Sci. 23: suppl. 483-501.
74. Okasaki, K., Baba, S. and Ikeda, H. et al. 1997. A 26-week oral repeated dose toxicity study of (±) -4-diethylamino-1, 1-dimethylbut-2-yn-1-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate monohydrochloride monohydrate (NS-21), a novel drug for urinary frequency and incontinence, in rats followed by a 9-week recovery test. J. Toxicol. Sci (Jpn). 22: suppl. Ⅰ. 59-92.
75. Onimaru, T., Chihaya, Y. and Nagata, R. et al. 1995. Four-week subacute intravenous toxicity study of MC-7 in rats followed by 4-week recovery test. Clin Report (Jpn) 29: 4667-4705.
76. Osborne, C. A., and Stevens, 1. B. 1981. Handbook of Canine and Feline Urinalysis, pp. 57-120. Ralston Purina, St. Louis, MO.
77. Paquignon, A., Tran, G. and Provost, J. P. 1993. Evaluation of the Clinitek 200 urinary test-strip reader in the analysis of dog and rat urines in pre-clinical toxicology studies. Lab. Anim. 27: 240-246.
78. Perry, S. W. 1965. Proteinuria in the Wistar rat. J. Path. Bact. 89: 729-733.
79. Plummer, D. T., Leathwood. P. D. and Blake, M. E. 1975. Urinary enzymes and kidney damage by aspirin and phenacetin. Chem. -Biol. Interact. 10: 277-284.
80. Pons, M., Schecko, A. and Witte, K. et al. 1996. Circadian rhythms in renal function in hypertensive TGR (mRen-2) 27 rats and their normotensive controls. Am. Physiol. Soci. 271: R1002−R1008.
81. Price, R. and Ellis, B. G. 1976. Urinary enzyme excretion in amino-nucleoside nephrosis in rats. Chem-Biol. Int. 13: 353-358.
82. Price, R. G. 1982. Urinary enzymes, nephrotoxicity and renal disease. Toxicol., 23: 99-134.
83. Raab, W. P. 1972. Diagnostic value of urinary enzyme determinations. Clin. Chemist 18: 5-25.
84. Raab, W., et al. 1976. Renal effects of gentamicin and cephaloridine. Arzneim. -Forsch. 26: 377-379.
85. Ragan, H. A. 1989. Markers of renal function and injury. In Loeb WF and Quimby FW (eds), The clinical chemistry of laboratory animals. pp. 321-343. Pergamon, New York,
86. Sands, J.M., Naruse, M. and Jacobs, J. D. et al. 1996. Changes in aquaporin-2 protein contribute to the urine concentrating defect in rats fed a low-protein diet. J. Clin. Invest. 97: 2808-2814.
87. Sakamaki, Y., Matsuzawa, T. and Koizumi, T. et al. 1995. 1-month oral toxicity study of balofloxacin (Q-35) in rats. Jpn. Pharmacol Ther. 23: suppl. 1529-1549. (Jpn)
88. Sakamaki, Y., Matsuzawa, T. and Koizumi, T. et al. 1995. 6-month oral toxicity study of balofloxacin (Q-35) in rats. Jpn. Pharmacol Ther (Jpn). 23: suppl. 1551-1568.
89. Sato, M. Tuchida, T. and Kimura, M. et al. 19995. Toxicity study of TTC-909 (4th Report). -52- week chronic intravenous toxicity study of TTC-909 in rats. Clin Report (Jpn). 29: 3027-3056.
90. Stonard. M. D., Gore, C. W. and Oliver, G. J. A. 1987. Urinary enzyme and protein patterns as indicators of injury to different regions of the kidney. Fund. Appl. Toxicol. 9: 339-351.
91. Stonard, M. D. 1990. Assessment of renal function and damage in animal species. J. Appl Toxicol 10: 267-274.
92. Stroo, W. E., et al. 1977. Enzymes of renal origin in urine as indicators of nephrotoxicity. Toxicol. Appl. Pharmacol. 39: 423-434.
93. Suetake, K., Furukawa, H. and Ohta, H. et al. 1996. Six-month subcutaneous toxicity study of SM-10902 in rats. Clin Report (Jpn) 30: 1273-1308 (Jpn).
94. Suetake, K., Furukawa, H. and Ohata, H. et al. 1996. One-month toxicity study of SM-10902 and SM-10902 ointment in rats. Clin Report (Jpn) 30: 1191-1227 (Jpn).
95. Sugihira, N., Sagai, M. and Suzuki, K. T. 1987. Renal damage induced by cadmium-metallothionein: effects on biochemical indicators. Toxicol. 44: 1-11
96. Takahashi, K., Iso, K. and Okabe, E. 1997. et al. Six-

month repeated dose oral toxicity study of rolipram in rats. Clin Report (Jpn) 31: 1465-1490.

97. Taylor, D. M., and Neal, D. L. 1982. False negative hypergiucosuria test-strip reactions in laboratory mice. Lab. Anim. 16, 192-197.
98. Vandoren, G., Mertens, B. and Heyns, W. et al. 1983. Differene forms of α_{2u}-globulin in male and female rat urine. Eur. J. Biochem. 134: 175-181.
99. Walker, R. L and Olson, M. E. 1995. Renal function in the laboratory rat: A student exercise. Am. Physiol. Soc. 13: S49-S55.
100. Weaver, R. V., Gray, J. E. and Schultz, T. R. 1975. Urinary proteins in Sprague-Dawley rats with chronic progressive nephrosis. Lab. Anim. Sci. 251: 705-710.
101. Weingand, K., Bloom, J., Carakostas, M., Hall, R., Heifrich, M., Latimer, K., Levine, B., Neptun, D., Rebar, Stitzel, K., and Troup, C. 1992. Clinical pathology testing recommendations for nonclinical toxicity and safety studies. Toxicol Pathol. 20 (3), Part 2, 539-543.
102. Weingand, K., Brown, G., and Hall. R. et al. 1996. Harmonization of animal clinical pathology testing in toxicity and safety studies, Fund, Appl. Toxicol. 29: 198-201.
103. West, R. W. and Jackson, C. D. 1981. Saccharin effects on the urinary physiology and urothelium of the rat when administered in diet or drinking water. Toxicol. Letter. 7: 409-416.
104. Whiting, P. H. and Brown, P. A. J. 1996. The relationship between enzymuria and kidney enzyme activities in experimental gentamicin nephrotoxicity. Renal Failure 18: 899-909.
105. Wright, P. J, Leathwood, P. D. and Plummer, D. T. 1972. Enzyme in rat urine: alkaline phosphatase 317-327.
106. Yagi, K., Tuchida, T. and Kimura, M. et al. 1995. Toxicity study of TTC-909 (2nd Report). -13- week subacute intraveous toxicity study of TTC-909 in rats. Clin Report (Jpn). 29: 2973-3000.
107. Yagi, K., Takenaka, C. and Sakai, S. et al. 1998. Toxicity studies of (+)-(5Z, 7E)-26, 26, 26, 27, 27, 27-hexafluoro-9, 10-secocholesta-5, 7, 10 (19)-triene-1α, 3β, 25-triol (ST-630) (the 2nd report)-Three-month oral repeated dose toxicity study in rats-. Clin Report (Jpn) 30: 2709-2738.
108. Yagi, K., Shirane, R., and Kimura, M. et al. 1998. Toxicity studies of (+)-(5Z, 7E)-26, 26, 26, 27, 27, 27-hexafluoro-9, 10-secocholesta-5, 7, 10 (19)-triene-1α, 3β, 25-triol (ST-630) (the 4th report)-Twelve-month oral repeated dose toxicity study in rats-. Clin Report (Jpn) 30: 2759-2789.
109. Yamada, T., Yamaguchi, T. and Miyata, K. et al. 1997. Twelve-month repeated oral toxicity study of SM-9018 in rats. Clin Report (Jpn). 31: 309-358.
110. Yamaguchi, K., Yanagi, H. and Shimizu, K. et al. 1997. Toxicity studies of landiolol hydrochloride (ONO-1101) (2) 4-week repeated dose intravenous toxicity study in rats with 4-week recovery test. J. Toxicol. Sci (Jpn). 22:suppl. Ⅲ, 443-461.
111. Yamaguchi, K., Yanagi, H. and Shimzu, K. et al. 1998. Toxicity study of sodium n-[2-[4-(2, 2-dimethylpropionyloxy) phenylsulfonylamino] benzoyl] aminoacetate tetrahydrate (ONO-5046・Na) (2) 4-week repeated dose intravenous toxicity study in rats with 4-week recovery test. J. Toxicol. Sci (Jpn). 23: suppl. Ⅲ 416-434.
112. Yamamoto, M., Takashima, S. and Hayashi, T. et al. 1995. Toxicity studies of GA-1080, a new TPN solution (9). - Three-month intravenous dose toxicity study of GA-1080 (H) in rats-. Jpn. Pharmacol. Ther (Jpn). 23: suppl. 2531-2552.
113. Yanagisawa, T., Maemura, S. and Sasaki, H. et al 1998. Subacute and chronic toxicity studies of triethylenetetramine dihydrochloride (TJA-250) by oral adminstration to F-344 rats. J. Toxicol. Sci. 23 (Jpn): suppl. Ⅳ, 619-642.
114. Yoshinaga, K., Maemura, S. and Atkinson, C. et al. 1995. A 13-week repeated oral dose toxicity study of TUMURA sho-seiryou-to (TJ-19) in rats with a 4-week recovery period. Jpn. Pharmacol. Ther. 23 (Jpn). suppl. 1777-1792.

CHAPTER 2

General Toxicology Related To

Spontaneous Lesions in Crj:CD(SD)IGS Rats Versus in Jcl:SD Rats Used in Toxicity Studies - Histopathological Findings -

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ABSTRACT. To clarify the pathological characteristics of Crj:CD(SD)IGS rats, the histopathological background data on toxicity studies carried out at Toxicology Research Laboratories in Fujisawa Pharmaceutical Co., Ltd. for safety assessment of new compounds were compared with those of Jcl:SD rats. The results indicated inflammatory lesions in various organs and tissues except the harderian gland were less frequent in IGS rats than in SD rats, whereas on the contrary, focal necrosis in the liver was conspicuous in IGS males. － Key words: Crj:CD(SD)IGS rats, Histopathological data, Jcl:SD rats, Spontaneous lesion.

CD(SD)IGS-1999: 23-28

INTRODUCTION

Toxicity studies of drugs, agricultural and industrial chemicals are the cornerstone of human safety evaluation in the development and regulation of these chemicals. Those who evaluate the pathology of these studies need to know the histopathological background data of animals used. Crj:CD(SD)IGS rats were produced by a new animal breeding system for the purpose of providing experimental animals having a worldwide uniform quality. It was developed by Charles River Inc. for internationalization of scientific research and development of new drugs by supplying uniform experimental animals by minimizing genetic ramifications as much as possible. We have accumulated histopathological data of the Crj:CD(SD)IGS rats and Jcl:SD rats used in our toxicity studies and compared the data of both strains.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats (IGS) and Jcl:SD rats (SD) were used in toxicity studies from Jun. 1997 to Feb. 1999, and from Apr. 1988 to Aug. 1996, respectively. For the control groups of these studies, histopathological data were gathered on 280 males and 230 females of 8-17 weeks old IGS rats in 53 studies, and on 230 males and 228 females of SD rats of the same age in 37 studies. All studies were carried out in accordance with Guidelines for "Animal Experiments at Fujisawa Pharmaceutical Co., Ltd.". IGS rats were purchased from Charles River Japan, Inc. (Hino farm, Japan) and SD rats were purchased from Clea Japan, Inc. (Ishibe farm, Japan). After more than 1 week of acclimation including quarantine has done, the animals were used in toxicity studies at the age of 6-7 weeks. Room temperature and relative humidity were controlled at respectively 22.0-23.8℃ and 44-69% and ventilation was set at 14-20 changes per hour. The room was lighted automatically from 07:00 h to 19:00 h. IGS rats and SD rats were allowed free access to standard laboratory food (CRF-1, autoclaved, Oriental Yeast Co., Ltd., Tokyo Japan) and filtered tap water containing 2 ± 1 ppm chlorine adjusted with sodium hypochlorite from an automatic dispenser.

The animals were sacrificed by bleeding under deep anesthesia with ether according to each study plan, and were necropsied completely. The organs and tissues were fixed in 10% neutral buffered formalin except for testes and eyes, which were fixed in formalin-sucrose-acetic acid fixation and glutaraldehyde fixation, respectively. All organs and tissues were embedded in paraffin, sectioned at 3μm, and stained with hematoxylin and eosin for routine histopathological examinations. The following organs and tissues were selected according to each study protocol and examined microscopically by certified pathologists; heart, thoracic aorta, sternal bone marrow, femoral bone marrow, spleen, thymus, mandibular lymph node, mesenteric lymph node, trachea, bronchus, lungs, tongue, sublingual gland, submandibular gland, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, liver, pancreas, kidneys, urinary bladder, pituitary, thyroids, parathyroids, adrenals, testes, epididymis, seminal vesicle, prostate, brain (cerebrum and cerebellum) , optic nerve, spinal cord, sciatic nerve, eye, harderian gland, skeletal muscle, sternum, femur, skin, mammary gland and any other tissues with gross lesions. The incidence of pathological findings was statistically evaluated between males or females of both strains using chi-square test.

RESULTS

The incidence of spontaneous lesions and the number of tissues examined are summarized in Table 1.

Cardiovascular system

Small foci of myocardial inflammation were observed only in SD rats and were frequently seen in males ($P<0.01$). The lesion was characterized by focal aggregates of large mononuclear cells and scattered phagocytic cells that were separated from the myocardial fibers. With the lesion, myocardial degeneration and/or fibrosis were sometimes seen.

Lymphohematopoietic system

There were no remarkable lesions in the bone marrow, thymus or lymph nodes except for the spleen in either strain. In the spleen, extramedullary hematopoiesis was a more common finding in male rats of both strains compared with females. There was no significant difference between either strain in incidence of extramedullary hematopoiesis, but the incidence was significantly higher in SD females than in IGS females ($P<0.01$). On the contrary, splenic hemosiderosis was more frequent in females of both strains than in the males, and the incidence was significantly higher in SD rats than in IGS rats in the males ($P<0.01$) and females ($P<0.01$).

Table 1. Incidence of Spontaneous lesions in IGS and SD rats

	Male		Female	
No. of animals	n=280 IGS (%)	n=230 SD (%)	n=280 IGS (%)	n=230 SD (%)
Cardiovascular system				
Heart	115	166	116	165
Inflammation, focal	0	18 (10.8)**	0	2 (1.2)
Thrombus, tricuspid valve	0	0	0	1 (0.6)
Necrosis	1 (0.9)	0	0	0
Thorancic aorta	35	138	35	137
Lymphohematopoietic system				
Sternal bone marrow	55	138	55	137
Hypocellularity	0	0	0	1 (0.7)
Femoral bone marrow	35	150	35	149
Spleen	160	174	160	172
Extramedullary hematopoiesis	73 (46.0)	66 (38.0)	5 (3.1)	31 (18.0)**
Deposition, hemosiderin	1 (0.6)	12 (6.9)**	10 (6.3)	47 (27.3)**
Necrosis, focal	1 (0.6)	0	0	0
Congestion	0	3 (1.7)	1 (0.6)	2 (1.2)
Capsular thickening	1 (0.6)	0	0	0
Thymus	40	156	40	155
Ductal structure	1 (2.5)	0	1 (2.5)	0
Hemorrhage	2 (5.0)	2 (1.3)	1 (2.5)	2 (1.3)
Mandibular lymph node	34	150	35	149
Cyst	0	2 (1.3)	0	0
Mesenteric lymph node	35	138	35	128
Sinal erythrocyte	0	0	0	1 (0.8)
Respiratory system				
Trachea	35	138	35	137
Epithelial hyperplasia	0	1 (0.7)	0	0
Glandular dilatation	0	4 (2.9)	0	2 (1.5)
Inflammatory cell infiltration	0	5 (3.6)	0	8 (5.8)
Bronchus	35	136	35	136
Inflammatory cell infiltration	0	0	0	2 (1.5)
Lung	40	158	40	155
Inflammatory cell infiltration	1 (2.5)	28 (17.7)*	0	20 (12.9)*
Hemorrhage, focal	0	27 (17.1)*	0	10 (6.5)
Alveolar form cell	2 (5.0)	2 (1.3)	0	5 (3.2)
Glanulation, focal	1 (2.5)	3 (1.9)	1 (2.5)	0
Spicle	2 (5.0)	1 (0.6)	0	1 (0.6)
Digestive system				
Tongue	35	137	35	137
Sublingual gland	35	137	35	137
Submandibular gland	35	137	35	137
Necrosis, focal	0	1 (0.7)	0	0
Esophagus	70	138	35	137
Stomach	35	145	70	143
Hyaline-droplets, glandular stomach epithelial cell	0	1 (0.7)	0	1 (0.7)
Inflammatory cell infiltration, forestomach	0	2 (1.4)	0	1 (0.7)
Hemorrhage, glandular stomach	0	4 (2.8)	0	0
Glandular dilatation	0	1 (0.7)	0	0
Vacuolar change, forestomach squamous epithelium	0	1 (0.7)	0	1 (0.7)
Duodenum	35	144	35	143
Ectopic pancreas	0	0	1 (2.9)	0
Erosion	0	3 (2.1)	0	0
Jejunum	60	144	60	143
Ileum	60	144	60	115
Colon	35	144	35	115
Rectum	35	144	35	115

Table 1. -continued.

	Male		Female	
No. of animals	n=280	n=230	n=280	n=230
	IGS (%)	SD (%)	IGS (%)	SD (%)
Liver	280	217	280	228
Focal necrosis	18 (6.4)*	2 (0.9)	2 (0.7)	2 (0.9)
Diapharagmatic hernia	1 (0.4)	0	0	1 (0.4)
Inflammatory cell infiltration	3 (1.1)	9 (4.1)	4 (1.4)	3 (1.3)
Mononuclear cell infiltration	0	6 (2.8)	0	1 (0.4)
Focal vacuolar change	3 (1.1)	2 (0.9)	7 (2.5)	0
Hemorrhage	0	2 (0.9)	0	1 (0.4)
Glanuloma	0	1 (0.4)	0	0
Pancreas	40	155	39	
Acinar atrophy	1 (203)	2(1.3)	0	
Acinar cell basophilic hypertrophy	0	1 (0.6)	0	
Inflammation	0	1 (0.6)	0	
interstitial lymphocyte infiltration	2 (5.0)	0	0.6 (0.9)	
Urinary system				
Kidney	280	230	280	228
Cyst	3 (1.1)	5 (2.2)	3 (1.1)	0
Outer medullary calcification	0	1 (0.4)	3 (1.1)	57 (25.0)**
Pelvic dilatation	2 (0.7)	0	0	0
Pelvic transitional epithelial hyperplasia	0	0	1 (0.4)	0
Protein cast	2 (0.7)	0	1 (0.4)	0
Pyelitis	0	22 (9.6)**	0	22 (9.6)**
Dysplasia	0	0	1 (0.4)	0
Inflammatory cell infiltration	0	1 (0.4)	0	0
Interstitial lymphocyte infiltration	0	3 (1.3)	0	1 (0.4)
Basophilic tubules	5 (1.8)	12 (5.2)	0	11 (4.8)**
Tubular atrophy	0	3 (1.3)	0	4 (1.8)
Tubular dilatation	0	0	0	4 (1.8)
Eosinophilic body	0	2 (0.9)	0	0
Urinary bladder	80	138	80	137
Cystitis	0	9 (6.5)*	0	7 (5.1)*
Lymphocyte infiltration	0	8 (5.8)	1 (1.3)	6 (4.4)
Lymphoid follicle development	0	0	0	2 (1.5)
Endocrine system				
Pituitary	40	158	40	197
Cyst	1 (2.5)	0	0	5 (2.5)
Glandular structure	1 (2.5)	10 (6.3)	0	1 (0.5)
Thyroid	274	141	274	141
Ectopic thymus	9 (3.3)	8 (5.7)	9 (3.3)	8 (5.7)
Follicular cell hypertrophy	1 (0.4)	2 (1.4)	1 (0.4)	2 (1.4)
Interstitial lymphocyte infiltration	0	1 (0.7)	0	0
Ultimobranchial body	0	1 (0.7)	0	1 (0.7)
Neutrophil infiltration	0	0	0	1 (0.7)
Lymphoid follicle	0	0	0	1 (0.7)
Parathyroid	33	128	33	123
Adrenal	275	158	275	197
Accessory cortical gland	3 (1.1)	1 (0.6)	1 (0.4)	3 (1.5)
Mononuclear cell infiltration	0	0	0	7 (3.6)
Vacuolar change, zona fasciculata	0	3 (1.9)	0	0
Genital system				
Testis	275	194	-	-
Tubular atrophy	6 (2.2)	5 (2.6)	-	-
Giant cell	1 (0.4)	0	-	-
Tubular dilatation	0	1 (0.5)	-	-
Epididymis	35	55	-	-
Spermatic glanuloma	1 (2.9)	0	-	-
Seminal vesicle	35	149	-	-
Prostate	35	148	-	-
Prostatitis	16 (45.7)	56 (37.8)	-	-

Table 1. -continued.

	Male		Female	
No. of animals	n=280 IGS (%)	n=230 SD (%)	n=280 IGS (%)	n=230 SD (%)
Ovary	-	-	35	183
Uterus	-	-	35	177
Vagina	-	-	35	177
Miscellaneous organs and tissues				
Brain	35	138	35	137
Hemosiderin deposition	0	0	1 (2.9)	0
Optic nerve	35	35	35	45
Spinal cord	35	138	35	134
Sciatic nerve	35	35	35	35
Eye	35	138	65	137
Retina folds/rosettes	1 (1.5)	7 (5.1)	1 (1.5)	0
Hemorrhage	0	1 (0.7)	0	0
Retina atrophy	0	1 (0.7)	0	0
Harderian gland	35	138	35	137
Lymphocyte infiltration	2 (5.7)*	0	6 (17.1)**	3 (2.2)
Porphyrin deposition	4 (11.4)**	1 (0.7)	1 (2.9)	4 (2.9)
Skeletal muscle	35	35	35	35
Sternum	35	138	35	137
Femur	35	143	35	142
Distal articular cartilage thickening	0	1 (0.7)	0	0
Skin	35	138	35	137
Mammary gland	5	134	35	137

* : differs from IGS or SD rats in each sex, $P < 0.05$
** : differs from IGS or SD rats in each sex, $P < 0.01$
Bold letters: number of tissues examined histopathologically

Respiratory system

Focal inflammatory cell infiltration in the lungs occurred in significantly high frequency in SD rats of both sexes (males and females, $P<0.05$). Perivascular and/or perilobular areas were the most common sites of this inflammatory change in the lungs. This lesion was only recorded in one of 80 IGS rats. The incidence of focal hemorrhage in the lung was significantly higher in SD males than in IGS males and no hemorrhage was found in any IGS rats. Mild dilatation of the respiratory gland and inflammation in the bronchus were only observed in SD rats.

Digestive system

Focal necrosis in the liver was more common in IGS males than in SD males ($P<0.05$). Grossly, the lesions were mainly observed in the fissure of the median lobe as dark red to yellowish white foci and the size were 1x1-10x10mm. Histopathological appearance of lesions was variable based on the pathological stage. Some exhibited hemorrhagic necrosis with scarce inflammatory response (Fig.1), while others showed inflammatory cell infiltration with foreign body giant cells as reaction to the necrotic and mineralized hepatocytes (Fig.2), and sometimes calcification in the center of the focus (Fig.3). A small number of diaphragmatic hernias and foci of cell infiltration in the liver was recorded as an unusual lesion of both strains. Some gastric mucosal abnormalities were recorded in SD rats but not in either sex of IGS rats. No abnormalities were found in the tongue, sublingual glands, esophagus, jejunum, ileum, colon or rectum in either strain.

Urinary system

The most remarkable difference in incidence of renal lesions in each strain was tubular calcification in the outermedullary zone restricted to SD females. Pyelitis occurred in male and female SD rats. Inflammatory changes such as cell infiltration, tubular atrophy, tubular dilatation, and eosinophilic body in the tubular epithelium were also limited in SD rats. None of the female IGS rats had basophilic tubules. As a similar tendency, inflammatory changes of the urinary bladder were detected only in SD rats.

Endocrine system

The most common finding of the thyroid glands was ectopic thymus, but there was no difference between sexes or strains in incidence. Inflammatory response of the adrenal glands was only detected in SD females.

Genital system

No abnormal findings were recorded in female genital organs or tissues in either strain, but relative high incidence of prostatitis was detected in IGS (45.7%) and SD (37.8%). Microscopically, suppurative inflammation in the glandular lumina and/or diffuse lymphocyte infiltration in the interstitial tissue were observed in that prostatitis. Spontaneous atrophy of the seminiferous tubules was found in both strains of rats, although the incidence was low (2.2-2.6%).

Miscellaneous organs and tissues

Lymphocyte infiltration in the harderian gland was more common in IGS rats than in SD rats in both sexes (males, $P<0.05$; females, $P<0.01$). Frequency of porphyrin deposition in the harderian gland was significantly higher in IGS males than in SD males ($P<0.05$). Retinal folds or rosettes formations were found in small numbers of both strains. No histopahological changes were recorded in the optic nerve, spinal cord, skeletal muscle, sternum bone, skin or mammary gland.

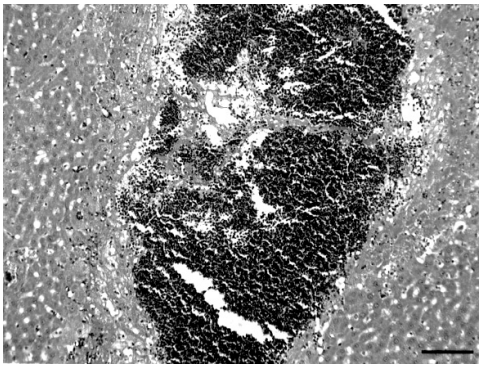

Fig.1. Liver, Male IGS rat. Focal necrosis. No inflammatory response is seen. H&E. Bar = 100 μm

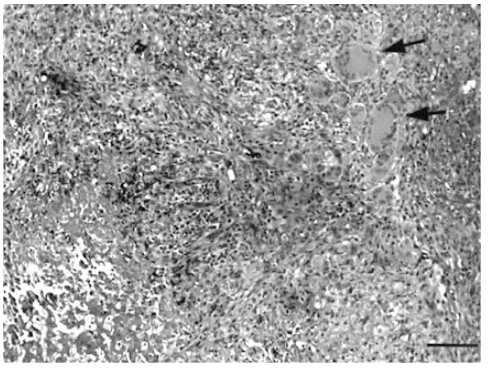

Fig.2. Liver, Male IGS rat. Focal necrosis. Giant cells (arrows) are prominent. H&E. Bar = 100 μm

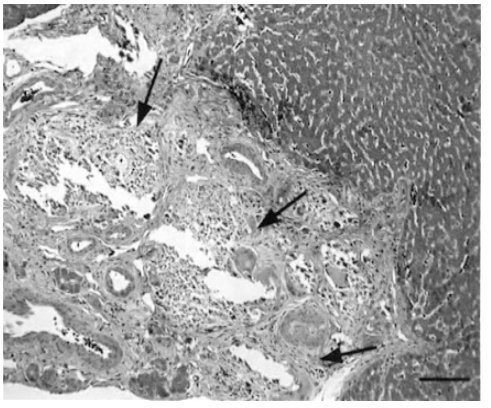

Fig.3 Liver, Male IGS rat. Focal necrosis. Calcification (arrows) at the center of the focus. H&E. Bar = 100 μm

DISCUSSION

From a view point of making a comparison with the same kind of Sprague-Dawley rats, spontaneous histopathologic changes in a number of organs and tissues were investigated in SD and IGS rats according to the same criteria.

Focal inflammation in the heart was frequently observed in SD males. It is well known that fibrosis and inflammation of the heart are common lesions in aged SD males [6]. The etiology of this myocardial disease is unknown; however, it is generally believed that the lesions are due to focal ischemia [1].

Hemosiderin deposition is commonly seen in the spleen of the aged rat [3]. In young Fischer rats, hemosiderin deposition is generally more prominent in females than in males [8], and a similar tendency was observed in SD and IGS rats in this study.

Extramedullary hematopoiesis was more prominent in males than in females in both strains. In F-344 rats, the incidence of extramedullary hematopoiesis was higher in males than in females at 7-18 weeks old; however, in aged 31-week old rats, the lesion was more common in females than in males [9].

In the lung, hemorrhage and inflammatory cell infiltration were more common in SD rats than in IGS rats in both sexes. Hemorrhage of the lung is often an agonal phenomenon related to mode of death [4]. Inflammation in the lung is well-documented [4]; however, the cause of changes is not clear.

It is worth mentioning that focal necrosis in the liver was frequently observed in IGS males. The cause of this lesion might be deficiency of microcirculation because we often observed hemorrhagic necrosis. The animals with large focus of necrosis often showed higher values of blood chemistry parameters including GOT and GPT (data not shown). In toxicity studies, it is important to be aware that this lesion may modify the effect of compounds.

Calcification occurred frequently in the outer-medullary tubules of the kidney in SD rats, but it was rare in IGS rats. It is reported that outer-medullary calcification was far more common in females [6], and would be prevented by ovariectomy or administrating oestrogen to castrated male and female rats [6]. Hormonal circumstance may be different between SD and IGS females.

Pyelitis was often observed in SD rats and associated with cystitis and lymphocyte infiltration in the urinary bladder.

Lymphocyte infiltration of the harderian gland occurred frequently in IGS rats. This is the only inflammatory lesion that was more frequent in IGS rats than in SD rats.

Greaves [2] described that porphyrin deposition in the harderian gland was more prominent in female rodents than in the males and castration, administration of androgens or estrogens moderated the pigment contents; however, the lesion was more frequent in male IGS rats than in the females.

As described above, inflammatory lesions in the heart, liver, lung, kidneys and urinary bladder were less prominent in IGS rats than in SD rats, except for in the harderian gland. Hemosiderin deposition in the spleen and calcification in the kidney were rarely observed in IGS rats. On the contrary, focal necrosis in the liver was a characteristic lesion of IGS males. It is surprising that there are interesting differences in spontaneous lesions between SD and IGS rats in spite of their being the same kind of Sprague-Dawley rats.

REFERENCES

1. Greaves, P. 1990. Heart and pericardium pp229-249. In: Histopathology of Preclinical Toxicity Studies. (Greaves, P. ed) , Elsevier, Amsterdam
2. Greaves, P. 1990. Eye pp781-798. In: Histopathology of Preclinical Toxicity Studies. (Greaves, P. ed), Elsevier, Amsterdam
3. Greaves, P. and Faccini, J.M. 1992. Spleen pp.45-47. In: Rat Histopathology, 2nd ed. (Greaves, P. and Faccini, J.M. eds.), Elsevier, Amsterdam.
4. Greaves, P. and Faccini, J.M. 1992. Bronchi/Lungs pp.79-83. In: Rat Histopathology, 2nd ed. (Greaves, P. and Faccini, J.M. eds.), Elsevier, Amsterdam.
5. Greaves, P. and Faccini, J.M. 1992. Urinary tract pp.170-189. In: Rat Histopathology, 2nd ed. (Greaves, P. and Faccini, J.M. eds.), Elsevier, Amsterdam
6. Muraoka,Y., Itoh, M., Maeda, Y. and Hayashi, Y. 1977. Histological changes of various organs in aged SD-JCL rats. Exp. Anim. 26: 1-12.
7. Peter, C.P., Burek, J.D. and Van Zwieten, M.J. 1986. Spontaneous nephropathies in rats., Toxicol. Pathol. 14: 91-100.
8. Stefanski, S. A., Elwell, M.R. and Stromberg, P.C. 1990. Spleen, Lymph Node, and Thymus. pp369-394. In: Pathology of the Fischer Rats. (Boorman, G. A., Eustis, S.l., Elwell, M.R., Montgomery, C.A., and Mackenzie, W.F. eds.), Academic press, San Diego
9. Tanakamaru Z. Morishima, H., Ito, T., Chatani, F., Sasaki, S. and Miyajima, H. 1991. Spontaneous lesions in F344/Jcl rats used in toxicity studies: Histopathological changes. J. Takeda Res. Lab. 50: 108-125.

Toxicological Data on the Rat Strains Crl:CD(SD)BR, Crl:CD(SD)IGSBR and Hannover Wistar - A Comparison of Body Weight, Food Consumption, Clinical Pathology and Survival Data.

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ABSTRACT. In the early 1990s, the Crl:CD(SD)BR strain of rat was the principal rat strain used in toxicology and oncogenicity studies at Covance Laboratories Europe (formerly Hazleton Europe). Due to decreasing survival in this strain a number of alternatives were investigated for their longevity, in-life parameters and background pathology. The data presented in this paper are from the Crl:CD(SD)IGSBR and the Hannover Wistar strains in comparison to the original Crl:CD(SD)BR strain. All animals were obtained at approximately 4 weeks of age, commencing on study at approximately 6 weeks of age and maintained under standard CLE conditions. The results show that when comparing the Crl:CD(SD)IGSBR and Hannover Wistar strains with the original Sprague Dawley strain, differences in body weight, survival and background pathology are noted. Initial data from the Crl:CD(SD)IGSBR strain showed no differences from the original strain. However, as data continues to be generated small differences in body weight and survival have become apparent, although these parameters remain significantly different from the Hannover Wistar. Therefore, although the Charles River Sprague Dawley (Crl:CD(SD)IGSBR) is also used at this laboratory, the Hannover Wistar rat was chosen as the alternative strain at Covance Europe. — Key words: Sprague Dawley, Hannover Wistar, Comparative data.

CD(SD)IGS-1999: 29 -33

INTRODUCTION

Historically, the Crl:CD(SD)BR strain of rat has been the principal rat strain used in toxicology and oncogenicity studies at Covance Laboratories Europe (formerly Hazleton Europe). During the early to mid 1990s survival in this strain was decreasing at this laboratory and elsewhere. As part of the continual programme of review and improvement conducted at this laboratory, a number of alternative strains were investigated for their longevity, in-life parameters and background pathology. The data presented here cover the two principal strains now in use at Covance Laboratories Europe in comparison to historical data from the Crl:CD(SD)BR strain. The Crl:CD(SD)IGSBR strain was introduced at this laboratory in replacement of the Crl:CD(SD)BR from 1996. The Hannover Wistar strain was first used at this laboratory in 1995.

MATERIALS AND METHODS

Animals and maintenance conditions: the data is obtained from the control animals of 50 studies each for the original Crl:CD(SD)BR strain, the new Crl:CD(SD)IGSBR strain and the Hannover Wistar strain. The studies were from 4 weeks to 2 years in duration. The animals were all supplied by Charles River UK LTD, Margate, UK.

All animals were obtained at approximately 4 weeks of age, commencing on study at approximately 6 weeks of age. Maintenance conditions were consistent in all studies. Animals on all studies were housed in groups of 5 in stainless steel cages with *ad libitum* access to SQC Rat and Mouse Maintenance Diet No 1 (Special Diet Services Ltd, Witham, UK) and filtered mains water. The dosing route was oral gavage.

Room temperature was maintained in the range 19 to 25℃ and humidity was maintained at 40 to 70%. Fluorescent lighting was controlled automatically to give a cycle of 12 hours light (0600 to 1800) and 12 hours dark.

Observations: Clinical observations and mortality checks were performed on all animals daily. Body weight was recorded weekly in all studies except oncogenicity studies where body weight was recorded weekly up to Week 16 and monthly thereafter. Food consumption was measured at the same time intervals as body weight.

Clinical pathology parameters were measured at intervals appropriate to the study type. Haematological parameters were measured on blood taken into tubes containing EDTA anticoagulant and clinical chemistry parameters were measured on plasma derived from blood taken into tubes containing Lithium Heparin anticoagulant. Blood samples were withdrawn from the tail vein (Crl:CD(SD)IGSBR and Hannover Wistar) or the orbital sinus (Crl:CD(SD)BR) after an overnight period without food. The parameters measured and the methods used are presented in Tables 1 and 2.

At the end of the study period all animals were weighed prior to necropsy. At necropsy, animals were given an intraperitoneal injection of sodium pentobarbitone followed by exanguination. Food was removed overnight prior to necropsy. A macroscopic examination was performed and the appropriate organs were dissected free from fat and other contiguous tissue and weighed before sampling for histopathology. Tissues were fixed in 10% buffered formalin with the exception of the eyes which were fixed in Bouins fluid.

RESULTS

Clinical observations: The nature and frequency of daily observations were similar for all three strains and dependent on the duration of the study. The most commonly seen observations included fur staining, hairloss, rough haircoat and sores and lesions.

Body weight: Body weight of the original strain Crl:CD(SD)BR was approximately 200g (males) and 160g (females) at the start

of the study with a weekly weight gain approaching 50g during the initial study period. On completion of 104 weeks, animals from this strain were often obese, with the body weight of some animals exceeding 1000g.

When first introduced, the body weight of the new strain Crl:CD(SD)IGSBR was comparable to that recorded among animals of the old strain. More recently, the body weight of animals from the new strain has been slightly lower than that of the old strain with a study starting weight of approximately 180g (males) and 140g (females). Body weight gain during the initial study period is also slightly lower than that of the original strain at about 40g per week.

The body weight and body weight gain of animals of the Hannover Wistar strain are significantly lower than the Sprague Dawley strains.

Food consumption: The data presented here is from animals which were group housed. Food consumption in the new and old Sprague Dawley strains is comparable. The food consumption of animals in the Hannover Wistar strain is lower than the Sprague Dawley strains. However, the differences in food consumption between the Sprague Dawley strains and the Hannover Wistar strains are small and would not alone account for the large difference in body weight.

Clinical pathology: Minor variations between the strains are apparent. For haematology for example, the red blood cell count, haemoglobin concentration and pack cell volume of the Hannover Wistar strain are higher than those in the Sprague Dawley strains after 4 weeks on study. At 26 weeks this difference is no longer apparent. For clinical chemistry, alkaline phosphatase activity is higher and the aspartate aminotransferase activity is lower in original strain animals than the other strains. However, there were no differences between the strains which could be considered likely to affect the interpretation of results. Key parameters from each strain are presented in Tables 5 and 6.

Although there are no differences in the results, the difference in body weight between the Sprague Dawley strains and the Hannover Wistar strains result in lower total blood volumes and therefore the amount of blood that can be taken from Hannover Wistar rats is lower than that in either of the Sprague Dawley strain.

Organ weights: The absolute weight of major organs after 4 weeks on study and after 26 weeks on study (i.e. approximately age 10 and 32 weeks) are presented in Table 7. The absolute weights are lower in the Hannover Wistar strain than the Sprague Dawley strains, reflecting the lower body weight of this strain. For organs whose weight is linked to body weight there are no interstrain differences in relative organ weight.

Survival: Survival during oncogenicity studies using animals of the Crl:CD(SD)BR strain declined year by year in the early to mid 1990s. To counter this declining survival, the number of animals allocated to oncogenicity studies at our laboratory was increased from 50/sex/group to up to 70/sex/group. Using larger numbers of animals ensured that the study would achieve 104 weeks of treatment with sufficient animals surviving to termination to permit statistical analysis.

Initial studies conducted using the Crl:CD(SD)IGSBR strain showed no difference in survival from the original strain. However, ongoing studies indicate that survival is increasing slightly in the new strain. The survival in current studies would indicate that the number of animals required per group to achieve acceptable absolute survival at 104 weeks will reduce back to 50/sex/group.

In contrast, survival at 104 weeks in oncogencity studies using the Hannover Wistar strain is high. Despite this high rate of survival, the experience at this laboratory is that submissions to the regulatory authorities are unaffected.

Among each of the strains survival is lower in females than in males.

Table 1 Haematology abbreviations and methods

Code	Parameter	Analyser	Reagents/Kit	Method of determination
Hb	Haemoglobin concentration	Technicon H1	Bayer Diagnostics Ltd.	Spectrophotometric measurement of cyanmethaemoglobin
RBC	Red blood cell count	Technicon H1	Bayer Diagnostics Ltd.	Flow cytometry
PCV	Packed cell volume	Calculation	N/A	PCV = (MCV × RBC) ÷ 10%
MCV	Mean cell volume	Technicon H1	Bayer Diagnostics Ltd.	Flow cytometry
MCH	Mean cell haemoglobin	Calculation	N/A	MCH = (Hb ÷ RBC) × 10
PLAT	Platelets	Technicon H1	Bayer Diagnostics Ltd.	Flow cytometry
WBC	White blood cell count	Technicon H1	Bayer Diagnostics Ltd.	Flow cytometry
N L	Neutrophils Lymphocytes	Manual: Blood smear	Manual: Modified Wright stain on an Ames Haema-tek staining machine	Manual: Visual appraisal of a blood smear using a Romanowski-type stain

Table 2 Clinical chemistry abbreviations and methods

Code	Parameter	Analyser	Reagents/Kit	Method of determination
ALK PHOS	Alkaline phosphatase	Hitachi 747	Boehringer Mannheim UK Ltd.	Colorimetric method using p-nitrophenyl phosphate as substrate
ALT	Alanine aminotransferase	Hitachi 747	Boehringer Mannheim UK Ltd.	Optimised UV method using L-Alanine and alpha-oxoglutarate as primary substrates
AST	Aspartate aminotransferase	Hitachi 747	Boehringer Mannheim UK Ltd.	Optimised UV method using L-aspartate and alpha-oxoglutarate as primary substrates
GLUC	Glucose	Hitachi 747	Boehringer Mannheim UK Ltd.	UV method using a coupled hexokinase procedure
T PROT	Total protein	Hitachi 747	Boehringer Mannheim UK Ltd.	Colorimetric method based on biuret reaction
TOT CHOL	Total cholesterol	Hitachi 747	Boehringer Mannheim UK Ltd.	Enzymatic method using cholesterol oxidase/esterase
TRIGS	Triglycerides	Hitachi 747	Boehringer Mannheim UK Ltd.	Enzymatic method using lipase and glycerol kinase as primary enzymes

Table 3 Comparison of body weight (mg)

	Start of dosing		Week 13		Week 52		Week 104	
Strain	Male	Female	Male	Female	Male	Female	Male	Female
Crl:CD(SD)BR	196	159	541	304	748	426	815	560
Crl:CD(SD)IGSBR	178	142	510	351	701	398	761	516
Hannover Wistar	156	143	372	231	500	280	555	355

Table 4 Comparison of food consumption(g)

	Start		Week 13		Week 52		Week 104	
Strain	Male	Female	Male	Female	Male	Female	Male	Female
Crl:CD(SD)BR	186	142	189	139	185	142	188	144
Crl:CD(SD)IGSBR	184	135	186	138	185	144	199	163
Hannover Wistar	149	102	151	121	147	120	140	136

Table 5.1 Comparison of haematological parameters - subchronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Red blood cell count mil.cmm	6.98	7.10	7.79	7.65	8.61	7.90
Haemoglobin concentration g/dl	15.3	15.7	15.1	14.5	16.4	15.7
PCV %	42.1	43.3	46.8	44.3	48.5	46.7
MCV fl	60.0	61.0	60.1	57.9	56.4	59.4
MCH pg	22.0	22.2	19.3	19.0	19.1	20.0
Platelet 1000/cmm	909	936	1057	1013	877	840
White blood cell count 1000/cmm	11.8	14.8	13.5	9.9	13.6	10.1
Neutrophil %	11	12	17	15	17	16
Lymphocyte %	88	87	79	79	78	80

Table 5.2 Comparison of haematological parameters - chronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Red blood cell count mil.cmm	8.08	7.69	8.61	7.90	8.48	7.92
Haemoglobin concentration g/dL	16.3	15.9	16.4	15.7	15.3	14.4
PCV %	44.0	43.2	48.5	46.7	44.4	42.1
MCV fl	55	56	56	59	52.0	53.0
MCH pg	20.2	21.4	19.1	20.0	17.9	18.2
Platelet 1000/cmm	946	841	877	840	866	884
White blood cell count 1000/cmm	15.8	10.8	13.6	10.1	8.0	6.1
Neutrophil %	13	12	17	16	19	18
Lymphocyte %	85	87	78	80	75	79

Table 6.1 Comparison of clinical chemistry parameters - subchronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Alkaline Phosphatase IU/L	459	307	316	135	333	209
Aspartate Aminotransferase IU/L	83	80	122	125	115	108
Alanine Aminotransferase IU/L	36	31	51	35	49	40
Glucose mmol/L	5.2	5.4	4.8	6.3	5.0	4.9
Total Protein g/L	59	64	60	67	55	60
Total Cholesterol mmol/L	1.5	1.5	1.6	1.3	1.6	1.6
Triglycerides mmol/L	0.89	0.57	0.96	0.61	0.96	0.54

Table 6.2 Comparison of clinical chemistry parameters - chronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Alkaline Phosphatase IU/L	190	121	212	119	235	107
Aspartate Aminotransferase IU/L	84	81	126	122	80	70
Alanine Aminotransferase IU/L	35	32	50	49	27	22
Glucose mmol/L	6.3	5.8	5.3	5.4	6.5	4.2
Total Protein g/L	61	62	64	69	63	65
Total Cholesterol mmol/L	1.2	1.1	1.6	1.8	1.3	1.3
Triglycerides mmol/L	0.87	0.77	1.20	0.85	0.96	0.54

Table 7.1 Comparison of absolute organ weights (gm) - subchronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Liver	9.313	6.444	9.412	7.130	7.755	5.261
Kidney	2.227	1.491	2.432	1.618	1.565	1.124
Adrenal	0.058	0.064	0.062	0.067	0.069	0.081
Spleen	0.781	0.561	0.677	0.547	0.578	0.429
Thymus	0.496	0.422	0.287	0.345	0.401	0.427
Brain	2.019	1.884	2.076	1.928	1.881	1.785

Table 7.2 Comparison of absolute organ weights (gm) - chronic administration

	Strain					
	Crl:CD(SD)BR		Crl:CD(SD)IGSBR		Hannover Wistar	
Parameter	Male	Female	Male	Female	Male	Female
Liver	12.73	7.363	12.879	8.203	9.956	6.077
Kidney	2.835	1.722	2.984	1.801	1.945	1.402
Adrenal	0.061	0.069	0.061	0.076	0.067	0.069
Spleen	0.976	0.546	1.021	0.603	0.805	0.351
Thymus	0.293	0.203	0.235	0.174	0.232	0.212
Brain	2.207	1.950	2.183	2.081	1.879	1.797

Table 8 Comparison of percentage survival at selected intervals

	Week 52		Week 80		Week 104	
Strain	Male	Female	Male	Female	Male	Female
Crl:CD(SD)BR	94	94	69	70	35	28
Crl:CD(SD)IGSBR	97	95	74	75	52	39
Hannover Wistar	99	99	90	89	89	77

DISCUSSION

During the early part of this decade, the use of the Crl:CD(SD)BR strain of rat for oncogenicity studies was under critical review. Animals of this strain were often seriously obese. Foot lesions were common as were mammary masses, which in combination with the high body weight contributed to inactivity of the animals and their decline in health. In addition to the health problems, there were practical problems with housing and handling the large animals.

A number of strategies were developed to ensure regulatory acceptance of studies. These included restricted feeding regimes, increasing animal numbers per group and the use of alternative strains. The restricted feeding regimes and increasing number of animals were accompanied by practical problems as well as being associated with animal welfare issues, particularly when group housing was preferred. Therefore, at Covance the use of an alternative strain was considered to be the best option.

A number of multinational pharmaceutical companies were already routinely using the Hannover Wistar strain. Other strains available for use by this laboratory were ruled out, either because of poor data, for example a high background incidence of a lesion or tumour type or because of inadequate availability of background data and regulatory acceptance.

Following a general review, the Hannover Wistar rat was chosen as the alternative strain at Covance Europe. The Charles River Sprague Dawley (Crl:CD(SD)IGSBR) is also used.

When comparing the two strains currently in use with the original Sprague Dawley strain, the significant differences are in body weight, survival and background pathology. Initial data from the Crl:CD(SD)IGSBR strain showed no differences from the original strain. However, as data continues to be generated small differences in body weight and survival have become apparent. These parameters remain significantly different from the Hannover Wistar.

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The Effects of Hydroxypropylcellulose (HPC) and Hydroxypropyl Methylcellulose Phthalate (HPMCP) on Toxicological Parameters in Crj:CD(SD)IGS Rats

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ABSTRACT. The effects of 6% hydroxypropylcellulose SL (HPC) and 15% hydroxypropyl methylcellulose phthalate HP-55 (HPMCP) suspensions at 10 mL/kg on toxicological parameters were investigated using Crj:CD(SD)IGS rats during a 4-week oral administration period and the reversibilities of changes were determined in selected animals during a subsequent 4 week recovery period. 5% gum arabic solution at 10 mL/kg was used as a control substance. No abnormalities in the general conditions or ophthalmology were observed in any group. Some gross and histopathological findings were observed in each group including the control group after the dosing and the recovery periods, but the frequency of occurrence was comparable among all groups. Although some differences between the HPC or HPMCP group and the control group were observed in body weight, food consumption, and some parameters relating to hematology, blood biochemistry, urinalysis and organ weight, these were very slight and were not observed after the completion of the recovery period. — Key words: Crj:CD(SD)IGS rats, Hydroxypropyl methylcellulose, Hydroxypropylcellulose

CD(SD)IGS-1999: 34-45

INTRODUCTION

The effects of hydroxypropylcellulose SL (HPC) and hydroxypropyl methylcellulose phthalate HP-55 (HPMCP), which were used as additives in some oral drugs, on toxic parameters were investigated in male and female Crj:CD(SD)IGS rats during a 4-week oral administration period and the reversibilities of changes were determined in selected animals during a subsequent 4 week recovery period.

MATERIALS AND METHODS

Animals and housing conditions: Fifty-seven male and 57 female 5-week-old Crj:CD(SD)IGS rats were purchased from the Hino Breeding Center of Charles River Japan Inc. The animals were acclimatized for 13-day preliminary housing period including a 7-day quarantine period. One female was sampled out for quarantine test and the remaining 57 males and 56 females were divided into 3 groups (17 animals of each sex per group) using a stratified random grouping method which ensured homogeneity of group means for body weights recorded on the day of grouping. Seven males and 7 females from each group were used in the 4-week recovery test. On the initial day of dministration, the animals were 6 to 7 weeks old, and the males and the females weighed 234-261g and 152-179g, respectively.

The animals were individually housed in stainless-steel cages (222W x 325D x 180H, mm; Nippon Cage Co., Ltd.) in a room that was maintained at 21-25 ℃ and 50-70 % RH, ventilated with an air-exchange rate 13-15 times hourly, lighted for 12 hours (07:00h-19:00h) daily. Each animal was freely provided with pelleted food (CRF-1, sterilized by radiation, Oriental Yeast Co., Ltd) and with Otsuka's in-house tap water by an automatic watering device. Animals were fasted during fresh urine sampling and then powder food was freely provided during 16hr-urine accumulating period in metabolic cages. The animals were identified by unique identification numbers punched in their ears after arrival. Animals were sacrificed following 16-21 hours fast.

Test substance: Hydroxypropylcellulose SL (HPC, Lot No.EG-0861, Nihon Soda Ltd.) and hydroxypropyl methylcellulose phthalate HP-55 (HPMCP, Lot No.12476, Shin-etsu Chemical Industry Ltd.) were used as test substances. They were stored at room temperature and protected from light until use. HPC was suspended at 6% and HPMCP at 15% in 5% gum arabic solution which were prepared with gum arabic (Sankyo Food Industry Ltd., Lot No.95112701) and water for injection (Otsuka Pharmaceutical Industry Ltd., Lot Nos.6L78 and 6J84) using mortar and pestle. 5% gum arabic solution was used as a control substance. All suspensions and the vehicle solution were prepared once a week, stored in a cool place (4℃) and protected from light until use. They were administered orally using a syringe and a gastric tube for rat once daily (08:00h-12:00h), 7 days a week, for 4 weeks. Selected animals of each group were subsequently maintained off-dose for a further 4 weeks.

Rationale for dose selection: The dose volume was set at 10 mL/kg that was considered to be an appropriate maximum volume used for repeated oral administration in rats. The concentration of HPC was set at 6%, maximum feasible concentration based on the high viscosity. The concentration of HPMCP was set at 15% to achieve dose high enough to investigate its effect.

Observations, measurements, and examinations: *General condition*: The general condition of each animal was observed twice a day, once before dosing and once about 4 hours post-dosing everyday, except on weekends and holidays at which time the animals were only observed before dosing. During the recovery period, observation was performed once daily in the morning.

Body weight: Body weight of each animal was measured on the day of grouping, on the initial day of dosing, once weekly just before dosing during the dosing period, and once weekly in the morning during the recovery period. Another measurement was performed on the day of necropsy to calculate organ-to-body weight ratio.

Food consumption: Weekly food consumption was measured by subtracting the amount remaining left from the amount allotted 7 days before throughout the study period and the daily consumption was calculated. Measurement was performed just before dosing during the dosing period and in the morning during the recovery period.

Hematology: On the day of necropsy after the 4-week dosing period or 4-week recovery period, blood samples of all animals

were obtained after the animals were fasted for 16 to 21 hours. The animals were laparotomized under anesthetization with ether and 1mL of blood was sampled from the posterior vein. 0.3mL of the sample was treated with EDTA-2K for general hematological tests, and serum was obtained form the remaining sample for measurements of total protein, A/G ratio, and protein fraction. Another 2mL of blood was sampled using a syringe treated with 0.2mL of 3.8% sodium citrate and plasma was prepared for coagulation tests. Hemoglobin (Hb), hematocrit (Ht), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plat), white blood cell count (WBC), and differential WBC ratio were measured by using hematological measurement device (H-1, Technicon). Reticulocyte ratio (RET) was examined using a light microscope with Brilliant Cresyl Blue stain. Prothrombin time (PT), and activated partial thromboplastin time (APTT) were measured with automated coagulation analyzer (Coagmaster, Hitachi Ltd.).

Blood biochemistry: As much as possible of blood sample for blood biochemical tests was obtained from each animal using a heparinized syringe, following blood sampling for the hematological tests. Creatine phosphokinase (CPK), lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GTP), total bilirubin (TBI), alkaline phosphatase (AlP), total cholesterol (CHO), triglycerides (TG), phospholipids (PL), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), creatinine (CRE), calcium (Ca) and inorganic phosphorus (P) were measured by an automated analyzer (Hitachi-7170, Hitachi Ltd.). Sodium (Na), potassium (K) and chloride (Cl) were measured by another automated analyzer (Hitachi-750, Hitachi Ltd.). Serum protein was separated by electrophoresis (cellulose acetate) into albumin (ALB), α_1-, α_2-, α_3-, β- and γ-globulin (GLB) fractions and A/G ratio (A/G) was measured with a densitometer (HELENA-CLINISCAN2, Helena Laboratories).

Urinalysis: In Week 4 of the dosing and recovery period, 5 animals from each group were fasted and placed in metabolism cages to collect fresh urine samples. Thereafter, the animals were fed, and urine was accumulated for about 16 hours to determine the following parameters. Fresh urine was used to determine the semiquantitative parameters (pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen) using test paper for urinalysis (Multistix SG-L; Bayer-Sankyo Ltd.) and Clinitec 200 (Ames). The urine sediment was obtained by centrifugation and was stained by modified Sternheiner method for microscopic observation. Accumulated urine was used to determine osmolality using automatic osmolality measurement device (OM-6030, Kyoto Daiichi Kagaku Co., Ltd.) and Na, K, Cl, CRE after the investigation of urine volume and gross color abnormality. The amount of water consumed during the urine accumulation was also measured.

Ophthalmology: Ophthalmological examinations of the 5 animals from each group were performed in Week 4 of the dosing and recovery period. Eyelid, cornea, conjunctiva, sclera, and iris of both eyes were examined by gross observation. Lens and fundi in both eyes were evaluated using an indirect ophthalmoscopy (All Pupil Indirect, Keeler Co.) and the right eye was photographed with a fundus camera (Kowa Genesis, Kowa Ltd.) after the pupil was dilated using mydriatic (Mydrin-P, Santen Pharmaceutical Co., Ltd.).

Necropsy: Following blood sampling from the posterior vein under ether anesthetization, the animals were euthanized by exsanguination from the abdominal veins and arteries on the day after the completion of the dosing and the recovery period, and macroscopic observation was performed.

Organ weights: Brain, submaxillary glands (including the sublingual glands), thymus, heart, lungs, liver, spleen, adrenal glands, kidneys, testes, seminal vesicle, prostate, ovaries and uterus were weighed on the day of necropsy. Thyroid and pituitary glands were weighed after being fixed in 10% neutral buffered formalin for 1 day. Bilateral organs were weighed together. Organ weights were obtained as absolute and relative weights (organ-to-body weight ratios).

Histopathology: The following organs or tissues of all the animals were fixed in 10% neutral buffered formalin: liver, kidneys, thymus, mandibular lymph nodes, mesenteric lymph nodes, spleen, heart, aorta, lungs, bronchi, trachea, esophagus, submaxillary glands, sublingual glands, parotid glands, tongue, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, seminal vesicle, prostate, epididymides, ovaries, uterus, vagina, pituitary gland, thyroid glands, parathyroid glands, adrenal glands, skin, mammary glands (the females), femoral skeletal muscle, brain, spinal cord (cervical), sciatic nerve, optic nerve, Harderian glands and bone marrow (sternum and femur). In addition, eyeballs were fixed in Davidson's solution and the testes were in Bouin's solution. The sternums and femurs were decalcified after fixation. According to the standard methods, the tissues were embedded in paraffin, thinly sectioned, and stained with hematoxylin and eosin. The kidneys and testes were further stained with periodic acid/Schiff for the evaluation.

Statistical analysis: Comparisons between control and HPC group and between control and HPMCP group were performed as below. The t-test with F-test for homogeneity of variance was used for inter-group comparisons of body weight, food consumption, hematology, blood biochemistry, and urinalysis (except semiquantitative test), and organ weights. The above data was subjected to Aspin-Welch's test when *F*-test for homogeneity of variance showed significant difference or to the t-test when it showed insignificant difference. Comparison of urinalysis data (except pH measured in semiquantitative) was performed using the exact rank-sum test for 2 x k tables. Wilcoxon's rank sum test was used for comparison of urine pH. Each test was performed in two-tailed and significance was presented at either the 5% or 1% level.

RESULTS

Mortality: No death was found in this study.

General condition: No abnormality was found in this study.

Body weight (Table 1): HPC group; No significant difference was observed when compared with the control in either sex. HPMCP group; After 4 weeks of the dosing period, the females showed increased body weights when compared with the control.

Food consumption (Table 2): HPC group; Females at Week 3 of the dosing period showed increased mean food consumptions when compared with the control. HPMCP group; No significant difference was observed when compared with the control.

Table 1. Body weight changes in Crj:CD(SD)IGS rats given HPC or HPMCP

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	17	17	17	17	17	17
Pre [a)]	250.3±11.9g	250.0±9.7	252.0±12.6	174.5±6.9	176.4±9.3	177.1±8.5
Week 1	294.9±18.2	297.1±13.5	302.0±20.0	193.6±7.8	198.6±11.9	198.5±9.2
Week 2	337.8±24.3	340.4±17.7	347.0±24.6	212.3±11.8	216.7±13.4	216.6±10.9
Week 3	373.7±30.4	376.6±21.6	387.4±28.5	228.7±12.7	236.5±16.4	234.4±11.8
Week 4	402.4±32.7	406.6±21.6	416.8±33.9	238.9±15.0	249.5±17.4	251.6±10.8**
No. of animals	7	7	7	7	7	7
R [b)]-Week 1	436.4±34.9	432.4±12.6	422.4±38.2	259.9±10.8	266.0±21.9	261.9±17.3
R-Week 2	463.3±39.8	460.1±15.8	450.4±38.7	272.6±13.7	281.0±23.5	271.7±15.5
R-Week 3	484.6±44.8	481.9±13.6	468.3±41.4	282.9±13.7	288.3±21.7	284.0±15.9
R-Week 4	509.9±51.4	505.3±14.8	489.4±44.2	289.9±14.0	296.0±26.7	294.6±18.4

a): Before administration b): Recovery period
**; P<0.01: Significant difference from the control

Table 2. Food consumption (g/day) in Crj:CD(SD)IGS rats given HPC or HPMCP

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	17	17	17	17	17	17
Week 1	25.7±2.7	26.1±1.4	26.4±2.5	18.2±1.1	19.2±2.0	18.6±1.1
Week 2	26.9±2.6	27.2±2.1	27.6±2.7	18.9±1.5	19.9±2.0	19.2±1.3
Week 3	27.8±2.6	28.1±2.4	29.1±2.7	19.5±1.6	21.1±2.2*	20.4±1.4
Week 4	28.1±2.7	28.2±2.2	29.1±2.9	19.8±1.7	20.8±1.6	20.6±1.8
No. of animals	7	7	7	7	7	7
R [a)]-Week 1	28.3±3.7	28.3±1.1	27.4±3.7	20.7±1.4	20.9±1.6	20.1±2.4
R-Week 2	29.7±4.2	29.1±1.3	28.0±3.3	22.1±2.0	22.7±2.4	21.1±2.5
R-Week 3	29.1±3.8	28.6±1.1	26.4±3.0	21.3±2.4	21.0±1.9	21.0±3.5
R-Week 4	29.6±4.2	29.1±1.1	27.0±2.6	21.0±1.7	21.3±2.9	20.6±2.9

a): Recovery period
*; P<0.05: Significant difference from the control

Hematology (Tables 3, 4): HPC group; After 4 weeks of the dosing period, decreased basophil (Bas) ratio in the males and increased mean corpuscular volume (MCV) in the females were observed when compared with the control. After 4 weeks of the recovery period, all parameters were comparable to those of the control. HPMCP group; After 4 weeks of the dosing period, shortened prothrombin time (PT) in the males, and increased mean corpuscular volume (MCV) in the females were observed. After 4 weeks of the recovery period, prolonged activated partial thromboplastin time (APTT) in the males and decreased platelet count (Plat) in the females were observed when compared with the control.

Blood chemistry (Tables 5, 6): HPC group; After 4 weeks of the dosing period, decreased potassium (K) in the females was observed when compared with the control. After 4 weeks of the recovery period, all parameters were comparable to those of the control. HPMCP group; After 4 weeks of the dosing period, increased phospholipid (PL) and total globulin (T-GLB), and decreased A/G ratio in the males, decreased ALB volume, A/G ratio and potassium (K), increased creatinine (CRE) in the females were observed when compared with the control. After 4 weeks of the recovery period, all parameters were comparable to those of the control.

Table 3. Hematology in Crj:CD(SD)IGS rats given HPC or HPMCP (Dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
Hb (g/dL)	15.59±0.54	15.60±0.56	15.62±0.60	15.30±0.54	15.24±0.62	15.27±0.70
Ht (%)	45.95±1.80	45.64±1.40	46.01±1.74	43.61±1.54	43.85±1.49	43.78±2.31
RBC (x10^6/mm^3)	8.166±0.390	8.177±0.193	8.049±0.390	8.029±0.354	7.834±0.315	7.831±0.421
MCV ($micro^3$)	56.30±1.63	55.84±1.33	57.21±1.22	54.38±1.70	56.00±1.61*	55.94±1.16*
MCH (pg)	19.10±0.58	19.09±0.55	19.42±0.67	19.07±0.77	19.47±.065	19.50±0.51
MCHC (%)	33.92±0.34	34.17±0.49	33.97±0.69	35.07±0.54	34.77±0.72	34.86±0.41
WBC (x10^3/mm^3)	10.546±2.474	10.769±2.459	12.216±2.266	8.307±3.314	9.304±3.219	7.943±1.396
Neut (%)	9.80±3.33	12.54±4.19	11.39±4.11	9.36±3.47	12.18±6.20	10.04±3.28
Lym (%)	85.06±3.83	81.96±4.80	83.05±4.33	85.08±3.85	82.07±6.47	84.09±3.74
Mon (%)	1.96±0.83	2.16±0.45	2.12±0.61	2.31±0.71	2.23±0.37	2.21±0.79
Eos (%)	0.80±0.35	0.87±0.28	0.95±0.30	1.18±0.36	1.17±0.39	1.54±0.44
Bas (%)	0.52±0.10	0.38±0.08**	0.47±0.09	0.41±0.14	0.40±0.12	0.43±0.13
Luc (%)	1.89±0.37	2.09±0.56	2.04±0.47	1.66±0.37	1.95±0.30	1.71±0.51
Plat (x10^3/mm^3)	1168.0±144.2	1089.5±89.0	1138.7±107.1	1181.1±83.0	1265.4±3573	1136.4±152.0
PT (Sec)	16.66±1.40	15.79±1.20	14.65±0.70**	13.46±0.19	13.38±0.16	13.48±0.30
APTT (Sec)	23.88±1.91	23.90±1.70	23.38±1.53	21.16±1.14	20.91±0.86	20.94±1.12

*; P<0.05, **; P<0.01: Significant difference from the control

Table 4. Hematology in Crj:CD(SD)IGS rats given HPC or HPMCP (Recovery period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
Hb (g/dL)	15.84±0.43	15.87±0.69	15.81±0.44	15.36±0.48	15.61±0.53	15.19±0.66
Ht (%)	46.33±0.91	46.60±1.42	46.66±1.11	44.14±1.29	44.87±1.73	44.00±2.20
RBC (x10^6/mm^3)	8.891±0.342	8.727±0.318	8.629±0.242	8.010±0.376	8.107±0.429	7.950±0.295
MCV ($micro^3$)	52.16±2.18	53.44±2.32	54.13±1.94	55.17±1.72	55.41±1.84	55.34±1.80
MCH (pg)	17.84±0.66	18.20±0.93	18.36±0.58	19.17±0.59	19.27±0.59	19.10±0.57
MCHC (%)	34.23±0.36	34.06±0.58	33.90±0.43	34.79±0.50	34.76±0.24	34.50±0.35
WBC (x10^3/mm^3)	12.061±1.894	10.926±2.372	9.609±2.881	7.636±2.610	8.686±5.017	6.401±2.168
Neut (%)	10.27±2.05	11.06±3.29	10.63±3.66	10.37±2.51	14.24±5.35	11.93±3.82
Lym (%)	84.11±2.18	83.93±3.85	84.13±4.47	84.61±2.61	80.19±5.88	82.23±4.98
Mon (%)	1.91±0.43	1.47±0.64	1.60±0.65	1.76±0.51	1.76±0.80	1.94±0.50
Eos (%)	1.24±0.33	1.23±0.50	1.37±0.43	1.24±0.48	1.43±0.47	1.36±0.72
Bas (%)	0.41±0.07	0.44±0.13	0.34±0.16	0.30±0.15	0.39±0.09	0.46±0.32
Luc (%)	2.07±0.42	1.87±0.38	1.86±0.37	1.73±0.29	1.96±0.21	2.09±0.57
Plat (x10^3/mm^3)	1095.1±107.4	1068.7±122.7	1137.9±112.7	1097.6±66.5	1064.1±84.6	980.9±116.2*
PT (Sec)	15.84±1.17	15.24±1.24	16.89±1.16	13.39±0.25	13.37±0.18	13.27±0.30
APTT (Sec)	23.10±0.94	23.57±2.00	24.59±1.12*	20.03±0.90	20.80±1.65	20.90±1.12

*; P<0.05: Significant difference from the control

Table 5. Biochemistry in Crj:CD(SD)IGS rats given HPC or HPMCP (Dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
CPK (IU/L)	114.6±15.3	128.6±27.4	117.1±24.1	86.7±15.7	89.3±15.7	90.1±28.9
LDH (IU/L)	52.8±9.3	58.0±19.0	57.6±15.0	72.4±20.1	72.8±13.6	66.8±13.9
GOT (IU/L)	63.5±7.1	61.9±6.8	60.4±5.5	65.0±12.8	59.3±6.9	60.8±9.4
GPT (IU/L)	27.8±2.8	26.5±3.2	26.8±4.4	28.7±12.0	26.8±5.9	23.2±6.3
ALP (IU/L)	369.1±85.1	350.0±51.2	320.2±68.6	173.0±47.3	209.5±43.3	182.1±24.9
GTP (IU/L)	0.0±0.0	0.0±0.0	0.0±0.0	3.1±0.6	2.8±0.4	2.8±0.4
TBI (mg/dL)	0.01±0.03	0.00±0.00	0.02±0.04	0.00±0.00	0.01±0.03	0.00±0.00
CHO (mg/dL)	54.6±9.3	52.8±8.0	61.6±11.1	65.8±18.0	60.3±10.6	61.6±9.8
PL (mg/dL)	89.1±10.6	88.5±11.5	104.4±16.4*	116.0±23.9	114.7±18.5	107.0±15.4
TG (mg/dL)	39.0±12.7	38.0±17.7	45.7±17.6	12.4±5.9	17.7±8.7	11.7±3.7
GLU (mg/dL)	137.7±12.8	135.0±11.1	140.7±11.1	124.5±14.7	119.8±11.2	123.8±11.1
TP (g/dL)	5.916±0.190	5.912±0.239	5.968±0.207	6.556±0.462	6.489±0.347	6.233±0.288
ALB (g/dL)	3.341±0.218	3.217±0.173	3.235±0.104	4.091±0.383	3.982±0.335	3.683±0.175**
α_1-G (g/dL)	1.040±0.117	1.147±0.118	1.132±0.129	0.823±0.137	0.781±0.129	0.846±0.108
$\alpha_{2,3}$-G (g/dL)	0.323±0.031	0.333±0.034	0.337±0.026	0.270±0.023	0.306±0.062	0.286±0.030
β-G (g/dL)	1.018±0.074	1.029±0.079	1.067±0.067	1.100±0.096	1.140±0.064	1.107±0.057
γ-G (g/dL)	0.194±0.047	0.188±0.047	0.200±0.043	0.271±0.038	0.281±0.057	0.311±0.059
T-GLB (g/dL)	2.575±0.145	2.695±0.169	2.733±0.180*	2.465±0.135	2.507±0.120	2.550±0.156
A/G	1.302±0.140	1.196±0.101	1.188±0.081*	1.660±0.139	1.591±0.157	1.448±0.081**
BUN (mg/dL)	13.43±1.67	13.70±1.63	14.35±2.35	16.70±2.27	15.68±3.36	18.15±3.21
CRE (mg/dL)	0.176±0.037	0.174±0.022	0.192±0.023	0.231±0.030	0.250±0.032	0.268±0.042*
Ca (mg/dL)	9.463±0.245	9.313±0.305	9.630±0.165	9.064±0.325	9.168±0.290	8.958±0.249
P (mg/dL)	7.315±0.344	7.067±0.550	7.693±0.669	5.413±0.831	5.604±0.657	5.663±0.614
Na (mEq/L)	145.3±1.3	144.4±1.3	145.6±1.3	143.4±0.8	144.1±1.7	144.2±1.3
K (mEq/L)	4.11±0.19	3.99±0.15	4.04±0.16	4.00±0.23	3.77±0.20*	3.81±0.17*
Cl (mEq/L)	104.9±1.5	104.9±1.3	104.3±0.8	108.3±2.2	107.7±1.9	108.4±2.1

*; P<0.05, **; P<0.01: Significant difference from the control

Table 6. Biochemistry in Crj:CD(SD)IGS rats given HPC or HPMCP (Recovery period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
CPK (IU/L)	93.3±17.7	85.9±16.2	88.9±15.2	75.1±16.1	70.1±6.6	73.0±12.1
LDH (IU/L)	73.4±26.1	63.3±19.5	61.6±21.2	57.7±14.1	60.4±10.7	74.3±32.9
GOT (IU/L)	59.6±4.5	58.1±3.4	60.7±3.8	60.0±10.3	56.7±6.1	72.4±22.1
GPT (IU/L)	28.3±2.2	26.3±4.5	26.9±2.5	28.0±10.5	27.7±8.4	30.6±10.4
ALP (IU/L)	238.3±43.1	208.3±27.2	253.7±19.1	123.1±34.0	116.7±22.8	109.0±25.5
GTP (IU/L)	0.1±0.4	0.4±0.5	0.1±0.4	0.7±0.5	0.9±0.7	0.9±0.4
TBI (mg/dL)	0.04±0.05	0.04±0.05	0.03±0.05	0.09±0.04	0.10±0.00	0.10±0.00
CHO (mg/dL)	64.7±12.0	62.9±10.5	53.1±11.6	65.3±10.7	63.7±7.1	65.3±11.9
PL (mg/dL)	110.4±14.6	106.6±17.1	94.9±17.5	126.9±22.8	125.9±15.1	130.3±19.6
TG (mg/dL)	82.1±45.7	59.7±22.7	58.6±35.3	31.0±8.1	32.1±25.5	31.4±14.9
GLU (mg/dL)	139.4±16.6	141.9±20.5	133.4±16.0	131.1±15.6	136.4±9.0	134.6±9.2
TP (g/dL)	6.363±0.208	6.369±0.224	6.171±0.218	6.913±0.461	6.919±0.388	6.910±0.181
ALB (g/dL)	3.264±0.178	3.229±0.150	3.211±0.085	4.209±0.370	4.157±0.382	4.231±0.228
α_1-G (g/dL)	1.183±0.129	1.250±0.143	1.224±0.096	0.826±0.134	0.884±0.154	0.820±0.065
$\alpha_{2,3}$-G (g/dL)	0.420±0.048	0.421±0.037	0.370±0.074	0.390±0.045	0.423±0.036	0.396±0.027
β-G (g/dL)	1.173±0.113	1.164±0.103	1.094±0.074	1.061±0.068	1.071±0.071	1.071±0.095
γ-G (g/dL)	0.317±0.060	0.306±0.088	0.274±0.059	0.429±0.069	0.380±0.034	0.390±0.048
T-GLB (g/dL)	3.099±0.098	3.140±0.173	2.960±0.210	2.704±0.121	2.761±0.148	2.679±0.129
A/G	1.051±0.065	1.033±0.077	1.090±0.091	1.556±0.103	1.513±0.168	1.586±0.138
BUN (mg/dL)	14.74±2.17	15.49±1.34	15.09±1.67	17.13±2.49	16.31±1.93	15.29±0.99
CRE (mg/dL)	0.224±0.026	0.231±0.048	0.253±0.027	0.286±0.021	0.291±0.027	0.306±0.037
Ca (mg/dL)	9.683±0.200	9.694±0.226	9.571±0.149	9.467±0.310	9.656±0.175	9.554±0.193
P (mg/dL)	6.124±0.214	6.366±1.123	5.933±0.349	4.714±0.455	5.007±0.623	4.393±0.537
Na (mEq/L)	140.9±0.7	141.1±1.2	141.1±1.1	141.1±1.1	140.9±1.5	141.3±0.5
K (mEq/L)	3.99±0.12	4.24±0.69	4.01±0.09	3.86±0.16	3.80±0.17	3.80±0.17
Cl (mEq/L)	105.1±1.2	104.4±1.7	105.1±1.5	107.4±2.1	106.0±2.1	107.3±2.0

Urinalysis (Tables 7, 8): HPC group; There was no abnormal parameter after 4 weeks of the dosing and the recovery periods when compared with the control. HPMCP group; After 4 weeks of the dosing period, decreased pH was observed in the females when compared with the control. After 4 weeks of the recovery period, decreased urine volume and increased white blood cell count in urine sediment were observed in the males when compared with the control.

Table 7. Urinalysis in Crj:CD(SD)IGS rats given HPC or HPMCP (Dosing period)

Sex		Male			Female			
Group		Control	HPC	HPMCP	Control	HPC	HPMCP	
No. of animals		5	5	5	5	5	5	
Water Cons.(mL)		48.2±13.7	57.0±4.5	58.2±5.4	50.8±3.3	56.6±5.7	49.4±13.1	
Urine Volume(mL)		12.16±1.88	11.88±3.25	10.12±2.63	8.92±2.23	11.88±3.75	10.12±2.75	
OSMO(mOsm)		1735.6±211.8	1773.6±385.1	2124.4±338.3	1857.6±323.3	1623.2±357.2	1904.8±461.9	
Na (mEq/day)		1.584±0.561	1.496±0.182	1.298±0.481	1.226±0.341	1.474±0.175	1.192±0.206	
K (mEq/day)		3.266±0.426	3.204±0.207	3.280±0.608	2.488±0.243	2.920±0.343	2.724±0.267	
Cl (mEq/day)		2.168±0.477	2.174±0.195	2.296±0.542	1.562±0.298	1.840±0.252	1.928±0.211	
CRE (mg/day)		10.022±0.740	9.960±0.672	9.808±0.662	6.134±0.869	6.360±0.626	7.094±0.552	
S-RBC		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
S-WBC		2.4±1.5	4.4±2.1	2.0±1.9	1.2±0.8	2.2±0.8	1.6±1.3	
EC		5.0±5.6	3.8±2.8	8.0±8.9	2.4±3.8	3.8±4.8	1.0±1.7	
CAST		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.0±2.2	
S-Others		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
pH	6	0	0	0	0	1	1	
	7.5	1	0	1	0	0	2	*
	8	2	2	3	1	1	2	
	8.5	2	3	1	4	3	0	
Protein	−	4	1	2	5	5	5	
	±	1	1	1	0	0	0	
	+	0	3	2	0	0	0	
Glucose	−	5	5	5	5	5	5	
Ketones	−	4	2	5	5	5	5	
	±	1	3	0	0	0	0	
Bilirubin	−	5	5	5	5	5	5	
Occult Blood	−	5	5	5	4	5	5	
	+	0	0	0	1	0	0	
Urobilinogen	±	5	5	5	5	5	5	

* ; P<0.05 : Significant difference from the control

Table 8. Urinalysis in Crj:CD(SD)IGS rats given HPC or HPMCP (Recovery period)

Sex		Male			Female		
Group		Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals		5	5	5	5	5	5
Water Cons.(mL)		24.2±5.7	24.0±3.1	21.4±7.5	22.4±2.9	26.0±4.8	20.4±3.9
Urine Volume(mL)		16.52±3.75	13.72±3.48	11.12±3.51*	10.72±3.16	12.32±5.84	10.36±2.42
OSMO(mOsm)		1436.0±307.5	1678.4±488.8	1821.2±314.5	1758.4±290.2	1574.0±501.0	1764.8±211.8
Na (mEq/day)		1.648±0.384	1.586±0.269	1.466±0.314	1.424±0.370	1.380±0.427	1.486±0.317
K (mEq/day)		3.424±0.303	3.476±0.387	3.024±0.518	2.800±0.499	2.656±0.555	2.634±0.377
Cl (mEq/day)		2.220±0.462	2.086±0.384	2.002±0.361	1.998±0.406	1.790±0.482	1.798±0.303
CRE (mg/day)		13.124±1.883	13.802±1.286	12.832±0.701	7.070±1.271	6.992±1.461	7.310±0.425
S-RBC		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
S-WBC		1.2±0.4	1.6±1.3	3.8±1.3**	0.8±1.1	0.8±0.8	1.0±1.0
EC		1.6±1.7	1.6±1.3	1.0±0.7	3.2±2.3	2.6±3.2	1.2±0.8
CAST		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
S-Others		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
pH	6	0	0	0	0	1	0
	6.5	0	0	0	0	2	0
	7	0	0	0	0	0	1
	7.5	0	0	0	1	0	0
	8	1	1	0	2	0	1
	8.5	4	4	4	2	2	3
	9	0	0	1	0	0	0
Protein	−	0	0	1	2	3	3
	±	1	1	0	0	1	1
	+	4	2	2	3	0	1
	++	0	2	2	0	1	0
Glucose	−	5	5	5	5	5	5
Ketones	−	3	1	2	4	4	4
	±	1	1	0	1	1	1
	+	1	3	3	0	0	0
Bilirubin	−	5	5	5	5	5	5
Occult Blood	−	5	4	5	4	5	5
	±	0	1	0	0	0	0
	+	0	0	0	1	0	0
Urobilinogen	±	5	5	5	5	5	5

* ; P<0.05 , ** ; P<0.01 : Significant difference from the control

Ophthalmology: No abnormality was observed in both sexes of each group.

Organ weights (Tables 9-12): HPC group; After 4 weeks of the dosing period, there was no difference in the relative liver weight although the absolute liver weight increased in the females when compared with the control. After 4 weeks of the recovery period, there was no difference in the relative ovaries weight although the absolute ovaries weight increased when compared with the control. HPMCP group; After 4 weeks of the dosing period, the absolute weights of the brain, heart and lungs of the males, and the submaxillary glands of the females showed no differences although their relative weights decreased when compared with the control. After 4 weeks of the recovery period, absolute spleen weight of the males showed no difference although relative spleen weight decreased when compared with the control.

Table 9. Organ weight in Crj:CD(SD)IGS rats given HPC or HPMCP (Dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
Final B.W. (g)	366.2±35.2	376.3±27.7	396.1±31.6	221.8±15.1	233.6±10.4	236.1±12.1*
Brain (g)	1.981±0.080	1.994±0.076	1.992±0.061	1.809±0.070	1.813±0.045	1.832±0.074
Pituitary (mg)	12.0±2.3	13.0±1.3	13.5±2.0	14.3±1.6	14.4±1.8	14.9±1.9
Submaxi. (g)	0.582±0.077	0.584±0.059	0.597±0.041	0.385±0.030	0.383±0.028	0.373±0.025
Thyroids (mg)	25.6±3.6	26.7±3.5	26.6±2.9	19.5±3.7	19.3±2.2	19.7±2.6
Thymus (mg)	482.9±139.7	509.1±167.2	450.0±110.0	392.5±75.5	409.0±130.9	419.0±63.3
Heart (g)	1.312±0.166	1.260±0.091	1.255±0.100	0.838±0.093	0.873±0.100	0.851±.055
Lungs (g)	1.242±0.141	1.262±0.103	1.283±0.120	0.956±0.056	0.993±0.061	0.995±0.071
Liver (g)	10.99±1.28	11.15±1.43	11.81±1.25	6.35±0.53	6.89±0.48*	6.70±0.49
Spleen (g)	0.666±0.127	0.617±0.092	0.681±0.054	0.445±0.062	0.488±0.053	0.458±0.050
Adrenals (mg)	58.3±7.9	59.2±9.6	63.1±7.6	63.9±6.5	67.4±7.7	62.2±6.7
Kidneys (g)	2.580±0.292	2.617±0.210	2.728±0.257	1.612±0.095	1.616±0.086	1.615±0.116
Testes (g)	3.161±0.265	3.312±0.190	3.121±0.186	NA	NA	NA
Semi. Ves. (g)	1.016±0.218	1.041±0.258	1.150±0.246	NA	NA	NA
Prostate (g)	0.932±0.135	0.957±0.081	1.009±0.147	NA	NA	NA
Ovaries (mg)	NA	NA	NA	91.0±13.0	97.5±15.3	92.1±14.5
Uterus (g)	NA	NA	NA	0.45±0.15	0.53±0.18	0.40±0.05

* ; P<0.05 : Significant difference from the control
NA: Not Available

Table 10. Relative organ weight in Crj:CD(SD)IGS rats given HPC or HPMCP (Dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
Final B.W. (g)	366.2±35.2	376.3±27.7	396.1±31.6	221.8±15.1	233.6±10.4	236.1±12.1*
Brain (%)	0.544±0.040	0.532±0.036	0.503±0.033*	0.820±0.064	0.779±0.032	0.780±0.047
Pituitary (mg%)	3.31±0.76	3.46±0.41	3.43±0.50	6.45±0.60	6.16±0.63	6.31±0.66
Submaxi. (%)	0.159±0.011	0.157±0.016	0.151±0.017	0.174±0.010	0.164±0.013	0.159±0.009**
Thyroids (mg%)	7.03±1.09	7.10±0.79	6.74±0.66	8.85±1.79	8.25±0.90	8.35±1.19
Thymus (mg%)	130.0±27.5	135.9±42.1	113.6±25.6	177.8±35.4	175.0±54.2	177.8±27.1
Heart (%)	0.359±0.032	0.336±0.020	0.317±0.023**	0.379±0.034	0.373±0.042	0.361±0.013
Lungs (%)	0.340±0.016	0.337±0.022	0.324±0.017*	0.430±0.023	0.425±0.025	0.421±0.027
Liver (%)	3.01±0.09	2.97±0.23	2.98±0.12	2.88±0.19	2.96±0.16	2.84±0.16
Spleen (%)	0.182±0.027	0.163±0.024	0.173±0.013	0.200±0.026	0.210±0.021	0.195±0.024
Adrenals (mg%)	15.99±2.09	15.71±2.16	16.01±2.14	28.86±2.72	28.86±3.19	26.45±3.54
Kidneys (%)	0.704±0.031	0.696±0.037	0.690±0.034	0.731±0.055	0.692±0.034	0.684±0.049
Testes (%)	0.871±0.124	0.885±0.084	0.793±0.075	NA	NA	NA
Semi. Ves. (%)	0.278±0.054	0.278±0.070	0.290±0.066	NA	NA	NA
Prostate (%)	0.257±0.032	0.255±0.020	0.255±0.035	NA	NA	NA
Ovaries (mg%)	NA	NA	NA	41.4±7.2	41.9±6.1	39.1±6.1
Uterus (%)	NA	NA	NA	0.203±0.061	0.226±0.074	0.170±0.025

* ; P<0.05, ** ; P<0.01 : Significant difference from the control
NA: Not Available

Table 11. Organ weight in Crj:CD(SD)IGS rats given HPC or HPMCP (Recovery period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
Final B.W (g)	480.7±47.2	475.4±15.6	462.7±41.9	271.1±13.5	281.4±25.5	274.1±15.9
Brain (g)	2.071±0.067	2.056±0.064	2.037±0.083	1.887±0.055	1.894±0.066	1.870±0.048
Pituitary (mg)	12.6±2.4	14.3±1.5	12.9±0.7	16.1±2.1	15.6±2.1	16.4±1.8
Submaxi. (g)	0.651±0.085	0.691±0.034	0.661±0.085	0.417±0.049	0.427±0.019	0.407±0.043
Thyroids (mg)	28.6±2.0	26.9±4.2	29.3±5.0	20.9±2.7	22.4±2.9	21.6±2.9
Thymus (mg)	439.4±116.7	359.0±121.2	328.6±92.3	281.0±61.7	356.0±98.7	239.0±71.6
Heart (g)	1.444±0.149	1.473±0.105	1.424±0.120	0.884±0.056	0.911±0.060	0.926±0.051
Lungs (g)	1.384±0.116	1.376±0.077	1.340±0.118	1.087±0.080	1.103±0.095	1.059±0.075
Liver (g)	13.47±1.66	13.34±0.71	12.14±1.62	6.70±0.50	7.26±0.53	7.01±0.38
Spleen (g)	0.796±0.140	0.724±0.047	0.693±0.088	0.446±0.069	0.503±0.082	0.430±0.061
Adrenals (mg)	56.6±6.3	63.1±6.0	53.6±6.0	64.1±5.1	71.4±9.3	65.4±10.3
Kidneys (g)	2.867±0.273	3.054±0.167	2.760±0.167	1.683±0.149	1.757±0.222	1.680±0.089
Testes (g)	3.433±0.283	3.411±0.196	3.313±0.202	NA	NA	NA
Semi. Ves (g)	1.399±0.345	1.399±0.365	1.274±0.340	NA	NA	NA
Prostate (g)	1.181±0.207	1.141±0.185	1.200±0.120	NA	NA	NA
Ovaries (mg)	NA	NA	NA	77.7±12.3	94.1±14.6*	89.1±12.9
Uterus (g)	NA	NA	NA	0.67±0.23	0.57±0.16	0.81±0.25

* ; P<0.05 : Significant difference from the control
NA: Not Available

Table 12. Relative organ weight in Crj:CD(SD)IGS rats given HPC or HPMCP (Recovery period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
Final B.W. (g)	480.7±47.2	475.4±15.6	462.7±41.9	271.1±13.5	281.4±25.5	274.1±15.9
Brain (%)	0.434±0.047	0.433±0.016	0.443±0.031	0.696±0.029	0.677±0.055	0.684±0.048
Pituitary (mg%)	2.63±0.43	3.03±0.35	2.80±0.39	5.97±0.91	5.59±1.04	6.01±0.84
Submaxi. (%)	0.136±0.013	0.146±0.010	0.144±0.014	0.154±0.019	0.151±0.012	0.149±0.009
Thyroids (mg%)	5.97±0.60	5.66±0.97	6.31±0.81	7.71±1.12	8.03±1.20	7.87±0.95
Thymus (mg%)	91.7±23.5	75.4±24.1	71.4±18.9	103.9±23.2	125.1±25.8	86.3±21.9
Heart (%)	0.301±0.025	0.313±0.026	0.310±0.025	0.326±0.028	0.326±0.030	0.337±0.030
Lungs (%)	0.289±0.021	0.290±0.021	0.290±0.008	0.401±0.040	0.393±0.031	0.387±0.018
Liver (%)	2.83±0.28	2.81±0.19	2.63±0.21	2.49±0.21	2.60±0.14	2.57±0.14
Spleen (%)	0.166±0.015	0.153±0.008	0.149±0.011*	0.164±0.022	0.177±0.014	0.157±0.021
Adrenals (mg%)	11.83±1.38	13.29±1.28	11.63±1.35	23.67±1.83	25.74±5.16	23.84±3.41
Kidneys (%)	0.599±0.043	0.643±0.048	0.600±0.027	0.624±0.065	0.626±0.068	0.614±0.031
Testes (%)	0.720±0.079	0.719±0.047	0.720±0.061	NA	NA	NA
Semi. Ves. (%)	0.290±0.061	0.294±0.081	0.277±0.082	NA	NA	NA
Prostate (%)	0.247±0.035	0.241±0.036	0.261±0.036	NA	NA	NA
Ovaries (mg%)	NA	NA	NA	28.6±4.7	33.7±6.1	32.7±5.0
Uterus (%)	NA	NA	NA	0.249±0.089	0.200±0.054	0.296±0.091

* ; P<0.05 : Significant difference from the control
NA: Not Available

Necropsy (Tables 13, 14): Macroscopical observations did not show any difference among the groups.

Histopathology (Tables 15, 16): Histopathological observations did not show any difference among the groups.

Table 13. Macropathological findings in Crj:CD(SD)IGS rats given HPC or HPMCP (dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
Liver						
Diaphragmatic nodule	0	0	1	0	0	0
Kidney						
Dilatation of renal pelvis	1	1	0	0	0	0
Thymus						
Focal change	0	0	0	2	0	0
Spleen						
Nodule	1	0	0	0	0	0
Lung						
Focal change	0	1	0	0	0	0

Table 14. Macropathological findings in Crj:CD(SD)IGS rats given HPC or HPMCP (recovery period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
Kidney						
Dilatation of renal pelvis	0	0	1	0	0	0
Thymus						
Focal change	0	0	0	0	0	1
Lung						
Focal change	0	0	1	0	0	0

Table 15. Histopathological findings in Crj:CD(SD)IGS rats given HPC or HPMCP (dosing period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	10	10	10	10	10	10
Trachea						
Small round cells infiltration in submucosa	1	2	0	0	0	0
Lung and Bronchus						
Calcification of pulmonary artery	1	0	1	0	0	0
Focal hemorrhage	0	0	1	0	0	0
Osseous metaplasia	1	0	0	0	0	0
Heart						
Focal necrosis in myocardium	0	1	1	1	0	0
Stomach						
Inflammatry cell infiltration in submucosal layer	0	0	0	0	0	1
Liver						
Focal infiltration of small round cells	3	3	4	2	3	4
Peripheral fatty change	1	0	1	3	3	2
Pancreas						
Focal atrophy of acini	0	0	0	0	1	0
Focal infiltration of small round cells into interstitium	0	0	0	0	0	2
Parotid gland						
Focal infiltration of small round cells	0 [a)]	0	1	0	0	0
Kidney						
Focal infiltration of small round cells into interstitium	3	2	0	1	2	0
Tubular basophilia	2	2	0	0	0	0
Pelvis dilatation / unilateral	1	1	0	0	0	0
Hyaline droplets deposition in proximal tubular epithelium	2	0	1	0	0	0
Eosinophilic bodies in proximal tubular epithelium	0	1	0	0	0	0
Calcium deposition in papilla	0	0	0	0	1	0
Prostate						
Lymphocytic prostatitis	1	1	1	0	0	0
Thyroid						
Ultimobranchial rest	0	0	0	1	1	0
Ectopic thymus	1	1	1	0	0	1
Harderian gland						
Focal small round cell infiltration	0 [a)]	1	0	1	0	1
Focal atrophy of acini	0 [a)]	1	0	0	0	0
Thymus						
Hemorrhage	2	0	0	0	0	0
Trachea						
Small round cells infiltration in submucosa	1	2	0	0	0	0
Lung and Bronchus						
Calcification of pulmonary artery	1	1	1	0	0	0
Osseous metaplasia	0	1	0	0	0	0

a) : 9 animals were examined.

Table 16. Histopathological findings in Crj:CD(SD)IGS rats given HPC or HPMCP (recover period)

Sex	Male			Female		
Group	Control	HPC	HPMCP	Control	HPC	HPMCP
No. of animals	7	7	7	7	7	7
Thymus						
Cyst	0	0	0	0	0	1
Hemorrhage	0	0	0	0	0	1
Trachea						
Small round cells infiltration in submucosa	0	2	1	2	0	0
Lung and Bronchus						
Calcification of pulmonary artery	4	0	2	3	0	1
Focal hemorrhage	0	0	1	0	0	0
Heart						
Focal necrosis in myocardium	1	1	2	0	1	1
Liver						
Focal infiltration of small round cells	0	1	1	3	1	2
Focal fatty change of hepatocytes	0	0	0	1	0	2
Peripheral fatty change	0	0	0	0	0	1
Kidney						
Calcium deposition in corticomedulla	0	0	0	1	0	0
Focal infiltration of small round cells into interstitium	0	1	0	1	0	1
Pelvis dilatation / unilateral	1	0	0	0	0	0
Tubular basophilia	0	0	1	0	0	0
Prostate						
Lymphocytic prostatitis	3	2	3	0	0	0
Pituitary						
Cyst	0	1	0	0	0	0
Thyroid						
Diffuse follicular cell hypertrophy and hyperplasia	1	0	0	0	0	0
Ectopic thymus	0	1	0	0	0	0
Eye						
Rosette formation in outer granular layer of retina, bilateral	1	0	1	0	0	0
Harderian gland						
Focal small round cell infiltration	1	2	1	1	0	0 [a)]

a): 6 animals were examined.

DISCUSSION

Changes due to additives dosed are postulated as follows. In 6% HPC group, increased food consumption and decreased plasma potassium (K) were observed. In 15% HPMCP group, decreased serum A/G ratio accompanied by increased T-GLB volume and plasma PL in the males, increased body weight, decreased serum A/G ratio accompanied by decreased ALB volume, increased plasma CRE, decreased plasma K and decreased urine pH in the females were observed. Changes mentioned above were thought to be insignificant because they could not be detected after the recovery period and no related histological changes were observed.

In conclusion, the effect of high dose of additives on rats could not completely denied, but it was not thought to be of toxicological significance under this study condition.

Background Data of General Toxicological Parameters in Crj:CD(SD)IGS Rats at 9 and 13 Weeks of Age

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ABSTRACT. Sixty male and sixty female Crj:CD(SD)IGS rats at 5 weeks of age were reared for either 4 or 8 weeks to obtain biological background data in our facility. At 9 or 13 weeks of age, 30 males and 30 females were examined for clinical signs, body weights, urinalysis, hematology, and blood biochemistry. All animals were euthanized for necropsy and organ weights. In comparison with males, females had lower urinary volume and excretion of urinary K and Cl, lower PT, APTT, NEFA, TG, and ALP values as well as a tendency of A/G ratio, T-CHO, F-CHO, PL, and CHE values to be higher. In comparison with rats at 9 weeks of age, rats at 13 weeks of age had higher RBC, TP, γ -globulin ratio, GLU, T-CHO, F-CHO, NEFA, TG, and PL values as well as a tendency of A/G ratio, GOT, ALP, LDH, and γ -GTP values to be lower. At necropsy, a large size of the spleen was found in one female at 13 weeks of age. ─ Key words: Crj:CD(SD)IGS, General toxicological parameters, Rats

CD(SD)IGS-1999: 46-51

INTRODUCTION

Crj:CD(SD)IGS rats, developed as an experimental animal by Charles River Inc. in response to the internationalization of new drug R & D, were reared for 4 or 8 weeks in our facility under the standard housing conditions to obtain data on a variety of parameters required for the evaluation of toxicities of drugs and chemicals.

MATERIALS AND METHODS

Animals and housing conditions

Sixty male and sixty female Crj:CD(SD)IGS rats at 4 weeks of age were supplied by Charles River Japan Inc.(Tsukuba, Japan) on September 3, 1997. Animals were quarantined and acclimated for 7 days, and those animals having favorable body weight gains without abnormal clinical signs were selected for study when they were at 5 weeks of age. Animals were housed in metal cages (W 280 × D 370 × H 180mm), 2 rats of either sex per cage, in a room maintained at temperature of 22 to 26℃, humidity of 44 to 63%, 10 to 15 times air-changes/hr, and a 12-hr lighting cycle (8:00 to 20:00). Pelleted food CE-2 (CLEA Japan, Inc) was given at all times, and drinking water (Ishikari-shi public water) was supplied using automatic watering nozzles. Animals were individually identified by the animal No. tattooed on the right and left ears.

Observations and examinations

1) Clinical signs: Physical signs and behaviors were examined once weekly.
2) Body weights: Animals were weighed on the day of the study initiation and once weekly thereafter.
3) Urinalysis: After 4 weeks (at 9 weeks of age) or 8 weeks (at 13 weeks of age), animals were housed individually in metabolism cages, and 3-hr or 16-hr urine samples were collected to examine the parameters shown in Table 1.
4) Hematological examination: After 4 weeks (at 9 weeks of age) or 8 weeks (at 13 weeks of age), animals were fasted with a supply of water for 19 hrs or more and then bled from the abdominal aorta under ether anesthesia for hematological and biochemical examinations. A part of the blood was treated with EDTA-2K. Another part of the blood was treated with 3.8% sodium citrate to obtain plasma after centrifugation at 3000 rpm for 10 min. The blood or plasma samples were examined on the parameters shown in Table 1.
5) Blood biochemical examination: After 4 weeks (at 9 weeks of age) or 8 weeks (at 13 weeks of age), animals were fasted with a supply of water for 19 hrs or more and then bled from the abdominal aorta under ether anesthesia. A part of the blood was used for hematological examination as mentioned before, and most of the remaining blood was stood still for 30 min or more at room temperature and then centrifuged at 3000 rpm for 10 min. The serum sample obtained was examined on the parameters shown in Table 1. Another part of the remaining blood was treated with heparin and centrifuged for 3000 rpm for 10 min to obtain plasma for LDH and CPK determinations.
6) Necropsy: After 4 weeks (at 9 weeks of age) or 8 weeks (at 13 weeks of age), animals were fasted with a supply of water for 19 hrs or more. Animals were exsanguinated to death under ether anesthesia, and the cranial, thoracic, and abdominal organs and tissues were examined grossly.
7) Organ weights: The following organs collected at necropsy were weighed; brain, pituitary, thyroids, salivary glands, thymus, heart, lungs, liver, spleen, kidneys, adrenals, testes, epididymides, seminal vesicle, prostate, ovaries, and uterus. Organ weights relative to body weights were calculated

RESULTS

No abnormal clinical signs were observed throughout the study period.

Body weights of males and females are shown in Table 2. Mean body weight gains for males (females) were 216 g (91g) at 9 weeks of age and 304 g (135 g) at 12 weeks of age, respectively.

Results of urinalysis are shown in Table 3. Urinary pH was 7 for more than half of the males with incidences decreasing in the

Table 1. Parameter, method and instrument for clinical examination

Examination		Parameter (Abbreviation)	Method	Instrument
Urinalysis	(3-hour urine)	pH, Protein, Glucose, Ketone bodies, Bilirubin, Urobilinogen, Occult blood	Test paper method (Pretest 8a, Wako Pure Chemical Ind., Ltd.)	
	(16-hour urine)	Urinary volume	Measuring cylinder	
		Specific gravity	Refractometry (ERMA)	
		Sodium (Na)	Ion-electrode method	Automated electrolyte analyzer (Synchron EL-ISE, Beckman)
		Potassium (K)	Ion-electrode method	
		Chloride (Cl)	Ion-electrode method	
Hematology		Red blood cell (RBC)	Electrical resistance method	Automated blood cell counter (System 9000, Baker Instrument)
		White blood cell (WBC)	Electrical resistance method	
		Platelet count (PLT)	Electrical resistance method	
		Mean corpuscular volume (MCV)	Electrical resistance method	
		Hemoglobin content (Hb)	Cyanmethemoglobin method	
		Hematocrit (Ht)	Calculated from RBC and MCV	
		Mean corpuscular hemoglobin (MCH)	Calculated from RBC and Hb	
		Mean corpuscular hemoglobin concentration (MCHC)	Calculated from Hb and Ht	
		Reticulocyte count (Ret)	Brecher's method, Microscopy	
		Differential leucocyte count	Giemsa stain, Microscopy	
		Prothrombin time (PT)	Clot method	Automated blood coagulation analyzer (ACL 100, Instrumentation laboratory)
		Activated partial thromboplastin time (APTT)	Clot method	
Blood chemistry		Total protein (TP)	Biuret method	Automated chemical analyzer (TBA-480, TOSHIBA)
		Albumin (ALB)	BCG method	
		Glucose (GLU)	UV method	
		Total cholesterol (T-CHO)	Enzyme method	
		Free cholesterol (F-CHO)	Enzyme method	
		Triglyceride (TG)	Enzyme method	
		Phospholipid (PL)	Enzyme method	
		Free fatty acid (NEFA)	Enzyme method	
		Urea nitrogen (BUN)	Urease-GLDH method	
		Creatinine (CRE)	Jaffé method	
		Glutamic oxaloacetic transaminase (GOT)	JSCC method	
		Glutamic pyruvic transaminase (GPT)	JSCC method	
		γ-Glutamyl transferase (γ-GTP)	Orlowski method	
		Alkaline phosphatase (ALP)	GSCC method	
		Leucine aminopeptidase (LAP)	Nagel method	
		Lactate dehydrogenase (LDH)	JSCC method	
		Creatine phosphokinase (CPK)	GSCC method	
		Total bilirubin (T-BIL)	Azobilirubin method	
		Calcium (Ca)	OCPC method	
		Inorganic phosphorus (iP)	Molybdenum blue method	
		Albumin globulin ratio (A/G)	Calculated from TP and ALB	
		Cholesterol ester ratio (E/T)	Calculated from T-CHO and F-CHO	
		Protein fractions	Cellulose acetate electrophoresis	Electrophoretic apparatus (PAV-50), automatic current voltage regulator, Densitron (CR-20)
		Sodium (Na)	Ion-electrode method	Automated electrolyte analyzer (Synchron EL-ISE, Beckman)
		Potassium (K)	Ion-electrode method	
		Chloride (Cl)	Ion-electrode method	

Table 2. Body weight changes

Age (weeks)	Male	Female
5	153.0±6.1 (60)	129.0±5.5 (60)
6	215.5±9.0 (60)	160.5±10.6 (60)
7	275.6±12.1 (60)	181.8±14.2 (60)
8	329.7±17.1 (60)	202.9±17.2 (60)
9	369.4±21.8 (60)	220.0±21.1 (60)
10	398.4±30.6 (30)	241.6±26.6 (30)
11	427.9±32.2 (30)	251.3±28.0 (30)
12	457.3±35.2 (30)	264.2±27.5 (30)

Values are expressed as Mean ± S.D., g
( ): Number of animals

Table 3. Urinalysis

	Male		Female	
Age (weeks)	9	13	9	13
Number of animals	30	30	30	30
pH				
6	9	13	5	5
7	16	15	17	17
8	5	2	6	8
9	0	0	2	0
Protein				
−(0 mg/dL)	0	0	16	12
±(10-20mg/dL)	3	3	7	13
+(30mg/dL)	23	22	7	5
++(100mg/dL)	4	5	0	0
Glucose				
−(0mg/dL)	30	30	30	30
Ketone bodies				
−(0mg/dL)	30	30	30	30
Bilirubin				
−(0mg/dL)	30	30	30	30
Urobilinogen				
±(normal)	30	30	30	30
Occult blood				
−(0mg/dL)	30	29	30	30
+(0.06mg/dL)	0	1	0	0
Urinary volume (mL/16hr)	14.4±7.9[a)]	14.0±5.3	7.2±2.7	10.7±5.8
Specific gravity	1.031±0.012	1.032±0.014	1.036±0.016	1.031±0.017
Electrolytes (mEq/16hr)				
Na	0.377±0.169	0.343±0.227	0.324±0.134	0.319±0.111
K	1.563±0.274	1.497±0.230	0.725±0.179	0.757±0.147
Cl	0.442±0.186	0.364±0.142	0.290±0.096	0.252±0.093

a): Values are expressed as Mean ± S. D.

order of pH 6 and pH 8. No apparent difference in this parameter was found between males at 9 and 13 weeks of age. For females, pH was 7 for more than half of the animals with incidences decreasing in the order of pH 8 and pH 6. Cases of pH 9 were found in 2 females at 9 weeks of age, but not at 13 weeks of age. Positive protein was found in all males, with (+) most frequently and incidences decreasing in the order of (++) and (±). No apparent differences in this parameter were found between males at 9 and 13 weeks of age. For females, incidences of (−) were most frequently noted at 9 weeks of age, but incidences of (−) and (±) were almost equal at 13 weeks of age. No gender- or age-related differences were found in urinary glucose, ketone bodies, bilirubin, urobilinogen, or occult blood. Occult blood was positive in 1 male only at 13 weeks of age. Urinary volume was lower in females than in males at both times, but no apparent age-related differences were found.

Urinary specific gravity showed no apparent gender- or age-related differences. For urinary electrolytes, Na excretion had no apparent gender- or age-related differences, but K and Cl excretions were lower in females than in males at both times, but no apparent age-related differences were found.

Results of hematological examinations are shown in Table 4. Comparison of data from animals of different gender and ages revealed higher RBC counts (males and females) and WBC count (males) at 13 weeks of age than those at 9 weeks of age and lower PT and APTT values in females than in males at both times. Other parameters showed no apparent gender- or age-related differences.

Table 4. Hematology

	Male		Female	
Age (weeks)	9	13	9	13
Number of animals	30	30	30	29
RBC ($\times 10^6$/mm^3)	6.22±0.26	7.37±0.60	6.92±0.30	7.12±0.42
Hb (g/dL)	14.2±0.4	15.2±0.8	15.3±0.5	14.7±0.6
Ht (%)	37.2±1.0	38.8±2.3	41.0±1.9	38.1±1.9
MCV (μ m^3)	59.8±1.7	53.2±1.8	59.3±1.3	53.6±1.3
MCH (pg)	22.9±0.8	20.8±0.8	22.1±0.7	20.7±0.8
MCHC (%)	38.3±0.6	39.2±0.7	37.3±1.0	38.6±0.9
PLT ($\times 10^3$/mm^3)	1139±106	1123±110	1122±119	1097±101
Ret (‰)	23±5	20±57	17±5	25±8
WBC ($\times 10^3$/mm^3)	4.5±1.3	8.1±2.7	4.6±1.5	4.4±2.1
Differential leucocyte count (%)				
Neutropils Stab	0.2±0.6	0.1±0.3	0.1±0.2	0.1±0.3
Segment	10.4±4.3	11.0±4.5	8.0±3.6	10.3±5.1
Basophils	0.0±0.0	0.1±0.3	0.1±0.3	0.2±0.5
Eosinophils	1.0±1.3	0.8±0.9	0.7±0.7	1.8±1.6
Monocytes	3.9±2.4	5.3±2.4	4.7±1.8	3.3±2.5
Lymphocytes	84.6±4.8	82.7±5.6	86.5±3.7	84.2±5.3
PT (sec)	13.8±1.9	14.2±2.2	11.8±0.6	11.5±0.5
APTT (sec)	19.8±1.9	18.5±1.8	14.8±1.2	14.1±1.1

Values are expressed as Mean ± S. D.

Table 5. Blood biochemistry

	Male		Female	
Age (weeks)	9	13	9	13
Number of animals	30	30	30	29
TP (g/dL)	5.8±0.2	7.0±0.4	6.3±0.4	7.2±0.3
ALB (g/dL)	3.6±0.1	4.0±0.2	4.0±0.2	4.2±0.2
Protein fraction (%)				
Albumin	59.7±4.5	55.1±4.6	57.8±2.9	60.4±3.5
Globurin				
α 1	24.6±3.2	21.7±3.6	24.0±1.7	18.3±3.4
α 2	4.4±1.7	8.7±1.6	4.6±0.7	5.7±1.6
β	10.2±1.1	11.6±1.2	11.5±1.2	11.9±2.2
γ	1.1±0.7	3.0±0.9	2.2±0.9	3.8±1.4
A/G ratio	1.64±0.12	1.33±0.10	1.71±0.13	1.37±0.08
GLU (mg/dL)	102±16	136±21	107±18	148±20
T-CHO (mg/dL)	54±8	66±13	68±11	88±12
F-CHO (mg/dL)	4.0±1.4	5.7±1.8	6.8±2.6	9.5±3.0
E/T ratio	0.93±0.02	0.92±0.02	0.90±0.03	0.89±0.02
NEFA (μ Eq/L)	860±175	916±147	767±128	820±157
TG (mg/dL)	52±18	65±24	31±12	42±20
PL (mg/dL)	80±11	92±15	115±18	148±19
GOT (U/L)	134±18	118±24	152±24	90±16
GPT (U/L)	30±4	33±6	25±4	26±6
ALP (IU/L)	546±113	296±68	303±75	178±43
LDH (U/L)	189±51	178±76	349±63	117±42
γ -GTP (U/L)	1.2±1.1	0.5±0.6	1.1±1.4	0.6±0.9
CHE (IU/L)	440±94	411±106	2071±504	2547±717
LAP (IU/L)	34±5	34±3	37±5	33±4
CPK (IU/L)	112±20	112±22	138±24	94±18
T-BIL (mg/dL)	0.04±0.02	0.05±0.02	0.04±0.02	0.10±0.02
BUN (mg/dL)	16.7±1.9	14.0±1.1	14.2±1.9	14.6±2.3
CRE (mg/dL)	0.60±0.04	0.56±0.06	0.59±0.06	0.64±0.04
Na (mEq/L)	144.7±1.7	143.1±1.1	142.2±1.7	140.2±1.3
K (mEq/L)	4.61±0.26	4.74±0.25	4.33±0.24	4.58±0.29
Cl (mEq/L)	109.6±1.5	110.6±1.4	109.6±1.6	112.7±1.7
Ca (mg/dL)	10.9±0.4	11.7±0.4	10.6±0.6	12.3±0.4
iP (mg/dL)	9.6±0.5	9.0±0.6	9.2±0.6	8.0±0.4

Values are expressed as Mean±S. D.

Table 6. Absolute organ weight

	Male		Female	
Age (weeks)	9	13	9	13
Number of animals	30	30	30	30
Body weight at necropsy (g)	347.0±18.2	448.7±35.8	197.7±15.3	254.5±27.6
Brain (g)	2.06±0.08	2.14±0.07	1.89±0.06	1.98±0.08
Pituitary gland (mg)	12.5±1.7	12.9±2.2	12.8±2.1	15.1±2.7
Thyroids (mg)	18.2±3.5	20.8±3.3	13.9±1.7	15.2±2.3
Right salivary gland. (mg)	315±28	353±44	202±23	219±21
Left salivary gland (mg)	310±30	349±36	202±20	214±20
Thymus (mg)	595±92	419±96	438±72	357±76
Heart (g)	1.19±0.09	1.40±0.13	0.74±0.06	0.88±0.09
Lungs (g)	1.23±0.07	1.35±0.12	0.92±0.07	0.99±0.10
Liver (g)	10.63±1.07	12.31±1.3	6.09±0.70	6.80±1.04
Spleen (g)	0.70±0.08	0.77±0.10	0.46±0.07	0.49±0.08
Right kidney (g)	1.35±0.11	1.59±0.16	0.84±0.08	0.92±0.10
Left kidney (g)	1.34±0.11	1.61±0.15	0.81±0.08	0.88±0.10
Right adrenal gland (mg)	27±4	27±4	30±4	30±5
Left adrenal gland (mg)	28±4	29±4	31±4	31±5
Right testis (g)	1.49±0.11	1.67±0.15		
Left testis (g)	1.50±0.12	1.68±0.14		
Right epididymis (g)	0.34±0.02	0.56±0.04		
Left epididymis (g)	0.33±0.02	0.55±0.04		
Seminal vesicle (g)	0.89±0.14	1.33±0.14		
Prostate (mg)	411±83	576±116		
Right ovary (mg)			41±6	41±6
Left ovary (mg)			40±7	41±8
Uterus (g)			0.40±0.09	0.47±0.08

Values are expressed as Mean±S. D.

Table 7. Relative organ weight

	Male		Female	
Age (weeks)	9	13	9	13
Number of animals	30	30	30	30
Brain (g%)	0.59±0.03	0.48±0.04	0.96±0.06	0.78±0.07
Pituitary gland (mg%)	3.6±0.4	2.9±0.5	6.5±0.9	5.9±0.9
Thyroids (mg%)	5.3±1.0	4.7±0.8	7.0±0.9	6.0±1.1
Right salivary gland. (mg%)	90±7	79±10	103±12	87±10
Left salivary gland (mg%)	90±7	78±9	103±116	85±9
Thymus (mg%)	172±27	93±21	222±32	141±26
Heart (g%)	0.34±0.02	0.31±0.03	0.38±0.02	0.35±0.02
Lungs (g%)	0.36±0.02	0.30±0.02	0.46±0.02	0.39±0.04
Liver (g%)	3.06±0.19	2.75±0.22	3.08±0.23	2.67±0.25
Spleen (g%)	0.20±0.02	0.17±0.02	0.23±0.03	0.19±0.02
Right kidney (g%)	0.39±0.03	0.36±0.04	0.42±0.03	0.36±0.03
Left kidney (g%)	0.39±0.03	0.36±0.04	0.41±0.03	0.35±0.03
Right adrenal gland (mg%)	8±1	6±1	15±2	12±2
Left adrenal gland (mg%)	8±1	6±1	16±2	12±2
Right testis (g%)	0.43±0.03	0.37±0.04		
Left testis (g%)	0.43±0.04	0.38±0.04		
Right epididymis (g%)	0.10±0.01	0.13±0.01		
Left epididymis (g%)	0.10±0.01	0.12±0.01		
Seminal vesicle (g%)	0.26±0.04	0.30±0.04		
Prostate (mg%)	119±23	129±28		
Right ovary (mg%)			21±3	16±3
Left ovary (mg%)			20±3	16±3
Uterus (g%)			0.20±0.05	0.19±0.04

Values are expressed as Mean±S. D.

Results of blood biochemical examinations are shown in Table 5. A clear gender-related difference was found in CHE value, which was about 5 to 6 times higher in females at both times. ALP value was lower in females than in males at both times and lower in both sexes of animals at 13 weeks of age than that at 9 weeks of age. Males and females at 13 weeks of age had higher TP, GLU, T-CHO, F-CHO, NEFA, TG, and PL values, γ-globulin ratio, and lower A/G ratio, GOT, LDH, and γ-GTP values than those at 9 weeks of age. Other parameters had no apparent gender- or age-related differences.

At necropsy, a large size of the spleen was found only in 1 female at 13 weeks of age, and no other findings were obtained in any animal.

Organ weights are shown in Tables 6 (absolute weight) and 7 (relative weight). Adrenal weights, both in absolute and relative, were higher in females than in males at both times. Both males and females at 13 weeks of age generally had higher absolute weights and lower relative weights for most organs than those of males and females at 9 weeks of age.

DISCUSSION

Biological background data on Crj:CD(SD)IGS rats at 9 or 13 weeks of age were collected in our facility. No abnormal clinical signs or changes in body weight were found in this study. An increase in urinary protein with age after birth and with aging, which is reported to occur [1,4], was not found in males or females of the present study. Higher excretions of urinary K and Cl in males than in females may reflect the difference in food consumption between males and females. Hematological examination revealed a tendency of PT and APTT values to be lower in females than in males at both times. For blood biochemical examination, it is known that ALP, GLU, and TG values are generally higher in males and CHE and PL values in females [2]. Although there are some variations in these parameters depending on the strain and age, above data corresponds fairly well with those of the present study except for GLU. In addition, T-CHO and F-CHO values were higher in females than in males at both times in the present study. Gender-related difference reported so far in serum CHE [3] is also confirmed, with 5 to 6 times higher in females than in males at both times in the present study.

One female at 13 weeks of age which had a large size of the spleen at necropsy also had hematological and blood biochemical changes. These changes consisted of abnormally high values of WBC, PLT, and differential neutrophils; low values of differential lymphocytes, ALB, A/G ratio, PL, NEFA, and CHE; and abnormally low values of ALP, α_1-, α_2-, β-, and γ-globurin ratios.

Organ weights tended to increase with age, more remarkably in males than in females.

REFERENCES

1. Neuhaus, O.W., and Flory, W. 1978. Age-dependent changes in the excretion of urinary proteins by rat. Nephron, 22: 570-576.
2. Tanimoto, Y. 1988. Biochemistry of blood and urine in experimental animals. pp 5, Soft Science Inc., Tokyo (in Japanese).
3. Tanimoto, Y. 1988. Biochemistry of blood and urine in experimental animals. pp 97-98, Soft Science Inc., Tokyo (in Japanese).
4. Weaver, R.N., Gray, J.E. and Schulz, J.R. 1975. Urinary proteins in Sprague-Dawley rats with chronic progressive nephrosis. Lab. Anim. Sci., 25: 705-710.

The General Toxicology Background Data in Crj:CD(SD)IGS Rats

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ABSTRACT. This study was performed to obtain general toxicity data of male and female Crj:SD(SD)IGS rats. Three different lots of animals at two different ages were used in the study to confirm variability of these data. It was concluded that the overall results in the study were almost compatible to those in the previous reports while slight variability was found in some parameters examined. The study would provide data to use the strain for the routine general toxicity studies. — Key words: General toxicity, Background data, IGS rats

CD(SD)IGS-1999: 52-59

INTRODUCTION

Crj:CD(SD)IGS strain rat (IGS rat) was produced by International Genetic Standard system and supplied from Charles River instead of Crj:CD(SD) rat. To confirm variability of general toxicology data among different lots of IGS rats, clinical signs, body weights, food consumption, the routine parameters of hematology, clinical chemistry and urinalysis, ophthalmology, macropathology, organ weights and histopathology were examined in the study.

MATERIALS AND METHODS

Animal Management: Three lots of male and female Crj:CD(SD)IGS rats were supplied from Charles River Japan Inc, Hino. Each lot contained 12 males and 12 females. Age at receipt was 4, 5 or 6 week-old, and called as Lot 1, 2 and 3 in the study, respectively. Animals of all lots were quarantined and acclimatized for 1-3 weeks and no abnormalities were found during these periods. Animals were 7-week-old at beginning of the experiment. The animals were housed in steel cages (W300 mm × D420 mm × H200 mm), and each cage contained 3 animals. Animal room temperature and relative humidity were within 23 ± 3 ℃ and 55 ± 20%, respectively. Lighting was controlled to give 12 hours of light (7:00 to 19:00) and 12 hours of darkness per day. Ventilation provided more than 10 changes of air per hour. The animals were provided with solid chow MF (Oriental Yeast Co. Ltd.) *ad libitum*. Water was sterilized by the addition of sodium hypochlorite and irradiation with ultraviolet light, and was available *ad libitum* via an automatic water dispenser.

Observations, Clinical and Laboratory Investigations:

Animals were housed during 13 and 26 weeks. At termination of study, animals were 20- and 33-week-old, respectively.

General Observations: Mortality and clinical signs were observed daily, and body weight and food consumption were measured weekly during 13 or 26 weeks.

Laboratory Investigations: After 13 or 26 weeks of observation period, animals were fasted overnight and then blood was taken from the abdominal aorta and the external jugular vein under anesthesia with sodium pentobarbital. Blood samples from the abdominal aorta were used for blood chemistry, while blood samples from the external jugular vein were used for hematology and serum protein fractions. EDTA and sodium citrate were added as anticoagulants to blood samples for hematological examinations and coagulation test, respectively. Sodium heparin was added to blood samples for blood chemistry.

1) Hematology: Red blood cell count (RBC), Hematocrit (Ht), Hemoglobin (Hb), Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Platelet count (Plat), White blood cell count (WBC), Differential WBC count (neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells), Reticulocyte count (Ret) were performed with the Total Hematology Management System (Technicon H・1E, Bayer Corporation, USA). Prothrombin time (PT), Activated partial thromboplastin time (APTT), Fibrinogen (Fbg) were measured using an automated coagulation analyzer (KC10A, Amelung GmbH, Germany).
2) Blood Chemistry: Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Creatine kinase (CK), Lactate dehydrogenase (LD), Total protein (TP), Total cholesterol (TCOL), Phospholipid (PL), Triglyceride (TG), Glucose (GLU), Urea nitrogen (UN), Creatinine (CRNN), Calcium (Ca), Inorganic phosphorus (IP), Chloride (Cl), Sodium (Na), Potassium (K) were examined with an automated analyzer (7170, Hitachi, Ltd., Japan). Serum protein fractions (albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin fractions) were analyzed using an automatic electrophoresis system (CTE 700 , Jokoh Co., Ltd., Japan). The albumin to globulin ratio (A/G) was calculated from the above values.
3) Urinalysis: Urine samples were collected for 22 hours before necropsy and determined urine volume. Osmolality was examined with an osmometer (3C2, Advanced Instruments, Inc., USA) by the freezing points method. Urine sodium and potassium concentrations were examined with an automated flame photometry (480, Ciba Corning, USA). Chloride concentration was examined with a chloride meter (925, Ciba Corning, USA). Urine sodium , potassium and chloride excretion rate was calculated from the concentrations and the urine volume. Urine creatinine concentration was examined with an automated analyzer (7170, Hitachi, Ltd., Japan), and its excretion rate was calculated from the concentration and the urine volume. Protein, pH,

glucose, ketone bodies, occult blood, bilirubin and urobilinogen were analyzed using multiple test papers, BM Test 10A (Boehringer Mannheim GmbH, Germany) followed by urine automatic analyzer Super UA (Hitachi , Ltd., Japan). For the urine sediment analysis, urine sediments were stained with Sternheimer-Malbin stain and observed microscopically.

Ophthalmological Examinations: Ophthalmological examinations were performed in all animals on Week 12 or Week 25. Appearance, conjunctiva, sclera, pupil, iris, cornea and palpebral reflex were examined macroscopically. Pupil, iris and cornea were observed with a surgical microscopy (KOM300 Kohnan Co., Japan). When necessary, sclera, pupil, iris, cornea, anterior chamber, lens and vitreous body were examined precisely using a pen light, a direct ophthalmoscope (11125 and 13010, Welch Allyn Inc., USA) and/or a slit lamp (Kowa Co., Ltd., Japan). Light reflex was examined in a light-protecting container using a pen light when necessary. An examination of fundus was carried out using an inverted image binophthalmoscope (IO-a, Neiz Instruments Co., Ltd., Japan) and fundus photos of both eyes were taken by a fundus camera (GENESIS K9L29, Kowa Co., Ltd., Japan). Prior to the ophthalmoscopic examinations, pupils were dilated by topical application of 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin P®, Santen Pharmaceutical Co., Ltd., Japan) .

Macroscopic examination and organ weight measurement: After 13-week or 26-week observation periods, animals were killed by exsanguination under anesthesia with sodium pentobarbital after overnight fast. External surface, subcutaneous tissue and cranial, cervical, thoracic and abdominal organs and tissues were examined macroscopically at necropsy. Liver, kidneys, heart, lungs, thyroids (including parathyroids), spleen, thymus, brain, testes, adrenals, pituitary, prostate and submaxillary glands (including sublingual glands) from all animals were excised at necropsy, were weighed and the relative organ weights (organ to 100g of body weight ratio) were calculated.

Histopathological Examination: The following organs were excised from all animals at necropsy.

liver, kidneys, brain, spinal cord, sciatic nerves, trachea, lungs (including bronchus), heart, aorta, spleen, thymus, adrenals, thyroids, parathyroids, pituitary, testes, epididymides, sublingual glands, submaxillary glands, Harderian glands, eyes, optic nerves, tongue, lymph nodes (mesenteric and submaxillary), pancreas, prostate, seminal vesicles, coagulating glands, urinary bladder, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (cecum, colon and rectum), bone marrow (sternum and femur), sternum, femurs, thigh muscle, mammary glands, skin

These organs and tissues were preserved in 10% neutral-buffered formalin (except for eyes, testes, epididymides, Harderian glands and optic nerves), embedded in paraffin wax, sectioned at 2-4 μm thickness, and stained with hematoxylin and eosin. Eyes, Harderian glands and optic nerves were preserved in formaldehyde-glutaraldehyde fixation solution. Testes and epididymides were fixed in formalin sucrose acetic acid (FSA) fixation solution and prepared histology slides by routine procedure. All above organs and tissues of all animals were examined by light microscopy.

RESULTS AND DISCUSSION

General Observations: There were no deaths during the observation period. Three females of Lot 1 showed alopecia as clinical signs from week 18 and hereafter.

Body weights and Food consumption (Figs. 1 and 2): Variation of mean body weights among three different lots of both males and females was almost within 10% of values. During observation period, mean food consumption during observation period was around 25 and 18 g/rat/day in males and females, respectively.

Hematology (Tables 1-1 and 1-2): Relatively variability in neutrophil count was found among three lots at 20-week old animals. No notable changes in hematological parameters were found between two different ages.

Clinical chemistry *(Tables 2-1 and 2-2)*: Data in AST, TG, LD, CK in males and females varied among three different lots than those of the other parameters. Values of TCHO in males and those of TCHO, TG, PL in females were higher in 33-week old animals when compared with those in 20-week-old rats, as reported previously [1].

Urinalysis (Tables 3-1 and 3-2): There were no remarkable differences in all parameters among three lots of animals at the same age. No age related changes were also recognized in any parameters of urinalysis.

Ophthalmology: One female killed at 20-week-old showed persistent pupillary membranes in iris at pre-test and during observation period. The other animals did not show any abnormalities.

Organ weights (Tables 4-1 to 4-4): There were substantial differences among lots at the same age. Marked variations among lots at the same age were found in thymus, pituitary, prostate and ovaries. An age-related decrease in thymic weights were evident in males and females. The decrease of thymic weight was somewhat prominent in the Lot 3 animals.

Macroscopic findings (Table 5): In 20-week-old animals, one male of the Lot 1 showed white spots in liver, and the another one male showed unilateral atrophy in testis and epididymis. In 33-week-old animals, thymic atrophy was observed in three males and one female of the Lot 3. The finding supported evidence of decreased thymus weight in the group. Mucosal thickening in ileum was found in one female of the Lot 3.

Histopathological findings (Tables 6-1 and 6-2): As spontaneous lesions, histopathological findings were mainly observed in liver, heart, kidneys in both male and females, and pancreas in males. Increased incidence of focal mononuclear cell infiltration of heart both in males and females, and of liver in males was evident at 33-week-old when compared with that of 20-week-old animals. Fibrosis of pancreatic islets associated with hemosiderin deposition was found in 5/18 males at 33-week-old. An increased incidence and severity of basophilic tubules in kidneys were also found in 33-week-old males. These findings were similar to the previous reports [2]. One female of the Lot 3 at age of 33 weeks showing macroscopic mucosal thickening revealed marked inflammation in ileum and marked lymphadenitis in mesenteric lymph node.

In conclusion, the overall results in the study were almost compatible to those in the previous reports while slight variability

was found in some parameters examined. The study would provide data to use the strain for the routine general toxicity studies.

REFERENCES

(1) S. Iwaki, et al. 1998. Background data of Crj:CD(SD)IGS rats dosed with distilled water orally for 4, 13 and 26 weeks, Biological reference data on CD(SD)IGS rats 1998, T. Matsuzawa and H. Inoue ed. pp. 64-70, BEST PRINTING, Tokyo.

(2) A. Tanaka, et al. 1998.Response to commercial low protein diet in Crj:CD(SD)IGS rats, T. Matsuzawa and H. Inoue ed. pp. 19-30, BEST PRINTING, Tokyo.

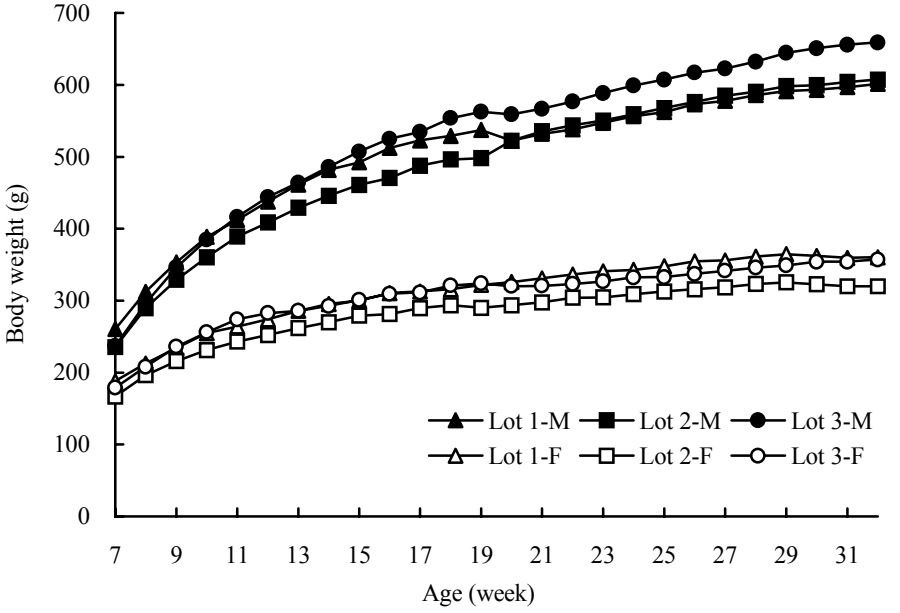

Fig. 1. Body weight changes in Crj:CD(SD)IGS rats

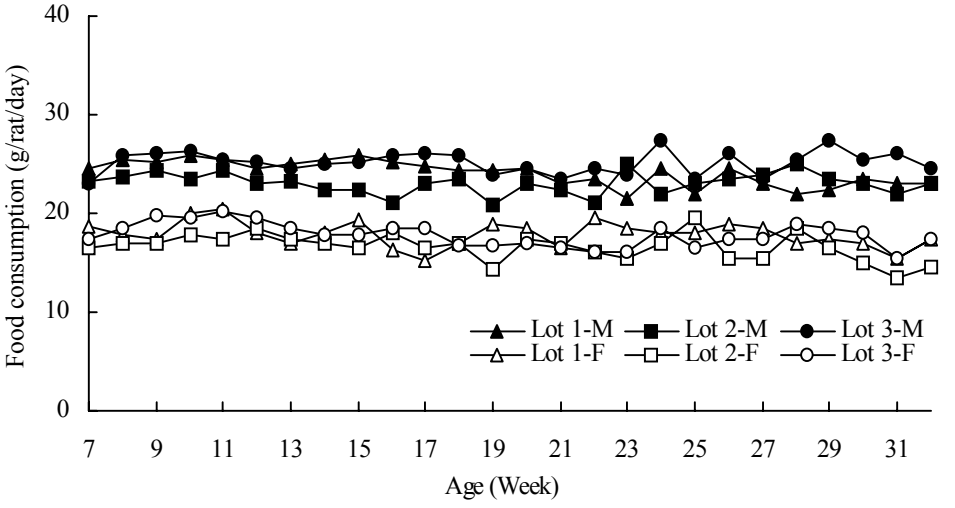

Fig.2. Food consumption in Crj:CD(SD)IGS rats

Table 1-1. Hematology in Crj:CD(SD)IGS male rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
WBC	($x10^3/\mu L$)	7.498±1.086	7.978±1.334	8.015±1.337	6.592±1.543	6.497±0.684	7.172±2.112
RBC	($x10^3/\mu L$)	8.508±0.505	8.268±0.147	8.148±0.469	8.372±0.375	8.200±0.358	8.002±1.055
Hb	(g/dl)	15.50±0.75	15.05±0.42	14.62±0.92	15.38±0.60	14.90±0.80	14.48±1.70
Ht	(%)	45.68±2.02	44.95±1.07	43.27±2.80	45.60±2.16	44.67±2.37	43.52±4.50
MCV	(fl)	53.73±2.19	54.35±1.32	53.03±0.44	54.45±0.60	54.50±2.74	54.60±2.33
MCH	(pg)	18.23±0.79	18.22±0.50	17.92±0.37	18.38±0.44	18.18±0.95	18.17±0.59
MCHC	(g/dl)	33.92±0.37	33.48±0.49	33.77±0.52	33.75±0.68	33.35±0.23	33.28±0.83
Plat	($x10^3/\mu L$)	926.0±97.4	982.3±58.9	991.2±92.6	1000.8±118.0	968.5±102.5	1072.8±214.5
Ret	($x10^4/\mu L$)	14.898±1.324	14.432±1.921	15.223±2.450	11.110±1.671	13.293±2.870	18.070±14.723
NEUT	($x10^3/\mu L$)	0.817±0.152	0.755±0.143	1.085±0.435	1.047±0.424	1.002±0.156	1.140±0.486
LYMP	($x10^3/\mu L$)	6.267±1.003	6.898±1.287	6.480±0.942	5.260±1.424	5.135±0.646	5.632±1.603
MONO	($x10^3/\mu L$)	0.227±0.045	0.167±0.101	0.213±0.094	0.163±0.080	0.197±0.048	0.200±0.075
EOS	($x10^3/\mu L$)	0.102±0.033	0.095±0.037	0.175±0.067	0.137±0.059	0.098±0.018	0.092±0.052
BASO	($x10^3/\mu L$)	0.017±0.008	0.015±0.005	0.018±0.008	0.013±0.010	0.010±0.000	0.015±0.005
LUC	($x10^3/\mu L$)	0.075±0.018	0.048±0.033	0.043±0.024	0.047±0.021	0.055±0.023	0.093±0.035
PT	(sec)	14.117±2.460	14.333±2.497	13.700±1.468	15.500±1.041	14.050±3.273	14.683±1.522
APTT	(sec)	21.87±1.69	22.15±1.20	22.63±0.53	22.67±2.07	21.95±1.22	23.23±1.68
Fbg	(g)	2.25±0.15	2.17±0.18	2.16±0.24	2.02±0.09	1.98±0.20	2.24±0.11

Table 1-2. Hematology in Crj:CD(SD)IGS female rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
WBC	($x10^3/\mu L$)	4.353±0.777	4.348±1.213	3.640±1.192	3.200±0.294	3.313±0.846	6.190±5.244
RBC	($x10^3/\mu L$)	7.775±0.291	7.682±0.117	7.395±0.479	7.222±0.494	7.408±0.200	7.513±0.563
Hb	(g/dl)	15.35±0.57	14.62±0.31	14.33±1.05	14.78±0.78	14.75±0.35	14.55±1.42
Ht	(%)	43.88±1.46	43.32±1.23	42.12±2.99	43.55±2.46	42.72±0.66	43.30±3.88
MCV	(fl)	56.50±1.63	56.40±1.44	56.98±2.21	60.35±2.30	57.70±1.49	57.57±1.03
MCH	(pg)	19.75±0.58	19.02±0.43	19.40±0.88	20.50±0.87	19.92±0.48	19.33±0.67
MCHC	(g/dl)	35.00±0.36	33.75±0.40	34.08±0.46	33.97±0.62	34.48±0.63	33.55±0.65
Plat	($x10^3/\mu L$)	941.7±49.9	913.2±70.0	858.5±74.6	951.7±116.5	851.8±61.9	1167.0±503.2
Ret	($x10^4/\mu L$)	14.345±2.850	13.500±2.721	12.650±2.175	9.573±2.079	11.597±2.064	11.750±4.266
NEUT	($x10^3/\mu L$)	0.587±0.121	0.737±0.384	0.432±0.142	0.485±0.166	0.578±0.092	2.527±4.746
LYMP	($x10^3/\mu L$)	3.483±0.750	3.433±1.074	3.002±1.182	2.510±0.339	2.530±0.758	3.287±0.650
MONO	($x10^3/\mu L$)	0.127±0.037	0.080±0.026	0.082±0.025	0.095±0.018	0.092±0.029	0.182±0.185
EOS	($x10^3/\mu L$)	0.113±0.035	0.065±0.012	0.092±0.026	0.078±0.025	0.075±0.020	0.105±0.022
BASO	($x10^3/\mu L$)	0.007±0.005	0.003±0.005	0.003±0.005	0.005±0.005	0.003±0.005	0.007±0.008
LUC	($x10^3/\mu L$)	0.038±0.010	0.028±0.015	0.028±0.008	0.025±0.010	0.030±0.015	0.082±0.065
PT	(sec)	9.433±0.163	9.650±0.207	9.467±0.240	9.550±0.187	9.467±0.121	9.717±0.286
APTT	(sec)	16.45±1.03	17.60±0.95	17.13±0.17	17.15±0.62	16.92±1.21	17.30±0.95
Fbg	(g)	1.69±0.07	1.62±0.08	1.61±0.13	1.45±0.12	1.44±0.11	1.70±0.50

Table 2-1. Clinical chemistry in Crj:CD(SD)IGS male rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
AST	(mU/mL)	68.5±9.7	84.2±22.2	91.2±71.1	92.5±25.1	71.7±14.2	78.0±9.5
ALT	(mU/mL)	30.7±5.3	32.2±6.6	41.3±34.0	46.5±10.3	32.2±8.9	37.8±5.8
TCHO	(mg/dL)	40.2±6.9	45.3±13.2	44.8±7.9	46.5±10.4	51.5±15.6	59.7±9.4
UN	(mg/dL)	13.62±2.02	15.00±1.03	13.73±1.91	13.22±1.48	12.98±1.12	12.97±1.36
GLU	(mg/dL)	149.0±17.9	141.7±9.2	155.3±18.7	144.2±8.8	141.7±18.2	155.8±19.1
CRNN	(mg/dL)	0.348±0.049	0.340±0.026	0.370±0.039	0.388±0.056	0.373±0.042	0.347±0.037
TP	(g/dL)	6.12±0.26	6.07±0.28	6.00±0.18	6.13±0.14	6.00±0.17	6.28±0.16
TG	(mg/dL)	43.3±10.9	28.5±6.8	56.3±49.9	44.2±8.5	52.5±28.2	47.2±23.2
ALP	(mU/mL)	163.8±22.0	163.8±28.3	159.2±19.4	145.7±44.8	126.5±34.4	151.2±21.1
LD	(mU/mL)	108.5±32.9	115.7±52.1	163.3±165.1	156.2±42.1	128.8±82.4	161.7±84.3
CK	(mU/mL)	158.2±67.0	117.5±28.2	210.0±171.9	262.0±235.7	175.5±58.8	402.8±613.3
PL	(mg/dL)	71.7±4.6	73.5±15.5	78.7±18.8	76.2±9.4	83.7±18.2	89.5±8.8
Alb	%	51.93±3.13	50.15±1.84	50.37±1.97	53.22±2.52	50.35±3.53	48.75±2.08
α 1-Glb	%	22.57±2.17	22.83±2.55	23.83±1.21	20.60±2.29	24.20±4.02	24.55±3.21
α 2-Glb	%	5.08±0.49	5.77±0.69	5.58±0.71	4.45±1.22	5.27±1.07	4.33±0.42
β -Glb	%	16.78±1.23	16.98±1.58	16.60±0.96	16.02±1.46	15.78±0.59	17.95±1.09
γ -Glb	%	3.63±0.96	4.27±1.03	3.62±0.93	5.72±1.98	4.40±1.05	4.42±1.42
A/G		1.090±0.140	1.008±0.077	1.017±0.076	1.143±0.115	1.022±0.144	0.955±0.081
IP	(mg/dL)	6.25±0.29	6.30±0.33	5.92±0.53	5.18±0.41	5.67±0.27	5.48±0.57
Ca	(mg/dL)	9.27±0.19	9.12±0.21	9.05±0.19	9.22±0.04	9.18±0.18	9.37±0.16
Na	(mEq/L)	141.2±1.0	142.8±0.8	141.0±1.3	144.2±1.0	143.3±1.4	142.3±0.5
K	(mEq/L)	3.65±0.15	3.83±0.22	3.68±0.27	3.57±0.12	3.53±0.08	3.72±0.40
Cl	(mEq/L)	105.0±1.1	106.7±0.8	104.3±0.8	107.8±0.8	105.8±1.2	104.0±1.3

Table 2-2. Clinical chemistry in Crj:CD(SD)IGS female rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
AST	(mU/mL)	60.5±6.8	70.5±18.6	71.8±17.6	60.5±9.7	65.2±8.0	98.0±45.5
ALT	(mU/mL)	22.3±3.1	31.8±19.0	30.5±10.7	30.3±8.5	27.5±5.4	38.8±14.4
TCHO	(mg/dL)	50.2±9.3	56.0±7.9	49.0±14.4	66.2±19.9	77.0±16.6	64.5±5.1
UN	(mg/dL)	16.88±2.02	15.07±1.60	15.03±1.13	16.40±3.00	15.17±1.38	15.28±2.67
GLU	(mg/dL)	130.0±12.4	141.0±17.8	119.0±14.2	127.3±14.9	136.3±20.9	114.5±12.6
CRNN	(mg/dL)	0.468±0.109	0.427±0.062	0.477±0.089	0.462±0.083	0.463±0.049	0.405±0.079
TP	(g/dL)	6.28±0.28	6.52±0.39	6.70±0.86	6.92±0.53	6.77±0.61	6.42±0.68
TG	(mg/dL)	28.2±9.6	33.0±16.6	24.7±3.7	45.5±12.4	36.5±11.3	49.3±32.5
ALP	(mU/mL)	84.2±9.6	73.0±18.1	71.7±18.0	62.3±16.8	50.7±14.9	75.2±70.3
LD	(mU/mL)	99.0±31.0	85.5±19.4	124.2±97.4	83.2±20.0	90.5±36.5	124.2±43.3
CK	(mU/mL)	255.3±194.0	115.5±50.2	117.8±35.9	84.3±10.6	185.8±233.6	101.2±14.6
PL	(mg/dL)	102.5±9.8	111.8±15.1	102.0±28.6	133.3±31.8	146.0±25.3	130.3±8.3
Alb	%	61.90±0.60	62.30±1.75	62.23±2.23	67.48±3.17	65.20±2.70	60.02±7.17
α 1-Glb	%	14.82±1.33	15.65±1.08	15.65±2.74	10.50±1.53	12.47±1.88	14.03±2.77
α 2-Glb	%	4.95±0.38	4.12±0.25	4.72±0.51	4.15±2.00	3.73±0.48	5.23±3.37
β -Glb	%	14.25±0.93	13.73±1.35	13.28±1.43	12.32±0.51	12.97±1.90	15.75±2.96
γ -Glb	%	4.08±1.19	4.20±1.00	4.12±0.71	5.55±1.43	5.63±1.95	4.97±1.55
A/G		1.625±0.040	1.657±0.129	1.657±0.168	2.100±0.316	1.888±0.228	1.558±0.383
IP	(mg/dL)	5.67±1.24	4.67±1.42	5.52±0.99	4.83±0.95	4.73±1.23	5.13±0.72
Ca	(mg/dL)	9.50±0.18	9.18±0.17	9.47±0.27	9.72±0.35	9.63±0.39	9.60±0.23
Na	(mEq/L)	141.2±0.8	142.8±1.9	143.0±1.3	144.0±1.4	143.0±1.1	143.5±0.5
K	(mEq/L)	3.72±0.17	3.47±0.20	3.48±0.13	3.60±0.20	3.57±0.34	3.77±0.24
Cl	(mEq/L)	107.0±1.4	108.3±2.2	106.8±2.6	109.2±1.7	106.5±1.5	109.0±2.3

Table 3-1. Urinalysis in Crj:CD(SD)IGS male rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
Urine volume	(mL/hr)	0.825±0.340	0.687±0.138	0.875±0.359	0.867±0.369	0.767±0.328	0.722±0.279
Osmolarity	(mOsm/kg)	945.3±254.6	927.0±177.8	913.7±320.1	862.5±303.0	976.2±376.8	1067.3±270.1
Na concentration	(mEq/L)	20.00±4.28	14.58±5.94	22.83±11.09	23.17±11.23	30.75±19.97	25.33±6.85
Na excretion	(mEq/hr)	0.0161±0.0065	0.0102±0.0055	0.0175±0.0044	0.02±0.01	0.0189±0.0076	0.0174±0.0058
K concentration	(mEq/L)	100.33±25.04	98.08±20.78	101.33±29.66	100.00±30.33	106.00±43.14	121.17±36.17
K excretion	(mEq/hr)	0.0761±0.0140	0.0652±0.0080	0.0806±0.0159	0.0780±0.0125	0.0713±0.0103	0.0803±0.0100
Cl concentration	(mEq/L)	25.5±9.5	20.2±6.0	20.2±5.0	20.7±6.2	31.8±22.3	34.0±10.7
Cl excretion	(mEq/hr)	0.0197±0.0075	0.0138±0.0047	0.0168±0.0049	0.0173±0.0079	0.0192±0.0070	0.0228±0.0055
CRNN	(mg/dl)	105.8±31.5	96.8±22.3	104.0±36.7	94.7±32.2	114.2±44.7	120.2±37.7
CRNN excretion	(mg/hr)	0.079±0.008	0.064±0.005	0.081±0.009	0.073±0.009	0.076±0.007	0.079±0.006

Table 3-2. Urinalysis in Crj:CD(SD)IGS female rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
Urine volume	(mL/hr)	0.818±0.378	0.757±0.489	0.582±0.221	0.665±0.338	0.505±0.277	0.502±0.195
Osmolarity	(mOsm/kg)	829.3±553.1	891.0±371.7	960.5±327.3	932.3±356.2	1075.3±395.7	963.5±262.4
Na concentration	(mEq/L)	32.08±28.60	36.17±20.02	36.67±7.99	31.33±14.17	31.50±14.53	30.75±10.81
Na excretion	(mEq/hr)	0.0189±0.0039	0.0209±0.0049	0.0208±0.0069	0.0176±0.0058	0.0151±0.0082	0.0145±0.0056
K concentration	(mEq/L)	75.83±43.62	87.50±37.17	95.00±41.53	106.00±41.58	104.58±43.46	94.25±27.01
K excretion	(mEq/hr)	0.0495±0.0099	0.0515±0.0086	0.0484±0.0057	0.0593±0.0124	0.0466±0.0172	0.0431±0.0042
Cl concentration	(mEq/L)	34.2±21.0	38.0±20.6	39.0±12.5	34.2±14.6	42.0±18.1	31.7±9.2
Cl excretion	(mEq/hr)	0.0221±0.0039	0.0217±0.0053	0.0208±0.0049	0.0188±0.0046	0.0174±0.0066	0.0150±0.0045
CRNN	(mg/dl)	70.7±47.1	65.7±30.6	83.7±26.0	78.5±35.4	97.0±38.8	91.5±30.0
CRNN excretion	(mg/hr)	0.044±0.005	0.038±0.005	0.044±0.005	0.043±0.005	0.041±0.008	0.041±0.003

Table 4-1. Absolute organ weights in Crj:CD(SD)IGS male rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
Liver	(g)	13.626±2.551	11.416±1.305	13.422±2.103	12.592±0.730	13.384±2.909	14.687±1.313
Kidneys	(g)	3.113±0.507	2.986±0.217	3.118±0.157	3.353±0.335	3.128±0.362	3.626±0.260
Brain	(g)	2.134±0.067	2.085±0.093	2.163±0.078	2.231±0.063	2.205±0.056	2.189±0.097
Lungs	(g)	1.566±0.134	1.430±0.104	1.474±0.117	1.629±0.084	1.523±0.105	1.688±0.101
Heart	(g)	1.599±0.169	1.462±0.141	1.521±0.145	1.640±0.170	1.667±0.163	1.689±0.166
Spleen	(g)	0.810±0.103	0.738±0.070	0.811±0.061	0.829±0.042	0.936±0.505	0.884±0.129
Thymus	(mg)	416±86	330±132	355±102	256±41	284±79	160±102
Adrenals	(mg)	60±4	58±10	66±13	60±5	58±9	60±12
Thyroids	(mg)	23±4	22±3	24±6	30±3	29±7	34±7
Pituitary	(mg)	13±2	13±3	13±2	13±2	15±2	14±1
Submaxillary glands	(mg)	756±78	686±74	713±73	772±66	717±79	779±115
Testes	(g)	3.121±0.282	3.123±0.243	3.125±0.216	3.438±0.178	3.389±0.317	3.361±0.241
Prostate	(mg)	1224±249	1251±278	1116±227	1178±365	1027±252	1435±191

Table 4-2. Absolute organ weights in Crj:CD(SD)IGS female rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
Liver	(g)	6.992±0.492	6.879±0.959	7.477±0.878	8.045±0.908	7.200±0.491	8.403±1.390
Kidneys	(g)	1.815±0.183	1.782±0.201	1.966±0.071	1.979±0.168	1.903±0.146	2.044±0.114
Brain	(g)	1.950±0.067	1.928±0.044	2.050±0.088	2.069±0.037	1.984±0.050	2.045±0.132
Lungs	(g)	1.167±0.086	1.092±0.111	1.208±0.093	1.295±0.161	1.170±0.098	1.243±0.059
Heart	(g)	0.932±0.033	0.885±0.069	1.003±0.051	1.003±0.070	0.968±0.071	1.014±0.046
Spleen	(g)	0.560±0.058	0.498±0.094	0.581±0.078	0.545±0.085	0.542±0.059	0.672±0.136
Thymus	(mg)	302±44	247±24	274±76	263±23	195±41	186±58
Adrenals	(mg)	70±9	64±7	69±7	71±7	70±9	69±13
Thyroids	(mg)	17±3	17±3	18±5	19±7	20±4	21±4
Pituitary	(mg)	13±5	18±3	18±4	19±4	19±4	19±3
Submaxillary glands	(mg)	498±44	431±65	495±81	480±62	448±24	438±39
Ovaries	(mg)	87±21	75±6	94±16	90±25	59±18	88±24

Table 4-3. Relative organ weights in Crj:CD(SD)IGS male rats

Age		20w			33w		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6
Liver	(g%)	2.462±0.229	2.458±0.180	2.433±0.221	2.184±0.128	2.272±0.267	2.304±0.085
Kidneys	(g%)	0.564±0.054	0.648±0.080	0.570±0.047	0.580±0.034	0.536±0.030	0.573±0.070
Brain	(g%)	0.392±0.040	0.452±0.047	0.396±0.044	0.388±0.031	0.381±0.044	0.345±0.021
Lungs	(g%)	0.286±0.028	0.309±0.019	0.270±0.033	0.283±0.011	0.262±0.023	0.265±0.011
Heart	(g%)	0.291±0.015	0.315±0.025	0.277±0.017	0.283±0.010	0.287±0.029	0.266±0.026
Spleen	(g%)	0.148±0.021	0.159±0.009	0.148±0.014	0.144±0.012	0.157±0.070	0.139±0.018
Thymus	(g%)	0.076±0.018	0.073±0.034	0.064±0.017	0.044±0.007	0.049±0.015	0.025±0.016
Adrenals	(g%)	0.011±0.001	0.013±0.002	0.012±0.001	0.010±0.001	0.010±0.001	0.009±0.002
Thyroids	(g%)	0.004±0.001	0.005±0.001	0.004±0.001	0.005±0.000	0.005±0.002	0.005±0.001
Pituitary	(g%)	0.002±0.000	0.003±0.001	0.002±0.001	0.002±0.000	0.003±0.000	0.002±0.000
Submaxillary glands	(g%)	0.138±0.012	0.149±0.018	0.131±0.018	0.135±0.019	0.123±0.012	0.122±0.016
Testes	(g%)	0.573±0.083	0.675±0.053	0.573±0.072	0.598±0.057	0.586±0.082	0.528±0.020
Prostate	(g%)	0.222±0.036	0.268±0.047	0.201±0.022	0.206±0.071	0.178±0.047	0.226±0.030

Table 4-4. Relative organ weights in Crj:CD(SD)IGS female rats

Age		20w			33w		
		4w	5w	6w	4w	5w	6w
No. of animals		6	6	6	6	6	6
Liver	(g%)	2.293±0.248	2.467±0.142	2.465±0.307	2.350±0.196	2.374±0.133	2.510±0.435
Kidneys	(g%)	0.594±0.067	0.645±0.089	0.648±0.038	0.581±0.066	0.627±0.034	0.610±0.026
Brain	(g%)	0.638±0.023	0.699±0.067	0.676±0.042	0.608±0.056	0.656±0.058	0.613±0.073
Lungs	(g%)	0.383±0.042	0.394±0.039	0.398±0.028	0.381±0.065	0.386±0.032	0.371±0.020
Heart	(g%)	0.305±0.014	0.319±0.019	0.330±0.011	0.294±0.024	0.319±0.023	0.303±0.027
Spleen	(g%)	0.184±0.025	0.181±0.044	0.191±0.023	0.159±0.022	0.179±0.023	0.200±0.039
Thymus	(g%)	0.099±0.015	0.090±0.014	0.090±0.022	0.077±0.005	0.064±0.012	0.055±0.015
Adrenals	(g%)	0.023±0.004	0.023±0.004	0.023±0.003	0.021±0.002	0.023±0.003	0.021±0.006
Thyroids	(g%)	0.006±0.001	0.006±0.002	0.006±0.002	0.006±0.002	0.007±0.001	0.006±0.001
Pituitary	(g%)	0.004±0.001	0.007±0.001	0.006±0.001	0.006±0.001	0.006±0.001	0.006±0.001
Submaxillary glands	(g%)	0.163±0.013	0.155±0.014	0.163±0.026	0.140±0.018	0.149±0.019	0.131±0.009
Ovaries	(g%)	0.028±0.007	0.027±0.003	0.031±0.005	0.026±0.008	0.020±0.007	0.026±0.007

Table 5. Macroscopic findings in Crj:CD(SD)IGS male and female rats

Sex		Male						Female					
Organ	Age	20w			33w			20w			33w		
	Findings	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No. of animals		6	6	6	6	6	6	6	6	6	6	6	6
Liver													
	White spots	0	0	1	0	0	0	0	0	0	0	0	0
Testis													
	Atrophy, unilateral	1	0	0	0	0	0	—	—	—	—	—	—
Epididymis													
	Atrophy, unilateral	1	0	0	0	0	0	—	—	—	—	—	—
Thymus													
	Atrophy	0	0	0	0	0	3	0	0	0	0	0	1
Ileum													
	Mucosal thickening	0	0	0	0	0	0	0	0	0	0	0	1

Table 6-1. Histopathological findings in Crj:CD(SD)IGS male rats

Age		20w																33w											
		Lot 1				Lot 2				Lot 3				Lot 1				Lot 2				Lot 3			
Organ / Findings	Grade	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++
Heart																									
Mononuclear cell infiltration, focal		6	0	0	0	4	2	0	0	4	2	0	0	3	3	0	0	2	4	0	0	5	1	0	0
Liver																									
Mononuclear cell infiltration, focal		5	1	0	0	6	0	0	0	5	1	0	0	4	2	0	0	2	4	0	0	3	3	0	0
Vacuolation, hepatocytes, focal		6	0	0	0	6	0	0	0	5	1	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Pancreas																									
Fibrosis, Langerhans islets		5	1	0	0	6	0	0	0	5	1	0	0	4	2	0	0	4	1	1	0	5	1	0	0
Hemosiderin deposition		6	0	0	0	6	0	0	0	5	1	0	0	5	1	0	0	5	1	0	0	5	1	0	0
Kidney																									
Basophilic tubules		6	0	0	0	4	2	0	0	6	0	0	0	5	0	1	0	4	1	1	0	2	4	0	0
Testis																									
Atrophy, seminiferous tubules, unilateral		5	0	0	1	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Hyperplasia, Leydig cells		5	1	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Epididymis																									
Decreased number of sperm, unilateral		5	0	0	1	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Cell debris, unilateral		5	0	1	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Prostate																									
Mononuclear cell infiltration		6	0	0	0	6	0	0	0	5	0	1	0	6	0	0	0	6	0	0	0	6	0	0	0

−: Negative ±: Slight +: Moderate ++: Marked

Table 6-2. Histopathological findings in Crj:CD(SD)IGS female rats

Age		20w																33w							
		Lot 1				Lot 2				Lot 3				Lot 1				Lot 2				Lot 3			
Organ / Findings	Grade	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++
Heart																									
Mononuclear cell infiltration, focal		6	0	0	0	6	0	0	0	6	0	0	0	3	3	0	0	5	1	0	0	6	0	0	0
Mesenteric lymph node																									
Lymphadenitis		6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	5	0	0	1
Stomach																									
Erosion		6	0	0	0	6	0	0	0	5	1	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Edema, submucosa		6	0	0	0	6	0	0	0	5	0	1	0	6	0	0	0	6	0	0	0	6	0	0	0
Cell infiltration, submucosa		6	0	0	0	6	0	0	0	5	1	0	0	6	0	0	0	6	0	0	0	6	0	0	0
Ileum																									
Inflammation, focal		6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	5	0	0	1
Liver																									
Mononuclear cell infiltration, focal		5	1	0	0	5	1	0	0	6	0	0	0	5	1	0	0	5	1	0	0	6	0	0	0
Kidney																									
Basophilic tubules		6	0	0	0	6	0	0	0	5	1	0	0	5	1	0	0	6	0	0	0	6	0	0	0
Vagina																									
Epidermal cyst		6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0	5	1	0	0
Adrenal																									
Vacuolation, zona fasciculata, focal		6	0	0	0	5	1	0	0	6	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0

−: Negative ±: Slight +: Moderate ++: Marked

Background Data on Spontaneous Ophthalmic Lesions in Crj:CD(SD)IGS Rats

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ABSTRACT. To accumulate background data pertaining to spontaneous ophthalmic lesions, 90 male and 90 female Crj:CD(SD)IGS (IGS) rats were observed ophthalmoscopically at 5, 9, 18, and 31 weeks of age, and examined histopathologically at 9, 18, and 31 weeks of age. Ophthalmoscopically, there were corneal opacity and abnormal retinal vasculature in males, vitreous hemorrhage in females, and persistent hyaloid artery and retinal atrophy in both sexes. The incidences of all the ophthalmoscopic lesions were low (10.0% and less). Histopathologically, preretinal arteriolar loop, retinal degeneration, and retinal hypoplasia were noticed in males, and microgranuloma with calcification in the bulbar conjunctiva, corneal degeneration, persistent hyaloid artery, and retinal dysplasia were observed in both sexes. Corneal degeneration, resulting in corneal opacity, was detected in unilateral and bilateral globes of the rats at 18 and 31 weeks of age with high incidences (76.7% in males and 50.0% in females at 31 weeks of age). The incidences of other ocular lesions were low (10.0% and less). — Key words: Crj:CD(SD)IGS, Ocular lesions, Rat.

CD(SD)IGS-1999: 60-62

INTRODUCTION

The international genetic standard system, which has been developed by Charles River, Inc., is a new breeding procedure of laboratory rats. The system makes it possible to produce uniform laboratory rats owing to the genetic ramification control. Crj:CD(SD)IGS (IGS) rats have been induced by the international genetic standard system and begun to be widely used in various toxicity studies. The animals are expected to meet internationalization of research and development of new drugs. However, background data on spontaneous ophthalmic lesions of IGS rats have not yet been fully accumulated. Therefore, we examined spontaneous ophthalmic lesions of the IGS rats by means of ophthalmoscopy and histopathology.

MATERIALS AND METHODS

Ninety male and 90 female Crj:CD(SD)IGS (IGS) rats were purchased from Tsukuba Breeding Center (Charles River Japan, Inc., Ibaraki) at 4 weeks of age. The rats were housed individually in a wire-mesh cage (21 x 35 x 20 cm) and were maintained in a barrier-sustained room controlled at 21 - 23℃ and 30 - 63% relative humidity, and ventilated 10 times per hr, with a 12-hr light-dark cycle. The animals had free access to a low protein commercial diet (18% protein content) for rats (CR-LRF with γ-ray irradiation, Oriental Yeast Co., Tokyo) and tap water. They cared for and were treated humanely in accordance with the *Guidelines for Animal Experimentation,* published by the Japanese Association for Laboratory Animal Science (Exp. Anim. 36: 285-288, 1987).

All IGS rats were observed for clinical signs and mortality twice daily and weighed weekly. Food consumption for 24 hrs was measured weekly. The rats were examined ophthalmoscopically at 5, 9, 18, and 31 weeks of age. Prior to the examination, a mydriatic solution (Mydrin P, Santen, Tokyo) was dropped to dilate the pupil. An ophthalmoscope (AU-12205, Welch Allyn, NY, U.S.A.) and an indirect ophthalmoscopy (All Pupil Indirect, Keeler Ltd., Berkshire, UK) with a 28 diopter aspheric lens (Nikon, Tokyo) were used for the examination. Fundus lesions were recorded using a fundus camera (RC-2 model-621, Kowa Co., Ltd., Tokyo). These rats were euthanatized by ether inhalation and subjected to a complete necropsy at 9, 18, and 31 weeks of age. The globes were fixed in Davidson's solution and the brain and other organs were fixed in 10% neutral buffered formalin. These tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined histopathologically.

RESULTS AND DISCUSSIONS

The incidences of spontaneous ocular lesions in IGS rats detected ophthalmoscopically and histopathologically are shown in Tables 1, 2, 3, and 4.

Ophthalmoscopically, corneal opacity was observed in unilateral and bilateral globes of males with the incidences of 5.0% and 10.0% at 18 and 31 weeks of age, respectively. The lesion consisted of multiple deposits of white fine granules on the corneal surface. The deposits were seen as a broad band in the central part of the cornea. Histologically, the lesions were confirmed as corneal degeneration and characterized by deposits of basophilic fine granules and basophilic laminated plaques in the corneal epithelial basement membrane (Bowman's membrane). The lesions occurred in both sexes and the incidences were 13.3% and 76.7% in males and 16.7% and 50.0% in females at 18 and 31 weeks of age, respectively. Corneal lesions were detected at higher frequent by the microscopic examination of the globes than by the ophthalmoscopic examinations. Corneal degeneration has been observed as superficial punctate opacities in Sprague-Dawley and Wistar rats of both sexes and varying ages [1], and has also been described as band keratopathy [3], corneal calcification [3], corneal crystal [7], and corneal dystrophy [2, 8] in rats. The incidences of corneal opacities and dystrophies in Sprague-Dawley rats have been reported to increase with age [13], coinciding with the present results.

Microgranuloma in the bulbar conjunctiva was detected histologically in unilateral globes of one male (3.3%) and 2 females (6.7%) at 31 weeks of age. The lesion was characterized by small accumulations of macrophages with calcific deposits beneath the conjunctival epithelium. Calcific microgranulomas in the bulbar conjunctiva have been observed in rats with the corneal calcification [2] and corneal dystrophy [3].

Persistent hyaloid artery was observed ophthalmoscopically in unilateral and bilateral globes of 7 males (7.7%) and 6 females (6.6%) at 5 weeks of age, 3 males (3.3%) and 6 females (6.6%) at 9 weeks of age, and 2 females (3.3%) at 18 weeks of age. In the present study, atypical vessels containing blood within their cavities in the vitreous were interpreted as a pathologic change. The lesions were detected histologically in 3 males (10.0%) and 4 females (13.3%) at 9 weeks of age and 2 females (6.7%) at 18 weeks of age. Since these abnormal vascular structures were present in the limited area of the globes, it seemed to be difficult to detect all abnormal cases histologically. Remnant of the hyaloid arterial system has been observed in young Sprague-Dawley rats [5, 7]. Incidence of the lesion has been reported to decrease with age [5], coinciding with the present results. The hyaloid artery system is well known as normal structures in the developing globes of rats. Remnant of the hyaloid arteries can be recognized ophthalmoscopically in young rats and in rats of 3 weeks it still contains some blood [4].

Table 1. Numbers of Male IGS Rats with Ophthalmoscopic Lesions (%)

Tissues and lesions	Weeks of age			
	5	9	18	31
No. of rats examined	90	90	60	30
Eyelid	—	—	—	—
Conjunctiva	—	—	—	—
Cornea				
Opacity, U	0(0)	0(0)	1(1.7)	1(3.3)
Opacity, B	0(0)	0(0)	2(3.3)	2(6.7)
Anterior chamber	—	—	—	—
Iris	—	—	—	—
Lens	—	—	—	—
Vitreous				
Persistent hyaloid artery, U	3(3.3)	3(3.3)	0(0)	0(0)
Persistent hyaloid artery, B	4(4.4)	0(0)	0(0)	0(0)
Retina				
Abnormal vasculature, U	0(0)	1(1.1)	1(1.7)	1(3.3)
Atrophy, U	0(0)	0(0)	1(1.7)	1(3.3)
Focal atrophy, U	0(0)	0(0)	3(5.0)	2(6.7)
Optic disc	—	—	—	—
Choroid	—	—	—	—

U : unilateral.
B : bilateral.
—: No lesions.

Table 2. Numbers of Female IGS Rats with Ophthalmoscopic Lesions (%)

Tissues and lesions	Weeks of age			
	5	9	18	31
No. of rats examined	90	90	60	30
Eyelid	—	—	—	—
Conjunctiva	—	—	—	—
Cornea	—	—	—	—
Anterior chamber	—	—	—	—
Iris	—	—	—	—
Lens	—	—	—	—
Vitreous				
Persistent hyaloid artery, U	3(3.3)	3(3.3)	0(0)	0(0)
Persistent hyaloid artery, B	3(3.3)	3(3.3)	2(3.3)	0(0)
Hemorrhage, U	1(1.1)	1(1.1)	0(0)	0(0)
Retina				
Focal atrophy, U	1(1.1)	2(2.2)	1(1.7)	0(0.0)
Optic disc	—	—	—	—
Choroid	—	—	—	—

U : unilateral.
B : bilateral.
—: No lesions.

Table 3. Numbers of Male IGS Rats with Histopathological Ocular Lesions (%)

Tissues and lesions	Weeks of age		
	9	18	31
No. of rats examined	30	30	30
Eyelid	—	—	—
Conjunctiva			
Microgranuloma, U	0(0)	0(0)	1(3.3)
Cornea			
Degeneration, U	0(0)	3(10.0)	11(36.7)
Degeneration, B	0(0)	1(3.3)	12(40.0)
Anterior chamber	—	—	—
Iris	—	—	—
Lens	—	—	—
Vitreous			
Persistent hyaloid artery, U	3(10.0)	0(0)	0(0)
Preretinal arteriolar loop, U	0(0)	0(0)	1(3.3)
Retina			
Dysplasia, U	0(0)	3(10.0)	3(10.0)
Hypoplasia, U	0(0)	0(0)	1(3.3)
Degeneration, U	0(0)	0(0)	1(3.3)
Optic disc	—	—	—
Choroid	—	—	—

U : unilateral.
B : bilateral.
—: No lesions.

Table 4. Numbers of Female IGS Rats with Histopathological Ocular Lesions (%)

Tissues and lesions	Weeks of age		
	9	18	31
No. of rats examined	30	30	30
Eyelid	—	—	—
Conjunctiva			
Microgranuloma, U	0(0)	0(0)	2(6.7)
Cornea			
Degeneration, U	0(0)	3(10.0)	7(23.3)
Degeneration, B	0(0)	2(6.7)	8(26.7)
Anterior chamber	—	—	—
Iris	—	—	—
Lens	—	—	—
Vitreous			
Persistent hyaloid artery, U	3(10.0)	0(0)	0(0)
Persistent hyaloid artery, B	1(3.3)	2(6.7)	0(0)
Retina			
Dysplasia, U	4(13.3)	0(0)	0(0)
Optic disc	—	—	—
Choroid	—	—	—

U : unilateral.
B : bilateral.
—: No lesions.

The abnormal vasculature, tortuosity of retinal arterioles, was observed in unilateral globe of one male each at 9, 18, and 31 weeks of age. The lesions have been observed in young, 8-25 weeks old, Sprague-Dawley rats [9]. The lesion was confirmed histologically as preretinal arteriolar loop. Preretinal anterior loops have been observed in 10- and 13-week-old female Sprague-Dawley rats [12].

A small hemorrhagic focus was observed in the preretinal vitreous portion of unilateral globe of one female when the animal was 5 and 9 weeks of age, at 18 weeks of age, however, the lesion was no longer detected ophthalmoscopically and histologically. Vitreous hemorrhage has been observed occasionally in Sprague-Dawley rats [9] and the lesions have been suggested to occur in association with remnant of the hyaloid system [13].

Focal and diffuse retinal atrophies were observed in unilateral globe of one female (1.1%) at 5 weeks of age, 2 females (2.2%) at 9 weeks of age, 4 males (6.7%) and one female (1.7%) at 18 weeks of age, and 3 males (10.0%) at 31 weeks of age. Focal atrophic lesions showed atypical limited areas or linear-shaped foci with increased reflectivity. These lesions were not accompanied by any abnormal changes in the retinal vasculatures. Histologically, focal retinal atrophies were observed as focal retinal dysplasias, characterized by focal loss and irregular arrangement of the rods and cones, the external limiting membrane, the outer nuclear, outer plexiform and inner nuclear layers and rosette formation in the posterior or peripheral areas of the retina. These lesions appeared similar to linear focal retinopathy reported previously in Sprague-Dawley rats [6]. Diffuse retinal atrophy was ophthalmoscopically observed to increase reflectivity of the whole retina with narrowness of the retinal vessels. Histologically, moderately degenerative changes were noticed in the whole retinal area. The retinal atrophy may be associated with developmental defects of the eye and visual pathways, because the lesions occurred in the unilateral globe and in the young rat. Unilateral degeneration and gliosis of the optic nerve and atrophy and gliosis of the associated optic tract have been reported in Wistar rats [11]. On the other hand, hypoplasia of the peripheral retina in the unilateral globe was observed histologically in one male at 31 weeks of age, which had neither degenerative nor reactive changes in the affected visual pathways. These findings were similar to those in a young F344 rat with unilateral optic nerve aplasia [10].

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REFERENCES

1. Bellhorn, R. W., Korte, G. E., and Abrutyn, D. 1988. Spontaneous Corneal Degeneration in the rat. Lab. Anim. Sci. 38: 46-50.
2. Bruner, R. H., Keller, W. F., Stitzel, K. A., Sauers, L. J., Reer, P. J., Long, P. H., Bruce, R. D., and Alden, C. L. 1992. Spontaneous corneal dystrophy and generalized basement membrane changes in Fischer-344 rats. Toxicol. Pathol. 20: 357-366.
3. Carlton, W. W. and Render, J. A. 1991. Calcification of the cornea. pp. 16-20. In: Monographs on Pathology of Laboratory Animals: Eye and Ear (Jones, T. C., Mohr, U., and Hunt. R. D. eds.). Springer-Verlag, Berlin.
4. Hebel, R. and Stromberg, M. W. 1986. Anatomy and Embryology of the Laboratory Rat. BioMed Verlag, Worthsee.
5. Heywood, R. 1973. Some clinical observations on the eyes of Sprague-Dawley rats. Lab. Anim. 7: 19-27.
6. Hubert, M-F., Gillet, J-P., and Durand-Cavagna, G. 1994. Spontaneous retinal changes in Sprague Dawley rats. Lab. Anim. Sci. 44: 561-567.
7. Kuno, H., Usui, T., Eydelloth, R. S., and Wolf, E. D. 1991. Spontaneous ophthalmic lesions in young Sprague-Dawley rats. J. Vet. Med. Sci. 53: 607-614.
8. Losco, P. E. and Troup, C. M. 1988. Corneal dystrophy in Fischer 344 rats. Lab. Anim. Sci. 38: 702-710.
9. Shibuya, K., Satou, K., Fujii, T., Nunoya, T., and Tajima, M. 1997. Spontaneous ophthalmic lesions in Sprague-Dawley, Wistar, and Fischer 344 rats. Anim. Eye Res. 16: 1-10.
10. Shibuya, K., Tajima, M., and Yamate, J. 1992. Unilateral optic nerve aplasia in an F344 rat with special reference to pathomorphological changes of the optic nerve pathways. J. Vet. Med. Sci. 54: 571-574.
11. Shibuya, K., Tajima, M., and Yamate, J. 1993. Unilateral atrophy of the optic nerve associated with retrograde and anterograde degenerations in the visual pathways in Slc: Wistar rats. J. Vet. Med. Sci. 55: 905-912.
12. Tanaka, K., Inagaki, S., and Doi, K. 1994. Preretinal arteriolar loops in rats. Lab. Anim. Sci. 44: 71-72.
13. Taradach, C. and Greaves, P. 1984. Spontaneous eye lesions in laboratory animals: Incidence in relation to age. CRC Crit. Rev. in Toxicol. 12: 121-147.

Comparison of Plasma Biochemical Parameters between Crj:CD(SD)IGS and Crj:CD(SD) rats

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ABSTRACT. Plasma biochemical data of Crj:CD(SD)IGS (IGS) rats were collected and compared with those of Crj:CD(SD)(CD) rats that had been accumulated in our institute. The male and female IGS rats, four-week-old, were purchased and maintained in our facility by 31 weeks of age. Blood samples were collected at 9, 18 and 31 weeks of age and examined. The results obtained indicated that both IGS and CD rats had similar biological values in almost all the parameters examined. However, values of glucose (GLU), alkaline phosphatase (ALP) and chloride (CL) in males and/or females of IGS rats were greater than those of CD rats; in contrast, values of total bilirubin (TB) and creatinine (CRE) in both sexes of IGS rats were smaller than those of CD rats. The plasma biochemical parameters in IGS rats obtained in the present study may be useful for toxicity studies. — Key words: Crj:CD(SD)IGS, Plasma biochemistry, Rat

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INTRODUCTION

The international genetic standard system, which has been developed by Charles River, Inc., is a new breeding procedure of laboratory rats. The system has been expected to produce uniform laboratory rats by its genetic ramification control. Crj:CD(SD)IGS (IGS) rats have been produced through the international genetic standard system and begun to be used widely in various toxicity studies. The IGS rats are anticipated to be suitable for internationalization of research and development of new drugs. At present, however, background data of plasma biochemical parameters of IGS rats have not yet been fully accumulated. Therefore, we investigated plasma biochemical parameters of IGS rats, and the results obtained were compared with those of CD rats.

MATERIALS AND METHODS

Animals: A total of 90 male and 90 female IGS rats, four-week-old, were purchased from Charles River Japan Inc. (Tsukuba Breeding Center, Ibaraki, Japan). The animals were acclimated to laboratory animal facilities at our institute for 12 days. The general conditions of all rats were a good state of health during the acclimatization period. The animals were housed individually in a stainless wire cage (21W ×35D ×20H cm) during the acclimatization and study periods. The animal room was maintained at a temperature of 21-23℃ and relative humidity of 30-63% during the acclimatization and study periods. The room was ventilated more than 10 times per hr (all fresh air), and the illuminated for 12 hr per day (from 07:00 to 19:00). The animals had free access to a low protein commercial diet (18% protein content) for rats (CR-LPF with γ-ray irradiation, Oriental Yeast Co., Tokyo, Japan) and tap water. They cared for and were treated humanely in accordance with the *Guidelines for Animal Experimentation*, published by the Japanese Association for Laboratory Animal Science (Exp. Anim, 36: 285-288. 1987)

Plasma biochemical examination: The 30 male and 30 female rats were sacrificed at 9, 18 and 31 weeks of age, respectively. The animals were fasted for approximately 17 hr before bleeding. Blood samples were collected from the abdominal aorta of the rats anesthetized with ether using heparin sodium salt as anticoagulant. Plasma was separated from blood samples by centrifugation (1500×g, 15 min) at 4℃ and stored at -70℃ until analysis. Reagent for Glucose (GLU) was obtained from Roche Diagnostics (Tokyo, Japan). Reagents for total protein (TP) and albumin (ALB) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Reagents for total cholesterol (TCHO), free cholesterol (FCHO), phospholipid (PL), triglyceride (TGL,), high-density lipoprotein cholesterol (HDLC) and calcium (Ca) were obtained from Daiichi Pure Chemical Co., Ltd., (Tokyo, Japan). Regents for total bilirubin (TB), choline esterase (CHE), inorganic phosphorus (IP), nonesterified fatty acid (NEFA), beta-lipoprotein (β-LP) and uric acid (UA) were purchased from Dia-Iatron Laboratories (Tokyo, Japan). Reagents for creatinine (CRE), urea nitrogen (UN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), creatine kinase (CK), gamma-glutamyltransferase (γ-GT) were purchased from Shino-Test (Tokyo, Japan). The above plasma biochemical parameters were determined using an automated analyzer (Model 7060, Hitachi Ltd., Tokyo, Japan). Albumin/globulin (A/G) ratio and ester ratio (E/T) were calculated using the following equation: ALB/(TP-ALB) and (TCHO-FCHO)/TCHO, respectively. Sodium (Na), potassium (K) and chloride (CL) were determined using an automated analyzer (Model STAX-1, Techno Medica Co., Ltd., Yokohama, Japan). Methods for the determination of plasma biochemical parameters in IGS and CD rats are shown in Table 1.

Statistical Analysis: Data were first analyzed by the F-test for homogeneity of variance with significant levels of 5%. If the test revealed homogeneity of variance, a comparison was made between the IGS and CD rats using the Student's *t*-test. In the case of heterogeneity of variance, a comparison was made between the IGS and CD rats using the Aspin-Welch *t*-test. Differences were considered significant at $p<0.05$ and $p<0.01$.

RESULTS

The plasma biochemical parameters in the male and female IGS rats determined in the present study are shown in Tables 2 and 3.

The plasma biochemical parameters in male and female CD rats that have been determined on the other occasions at our institute are shown in Tables 4 and 5. The results obtained by plasma biochemical examinations of IGS rats at 9, 18 and 31 weeks of age were compared with those of CD rats at 8 - 12, 14 - 18 and 20 - 24 weeks of age, respectively.

In male IGS rats at nine weeks of age, the values of GLU, TCHO, NEFA, ALP, Ca, IP, K and CL were significantly larger than those of their CD counterparts; conversely, the values of TGL, TB, CRE, UN, AST, ALT, LDH and Na were significantly smaller than those of CD rats. In male IGS rats at 18 weeks of age, the values of GLU, A/G, NEFA, UN, AST, LDH, ALP, CK, IP and CL were significantly larger than the corresponding values of CD rats; on the other hand, the values of TP, PL, TGL, TB, CRE and ALT were significantly smaller than those of CD rats. In male IGS rats at 31 weeks of age, the values of GLU, LDH, ALP, Ca, IP, K and CL were significantly larger than the corresponding values of CD rats; in contrast, the values of TB, CRE and Na were significantly smaller than those of CD rats (Tables 2 and 4).

In female IGS rats at nine weeks of age, the values of GLU, A/G, TCHO, ALP, CK, Ca, K and CL were significantly larger than the corresponding values of CD rats; conversely, the values of TP, TGL, TB, CRE, AST, ALT, LDH, and Na counts were significantly smaller in IGS rats. At 18 weeks of age, the values of GLU, A/G, ALP and CL were significantly larger in IGS rats than in CD rats; in contrast, the values of TP, PL, TGL,TB and CRE were significantly smaller in IGS rats. At 31 weeks of age, the values of GLU, A/G, UN, LDH, ALP and CL were significantly larger in IGS rats; on the other hand, the values of TCHO, TB, CRE, ALT and Na were significantly smaller in IGS rats (Tables 3 and 5). GLU, TP, TCHO, FCHO, PL, TGL and β-LP in both sexes of both strains tended to increase with age. On the other hand, ALP, CK and IP in both sexes of both strains tended to decrease with age.

DISCUSSION

The results obtained in the present study revealed that many of plasma biochemical parameters of IGS rats differed significantly from those of CD rats. The values of plasma biochemical parameters in IGS rats differing more than mean $\pm$ 1.96SD from those in CD rats were as follows: At 9, 18 and 31 weeks of age, the values of CL in males and GLU in females of IGS rats were greater than those of CD rats, whereas CRE values in both sexes of IGS rats were smaller than those of CD rats. At 9 and 18 weeks of age, the values of TB in both sexes of IGS rats were smaller than those of CD rats. At 18 weeks of age, A/G and ALP in both sexes of IGS rats were greater than those of CD rats, however TP in males

Table 1. Methods used for the plasma biochemical examination

Parameters	IGS rats	CD rats
GLU	Hexokinase	Glucose dehydrogenase
TP	Biuret	Biuret
ALB	Bromcresol green	Bromcresol green
A/G	Calculation; ALB/(TP-ALB)	Calculation; ALB/(TP-ALB)
TCHO	Cholesterol esterase/Cholesterol oxidase	Cholesterol esterase/Cholesterol oxidase
FCHO	Cholesterol oxidase	N.E.
E/T	Calculation; (TCHO-FCHO)/TCHO	N.E.
PL	Phospholipase D/Choline oxidase	Phospholipase D/Choline oxidase
TGL	Lipoprotein lipase/Glycerol kinase/GPO	Lipoprotein lipase/Glycerol kinase/GPO
NEFA	Acyl-CoA synthetase	Acyl-CoA synthetase/Acyl-CoA oxidase
β-LP	Immunonephelometry	N.E.
HDLC	Selective inhibition	N.E.
TB	Bilirubin oxidase	Azobilirubin
CRE	Creatinase/Sarcosine oxidase/Creatininase	Jaffe
UN	Urease/Glutamate dehydrogenase	Urease/Glutamate dehydrogenase
UA	Uricase	N.E.
AST	JSCC recommended	UV rate
ALT	JSCC recommended	UV rate
LDH	JSCC recommended	UV rate
ALP	JSCC recommended	*p*-Nitrophenylphosphate
LAP	*L*-Leucine-*p*-nitroanilide	N.E.
CK	JSCC recommended	UV rate
γ-GT	JSCC recommended	N.E.
CHE	Butylthiocholine	N.E.
Ca	*o*-Cresolphthalein complexone	*o*-Cresolphthalein complexone
IP	Purine nucleotide phosphorylase/Xanthine oxidase	Ammonium moribdate (UV)
Fe	Nitroso-PSAP	N.E.
Na	Ion-specific electrode	Ion-specific electrode
K	Ion-specific electrode	Ion-specific electrode
CL	Ion-specific electrode	Ion-specific electrode

GPO, Glycerol-3-phosphate oxidase; CoA, Coenzyme A; JSCC, Japan Society of Clinical Chemistry; Nitroso-PSAP, 2-Nitroso-5-(*N*-propyl-*N*-sulfopropylamino)-phenol; N.E., Not examined; UV, Ultraviolet

of IGS rats was smaller than those of CD rats. At 31 weeks of age, ALP in males and, UN and CL in females of IGS rats were greater than those of CD rats. The remaining parameters in IGS rats appeared to be in the biological range of CD rats.

The values of TB and CRE in both sexes of IGS rats were lower than those of CD rats. In CD rats, TB and CRE were determined by the azobilirubin method (diazo reaction) and Jaffe method, respectively. In IGS rats, on the other hand, those parameters were measured by the enzymatic method. It was considered that the lower values of TB and CRE in IGS rats might be ascribed mainly to a difference in measuring method, because specificity of the enzymatic method is higher than that of the azobilirubin and Jaffe method.

Wolford et al. [3] have reported the reference range data for serum chemical values (GLU, TP, TCHO, TGL, UN, CRE, TB, ALP, AST, ALT, Ca, IP, Na, K and CL) in Crl:COBSCD(SD) rats in the age groups of <6 months, 6 - 18 months and >18 months. Those parameter values in IGS rats obtained in the present study were in their mean ± 1.96SD range except for GLU, CRE, ALP, IP, Na, K and/or CL. The plasma or serum biochemical reference data for rats have also been presented by the other authors [1, 2]. When compared with their data, the plasma biochemical parameters of IGS rats were almost comparable to their values except for GLU, TGL, CRE, AST ALP, LAP, CHE, Na and/or CL. In comparison with the aforementioned three reference range data, there were no constant trends in an appearance of variation in the plasma biochemical values of IGS rats. The values of the plasma biochemical parameters observed in IGS rats seemed to be in the biological range of the Sprague-Dawley strain.

Age-related changes in serum biochemical parameters in Sprague-Dawley rats have been well documented by Wolford et al. [4]. According to their report, TP increased during the ages of 12 months, and subsequently decreased with age; TCHO and TG increased with age, however, TG began to decrease after 30 months; IP decreased quickly during the age of 6 months; ALP declined gradually with age. In the plasma biochemical data of IGS rats and our background data of CD rats, TP, TCHO, TG ALP and IP were consistent with their data. Sex-related changes in biochemical parameters in rats are also well known. In general, the values of LDH and ALP are greater in males than in females; in contrast, the values of TP, ALB, PL, NEFA, CHE and Fe are greater in females than in males. Similar tendencies were observed in IGS and CD rats examined in the present study.

In conclusion, plasma biochemical parameters of IGS rats were substantially consistent with those of CD rats. The plasma biochemical information on the IGS strain presented here may be used as the reference data for toxicity studies.

Table 2. Plasma biochemical parameters of male Crj:CD(SD)IGS rats

Parameters	Weeks of age (Number of rats)		
	9 (30)	18 (30)	31 (30)
GLU (mg/dL)	187±16**	221±16**	231±31**
TP (g/dL)	5.71±0.21	6.00±0.25**	6.26±0.24
ALB (g/dL)	3.73±0.16	3.71±0.17	3.63±0.17
A/G	1.90±0.22	1.63±0.13**	1.39±0.10
TCHO (mg/dL)	63.1±12.5**	70.2±18.1	83.3±16.6
FCHO (mg/dL)	14.4±4.1	16.7±5.5	21.6±8.4
E/T	0.78±0.04	0.77±0.02	0.75±0.06
PL (mg/dL)	104±16	105±18**	131±31
TGL (mg/dL)	28.9±16.4**	37.0±11.3**	83.7±40.0
NEFA (mEq/L)	0.874±0.146**	0.730±0.107**	0.763±0.151
β -LP (mg/dL)	0.11±0.16	2.05±1.18	6.87±12.02
HDLC (mg/dL)	28.0±5.2	28.6±5.9	29.6±5.3
TB (mg/dL)	0.059±0.014**	0.063±0.015**	0.157±0.457**
CRE (mg/dL)	0.261±0.021**	0.298±0.036**	0.300±0.038**
UN (mg/dL)	14.4±1.7**	15.9±2*	13.8±1.2
UA (mg/dL)	0.888±0.302	0.907±0.468	0.920±0.994
AST (IU/L)	69.2±5.6**	73.3±12.1**	117.7±176.9
ALT (IU/L)	26.1±3.9**	29.3±4.6**	54.5±89.3
LDH (IU/L)	156±102**	244±94**	201±134**
ALP (IU/L)	551±103**	269±52**	233±224*
LAP (IU/L)	76.4±7.0	68.1±4.9	63.2±10.0
CK (IU/L)	166±33	142±29**	107±35
γ -GT (IU/L)	0.57±0.28	0.55±0.38	0.63±0.71
CHE (IU/L)	71±26	52±15	66±18
Ca (mg/dL)	10.21±0.22**	10.07±0.38	10.29±0.24**
IP (mg/dL)	7.58±0.74*	5.54±0.48*	4.88±0.39*
Fe (μ g/dL)	148±56	141±24	135±29
Na (mmol/L)	140±1**	140±1	140±1**
K (mmol/L)	4.12±0.34**	3.75±0.26	3.75±0.22**
CL (mmol/L)	112±1**	112±2**	112±2**

Each value represents mean±S.D.
*: Significantly different from the CD rats, p<0.05.
**: Significantly different from the CD rats, p<0.01.

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REFERENCES

1. Matsuzawa, T., Nomura, M., and Unno, T. 1993. Clinical pathology reference ranges of laboratory animals. J. Vet. Med. Sci. 55: 351-362.
2. Tanimoto, Y. 1989. pp. 164-163. In: Biological reference data book on experimental animals (Tajima, Y. ed.). Soft Science Inc., Tokyo (in Japanese).
3. Wolford, S. T., Schroer, R. A., Gohs, F. X., Gallo, P. P., Brodeck, M., Falk, H. B., and Ruhren, R. 1986. Reference range data base for serum chemistry and hematology values in laboratory animals. J. Toxicol. Environm. Health 18: 161-168.
4. Wolford, S. T., Schroer, R. A., Gohs, F. X., Gallo, P. P., Brodeck, M., Falk, H. B., and Ruhren, R. 1987. Age-related change in serum chemistry and hematology values in Sprague-Dawley rats. Fund. Appl. Toxicol. 8: 80-88.

Table 3. Plasma biochemical parameters of female Crj:CD(SD)IGS rats

Parameters	Weeks of age (Number of rats)		
	9 (30)	18 (30)	31 (30)
GLU (mg/dL)	171±16**	189±25**	208±23**
TP (g/dL)	6.04±0.3*	6.28±0.32**	6.88±0.71
ALB (g/dL)	4.28±0.26	4.36±0.32	4.74±0.50*
A/G	2.45±0.27**	2.28±0.25**	2.30±0.45**
TCHO (mg/dL)	64.1±14.8**	71.6±12.2	77.3±12.9*
FCHO (mg/dL)	14.4±6.1	18.0±4.3	20.1±4.1
E/T	0.78±0.06	0.75±0.02	0.74±0.02
PL (mg/dL)	118±22	133±21**	156±27
TGL (mg/dL)	7.3±3.9**	15.9±15.4**	28.9±19.1
NEFA (mEq/L)	0.785±0.146	0.828±0.159	0.979±0.192
β-LP (mg/dL)	0.08±0.19	0.31±0.45	2.46±3.68
HDLC (mg/dL)	31.6±6.9	35.0±5.2	37.8±5.4
TB (mg/dL)	0.053±0.013**	0.067±0.017**	0.075±0.024**
CRE (mg/dL)	0.300±0.045**	0.339±0.035**	0.374±0.042**
UN (mg/dL)	19.3±3.1	17.7±2.2	17.6±3.2*
UA (mg/dL)	0.683±0.180	0.678±0.196	0.759±0.257
AST (IU/L)	66.3±6.3**	72.5±17.8	93.8±65.0
ALT (IU/L)	22.0±3**	31.2±12.8	33.0±22.1*
LDH (IU/L)	99±44*	82±27	232±183**
ALP (IU/L)	345±78**	138±32**	109±55**
LAP (IU/L)	70.4±7.4	62.2±7.8	55.2±6.9
CK (IU/L)	122±25*	68±10	97±42
γ-GT (IU/L)	0.63±0.36	0.70±0.30	0.60±0.36
CHE (IU/L)	375±102	621±157	603±117
Ca (mg/dL)	10.15±0.30**	10.05±0.37	10.26±0.41
IP (mg/dL)	5.99±0.43	4.03±0.70	3.41±0.64
Fe (μg/dL)	243±55	314±60	311±65
Na (mmol/L)	140±1**	140±1	140±1**
K (mmol/L)	4.04±0.41**	3.61±0.29	3.38±0.34
CL (mmol/L)	113±1**	114±2**	114±1**

Each value represents mean±S.D.
*: Significantly different from the CD rats, $p<0.05$.
**: Significantly different from the CD rats, $p<0.01$.

Table 4. Plasma biochemical parameters of male Crj:CD(SD) rats

Parameters	Weeks of age		
	8 - 12	20 - 24	32 - 36
GLU (mg/dL)	147±26 (74) [a]	189±30 (35)	198±35 (30)
TP (g/dL)	5.83±0.44 (86)	6.63±0.31 (41)	6.42±0.37 (30)
ALB (g/dL)	3.70±0.38 (86)	3.62±0.31 (41)	3.72±0.27 (30)
A/G	1.84±0.34 (86)	1.21±0.20 (35)	1.39±0.17 (30)
TCHO (mg/dL)	51.3±13.1 (74)	75.5±17.5 (35)	88.3±23.0 (30)
FCHO (mg/dL)	N.E.	N.E.	N.E.
E/T	N.E.	N.E.	N.E.
PL (mg/dL)	101±18 (34)	118±19 (35)	N.E.
TGL (mg/dL)	44.9±25.2 (34)	81.6±40.5 (35)	N.E.
NEFA (mEq/L)	0.559±0.213 (22)	0.463±0.090 (25)	N.E.
β-LP (mg/dL)	N.E.	N.E.	N.E.
HDLC (mg/dL)	N.E.	N.E.	N.E.
TB (mg/dL)	0.145±0.040 (74)	0.169±0.045 (35)	0.174±0.045 (30)
CRE (mg/dL)	0.489±0.092 (86)	0.601±0.054 (35)	0.525±0.045 (30)
UN (mg/dL)	18.2±3.7 (86)	14.5±2.3 (35)	13.1±1.7 (30)
UA (mg/dL)	N.E.	N.E.	N.E.
AST (IU/L)	79.4±23.1 (84)	59.9±12.4 (35)	51.7±22.8 (30)
ALT (IU/L)	40.0±15.1 (84)	31.6±7.2 (35)	34.0±16.4 (30)
LDH (IU/L)	250±158 (34)	132±94 (25)	99±52 (30)
ALP (IU/L)	354±103 (62)	154±27 (35)	129±36 (30)
LAP (IU/L)	N.E.	N.E.	N.E.
CK (IU/L)	139±72 (12)	100±38 (25)	N.E.
γ-GT (IU/L)	N.E.	N.E.	N.E.
CHE (IU/L)	N.E.	N.E.	N.E.
Ca (mg/dL)	9.68±0.75 (86)	9.88±0.52 (35)	9.83±0.34 (30)
IP (mg/dL)	7.15±0.93 (76)	5.03±0.71 (35)	4.60±0.52 (30)
Fe (μg/dL)	N.E.	N.E.	N.E.
Na (mmol/L)	143±3 (86)	141±3 (41)	144±2 (30)
K (mmol/L)	3.83±0.31 (86)	3.68±0.22 (41)	3.60±0.20 (30)
CL (mmol/L)	107±2 (86)	108±2 (41)	106±2 (30)

Each value represents mean±S.D.
a: Number of rats.
N.E.: Not examined.

Table 5. Plasma biochemical parameters of female Crj:CD(SD) rats

Parameters	Weeks of age		
	8 - 12	20 - 24	32 - 36
GLU (mg/dL)	120±25 (73) [a]	151±18 (35)	153±20 (30)
TP (g/dL)	6.27±0.72 (85)	6.72±0.38 (41)	6.87±0.53 (30)
ALB (g/dL)	4.20±0.48 (85)	4.23±0.36 (41)	4.51±0.46 (30)
A/G	2.06±0.28 (85)	1.72±0.22 (35)	1.95±0.33 (30)
TCHO (mg/dL)	53.5±14.6 (73)	74.1±16.0 (35)	88.3±23.6 (30)
FCHO (mg/dL)	N.E.	N.E.	N.E.
E/T	N.E.	N.E.	N.E.
PL (mg/dL)	109±23 (33)	151±29 (35)	N.E.
TGL (mg/dL)	32.7±12.6 (33)	74.0±35.8 (35)	N.E.
NEFA (mEq/L)	0.669±0.262 (22)	0.887±0.198 (25)	N.E.
β-LP (mg/dL)	N.E.	N.E.	N.E.
HDLC (mg/dL)	N.E.	N.E.	N.E.
TB (mg/dL)	0.171±0.043 (73)	0.183±0.055 (35)	0.216±0.074 (30)
CRE (mg/dL)	0.510±0.086 (85)	0.633±0.043 (35)	0.524±0.031 (30)
UN (mg/dL)	20.6±4.1 (85)	16.4±1.7 (35)	13.9±1.8 (30)
UA (mg/dL)	N.E.	N.E.	N.E.
AST (IU/L)	84.8±35.4 (85)	65.1±22.5 (35)	105.1±79.4 (30)
ALT (IU/L)	39.8±17.0 (85)	34.5±20.9 (35)	56.6±42.8 (30)
LDH (IU/L)	141±80 (33)	80±36 (25)	122±115 (30)
ALP (IU/L)	256±87 (61)	97±20 (35)	75±30 (30)
LAP (IU/L)	N.E.	N.E.	N.E.
CK (IU/L)	93±45 (12)	68±20 (25)	N.E.
γ-GT (IU/L)	N.E.	N.E.	N.E.
CHE (IU/L)	N.E.	N.E.	N.E.
Ca (mg/dL)	9.58±0.87 (85)	9.90±0.45 (35)	10.00±0.60 (30)
IP (mg/dL)	5.97±1.23 (76)	4.09±0.77 (35)	3.62±0.82 (30)
Fe (μg/dL)	N.E.	N.E.	N.E.
Na (mmol/L)	142±3 (85)	140±2 (41)	143±3 (30)
K (mmol/L)	3.75±0.37 (85)	3.69±0.34 (41)	3.43±0.31 (30)
CL (mmol/L)	108±4 (85)	111±2 (41)	106±2 (30)

Each value represents mean±S.D.
a: Number of rats.
N.E.: Not examined.

Background Data for Repeated-Dose Toxicity Studies Using Crj:CD(SD)IGS Rats (II)

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ABSTRACT. Background data for use in repeated-dose toxicity studies were collected when Crj:CD(SD)IGS rats were fed a commercial low protein diet (CR-LPF, protein content; 18%) for 2, 4 or 13 weeks starting at 6 weeks of age. Evaluation was based on gross pathology, organ weights and histopathology. Age-related changes were observed in the incidence or severity of cardiomyopathy, renal basophilic tubules, hematopoietic cell proliferation and pigmentation in the spleen upon histopathology. Sex-related changes were observed in the adrenal grand weight and incidence of the cadiovascular and renal lesions upon histopathology. No age- or sex-related changes were seen upon gross pathology because the incidence of the macroscopic findings was low. These changes have also been observed in Sprague-Dawley rats including Crj:CD(SD) rats used commonly in repeated-dose toxicity studies — Key words: background data, CD(SD)IGS rats, Low protein diet

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INTRODUCTION

Sprague-Dawley (SD) rats have been commonly used in toxicity and carcinogenicity studies. Over the past two decades, a decrease in survival and increases in degenerative diseases and tumors have been observed in this strain, especially CD(SD) rats (Charles River Inc.), in the United States [1, 2]. These adverse changes have been associated with an increase in body weight which is influenced by selection for more rapid growth and greater fecundity and excessive food intake [3]. CD(SD)IGS rats are being produced by the gold standard system, a new breeding system which has been developed by Charles River Inc. to help ensure uniform experimental animals with minimized genetic ramifications and to promote internationalization of the research and development of new drugs [4].

It has been reported that background data on examinations in animal rooms and clinical pathology for repeated-dose toxicity studies using Crj:CD(SD)IGS rats [5, 6]. This study was designed to collect and evaluate gross pathological, organ weights and histopathological data on Crj:CD(SD)IGS rats from the age of 6 to 19 weeks for use as historical control data in future repeated-dose toxicity studies using this particular strain.

MATERIALS AND METHODS

1. Animals

One hundred male and 100 female Crj:CD(SD)IGS rats (SPF animals) aged 4 weeks were obtained from Charles River Japan Inc. on November 14, 1995, January 9, 1996 and March 5, 1996 and acclimatized to the environmental conditions of the Takatsuki Laboratories for 1 week. Sixty animals, 30 per sex, in the 1st lot and 120 animals, 60 per sex, in the 2nd and 3rd lot in good condition were selected for this study on the basis of clinical signs and body weight. They were allocated randomly to 3 groups each comprised of 10 males and 10 females in the 1st lot and 20 males and 20 females in the 2nd and 3rd lot and then acclimatization continued for an additional week. At the start of the experiment, the animals were 6 weeks old and ranged in weight from 169 to 226 g (males) and from 137 to 168 g (females).

2. Animal husbandry

Animals were individually housed in metal, mesh-bottom cages. Each cage in each group was allocated randomly to a position on the shelves in a clean booth. The booth was placed in an animal room with a room temperature of 20-26℃, a relative humidity of 40-70%, air exchange 8-25 times/hour and a 12-hour light/dark cycle (light on from 7:00 to 19:00). Before selection, all the animals were allowed free access to tap water and the standard powdered laboratory animal diet (CRF-1, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source). After selection, the animals were switched to a powdered laboratory animal diet having a lower protein content (CR-LPF, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source).

3. Grouping and diet components

The experimental groups were as follows.

The 30 males and 30 females in the 1st lot and 60 males and 60 females in the 2nd and 3rd lot were randomly divided into 3 groups

Lot	No. of animals (Animal No.)					
	Male			Female		
	2 weeks	4 weeks	13 weeks	2 weeks	4 weeks	13 weeks
1	10 (1-10)	10 (11-20)	10 (21-30)	10 (31-40)	10 (41-50)	10 (51-60)
2, 3	20 (1-20)	20 (21-40)	20 (41-60)	20 (61-80)	20 (81-100)	20 (101-120)

of 10 and 20, respectively, for the 2-, 4- and 13-week study. Eight, 10 and 19 weeks of age are abbreviated as 8w, 10w and 19w, respectively.

The diet components were as follows.

Component	CR-LPF
Gross energy (kcal/kg)	3490
Moisture (%)	7.5
Crude protein (%)	18.4
Crude fat (%)	4.8
Crude fiber (%)	5.0
Crude ash (%)	6.3
Nitrogen-free extract (%)	58.0

4. Examinations and methods

Macroscopic observations, organ weight measurement and microscopic observations were conducted for each of the 10 males and 10 females or 20 males and 20 females in each subgroup.

1) Necropsy and organ weights

Before necropsy, all animals in each group were fasted for about 20 hours and were weighed before they were exsanguinated under ether anesthesia. For each animal, the visceral organs were examined visually, and the following organs were weighed with electronic balances (AE160, AE163 and PM4800, Mettler GmbH): brain, heart, liver, kidneys, spleen, lungs, thymus, pituitary, adrenals, testes, ovaries and ventral prostate. Relative organ weights were calculated as a percentage of body weight.

2) Histopathology

The liver, kidneys, heart, lungs, testes, prostate, ovaries, pituitary and adrenals from all animals in each group were fixed in 10% neutral buffered formalin. The testes from all animals in each group were fixed in Bouin's solution. These organs were embedded in paraffin, sectioned at 4- μm thick, stained with hematoxylin-eosin and examined by light microscopy.

5. Statistical analysis

The data on organ weighs were analyzed statistically as follows. An analysis of variance was performed to see whether there were effects of age, sex and/or lot [7].

RESULTS

1. Gross pathology (Table 1)

The various changes shown in Tables 1 were observed, but each incidence and severity of the change were low and no age- or sex-related changes were observed.

Table 1. Incidences of gross pathological findings

Male												
Age	8 weeks				10 weeks				19 weeks			
Grade of the finding	−	+	++	+++	−	+	++	+++	−	+	++	+++
Seminal vesicle												
Small	50	0	0	0	50	0	0	0	49	1	0	0
Thymus												
Focus, Red, Multiple	50	0	0	0	50	0	0	0	49	1	0	0
Lung												
Focus, Posterior lobe Red, Punctate	50	0	0	0	49	1	0	0	50	0	0	0
Bone												
Discoloration, Cranium Dark, Red, Motteled	50	0	0	0	50	0	0	0	49	1	0	0
Discoloration, Cranium Gray, White, Focus	50	0	0	0	50	0	0	0	49	1	0	0
Thick, Cranium	50	0	0	0	50	0	0	0	49	1	0	0
Submandibular lymph node												
Cyst, Clear	50	0	0	0	49	1	0	0	50	0	0	0
Eye												
Small	50	0	0	0	49	1	0	0	50	0	0	0
Skin												
Crust, Axillary	49	1	0	0	50	0	0	0	50	0	0	0
Crust, Back	49	1	0	0	50	0	0	0	50	0	0	0
Ulcer, Back	49	1	0	0	50	0	0	0	50	0	0	0
Kidney												
Dilatation, Pelvis	49	0	1	0	50	0	0	0	49	1	0	0
Female												
Age	8 weeks				10 weeks				19 weeks			
Grade of the finding	−	+	++	+++	−	+	++	+++	−	+	++	+++
Ovary												
Cyst	50	0	0	0	50	0	0	0	49	1	0	0
Cyst, Capsule	49	1	0	0	50	0	0	0	50	0	0	0

Grades of the findings were recorded as follows: −, none; +, mild; ++, moderate; +++, marked.

2. Organ weights (Tables 2, 3)

The thymus weight in both sexes was tended to decrease with age. The weights of the ventral prostate in males and pituitary gland in females were increased with increasing body weight. The weights of other organs were tended to increase with age; the weight gains in the brain, adrenal glands and ovaries were relatively low (<10%). The adrenal gland weights was higher in females than in males at any age.

3. Histopathology (Table 4)

1) Liver

Mononuclear cell infiltration were observed at the highest rate of incidence (62-72%) in both sexes at any age. Vacuolization of the intracytoplasmic hepatocytes were seen at a high rate at 8w and 10w, especially in females; however, the incidence decreased in both sexes at 19w. Hematopoietic cell proliferation was seen in males at 8w and incidence of the change in males was decreased with age. Incidence of this in females was low at any age (<2%).

2) Heart

Cadiomyopathy, characterized by the inflammatory cell infiltration and proliferation of the tissues, was observed in males in any age and incidence of the change tented to increase with age.

3) Adrenal gland

Hyperplasia of the zona fasciculata and cystic degeneration of the cortex were seen each in a male at 19w.

4) Prostate

Mononuclear cell infiltration were observed at any age and incidence of the change tended to increase with age.

5) Spleen

Hematopoietic cell proliferation was observed in males at any age and incidence of the change tended to decrease with age. Pigmentation was seen at 19w in males and at any age in females and incidence of this change was higher in females than in males at 19w.

6) Kidney

Basophilic tubules was seen in both sexes at any age and incidence of the change tended to decrease at 13w. Mononuclear cell infiltration was seen in both sexes and incidence of the change in females tended to decrease at 19w. Hyaline droplets in the proximal tubules were seen in males at any age and incidence of the change was relatively high at 10w and 19w. Calcification in the C-M junction or medulla was observed in both sexes at any age. Hyperplasia of the pelvis epithelium was seen at any age in males

Table 2. Organ weights

Sex	Age (weeks)	No. of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Spleen (g)
Male	8	50	271±12(49)	1.91±0.07(49)	1.03±0.07(49)	1.10±0.08(49)	8.45±0.55(49)	2.25±0.16(49)	0.57±0.09(49)
	10	50	354±25	2.00±0.07	1.19±0.10	1.24±0.10	10.34±1.04	2.59±0.26	0.67±0.12
	19	50	493±45	2.12±0.08	1.40±0.14	1.36±0.12	12.16±1.29	2.91±0.26	0.72±0.10

Sex	Age (weeks)	No. of animals	Pituitary (mg)	Adrenals (mg)	Thymus (mg)	Testes (g)	Ventral prostate (mg)
Male	8	50	9.2±1.3(49)	48.6±7.8(49)	602.4±95.7(49)	2.83±0.20(49)	316.5±55.8(49)
	10	50	10.9±2.0(49)	55.2±9.3	530.3±68.4	3.19±0.24	474.4±94.5
	19	50	12.0±2.3	53.1±8.7	273.6±64.7	3.50±0.24	585.6±151.5

Sex	Age (weeks)	No. of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Spleen (g)
Female	8	50	175±12	1.79±0.07	0.69±0.07	0.84±0.06	5.59±0.58	1.48±0.10	0.38±0.06
	10	50	208±16	1.84±0.07	0.75±0.06	0.92±0.07	5.97±0.56	1.57±0.12	0.43±0.07
	19	50	270±19	1.59±0.08	0.86±0.06	1.01±0.08	6.59±0.57	1.66±0.12	0.46±0.07

Sex	Age (weeks)	No. of animals	Pituitary (mg)	Adrenals (mg)	Thymus (mg)	Ovaries (mg)
Female	8	50	9.4±2.0	55.6±6.9	477.4±76.4	72.9±10.3
	10	50	11.8±2.4	62.0±9.4	433.3±93.3	81.0±12.5
	19	50	15.0±2.8	58.1±7.4	258.4±62.4	74.5±13.1

Number in parentheses indicates the number of animals examined.

Table 3. Organ weights (body weight ratio)

Sex	Age (weeks)	No. of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys	Spleen
Male	8	50	271±12(49)	0.71±0.04(49)	0.38±0.02(49)	0.03±0.08(49)	0.15±0.55(49)	0.83±0.05(49)	0.21±0.03(49)
	10	50	354±25	0.57±0.04	0.34±0.02	0.35±0.03	2.92±0.14	0.73±0.05	0.19±0.03
	19	50	493±45	0.43±0.04	0.29±0.02	0.28±0.02	2.47±0.12	0.59±0.06	0.15±0.02

Sex	Age (weeks)	No. of animals	Pituitary ($\times 10^{-3}$)	Adrenals ($\times 10^{-3}$)	Thymus ($\times 10^{-3}$)	Testes	Ventral prostate ($\times 10^{-3}$)
Male	8	50	3.4±0.5(49)	17.9±2.8(49)	222.5±37.0(49)	1.05±0.09(49)	116.7±19.8(49)
	10	50	3.1±0.5(49)	15.6±2.6	150.3±19.3	0.91±0.10	134.5±26.8
	19	50	2.4±0.4	10.8±1.7	55.7±13.5	0.71±0.07	119.1±29.4

Sex	Age (weeks)	No. of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys	Spleen
Female	8	50	175±12	1.03±0.07	0.40±0.03	0.48±0.02	3.19±0.18	0.85±0.05	0.22±0.03
	10	50	208±16	0.89±0.06	0.36±0.02	0.44±0.03	2.87±0.15	0.76±0.06	0.21±0.03
	19	50	270±19	0.73±0.05	0.32±0.02	0.38±0.03	2.45±0.01	0.62±0.04	0.17±0.02

Sex	Age (weeks)	No. of animals	Pituitary ($\times 10^{-3}$)	Adrenals ($\times 10^{-3}$)	Thymus ($\times 10^{-3}$)	Ovaries ($\times 10^{-3}$)
Female	8	50	5.4±1.1	32.0±4.5	273.4±41.1	41.8±5.6
	10	50	5.7±1.1	29.9±5.1	207.3±36.8	39.0±6.4
	19	50	5.6±1.0	21.7±3.1	96.0±22.8	27.7±5.2

Number in parentheses indicates the number of animals examined.

and at 10w and 19w in females. Cyst in the cortex, medulla or papilla was seen at 8w.

DISCUSSION AND CONCLUSION

The common causes of death in Sprague-Dawley rats are pituitary tumor in both sexes, the renal and cardiovascular disease and mammary gland tumor. The age-related lesions are known to be chronic progressive nephrosis and basophilic tubules in the kidney and cardiomyopathy characterized by mononuclear cell infiltration, myocardial degeneration and myocardial fibrosis [1-3, 8]. In the present study, Crj:CD(SD)IGS rats aged 8-19 weeks were used and the experimental period was not enough long to estimate the age-related changes in this strain. However, the age-related increase in incidence of the renal basophilic tubules and cardiomyopathy and the sex-difference of the renal and cadiovascular lesions were observed and these changes were similar as those in CD(SD) rats [9]. Also, the decreased incidence of the hemopoietic proliferation and increased incidence of the pigmentation were observed in the spleen; these were also common changes in Sprague-Dawley rats [9].

No age- or sex-related changes upon gross pathology could not be estimated because incidence of each lesion was low. The age-related decrease in the thymus weight and age-related increase in the other organ weight were observed and sex-related change in the adrenal weights; these were similar changes as those in other strains of rats and Sprague-Dawley rats [9].

In the previous paper [5, 6], there were no great differences among Crj:CD(SD)IGS rats, Crj:CD(SD) rats and other breeders' Sprague-Dawley rats, Jcl:SD and Slc:SD rats, with regard to the biological parameters examined in repeated-dose toxicity studies.

From these results, it was concluded that there are no great differences among Crj:CD(SD)IGS rats, Crj:CD(SD) rats and other breeders' Sprague-Dawley rats, Jcl:SD and Slc:SD rats, with regard to the biological parameters examined in repeated-dose toxicity studies.

REFERENCES

1. Keenan, K. P. et al.: The effects of overfeeding and dietary restriction on Sprague-Dawley rat survival and early pathology biomarkers of aging. Toxicol. Pathol., 22, 300-315 (1994)
2. Keenan, K. P. et al.: Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: II. Effects on age-related proliferative and degenerative lesions. Toxicol. Pathol., 23, 287-302 (1995)
3. Keenan, K. P. et al.: The effects of diet, overfeeding and moderate dietary restriction in Sprague-Dawley rat survival, disease and toxicology. J. Nutr., 127 (Suppl. 5), 851S-856S (1997)
4. Charles River Japan Letter, 6, 10 (1993)
5. Naeshiro, I. et al.: Crj:CD(SD)IGS rats and effects of a

Table 4. Incidences of histopathological findings (Male)

Duration	2 weeks		4 weeks		13 weeks	
Diet	CR-LPF		CR-LPF		CR-LPF	
Liver						
Hematopoietic cell proliferation	9/50	18%	0/50	0%	1/50	2%
Hemorrhage, Glisson sheath, Focal	4/50	8%	0/50	0%	1/50	2%
Hernia, Median lobe	0/50	0%	1/50	2%	0/50	0%
Hypertrophy, Sinusoidal cell	1/50	2%	0/50	0%	0/50	0%
Infiltrative cell, Mononuclear cell	32/50	64%	36/50	72%	36/50	72%
Necrosis, Hepatocyte, Focal	4/50	8%	1/50	2%	3/50	6%
Vacuolization intracytoplasmic, Hepatocyte, Focal	4/50	8%	2/50	4%	4/50	8%
Vacuolization intracytoplasmic, Hepatocyte, Periportal	6/50	12%	3/50	6%	2/50	4%
Heart						
Cardiomyopathy	4/50	8%	9/50	18%	13/50	26%
Granuloma, Epicardium, Atrium	1/50	2%	0/50	0%	0/50	0%
Adrenal						
Hyperplasia, Zona fasciculata, Focal	0/50	0%	0/50	0%	1/50	2%
Vacuolization intracytoplasmic, Zona fasciculata	0/50	0%	2/50	4%	2/50	4%
Cyst	1/50	2%	0/50	0%	0/50	0%
Degeneration, Cortex, Cystic, Focal	0/50	0%	0/50	0%	1/50	2%
Degeneration, Medulla, Cystic, Focal	0/50	0%	1/50	2%	0/50	0%
Dilatation, Sinus, Cortex	0/50	0%	0/50	0%	1/50	2%
Adenoma, Cortex	0/50	0%	0/50	0%	1/50	2%
Pituitary						
Cyst, Pars distalis	1/50	2%	3/48	6%	1/50	2%
Cyst, Pars intermedia	0/50	0%	0/48	0%	1/50	2%
Prostate						
Infiltrative cell, Mononuclear cell	8/50	16%	13/50	26%	20/50	40%
Testis						
Degeneration, Germinal epithelium	0/50	0%	2/50	4%	0/50	0%
Atrophy, Seminiferous tubule, Focal	2/50	4%	0/50	0%	2/50	4%
Atrophy, Seminiferous tubule, Diffuse	1/50	2%	0/50	0%	0/50	0%
Vacuolization intracytoplasmic, Sertoli cell	0/50	0%	1/50	2%	0/50	0%
Spleen						
Hematopoietic cell proliferation	32/50	64%	14/50	28%	2/50	4%
Pigmentation	0/50	0%	0/50	0%	24/50	48%
Lung						
Calcification, Artery	0/50	0%	0/50	0%	1/50	2%
Infiltrative cell, Eosinophil	0/50	0%	3/50	6%	1/50	2%
Infiltrative cell, Foam cell	0/50	0%	4/50	8%	0/50	0%
Infiltrative cell, Mononuclear cell	1/50	2%	0/50	0%	0/50	0%
Inflammation, Focal	2/50	4%	2/50	4%	0/50	0%
Metaplasia, Osseous	0/50	0%	1/50	2%	2/50	4%
Kidney						
Basophilic, Renal tubule	36/50	72%	28/50	56%	14/50	28%
Calcification, C-M junction/Medulla	3/50	6%	7/50	14%	3/50	6%
Calcification, Pelvis, Submucosa	0/50	0%	2/50	4%	1/50	2%
Cast	2/50	4%	1/50	2%	4/50	8%
Cyst, Cortex/Medulla/Papilla	2/50	4%	0/50	0%	0/50	0%
Dilatation, Renal tubule	0/50	0%	1/50	2%	2/50	4%
Fibrosis, Cortex	2/50	4%	0/50	0%	0/50	0%
Fibrosis, Papilla	0/50	0%	0/50	0%	2/50	4%
Hyaline droplet, Renal tubule	1/50	2%	7/50	14%	8/50	16%
Hydronephrosis, Unilateral/Bilateral	2/50	4%	0/50	0%	3/50	6%
Hyperplasia, Collecting tubule	0/50	0%	0/50	0%	1/50	2%
Hyperplasia, Epithelium, Pelvis	6/50	12%	5/50	10%	3/50	6%
Infiltrative cell, Mononuclear cell	14/50	28%	9/50	18%	8/50	16%
Submandibular lymph node						
Dilatation, Sinus	0/0	—	1/1		0/0	—
Thymus						
Hemorrhage	0/0	—	0/0	—	1/1	
Bone						
Exostosis, Cranium	0/0	—	0/0	—	1/1	

Table 4. Incidences of histopathological findings (Female)

Duration	2 weeks		4 weeks		13 weeks	
Diet	CR-LPF		CR-LPF		CR-LPF	
Liver						
Hematopoietic cell proliferation	1/50	2%	0/50	0%	0/50	0%
Infiltrative cell, Mononuclear cell	31/50	62%	34/50	68%	34/50	68%
Necrosis, Hepatocyte, Focal	1/50	2%	0/50	0%	3/50	6%
Thrombus, Sinusoid	0/50	0%	0/50	0%	1/50	2%
Vacuolization intracytoplasmic, Hepatocyte, Focal	2/50	4%	1/50	2%	0/50	0%
Vacuolization intracytoplasmic, Hepatocyte, Periportal	20/50	40%	26/50	52%	1/50	2%
Heart						
Cardiomyopathy	0/50	0%	2/50	4%	3/50	6%
Infiltrative cell, Atrium, Mononuclear cell	1/50	2%	0/50	0%	2/50	4%
Adrenal						
Infiltrative cell, Cortex, Mononuclear cell	0/50	0%	0/50	0%	1/50	2%
Pituitary						
Cyst, Pars intermedia	0/50	0%	0/50	0%	3/50	6%
Cyst, Pars nervosa	0/50	0%	0/50	0%	1/50	2%
Ovary						
Cyst	0/50	0%	0/50	0%	2/50	4%
Spleen						
Hematopoietic cell proliferation	1/50	2%	1/50	2%	1/50	2%
Pigmentation	0/50	0%	12/50	24%	47/50	94%
Lung						
Calcification, Artery	0/50	0%	1/50	2%	1/50	2%
Infiltrative cell, Mononuclear cell	1/50	2%	0/50	0%	0/50	0%
Metaplasia, Osseous	0/50	0%	0/50	0%	1/50	2%
Kidney						
Basophilic, Renal tubule	14/50	28%	17/50	34%	2/50	4%
Calcification, C-M junction/Medulla	4/50	8%	1/50	2%	6/50	12%
Calcification, Papilla	0/50	0%	1/50	2%	0/50	0%
Calcification, Pelvis, Submucosa	0/50	0%	0/50	0%	1/50	2%
Cast	0/50	0%	0/50	0%	1/50	2%
Cyst, Cortex/Medulla/Papilla	5/50	10%	0/50	0%	0/50	0%
Dilatation, Renal tubule	1/50	2%	1/50	2%	0/50	0%
Fibrosis, Cortex	1/50	2%	1/50	2%	0/50	0%
Fibrosis, Medulla	1/50	2%	0/50	0%	0/50	0%
Hydronephrosis, Unilateral	1/50	2%	0/50	0%	0/50	0%
Hyperplasia, Epithelium, Pelvis	0/50	0%	1/50	2%	6/50	12%
Infiltrative cell, Mononuclear cell	7/50	14%	6/50	12%	2/50	4%

commercial low protein diet on the biological parameters used in repeated dose-toxicity studies. Biological reference data on CD(SD)IGS rats - 1998, 108-120 (1998)

6. Naeshiro, I. et al.: Background data for repeated-dose toxicity studies using Crj:CD(SD)IGS rats. Biological reference data on CD(SD)IGS rats - 1998, 100-107 (1998)
7. Snedecor, G. W. and Cochran, W. G.: Statistical methods, 7th ed. pp215-237, The Iowa State University Press (1980)
8. Keenan, K. P. et al.: Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasmas. Toxicol. Pathol., 23, 269-286 (1995)
9. Mohr, U. et al.: Pathobiology of the aging rat. ILSI Press (1994)

Background Data for Repeated-Dose Toxicity Studies Using Crj:CD(SD)IGS Rats at the Hikari Branch, Drug Safety Research Laboratories in Takeda Chemical Industries, Ltd.

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ABSTRACT. Background data for use in repeated-dose toxicity studies were collected when Crj:CD(SD)IGS rats were fed a commercial low protein diet (CR-LPF, protein content; 18%) for 2, 4, 13 or 26 weeks starting at 6 weeks of age. Evaluation was based on clinical observation, body weight, food consumption, water intake, urine volume, ophthalmoscopy, urinalysis, urine chemistry, hematology, blood chemistry, activities of hepatic drug-metabolizing enzymes, gross pathology, organ weights and histopathology. Sex-related differences were observed in urinary pH, creatinine and protein; leukocyte count upon hematological examination; plasma A/G ratio, albumin, triglyceride, ALP, CK, thyroxine and triiodothyronine ; and hepatic aminopyrine-*N*-demethylase and aniline hydroxylase activities. Age-related changes were observed in urinary protein, calcium and creatinine; erythrocyte count, MCH, MCV, neutrophils count upon hematological examination; plasma total protein, albumin, A/G ratio, total and HDL cholesterol, phospholipids, total bilirubin, AST, ALT, CK, inorganic phosphorus and thyroxine; hepatic aniline hydroxylase activity and all organ weights. Upon histopathology; cardiomyopathy, mononuclear cell infiltration in the liver, kidney, pancreas, harderian gland, epididymis and prostate, dilatation of the gastric gland, calcification of the pulmonary artery, pigmentation in the spleen and basophilic renal tubule were observed age-relatedly. These changes have also been observed in Crj:CD(SD) rats and other breeders' Sprague-Dawley rats with regard to biological parameters examined in repeated-dose toxicity studies. — Key words: Background data, CD(SD)IGS rat, Low protein diet

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INTRODUCTION

Crj:CD(SD)IGS rats are produced by Charles River Inc. under a new breeding system to help endure uniform experimental animals. This study was designed to collect background data on Crj:CD(SD)IGS rats fed an 18% protein diet from the age of 6 to 32 weeks for use in future repeated-dose toxicity studies.

MATERIALS AND METHODS

1. Animals

Three lots of 100 male and 100 female Crj:CD(SD)IGS rats (SPF animals) aged 4 weeks were obtained from Charles River Japan Inc. on November 14, 1995, January 9, 1996 and March 5, 1996, and acclimatized to the environmental conditions of our laboratories for 1 week. Eighty animals, 40 per sex, from the 1st lot and 160 animals, 80 per sex, each from the 2nd and 3rd lots in good condition were selected for this study on the basis of clinical signs and body weight. They were allocated randomly to 4 groups each comprised of 10 males and 10 females in the 1st lot and 20 males and 20 females in the 2nd and 3rd lot and then acclimatization continued for an additional week. The 4 groups were used for the 2-, 4-, 13- and 26-week studies, respectively. Weeks of age are abbreviated as w, such as 6w means 6 weeks of age. At the start of the experiment, the animals were 6 weeks old and ranged in weight from 177 to 231 g (males) and from 129 to 178 g (females).

The experimental groups were as follows.

Lot	No. of animals							
	Male				Female			
	2 weeks	4 weeks	13 weeks	26 weeks	2 weeks	4 weeks	13 weeks	26 weeks
1	10	10	10	10	10	10	10	10
2	20	20	20	20	20	20	20	20
3	20	20	20	20	20	20	20	20

2. Animal husbandry

Animals were individually housed in metal, mesh-bottom cages. Each cage in each group was allocated randomly to a position on the shelves in a clean booth. The booth was placed in an animal room with a room temperature of 20-26℃, a relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (lights on from 07:00 to 19:00). Before selection, all animals were allowed free access to tap water and a standard powdered laboratory animal diet (CRF-1, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source). After selection, the animals were switched to a powdered laboratory animal diet having a lower protein content (CR-LPF, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source).

The diet components were as follows.

Type	CRF-1	CR-LPF
Gross energy (kcal/kg)	3600	3490
Moisture (%)	7.7	7.5
Crude protein (%)	23.1	18.4
Crude fat (%)	5.9	4.8
Crude fiber (%)	3.3	5.0
Crude ash (%)	6.5	6.3
Nitrogen-free extract (%)	53.5	58.0

3. Examinations and methods

1) Clinical signs

All animals were observed for mortality, morbidity and clinical signs once a day during the experimental period. In addition, detailed examinations for clinical signs were conducted for all animals once a week during the experimental period.

2) Body weight

Each animal was weighed using an electronic balance (PM4600, Mettler GmbH) twice a week from 6w to 10w and once a week thereafter.

3) Food consumption

At each feeding (once a week), the weight of animal diet given and that of the diet remaining were measured for each animal in each group with an electronic balance (PM4600, Mettler GmbH), and food consumption values for the week were calculated.

4) Water intake and urine output

Water intake and urine output for 10 animals of each sex in the 1st lot and for 20 animals of each sex in the 2nd and 3rd lots were measured at 7w, 9w, 18w and 31w. Water intake was calculated as the difference between the weight of water given and that of the water remaining 24 hr later. Urine output was taken as the weight of urine collected over 24 hr. The water and urine were weighed using an electronic balance (PM4600, Mettler GmbH).

5) Ophthalmoscopy

Ophthalmoscopic examination was performed for all animals in each group once before the experiment commenced (5w) and at 9w, 18w and 31w. The cornea, anterior chamber, iris, lens and fundus in both eyes were examined with an ophthalmoscope (BETA200, Heine Optotechnik) and a fundus camera (Kowa RC-2, model-621, Kowa Co., Ltd.). Ophthalmoscopic examination of the fundus was performed 5-10 min after the instillation of a mydriatic (Mydrin-P®, Santen Pharmaceutical Co., Ltd.).

6) Urinalysis

Urinalysis was performed for 10 animals of each sex in the 1st lot and for 20 animals of each sex in the 2nd and 3rd lots at 7w, 9w, 18w and 31 or 32w. Animals were denied access to food and water, and a 4-hr morning urine sample was collected and centrifuged at 400×g for 5 min. The supernatant was examined using a reagent strip (Multistix® SG-L, Bayer-Sankyo Co., Ltd.) and an automated urine analyzer (Clinitek 200, Bayer-Sankyo Co., Ltd.), and the following were determined: pH, protein, glucose, occult blood, ketone bodies and urobilinogen. The urinary sediment was fixed in 20% buffered neutral formalin and stained with a urinary sediment stain (URI-CEL®, Cambridge Chemical Products, Inc.), and casts, epithelial cells, leukocytes and erythrocytes were counted under a microscope.

7) Urine chemistry

Urine chemical analysis was conducted for 10 animals of each sex in the 1st lot and for 20 animals of each sex in the 2nd and 3rd lots at 7w, 9w, 18w and 31 or 32w. Animals were denied access to food and water, and a 4-hr morning urine sample was collected and centrifuged at 400 ×g for 5 min. The values of the following in the supernatant were determined with automated blood chemistry analyzers (Hitachi 7150, Hitachi, Ltd. and System E3A, Beckman Instruments, Inc.) and standard reagents (Wako Pure Chemical Industries, Ltd. or Shionogi Co., Ltd.). Osmotic pressure was determined with an osmometer (OSM-1, Shimadzu).

- creatinine (method of Jaffé)
- total protein (pyrogallol red method)
- *N*-acetyl- β -D-glucosaminase (CPR-NAG method)
- calcium (OCPC method)
- inorganic phosphorus (molybdic acid direct method)
- sodium (ion-selective electrode method)
- potassium (ion-selective electrode method)
- chloride (ion-selective electrode method)
- osmotic pressure (freezing point depression method)

8) Hematology and blood chemistry

Hematological and blood chemical analyses were conducted for each of the 10 males and 10 females (1st lot) or 20 males and 20 females (2nd and 3rd lots) at 8w, 10w, 19w and 32w. Before necropsy, the animals were fasted for about 20 hr, and blood was withdrawn from the abdominal aorta with a vacuum blood collecting tube containing EDTA2K (hematology) or heparin sodium (blood chemistry) under ether anesthesia. The heparinized blood was centrifuged at 7500×g for 10 min to obtain plasma for blood chemistry.

a) Hematology

The values of the following were determined or calculated with an automated hematology analyzer (E-5000, Sysmex Corp.) and an automated reticulocyte analyzer (R-2000, Sysmex Corp.). The differential leukocyte count was performed by microscopy after May-Giemsa staining.

- erythrocyte count (electric resistance detection method)
- leukocyte count (electric resistance detection method)
- platelet count (electric resistance detection method)
- hematocrit value (cumulative pulse height detection method)
- hemoglobin concentration (SLS-hemoglobin method)
- mean corpuscular hemoglobin (MCH; calculated)
- mean corpuscular hemoglobin concentration (MCHC; calculated)
- mean corpuscular volume (MCV; calculated)
- reticulocyte count (flow cytometry using the argon laser method)
- differential leukocyte count

b) Blood chemistry

The values of the following were determined with automated blood chemistry analyzers (Hitachi 7150, Hitachi, Ltd. and System E3A, Beckman Instruments, Inc.) and standard reagents (Wako Pure Chemical Industries, Ltd. and Sigma Co.), except for the A/G ratio which was calculated from the total protein and albumin values. At 8w in the 1st lot, albumin and A/G ratio were not determined because of shortage of sample. Triiodothyronine and thyroxine were determined with a commercial kit (Boehringer-Mannheim GmbH).

- total protein (biuret method)
- albumin (BCG method)
- A/G ratio
- glucose (glucokinase-G-6-PDH method)
- total cholesterol (COD-DAOS method)
- HDL-cholesterol (phosphotungstate-magnesium precipitation method)
- triglyceride (GPO-DAOS method)
- phospholipids (oxidase-DAOS method)
- urea nitrogen (urease-GlDH method)

- creatinine (method of Jaffé)
- total bilirubin (alkaline azobilirubin method)
- direct bilirubin (alkaline azobilirubin method)
- aspartate aminotransferase (AST: modified JSCC method)
- alanine aminotransferase (ALT: modified JSCC method)
- alkaline phosphatase (ALP: *p*-nitrophenylphosphate substrate method)
- lactate dehydrogenase (LDH: Wróblewski-LaDue method)
- creatine kinase (CK: GSCC method)
- sodium (ion-selective electrode method)
- potassium (ion-selective electrode method)
- chloride (ion-selective electrode method)
- calcium (OCPC method)
- inorganic phosphorus (molybdic acid direct method)
- triiodothyronine (T3, EIA)
- thyroxine (T4, EIA)

9) Activity of hepatic drug-metabolizing enzymes

At necropsy, small pieces (ca. 1 g) of the liver were removed from all animals in each group and were frozen. After thawing, the pieces were homogenized individually in 4 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 9000 × g for 20 min, and the supernatant was assayed for aminopyrine-*N*-demethylase (Cochin & Axelrod method) and aniline hydroxylase (modified method of Wills) activity.

10) Necropsy and organ weights

Necropsy and organ weight measurement were conducted for 10 animals/sex (1st lot) or 20 animals/sex (2nd and 3rd lots) at 8w, 10w, 19w and 32w. Before necropsy, all animals in each group were fasted for about 20 hr and were weighed before they were exsanguinated under ether anesthesia. For each animal, the visceral organs were examined visually, and the following organs were weighed with electronic balances (AE260 and PM2000, Mettler GmbH): brain, heart, liver, kidneys, spleen, lungs, thymus, pituitary, adrenals, testes, ovaries and ventral prostate. Relative organ weights were calculated as a percentage of body weight.

11) Histopathology

The adrenals, aorta, brain, cecum, cervical spinal cord, colon, duodenum, epididymides, esophagus, femurs, Harder's glands, heart, ileum, jejunum, kidneys, liver, lumbar spinal cord, lung, mammary glands, mesenteric lymph nodes, ovaries, pancreas, parathyroids, pituitary, sciatic nerves, seminal vesicles, skeletal muscles, skin, spleen, sternum, stomach, sublingual glands, submandibular glands, submandibular lymph nodes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina and ventral prostate from all animals necropsied were fixed in 10% neutral buffered formalin. The eyes were pre-fixed in Davidson's solution containing 3% glutaraldehyde and post-fixed in 10% neutral buffered formalin. The testes were fixed in Bouin's solution. These organs were embedded in paraffin, sectioned at 4- μm thick, stained with hematoxylin-eosin and examined by light microscopy.

4. Statistical analysis

The data on body weight, food consumption, water intake, urine output, hematology, blood chemistry, activity of hepatic drug-metabolizing enzymes and organ weights were analyzed statistically using an analysis of variance [8] to see whether there were effects of age, sex and/or lot.

RESULTS

Data from the 3 lots of animals were combined and evaluated.

1. Mortality

No animals died during the experimental period.

2. Clinical signs (Table 1)

Hair loss was observed during the first half of the experimental period in males and at 10w and thereafter in females. Malinterdigitation was observed in 2 males and 1 females. Wound, lacrimation and hemorrhage were observed in 1 male and 1 female. Stained fur around the eyes and small testis were observed in 1 male each, and decreased feces was observed in 1 female.

3. Body weight (Table 2)

In both sexes, body weight increased rapidly up to 9w, then increased gradually, and almost reached a plateau level at 18w.

4. Food consumption (Table 3)

No significant changes were observed during the experimental period. The average weekly food consumption value was 187 g in males and 124 g in females.

5. Water intake and urine output (Table 4)

Urine output was lower at 7w than at 9w, 18w and 31w in males, and it was lower at 7w and 9w than at 18w and 31w in females. No significant changes in water intake were observed at any age.

6. Ophthalmoscopy

There were no abnormalities in any group.

7. Urinalysis (Table 5)

The pH level was higher in males than in females. An age-related increase in the grade of protein was observed in both sexes.

8. Urine chemistry (Table 6)

Creatinine and total protein were higher in males than in females, and increased with age. Inorganic phosphorus was higher in females than in males. The calcium levels decreased at 9w as compared with those at 7w; however, they increased with age after 9w.

9. Hematology (Table 7)

The leukocyte count was higher in males than in females at each age examined. Age-related increases in the erythrocyte count in males were observed until 19w. Age-related decreases in MCH and MCV and age-related increases in the neutrophil and eosinophil counts were observed in both sexes.

10. Blood chemistry (Table 8)

The glucose, triglyceride, ALP, CK and thyroxine values were higher in males than in females at each age examined. The albumin, A/G ratio, urea nitrogen, phospholipids and triiodothyronine values were lower in males than in females. The total protein, albumin, total and HDL cholesterol, phospholipids, total bilirubin, AST and ALT values increased slightly with age. The A/G ratio, ALP, CK, inorganic phosphorus and thyroxine values decreased with age.

11. Activity of the hepatic drug-metabolizing enzymes (Table 9)

Aminopyrine-*N*-demethylase and aniline hydroxylase activities were higher in males than in females at each age examined. Aniline hydroxylase activity decreased after 10w.

12. Organ weights (Tables 10, 11)

In all organs the absolute weights increased and the relative weights to body weight decreased with age, except for the thymus and ovary. The thymus weight decreased with age. The

ovary weight increased slightly at 10w as compared with the values at 8w; however, it tended to decrease after 19w.

13. Gross pathology (Table 12)

The incidence of dilatation of the renal pelvis increased slightly with age in males. Small thymus was observed 2 males and 2 females at 32w. Other changes were seen occasionally as shown in Table 12; however, they were low in incidence and slight in grade.

14. Histopathology (Table 13)

Heart: Cardiomyopathy increased with age markedly in males and slightly in females.

Liver: Mononuclear cell infiltration was observed in most animals and the incidence tended to increased with age.

Kidney: Basophilic tubules was observed in more than half animals at 8w; however, the incidence decreased with age. Mononuclear cell infiltration increased with age except in females at 32w

Spleen: Pigmentation appeared in most animals at 19w and the incidence further increased at 32w. Hematopoietic cell proliferation was observed in most males at each age examined and in 4 femalesat 8 and 10w in most females at 19 and 32w.

Pancreas: The incidence of mononuclear cell infiltration was observed at 19 and 32w and increased at 32w.

Harderian gland: The incidence of mononuclear cell infiltration increased with age.

Epididymis: The incidence of mononuclear cell infiltration increased with age.

Prostate: The incidence of mononuclear cell infiltration increased with age.

Lung: The incidence of calcification in the artery increased with age in males.

Stomach: Dilatation of the glands was observed at 19 and 32w and the incidence increased at 32w.

Other organs: Several changes were observed as shown in Table 13, but they were low in incidence or there were no difference between sexes or among ages examined.

DISCUSSION

Crj:CD(SD)IGS rats were fed a low protein diet for 26 weeks and the biological parameters used in repeated dose toxicity studies were examined.

Sex-related differences were observed in urinary pH, creatinine and protein; leukocyte count in blood; plasma A/G ratio, albumin, triglyceride, ALP, CK, thyroxine and triiodothyronine; and hepatic aminopyrine-*N*-demethylase and aniline hydroxylase activities. These differences were also observed in a previous study using the same lots of Crj:CD(SD)IGS rats conducted in our Osaka laboratory [5].

Age-related changes were observed in urinary protein, calcium and creatinine; erythrocyte count, MCH, MCV and neutrophil and eosionphil counts in blood; plasma total protein, albumin, A/G ratio, total and HDL cholesterol, phospholipids, total bilirubin, AST, ALT, CK, inorganic phosphorus and thyroxine; and hepatic aniline hydroxylase activity. Almost all the changes were also observed in the previous study in our Osaka laboratory [5], as well as in various strains of rats used commonly in repeated-dose toxicity studies [4, 6, 7, 9]. In the previous study in our Osaka laboratory, plasma ALT, AST and LDH increased at 19w in Crj:CD(SD)IGS rat [5]. In the present study, more marked increases in AST, ALT and LDH were observed in females at 32w. The increases in these enzyme activities are also observed in SD rats [4, 9], but they are not so marked. Therefore, the marked increases in the plasma enzyme activities without hepatic lesions are considered to be unique changes in aged Crj:CD(SD)IGS rats.

In contrast to almost all organs increasing in weight with age, the thymus and ovary decreased in weight with age. It is well known that the thymus atrophy with age and the ovary mature at 10w. In histopathology, several age-related changes, mononuclear cell infiltration in several organs, cardiomyopathy, pigmentation in the spleen and calcification in the pulmonary artery, were observed. The same findings are observed in SD rats [1, 2, 3].

From these results, it is concluded that there are no great differences among Crj:CD(SD)IGS rats, Crj:CD(SD) rats and other breeders' Sprague-Dawley rats, Jcl:SD and Slc:SD rats, with regard to the biological parameters examined in repeated-dose toxicity studies.

REFERENCES

1. Keenan, K. P. et al. 1994. The effects of overfeeding and dietary restriction on Sprague-Dawley rat survival and early pathology biomarkers of aging. Toxicol. Pathol., 22: 300-315.
2. Keenan, K. P. et al. 1995. Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms. Toxicol. Pathol., 23: 269-286.
3. Keenan, K. P. et al. 1995. Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: II. Effects on age-related proliferative and degenerative lesions. Toxicol. Pathol., 23: 287-302.
4. Nagase, S. et al. 1976. Clinico-biochemical data on laboratory animals. Exp. Anim., 25: 149-210.
5. Neshiro, I. et al. 1998. Background data for repeated-dose toxicity studies using Crj:CD(SD)IGS rats. Biological Reference Data on CD(SD)IGS Rats - 1998, 100-107.
6. Nakagawa, I. and Masana Y. 1971. Effect of protein nutrition on growth and life span in the rat. J. Nutr., 101: 613-620.
7. Neuhaus, W. O. 1978. Age-dependent changes in the excretion of urinary protein by the rats. Nephron, 22: 570-576.
8. Snedecor, G. W. and Cochran, W. G. 1980. Statistical methods, 7th ed. pp 215-237, The Iowa State University Press.
9. Wolford, S. T. et al. 1986. Reference range data base for serum chemistry and hematology values in laboratory animals. J. Toxicol. Environm. Health.,18: 161-188.

Table 1. Clinical signs (incidence of individual signs) in Crj:CD(SD)IGS rats fed a low protein diet

Age (weeks)	Number of animals	Hair loss		Wound		Malinterdigitation		Fur around eye, stained		Lacrimation		Hemorrhage		Testis, small		Feces, decreased	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
6	200	0	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0
7	200	2	0	1	1	0	0	0	0	0	0	0	0	0	—	0	1
8	150	1	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0
9	150	1	0	1	0	0	0	0	0	0	0	0	0	0	—	0	0
10	100	3	1	1	0	0	0	0	0	0	0	0	0	0	—	0	0
11	100	3	1	1	0	0	0	0	0	0	0	1	1	1	—	0	0
12	100	2	1	1	0	1	0	0	0	0	0	0	1	1	—	0	0
13	100	2	2	1	0	2	1	1	0	0	1	0	0	1	—	0	0
14	100	3	3	1	0	2	1	1	0	1	1	0	0	1	—	0	0
15	100	4	4	0	0	2	1	0	0	1	0	0	0	1	—	0	0
16	100	3	3	0	0	2	1	1	0	1	1	0	0	1	—	0	1
17	100	3	2	0	0	2	1	1	0	1	1	0	0	1	—	0	0
18	100	3	3	0	0	2	1	1	0	1	0	0	0	1	—	0	0
19	50	0	2	0	0	1	0	1	0	1	0	0	0	1	—	0	0
20	50	0	2	0	0	1	0	1	0	1	0	0	0	1	—	0	0
21	50	0	2	0	0	1	0	1	0	1	0	0	0	1	—	0	0
22	50	0	2	0	0	1	0	1	0	1	0	1	1	1	—	0	0
23	50	0	2	0	0	1	0	0	0	1	0	0	0	1	—	0	0
24	50	0	2	0	0	1	0	0	0	1	0	0	0	1	—	0	0
25	50	0	2	0	0	1	0	0	0	1	0	0	0	1	—	0	0
26	50	0	1	0	0	1	0	0	0	1	0	0	0	1	—	0	0
27	50	0	1	0	0	1	0	0	0	1	0	0	0	1	—	0	1
28	50	0	1	0	0	1	0	0	0	1	0	0	0	1	—	0	1
29	50	0	1	0	0	1	0	0	0	1	0	0	0	1	—	0	1
30	50	0	1	0	0	1	0	0	0	1	0	0	0	1	—	0	1
31	50(49)	0	2	0	0	1	0	0	0	1	0	0	0	1	—	0	0

Number in parentheses indicates the number of female animals examined.

Table 2. Body weight in Crj:CD(SD)IGS rats fed a low protein diet

Age (weeks)	Number of animals	Male	Female
6	200	203.5±10.5	153.0±8.8
7	200	260.3±14.1	172.7±13.7
8	200	308.9±18.6	190.6±17.3
9	150	347.0±23.1	206.2±20.0
10	150	379.0±26.6	216.0±23.4
11	100	408.5±30.3	228.5±23.4
12	100	434.2±32.0	240.8±23.9
13	100	456.9±33.0	252.2±23.6
14	100	475.9±34.6	258.8±23.6
15	100	494.0±36.7	266.5±24.7
16	100	510.4±38.2	273.2±24.5
17	100	522.0±39.1	278.4±25.1
18	100	532.6±40.5	281.2±25.2
19	100	541.9±40.6	282.9±25.0
20	50	560.0±33.4	284.6±24.2
21	50	569.1±35.0	291.6±24.9
22	50	578.2±35.7	294.3±26.3
23	50	586.2±36.6	297.6±26.3
24	50	594.6±38.3	302.5±27.6
25	50	602.3±39.4	306.3±28.2
26	50	609.1±41.0	309.0±28.7
27	50	614.8±39.8	311.6±29.8
28	50	621.0±39.9	314.4±29.7
29	50	625.9±39.7	317.0±30.6
30	50	632.3±40.8	318.1±31.2
31	50	637.3±40.2	321.6±32.2 (49)
32	50	644.4±42.5	323.7±33.1 (49)

Data are expressed as mean±S.D. (g).
Number in parentheses indicates the number of animals examined.

Table 3. Food consumption in Crj:CD(SD)IGS rats fed a low protein diet

Age (weeks)	Number of animals	Male	Female
6	200	170.9±10.9	117.4±11.6
7	200	182.4±13.4	119.8±12.5
8	150	183.2±15.9	121.2±15.4
9	150	183.0±16.2	118.3±17.2
10	100	184.7±14.8	121.8±16.4
11	100	188.4±13.7	125.4±15.0
12	100	191.5±13.7	129.3±13.3
13	100	187.6±15.0	125.2±11.8
14	100	187.3±15.4	126.4±12.3
15	100	187.0±15.2	125.5±10.9
16	100	185.0±16.2	122.6±12.1
17	100	183.7±17.7	119.6±14.2
18	100	180.2±16.4	117.3±12.6
19	50	187.9±14.0	124.9±10.6
20	50	192.9±15.8	127.4±11.2
21	50	191.7±14.2	127.5±13.5
22	50	188.9±13.8	127.0±15.1
23	50	187.8±14.9	125.4±13.3
24	50	187.2±13.8	125.0±13.0
25	50	189.0±14.8	125.3±13.5
26	50	190.0±14.3	126.4±13.1
27	50	191.0±13.4	126.2±16.9
28	50	191.2±14.3	124.5±17.1
29	50	191.3±13.8	125.6±15.4
30	50	187.4±13.3	126.6±13.5 (49)
31	49	184.8±15.0	122.7±12.6

Data are expressed as mean±S.D. (g/animal/week).
Number in parentheses indicates the number of animals examined

Table 4. Water intake and urine output in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Water intake (g/day)	Urine output (g/day)
Male	7	50	38.70±6.05	16.30±4.90
	9	50	41.76±7.51	19.65±5.78
	18	50	36.74±6.57	20.88±5.60
	31	50	34.56±8.65	19.52±8.49
Female	7	50	28.09±5.56	11.81±4.49
	9	50	29.45±7.12	12.68±5.98
	18	49	32.36±8.20	16.63±7.30 (47)
	31	49	33.90±9.43	18.97±8.53

Data are expressed as mean±S.D.
Number in parentheses indicates the number of animals examined.

Table 5. Urinalysis in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	pH					Protein					Glucose		Ketones			Occult blod					Urobilinogen
			5	6	7	8	9	−	±	+	++	+++	−	±	−	±	+	−	±	+	++	+++	±
Male	8	50	0	0	2	6	42	0	3	39	8	0	50	0	19	26	5	48	2	0	0	0	50
	10	50	0	0	3	6	41	0	7	35	8	0	49	1	5	21	24	48	0	2	0	0	50
	19	50	0	0	13	9	28	0	0	29	21	0	50	0	0	18	32	50	0	0	0	0	50
	31-32	49	0	1	6	11	31	1	0	22	24	2	47	2	5	29	24	46	0	0	1	2	49
Female	8	50	0	3	10	16	21	24	10	16	0	0	50	0	41	8	1	50	0	0	0	0	50
	10	50	0	8	13	13	16	22	8	18	2	0	50	0	32	15	3	50	0	0	0	0	50
	19	49	1	10	14	11	13	18	3	25	3	0	49	0	36	10	3	49	0	0	0	0	49
	31-32	49	1	9	16	14	9	13	11	22	3	0	49	0	32	16	0	49	0	0	0	0	49

Sex	Age (weeks)	Number of animals	Casts		Epithe. cells		Leukocytes		Erythrocytes			
			−	+	−	+	−	+	−	+	++	+++
Male	8	50	49	1	49	1	50	0	50	0	0	0
	10	50	49	1	44	6	49	1	48	0	2	0
	19	50	48	2	49	1	50	0	50	0	0	0
	31-32	49	43	6	40	9	48	1	46	0	0	3
Female	8	50	50	0	49	1	40	10	50	0	0	0
	10	50	50	0	50	0	46	4	49	1	0	0
	19	50	49	1	50	0	43	7	50	0	0	0
	31-32	49	49	0	47	2	44	5	49	0	0	0

Table 6. Urine chemistry in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Creatinine (mg/dL)	Total protein (mg/dL)	N-acethyl- β -D-glucosaminidase (U/L)	Sodium (mmol/L)	Potassium (mmol/L)
Male	7	50	53.8±17.3	50.3±36.1	14.5±4.7	115.0±38.3	212.9±64.8
	9	49	75.2±33.0	71.6±38.8	14.9±8.9	81.8±41.8	195.4±72.0
	18	50	132.6±40.5	89.0±43.9	21.6±7.8	71.6±47.0	183.1±111.4
	30-31	48	145.3±54.1	89.9±61.2	23.7±9.1	101.4±42.3	248.6±105.6
Female	7	48	44.1±19.3	1.3±4.0	11.5±5.8	79.6±40.0 (47)	169.7±66.8 (47)
	9	43	69.6±31.3	6.1±8.9 (39)	14.3±7.9	70.9±30.2	181.7±72.7
	18	40	94.4±46.6	5.8±7.6	16.6±7.6	60.6±40.3 (39)	165.1±131.7 (39)
	30-31	47	95.6±44.3	7.8±10.7	15.6±6.4	87.1±41.2 (46)	186.2±86.3 (45)

Sex	Age (weeks)	Number of animals	Chloride (mmol/L)	Osmolality (mOsmol/kg)	Glucose (mg/dL)	Calcium (mg/dL)	Inorganic phosphorus (mg/dL)
Male	7	50	154.4±52.0	1055±345	12.7±3.6	10.0±5.4	18.1±23.2
	9	49	111.7±53.0	984±354	13.0±5.2	6.3±3.6	18.9±25.1
	18	50	100.1±66.9	1236±473	17.5±5.3	8.7±5.0	39.6±45.4
	30-31	48	140.1±64.2	1480±421 (47)	17.2±6.1	13.8±7.0	20.7±21.3
Female	7	48	115.6±58.1 (46)	984±438	11.2±3.7	9.5±5.5	41.3±49.3 (47)
	9	43	91.0±44.0	1153±514 (42)	14.2±6.0	6.1±3.8 (42)	86.7±117.3
	18	40	86.0±68.4 (39)	1127±475 (35)	16.2±6.8	14.2±8.2	96.5±96.6
	30-31	20	110.3±51.4	1248±379 (40)	16.3±6.0	29.3±19.0	89.9±84.8 (45)

Data are expressed as mean±S.D.
Number in parentheses indicates the number of animals examined.

Table 7. Hematology in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Erythrocytes ($\times 10^4/\mu$L)	Hematocrit (%)	Hemoglobin (g%)	MCH (pg)	MCHC (%)	MCV (cμ)	Platelets ($\times 10^4/\mu$L)
Male	8	50	780±39	46.9±2.1	15.5±0.8	19.9±0.6	33.2±0.5	60±1.6	136.1±14.4
	10	50	846±42	47.8±2.1	16.0±0.7	19.0±0.8	33.5±0.7	57±1.6	125.0±11.4
	19	50	908±36	47.7±1.7	15.9±0.6	17.5±0.6	33.3±0.6	53±1.5	121.5±22.9
	32	49	904±34	47.3±1.6	15.6±0.6	17.3±0.6	33.0±0.6	52±1.7	117.0±18.3
Female	8	50	815±32	46.7±1.7	16.0±0.6	19.7±0.6	34.3±0.5	57±1.5	128.9±13.1
	10	50	835±29	46.1±1.6	15.8±0.5	18.9±0.6	34.3±0.7	55±1.7	122.5±16.3
	19	50	837±48	45.7±2.0	15.4±0.8	18.5±0.6	33.8±0.6	55±2.1	111.1±19.8
	32	49	810±45	44.5±2.2	14.9±0.8	18.4±0.6	33.5±0.6	55±1.5	106.4±13.1

Sex	Age (weeks)	Number of animals	Leukocytes ($\times 10^2/\mu$L)	Reticulocytes (%)	Leukocytes, differential count(%)				
					Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Male	8	50	111±33	4.2±0.7	7.0±5.4	92.3±5.5	0.2±0.4	0.5±0.6	0.0±0.0
	10	50	111±32	2.6±0.4	8.9±4.4	90.0±4.4	0.2±0.5	0.9±1.0	0.0±0.0
	19	50	113±31	2.1±0.3	14.2±5.6	84.3±5.6	0.3±0.5	1.3±1.0	0.0±0.0
	32	49	111±30	2.3±0.5	18.6±6.6	79.3±6.7	0.2±0.5	1.8±1.8	0.0±0.0
Female	8	50	87±22	2.4±0.6	7.0±3.3	91.9±3.6	0.2±0.5	0.9±1.1	0.0±0.0
	10	50	88±27	2.1±0.5	6.7±3.8	92.3±4.1	0.1±0.4	0.9±1.1	0.0±0.0
	19	50	73±20	2.1±1.9	11.2±6.1	87.1±6.0	0.2±0.5	1.4±1.4	0.0±0.0
	32	49	68±18	2.2±0.7	15.1±7.1	82.7±7.6	0.2±0.5	2.0±1.6	0.0±0.0

Data are expressed as mean±S.D.

Table 8. Blood chemistry in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Total protein (g/dL)	Albmin (g/dL)	A/G ratio	Haptoglobin (mg/dL)	Glucose (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)
Male	8	50	5.81±0.18	3.15±0.29(40)	1.21±0.20(40)	14.92±5.39	143±18	67±12	40±8
	10	50	5.96±0.22	3.28±0.14	1.23±0.08	11.51±5.18	139±18	70±12	42±7
	19	50	6.31±0.30	3.20±0.16	1.04±0.12	16.26±4.74	163±14	81±16	50±9
	32	48	6.40±0.23	3.26±0.16	1.04±0.22	14.03±5.08	167±14	88±22	52±13
Female	8	50	5.96±0.24	3.48±0.29(40)	1.43±0.23(40)	12.01±4.72	120±15	70±16	43±9
	10	49	6.16±0.34	3.66±0.29	1.47±0.16	9.00±4.22	129±14	77±15	48±9
	19	50	6.75±0.47	3.93±0.37	1.40±0.17	13.00±4.01	139±17	85±16	56±11
	32	49	6.98±0.43	4.18±0.35	1.49±0.28	13.78±4.11	146±14	102±21	63±12

Sex	Age (weeks)	Number of animals	HDL cholesterol (%)	Triglyceride (mg/dL)	Phospholipids (mg/dL)	Urea nitrogen (mg/dL)	Creatinine (mg/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Male	8	50	60.5±5.7	49±11	99.9±14	14.0±2.0	0.4±0.1	0.06±0.01	0.05±0.01
	10	50	61.1±5.9	42±12	102.2±16	13.6±1.8	0.5±0.1	0.06±0.16	0.05±0.01
	19	50	62.5±5.2	64±19	117.6±18	14.4±1.5	0.5±0.1	0.07±0.02	0.06±0.02
	32	48	59.3±5.3	84±26	123.7±28	14.0±1.8	0.6±0.1	0.10±0.02	0.07±0.18
Female	8	50	62.6±6.1	34±8	119.7±21	16.5±2.9	0.5±0.1	0.06±0.01	0.05±0.01
	10	49	63.2±6.5	30±8	135.0±24	17.2±2.4	0.5±0.1	0.08±0.02	0.05±0.01
	19	50	65.7±4.2	43±12	159.2±28	16.9±2.7	0.6±0.1	0.09±0.02	0.07±0.02
	32	49	62.7±7.2	54±22	185.5±35	16.0±2.0	0.6±0.1	0.13±0.04	0.09±0.03

Sex	Age (weeks)	Number of animals	AST (U/L)	ALT (U/L)	LAP (U/L)	LDH (U/L)	ALP (U/L)	CK (U/L)	Sodium (mmol/L)
Male	8	50	63±7	23±4	73±5	100±24	539±99	177±24	144±1
	10	50	60±6	24±4	72±7	85±23	417±71	118±28	144±1
	19	50	59±11	26±5	63±6	83±28	179±27	82±18	143±1
	32	48	67±36	32±20	58±6	104±49	138±27	75±15	142±1
Female	8	50	60±10	17±3	67±7	97±25	313±61	126±71	142±1
	10	49	58±9	20±5	61±6	83±17	216±44	91±21	142±1
	19	50	79±55	34±29	54±6	106±85	90±25	67±19	143±1
	32	49	109±110	52±68	51±6	116±58	61±19	61±20	142±1

Sex	Age (weeks)	Number of animals	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mmol/L)	Inorganic phosphorus (mg/dL)	Thyroxine (T4) (μg/dL)	Triiodothyronine (T3) (ng/mL)
Male	8	50	3.5±0.2	114±2	9.93±0.26	8.5±0.5	4.3±0.5	1.3±0.2
	10	50	3.5±0.2	114±2	9.70±0.31	7.7±0.5	4.1±0.4	1.4±0.2
	19	50	3.6±0.2	114±2	9.69±0.54	6.3±0.5	4.3±0.7	1.4±0.2(49)
	32	48	3.6±0.2	114±2	9.93±0.32	5.7±0.4	3.9±0.4	1.5±0.4
Female	8	50	3.5±0.3	116±2	9.83±0.30	7.7±0.6	3.6±0.7	1.6±0.3
	10	49	3.4±0.4	116±3	9.63±0.33	7.2±0.7	3.4±0.7	1.4±0.2
	19	50	3.4±0.5	116±2	9.92±0.33	6.3±0.9	3.4±0.7	1.6±0.3(49)
	32	49	3.5±0.4	117±2	10.05±0.41	5.5±0.6	3.1±0.6	1.5±0.4

Data are expressed as mean±S.D.
Number in parentheses indicates the number of animals examined.

Table 9. Activity of hepatic drug-metabolizing enzymes in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Liver weight (g)	Relative liver weight (%)	Aminopyrine-N-demethylase (mU/g liver)	Aminopyrine-N-demethylase (mU/mg pr.)	Aniline hydroxylase (mU/g liver)	Aniline hydroxylase (mU/mg pr.)
Male	8	50	8.36±0.87	3.05±0.19	156.9±39.7	0.916±0.202	12.8±2.9	0.075±0.016
	10	50	9.71±0.83	2.78±0.16	187.2±45.4	1.129±0.334	15.2±2.7	0.090±0.015
	19	50	12.35±1.61	2.46±0.22	170.0±35.9	1.048±0.204	10.5±2.9	0.064±0.015
	32	50	14.00±1.43	2.29±0.14	167.6±42.6	1.061±0.257	8.7±3.4	0.054±0.020
Female	8	50	5.34±0.58	3.11±0.17	117.1±28.0	0.654±0.135	11.0±3.5	0.062±0.018
	10	50	5.75±0.72	2.85±0.16	113.0±26.4	0.687±0.192	11.1±3.2	0.066±0.017
	19	50	6.46±0.63	2.42±0.15	113.1±22.7	0.689±0.136	9.0±2.5	0.054±0.013
	32	49	7.06±0.95	2.33±0.15	108.6±18.9	0.666±0.112	7.9±2.1	0.048±0.010

Data are expressed as mean±S.D.

Table 10. Organ weights in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Spleen (g)
Male	8	50	274±18	1.95±0.07	1.01±0.09	1.05±0.08	8.36±0.87	2.27±0.22	0.55±0.09
	10	50	350±22	2.00±0.07	1.15±0.08	1.16±0.07	9.71±0.83	2.60±0.21	0.63±0.10
	19	50	501±44	2.14±0.09	1.40±0.14	1.32±0.12	12.35±1.61	3.12±0.37	0.73±0.13
	32	50	610±42	2.16±0.10	1.53±0.11	1.45±0.12	14.00±1.43	3.32±0.25	0.80±0.12
Female	8	50	171±14	1.80±0.07	0.68±0.07	0.82±0.06	5.34±0.58	1.50±0.17	0.37±0.06
	10	50	202±21	1.85±0.07	0.74±0.06	0.86±0.09	5.75±0.72	1.58±0.19	0.43±0.23
	19	50	267±24	1.94±0.07	0.85±0.08	0.97±0.08	6.46±0.63	1.74±0.14	0.45±0.05
	32	49	303±31	1.98±0.08	0.93±0.10	1.02±0.07	7.06±0.95	1.88±0.20	0.47±0.07

Sex	Age (weeks)	Number of animals	Thymus (mg)	Pituitary gland (mg)	Adrenal glands (mg)	Testes (g)	Ventral prostate (mg)	Ovaries (mg)
Male	8	50	561.8±113.7	9.1±2.1	49.0±6.7	2.83±0.20	296.8±66.5	—
	10	50	519.9±110.2	9.9±1.5	51.0±8.8	3.26±0.24	410.7±84.1	—
	19	50	294.1±77.7	11.7±1.8	54.5±8.2	3.52±0.31	529.7±118.4	—
	32	50	161.0±52.9	12.3±3.6	52.3±9.6	3.56±0.47	536.7±153.1	—
Female	8	50	435.4±82.2	9.9±1.8	55.1±7.2	—	—	70.1±11.2
	10	50	418.4±98.5	10.8±1.8	58.8±7.1	—	—	75.2±16.7
	19	50	246.2±53.5	13.8±2.5	58.2±7.5	—	—	70.4±16.4
	32	49	143.2±35.6	14.6±4.1	60.4±8.6	—	—	65.8±12.6

Data are expressed as mean±S.D.

Table 11. Organ weights (body weight ratio) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Age (weeks)	Number of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys	Spleen
Male	8	50	274±18	0.72±0.05	0.37±0.02	0.38±0.02	3.05±0.19	0.83±0.05	0.20±0.03
	10	50	350±22	0.57±0.04	0.33±0.20	0.33±0.02	2.78±0.16	0.74±0.05	0.18±0.03
	19	50	501±44	0.43±0.03	0.28±0.02	0.26±0.02	2.46±0.22	0.62±0.05	0.15±0.02
	32	50	610±42	0.36±0.03	0.25±0.02	0.24±0.02	2.29±0.14	0.55±0.04	0.13±0.02
Female	8	50	171±14	1.06±0.09	0.39±0.02	0.48±0.03	3.11±0.17	0.88±0.07	0.21±0.03
	10	50	202±21	0.93±0.10	0.37±0.03	0.43±0.03	2.85±0.16	0.79±0.06	0.21±0.12
	19	50	267±24	0.73±0.07	0.32±0.02	0.37±0.03	2.42±0.15	0.65±0.05	0.17±0.02
	32	49	303±31	0.66±0.07	0.31±0.03	0.34±0.03	2.33±0.15	0.62±0.05	0.16±0.02

Sex	Age (weeks)	Number of animals	Thymus ($\times 10^{-3}$)	Pituitary gland ($\times 10^{-3}$)	Adrenal glands ($\times 10^{-3}$)	Testes	Ventral prostate ($\times 10^{-3}$)	Ovaries ($\times 10^{-3}$)
Male	8	50	205±40	3.3±0.7	17.9±2.4	1.04±0.09	109±24	—
	10	50	149±31	2.8±0.3	14.6±2.7	0.94±0.10	118±26	—
	19	50	59±15	2.3±0.3	10.9±1.5	0.71±0.07	106±23	—
	32	50	26±9	2.0±0.6	8.6±1.6	0.59±0.09	88±26	—
Female	8	50	254±41	5.8±0.9	32.3±4.1	—	—	41.0±6.1
	10	50	206±37	5.4±0.6	29.3±3.6	—	—	37.2±6.9
	19	50	92±19	5.2±0.9	21.9±3.0	—	—	26.5±6.0
	32	49	48±13	4.8±1.1	20.0±2.8	—	—	21.9±4.4

Data are expressed as mean±S.D.

Table 12. Gross pathology (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Male				Female			
Age (weeks)	8	10	19	32	8	10	19	32
Number of animals	50	50	50	50	50	50	50	50
Cecum								
Focus	0	0	0	1	0	0	0	0
Ileum								
Diverticulum	1	0	0	0	0	0	0	0
Focus, Multiple	0	0	0	0	0	0	0	1
Jejunum								
Focus, Multiple	0	0	0	0	0	0	0	1
Liver								
Enlarged	0	0	0	0	0	0	0	1
Focus, Median, lobe	2	0	0	2	0	1	0	2
Focus, Right, lobe	1	0	0	0	0	0	0	1
Heart								
Congenital defect, Ventricle	1	0	0	0	0	0	0	0
Adrenal gland								
Enlarged, Bilateral	0	0	0	1	0	0	0	0
Focus, Unilateral, White	1	0	0	0	0	0	0	0
Pituitary gland								
Enlarged	0	0	0	0	0	0	0	1
Focus, Red	0	0	0	1	0	0	0	0
Spleen								
Enlarged	0	0	0	0	0	1	0	1
Focus, White, Multiple	0	0	0	0	0	1	0	0
Focus, Yellow	0	1	0	0	0	1	0	0
Thymus								
Discoloration, Red	0	0	0	1	0	0	0	0
Small	0	0	0	2	0	0	0	2
Tissue NOS								
Hydrothorax	1	1	0	0	0	0	0	1
Uterus								
Distension, Bilateral, Horn	—	—	—	—	0	0	0	1
Lymph node								
Discoloration, Mandibular, Red	0	0	0	0	0	0	0	1
Discoloration, Mesenteric, Red	0	0	0	0	0	0	0	1
Enlarged, Mandibular	0	0	0	0	0	0	0	1
Lung								
Focus, Multiple	0	0	0	0	0	0	0	1
Eye								
Discoloration, Unilateral, White	0	0	0	0	0	0	0	1
Brain								
Focus, Cerebrum	0	0	0	0	0	0	1	0
Ovary								
Cyst, Unilateral	—	—	—	—	1	0	0	0
Skin								
Scar, Neck	1	0	0	0	0	0	0	0
Epididymis								
Small, Bilateral	0	0	0	1	—	—	—	—
Small, Unilateral	0	0	1	0	—	—	—	—
Prostate								
Discoloration, Yellow	0	0	1	0	—	—	—	—
Testis								
Small, Bilateral	0	0	1	1	—	—	—	—
Small, Unilateral	0	0	1	0	—	—	—	—
Nose								
Hemorrhage	0	0	1	0	0	0	1	0
Kidney								
Cyst, Unilateral	0	0	0	0	1	0	0	0
Dilatation, Pelvis, Bilateral	0	1	0	1	1	0	0	0
Dilatation, Pelvis, Unilateral	0	0	2	2	0	1	1	0
Focus, Unilateral	0	0	0	0	0	0	0	0
Urinary bladder								
Nodule, Yellow	0	0	1	0	0	0	0	0
Subcutaneous Tissue								
Focus, Neck	0	0	0	0	1	0	0	0

Table 13-1. Histopathology (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Male				Female			
Age (weeks)	8	10	19	32	8	10	19	32
Number of animals	50	50	50	50	50	50	50	50
Cecum								
Dilatation, Crypt	1	0	0	0	0	0	0	0
Hemorrhage, Mucosa	0	0	0	1	0	0	0	0
Ileum								
Calcification, Lymphoid tissue	0	0	1	0	0	0	3	0
Diverticulum	1	0	0	0	0	0	0	0
Colon								
Dilatation, Crypt	0	0	0	0	0	0	1	0
Malignant lymphoma	0	0	0	0	0	1	0	0
Duodenum								
Calcification, Mucosa	0	0	0	1	0	0	0	0
Infiltrative cell, Mucosa, Mononuclear cell	0	0	1	1	0	0	0	0
Jejunum								
Infiltrative cell, Mononuclear cell	0	0	0	1	0	0	0	0
Calcification, Lymphoid tissue	0	0	1	2	0	0	1	0
Malignant lymphoma	0	0	0	0	0	1	0	0
Esophagus								
Infiltrative cell, Mononuclear cell	1	0	0	0	0	0	0	0
Liver								
Atrophy, Hepatocyte, Diffuse	0	0	0	0	0	0	0	1
Basophilic focus	0	0	0	1	0	0	0	1
Calcification	0	0	0	1	0	0	0	0
Degeneration, Hepatocyte, Cystic	0	0	0	1	0	0	0	0
Dilatation, Sinusoid, Focal	0	0	0	0	0	0	0	1
Granuloma	2	1	3	2	0	1	5	2
Hemorrhage, Focal	0	0	0	1	0	0	2	2
Infiltrative cell, Mononuclear cell	42	41	46	48	30	39	46	46
Necrosis, Hepatocyte, Focal	1	3	0	3	0	0	1	4
Peliosis, Focal	0	0	0	0	0	0	0	1
Proliferation, Bile duct	1	0	1	5	0	0	0	1
Vacuolization intracyto, Hepatocyte. Diffuse	1	3	0	1	7	6	1	2
Vacuolization intracyto ,Hepatocyte, Focal	6	2	10	12	5	5	5	6
Vacuolization intracyto, Hepatocyte, Periportal	1	4	3	0	27	15	2	0
Malignant lymphoma	0	0	0	0	0	1	0	1
Pancreas								
Atrophy, Acinus, Focal	0	0	6	7	0	0	2	2
Basophilic focus, Acinus	0	0	1	0	0	0	0	0
Fibrosis	0	0	1	0	0	0	0	0
Granuloma	0	0	0	2	0	0	0	0
Hyperplasia, Islet cell, Diffuse	0	0	0	1	0	0	0	0
Hyperplasia, Islet cell, Focal	0	0	3	4	0	0	0	1
Infiltrative cell, Fatty	0	0	0	2	0	0	0	0
Infiltrative cell, Inflammatory cell	0	0	1	0	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	3	17	0	0	6	14
Inflammation, Focal	0	0	1	0	0	0	1	0
Necrosis, Acinus, Single cell	0	0	0	0	0	0	0	1
Pigmentaion	0	0	1	1	0	0	0	0
Stomach								
Dilatation, Glands	1	1	11	22	0	0	7	19
Hyperplasia, Basal cell, Diffuse	0	0	0	0	0	0	0	1
Hyperkeratosis, Forestomach	0	0	0	0	0	0	1	0
Malignant lymphoma	0	0	0	0	0	0	0	1
Sublingual gland								
Atrophy, Acinus, Focal	0	0	0	0	0	0	1	0
Submandibular gland								
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	1	0

Table 13-2. Histopathology (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Male				Female			
Age (weeks)	8	10	19	32	8	10	19	32
Number of animals	50	50	50	50	50	50	50	50
Tongue								
Calcification, Muscularis	1	0	0	0	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	0	1	0	0	0	1
Heart								
Calcification, Artery	0	0	0	1	0	0	0	0
Cardiomyopathy	0	2	19	29	0	0	7	9
Fibrosis, Valve	0	0	1	2	0	0	0	1
Infiltrative cell, Mononuclear cell	3	3	3	6	1	0	0	4
Vacuolization intracytoplasmic, Myocardium, Focal	0	0	0	1	0	0	0	0
Pigmentaion	0	0	0	1	0	0	0	0
Necrosis, Myocardium, Focal	0	0	1	0	0	0	0	0
Adrenal gland								
Accessory, Adrenal, Cortical, Nodule	0	0	0	0	0	0	1	0
Degeneration, Cortex, Eosinophilic, Focal	0	0	0	0	0	0	0	1
Hemorrhage, Cortex,Focal	0	0	0	0	0	0	0	1
Hyperplasia, Cortex, Focal	0	0	0	0	0	0	0	2
Hypertrophy, Cortex, Focal	0	1	0	0	0	0	0	0
Malignant lymphoma	0	0	0	0	0	0	0	1
Vacuolization intracytoplasmic, Cortex, Diffuse	0	0	2	4	0	0	0	0
Vacuolization intracyto, Cortex, Focal	0	0	0	1	0	0	0	1
Parathyroid								
Hyperplasia, Focal	0	0	0	0	0	0	0	1
Infiltrative cell, Mononuclear cell	0	0	0	1	0	0	1	0
Pituitary gland								
Adenoma, Pars distalis	0	0	0	1	0	0	0	0
Cyst, Pars distalis	0	0	1	0	0	0	0	0
Cyst, Pars intermedia	0	0	1	1	0	1	1	1
Infiltrative cell, Mononuclear cell	0	1	0	0	0	0	0	0
Thyroid gland								
Calcification, Follicle	1	0	0	0	0	0	0	0
Ectopic thymus	5	5	5	1	10	4	4	1
Hyperplasia, C-cell, Diffuse	0	0	0	1	0	0	0	7
Hyperplasia, C-cell, Focal	0	0	0	2	0	0	1	0
Hypertrophy, Follicular cell, Focal	0	0	0	1	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	1	1
Ultimobranchial body	7	6	17	13	13	5	18	11
Ovary								
Cyst	—	—	—	—	1	0	1	1
Malignant lymphoma	—	—	—	—	0	0	0	1
Uterus								
Dilatation, Lumen, (Horn)	—	—	—	—	0	1	3	5
Malignant lymphoma	—	—	—	—	0	0	0	1
Vagina								
Cornification	—	—	—	—	0	0	4	6
Mucification	—	—	—	—	0	0	0	4
Mesenteric lymph node								
Hemorrhage, Focal	0	0	0	0	0	0	1	0
Malignant lymphoma	0	0	0	0	0	0	0	1
Epididymis								
Atrophy, Diffuse	0	0	1	0	—	—	—	—
Cell debris	0	3	1	0	—	—	—	—
Depletion, Sperm	1	0	0	0	—	—	—	—
Infiltrative cell, Mononuclear cell	3	4	19	34	—	—	—	—
Inflammation	1	0	0	0	—	—	—	—
Vacuolization intracyto., Epithelium	0	0	0	1	—	—	—	—

Table 13-3. Histopathology (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Male				Female			
Age (weeks)	8	10	19	32	8	10	19	32
Number of animals	50	50	50	50	50	50	50	50
Prostate								
Hyperplasia, Focal	0	0	0	1	—	—	—	—
Infiltrative cell, Mononuclear cell	1	11	24	17	—	—	—	—
Inflammation	4	6	8	8	—	—	—	—
Testis								
Atrophy, Seminiferous tubule, Diffuse	0	0	1	1	—	—	—	—
Atrophy, Seminiferous tubule, Focal	1	0	3	1	—	—	—	—
Degeneration, Seminiferous epithelium	0	0	0	6	—	—	—	—
Bone marrow								
Granuloma	0	0	0	1	0	0	0	1
Malignant lymphoma	0	0	0	0	0	1	0	1
Spleen								
Hematopoietic cell proliferation	34	18	36	47	4	4	24	36
Pigmentation	0	0	36	49	0	0	46	48
Thick, Capsule	0	0	0	0	0	1	0	0
Malignant lymphoma	0	0	0	0	0	1	0	1
Thymus								
Atrophy	0	0	0	1	0	0	0	0
Hemorrhage	0	0	0	1	0	0	0	0
Infiltrative cell, Mononuclear cell	1	0	0	0	0	0	0	0
Submandibular lymph node								
Hyperplasia, Lymph follicle	0	0	0	0	0	0	1	0
Hyperplasia, Plasma cell	0	0	2	3	0	0	1	1
Hypertrophy, Lymph follicle	0	0	0	1	0	0	0	0
Malignant lymphoma	0	0	0	0	0	0	0	1
Skeletal muscle								
Infiltrative cell, Mononuclear cell	0	0	1	2	0	0	0	0
Femur								
Osteosclerosis	0	0	0	0	0	1	0	0
Mammary gland								
Dilatation, Duct, Focal	0	0	0	0	0	0	0	1
Fibroadenoma, Inguinal	0	0	0	0	0	0	0	1
Skin								
Ulcer, Neck	0	0	0	0	1	0	0	0
Brain								
Calcification, Cerebrum	0	0	0	0	0	0	1	0
Dilatation, Vein, Meninges	0	0	0	0	0	0	1	0
Gliosis, Cerebrum	1	0	0	0	0	0	0	0
Malignant Reticulosis, Cerebrum	0	0	0	1	0	0	0	0
Spinal cord								
Calcification, White matter	0	0	0	0	1	0	0	0
Lung								
Calcification, Artery	2	11	17	20	3	8	15	8
Granuloma	0	0	1	1	1	0	0	0
Hemorrhage	1	1	1	0	0	1	1	0
Hypertrophy, Artery, Media	0	1	0	0	0	1	0	0
Infiltrative cell, Foam cell	2	4	8	6	0	0	4	4
Infiltrative cell, Mononuclear cell	1	0	4	3	0	0	3	2
Inflammation, Focal	0	0	0	1	0	0	0	0
Metaplasia, Osseous	0	7	3	5	0	0	4	2
Malignant lymphoma	0	0	0	0	0	1	0	1
Trachea								
Dilatation, Gland	0	2	0	0	0	1	0	0
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	2	4
Infiltrative cell, (Submucosa), Mononuclear cell	0	0	3	3	0	0	0	0

Table 13-4. Histopathology (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein diet

Sex	Male				Female			
Age (weeks)	8	10	19	32	8	10	19	32
Number of animals	50	50	50	50	50	50	50	50
Nose								
Inflammation, Nasopharyngeal duct	0	0	0	0	0	0	2	0
Eye								
Atrophy, Retina, Diffuse	0	0	0	0	0	1	0	0
Atrophy, Retina, Focal	0	0	1	0	0	0	0	1
Calcification, Cornea	0	0	0	1	0	0	0	0
Retina rosette	0	0	1	0	1	0	1	1
Harderian gland								
Atrophy, Acinus	0	0	0	0	0	1	0	0
Degeneration, Acinus, Vacuolated	0	1	0	0	0	0	0	0
Infiltrative cell, Mononuclear cell	1	1	8	11	0	2	13	13
Malignant lymphoma	0	0	0	0	0	0	0	1
Kidney								
Basophilic, Renal tubule	34	28	27	20	26	16	5	6
Calcification, Cortex	0	0	3	1	0	1	1	0
Calcification, Medulla	2	0	0	0	1	2	0	0
Calcification, Outer medulla	0	0	0	2	1	4	3	4
Calcification, Papilla	2	2	6	3	1	1	12	6
Calcification, Pelvis	1	0	3	2	0	1	0	14
Cast, Renal tubule	2	1	8	7	0	0	3	3
Cyst	4	1	0	0	1	1	0	0
Dilataion, Renal tubule	0	0	1	0	0	0	0	0
Dilataion, Pelvis	1	0	0	2	0	0	0	0
Eosino body, Renal tubule	0	1	2	0	0	0	0	0
Hyaline droplet, Renal tubule	8	8	2	2	0	0	0	0
Hydronephrosis	0	0	1	0	1	0	1	0
Hyperplasia, Transitional epithelium, Focal	0	0	2	4	0	1	0	1
Infiltrative cell, Mononuclear cell	11	13	20	31	8	3	19	16
Malignant lymphoma	0	0	0	0	0	0	0	1
Metaplasia, Papilla, Osseous	0	1	0	0	0	0	0	0
Pigmentation, Renal tubule	0	0	0	1	0	0	0	0
Urinary bladder								
Calculus	3	4	1	0	0	0	0	0
Infiltrative cell, Mononuclear cell	0	3	3	4	0	0	0	0

Crj:CD(SD)IGS Rats and the Effects of a Commercial Low Protein Diet on the Biological Parameters Used in Repeated-Dose Toxicity Studies at the Hikari Branch, Drug Safety Research Laboratories in Takeda Chemical Industries, Ltd.

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ABSTRACT. Crj:CD(SD)IGS rats were fed either a commercial low protein (CR-LPF, 18%) or normal protein (CRF-1, 24%) diet for 2, 4, 13 or 26 weeks starting at 6 weeks of age (6w), and the effects of the 2 different dietary protein contents on the biological parameters used in repeated-dose toxicity studies were examined. No significant biological differences were observed between the CR-LPF (LPF) group and the CRF-1 (F-1) group in clinical signs, body weight, water intake, urine volume, ophthalmoscopy, hematology, hepatic drug-metabolizing enzyme activities or gross pathology. Food consumption was higher in the LPF group during the experimental period. An increase in urinary ketone bodies and a decrease in urinary calcium were observed in the LPF group. Additionally, a urinary protein level increased with age in males in the F-1 group. In blood chemistry, increases in total and HDL cholesterols, total protein, albumin, phospholipids and thyroxine, and a decrease in triiodothyronine were observed in the LPF group compared with the F-1 group. Upon histopathological examination, cardiomyopathy was observed more frequently in males in the F-1 group than in the LPF group, and calcification in the pelvis of the kidney was observed more frequently in females in the LPF group than the F-1 group. In conclusion, there were no great differences in the biological parameters used in repeated-dose toxicity studies between Crj:CD(SD)IGS rats fed commercial low and normal protein diets for up to 26 weeks. — Key words: Biological parameter, CD(SD)IGS rat, Low protein diet

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INTRODUCTION

We have used a 23-24% protein diet for repeated-dose toxicity and carcinogenicity studies in rats. However, it has been suggested that lowering dietary protein levels reduces the incidence of spontaneous lesions and prolongs their life span [5,9]. In a previous study [6], an 18% protein diet was fed to Crj:CD(SD)IGS rats at 6-19 weeks of age and the biological parameters commonly used in repeated-dose toxicity studies were compared with those in rats fed with the conventional diet. In the present study, the 18% protein diet was fed to rats at up 32 weeks of age and effects of the diet on the parameters were further investigated. Crj:CD(SD)IGS rats are produced by Charles River Inc. under a new breeding system to help endure uniform experimental animals. This study was also designed to collect background data on Crj:CD(SD)IGS rats at 6-32 weeks of age for repeated-dose toxicity studies.

MATERIALS AND METHODS

1. Animals

One hundred male and 100 female Crj:CD(SD)IGS rats (SPF animals) aged 4 weeks were obtained from Charles River Japan Inc. on November 14, 1995 and acclimatized to the environmental conditions of our laboratories for 1 week. One hundred and sixty animals, 80 per sex, in good conditions were selected for this study on the basis of clinical signs and body weight. They were allocated randomly to 2 groups each comprised of 40 males and 40 females on the basis of body weight stratification, and then acclimatization continued for 1 week. At the start of the experiment, the animals were 6 weeks old and ranged in body weight from 200 to 232 g (males) and from 144 to 177 g (females).

2. Animal husbandry

Animals were individually housed in metal, mesh-bottom cages. Each cage in each group was allocated randomly to a position on the shelves in a clean booth. The booth was placed in an animal room with a room temperature of 20-26℃, a relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (lights on from 07:00 to 19:00). Before grouping, all the animals were allowed free access to tap water and the standard powdered laboratory animal diet (CRF-1, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source). After grouping, one group continued to receive the standard diet, and the other group was switched to a powdered laboratory animal diet having a lower protein content (CR-LPF, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source).

3. Grouping and diet components

The experimental groups were as follows.

Group	Diet	Protein content (%)	No. of animals							
			Male				Female			
			2 weeks	4 weeks	13 weeks	26 weeks	2 weeks	4 weeks	13 weeks	26 weeks
1	CRF-1	24	10	10	10	10	10	10	10	10
2	CR-LPF	18	10	10	10	10	10	10	10	10

The 40 males and 40 females in each group were randomly divided into 4 groups of 10 for the 2-, 4-, 13- and 26-week study. Eight, 10, 19 and 32 weeks of age are abbreviated as 8w, 10w, 19w and 32w, respectively.

The diet components were as follows.

Group	Normal protein diet	Low protein diet
Type	CRF-1	CR-LPF
Gross energy (kcal/kg)	3600	3490
Moisture (%)	7.7	7.5
Crude protein (%)	23.1	18.4
Crude fat (%)	5.9	4.8
Crude fiber (%)	3.3	5.0
Crude ash (%)	6.5	6.3
Nitrogen-free extract (%)	53.5	58.0

The CRF-1 and CR-LPF groups are abbreviated as the F-1 and LPF group, respectively.

4. Examinations and methods

1) Clinical signs

All animals were observed for mortality, morbidity and clinical signs once a day during the experimental period. In addition, detailed examinations for clinical signs were conducted for all animals once a week during the experimental period.

2) Body weight

Each animal was weighed using an electronic balance (PM4600, Mettler GmbH) twice a week from 6w to 10w and once a week thereafter.

3) Food consumption

At each feeding (once a week), the weight of animal diet given and that of the diet remaining were measured for each animal in each group with an electronic balance (PM4600, Mettler GmbH), and food consumption values for the week were calculated.

4) Water intake and urine output

Water intake and urine output for 10 animals of each sex in each group were measured at 7w, 9w, 18w and 31w. Water intake was calculated as the difference between the weight of water given and that of the water remaining 24 hr later. Urine output was taken as the weight of urine collected over 24 hr. The water and urine were weighed using an electronic balance (PM4600, Mettler GmbH).

5) Ophthalmoscopy

Ophthalmoscopic examination was performed for all animals in each group once before the experiment commenced (5w) and at 9w, 18w and 31w. The cornea, anterior chamber, iris, lens and fundus in both eyes were examined with an ophthalmoscope (BETA200, Heine) and a fundus camera (Kowa RC-2, model-621, Kowa Co., Ltd.). Ophthalmoscopic examination of the fundus was performed 5-10 minutes after the instillation of a mydriatic (Mydrin-P®, Santen Pharmaceutical Co., Ltd.).

6) Urinalysis,

Urinalysis was performed for 10 animals of each sex in each group at 7w, 9w, 18w and 31w. Animals were denied access to food and water, and a 4-hr morning urine sample was collected and centrifuged at 400 × g for 5 min. The supernatant was examined using a reagent strip (Multistix® SG-L, Bayer-Sankyo Co., Ltd.) and an automated urine analyzer (Clinitek 200, Bayer-Sankyo Co., Ltd.), and the following were determined: pH, protein, glucose, occult blood, ketone bodies and urobilinogen. The urinary sediment was fixed in 20% buffered neutral formalin and stained with a urinary sediment stain (URI-CEL®, Cambridge Chemical Products, Inc.), and casts, epithelial cells, leukocytes and erythrocytes were counted under a microscope.

7) Urine chemistry

Urine chemical analysis was conducted for 10 animals of each sex in each group at 7w, 9w, 18w and 31w. Animals were denied access to food and water, and a 4-hr morning urine sample was collected and centrifuged at 400 × g for 5 min. The values of the following in the supernatant were determined with automated blood chemistry analyzers (Hitachi 7150, Hitachi, Ltd. and System E3A, Beckman Instruments, Inc.) and standard reagents (Wako Pure Chemical Industries, Ltd. or Shionogi Co., Ltd.). Osmotic pressure was determined with an osmometer (OSM-1, Shimadzu).

- creatinine (method of Jaffé)
- total protein (pyrogallol red method)
- *N*-acetyl- β -D-glucosaminase (CPR-NAG method)
- calcium (OCPC method)
- inorganic phosphorus (molybdic acid direct method)
- sodium (ion-selective electrode method)
- potassium (ion-selective electrode method)
- chloride (ion-selective electrode method)
- osmotic pressure (freezing point depression method)

8) Hematology and blood chemistry

Hematological and blood chemical analyses were conducted for each of the 10 males and 10 females in each subgroup. Before necropsy, the animals were fasted for about 20 hr, and blood was withdrawn from the abdominal aorta with a vacuum blood collecting tube containing EDTA2K (hematology) or heparin sodium (blood chemistry) under ether anesthesia. The heparinized blood was centrifuged at 7500 × g for 10 min to obtain plasma for blood chemistry.

a) Hematology

The values of the following were determined or calculated with an automated hematology analyzer (E-5000, Sysmex Corp.) and an automated reticulocyte analyzer (R-2000, Sysmex Corp.). The differential leukocyte count was determined by microscopy after May-Giemsa staining.

- erythrocyte count (electric resistance detection method)
- leukocyte count (electric resistance detection method)
- platelet count (electric resistance detection method)
- hematocrit value (cumulative pulse height detection method)
- hemoglobin concentration (SLS-hemoglobin method)
- mean corpuscular hemoglobin (MCH; calculated)
- mean corpuscular hemoglobin concentration (MCHC; calculated)
- mean corpuscular volume (MCV; calculated)
- reticulocyte count (flow cytometry using the argon laser method)

- differential leukocyte count

b) Blood chemistry

The values of the following were determined with automated blood chemistry analyzers (Hitachi 7150, Hitachi, Ltd. and System E3A, Beckman Instruments, Inc.) and standard reagents (Wako Pure Chemical Industries, Ltd. and Sigma Co.), except for the A/G ratio which was calculated from the total protein and albumin values. In 8w, albumin and A/G ratio were not determined because of shortage of sample. Triiodothyronine and thyroxine were determined with a commercial kit (Boehringer-Mannheim GmbH).

- total protein (biuret method)
- albumin (BCG method)
- A/G ratio
- glucose (glucokinase-G-6-PDH method)
- total cholesterol (COD-DAOS method)
- HDL-cholesterol (phosphotungstate-magnesium precipitation method)
- triglyceride (GPO-DAOS method)
- phospholipids (oxidase-DAOS method)
- urea nitrogen (urease-GlDH method)
- creatinine (method of Jaffé)
- total bilirubin (alkaline azobilirubin method)
- direct bilirubin (alkaline azobilirubin method)
- aspartate aminotransferase (AST: modified JSCC method)
- alanine aminotransferase (ALT: modified JSCC method)
- alkaline phosphatase (ALP: *p*-nitrophenylphosphate substrate method)
- lactate dehydrogenase (LDH: Wróblewski-LaDue method)
- creatine kinase (CK: GSCC method)
- sodium (ion-selective electrode method)
- potassium (ion-selective electrode method)
- chloride (ion-selective electrode method)
- calcium (OCPC method)
- inorganic phosphorus (molybdic acid direct method)
- triiodothyronine (T3: EIA)
- thyroxine (T4: EIA)

9) Activity of hepatic drug-metabolizing enzymes

At necropsy, small pieces (ca. 1 g) of the liver were removed from all animals in each group and were frozen. After thawing, the pieces were homogenized individually in 4 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 9000 $\times$ g for 20 min, and the supernatant was assayed for aminopyrine-*N*-demethylase (Cochin & Axelrod method) and aniline hydroxylase (modified method of Wills) activities.

10) Necropsy and organ weights

Before necropsy, all animals in each group were fasted for about 20 hr and were weighed before they were exsanguinated under ether anesthesia. For each animal, the visceral organs were examined visually, and the following organs were weighed with electronic balances (AE260 and PM2000, Mettler GmbH): brain, heart, liver, kidneys, spleen, lungs, thymus, pituitary, adrenals, testes, ovaries and ventral prostate. Relative organ weights were calculated as a percentage of body weight.

11) Histopathology

The following organs/tissues from all animals in each group were fixed in 10% neutral buffered formalin; adrenals, aorta, bone marrow, brain, cecum, cervical spinal cord, colon, duodenum, epididymides, esophagus, femurs, Harder's glands, heart, ileum, jejunum, kidneys, liver, lumbar spinal cord, lung, mammary glands, mesenteric lymph nodes, ovaries, pancreas, parathyroids, pituitary, sciatic nerves, seminal vesicles, skeletal muscles, skin, spleen, sternum, stomach, sublingual glands, submandibular glands, submandibular lymph nodes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina and ventral prostate. The eyes were pre-fixed in Davidson's solution containing 3% glutaraldehyde and post-fixed in 10% neutral buffered formalin. The testes were fixed in Bouin's solution. These organs were embedded in paraffin, sectioned at 4- μm thick, stained with hematoxylin-eosin and examined by light microscopy.

5. Statistical analysis

The data on body weight, food consumption, water intake, urine output, hematology, blood chemistry, activity of hepatic drug-metabolizing enzymes and organ weights were analyzed statistically as follows. Statistical analyses were performed using the F test for homogeneity of variance followed by Student's t test or the Aspin & Welch t test [1]. All statistical tests were conducted at the 5% and 1% two-tailed probability levels.

RESULTS

1. Mortality

No animals died in either group during the experimental period.

2. Clinical signs (Table 1)

Hair loss was observed in males and females in each group. Malinterdigitation was observed in 2 males, missing incisor was observed in 1 male and decreased feces was observed in 1 female in the F-1 group; however, no significant differences were observed between the 2 groups because of the low incidence of each finding.

3. Body weight (Table 2)

Body weight was increased with age in both sexes in both groups. There were no significant differences between the 2 groups.

4. Food consumption (Table 3)

Food consumption in the LPF group was increased or tended to be increased when compared with the value in the F-1 group throughout the experimental period.

5. Water intake and urine output (Table 4)

There were no significant differences between the 2 groups during the experimental period.

6. Ophthalmoscopy

There were no abnormalities in any group. A slight opacity of the lens was observed in one male before the experiment commenced (5w); however, it recovered by 9w.

7. Urinalysis (Table 5)

In males in the LPF group, an increased level of ketone bodies at 7w was observed when compared with the corresponding levels in the F-1 group. The level of protein increased with age in males in the F-1 group.

8. Urine chemistry (Table 6)

In the LPF group, an decrease in calcium and an increase in inorganic phosphorus were observed compared with the F-1 group

at each examined week. Increases in sodium, potassium and chloride were observed in males at 7w and in females at 7w and 9w in the LPF group compared with the F-1 group. At 7w in males, an increase in creatinine was observed in the LPF group compared with the F-1 group. The urinary protein increased with age in males in the F-1 group.

9. Hematology (Table 7)

There were no significant differences between the 2 groups. The erythrocyte count increased with age in males in both groups. The MCH and MCV decreased with age and neutrophil and eosinophil counts increased with age in both sexes in both groups.

10. Blood chemistry (Table 8)

In the LPF group, increases in total cholesterol and HDL cholesterol were observed in males at 10w compared with F-1 group. At 32w, an increase in total cholesterol in both sexes, increases in total protein, albumin and phospholipids in females and an increase in thyroxine (T4) and a decrease in triiodothyronine (T3) in males were observed in the LPF group compared with the F-1 group. ALP decreased with age in both sexes and both groups.

11. Activity of hepatic drug-metabolizing enzymes (Table 9)

There were no significant differences between the 2 groups.

12. Organ weights (Tables 10, 11)

In males, there were no significant differences between the 2 groups. In females at 32w, the ovary weight was slightly lower in the LPF group than in the F-1 group. The thymus weight decreased with age in both sexes in both groups.

13. Gross pathology (Table 12)

Dilatation of the pelvis in the kidney was observed in 1 female in LPF group at 8w and 2 males and 1 female each in both groups at 19w. A focus in the kidney was observed in 1 male in the F-1 group at 10w. Foci in the liver were observed in 1 male and 1 female in the F-1 group at 19w, and in 2 females in the F-1 group and 1 female in the LPF group at 32w. Small thymus was observed in 2 males and 2 females in the F-1 group and 1 male and 1 female in the LPF group at 32w. However, no significant differences were observed between the 2 groups because of the low incidence of these findings.

14. Histopathology (Table 13)

Heart: The incidence of cardiomyopathy in males was higher in the F-1 group (9 males) than in the LPF group (4 males) at 32w.

Kidney: Basophilic tubules and mononuclear cell infiltration were occasionally observed in both sexes in both groups. Calcification in the pelvis in females was observed more frequently in the LPF group (7 females) than in the F-1 group (2 females).

Harderian gland: Mononuclear cell infiltration were observed 5 females in the F-1 group and 6 males and 2 females in the LPF group at 32w.

Liver: Vacuolization of the centrilobular hepatocytes and mononuclear cell infiltration were observed frequently in both sexes in both groups. However, no significant differences were observed between the 2 groups.

Others: The other changes shown in Table 13 were observed occasionally, however no significant differences were observed between the 2 groups.

DISCUSSION

Crj:CD(SD)IGS rats were fed either a low protein or normal protein diet, and the effects of the different protein contents on the biological parameters used in repeated dose toxicity studies were examined.

No significant differences were observed between the low and normal protein diet groups in clinical signs, body weight, water intake, urine volume, ophthalmoscopy, hematology, hepatic drug-metabolizing enzyme activities or gross pathology.

Food consumption in the LPF group was higher than in the F-1 group during the experimental period, although no significant differences were observed in body weight. It has been reported that food consumption levels for the low protein diet were higher than for the normal protein diet, and this may be due to higher fiber content of the low protein diet [2, 8].

In urinalysis, urinary ketone bodies in females at 7w was higher in the LPF group than in the F-1 group. A similar change was observed in our Osaka laboratory in the same lot of Crj:CD(SD)IGS rats [6]; however, the reason for the difference between the 2 groups is obscure. Urinary calcium was decreased in the LPF group when compared with the values in the F-1 group. It has been reported that dietary protein restriction results in a reduction in urinary calcium excretion. The cause for hypocalciuria is unclear, but the change could represent an adaptation to low dietary protein availability and the protection against bone mass loss [7]. In the LPF group, an increase in inorganic phosphorus at each week examined, increases in sodium, potassium and chloride in males and/or in females at 7w and 9w and an increase in creatinine in males at 7w were observed compared with the F-1 group. The reason for the difference between the 2 groups is obscure; however, the difference is not considered to be significant because no significant differences in these parameters between the 2 groups were observed in Osaka laboratory [6], except potassium levels.

In blood chemistry, increases in total cholesterol and HDL cholesterol in males were observed at 10w in the LPF group compared with F-1 group. At 32w, increases in total cholesterol (both sexes), in total protein, albumin and phospholipids (females) and in thyroxine (males) and a decrease in triiodothyronine (males) were observed in the LPF-group compared with the F-1 group. It has been reported that an increase in cholesterol in males and decreases in total protein and albumin in females were observed when rats were fed a low protein diet [8]. The reasons for these changes are obscure.

The ovary weight in the LPF group at 32w was lower than that in the F-1 group. The difference between the 2 groups was small, and no abnormalities were observed in histopathological examination of the ovary in the LPF group. Therefor, this change may be incidental.

It has been reported that a low protein diet decreases the severity of cardiomyopathy, prevents nephrocalcinosis and decreases the incidence and severity of nephropathy in F344 rats [8]. In other reports, however, dietary restriction lowered the progression of cardiomyopathy and nephropathy in female SD rats, but protein restriction did not [2, 3, 4]. In this study, cardiomyopathy in males at 32w was observed more frequently in the F-1 group than the LPF group. Therefore, the low protein diet was consid-

ered to have a tendency to prevent the incidence of cardiomyopathy. Urinary protein levels in males increased with age in the F-1 group, but not in the LPF group. However, nephropathy was not observed in this study, and calcification in the pelvis in the kidney was observed more frequently in females in the LPF group than in the F-1 group. Further studies are needed to evaluate the effects of the low protein diet on the severity and incidence of nephrocalcinosis and nephropathy.

In conclusion, there were no great differences in the biological parameters used in general toxicity studies between Crj:CD(SD)IGS rats fed a commercial low diet and those fed a normal protein diet for 26 weeks.

REFERENCES

1. Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Statist. Assoc., 50: 1096-1121.
2. Keenan, K. P. et al. 1994. The effects of overfeeding and dietary restriction on Sprague-Dawley rat survival and early pathology biomarkers of aging. Toxicol. Pathol., 22: 300-315.
3. Keenan, K. P. et al. 1995. Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: Ⅰ. Effects on spontaneous neoplasms. Toxicol. Pathol., 23: 269-286.
4. Keenan, K. P. et al. 1995. Diet overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: Ⅱ. Effects on age-related proliferative and degenerative lesions. Toxicol. Pathol., 23: 287-302.
5. Masoro, E. J. et al. 1989. Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. Am. J. Clin. Nutr., 49: 1217-1227.
6. Naeshiro, I. et al. 1998. Crj:CD(SD)IGS rats and effects of a commercial low protein diet on the biological parameters used in repeated-dose toxicity studies. Biological Reference Data on CD(SD)IGS Rats - 1998, 108-120.
7. Orwoll, E. et al. 1992. Effects of dietary protein deficiency on mineral metabolism and bone mineral density. Am. J. Clin. Nutr., 56: 314-319.
8. Rao, G. N. et al. 1996. New diet (NTP-2000) for rats in the national toxicology program toxicity and carcinogenicity studies. Fundam. Appl. Toxicol., 32: 102-108.
9. Tucker, S. M. et al. 1976. Influence of diet and feed restriction on kidney function of aging male rats. J. Gerontol., 31: 264-270.

Table 1. Clinical signs (incidence of individual signs) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age (weeks)	Number of animals	Hair loss				Malinterdigitation				Feces, decreased				Incisor, missing			
		Male		Female		Male		Female		Male		Female		Male		Female	
		CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF
6	40	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
7	40	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
8	30	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
9	30	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0
10	20	2	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
11	20	2	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
12	20	2	1	1	1	2	0	0	0	0	0	0	0	1	0	0	0
13	20	2	1	1	1	2	0	0	0	0	0	0	0	1	0	0	0
14	20	3	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0
15	20	3	1	2	2	2	0	0	0	0	0	0	0	0	0	0	0
16	20	4	1	2	2	2	0	0	0	0	0	0	0	0	0	0	0
17	20	4	1	2	2	2	0	0	0	0	0	0	0	0	0	0	0
18	20	5	1	1	2	2	0	0	0	0	0	0	0	0	0	0	0
19	10	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
20	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
21	10	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
22	10	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
23	10	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
24	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
25	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
26	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
27	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
28	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
29	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
30	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
31	10	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Table 2. Body weight in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age (weeks)	Number of animals	Male		Female	
		CRF-1	CR-LPF	CRF-1	CR-LPF
6	40	216±10	215±9	157±7	159±7
7	40	276±14	272±12	179±11	180±11
8	40	326±18	322±17	200±13	200±14
9	30	365±19	358±22	217±17	217±17
10	30	395±23	389±28	229±21	227±19
11	20	426±23	417±30	244±26	242±19
12	20	452±26	446±34	257±26	254±21
13	20	475±25	469±37	269±25	265±21
14	20	495±28	488±40	274±26	270±22
15	20	513±28	505±43	278±26	279±22
16	20	527±29	520±47	286±27	284±23
17	20	538±32	528±48	288±28	287±25
18	20	546±33	537±50	284±28	283±25
19	20	550±35	544±51	288±28	284±26
20	10	552±39	556±34	301±34	298±29
21	10	562±39	565±35	304±33	304±31
22	10	566±42	570±34	306±37	307±33
23	10	572±44	578±36	311±35	314±34
24	10	579±44	584±37	317±35	320±37
25	10	588±47	594±36	319±37	324±37
26	10	598±50	599±39	323±39	326±37
27	10	603±52	608±40	326±38	331±39
28	10	606±48	611±39	332±36	336±38
29	10	613±46	620±40	336±39	340±40
30	10	621±48	632±42	338±41	338±40
31	10	627±50	640±40	343±41	344±42
32	10	630±53	645±42	343±41	345±42

Data are expressed as mean±S.D.(g).
Not significantly different between CRF-1 and CR-LPF group

Table 3. Food consumption in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age (weeks)	Number of animals	Male		Female	
		CRF-1	CR-LPF	CRF-1	CR-LPF
6	40	163±11	173±10**	109±8	120±8**
7	40	170±13	181±14**	112±9	122±12**
8	30	173±11	182±13**	114±9	125±12**
9	30	168±12	181±16**	111±10	121±13**
10	20	173±12	182±13*	117±13	126±10*
11	20	174±13	184±13*	116±10	126±12**
12	20	174±13	188±15**	114±9	128±11**
13	20	171±11	183±16**	108±8	123±11**
14	20	171±11	183±17*	109±8	127±14**
15	20	169±11	181±18*	107±8	122±10**
16	20	167±14	174±17	103±8	117±13**
17	20	161±12	171±20	92±8	103±12**
18	20	156±11	167±17*	93±8	105±12**
19	10	165±12	178±11*	107±6	126±15**
20	10	173±10	185±8**	113±10	133±15**
21	10	170±15	184±12*	120±10	137±19*
22	10	169±11	182±12*	119±7	140±23*
23	10	170±13	183±12*	120±6	137±20*
24	10	173±17	187±10*	118±7	136±19*
25	10	171±17	185±13*	116±6	135±20*
26	10	170±12	188±15**	117±6	137±19**
27	10	171±11	186±13*	119±6	136±19*
28	10	169±13	192±17**	119±7	135±18*
29	10	169±14	193±14**	114±6	131±15**
30	10	168±16	186±11*	115±8	134±18**
31	10	156±19	177±10**	108±41	125±16**

Data are expressed as mean±S.D.(g/animal/week)
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 4. Water intake and urine output in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Water intake (g/day)	Urine output (g/day)
Male	CRF-1	7	10	36.0±6.3	15.5±5.5
		9	10	40.2±8.6	17.5±7.3
		18	10	36.3±8.3	18.2±7.4
		31	10	32.6±9.3	19.0±8.5
	CR-LPF	7	10	38.3±6.6	14.5±4.6
		9	10	42.2±7.5	17.4±5.2
		18	10	36.8±7.2	19.2±6.6
		31	10	30.2±5.7	17.2±5.6
Female	CRF-1	7	10	28.3±5.6	12.5±4.2
		9	10	28.3±3.6	11.9±2.4
		18	9	32.7±3.8	16.7±3.4
		31	10	34.4±9.7	21.1±7.9
	CR-LPF	7	10	31.1±4.4	12.6±4.8
		9	10	31.3±6.0	11.4±4.5
		18	9	33.3±7.9	13.5±4.4
		31	10	37.1±11.9	21.7±9.9

Data are expressed as mean±S.D.
Not significantly different between CRF-1 and CR-LPF group

Table 5. Urinalysis in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	pH				Protein					Glucose		Ketones				Occult blod				
				6	7	8	9	−	±	+	++	+++	−	±	−	±	+	++	−	±	+	++	+++
Male	CRF-1	7	10	0	0	1	9	0	0	8	2	0	10	0	5	4	1	0	10	0	0	0	0
		9	10	0	0	1	9	0	0	6	4	0	9	1	1	2	7	0	10	0	0	0	0
		18	9	0	0	0	9	0	0	3	5	1	9	0	2	1	4	2	9	0	0	0	0
		31	10	0	0	1	9	0	0	5	3	2	10	0	1	4	5	0	10	0	0	0	0
	CR-LPF	7	10	0	1	1	8	0	0	7	3	0	10	0	1	5	4	0	9	1	0	0	0
		9	10	0	1	2	7	0	0	8	2	0	10	0	0	5	5	0	9	0	1	0	0
		18	10	0	0	0	10	0	0	5	5	0	10	0	0	3	7	0	10	0	0	0	0
		31	10	0	1	0	9	0	0	3	7	0	8	2	0	5	5	0	9	0	0	0	1
Female	CRF-1	7	10	0	4	0	6	1	5	4	0	0	10	0	6	4	0	0	9	1	0	0	0
		9	10	2	2	3	3	1	4	4	1	0	10	0	5	4	1	0	10	0	0	0	0
		18	10	0	4	2	4	5	4	1	0	0	10	0	8	2	0	0	10	0	0	0	0
		31	10	2	1	1	6	1	1	8	0	0	10	0	6	4	0	0	10	0	0	0	0
	CR-LPF	7	10	0	4	4	2	0	4	6	0	0	10	0	5	5	0	0	10	0	0	0	0
		9	10	2	3	4	1	0	3	5	2	0	10	0	3	5	2	0	10	0	0	0	0
		18	9	2	2	3	2	6	0	2	1	0	9	0	6	2	1	0	9	0	0	0	0
		31	10	2	3	1	4	1	2	6	1	0	10	0	6	4	0	0	10	0	0	0	0

Sex	Diet	Age (weeks)	Number of animals	Urobilinogen	Casts		Epithe. cells		Leukocytes		Erythrocytes			
				±	−	+	−	+	−	+	−	+	++	+++
Male	CRF-1	7	10	10	10	0	10	0	10	0	10	0	0	0
		9	10	10	10	0	10	0	10	0	10	0	0	0
		18	10	10	10	0	10	0	10	0	9	1	0	0
		31	10	10	10	0	10	0	9	1	10	0	0	0
	CR-LPF	7	10	10	9	1	10	0	10	0	10	0	0	0
		9	10	10	10	0	10	0	9	1	9	0	1	0
		18	10	10	10	0	10	0	10	0	10	0	0	0
		31	10	10	10	0	10	0	10	0	9	0	0	1
Female	CRF-1	7	10	10	10	0	10	0	6	4	10	0	0	0
		9	10	10	10	0	9	1	10	0	10	0	0	0
		18	10	10	10	0	10	0	10	0	10	0	0	0
		31	10	10	10	0	10	0	8	2	10	0	0	0
	CR-LPF	7	10	10	10	0	9	1	6	4	10	0	0	0
		9	10	10	10	0	10	0	8	2	10	0	0	0
		18	10	10	10	0	10	0	9	1	10	0	0	0
		31	10	10	10	0	10	0	7	3	10	0	0	0

Table 6. Urine chemistry in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Creatinine (mg/dL)	Total protein (mg/dL)	N-acethyl-β-D-glucosaminidase (U/L)	Sodium (mmol/L)	Potassium (mmol/L)
Male	CRF-1	7	10	48.4±15.6	57.5±32.3	14.6±3.9	107.6±45.1	145.7±59.0
		9	9	77.1±25.7	104.8±43.7	4.6±6.9	105.6±34.2	166.4±49.8
		18	9	146.1±55.1	147.7±141.9	16.8±8.5	84.4±39.9	194.7±89.5
		31	10	125.3±46.8	217.7±288.4	18.9±7.4	112.5±60.1	186.5±71.6
	CR-LPF	7	10	68.4±19.2*	78.6±49.2	17.2±5.7	144.6±30.5*	262.3±74.0**
		9	10	82.7±38.1	79.1±24.1	11.5±11.6	81.9±36.0	183.6±46.9
		18	10	125.2±46.5	64.2±32.2	15.9±5.4	88.7±40.3	206.2±62.9
		31	9	135.4±43.2	90.1±48.0	20.1±8.0	101.3±38.4	226.3±98.4
Female	CRF-1	7	10	62.8±20.5	3.5±5.7	15.8±4.6	68.6±18.9	136.2±44.2
		9	10	93.8±59.0	12.2±10.9	21.7±15.7	48.5±21.7	140.3±46.9
		18	8	80.3±29.3	4.3±5.9	13.3±6.8	58.6±26.5	110.7±38.3
		31	10	110.2±46.8	9.5±8.9	17.4±9.3	101.6±60.3	164.4±71.9
	CR-LPF	7	10	59.1±14.0	3.3±5.3	15.8±4.8	107.2±52.3	215.0±54.3**
		9	8	85.5±33.6	8.8±10.2	19.8±8.4	83.4±24.4**	199.5±45.2*
		18	7	71.6±49.4	4.3±7.4	11.6±8.0	52.4±30.0	105.5±78.6
		31	10	104.6±35.8	11.0±12.0	16.6±6.8	93.6±32.3	211.0±89.7

Sex	Diet	Age (weeks)	Number of animals	Chloride (mmol/L)	Osmolality (mOsmol/kg)	Glucose (mg/dL)	Calcium (mg/dL)	Inorganic phosphorus (mg/dL)
Male	CRF-1	7	10	143.4±52.2	1023±380	12±4	37.9±19.9	0.9±0.3
		9	9	135.3±46.1	1256±470	13±3	20.8±12.6	2.3±1.3
		18	9	93.0±61.3	1385±628	17±6	7.8±4.6	10.5±11.3
		31	10	131.5±51.7	1613±571	15±5	38.0±16.7	2.9±1.8
	CR-LPF	7	10	201.5±45.7*	1350±357	15±4	12.9±5.2**	12.3±19.2
		9	10	119.5±36.1	1180±246	13±4	8.3±3.9*	17.9±18.8*
		18	10	105.0±35.6	1488±445	16±5	4.1±2.1*	21.0±14.6
		31	9	149.8±68.6	1492±488	17±6	15.0±7.3**	11.5±9.6*
Female	CRF-1	7	10	106.9±36.8	1270±358	13±4	20.3±12.2	6.4±8.2
		9	10	87.1±52.1	1345±517	17±9	16.8±7.8	25.3±50.7
		18	8	77.9±39.5	1084±277	13±4	38.2±16.4	36.8±27.7
		31	10	129.1±73.4	1356±472	16±6	75.8±21.7	14.5±21.6
	CR-LPF	7	10	158.1±61.5*	1380±395	14±3	12.5±5.0	54.6±59.0*
		9	8	125.5±45.2	1481±399	17±6	10.7±5.0	73.2±67.3
		18	7	41.3±18.2*	948±527	13±7	11.1±4.4**	65.5±60.6
		31	10	120.1±63.7	1379±397	17±5	54.1±28.8	72.9±79.6*

Data are expressed as mean±S.D.(g/animal/week).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 7. Hematology in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Erythrocytes ($\times 10^4/\mu$L)	Hematocrit (%)	Hemoglobin (g%)	MCH (pg)	MCHC (%)	MCV (cμ)	Platelets ($\times 10^4/\mu$L)
Male	CRF-1	8	10	791±12	47.6±1.2	15.7±0.5	19.9±0.5	33.0±0.5	60±1	135.7±11.3
		10	10	877±41	49.5±1.8	16.6±0.7	19.0±0.7	33.6±0.5	57±1	120.2±14.4
		19	10	917±23	47.2±1.1	15.7±0.5	17.2±0.6	33.3±0.5	52±2	113.5±15.5
		32	10	923±22	47.3±1.3	15.7±0.7	17.1±0.4	33.3±0.7	51±1	118.8±11.0
	CR-LPF	8	10	808±33	48.6±1.9	16.0±0.7	19.8±0.7	32.9±0.6	60±2	137.5±17.8
		10	10	874±40	49.8±1.5	16.9±0.4	19.4±0.8	33.9±0.6	57±2	129.0±12.8
		19	10	890±47	47.0±2.4	15.5±0.9	17.4±0.5	33.0±0.5	53±2	113.4±13.9
		32	10	906±31	47.7±1.9	15.7±0.5	17.4±0.6	32.9±0.6	53±2*	117.2±11.5
Female	CRF-1	8	10	832±27	47.6±1.4	16.0±0.4	19.2±0.4	33.6±0.5	57±1	131.7±10.2
		10	10	876±37	47.8±1.7	16.6±0.5	19.0±0.5	34.8±0.4	55±1	128.3±9.9
		19	10	835±26	44.1±1.3	14.8±0.5	17.7±0.5	33.5±0.5	53±1	117.0±11.3
		32	10	835±43	44.9±1.9	15.0±0.6	18.0±0.4	33.3±0.3	54±1	106.7±13.8
	CR-LPF	8	10	828±27	47.2±2.2	16.1±0.7	19.5±0.6	34.2±0.3**	57±2	132.0±12.9
		10	10	846±26*	46.9±1.3	16.1±0.5*	19.1±0.7	34.4±0.5	55±2	125.5±12.6
		19	10	836±41	45.0±2.1	15.3±0.7	18.3±0.4*	34.0±0.5	54±2	120.5±14.4
		32	10	783±60*	43.2±3.0	14.4±1.1	18.3±0.6	33.2±0.6	55±1*	103.9±9.8

Sex	Diet	Age (weeks)	Number of animals	Leukocytes ($\times 10^2/\mu$L)	Reticulocytes (%)	Leukocytes, differential count (%)				
						Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Male	CRF-1	8	10	98±19	3.8±0.5	5.7±2.3	93.3±2.2	0.3±0.7	0.7±0.7	0.0±0.0
		10	10	128±24	2.3±0.4	5.8±1.5	93.3±1.3	0.1±0.3	0.8±0.9	0.0±0.0
		19	10	106±34	2.0±0.3	13.6±9.5	85.3±9.3	0.0±0.0	1.1±0.9	0.0±0.0
		32	10	89±33	2.0±0.5	20.1±7.9	77.8±7.6	0.5±0.5	1.6±1.3	0.0±0.0
	CR-LPF	8	10	127±23**	4.0±0.6	5.0±2.7	93.8±3.1	0.5±0.7	0.7±0.9	0.0±0.0
		10	10	143±34	2.5±0.5	8.3±4.7	90.3±4.6	0.1±0.3	1.3±1.2	0.0±0.0
		19	10	97±35	2.2±0.4	14.6±6.4	83.6±6.8	0.5±0.7	1.3±0.7	0.0±0.0
		32	10	103±20	2.2±0.2	21.1±8.5	77.6±8.4	0.2±0.6	1.1±1.3	0.0±0.0
Female	CRF-1	8	10	98±25	2.6±0.6	5.6±2.8	93.6±2.7	0.5±0.7	0.3±0.5	0.0±0.0
		10	10	94±17	1.9±0.3	4.4±2.2	94.6±2.5	0.4±0.5	0.6±0.7	0.0±0.0
		19	10	63±26	1.7±0.4	9.9±4.4	88.5±4.8	0.0±0.0	1.6±0.7	0.0±0.0
		32	10	58±11	1.8±0.3	19.4±7.5	77.8±6.7	0.3±0.7	2.5±1.8	0.0±0.0
	CR-LPF	8	10	87±14	2.4±0.5	5.2±2.8	93.5±2.9	0.3±0.7	1.0±0.9	0.0±0.0
		10	10	98±23	2.2±0.5	6.2±1.5*	92.4±1.9*	0.4±0.7	1.0±1.1	0.0±0.0
		19	10	59±12	1.8±0.3	11.0±4.8	87.3±4.2	0.3±0.7	1.4±1.5	0.0±0.0
		32	10	67±8	2.0±0.3	15.3±4.6	82.8±5.2	0.1±0.3	1.8±1.6	0.0±0.0

Data are expressed as mean±S.D.(g/animal/week).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 8-1. Blood chemistry in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Total protein (g/dL)	Albmin (g/dL)	A/G ratio	Haptoglobin (mg/dL)	Glucose (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)
Male	CRF-1	8	10	5.83±0.22	ND	ND	12.46±3.93	142±16	58±10	38±5
		10	9	6.13±0.20	3.38±0.09	1.24±0.09	14.89±1.89	145±8	53±10	37±7
		19	10	6.41±0.23	3.24±0.25	1.03±0.14	20.14±1.86	159±10	76±15	54±11
		32	10	6.42±0.25	3.24±0.20	1.03±0.12	11.42±4.69	156±7	76±15	50±11
	CR-LPF	8	10	5.85±0.24	ND	ND	14.58±4.23	156±17	65±16	42±11
		10	10	6.18±0.13	3.44±0.08	1.26±0.09	14.70±4.32	130±15*	72±15**	47±8**
		19	10	6.22±0.29	3.13±0.14	1.02±0.12	14.45±7.23*	155±10	80±14	49±7
		32	10	6.56±0.24	3.28±0.09	1.00±0.06	11.86±4.07	165±8*	89±8*	53±5
Female	CRF-1	8	10	6.07±0.24	ND	ND	13.60±3.94	120±12	62±12	42±7
		10	10	6.42±0.35	3.87±0.30	1.52±0.15	11.88±3.40	125±12	65±10	47±7
		19	10	7.03±0.26	4.07±0.20	1.38±0.10	13.21±4.71	141±10	86±16	60±9
		32	10	6.88±0.38	4.00±0.30	1.39±0.08	12.19±2.39	149±12	86±19	58±15
	CR-LPF	8	10	6.03±0.20	ND	ND	13.66±4.47	118±17	72±15	46±7
		10	10	6.14±0.39	3.66±0.26	1.48±0.07	12.47±3.69	127±13	72±12	46±8
		19	10	7.24±0.28	4.25±0.24	1.43±0.14	11.07±3.73	134±15	98±17	65±10
		32	10	7.29±0.35*	4.29±0.30*	1.44±0.11	11.26±4.01	148±10	115±19**	68±11

Sex	Diet	Age (weeks)	Number of animals	HDL cholesterol (%)	Triglyceride (mg/dL)	Phospholipids (mg/dL)	Urea nitrogen (mg/dL)	Creatinine (mg/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Male	CRF-1	8	10	65.8±3.0	48±15	92±12	13.1±1.2	0.4±0.0	0.05±0.01	0.06±0.01
		10	9	69.9±3.3	64±16	92±14	15.0±2.0	0.5±0.1	0.05±0.01	0.04±0.01
		19	10	72.0±6.0	62±18	114±19	12.2±1.4	0.5±0.0	0.05±0.01	0.04±0.01
		32	10	65.0±3.6	77±21	111±17	12.5±2.7	0.5±0.1	0.08±0.03	0.04±0.02
	CR-LPF	8	10	65.6±3.6	47±10	100±17	14.1±1.7	0.4±0.0	0.05±0.01*	0.06±0.01
		10	10	66.4±5.9	52±16	107±21	15.6±1.5	0.5±0.0	0.07±0.02**	0.05±0.02
		19	10	61.6±6.0**	45±7*	113±15	14.4±1.6**	0.5±0.0	0.06±0.02	0.05±0.01
		32	10	59.6±4.2**	87±26	124±12	13.9±1.8	0.5±0.0	0.09±0.02	0.05±0.01
Female	CRF-1	8	10	67.7±3.9	39±7	116±18	15.7±1.8	0.5±0.1	0.05±0.01	0.06±0.01
		10	10	71.5±5.2	34±9	128±18	16.9±2.5	0.5±0.0	0.07±0.02	0.05±0.01
		19	10	70.1±4.8	40±7	163±23	19.3±2.2	0.6±0.1	0.08±0.02	0.06±0.01
		32	10	67.1±4.5	42±13	154±30	15.3±1.8	0.5±0.1	0.09±0.01	0.07±0.01
	CR-LPF	8	10	64.8±8.8	41±10	127±17	15.0±1.7	0.5±0.1	0.06±0.01**	0.06±0.01
		10	10	64.6±5.7*	35±8	129±22	18.5±1.8	0.5±0.1	0.08±0.01	0.05±0.01
		19	10	66.1±5.4	43±9	183±29	20.3±2.8	0.6±0.1	0.08±0.03	0.06±0.01
		32	10	59.6±7.5*	47±17	204±29**	14.5±2.0	0.6±0.0	0.13±0.05	0.09±0.03*

Data are expressed as mean±S.D.
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 8-2. Blood chemistry in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	AST (U/L)	ALT (U/L)	LAP (U/L)	LDH (U/L)	ALP (U/L)	CK (U/L)	Sodium (mmol/L)
Male	CRF-1	8	10	65±7	25±3	69±5	101±17	477±110	164±15	144±1
		10	9	60±9	26±4	71±6	75±13	363±85	119±14	144±1
		19	10	54±5	24±4	60±4	89±18	164±25	71±8	143±1
		32	10	59±7	30±5	60±6	90±26	144±33	66±10	142±1
	CR-LPF	8	10	58±4*	22±3*	71±4	87±15	513±92	168±12	144±1
		10	10	57±8	23±3	73±4	90±22	394±51	113±13	144±1
		19	10	55±7	23±3	66±6*	95±33	175±22	71±15	143±1
		32	10	56±10	25±4*	58±5	83±18	129±23	69±6	142±1
Female	CRF-1	8	10	56±7	16±2	62±7	88±16	304±57	116±10	143±1
		10	10	64±14	22±5	65±5	99±20	234±87	93±10	143±1
		19	10	76±41	36±31	52±5	116±70	71±11	57±5	143±1
		32	10	67±23	30±11	51±5	80±31	50±9	46±4	142±1
	CR-LPF	8	10	54±5	16±1	67±7	89±17	322±44	121±16	142±1
		10	10	62±12	21±4	64±6	93±11	196±42	89±11	142±1**
		19	10	106±105	51±55	52±4	161±162	79±18	57±6	143±1
		32	10	177±193	95±131	53±9	132±75	61±19	51±6	142±1

Sex	Diet	Age (weeks)	Number of animals	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mg/dL)	Inorganic phosphorus (mg/dL)	Thyroxine (T4) (μg/dL)	Triiodothyronine (T3) (ng/mL)
Male	CRF-1	8	10	3.7±0.2	114±1	10.26±0.27	8.6±0.5	4.49±0.45	1.26±0.22
		10	9	3.3±0.3	114±2	9.96±0.20	8.1±0.4	4.02±0.28	1.19±0.09
		19	10	3.5±0.2	116±1	9.68±0.21	6.0±0.5	3.86±0.61	1.42±0.32
		32	10	3.7±0.2	112±1	10.10±0.22	5.6±0.5	3.12±0.35	1.36±0.12
	CR-LPF	8	10	3.6±0.2	114±2	10.18±0.21	8.2±0.4	4.55±0.47	1.14±0.09
		10	10	3.4±0.2	114±1	10.11±0.23	7.4±0.3**	4.05±0.54	1.21±0.10
		19	10	3.6±0.3	118±1	9.53±0.34	6.2±0.4	3.77±0.56	1.20±0.10
		32	10	3.7±0.2	112±1	10.15±0.15	5.6±0.3	3.68±0.30**	1.17±0.08**
Female	CRF-1	8	10	3.8±0.3	118±1	10.15±0.23	7.5±0.4	3.37±0.47	1.43±0.19
		10	10	3.4±0.4	118±1	9.92±0.36	6.7±0.5	3.60±0.81	1.44±0.14
		19	10	3.1±0.3	121±1	9.88±0.16	5.4±0.4	3.16±0.44	1.23±0.10
		32	10	3.7±0.3	115±1	10.09±0.23	5.8±0.5	2.75±0.38	1.29±0.19
	CR-LPF	8	10	3.7±0.4	117±1	10.11±0.18	7.4±0.5	3.20±0.72	1.32±0.23
		10	10	3.7±0.4	116±2**	9.94±0.30	6.8±0.9	3.45±0.58	1.36±0.17
		19	10	3.0±0.4	120±2	9.97±0.16	5.7±0.4	2.85±0.34	1.15±0.10
		32	10	3.5±0.3	115±1	10.39±0.39	5.7±0.8	3.04±0.46	1.31±0.17

Data are expressed as mean±S.D.
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 9. Activity of hepatic drug-metabolizing enzymes in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Liver weight (g)	Relative liver weight (%)	Aminopyrine-N-demethylase		Aniline hydroxylase	
						(mU/g liver)	(mU/mg pr.)	(mU/g liver)	(mU/mg pr.)
Male	CRF-1	8	10	9.42±0.86	3.07±0.13	138.3±24.3	0.876±0.147	11.6±1.6	0.073±0.008
		10	10	10.84±1.45	2.94±0.22	151.3±47.1	0.983±0.264	11.9±3.2	0.078±0.018
		19	10	13.21±1.37	2.46±0.17	151.4±25.1	1.135±0.173	11.2±2.3	0.084±0.016
		32	10	13.70±1.64	2.29±0.16	155.8±31.0	0.877±0.129	12.2±3.8	0.068±0.018
	CR-LPF	8	10	8.90±0.80	3.09±0.24	165.9±31.0*	1.032±0.196	14.4±3.6*	0.090±0.022
		10	10	9.80±0.73	2.71±0.13*	170.1±38.2	1.015±0.216	16.1±2.1**	0.096±0.011*
		19	10	11.93±1.47	2.32±0.15	170.9±28.4	1.218±0.176	13.7±2.3*	0.098±0.014
		32	10	14.10±0.85	2.32±0.12	128.1±30.4	0.750±0.153	9.1±3.6	0.052±0.018
Female	CRF-1	8	10	5.82±0.49	3.12±0.20	101.8±15.7	0.628±0.097	10.3±2.2	0.064±0.012
		10	10	5.79±0.45	2.74±0.17	98.0±15.8	0.607±0.089	11.9±2.3	0.074±0.013
		19	10	6.25±0.53	2.33±0.17	103.3±12.0	0.755±0.107	12.5±2.2	0.092±0.018
		32	10	7.05±0.64	2.21±0.19	115.5±24.7	0.619±0.123	14.4±4.3	0.077±0.022
	CR-LPF	8	10	5.68±0.48	3.14±0.12	123.4±16.6**	0.724±0.085*	12.1±3.4	0.071±0.018
		10	10	5.73±0.39	2.78±0.18	117.4±15.2*	0.718±0.093*	13.3±3.6	0.081±0.021
		19	10	6.29±0.44	2.39±0.07	113.8±23.7	0.820±0.161	11.8±1.6	0.085±0.010
		32	10	7.69±1.23	2.39±0.19	103.7±22.4	0.559±0.107	9.8±8.0	0.053±0.042

Data are expressed as mean±S.D.(g/animal/week).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 10. Organ weights in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Spleen (g)
Male	CRF-1	8	10	306±19	1.95±0.09	1.11±0.09	1.13±0.08	9.42±0.86	2.50±0.27	0.60±0.10
		10	10	368±26	2.03±0.08	1.25±0.12	1.18±0.08	10.84±1.45	2.77±0.28	0.62±0.09
		19	10	536±25	2.13±0.08	1.40±0.12	1.32±0.09	13.21±1.37	3.17±0.34	0.75±0.13
		32	10	597±50	2.19±0.08	1.53±0.12	1.46±0.16	13.70±1.64	3.60±0.43	0.81±0.11
	CR-LPF	8	10	288±17*	1.97±0.06	1.04±0.09	1.07±0.08	8.90±0.80	2.45±0.24	0.58±0.08
		10	10	362±22	2.00±0.08	1.17±0.08	1.21±0.07	9.80±0.73	2.66±0.26	0.62±0.11
		19	10	514±61	2.19±0.12	1.43±0.13	1.33±0.14	11.93±1.47	3.11±0.41	0.77±0.15
		32	10	609±40	2.17±0.07	1.54±0.08	1.43±0.09	14.10±0.85	3.37±0.23	0.79±0.05
Female	CRF-1	8	10	186±6	1.79±0.07	0.72±0.05	0.85±0.05	5.82±0.49	1.57±0.12	0.40±0.06
		10	10	211±11	1.85±0.07	0.76±0.03	0.89±0.05	5.79±0.45	1.62±0.09	0.41±0.04
		19	10	268±21	1.91±0.07	0.83±0.06	0.96±0.09	6.25±0.53	1.61±0.25	0.41±0.06
		32	10	321±41	1.99±0.08	0.95±0.07	1.03±0.08	7.05±0.64	1.87±0.11	0.46±0.04
	CR-LPF	8	10	180±10	1.81±0.06	0.72±0.06	0.86±0.05	5.68±0.48	1.55±0.14	0.40±0.05
		10	10	207±18	1.85±0.06	0.75±0.04	0.91±0.05	5.73±0.39	1.57±0.13	0.42±0.05
		19	10	263±21	1.91±0.07	0.83±0.07	0.96±0.07	6.29±0.44	1.71±0.12	0.43±0.04
		32	10	321±39	1.95±0.08	1.01±0.12	1.05±0.07	7.69±1.23	1.99±0.20	0.51±0.08

Sex	Diet	Age (weeks)	Number of animals	Thymus (mg)	Pituitary gland (mg)	Adrenal glands (mg)	Testes (g)	Ventral prostate (mg)	Ovaries (mg)
Male	CRF-1	8	10	600.9±100.5	10.2±1.8	52.3±5.0	2.97±0.26	352.0±43.0	—
		10	10	498.4±91.0	11.0±2.2	61.1±13.6	3.21±0.23	487.7±102.0	—
		19	10	339.9±123.2	12.4±0.8	52.4±8.5	3.34±0.39	524.3±195.3	—
		32	10	157.8±31.1	12.1±2.8	53.9±12.0	3.46±0.27	499.0±166.3	—
	CR-LPF	8	10	561.3±155.8	10.4±2.4	52.7±7.2	2.85±0.08	354.1±93.3	—
		10	10	504.2±92.1	11.5±0.8	57.0±10.0	3.28±0.23	455.2±82.0	—
		19	10	328.9±100.9	12.9±2.2	53.8±10.7	3.61±0.25	522.2±128.3	—
		32	10	164.3±39.5	11.4±1.0	53.2±7.7	3.73±0.25*	498.0±135.2	—
Female	CRF-1	8	10	471.3±67.7	10.4±1.1	56.6±5.9	—	—	73.0±10.4
		10	10	443.0±80.3	10.9±1.3	62.5±6.0	—	—	78.2±10.7
		19	10	265.1±59.9	16.1±2.9	54.7±8.1	—	—	55.4±13.1
		32	10	152.9±39.0	13.9±3.5	62.8±6.6	—	—	75.1±16.3
	CR-LPF	8	10	470.8±66.1	11.2±2.0	55.3±8.1	—	—	72.2±11.0
		10	10	409.6±77.4	11.6±1.5	61.3±7.0	—	—	78.3±10.2
		19	10	241.5±57.8	14.9±1.9	54.9±5.0	—	—	53.3±13.0
		32	10	147.9±42.2	16.9±7.1	64.5±11.1	—	—	59.1±16.8*

Data are expressed as mean±S.D.(g/animal/week).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 11. Organ weights (body weight ratio) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Diet	Age (weeks)	Number of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys	Spleen
Male	CRF-1	8	10	306±19	0.64±0.04	0.36±0.02	0.37±0.03	3.07±0.13	0.82±0.06	0.20±0.03
		10	10	368±26	0.55±0.03	0.34±0.02	0.32±0.01	2.94±0.22	0.75±0.06	0.17±0.02
		19	10	536±25	0.40±0.02	0.26±0.02	0.25±0.02	2.46±0.17	0.59±0.06	0.14±0.02
		32	10	597±50	0.37±0.04	0.26±0.02	0.24±0.02	2.29±0.16	0.61±0.07	0.14±0.02
	CR-LPF	8	10	288±17*	0.69±0.05*	0.36±0.02	0.37±0.01	3.09±0.24	0.85±0.06	0.20±0.02
		10	10	362±22	0.57±0.04	0.32±0.02	0.33±0.02	2.71±0.13*	0.74±0.05	0.17±0.03
		19	10	514±61	0.43±0.03*	0.28±0.02	0.26±0.01	2.32±0.15	0.61±0.06	0.15±0.02
		32	10	609±40	0.36±0.03	0.25±0.02	0.24±0.02	2.32±0.12	0.56±0.04	0.13±0.01
Female	CRF-1	8	10	186±6	0.96±0.03	0.38±0.02	0.45±0.02	3.12±0.20	0.84±0.05	0.21±0.03
		10	10	211±11	0.88±0.04	0.36±0.02	0.42±0.02	2.74±0.17	0.77±0.04	0.19±0.02
		19	10	268±21	0.71±0.06	0.31±0.02	0.36±0.02	2.33±0.17	0.60±0.09	0.15±0.02
		32	10	321±41	0.63±0.08	0.30±0.03	0.32±0.03	2.21±0.19	0.59±0.05	0.15±0.01
	CR-LPF	8	10	180±10	1.01±0.06	0.40±0.02	0.48±0.01**	3.14±0.12	0.86±0.05	0.22±0.03
		10	10	207±18	0.90±0.10	0.37±0.04	0.44±0.03	2.78±0.18	0.76±0.06	0.20±0.03
		19	10	263±21	0.73±0.06	0.31±0.02	0.37±0.03	2.39±0.07	0.65±0.05	0.16±0.02
		32	10	321±39	0.62±0.08	0.32±0.03	0.33±0.04	2.39±0.19	0.62±0.05	0.16±0.02

Sex	Diet	Age (weeks)	Number of animals	Thymus ($\times 10^{-3}$)	Pituitary gland ($\times 10^{-3}$)	Adrenal glands ($\times 10^{-3}$)	Testes (g)	Ventral prostate ($\times 10^{-3}$)	Ovaries ($\times 10^{-3}$)
Male	CRF-1	8	10	197±33	3.3±0.4	17.1±1.8	0.97±0.07	115±11	—
		10	10	135±22	3.0±0.5	16.7±3.6	0.88±0.07	133±27	—
		19	10	63±22	2.3±0.2	9.9±2.0	0.62±0.07	98±35	—
		32	10	26±5	2.0±0.4	9.0±1.8	0.59±0.08	84±29	—
	CR-LPF	8	10	194±50	3.6±0.7	18.4±2.9	0.99±0.05	123±30	—
		10	10	140±28	3.2±0.2	15.8±2.7	0.91±0.09	126±24	—
		19	10	64±17	2.5±0.4	10.5±1.9	0.71±0.07*	102±22	—
		32	10	27±7	1.9±0.1	8.8±1.5	0.61±0.04	82±21	—
Female	CRF-1	8	10	253±31	5.6±0.6	30.4±2.5	—	—	39.3±5.5
		10	10	209±30	5.2±0.5	29.6±2.5	—	—	37.1±5.7
		19	10	99±25	6.0±1.0	20.4±3.1	—	—	20.5±3.7
		32	10	48±10	4.3±1.0	19.8±3.1	—	—	23.4±4.2
	CR-LPF	8	10	261±34	6.2±1.1	30.6±3.9	—	—	40.1±6.1
		10	10	199±39	5.6±0.6	29.8±3.9	—	—	38.3±7.0
		19	10	91±17	5.7±1.0	21.0±3.0	—	—	20.3±4.9
		32	10	47±15	5.2±1.6	20.2±3.9	—	—	18.6±5.2

Data are expressed as mean±S.D.(g/animal/week).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)

Table 12. Gross patholoty (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male								Female							
Diet	CRF-1				CR-LPF				CRF-1				CR-LPF			
Age (weeks)	8	10	19	32	8	10	19	32	8	10	19	32	8	10	19	32
Number of animals	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cecum																
Focus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Liver																
Focus, Median lobe	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0	0
Focus, Right lobe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pituitary gland																
Enlarged	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Thymus																
Small	0	0	0	2	0	0	0	1	0	0	0	2	0	0	0	1
Kidney																
Dilatation, Pelvis, Bilateral	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
Dilatation, Pelvis, Unilateral	0	0	2	0	0	0	2	0	0	0	0	0	0	0	1	0
Focus, Unilateral, White	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urinary bladder																
Nodule, Yellow	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Table 13-1. Histopathology patholoty (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male								Female							
Diet	CRF-1				CR-LPF				CRF-1				CR-LPF			
Age (weeks)	8	10	19	32	8	10	19	32	8	10	19	32	8	10	19	32
Number of animals	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cecum																
Hemorrhage, Mucosa	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Colon																
Dilatation, Crypt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hyperplasia, Lymphoid tissue	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Duodenum																
Infiltrative cell, Mucosa, Mononuclear cell	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Jejunum																
Infiltrative cell, Mononuclear cell	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Calcification, Lymph tissue	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Liver																
Dilatation, Sinusoid, Focal	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	1
Eosinophilic Focus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Granuloma	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1
Hemorrhage, Focal	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Infiltrative cell, Mononuclear cell	8	9	8	10	7	9	7	10	8	6	9	9	5	10	9	10
Inflammation, Bile duct	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Necrosis, Hepatocyte, Focal	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Peliosis, Focal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Proliferation, Bile duct	0	0	0	2	0	0	0	3	0	0	0	1	0	0	0	1
Vacuolization intracyto, Hepatocyte. Diffuse	0	1	0	0	0	2	0	0	2	0	0	0	0	0	0	0
Vacuolization intracyto, Hepatocyte, (Median lobe), Focal	0	1	3	3	1	0	0	2	2	1	1	4	4	1	1	1
Vacuolization intracyto, Hepatocyte, Periportal	3	6	8	0	0	2	3	0	6	8	4	0	7	7	2	0
Pancreas																
Atrophy, Acinus, Focal	0	0	1	0	0	0	2	2	0	0	1	1	0	0	1	1
Basophilic focus, Acinus, Focal	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Fibrosis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibrosis, Islets pancreatic	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Granuloma	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Duct	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Islet cell, Diffuse	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, Islet cell, Focal	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	1	3	0	0	0	6	0	1	0	4	0	0	0	6
Inflammation, Focal	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Necrosis, Acinus, Single cell	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Pigmentaion	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
Stomach																
Dilatation, Glands	0	0	1	2	0	0	0	2	0	1	0	3	0	0	0	3
Hyperplasia, Basal cell, Diffuse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hyperplasia, Basal cell, Focal	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tongue																
Calcification	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Heart																
Calcification	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cardiomyopathy	0	0	1	9	0	0	1	4	0	0	0	0	0	0	0	3
Infiltrative cell, Mononuclear cell	0	3	1	0	0	1	0	4	0	0	2	3	0	0	0	1
Vacuolization intracyto, Myocardium, Focal	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Pigmentaion	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0
Necrosis, Myocardium, Focal	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 13-2. Histopathology patholoty (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male								Female							
Diet	CRF-1				CR-LPF				CRF-1				CR-LPF			
Age (weeks)	8	10	19	32	8	10	19	32	8	10	19	32	8	10	19	32
Number of animals	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Adrenal gland																
Degeneration, Cortex, Eosinophilic, Focal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hemorrhage, Cortex, Focal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Hyperplasia, Cortex, Focal	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Hypertrophy, Cortex, Focal	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Parathyroid																
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pituitary gland																
Cyst, Pars intermedia	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Thyroid gland																
Cell debris	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ectopic thymus	1	3	0	0	0	1	0	0	1	0	0	0	2	1	2	0
Hyperplasia, C-cell, Diffuse	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	3
Hyperplasia, C-cell, Focal	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hypertrophy, Follicular cell, Focal	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Infiltrative cell, Mononuclear cell	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1
Ultimobranchial body	1	0	0	2	1	1	2	3	5	4	2	2	2	1	2	5
Vagina																
Comification	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	2
Cyst	—	—	—	—	—	—	—	—	1	0	0	0	0	0	0	0
Epididymis																
Depletion,Sperm	0	0	0	0	1	0	0	0	—	—	—	—	—	—	—	—
Infiltrative cell, Mononuclear cell	0	0	2	7	0	0	3	9	—	—	—	—	—	—	—	—
Vacuolization intracyto, Epithelium	0	0	0	0	0	0	0	1	—	—	—	—	—	—	—	—
Prostate																
Infiltrative cell, Mononuclear cell	0	2	5	2	0	2	7	3	—	—	—	—	—	—	—	—
Inflammation	0	0	0	4	0	0	0	6	—	—	—	—	—	—	—	—
Testis																
Atrophy, Seminiferous tubule	0	0	0	0	1	0	0	0	—	—	—	—	—	—	—	—
Degeneration, Seminiferous epithelium	0	0	0	0	0	0	0	3	—	—	—	—	—	—	—	—
Bone marrow																
Granuloma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Spleen																
Hematopoietic cell proliferation	9	6	5	9	10	5	5	10	2	1	2	7	2	2	2	10
Pigmentation	0	0	0	9	0	0	0	10	0	0	6	10	0	0	7	10
Submandibular lymph node																
Hyperplasia, Plasma cell	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Femur																
Inflammation, Synovial tissue, Patella	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Mammary gland																
Dilatation, Duct, Focal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Brain																
Gliosis, Cerebrum	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Lung																
Calcification, Artery	2	0	1	4	0	2	2	7	1	0	1	2	0	2	3	1
Hemorrhage	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Hemorrhage, Focal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hyperplasia, Epithelium, Bronchus, Focal	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypertrophy, Artery, Media	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrative cell, (focal), Foam cell	1	0	3	2	1	2	1	3	0	0	0	3	0	0	1	0
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0
Metaplasia, (Alveolus), Osseous	1	1	0	1	0	0	1	2	0	1	0	0	0	0	1	2

Table 13-3. Histopathology patholoty (summary incidence of findings) in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male								Female							
Diet	CRF-1				CR-LPF				CRF-1				CR-LPF			
Age (weeks)	8	10	19	32	8	10	19	32	8	10	19	32	8	10	19	32
Number of animals	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Trachea																
Infiltrative cell, Mononuclear cell	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Infiltrative cell, (Submucosa), Mononuclear cell	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
Eye																
Atrophy, Retina, Focal	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Calcification, Cornea	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Harderian gland																
Infiltrative cell, Mononuclear cell	0	0	2	0	0	0	1	6	0	0	1	5	0	0	2	2
Kidney																
Basophilic, Renal tubule	6	10	3	6	8	9	4	4	5	1	2	3	9	5	0	3
Calcification, Cortex	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Calcification, Corticomedullary junction	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Calcification, Medulla	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0
Calcification, Papilla	0	0	3	3	0	0	0	1	0	0	2	1	0	0	0	0
Calcification, Pelvis	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	7
Cast,(Renal tubule)	0	0	2	3	1	0	2	1	0	0	1	2	0	0	1	1
Cyst	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Dilataion, Collecting tubule	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Dilataion, Renal tubule	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dilataion, Pelvis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Hyaline droplet, Renal tubule	1	1	1	0	2	4	0	0	0	0	0	0	0	0	0	0
Hydronephrosis	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0
Hyperplasia, Transitional epithelium	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0
Hyperplasia, Transitional epithelium, (Pelvis), Focal	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Infiltrative cell, Mononuclear cell	2	3	4	5	2	4	5	8	2	4	1	3	2	1	5	5
Urinary bladder																
Infiltrative cell, Mononuclear cell	0	0	0	1	0	0	1	1	0	0	0	2	0	0	0	0

Comparison of Historical Control Data between Crj:CD(SD)IGS and Crj:CD(SD) rats

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ABSTRACT. A Comparison of the historical data obtained from Crj:CD(SD)IGS and Crj:CD(SD) rats used in the control groups of toxicity studies was performed. No death occurred in IGS or SD rats. The body weights were lower and the food consumption was higher in male and female IGS rats than those in age-matched SD rats. In urinalysis, low urinary volume, and low Na, K, and Cl concentrations were observed in IGS rats when compared to SD rats. In hematology, prothrombin time was reduced and activated partial thrombin time was extended in male and female IGS rats than in SD rats. In serum biochemistry, triglyceride and total bilirubin values in male and female IGS rats were lower than in SD rats. There were no apparent differences in organ weights or histopathological findings between IGS and SD rats.—Key words: Crj:CD(SD)IGS, Crj:CD(SD), Rats, General toxicological parameter

CD(SD)IGS-1999: 105-118

INTRODUCTION

Crj:CD(SD)IGS rats were developed by Charles River Inc. to eliminate international differences in the Crj:CD(SD) strain. We have been using Crj:CD(SD)IGS strain rats for 2 years in toxicology studies. In this study, we compared the differences in body weight, food consumption, urinalysis, hematology, serum biochemistry, organ weight, and histopathology data between Crj:CD(SD)IGS strain and Crj:CD(SD) strain rats.

MATERIALS AND METHODS

Animals: Crj:CD(SD)IGS (IGS hereafter) rats were obtained for separate toxicology studies conducted from June, 1996 to November, 1998 from Charles River Japan (Hino or Atsugi, Japan). Crj:CD(SD) (SD hereafter) rats were obtained from the same supplier for separate toxicology studies from April, 1987 to November, 1997. The animals were quarantined and acclimated for 1 week. During this period, animals showing healthy and favorable growth were selected for the toxicology studies. Study animals were assigned to the control groups by stratified randomization so as to achieve similar mean body weights in each group. The animals were identified by ear punching.

Maintenance conditions: The animals were housed individually in suspended stainless steel cages [32.5 cm (D) × 19.5 cm (W) × 18.0 cm (H)] in the SPF Animal Facility. Temperature and relative humidity in the animal rooms were set at 22 ± 2℃ and 55 ± 10%, respectively, with air changes (15 times/hour), and a 12 hr artificial light cycle (6:00 to 18:00). The animal rooms were cleaned daily, and cages were replaced by sterilized ones at least once every 4 weeks. Solid food (CE-2, Clea Japan, Inc.), and water, certified to meet the Water Quality Standard required by the Japanese Water Supply Law, were provided *ad libitum* using an automatic water supply apparatus (Edstrom Industries, Inc.).

Body weight: Body weights were measured using an electronic balance, once during the quarantine and acclimation period, and once every week thereafter.

Food consumption: Daily food consumption was calculated from the amount of food supplied and the amount left over, once during the quarantine and acclimation period, and once every week thereafter.

Urinalysis: Fresh urine was collected by the compulsory method and the color was examined visually, and pH, protein, glucose, ketone bodies, bilirubin, occult blood and urobilinogen were analyzed with a Clinitek 200 + urine analyzer (MILES Labs., Inc.) using MULTISTIX®. Four-hour urine collected using a metabolic cage under fasting condition and continued water supply was used for the following examinations: Urinary volume was measured using a measuring cylinder, specific gravity was measured with a urinary refractometer (URICON-S, Atago Co., Ltd.), urine sediment was examined microscopically after centrifugation (1500 r.p.m., 5 min.), sodium (Na), potassium (K) and chloride (Cl) values were measured using an electrolyte analyzer (PVA-α Ⅲ, A & T Co., Ltd.).

Hematology: The animals were fasted for 16 to 24 hours. Blood was collected from the caudal vena cava under sodium pentobarbital anesthesia. Red blood cell (RBC), white blood cell (WBC) and platelet counts, hematocrit value, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined or calculated using a multipurpose automatic cell counter (E-4000, Sysmex Co., Ltd.) with EDTA-2K as an anticoagulant. Reticulocyte (Brecher staining) and differential white blood cell counts (Wright staining) were determined using a blood cell autoanalyzer (MICROX HEG-120A, OMRON Co., Ltd.) with EDTA-2K as an anticoagulant. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using an automatic blood coagulation measuring apparatus (CA-5000, Sysmex Co., Ltd.) with sodium citrate as an anticoagulant.

Serum biochemistry: After blood sampling for hematology, as much blood as possible was also collected from the abdominal aorta as much as possible. Sera obtained by centrifugation (3000 r.p.m., 15 min.) were used to measure the following parameters: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), total bilirubin, total protein, albumin, total cholesterol, triglyceride (TGL), phospholipid (PL), glucose, blood urea nitrogen (BUN), creatinine, uric acid (UA), inorganic phosphorus (IP), Calcium (Ca), Sodium (Na), Potassium (K), and Chloride (Cl) were measured using an autoanalyzer (Clinalyzer RX-10, JEOL, Ltd.). Protein fractions (including A/G ratio) were calculated using automatic electrophoresis apparatus (MODEL FED- Ⅱ, Cosmo Co., Ltd.).

Organ weights: After blood sampling, animals were sacrificed by exsanguination from the abdominal aorta. The liver, kidneys (right and left), heart, lung, spleen, brain, thymus, submandibular glands (right and left), pituitary, thyroids (right and left), adrenals (right and left), testes (right and left), epididymides (right and left), prostate, seminal vesicle, ovaries (right and left), and uterus were weighed using electronic balances. Relative organ weights were calculated as a percentage of the final body weight.

Histopathology: The heart, thoracic aorta, spleen, thymus, bone marrow and bones (femur, sternum), submandibular and mesenteric lymph nodes, trachea, lungs (including bronchus), tongue, esophagus, stomach (forestomach and glandular stomach), duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, kidneys, urinary bladder, epididymides, seminal vesicle, prostate, ovaries, uterus, vagina, mammary gland (females only), pituitary, thyroids (including parathyroids), adrenals, cerebrum, cerebellum, brain stem, thoracic spinal cord, sciatic nerve, skeletal muscle (musculus quadriceps femoris), submandibular gland, sublingual gland, lacrimal gland, Harderian gland and skin were fixed in 10% neutral buffered formalin, the eyeballs (with optic nerves) were fixed in a glutaraldehyde solution, and the testes were fixed in 10% neutral buffered formalin or Bouin's solution. All the above tissues were dehydrated by a graded series of ethanol, cleared by xylene, embedded in paraffin, sectioned at a thickness of 4 μm, stained with hematoxylin-eosin, and examined microscopically.

Statistical analysis: Statistical analysis was not performed.

RESULTS AND DISCUSSION

Mortality: No death occurred in IGS and SD rats.

Body weights: The body weights of male and female IGS rats were constantly lower than those in age-matched SD rats (Fig. 1). The difference in body weight between IGS and SD rats was greater in male rats. Other authors have also reported this finding [2, 3, 4, 6, 7, 11]. When compared to previously reported body weight values for IGS rats fed the same food as in this study (CE-2), the body weight of male IGS rats was lower until Week 25 and higher thereafter, and for female IGS rats, it was constantly lower than the values reported by Tanaka et al. [10]. The body weights of both sexes were constantly lower than those reported by Isobe et al. [6].

Food consumption: In contrast with reported data [1, 3, 4, 6,11], food consumption in male and female IGS rats was higher than that in age-matched SD rats at almost all measurement points (Fig. 2). Food consumption in male and female IGS rats was higher when the results were compared to those in reports on CE-2-fed animals [6,10]. The cause of the discrepancies between this study and previous reports is unknown.

Urinalysis: Low values for urinary volume, and Na, K, Cl concentrations were seen in male and female IGS rats when compared to those in SD rats (Table 1). There were no apparent differences in other urinalysis parameters between IGS and SD rats.

Hematology: PT was reduced, and APTT extended in male and female IGS rats than those in SD rats at all measurement points (Table 2). Similar tendencies have also been reported [8], although the differences between IGS and SD rats were within reference range. There were no other conspicuous differences common to either sex or in measurement points between IGS and SD rats.

Serum biochemistry: Triglyceride and total bilirubin values in male and female IGS rats were lower than those in SD rats at all measurement points (Table 3), as has also been reported by other authors [5, 6].

Organ weights: There were no apparent differences in organ weights between IGS and SD rats. (Table 4).

Histopathology: There were no characteristic lesions in IGS rats when compared to SD rats, as has also been reported by other authors [7, 9]. Mononuclear cell infiltration in the esophageal adventitia and inflammatory cell infiltration in the meninx of the cerebellum were only observed in IGS rats. However, the significance of the lesions was not clear because of the small amount of background data on IGS rats (Table 5). The following findings, which occurred in more than 2 IGS rats, were also observed in SD rats: Mononuclear cell infiltration, necrosis and fibrosis in the heart, cyst in the thymus, foamy cell (alveolus), osseous metaplasia, mononuclear cell infiltration (perivascular), mineralization (vascular wall), hemorrhage (alveolus), inflammatory cell infiltration (alveolus) and focal congestion in the lung, squamous metaplasia in the trachea, dilatation of the gland (fundus) and hemorrhage in the lamina propria (fundus) in the stomach, mononuclear cell infiltration and brown pigment deposition in the pancreas, mononuclear cell infiltration (periportal), microgranuloma, vacuolation of periportal hepatocyte and necrosis in the liver, regenerating tubule, mononuclear cell infiltration, eosinophilic body (proximal tubule), hyaline cast, mineralization (cortex), cyst (cortex), mineralization (papilla), dilatation of the pelvis, focal glomerulosclerosis and inflammatory cell infiltration (papilla) in the kidney, atrophy, giant cell and vacuolation of the seminiferous tubule in the testis, mononuclear cell infiltration and decreased sperm in the epididymis, mononuclear cell infiltration (interstitium and lumen) and mineralization (lumen) in the prostate, cyst and mineralization (oocyte) in the ovary, hydrometra, cyst (pars distalis) in the pituitary, ultimobranchial body, ectopic thymus, mineralization (colloid) in the thyroid, mononuclear cell infiltration (meninx) in the cerebellum, retinal dysplasia, mononuclear cell infiltration in the lacrimal gland and harderian gland, mononuclear cell infiltration and necrosis in the skeletal muscle, mononuclear cell infiltration in the submandibular gland.

REFERENCES

1. Batham, P., Kangas, L., Farrell, D., Boyer, M. 1998. The effect of controlled feeding on the growth of Crl:CD(SD)IGS rats. Biological Reference Data on CD(SD)IGS Rats: 131-134.
2. Hooks, W.N. 1998. A comparison between the original and international genetic standard Charles River (UK) designations of Sprague-Dawley rat, at the 13-week time point. Biological Reference Data on CD(SD)IGS Rats: 50-56.
3. Hooks, W.N. 1998. An ongoing review of the mortality, bodyweight and food consumption of Charles River Sprague-Dawley international genetic standard rats in comparison with the original strain designation in long term studies. Biological Reference Data on CD(SD)IGS Rats: 57-63.

4. Inoue, I., Motomura, I., Takagi, S., Nagaoka, T., Takeuchi, M. 1998. Background data for clinical and pathological parameters in Crj:CD(SD)IGS rats bred for long time. Biological Reference Data on CD(SD)IGS Rats: 121-130.
5. Inoue, Y., Eguchi, F., Sugimoto, J., Mutai, M. 1998. Study on clinical chemistry values in Crj:CD(SD)IGS rats. Biological Reference Data on CD(SD)IGS Rats: 135-138.
6. Isobe, M., Ohtsubo, S., Sato, A., Ogawa, T., Ikeda, T., Morimoto, H., Eguchi, K., Nakayama, T. 1998. General toxicological parameters in Crj:CD(SD)IGS rats and Crj:CD(SD) rats. Biological Reference Data on CD(SD)IGS Rats: 143-154.
7. Okazaki, S., Suwa, K., Okazaki, K., Hatayama, K., Ichikawa, Y., Komatsu, M., Ogawa, T., Tamura, K. 1998. Comparison of general toxicological parameters between Crj:CD(SD) rats fed normal commercial diet and Crj:CD(SD)IGS rats fed normal or low protein commercial diet. Biological Reference Data on CD(SD)IGS Rats: 71-81.
8. Saitoh, T., Shibuya, K., Kizaki, H., Ihara, M., Shibuya, N., Kudow, S., Itabashi, M., Nunoya, T., Tajima, M. 1998. Comparison of hematological parameters between Crj:CD(SD)IGS and Crj:CD(SD) rats. Biological Reference Data on CD(SD)IGS Rats: 139-142.
9. Suwa, T., Murakami, N., Kitagaki, M., Yamaguchi, M., Sasa, H., Tanaka, C. 1998. Characteristics of Crj:CD(SD)IGS rats compared with Crj:CD(SD) rats based on a repeated dose toxicity study. Biological Reference Data on CD(SD)IGS Rats: 82-89.
10. Tanaka, A., Azegami, J., Kojima, K., and Imai, K. 1998. Response to commercial low protein diet in Crj:CD(SD)IGS rats. Biological Reference Data on CD(SD)IGS Rats: 19-30.
11. Yamamoto, T., Kakamu, S., Suzuki, K., Sugiyama, Y., Mukai, D., Ohba, K., Yamakawa, S., Iwata, H., Inoue, H. 1998. Mortality, body weight, food consumption, clinical laboratory test and pathological examination in Crj:CD(SD)IGS rats in subchronic and chronic toxicity studies. Biological Reference Data on CD(SD)IGS Rats: 39-42.

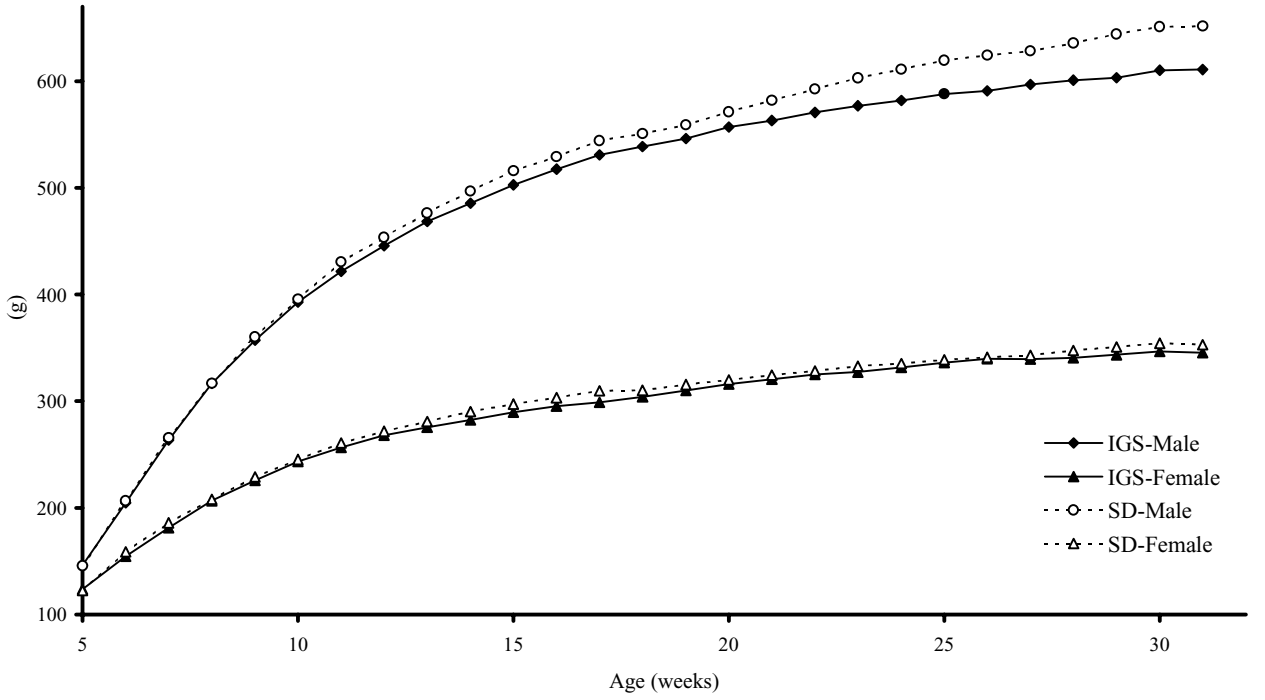

Figure 1: Body weight

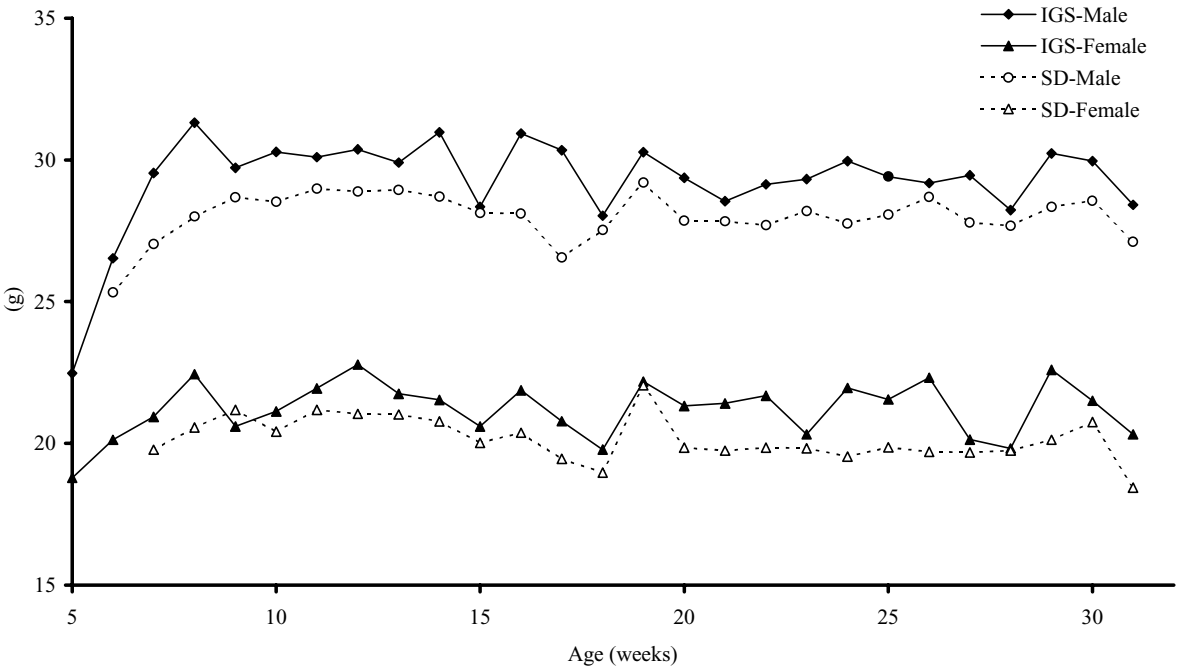

Figure 2: Food consumption

Table 1-1. Urinalysis (Male)

Age		7-8 Weeks		9-13 Weeks		16-23 Weeks		30-40 Weeks	
Strain		IGS	SD	IGS	SD	IGS	SD	IGS	SD
Color		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	Normal	35	35	132	92	10	70	22	67
pH		N=35	N=35	N=132	N=92	N=10	N=63	N=22	N=67
	5.0	0	0	0	1	0	0	0	0
	5.5	0	2	2	0	0	0	0	0
	6.0	0	1	3	7	1	4	3	9
	6.5	4	4	10	4	0	5	3	13
	7.0	15	8	36	27	3	18	9	25
	7.5	13	12	38	24	3	17	6	10
	8.0	3	6	26	18	1	11	1	8
	8.5	0	2	14	5	2	7	0	2
	9.0	0	0	3	6	0	1	0	0
Protein		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	−	5	5	31	8	0	12	1	4
	＋−	0	4	10	10	0	4	2	2
	＋	11	19	59	49	6	30	6	23
	++	19	7	31	18	4	17	8	21
	+++	0	0	1	7	0	7	5	17
	++++	0	0	0	0	0	0	0	0
Glucose		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	0	35	35	132	92	10	70	22	67
Ketone body		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	−	19	24	35	49	2	23	9	23
	＋−	15	9	65	42	5	22	11	29
	＋	1	2	32	1	3	25	2	15
Bilirubin		N=35	N=35	N=122	N=92	N=10	N=70	N=22	N=67
	−	35	35	121	92	10	70	22	62
	＋−	0	0	1	0	0	0	0	5
	＋	0	0	0	0	0	0	0	0
Occult blood		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	−	34	32	111	68	8	54	18	58
	＋−	0	3	16	18	1	10	1	4
	＋	1	0	5	5	0	3	1	5
	++	0	0	0	1	1	1	2	0
	+++	0	0	0	0	0	2	0	0
Urobilinogen		N=35	N=35	N=132	N=92	N=10	N=70	N=22	N=67
	−	14	19	112	61	6	39	15	42
	＋−	21	16	20	31	4	31	7	25

Table 1-1. Continued

Age	7-8 Weeks		9-13 Weeks		16-23 Weeks		30-40 Weeks	
Strain	IGS	SD	IGS	SD	IGS	SD	IGS	SD
RBC	N=11	N=35	N=10	N=0	N=5	N=20	N=5	N=25
−	11	35	10		5	19	4	25
＋−	0	0	0		0	1	0	0
＋	0	0	0		0	0	0	0
++	0	0	0		0	0	1	0
WBC	N=11	N=35	N=10	N=0	N=5	N=20	N=5	N=25
−	11	32	10		5	18	5	23
＋−	0	3	0		0	2	0	2
＋	0	0	0		0	0	0	0
++	0	0	0		0	0	0	0
Phosphate crystal	N=11	N=35	N=10	N=0	N=5	N=20	N=5	N=25
0	2	9	7		0	9	1	9
1-5/HPF	5	25	3		3	11	4	13
6-20/HPF	2	1	0		0	0	0	3
20</HPF	2	0	0		2	0	0	0
Epithelium	N=11	N=35	N=20	N=0	N=5	N=20	N=5	N=25
0	5	25	19		1	19	5	13
1-5/HPF	6	10	1		4	1	0	12
Sperm	N=11	N=35	N=10	N=0	N=5	N=20	N=5	N=25
0	10	29	3		2	8	2	5
1-5/HPF	1	6	7		3	12	3	20
Urinary volume (mL)	35	N=30	N=57	N=34	N=5	N=50	N=10	N=45
	3.38±1.52	4.94±4.88	3.65±1.45	5.22±2.53	3.98±0.73	11.72±8.06	3.42±1.67	7.90±4.67
Specific gravity	N=11	N=30	N=47	N=34	N=5	N=50	N=10	N=45
	1.0521±0.0162	1.0359±0.0150	1.0264±0.0121	1.0320±0.0176	1.0328±0.0148	1.0363±0.0183	1.0341±0.0194	1.0439±0.0186
Na	N=29	N=20	N=37	N=19	N=0	N=45	N=0	N=25
	61.1±46.0	87.4±41.4	33.4±22.3	72.8±49.1		59.2±44.7		87.5±28.5
K	N=29	N=20	N=37	N=19	N=0	N=45	N=0	N=25
	209.7±72.0	210.6±75.7	122.8±70.3	194.3±129.8		152.4±83.9		217.2±60.8
Cl	N=29	N=20	N=37	N=19	N=0	N=45	N=0	N=25
	91.4±49.8	118.4±42.5	51.2±36.4	94.1±56.1		69.6±47.3		76.3±36.3

Table 1-2. Urinalysis (Female)

Age		7-8 Weeks		9-13 Weeks		16-23 Weeks		30-40 Weeks	
Strain		IGS	SD	IGS	SD	IGS	SD	IGS	SD
Color		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	Normal	30	15	132	92	10	75	22	68
pH		N=30	N=15	N=132	N=92	N=10	N=68	N=22	N=68
	5.0	1	1	0	0	0	0	1	0
	5.5	1	1	3	1	0	0	0	1
	6.0	6	1	11	4	0	4	6	17
	6.5	5	1	23	11	2	13	7	13
	7.0	11	7	34	28	3	18	4	22
	7.5	3	2	33	23	5	23	4	8
	8.0	3	1	19	13	0	6	0	6
	8.5	0	1	7	8	0	3	0	1
	9.0	0	0	2	4	0	1	0	0
Protein		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	−	18	4	76	43	7	29	7	24
	＋−	1	5	9	15	0	16	0	15
	＋	10	5	42	33	2	14	12	21
	++	1	1	5	1	1	12	1	7
	+++	0	0	0	0	0	4	2	1
	++++	0	0	0	0	0	0	0	0
Glucose		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	0	30	15	132	92	10	75	22	68
Ketone body		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	−	14	11	91	83	4	70	14	56
	＋−	16	4	40	9	6	5	8	12
	＋	0	0	1	0	0	0	0	0
Bilirubin		N=30	N=15	N=122	N=92	N=10	N=75	N=22	N=68
	−	30	15	121	91	10	74	22	68
	＋−	0	0	1	1	0	0	0	0
	＋	0	0	0	0	0	1	0	0
Occult blood		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	−	27	13	111	74	10	65	22	61
	＋−	2	1	16	13	0	9	0	4
	＋	1	1	5	4	0	1	0	3
	++	0	0	0	1	0	0	0	0
	+++	0	0	0	0	0	0	0	0
Urobilinogen		N=30	N=15	N=132	N=92	N=10	N=75	N=22	N=68
	−	8	12	79	57	2	41	9	54
	＋−	22	3	53	35	8	34	13	14
RBC		N=35	N=25	N=20	N=0	N=5	N=20	N=5	N=25
	−	35	25	20		5	20	5	25
	＋−	0	0	0		0	0	0	0
	＋	0	0	0		0	0	0	0
	++	0	0	0		0	0	0	0
WBC		N=35	N=25	N=20	N=0	N=5	N=20	N=5	N=25
	−	33	21	14		5	20	5	18
	＋−	2	4	6		0	0	0	7
	＋	0	0	0		0	0	0	0
	++	0	0	0		0	0	0	0
Phosphate crystal		N=35	N=25	N=10	N=0	N=5	N=20	N=5	N=25
	0	20	9	9		1	16	2	9
	1-5/HPF	14	14	1		4	3	2	12
	6-20/HPF	1	2	0		0	1	1	4
	20</HPF	0	0	0		0	0	0	0
Epithelium		N=35	N=25	N=20	N=0	N=5	N=20	N=5	N=25
	0	24	18	15		4	20	5	25
	1-5/HPF	11	7	5		1	0	0	0
Urinary volume (mL)		N=30	N=10	N=57	N=34	N=5	N=55	N=10	N=45
		2.61±0.88	6.43±3.98	2.91±1.13	3.90±2.16	2.16±0.75	8.23±7.50	3.47±1.74	8.06±5.48
Specific gravity		N=6	N=10	N=47	N=34	N=5	N=55	N=10	N=45
		1.0212±0.0076	1.0389±0.0196	1.0211±0.0091	1.0254±0.0164	1.0290±0.0167	1.0362±0.0141	1.0222±0.0084	1.0444±0.0218
Na		N=24	N=10	N=37	N=19	N=0	N=50	N=0	N=25
		29.8±19.9	87.2±47.6	29.2±18.4	50.5±41.3		62.8±38.8		113.4±31.3
K		N=24	N=10	N=37	N=19	N=0	N=50	N=0	N=25
		141.2±66.2	178.2±86.0	89.9±44.7	144.7±119.8		143.5±70.0		266.6±62.6
Cl		N=24	N=10	N=37	N=19	N=0	N=50	N=0	N=25
		57.8±31.0	103.5±61.1	33.0±21.7	56.2±54.5		61.6±45.0		122.4±33.4

Table 2-1. Hematology (Male)

Age	7-8 Weeks				9-13 Weeks				16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD		IGS		SD		IGS		SD	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
RBC ($\times 10^4$/mm^3)	689±39	36	699±37	33	805±51	95	788±40	70	912±48	10	880±39	60	909±49	22	845±65	69
Hematocrit (%)	42.3±1.9	36	43.3±1.7	33	46.4±2.3	95	46.2±2.2	70	46.8±2.0	10	46.6±2.0	60	45.6±2.2	22	44.4±2.9	69
Hemoglobin (g/dL)	14.5±0.7	36	14.4±0.7	33	16.0±0.8	95	15.9±0.8	70	15.9±0.5	10	15.9±0.6	60	16.0±0.8	22	14.9±1.1	69
MCV (fL)	61.5±1.8	36	62.0±2.2	33	57.8±2.0	95	58.7±2.1	70	51.4±2.4	10	52.9±1.7	60	50.2±2.4	22	52.6±2.2	69
MCH (pg)	21.0±0.9	36	20.6±0.8	33	19.9±0.9	95	20.2±0.7	70	17.4±0.7	10	18.0±0.8	60	17.6±0.9	22	17.7±0.9	69
MCHC (%)	34.2±0.9	36	33.3±0.7	33	34.5±1.1	95	34.4±0.8	70	33.9±0.5	10	34.1±0.9	60	35.1±0.7	22	33.6±1.0	69
Reticulocyte (%)	3.53±1.32	16	4.31±1.76	30	1.63±0.72	95	3.06±1.31	65	1.25±0.40	10	1.6±0.6	60	1.2±0.7	22	2.2±1.1	49
WBC ($\times 10^2$/mm^3)	65.1±22.1	36	59.7±19.5	33	75.3±25.1	95	72.4±31.5	70	58.9±19.5	10	68.3±21.8	60	56.0±18.0	22	64.4±30.5	69
Stab Neutrophil (%)	0.0±0.0	16	0.2±0.5	30	0.1±0.2	95	0.3±0.5	65	0.0±0.0	10	0.3±0.6	60	0.0±0.0	22	0.3±0.7	69
Segmented Neutrophil (%)	7.4±4.3	16	7.9±4.9	30	6.7±3.7	95	8.5±5.9	65	10.2±3.2	10	12.6±5.8	60	13.0±9.4	22	18.5±12.2	69
Eosinophil (%)	0.4±0.6	16	0.7±0.8	30	0.6±0.8	95	0.9±1.2	65	0.7±0.8	10	1.2±1.1	60	1.6±1.0	22	1.5±1.3	69
Basophil (%)	0.0±0.0	16	0.0±0.0	30	0.0±0.0	95	0.0±0.0	65	0.0±0.0	10	0.0±0.0	60	0.0±0.0	22	0.0±0.3	69
Monocyte (%)	0.8±1.3	16	1.9±2.8	30	1.5±1.8	95	2.9±2.3	65	2.6±1.9	10	3.8±3.2	60	0.7±1.0	22	3.7±2.9	69
Lymphocyte (%)	91.3±4.0	16	89.3±7.0	30	91.1±4.5	95	87.5±7.2	65	86.5±4.4	10	82.1±6.6	60	84.7±9.8	22	76.0±13.0	69
Platelet ($\times 10^4$/mm^3)	143±23	36	145±26	33	123±16	95	133±19	70	103±30	10	117±28	60	111±26	22	114±30	69
PT (Sec)	9.7±0.5	16	11.7±3.4	20	10.0±1.5	95	16.9±5.8	70	10.5±0.5	10	15.9±7.2	60	10.1±0.9	22	13.5±4.1	69
APTT (Sec)	24.2±1.8	16	21.1±3.8	20	24.2±2.1	95	20.4±3.3	70	27.2±3.8	10	20.1±3.1	60	24.5±3.1	22	18.6±2.3	69

Table 2-2. Hematology (Female)

Age	7-8 Weeks				9-13 Weeks				16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD		IGS		SD		IGS		SD	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
RBC ($\times 10^4$/mm^3)	712±37	26	694±25	13	764±38	95	743±37	70	789±22	10	800±43	70	800±32	22	742±58	69
Hematocrit (%)	42.9±2.2	26	42.4±1.4	13	44.0±1.9	95	43.3±1.9	70	43.3±0.9	10	44.3±1.9	70	43.4±1.6	22	41.4±2.6	69
Hemoglobin (g/dL)	15.1±0.9	26	14.2±0.5	13	15.2±0.8	95	15.0±0.8	70	14.9±0.6	10	15.2±0.7	70	15.3±0.6	22	14.0±1.1	69
MCV (fL)	60.3±1.3	26	61.0±1.4	13	57.6±1.5	95	58.3±1.8	70	54.9±1.4	10	55.4±1.4	70	54.3±1.7	22	55.9±1.9	69
MCH (pg)	21.1±0.6	26	20.4±0.6	13	19.9±0.7	95	20.1±0.6	70	18.9±0.6	10	19.1±0.9	70	19.1±0.6	22	18.9±0.8	69
MCHC (%)	35.1±1.2	26	33.5±0.5	13	34.6±1.0	95	34.5±0.9	70	34.4±0.7	10	34.4±1.3	70	35.2±0.6	22	33.8±0.9	69
Reticulocyte (%)	2.9±1.3	6	2.1±0.4	10	1.59±0.72	95	3.04±1.13	65	0.8±0.4	10	1.4±0.7	70	1.3±0.5	22	2.0±0.9	49
WBC ($\times 10^2$/mm^3)	59.0±19.4	26	56.8±20.2	13	62.0±20.6	95	54.7±22.4	70	44.4±21.6	10	46.8±20.8	70	42.3±17.4	22	41.7±19.1	69
Stab Neutrophil (%)	0.0±0.0	6	0.0±0.0	10	0.0±0.3	95	0.2±0.5	65	0.0±0.0	10	0.6±0.9	70	0.1±0.3	22	0.3±0.7	69
Segmented Neutrophil (%)	3.5±1.5	6	7.1±6.3	10	6.6±6.2	95	7.2±4.5	65	12.5±5.9	10	9.8±5.0	70	9.8±4.3	22	15.9±11.8	69
Eosinophil (%)	1.0±1.3	6	1.3±1.3	10	0.8±0.9	95	1.5±1.5	65	1.6±1.7	10	1.1±1.4	70	1.2±1.6	22	1.4±1.2	69
Basophil (%)	0.0±0.0	6	0.0±0.0	10	0.0±0.0	95	0.0±0.1	65	0.0±0.0	10	0.0±0.0	70	0.0±0.0	22	0.1±0.4	69
Monocyte (%)	2.5±1.8	6	0.5±1.3	10	1.4±1.5	95	2.6±2.1	65	1.9±2.0	10	3.4±3.3	70	0.9±1.4	22	2.4±1.9	69
Lymphocyte (%)	93.0±3.7	6	91.1±7.0	10	91.2±6.6	95	88.5±5.7	65	84.0±7.5	10	85.1±7.0	70	88.1±4.9	22	79.9±12.5	69
Platelet ($\times 10^4$/mm^3)	136±14	26	137±12	13	124±20	95	129±20	70	95±20	10	111±29	70	107±18	22	106±20	69
PT (Sec)	8.8±0.1	6	9.6±0.3	10	8.6±0.6	95	14.6±4.6	70	9.2±0.8	10	12.9±3.9	70	8.7±0.5	22	12.8±3.9	69
APTT (Sec)	19.7±1.3	6	17.5±0.5	10	18.8±1.6	95	16.5±2.5	70	20.6±1.2	10	16.1±2.1	70	20.2±2.4	22	16.5±1.7	69

Table 3-1. Serum Biochemistry (Male)

Age	7-8 Weeks				9-13 Weeks			
Strain	IGS		SD		IGS		SD	
	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N
AST (IU/L)	95.4±24.0	36	115.3±23.5	33	84.3±31.8	95	117.5±23.1	76
ALT (IU/L)	27.8±7.4	36	23.8±6.1	33	26.7±5.6	95	27.2±7.0	76
ALP (IU/L)	453.7±78.5	36	457.2±71.7	33	317.6±62.6	95	366.7±113.2	70
LDH (IU/L)	615.2±661.5	16	2304.3±696.5	18	1122.3±1457.6	95	3075.3±1201.7	30
CPK (IU/L)	278.6±209.8	16	807.5±196.3	25	351.8±319.1	90	820.9±301.9	45
Total bilirubin (mg/dL)	0.138±0.020	36	0.182±0.053	33	0.152±0.040	95	0.237±0.055	70
Total protein (g/dL)	5.19±0.34	36	5.32±0.30	33	5.61±0.30	95	5.72±0.47	80
Albumin (g/dL)	3.86±0.23	36	3.82±0.21	30	3.95±0.20	95	3.80±0.32	76
Total cholesterol (mg/dL)	51.9±8.4	36	56.8±15.7	33	52.7±11.5	95	46.0±11.2	70
TGL (mg/dL)	29.7±10.1	36	53.0±20.9	33	30.9±12.0	95	52.9±33.6	70
PL (mg/dL)	94.7±16.7	6	97.9±22.2	25	83.3±14.5	30	82.3±18.1	51
Glucose (mg/dL)	135.4±21.9	36	116.4±22.0	33	146.7±27.5	95	142.2±53.2	80
BUN (mg/dL)	16.59±2.22	36	15.83±2.75	33	16.18±2.91	95	18.28±5.83	76
Creatinine (mg/dL)	0.423±0.084	36	0.409±0.051	33	0.427±0.083	95	0.476±0.161	76
UA (mg/dL)	1.214±0.390	16	1.365±0.307	25	0.619±0.401	40	1.307±0.362	50
IP (mg/dL)	9.999±0.732	36	9.404±0.749	33	8.173±1.037	95	8.588±0.870	76
Ca (mg/dL)	9.55±0.46	26	9.07±0.56	33	9.38±0.39	95	9.33±0.52	70
Na (mEq/L)	146.0±4.1	36	144.7±3.3	33	145.2±1.6	95	144.6±1.8	70
K (mEq/L)	4.13±0.35	36	4.27±0.30	33	3.93±0.48	95	4.22±0.31	70
Cl (mEq/L)	104.6±3.2	36	103.6±3.4	33	104.5±2.0	95	106.4±2.7	70
Albumin (%)	56.53±1.18	16	56.92±2.25	30	53.86±3.14	50	54.97±2.57	55
α 1 globulin (%)	16.09±1.18	16	19.01±1.70	30	18.29±2.26	50	19.26±3.27	55
α 2 globulin (%)	8.43±0.73	16	8.27±0.83	30	7.95±1.15	50	7.91±1.28	55
β globulin (%)	13.08±0.92	16	12.92±1.05	30	14.29±2.70	50	14.70±1.76	55
γ globulin (%)	5.88±0.62	16	2.88±1.11	30	5.61±1.94	50	3.17±0.81	55
A/G	1.302±0.064	16	1.327±0.120	30	1.177±0.152	50	1.227±0.126	55

Table 3-1. Continued

Age	16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD	
	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N
AST (IU/L)	98.2±27.7	10	89.5±26.3	60	110.2±35.9	22	111.7±45.6	69
ALT (IU/L)	35.5±8.6	10	29.7±8.8	60	41.1±14.4	22	38.3±16.6	69
ALP (IU/L)	151.5±27.9	10	156.7±33.4	60	124.3±23.0	22	146.1±41.7	69
LDH (IU/L)	1045.2±466.9	10	2493.3±1995.4	30	2507.7±951.3	12	2607.5±2147.8	44
CPK (IU/L)	219.2±49.0	10	693.4±408.1	30	578.0±315.1	22	503.4±359.5	54
Total bilirubin (mg/dL)	0.131±0.019	10	0.233±0.056	60	0.144±0.026	22	0.228±0.053	69
Total protein (g/dL)	6.11±0.22	10	6.07±0.30	60	6.21±0.41	22	6.17±0.42	69
Albumin (g/dL)	4.09±0.18	10	3.98±0.20	60	4.18±0.22	22	3.75±0.42	69
Total cholesterol (mg/dL)	47.5±13.8	10	49.4±18.5	60	70.0±25.5	22	72.0±26.2	69
TGL (mg/dL)	39.4±26.6	10	52.3±33.8	60	54.1±17.4	22	92.6±55.2	69
PL (mg/dL)	–	–	105.8±28.6	60	110.8±25.8	22	118.9±35.9	34
Glucose (mg/dL)	181.6±22.6	10	150.8±23.7	60	170.0±79.1	22	155.0±36.5	69
BUN (mg/dL)	16.64±0.78	10	16.50±3.03	60	17.13±2.73	22	19.44±3.58	69
Creatinine (mg/dL)	0.516±0.064	10	0.501±0.089	60	0.601±0.147	22	0.542±0.122	69
UA (mg/dL)	0.569±0.177	10	1.184±0.259	20	1.536±1.731	22	1.331±0.416	34
IP (mg/dL)	6.283±0.946	10	6.288±0.913	50	6.391±1.229	22	5.500±0.854	69
Ca (mg/dL)	9.23±0.50	10	9.51±0.48	60	10.40±1.05	22	9.50±0.67	69
Na (mEq/L)	142.5±1.3	10	144.2±1.7	60	144.7±1.8	22	145.3±3.8	70
K (mEq/L)	3.93±0.39	10	4.03±0.47	60	4.60±1.11	22	4.35±0.46	70
Cl (mEq/L)	103.4±1.5	10	105.8±2.7	60	103.4±2.0	22	106.1±3.3	70
Albumin (%)	—	—	48.41±2.00	50	48.09±2.68	22	46.43±6.25	34
α 1 globulin (%)	—	—	22.49±2.20	50	21.99±2.49	22	22.54±4.21	34
α 2 globulin (%)	—	—	7.12±1.14	50	6.99±1.66	22	7.46±1.75	34
β globulin (%)	—	—	18.43±2.57	50	15.75±1.70	22	16.28±2.29	34
γ globulin (%)	—	—	3.56±1.58	50	7.19±0.98	22	7.29±3.08	34
A/G	—	—	0.941±0.073	50	0.931±0.102	22	0.890±0.201	34

Table 3-2. Serum Biochemistry (Female)

Age	7-8 Weeks				9-13 Weeks			
Strain	IGS		SD		IGS		SD	
	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N
AST (IU/L)	86.3±16.2	26	103.8±15.3	13	79.2±39.1	95	112.2±22.9	70
ALT (IU/L)	21.3±5.3	26	18.2±3.4	13	23.0±12.2	95	23.4±7.1	70
ALP (IU/L)	276.7±55.2	26	268.0±53.1	13	196.9±42.2	95	208.8±62.3	70
LDH (IU/L)	812.3±322.3	6	2427.0±906.1	3	1142.7±1615.0	95	3676.0±1523.5	24
CPK (IU/L)	296.8±145.2	6	718.9±156.8	10	326.5±345.2	90	743.5±246.9	45
Total bilirubin (mg/dL)	0.136±0.018	26	0.149±0.025	13	0.154±0.038	95	0.257±0.049	70
Total protein (g/dL)	5.32±0.41	26	5.32±0.24	13	5.71±0.36	95	5.76±0.42	70
Albumin (g/dL)	4.04±0.33	26	3.89±0.14	10	4.10±0.22	95	3.96±0.37	70
Total cholesterol (mg/dL)	66.5±11.9	26	73.8±13.3	13	62.0±15.2	95	58.7±15.4	70
TGL (mg/dL)	22.1±8.3	26	44.5±22.9	13	16.2±5.2	95	33.6±30.0	70
PL (mg/dL)	107.8±30.6	6	139.7±20.3	10	106.5±18.8	30	110.3±26.8	45
Glucose (mg/dL)	132.5±17.5	26	119.1±18.7	13	137.6±26.2	95	126.8±15.6	70
BUN (mg/dL)	16.22±2.83	26	17.93±4.41	13	19.75±4.34	95	18.98±3.01	70
Creatinine (mg/dL)	0.454±0.102	26	0.386±0.044	13	0.463±0.093	95	0.450±0.073	70
UA (mg/dL)	1.068±0.173	6	1.473±0.535	10	0.584±0.459	40	1.245±0.418	50
IP (mg/dL)	8.865±0.900	26	9.121±1.304	13	7.878±1.263	95	8.125±1.152	70
Ca (mg/dL)	9.55±0.49	26	9.22±0.49	13	9.44±0.46	95	9.56±0.53	70
Na (mEq/L)	144.5±1.7	26	141.8±1.2	13	145.1±1.9	95	143.9±2.0	70
K (mEq/L)	3.83±0.22	26	4.33±0.85	13	3.80±0.54	95	4.08±0.32	70
Cl (mEq/L)	104.3±2.0	26	103.8±1.7	13	106.3±2.5	95	107.1±2.6	70
Albumin (%)	57.27±1.60	6	58.83±0.86	10	56.58±3.07	50	57.79±2.79	55
α 1 globulin (%)	14.05±1.54	6	17.20±0.69	10	15.55±1.65	50	16.52±3.06	55
α 2 globulin (%)	8.05±0.65	6	7.40±0.36	10	7.70±1.36	50	7.27±1.41	55
β globulin (%)	13.85±1.13	6	12.79±0.67	10	14.18±2.51	50	14.83±1.86	55
γ globulin (%)	6.78±0.96	6	3.78±0.33	10	6.00±1.74	50	3.59±0.84	55
A/G	1.342±0.086	6	1.431±0.049	10	1.315±0.171	50	1.379±0.159	55

Table 3-2. Continued

Age	16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD	
	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N	Mean ±SD	N
AST (IU/L)	70.4±24.8	10	78.0±30.0	70	120.6±52.4	22	109.3±56.6	69
ALT (IU/L)	33.5±19.5	10	29.5±14.7	70	49.2±27.6	22	43.8±39.4	69
ALP (IU/L)	69.0±16.2	10	78.8±24.3	70	48.1±16.0	22	71.9±32.5	69
LDH (IU/L)	277.5±141.1	10	1290.7±1500.2	40	2094.0±731.4	12	1620.3±1206.5	44
CPK (IU/L)	89.6±26.8	10	351.9±299.9	40	370.9±228.5	22	344.3±237.5	54
Total bilirubin (mg/dL)	0.171±0.019	10	0.253±0.068	70	0.185±0.042	22	0.254±0.075	69
Total protein (g/dL)	6.47±0.53	10	6.51±0.53	70	6.73±0.52	22	6.62±0.51	69
Albumin (g/dL)	4.58±0.34	10	4.44±0.40	70	4.87±0.37	22	4.37±0.49	69
Total cholesterol (mg/dL)	70.4±13.3	10	67.6±16.7	70	92.3±22.8	22	75.3±20.2	69
TGL (mg/dL)	23.1±8.2	10	33.3±29.6	70	44.4±31.4	22	64.4±49.0	69
PL (mg/dL)	–	–	147.6±35.5	40	172.1±36.0	22	153.1±32.5	34
Glucose (mg/dL)	148.0±12.8	10	151.0±23.9	70	139.2±13.4	22	134.4±31.1	69
BUN (mg/dL)	14.95±2.94	10	17.64±3.13	70	17.92±3.70	22	19.98±3.62	69
Creatinine (mg/dL)	0.426±0.061	10	0.518±0.073	70	0.649±0.134	22	0.555±0.080	69
UA (mg/dL)	0.183±0.074	10	0.782±0.205	30	0.930±0.227	22	1.132±0.306	34
IP (mg/dL)	5.552±0.471	10	5.762±1.881	60	4.965±1.639	22	5.362±1.319	69
Ca (mg/dL)	9.28±0.65	10	10.01±0.38	70	10.21±0.47	22	9.82±0.62	69
Na (mEq/L)	142.3±1.1	10	143.3±1.7	70	144.0±1.7	22	145.4±3.3	69
K (mEq/L)	3.40±0.18	10	3.84±0.43	70	3.74±0.26	22	3.89±0.35	69
Cl (mEq/L)	104.9±1.7	10	107.9±4.1	70	104.3±2.3	22	107.2±2.5	69
Albumin (%)	—	—	55.20±3.46	60	55.73±3.74	22	53.60±6.26	34
α 1 globulin (%)	—	—	16.18±2.52	60	15.02±1.90	22	17.29±2.79	34
α 2 globulin (%)	—	—	6.77±1.55	60	6.45±1.64	22	6.14±1.44	34
β globulin (%)	—	—	17.50±4.02	60	14.46±1.71	22	15.12±2.58	34
γ globulin (%)	—	—	4.35±1.77	60	8.33±1.15	22	7.85±2.70	34
A/G	—	—	1.244±0.165	60	1.273±0.186	22	1.190±0.263	34

Table 4-1. Organ weight (Male)

Age	7-8 Weeks				9-13 Weeks				16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD		IGS		SD		IGS		SD	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
Liver (g)	9.10±1.12	36	8.66±1.50	43	9.56±1.50	95	9.76±1.74	70	14.01±2.61	10	14.38±2.37	70	15.66±1.80	22	16.36±4.20	69
Liver (g%)	3.76±0.37		3.69±0.46		3.03±0.29		3.13±0.28		2.70±0.24		2.72±0.30		2.70±0.26		2.72±0.42	
Kidneys (R+L, g)	2.35±0.19	36	2.19±0.23	38	2.56±0.35	95	2.53±0.31	70	3.47±0.31	10	3.47±0.39	30	3.92±0.43	22	3.34±0.55	49
Kidneys (R+L, g%)	0.97±0.06		0.95±0.06		0.81±0.08		0.82±0.06		0.68±0.03		0.64±0.05		0.68±0.08		0.59±0.08	
Heart (g)	1.00±0.09	36	0.92±0.12	30	1.10±0.14	90	1.09±0.16	60	1.48±0.12	10	1.48±0.16	60	1.65±0.13	22	1.50±0.18	69
Heart (g%)	0.41±0.03		0.39±0.03		0.35±0.03		0.35±0.03		0.29±0.03		0.28±0.03		0.28±0.02		0.25±0.03	
Lung (g)	1.08±0.09	36	1.00±0.11	23	1.19±0.12	95	1.20±0.16	70	1.46±0.15	10	1.45±0.14	60	1.59±0.08	22	1.58±0.32	69
Lung (g%)	0.45±0.03		0.46±0.04		0.38±0.03		0.39±0.04		0.28±0.02		0.28±0.03		0.27±0.02		0.27±0.05	
Spleen (g)	0.70±0.13	36	0.58±0.13	33	0.63±0.12	95	0.63±0.12	70	0.84±0.18	10	0.80±0.12	60	0.83±0.12	22	0.92±0.45	69
Spleen (g%)	0.29±0.06		0.25±0.04		0.20±0.04		0.20±0.03		0.16±0.02		0.15±0.02		0.14±0.02		0.16±0.07	
Brain (g)	1.85±0.08	36	1.90±0.08	33	1.96±0.09	95	1.95±0.10	70	2.13±0.09	10	2.12±0.11	60	2.19±0.08	22	2.19±0.11	69
Brain (g%)	0.77±0.06		0.82±0.08		0.63±0.06		0.63±0.07		0.42±0.04		0.41±0.03		0.38±0.03		0.38±0.06	
Thymus (g)	0.65±0.16	36	0.63±0.14	30	0.47±0.13	90	0.57±0.17	60	0.36±0.10	10	0.40±0.13	60	0.22±0.06	22	0.32±0.12	69
Thymus (g%)	0.27±0.06		0.27±0.07		0.15±0.04		0.18±0.05		0.07±0.01		0.08±0.02		0.04±0.01		0.05±0.02	
Submandibular glands (R+L, g)	0.48±0.04	16	0.47±0.05	30	0.59±0.10	90	0.55±0.08	60	0.69±0.09	10	0.69±0.09	30	0.77±0.08	22	0.67±0.09	49
Submandibular glands (R+L, g%)	0.20±0.02		0.20±0.01		0.19±0.03		0.18±0.02		0.13±0.02		0.13±0.01		0.13±0.01		0.12±0.02	
Pituitary (mg)	10±1	36	10±2	30	11±2	90	10±2	60	14±2	10	13±2	70	14±1	22	13±3	69
Pituitary (mg%)	4.1±0.5		4.2±0.9		3.4±0.5		3.3±0.5		2.7±0.4		2.5±0.4		2.4±0.3		2.2±0.5	
Thyroids (R+L, mg)	17±4	16	17±4	20	18±5	90	21±4	60	24±7	10	26±5	30	28±5	22	28±7	46
Thyroids (R+L, mg%)	7.3±1.4		7.7±1.8		5.8±1.5		6.7±1.4		4.7±1.1		4.8±0.9		4.8±0.8		4.9±1.1	
Adrenals (R+L, mg)	56±8	36	52±7	33	59±9	95	58±12	70	60±8	10	60±9	40	61±7	22	57±13	49
Adrenals (R+L, mg%)	23.0±3.0		22.4±2.6		18.8±2.9		18.6±3.6		11.7±1.9		11.1±1.8		10.5±1.7		10.1±2.0	
Testes (R+L, g)	2.41±0.19	36	2.25±0.13	23	3.01±0.26	95	2.90±0.22	70	3.45±0.18	10	3.41±0.26	40	3.45±0.33	22	3.30±0.28	49
Testes (R+L, g%)	1.00±0.10		1.02±0.06		0.96±0.12		0.94±0.11		0.67±0.06		0.63±0.06		0.60±0.07		0.59±0.11	
Epididymides (R+L, g)	0.56±0.17	16	0.38±0.04	20	0.83±0.11	90	0.76±0.16	60	1.38±0.14	10	1.42±0.13	30	1.51±0.13	22	1.42±0.16	49
Epididymides (R+L, g%)	0.23±0.06		0.17±0.02		0.27±0.03		0.25±0.05		0.27±0.02		0.26±0.03		0.26±0.03		0.26±0.04	
Prostate (g)	0.56±0.19	36	0.50±0.11	20	0.85±0.20	90	0.86±0.23	60	1.36±0.30	10	1.42±0.27	60	1.40±0.27	22	1.11±0.37	69
Prostate (g%)	0.23±0.07		0.23±0.05		0.27±0.06		0.28±0.06		0.26±0.06		0.27±0.05		0.24±0.05		0.19±0.08	
Seminal vesicle (g)	0.61±0.21	16	0.42±0.10	20	0.99±0.20	80	0.91±0.27	60	—	0	1.69±0.30	60	1.95±0.29	22	1.70±0.30	49
Seminal vesicle (g%)	0.26±0.08		0.19±0.04		0.32±0.06		0.29±0.07		—		0.32±0.06		0.34±0.06		0.30±0.06	

Table 4-2. Organ weight (Female)

Age	7-8 Weeks				9-13 Weeks				16-23 Weeks				30-40 Weeks			
Strain	IGS		SD		IGS		SD		IGS		SD		IGS		SD	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
Liver (g)	6.71±0.98	26	6.40±1.25	23	6.11±0.76	95	6.28±0.87	70	7.33±0.86	10	7.59±1.16	80	8.32±1.71	22	8.72±2.35	69
Liver (g%)	3.79±0.36		3.93±0.63		2.97±0.24		3.12±0.21		2.59±0.17		2.64±0.32		2.54±0.26		2.66±0.41	
Kidneys (R+L, g)	1.74±0.18	26	1.53±0.15	18	1.64±0.23	95	1.66±0.20	70	2.09±0.20	10	1.90±0.23	40	2.11±0.28	22	1.97±0.25	49
Kidneys (R+L, g%)	0.98±0.07		0.95±0.07		0.80±0.08		0.83±0.06		0.74±0.05		0.66±0.06		0.65±0.06		0.65±0.07	
Heart (g)	0.76±0.08	26	0.64±0.06	10	0.74±0.08	90	0.73±0.09	60	0.93±0.05	10	0.92±0.11	70	1.06±0.12	22	0.96±0.11	69
Heart (g%)	0.43±0.03		0.41±0.02		0.36±0.03		0.37±0.03		0.33±0.02		0.32±0.03		0.33±0.03		0.30±0.03	
Lung (g)	0.93±0.08	26	0.86±0.08	13	0.95±0.08	95	0.98±0.10	70	1.07±0.07	10	1.11±0.10	70	1.16±0.12	22	1.17±0.18	69
Lung (g%)	0.53±0.04		0.54±0.06		0.47±0.04		0.49±0.04		0.38±0.02		0.39±0.03		0.36±0.04		0.37±0.06	
Spleen (g)	0.48±0.07	26	0.41±0.07	13	0.46±0.08	95	0.47±0.08	70	0.53±0.07	10	0.53±0.09	70	0.55±0.09	22	0.60±0.26	69
Spleen (g%)	0.27±0.04		0.25±0.04		0.22±0.03		0.23±0.03		0.19±0.03		0.19±0.03		0.17±0.03		0.19±0.08	
Brain (g)	1.72±0.07	26	1.77±0.06	13	1.83±0.08	95	1.83±0.07	70	1.91±0.05	10	1.94±0.08	70	1.98±0.10	22	2.02±0.11	69
Brain (g%)	0.98±0.08		1.11±0.06		0.90±0.08		0.92±0.09		0.68±0.05		0.68±0.06		0.62±0.08		0.64±0.10	
Thymus (g)	0.55±0.13	26	0.54±0.10	10	0.45±0.11	90	0.50±0.09	60	0.30±0.07	10	0.34±0.09	70	0.19±0.06	22	0.23±0.07	69
Thymus (g%)	0.31±0.07		0.34±0.07		0.22±0.04		0.26±0.05		0.11±0.03		0.12±0.02		0.06±0.02		0.07±0.02	
Submandibular glands (R+L, g)	0.35±0.02	6	0.34±0.02	10	0.41±0.07	90	0.39±0.04	60	0.41±0.04	10	0.42±0.04	40	0.47±0.05	22	0.45±0.05	49
Submandibular glands (R+L, g%)	0.23±0.02		0.22±0.01		0.20±0.03		0.20±0.02		0.14±0.01		0.15±0.02		0.15±0.02		0.15±0.02	
Pituitary (mg)	10±2	26	11±2	10	12±2	90	12±2	60	16±4	10	16±3	80	20±7	22	19±6	69
Pituitary (mg%)	5.9±1.3		6.9±1.2		6.1±1.2		6.2±1.0		5.5±1.1		5.7±1.2		6.1±1.4		6.1±1.7	
Thyroids (R+L, mg)	17±3	6	12±3	10	15±5	90	17±4	60	18±3	10	22±5	40	20±4	22	21±5	49
Thyroids (R+L, mg%)	11.3±1.9		7.5±2.0		7.4±2.3		8.7±1.9		6.4±1.1		7.6±1.7		6.2±1.4		7.0±1.6	
Adrenals (R+L, mg)	60±7	26	49±8	13	62±10	95	63±10	70	69±7	10	60±10	50	68±9	22	61±11	49
Adrenals (R+L, mg%)	33.8±4.1		31.1±4.5		30.5±5.0		31.7±4.7		25.6±2.6		21.0±3.4		21.1±3.7		20.3±4.4	
Ovaries (R+L, mg)	71±12	26	71±12	13	82±16	95	84±17	70	74±15	10	77±14	50	63±18	22	68±21	49
Ovaries (R+L, mg%)	40.4±6.8		45.1±7.4		39.9±7.3		41.9±7.3		26.3±5.4		27.0±4.5		19.5±6.2		22.4±6.4	
Uterus (g)	0.34±0.07	20	0.38±0.09	10	0.46±0.15	90	0.42±0.15	60	0.53±0.10	10	0.55±0.19	80	0.77±0.23	22	0.68±0.23	49
Uterus (g%)	0.19±0.04		0.24±0.06		0.23±0.08		0.22±0.08		0.19±0.03		0.19±0.07		0.24±0.06		0.23±0.09	

Table 5. Histopathology

		Male		Female	
		IGS N=174	SD N=3777	IGS N=164	SD N=3740
Heart					
	Mononuclear cell infiltration	30 (17.24%)	126 (3.34%)	7 (4.27%)	25 (0.67%)
	Necrosis	12 (6.90%)	53 (1.40%)	1 (0.61%)	17 (0.45%)
	Fibrosis	3 (1.72%)	36 (0.95%)	0 (0.00%)	8 (0.21%)
	Hemorrhage	1 (0.57%)	3 (0.08%)	0 (0.00%)	0 (0.00%)
	Thrombus, right atrium	0 (0.00%)	4 (0.11%)	0 (0.00%)	2 (0.05%)
	Thickening, endocardium	0 (0.00%)	2 (0.05%)	0 (0.00%)	1 (0.03%)
	Inflammatory cell infiltration	0 (0.00%)	2 (0.05%)	0 (0.00%)	1 (0.03%)
	Thickening, epicardium	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Mineralization	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.05%)
Aorta (Thoracic)					
	Mineralization, intima	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Spleen					
	Inflammatory cell infiltration, capsule	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Thymus					
	Cyst	1 (0.57%)	10 (0.26%)	3 (1.83%)	29 (0.78%)
	Thymoma, benign	0 (0.00%)	1 (0.03%)	1 (0.61%)	0 (0.00%)
	Hemorrhage	0 (0.00%)	10 (0.26%)	0 (0.00%)	6 (0.16%)
	Mononuclear cell infiltration, capsule	0 (0.00%)	5 (0.13%)	0 (0.00%)	3 (0.08%)
	Thickening, capsule	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Inflammatory cell infiltration, capsule	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Foreign body granuloma	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.05%)
Bone marrow (Sternal)					
	Fibrosis	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Submandibular lymph node					
	Giant cell, sinus	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
Trachea					
	Squamous metaplasia	2 (1.15%)	5 (0.13%)	0 (0.00%)	1 (0.03%)
Lung					
	Foamy cell, alveolus	50 (28.74%)	102 (2.70%)	34 (20.73%)	81 (2.17%)
	Osseous metaplasia	6 (3.45%)	0 (0.00%)	3 (1.83%)	1 (0.03%)
	Mononuclear cell infiltration, perivascular	6 (3.45%)	38 (1.01%)	2 (1.22%)	34 (0.91%)
	Mineralization, vascular wall	6 (3.45%)	22 (0.58%)	1 (0.61%)	16 (0.43%)
	Hemorrhage, alveolus	4 (2.30%)	4 (0.11%)	3 (1.83%)	1 (0.03%)
	Inflammatory cell infiltration, alveolus	3 (1.72%)	3 (0.08%)	1 (0.61%)	5 (0.13%)
	Congestion, focal	3 (1.72%)	2 (0.05%)	0 (0.00%)	0 (0.00%)
	Hematoidin crystal, alveolus	1 (0.57%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Arteritis	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Inflammatory cell infiltration, perivascular	0 (0.00%)	6 (0.16%)	1 (0.61%)	6 (0.16%)
	Foreign body granuloma	0 (0.00%)	44 (1.16%)	0 (0.00%)	26 (0.70%)
	Thickening, vascular wall	0 (0.00%)	5 (0.13%)	0 (0.00%)	2 (0.05%)
	Thrombus	0 (0.00%)	2 (0.05%)	0 (0.00%)	4 (0.11%)
	Thickening, pleura	0 (0.00%)	2 (0.05%)	0 (0.00%)	0 (0.00%)
	Squamous metaplasia, bronchus	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Tongue					
	Necrosis, muscle fiber	1 (0.57%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Regeneration, muscle fiber	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Thrombus	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Esophagus					
	Mononuclear cell infiltration, adventitia	3 (1.72%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Granulation tissue, adventitia	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 5. Continued

		Male		Female	
		IGS N=174	SD N=3777	IGS N=164	SD N=3740
Stomach					
	Dilatation, gland, fundus	2 (1.15%)	34 (0.90%)	1 (0.61%)	27 (0.72%)
	Inflammatory cell infiltration, submucosa, fundus	1 (0.57%)	13 (0.34%)	0 (0.00%)	1 (0.03%)
	Hyperkeratosis, limiting ridge	1 (0.57%)	4 (0.11%)	0 (0.00%)	1 (0.03%)
	Epidermal cyst, forestomach	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Erosion, forestomach	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Hyaline droplet, chief cell, fundus	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Hemorrhage, lamina propria, fundus	0 (0.00%)	0 (0.00%)	3 (1.83%)	1 (0.03%)
	Edema, submucosa, fundus	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Mineralization, lamina propria, fundus	0 (0.00%)	1 (0.03%)	0 (0.00%)	1 (0.03%)
	Granuloma, submucosa, fundus	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Mineralization, submucosa, fundus	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Duodenum					
	Inflammatory cell infiltration, lamina propria	1 (0.57%)	1 (0.03%)	0 (0.00%)	1 (0.03%)
	Erosion	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Dilatation, crypt	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Jejunum					
	Mineralization, lamina propria	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Ileum					
	Inflammatory cell infiltration, muscular layer	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cecum					
	Dilatation, gland	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Mineralization, epithelium	0 (0.00%)	1 (0.03%)	0 (0.00%)	2 (0.05%)
	Granuloma, submucosa	0 (0.00%)	1 (0.03%)	0 (0.00%)	1 (0.03%)
	Arteritis	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Hemorrhage, lamina propria	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Colon					
	Hemorrhage, lamina propria	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Hemorrhage, serosa	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Granuloma, lamina propria	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Mononuclear cell infiltration, serosa	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Fibrosis, mesentery	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
Pancreas					
	Mononuclear cell infiltration	11 (6.32%)	52 (1.38%)	8 (4.88%)	32 (0.86%)
	Brown pigment deposition	3 (1.72%)	55 (1.46%)	0 (0.00%)	4 (0.11%)
	Chronic insulitis	1 (0.57%)	73 (1.93%)	0 (0.00%)	7 (0.19%)
	Regenerating nodule	0 (0.00%)	25 (0.66%)	0 (0.00%)	8 (0.21%)
	Microgranuloma	0 (0.00%)	4 (0.11%)	0 (0.00%)	3 (0.08%)
	Inflammatory cell infiltration	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Liver					
	Mononuclear cell infiltration, periportal	104 (59.77%)	228 (6.04%)	83 (50.61%)	194 (5.19%)
	Microgranuloma	56 (32.18%)	184 (4.87%)	47 (28.66%)	163 (4.36%)
	Vacuolation, hepatocyte, periportal	12 (6.90%)	254 (6.72%)	15 (9.15%)	133 (3.56%)
	Necrosis	5 (2.87%)	12 (0.32%)	1 (0.61%)	4 (0.11%)
	Inflammatory cell infiltration, periportal	1 (0.57%)	2 (0.05%)	0 (0.00%)	1 (0.03%)
	Fibrosis	1 (0.57%)	3 (0.08%)	0 (0.00%)	1 (0.03%)
	Peliosis	1 (0.57%)	1 (0.03%)	0 (0.00%)	2 (0.05%)
	Bile duct proliferation	0 (0.00%)	14 (0.37%)	0 (0.00%)	6 (0.16%)
	Hypertrophy, Kupffer cell	0 (0.00%)	1 (0.03%)	0 (0.00%)	2 (0.05%)
	Foci	0 (0.00%)	1 (0.03%)	0 (0.00%)	1 (0.03%)
	Hepatocellular adenoma	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Microabscess	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)

Table 5: Continued

		Male		Female	
		IGS N=174	SD N=3777	IGS N=164	SD N=3740
Kidney					
	Regenerating tubule	90 (51.72%)	228 (6.04%)	36 (21.95%)	89 (2.38%)
	Mononuclear cell infiltration	58 (33.33%)	113 (2.99%)	25 (15.24%)	50 (1.34%)
	Eosinophilic body, proximal tubule	51 (29.31%)	102 (2.70%)	3 (1.83%)	7 (0.19%)
	Hyaline cast	21 (12.07%)	98 (2.59%)	7 (4.27%)	30 (0.80%)
	Mineralization, cortex	11 (6.32%)	18 (0.48%)	8 (4.88%)	13 (0.35%)
	Cyst, cortex	8 (4.60%)	9 (0.24%)	0 (0.00%)	2 (0.05%)
	Mineralization, papilla	5 (2.87%)	43 (1.14%)	8 (4.88%)	48 (1.28%)
	Dilatation, pelvis	5 (2.87%)	12 (0.32%)	0 (0.00%)	6 (0.16%)
	Glomerulosclerosis, focal	2 (1.15%)	12 (0.32%)	0 (0.00%)	1 (0.03%)
	Inflammatory cell infiltration, papilla	2 (1.15%)	2 (0.05%)	0 (0.00%)	3 (0.08%)
	Calculus, pelvis	1 (0.57%)	4 (0.11%)	5 (3.05%)	11 (0.29%)
	Hyperplasia, pelvic epithelium	1 (0.57%)	16 (0.42%)	3 (1.83%)	3 (0.08%)
	Fibrosis	1 (0.57%)	18 (0.48%)	1 (0.61%)	6 (0.16%)
	Cyst, medulla	1 (0.57%)	2 (0.05%)	0 (0.00%)	0 (0.00%)
	Amyloidosis	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Osseous metaplasia, pelvis	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Liposarcoma	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Urinary bladder					
	Mononuclear cell infiltration, lamina propria	1 (0.57%)	5 (0.13%)	0 (0.00%)	1 (0.03%)
	Hyperplasia, epithelium	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Inflammatory cell infiltration, lamina propria	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.05%)
Testis					
	Atrophy, seminiferous tubule	3 (1.72%)	7 (0.19%)	− (−)	− (−)
	Giant cell, seminiferous tubule	2 (1.15%)	1 (0.03%)	− (−)	− (−)
	Vacuolation, seminiferous tubule	1 (0.57%)	0 (0.00%)	− (−)	− (−)
Epididymis					
	Mononuclear cell infiltration	9 (5.17%)	14 (0.37%)	− (−)	− (−)
	Decreased sperm	2 (1.15%)	2 (0.05%)	− (−)	− (−)
	Spermatic granuloma	1 (0.57%)	0 (0.00%)	− (−)	− (−)
	Spermatogenic cell, lumen	1 (0.57%)	0 (0.00%)	− (−)	− (−)
	Fibrosis	1 (0.57%)	0 (0.00%)	− (−)	− (−)
	Inflammatory cell infiltration	(0.00%)	1 (0.03%)	− (−)	− (−)
Prostate					
	Mononuclear cell infiltration, interstitium	35 (20.11%)	50 (1.32%)	− (−)	− (−)
	Inflammatory cell infiltration, lumen	6 (3.45%)	18 (0.48%)	− (−)	− (−)
	Mineralization, lumen	3 (1.72%)	2 (0.05%)	− (−)	− (−)
	Fibrosis	0 (0.00%)	5 (0.13%)	− (−)	− (−)
	Hemorrhage	0 (0.00%)	1 (0.03%)	− (−)	− (−)
Ovary					
	Cyst	− (−)	− (−)	2 (1.22%)	9 (0.24%)
	Mineralization, oocyte	− (−)	− (−)	2 (1.22%)	1 (0.03%)
Uterus					
	Hydrometra	− (−)	− (−)	6 (3.66%)	25 (0.67%)
	Endometrial stromal polyp	− (−)	− (−)	0 (0.00%)	1 (0.03%)
Vagina					
	Cyst	− (−)	− (−)	0 (0.00%)	2 (0.05%)
Mammary gland					
	Necrosis, muscle fiber	− (−)	− (−)	1 (0.61%)	0 (0.00%)
	Inflammatory cell infiltration	− (−)	− (−)	1 (0.61%)	0 (0.00%)
	Fibroadenoma	− (−)	− (−)	0 (0.00%)	11 (0.29%)
	Fibrosis	− (−)	− (−)	0 (0.00%)	1 (0.03%)
Pituitary					
	Cyst, pars distalis	3 (1.72%)	8 (0.21%)	1 (0.61%)	5 (0.13%)
	Cyst, pars intermedia	1 (0.57%)	0 (0.00%)	0 (0.00%)	2 (0.05%)
	Cyst, pars nervosa	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Adenoma	0 (0.00%)	9 (0.24%)	0 (0.00%)	19 (0.51%)
	Mineralization, pars distalis	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
	Mineralization, pars intermedia	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)

Table 5. Continued

		Male		Female	
		IGS N=174	SD N=3777	IGS N=164	SD N=3740
Thyroid					
	Ultimobranchial body	25 (14.37%)	31 (0.82%)	25 (15.24%)	47 (1.26%)
	Ectopic thymus	8 (4.60%)	1 (0.03%)	6 (3.66%)	7 (0.19%)
	Mineralization, colloid	3 (1.72%)	6 (0.16%)	0 (0.00%)	0 (0.00%)
	Mononuclear cell infiltration	1 (0.57%)	7 (0.19%)	0 (0.00%)	2 (0.05%)
	C-cell hyperplasia	0 (0.00%)	8 (0.21%)	0 (0.00%)	11 (0.29%)
	Cyst	0 (0.00%)	1 (0.03%)	0 (0.00%)	1 (0.03%)
Adrenal					
	Cortical nodule	1 (0.57%)	12 (0.32%)	0 (0.00%)	18 (0.48%)
	Osseous metaplasia	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Accessory adrenal	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Peliosis	0 (0.00%)	3 (0.08%)	0 (0.00%)	26 (0.70%)
	Mineralization, capsule	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
	Mononuclear cell infiltration	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
	Mineralization, cortex	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Cerebrum					
	Meningioma	0 (0.00%)	0 (0.00%)	1 (0.61%)	0 (0.00%)
	Astrocytoma	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.05%)
	Hydrocephalus	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
	Gliosis	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Cerebellum					
	Mononuclear cell infiltration, meninx	1 (0.57%)	1 (0.03%)	2 (1.22%)	0 (0.00%)
	Inflammatory cell infiltration, meninx	0 (0.00%)	0 (0.00%)	2 (1.22%)	0 (0.00%)
Sciatic nerve					
	Mononuclear cell infiltration	0 (0.00%)	1 (0.03%)	0 (0.00%)	0 (0.00%)
Skeletal muscle					
	Mononuclear cell infiltration	3 (1.72%)	3 (0.08%)	1 (0.61%)	2 (0.05%)
	Necrosis	3 (1.72%)	2 (0.05%)	1 (0.61%)	1 (0.03%)
	Regeneration	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Eyeball					
	Retinal dysplasia	8 (4.60%)	4 (0.11%)	6 (3.66%)	8 (0.21%)
	Mineralization, corneal epithelium	1 (0.57%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Cataract	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
	Neuritis, optic nerve	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)
Lacrimal gland					
	Mononuclear cell infiltration	14 (8.05%)	30 (0.79%)	4 (2.44%)	6 (0.16%)
Harderian gland					
	Mononuclear cell infiltration	7 (4.02%)	18 (0.48%)	6 (3.66%)	30 (0.80%)
Submandibular gland					
	Mononuclear cell infiltration	2 (1.15%)	2 (0.05%)	0 (0.00%)	1 (0.03%)
	Mineralization, gland	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.03%)

A Comparison of Levels of Cytochrome P-450 Induced by Phenobarbital between Crj:CD(SD)IGS and Crj:CD(SD) Rats

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ABSTRACT. Male and female of both Crj:CD(SD)IGS and Crj:CD(SD) rats were subjected to repeated oral administration of sodium phenobarbital at a daily dose of 80 mg/kg for 7 days. After the last administration, body and liver weight, relative liver weight, and contents of microsomal protein and cytochrome P-450 were determined.

In male and female rats of both strains, significant increase in liver weight, relative liver weight, and contents of microsomal protein and cytochrome P-450 were observed in comparison with each negative control. The increase in cytochrome P-450 content in Crj:CD(SD)IGS rats was greater than that in Crj:CD(SD) rats in both sexes, and increase in cytochrome P-450 content was greater in male than in female rats. － Key words: Crj:CD(SD) rats, Crj:CD(SD)IGS rats, Comparison, Cytochrome P450

CD(SD)IGS-1999: 119-120

INTRODUCTION

International Genetic Standard System (IGS system) was designed by Charles River Inc. Group to minimize a genetic divergence of animals and to supply animals possessing homogeneous characteristics. The induction level of cytochrome P-450 of Crj:CD(SD)IGS rats thus produced were compared with that of Crj:CD(SD) rats. In this study, sodium phenobarbital, a typical inducer of cytochrome P-450, was orally administered to rats of both strains once a day for 7 days, and contents of microsomal protein and cytochrome P-450 were compared between Crj:CD(SD)IGS and Crj:CD(SD) rats.

MATERIALS AND METHODS

1. Test Compound

Sodium phenobarbital (Lot No. ACL1510) was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan).

2. Animals

Ten rats each for both sexes of Crj:CD(SD)IGS and Crj:CD(SD) strain produced by Charles River Japan Inc. were used in the study.

3. Housing Conditions

Rats were housed in polycarbonate cages after receipt, and were acclimated for 7 days. The animals were bred in the animal room in the Technical Center, Department of Production, Charles River Japan Inc. The study was carried out under the controlled environmental conditions of a temperature of 21 ～ 23℃ and a relative humidity of 50% ～ 60%, Rats were freely provided with solid feed (CRF-1) and tap water.

4. Animal Grouping

The Crj:CD(SD)IGS and Crj:CD(SD) rats were divided into a negative control group and a phenobarbital-treated group of 5 rats each. The animal grouping of female rats was the same as male rats.

5. Dosage

Sodium phenobarbital was orally administered to non-fasted rats at a daily dose of 80 mg/kg (10 mL/kg) once a day at 24-h intervals for 7 days. As negative control, formulation vehicle (5% arabic gum, Lot No. PAM0199, Wako Pure Chemical Industries Ltd.) was orally administered to non-fasted rats at a daily dose of 10 mL/kg once a day at 24-h intervals for 7 days. The preparation and administration of dosing formulation was carried out in Charles River Japan Inc.

6. Sample Collection, Preparation and Analysis

Rats were subjected to repeated oral administration of sodium phenobarbital or formulation vehicle once a day at 24-h intervals for 7 days. Twenty-four hours after the final administration, the rats fasted for 16 h were weighed and killed by decapitation and the liver was immediately dissected, weighed, perfused with 1.15% KC1 aqueous solution. These operations were carried out at 4℃ in Charles River Japan Inc. and the ice-chilled liver was immediately sent to Nemoto & Co., Ltd. The liver was homogenized in 3 volumes of 0.15 M KC1-10 mM EDTA (pH7.4). The homogenate was centrifuged at 9,000 × g for 20 min and the microsomal fraction was obtained by centrifugation of the supernatant at 105,000 × g for 60 min. The resulting pellet was suspended in 20% glycerol-10 mM potassium phosphate buffer (pH 7.25). The contents of protein and cytochrome P-450 in the microsome suspension were determined. All the operations were carried out at 4℃.

7. Measurement

The body and liver weight, relative liver weight (% of body weight), and contents of microsomal protein and cytochrome P-450 were determined. Microsomal protein was measured by the method of Lowry et al [1]. Content of cytochrome P-450 was determined according to the method of Omura and Sato [2].

8. Experimental Records

Each result was expressed as the mean value ± standard deviation (S.D.) for 5 animals. Statistical difference in mean values was analyzed by Student's t-test and 2-way ANOVA (SAS, SAS Institute Inc.).

RESULTS AND DISCUSSION

Male and female of both Crj:CD(SD)IGS and Crj:CD(SD) rats were subjected to repeated oral administration of sodium phenobarbital at a daily dose of 80 mg/kg for 7 days. After the last administration, body and liver weight, relative liver weight, and contens of microsomal protein and cytochrome P-450 were determined (Table 1, Table 2)

In both sexes and strains, significant increase in the liver weight,

relative liver weight, and contents of microsomal protein and cytochrome P-450 were observed in comparison with each negative control group. The result of Student's t-test indicated significant differences in the liver weight of male Crj:CD(SD)IGS rats (P<0.05) and in all other items of male and female rats of both strains (P<0.01) compared with the negative control. No significant difference in body weight was observed in both strains compared with the respective negative control.

As to the degree of increase in microsomal cytochrome P-450 content, the increase was greater in Crj:CD(SD)IGS rats than in Crj:CD(SD) rats in both sexes. In both strains, the increase was greater in male rats than in female rats. The difference in the degree of increase in cytochrome P-450 content between the sexes in each strain and the difference between the strains in each sex were analyzed statistically using 2-way ANOVA. A significant difference between both strains was observed in female rats (P<0.05) but not in male rats. In both strains, a significant difference was observed between the sexes (P<0.05).

The above results indicated sodium phenobarbital induced hepatic microsomal cytochrome P-450 in both sexes of Crj:CD(SD)IGS and Crj:CD(SD) rats. The degree of induction of cytochrome P-450 in female rats was significantly greater in Crj:CD(SD)IGS than that in Crj:CD(SD) strain. In both strains, male rats showed a significantly great degree of induction compared with female rats.

REFERENCES

1. Lowry, O.H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. 1951. Biol. Chem,* 193: 265-275.
2. Omura, T. and Sato, R., *1964. J. Biol. Chem.,* 239: 2379-2385.

Table 1. Effect of repeated administration of phenobarbital-Na once a day for 7 days to male rats on liver drug metabolizing enzymes

	Crj:CD(SD)IGS			Crj:CD(SD)		
	Control	Phenobarbital-Na		Control	Phenobarbital-Na	
Body weight (g)	232±19	230±8		215±4	222±15	
Liver weight (g)	7.96±0.72	10.6±1.2	*	7.19±0.45	9.70±0.92	**
(% of body weight)	3.44±0.24	4.59±0.41	**	3.34±0.17	4.36±0.24	**
Microsomes						
Protein (mg/g liver)	23.3±1.5	42.0±3.7	**	21.5±4.8	35.2±3.2	**
Cytochrome P-450 (nmol/mg protein)	0.687±0.046	1.97±0.27	**	0.549±0.051	1.56±0.40	**

Each value represents the mean±S.D. of 5 rats.
*: Significantly different from the value of the control (P<0.05)
**: Significantly different from the value of the control (P<0.01)

Table 2. Effect of repeated administration of phenobarbital-Na once a day for 7 days to female rats on liver drug metabolizing enzymes

	Crj:CD(SD)IGS			Crj:CD(SD)		
	Control	Phenobarbital-Na		Control	Phenobarbital-Na	
Body weight (g)	157±9	159±3		154±2	151±6	
Liver weight (g)	4.98±0.29	6.7±0.1	**	5.16±0.26	6.18±0.30	**
(% of body weight)	3.17±0.14	4.18±0.08	**	3.34±0.16	4.11±0.15	**
Microsomes						
Protein (mg/g liver)	20.4±1.4	30.5±2.9	**	20.3±1.7	26.2±2.2	**
Cytochrome P-450 (nmol/mg protein)	0.483±0.072	1.30±0.23	**	0.491±0.080	0.963±0.230	**

Each value represents the mean±S.D. of 5 rats.
**: Significantly different from the value of the control (P<0.01)

A Comparison of Drug-Metabolizing Enzymes Between Crj:CD(SD)IGS Rats and Crj:CD(SD) Rats

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ABSTRACT. Activities of several drug-metabolizing enzymes in rat liver without induction, including aminopyrine-N-demethylase, aniline-p-hydroxylase, 7-ethoxycoumarin-O-deethylase, UDP-glucuronyltransferase, and cytochrome P-450 in microsomal fraction, as well as glutathione-S-transferase in the cytosol fraction, were compared between Crj:CD(SD)IGS and Crj:CD(SD) rats. In the Crj:CD(SD)IGS male rats, activities of aminopyrine-N-demethylase, aniline-p-hydroxlase, and 7-ethoxycoumarin-O-deethylase were higher than those in male Crj:CD(SD) rats at 9, 18, and 32 weeks of age. Similar results were obtained from the 9-week-old females. These results suggest that Crj:CD(SD)IGS rat and Crj:CD(SD) rat might show a difference in toxicological response to certain chemicals due to differences in their drug-metabolizing abilities. — key words: Crj:CD(SD)IGS, rat, drug-metabolizing enzyme, background data.

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INTRODUCTION

In order to obtain background data on Crj:CD(SD)IGS rats [IGS], the general toxicological parameters at 4- and 26-week time points (when the rats were approximately 9 and 32 weeks of age, respectively) were collected and compared with those of Crj:CD(SD) rats [SD][1]. On the other hand, in the safety evaluation of drugs and other chemicals, it is also important to know the proportion of drug-metabolizing enzymes because the efficiency of drug metabolism and elimination highly contribute to those toxic responses. Basal activities of several drug-metabolizing enzymes of Crj:CD(SD)IGS rats at 9, 18, and 32 weeks of age were measured and compared with those of Crj:CD(SD) rats.

MATERIALS AND METHODS

IGS and SD were obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan) at 4 weeks of age. They were maintained individually in metal cages, and fed commercial laboratory chow (CE-2) and tap water *ad libitum*. The livers were removed at the ages of 9, 18, and 32 weeks. These ages corresponded to the 4-, 13-, and 26-week time points in the toxicity studies. Microsome and cytosol fractions were prepared from the left lateral lobes of their livers. Microsomes were used for determination of aminopyrine-N-demethylase[2], aniline-p-hydroxylase[3], 7-ethoxy-coumarin-O-deethylase[4], UDP-glucuronyltransferase[5], and cytochrome P-450[5], and the cytosol fractions were used for glutathione-S-transferase assays with several substrates[6].

RESULTS AND DISCUSSION

Tables 1 and 2 show the body weight, liver weight, and microsome recovery in male and female rats, respectively, at the each of the evaluation times. Several parameters in IGS were significantly lower than those in SD.

Monooxygenase activities and cytochrome P450 contents (the latter in terms of nanomoles per milligram of liver microsomal protein) for both strains are shown in Table 3, and those activities and contents per gram of body weight for both strains are shown in Table 4.

In the IGS male rats, activities of aminopyrine-N-demethylase, aniline-p-hydroxylase, and 7-ethoxy-coumarin-O-deethylase were higher than those in SD males at 9, 18, and 32 weeks of age. Similar results were obtained from the 9-week-old females. These activities were recalculated from per mg of microsome to g of body weight, because the dosages of drugs or other chemicals for usual toxicological study are decided based on the body weight at the time of administration. After this conversion, a few activities showed a significant difference between both strains.

Activities of hepatic UDP-glucuronyltransferase in male and female rats are shown in Table 5. A clear difference in this enzyme activity between the two strains was not observed.

Tables 6 and 7 show the activities of hepatic glutathione-S-transferase in male and female rats, respectively, tested with several substrates. The activities toward a few substrates in IGS rats were significantly lower than those in SD animals.

The expression of cytochrome P450 is dependent on maturation, sex hormones, etc. In the body weight growth curve and enzyme activities, the difference between the two strains was the largest at 9 weeks of age in this time point study.

These results suggest that Crj:CD(SD)IGS and Crj:CD(SD) rats might show a difference in toxicological responses to certain some chemicals due to strain differences in drug-metabolizing abilities, especially in the case of the usage of younger rats. Further studies are required to evaluate various elements of these findings.

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REFERENCES

1. Kojima, K., Azegami, J., and Tanaka, A. 1999. Response to commercial low protein diet in Crj:CD(SD)IGS rats. pp - . In : Biological Reference Data on CD(SD)IGS Rats - 1999 (Matsuzawa, T. and Inoue, H. eds.) Best Printing Co. Ltd., Tokyo
2. Matsubara, T., Touchi, A., and Tochino, Y. 1977. Hepatic aminopyrine N-demethylase system : further studies of assay procedure. Japanese Journal of Pharmacology 27: 127-136.

3. Matsubara, T. and Tochino, Y. 1975. Inhibitory action of cyanide on aniline hydroxylase system. FEBS Letters 52: 77-80.
4. Matsubara, T., Otsubo, S., Yoshihara, E., and Touchi, A. 1983. Biotransformation of coumarin derivatives (2) Oxidative metabolism of 7-alkoxycoumarin by microsomal enzymes and a simple assay procedure for 7-alkoxycoumarin O-dealkylase. Japanese Journal of Pharmacology 33: 41-56.
5. Omura, T. and Sato, R. 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. Journal of Biological Chemistry 239: 2370-2378.
6. Habig, W.H., Pabst, M.J., and Jakoby, W.B. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249: 7130-7139.

Table 1. Body weight, liver weight and microsome recovery in male rats

	9 weeks (n=10)		18 weeks (n=10)		32 weeks (n=10)	
	SD	IGS	SD	IGS	SD	IGS
Body weight (g)	351.1 [a)]	322.9**	578.7	533.0	796.7	612.2**
	15.7	15.6	42.8	67.8	84.2	96.5
Liver weight (g)	11.44	9.95**	15.89	13.55*	22.80	14.76**
	0.96	0.86	1.54	2.50	3.80	2.65
Relative liver weight	32.57	30.79	27.46	25.30*	28.54	24.05**
(mg/g body weight)	2.23	1.84	1.76	1.89	3.21	1.00
Microsome recovery	23.63	25.21	17.44	19.62	18.80	17.87
(mg/g Liver)	5.37	4.33	3.23	3.54	2.27	1.87
Microsome recovery	0.765	0.772	0.476	0.499	0.537	0.430**
(mg/g body weight)	0.159	0.111	0.081	0.112	0.089	0.045

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Values are expressed as mean±S.D.
* : Significantly different from SD rats $p < 0.05$
** : Significantly different from SD rats $p < 0.01$

Table 2. Body weight, liver weight and microsome recovery in female rats

	9 weeks (n=10)		32 weeks (n=10)	
	SD	IGS	SD	IGS
Body weight (g)	229.2 [a)]	193.6**	350.4	312.3
	30.0	21.3	54.0	37.4
Liver weight (g)	6.98	5.66**	8.61	7.26*
	1.12	0.63	1.74	0.95
Relative liver weight	30.38	29.29	24.70	23.27
(mg/g body weight)	1.64	1.82	3.95	1.69
Microsome recovery	21.14	20.74	18.59	17.37
(mg/g Liver)	3.29	2.45	1.44	2.87
Microsome recovery	0.641	0.606	0.459	0.402
(mg/g body weight)	0.100	0.069	0.084	0.065

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Values are expressed as mean±S.D.
* : Significantly different from SD rats $p < 0.05$
** : Significantly different from SD rats $p < 0.01$

Table 3. Monooxygenase activities and Cytochrome P-450 contents in liver microsomes from male and female rats

Sex		9 weeks (n=10)		18 weeks (n=10)		32 weeks (n=10)	
		SD	IGS	SD	IGS	SD	IGS
Male	7-Ethoxycoumarin-O-deethylase	1.12 [a)]	1.73**	1.47	1.74*	1.29	1.93**
	(nmol/min/mg potein)	0.16	0.34	0.20	0.33	0.18	0.47
	Aniline-*p*-hydroxylase	0.79	0.96**	0.90	1.06**	0.84	1.03**
	(nmol/min/mg potein)	0.10	0.16	0.08	0.11	0.08	0.15
	Aminopyrine-N-demethylase	10.59	14.12**	12.62	14.76*	11.39	15.22**
	(nmol/min/mg potein)	1.61	2.19	1.44	2.32	2.10	2.99
	Cytochrome P-450	0.67	0.76*	0.95	0.96	0.88	1.00*
	(nmol/mg potein)	0.09	0.07	0.07	0.10	0.11	0.13
Female	7-Ethoxycoumarin-O-deethylase	0.89	1.14**	—	—	1.21	1.27
	(nmol/min/mg potein)	0.11	0.17			0.15	0.17
	Aniline-*p*-hydroxylase	0.70	0.89**	—	—	0.76	0.81
	(nmol/min/mg potein)	0.11	0.12			0.11	0.11
	Aminopyrine-N-demethylase	5.13	6.56**	—	—	5.10	5.48
	(nmol/min/mg potein)	0.39	0.98			0.59	1.10
	Cytochrome P-450	0.55	0.56	—	—	0.66	0.72
	(nmol/mg potein)	0.10	0.07			0.06	0.08

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Values are expressed as mean±S.D.
* : Significantly different from SD rats $p < 0.05$
** : Significantly different from SD rats $p < 0.01$

Table 4. Monooxygenase activities and Cytochrome P-450 contents in liver microsomes from male and female rats

Sex		9 weeks (n=10)		18 weeks (n=10)		32 weeks (n=10)	
		SD	IGS	SD	IGS	SD	IGS
Male	7-Ethoxycoumarin-O-deethylase	0.86 [a)]	1.34**	0.69	0.87	0.69	0.82
	(nmol/min/g body weight)	0.23	0.34	0.11	0.25	0.15	0.18
	Aniline-*p*-hydroxylase	0.60	0.74*	0.43	0.53*	0.45	0.44
	(nmol/min/g body weight)	0.13	0.15	0.07	0.12	0.08	0.05
	Aminopyrine-N-demethylase	8.07	10.85**	5.95	7.38	6.06	6.50
	(nmol/min/g body weight)	1.94	2.04	0.93	2.01	1.27	1.26
	Cytochrome P-450	0.51	0.59	0.45	0.48	0.47	0.43
	(nmol/g body weight)	0.11	0.10	0.07	0.13	0.09	0.03
Female	7-Ethoxycoumarin-O-deethylase	0.57	0.69	—	—	0.55	0.51
	(nmol/min/g body weight)	0.13	0.12			0.11	0.09
	Aniline-*p*-hydroxylase	0.45	0.53	—	—	0.35	0.32
	(nmol/min/g body weight)	0.11	0.07			0.08	0.06
	Aminopyrine-N-demethylase	3.29	3.96*	—	—	2.35	2.17
	(nmol/min/g body weight)	0.55	0.58			0.52	0.32
	Cytochrome P-450	0.36	0.34	—	—	0.30	0.29
	(nmol/g body weight)	0.09	0.07			0.06	0.05

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Values are expressed as mean±S.D.
* : Significantly different from SD rats $p < 0.05$
** : Significantly different from SD rats $p < 0.01$

Table 5. Activities of hepatic UDP-glucuronyltransferase in male and female rats

	9 weeks (n=10)		18 weeks (n=10)		32 weeks (n=10)	
	SD	IGS	SD	IGS	SD	IGS
male	13.30 3.56	12.48 1.97	12.86 2.08	15.20 2.98	15.74 4.05	13.72 2.51
female	10.01 2.40	7.54** 0.71	—	—	12.49 2.62	9.23** 1.84

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Activities(nmol/min/mg protein) are expressed as mean±S.D.
** : Significantly different from SD rats $p < 0.01$

Table 6. Activities of hepatic glutathione-S-transferase in male rats

Substrate (nmol/min/mg protein)	9 weeks (n=10)		32 weeks (n=10)	
	SD	IGS	SD	IGS
Δ^5-Androsten-3,17-dione	52.9 [a)] 8.6	41.1* 12.6	42.9 9.6	42.2 11.6
1,2-Dichloro-4-nitrobenzene	48.8 6.4	36.4** 5.9	51.6 11.3	51.9 4.9
trans-4-Phenyl-3-buten-2-one	9.44 1.27	8.19 1.58	13.10 3.73	15.31 3.40
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	7.11 1.81	6.31 2.70	18.65 4.22	16.86 2.17
1-Chloro-2,4-dinitrobenzene	852 83	738* 106	622 128	729 124
Ethacrynic acid	4.62 1.05	4.29 0.86	7.30 0.97	6.50 0.73

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Activities are expressed as mean±S.D.
* : Significantly different from SD rats $p < 0.05$
** : Significantly different from SD rats $p < 0.01$

Table 7. Activities of hepatic glutathione-S-transferase in female rats

Substrate (nmol/min/mg protein)	9 weeks (n=10)		32 weeks (n=10)	
	SD	IGS	SD	IGS
Δ^5-Androsten-3,17-dione	33.4 [a)] 6.1	27.4 6.9	29.0 13.0	22.6 8.7
1,2-Dichloro-4-nitrobenzene	18.1 1.6	14.8** 2.4	15.6 3.6	13.5 1.1
trans-4-Phenyl-3-buten-2-one	4.15 1.26	3.96 1.05	5.18 0.91	6.21 1.31
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	12.53 2.41	10.52 3.18	17.42 1.74	13.64** 2.79
1-Chloro-2,4-dinitrobenzene	501 43	475 37	635 122	595 65
Ethacrynic acid	6.08 1.12	5.44 0.72	7.19 0.81	6.33 1.89

SD: Crj:CD(SD) rats IGS: Crj:CD(SD)IGS rats
a): Activities are expressed as mean±S.D.
** : Significantly different from SD rats $p < 0.01$

A Comparison of General Toxicological Parameters Between the Crj:CD(SD) Rats and Crj:CD(SD)IGS Rats, at the 4- and 26- week Time Points

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ABSTRACT. General toxicological data at 4- and 26-week time points (when the rats were approximately 9 and 32 weeks of age, respectively) were collected from Crj:CD(SD)IGS rats [IGS] and compared with those of Crj:CD(SD) rats [SD]. The body weight growth curve showed a marginally lower shape in both male and female IGS rats than in SD ones of either sex. In histopathological examination, age-related changes, such as deposition of brown pigment and fibrosis in pancreatic islets, periportal fatty changes in the liver, myocardial degeneration and fibrosis, and basophilic tubules and casts in the kidney, were observed in both strains. However, the incidence and severity in IGS animals were lower than those in SD ones. These results suggest that the onset of aging was delayed in IGS. In both sexes of IGS at 9 and 32 weeks of age, the number of WBC's and the concentrations of lipids (total and free cholesterol, phospholipids, triglycerides, etc.) were significantly lower than those in SD rats. At 32 weeks of age, the concentrations of plasma protein and electrolytes and enzyme activities were significantly different from those in SD. These data suggest that the differences of some parameters between IGS and SD should be considered for the evaluation of general toxicological studies. —Key words: Crj:CD(SD)IGS, rat, general toxicological parameter

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INTRODUCTION

The International Genetic Standard (IGS) is an animal breeding system developed by Charles River, Inc. in order to supply uniform quality animals by minimizing the genetic ramifications. The general toxicological data at 4- and 26-week time points (when the rats were approximately 9 and 32 weeks of age, respectively) were collected from Crj:CD(SD)IGS rats [IGS] and compared with those of Crj:CD(SD) rats [SD]. The data for the 26-week time point were also compared with our data in "Biological Reference Data on CD(SD)IGS Rats - 1998" [1] and evaluated for reproducibility.

MATERIALS AND METHODS

Both sexes of IGS and SD rats were obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan) at 4 weeks of age. The rats were 5 weeks of age at start of the study, housed individually, and maintained under standard laboratory conditions, with target ranges of 24 ± 1℃ for temperature and 50-65% for relative humidity. The room air was ventilated 15 times/hr, and a 12 hr-light/12 hr-dark cycle (lighting 7:00-19:00) was maintained. The animals were given CE-2 *ad libitum* throughout study and tap water was also supplied *ad libitum*. Each group contained 10 animals/each sex for the 4-week study and 20 animals/each sex for the 26-week study. Tests on urine and blood were done with the following analyzers: CLINITEK 200 + (Bayer-Sankyo Co., Ltd.), Coulter Counter Model S-Plus (Coulter Electronics, Inc.), COBAS-FARA (Roche Diagnostic Systems Inc.), Epalyzer (Helena Lab. Corp.), EA05 (A & T Corp.) and CA-1000 (Toa Medical Electronics Co., Ltd.).

In this study, general signs, body weight, food and water consumption, and other laboratory parameters (ophthalmologic, urinalysis, histopathological observation, hematology and blood biochemistry) were observed.

General signs were observed daily throughout the experimental period. The body weight of each animal was recorded once a week during the experimental period at the ages of 5 through 17, and 21, 25, 29, and 32 weeks, and the mean body weight and standard deviation for the group were calculated. The food consumption was recorded once a week at the ages of 5 through 17, and 21, 25, and 29 weeks. Water consumption was also recorded once a week at the ages of 5, 8, 17, and 30 weeks. The results for evidence of the pupillary reflex and the observations of the lens and cornea by slit lamp of all rats were recorded at the ages of 4, 8, 17, and 30 weeks. At the same time, a retina examination was performed with a fundas camera. The animals were deprived of food overnight on the day before the blood collection, and blood was collected from the abdominal caval vein under pentobarbital anesthesia by use of syringes containing one of the following: sodium citrate for the coagulation test, EDTA-2K for hematological observations, no additive for the serum protein fraction, and heparin for other blood biochemical observations.

After blood collection for the above purposes, the animals were killed by exsanguination and necropsied immediately.

RESULTS AND DISCUSSION

The body weight growth curve showed a marginally lower shape in male and female IGS's than in the corresponding SD's. Statistical significance was demonstrated from Week 3 (male, Table 1) or Week 2 (female, Table 2). The food consumption curve was similar to that of body weight growth (Tables 3 and 4). The body weight growth curve, the food consumption, and the water intake in this study showed a good reproducibility with respect to the former study [1].

As the results of the ophthalmology examination at 4, 8, 17, and 30 weeks of age, the appearance of corneal crystals in IGS rats occurred later than that in SD ones, and the disappearance of persistent hyaloid in arteries was faster in IGS than in SD in both sexes. Other signs were seen here and there; however no significant difference was found between the two strains.

Absolute and relative organ weights are shown in Tables 5 and 6. The relative weight of the liver in male IGS's was significantly

lower than that of the organ in SD males. However, relative organ weights of heart, lung, and testes in IGS were significantly higher than those of these organs in SD. In the female IGS, relative organ weight of liver or lung was lower or higher than the respective one in SD animals.

The incidence of each macroscopical findings showed no significant differences between IGS and SD strains. In histopathological examination, age-related changes, such as deposition of brown pigment and fibrosis in pancreatic islets, periportal fatty changes in the liver, myocardial degeneration and fibrosis, and basophilic tubules and casts in the kidney, were observed in both strains. However, the incidence and severity in IGS were lower than those in SD (Tables 7 and 8). These results suggest that the onset of aging was delayed in IGS rats.

In the urinalysis at 5-, 8-, 17- and 30-week-old rats, the concentrations of some electrolytes in male IGS's were significantly lower than those in male SD's.

In both sexes of IGS at 9 and 32 weeks of age, the number of WBC's and values for a few other parameters were significantly lower than those in SD's, and RBC's and a few other parameters were significantly higher than those in SD's (Tables 9 and 10). In the same experimental period, the concentrations of lipids (total and free cholesterol, phospholipids, triglycerides, etc.) in IGS were significantly lower than those in SD (Tables 11 and 12) in both sexes. These results showed a good reproducibility with respect to the former study [1]. At 32 weeks of age, IGS rats showed concentrations of plasma proteins and electrolytes and enzyme activities that were significantly different from those in SD animals; and at 9 weeks of age, the values for several parameters in IGS's also were significantly different from those of SD's.

The above data suggest that the differences in some parameters between IGS and SD strains should be considered for the evaluation of general toxicological studies.

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REFERENCE

1. Tanaka, A., Azegami, J., Kojima, K., and Imai, K. 1998. Response to commercial low protein diet in Crj:CD(SD)IGS rats. pp 19-30. In: Biological Reference Data on CD(SD)IGS Rats - 1998 (Matsuzawa, T. and Inoue, H. eds.) Best Printing Co. Ltd., Tokyo

Table 1-1. Body weight in males

Weeks of age	4-week study	
	CD(SD)	CD(SD)IGS
	n=10	n=10
5	140.2± 4.8	149.5± 4.7 **
6	205.4±10.1	207.2± 5.2
7	265.9±13.9	261.4± 7.5
8	330.0±16.1	313.5±12.1 *
Final body weight	373.0±16.1	344.6±16.2 **

Each value represents the Mean±S.D.(Unit: grams)
Significant difference from CD(SD), * $p<0.05$ and ** $p<0.01$ (by Student's t-test)

Table 1-2. Body weight in males

Weeks of age	26-week study	
	CD(SD)	CD(SD)IGS
	n=20	n=20
5	142.0± 6.2	149.4± 4.9 **
6	212.8±11.7	207.9±12.2
7	277.8±16.6	261.1±19.6 **
8	347.6±22.2	315.6±25.9 **
9	402.3±29.1	350.4±31.3 **
10	450.9±31.1	387.7±37.7 **
11	497.0±35.8	419.1±40.5 **
12	530.9±39.0	444.9±44.3 **
13	562.1±43.2	467.6±45.7 **
14	583.5±46.4	483.4±48.9 **
15	604.3±46.8	499.6±49.1 **
16	624.1±49.9	516.2±50.9 **
17	642.3±50.7	527.6±52.1 **
21	706.2±59.7	573.3±56.4 **
25	753.2±70.1	600.8±61.4 **
29	788.4±78.8 a)	617.0±69.6 **
Final body weight	798.5±82.7 a)	624.2±72.2 **

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), ** $p<0.01$ (by Student's t-test)
a):n=19

Table 2-1. Body weight in females

Weeks of age	4-week study		
	CD(SD)	CD(SD)IGS	
	n=10	n=10	
5	127.2± 6.0	127.4± 4.4	
6	168.8±12.6	158.1± 8.9	*
7	198.2±19.7	177.4±14.0	*
8	227.0±23.6	200.0±18.1	*
Final body weight	246.8±30.3	211.1±21.1	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * $p<0.05$ and ** $p<0.01$ (by Student's t-test)

Table 2-2. Body weight in females

Weeks of age	26-week study		
	CD(SD)	CD(SD)IGS	
	n=20	n=20	
5	129.4± 5.7	128.0± 5.8	
6	171.0±12.4	158.1±10.5	**
7	197.9±16.8	180.5±13.3	**
8	225.8±24.7	202.6±16.8	**
9	241.5±27.3	213.5±18.2	**
10	266.7±32.7	232.0±19.7	**
11	282.7±35.1	248.2±20.5	**
12	301.6±39.3	259.2±22.0	**
13	316.2±41.0	270.0±23.0	**
14	325.5±41.9	276.6±25.0	**
15	338.3±47.2	286.0±25.9	**
16	347.0±50.1	291.8±28.1	**
17	353.9±52.8	295.4±27.1	**
21	373.8±61.1	308.9±27.3	**
25	392.6±69.8	325.4±32.2	**
29	412.3±82.3	335.4±38.5	**
Final body weight	417.4±85.4	332.4±38.9	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), ** $p<0.01$ (by Student's t-test)

Table 3-1. Food consumption in males

Weeks of age	4-week study		
	CD(SD)	CD(SD)IGS	
	n=10	n=10	
5	22.6±1.7	22.3±1.2	
6	26.7±2.1	25.7±1.8	
7	28.8±2.5	26.8±1.3	*
8	29.7±2.3	26.7±2.3	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * $p<0.05$ and ** $p<0.01$ (by Student's t-test)

Table 3-2. Food consumption in males

Weeks of age	26-week study		
	CD(SD)	CD(SD)IGS	
	n=20	n=20	
5	22.4±1.7	22.0±1.5	
6	28.1±2.4	25.8±2.0	**
7	31.7±2.5	26.6±2.8	**
8	32.5±3.0	26.9±2.6	**
9	32.8±3.1	27.0±3.0	**
10	33.7±3.1	27.7±2.5	**
11	34.5±3.1	27.7±3.5	**
12	35.9±3.2	28.5±2.7	**
13	33.6±4.2	26.8±3.1	**
14	33.7±3.8	26.7±2.3	**
15	34.7±2.7	28.2±3.0	**
16	34.1±3.0	28.1±3.6	**
17	32.6±3.8	26.9±3.1	**
21	32.7±3.2	26.6±3.0	**
25	30.1±7.0	24.4±2.3	**
29	33.0±3.6 a)	26.1±3.4	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), **p<0.01 (by Student's t-test)
a):n=19

Table 4-1. Food consumption in females

Weeks of age	4-week study		
	CD(SD)	CD(SD)IGS	
	n=10	n=10	
5	19.1±2.0	17.5±1.0	*
6	21.3±3.8	18.3±2.0	*
7	21.5±4.2	18.5±2.0	
8	21.8±4.5	16.3±3.8	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * p<0.05 and ** p<0.01 (by Student's t-test)

Table 4-2. Food consumption in females

Weeks of age	26-week study		
	CD(SD)	CD(SD)IGS	
	n=20	n=20	
5	19.8±2.0	18.1±1.5	**
6	21.5±2.7	18.1±1.9	**
7	21.4±3.2	18.2±2.1	**
8	18.9±3.8	17.1±2.2	
9	21.7±3.1	17.4±2.8	**
10	21.4±4.3	18.7±2.7	*
11	23.4±4.0	17.9±2.9	**
12	24.4±4.5	20.8±2.3	**
13	22.9±4.1	19.3±2.0	**
14	22.5±4.2	19.4±2.2	**
15	22.8±4.6	18.5±2.6	**
16	22.8±4.5	18.9±1.6	**
17	20.3±4.2	15.9±3.0	**
21	21.2±4.1	18.9±2.8	*
25	21.1±3.9	16.1±2.4	**
29	22.3±5.0	18.3±2.2	**

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * p<0.05 and ** p<0.01 (by Student's t-test)

Table 5. Organ weight in males

	4-week study			26-week study		
	CD(SD) n=10	CD(SD)IGS n=10		CD(SD) n=19	CD(SD)IGS n=20	
Final body weight (g)	351.1 ±15.7	322.9 ±15.6	**	774.4 ±84.8	600.0 ±69.4	**
Absolute						
Brain (g)	1.92 ± 0.07	1.89 ± 0.07		2.10 ± 0.08	2.09 ± 0.09	
Thymus (g)	0.63 ± 0.11	0.49 ± 0.08	**	0.16 ± 0.07	0.15 ± 0.06	
Heart (g)	1.16 ± 0.06	1.07 ± 0.08	**	1.82 ± 0.17	1.50 ± 0.19	**
Lunge (g)	1.16 ± 0.07	1.09 ± 0.06	*	1.53 ± 0.14	1.43 ± 0.12	*
Liver (g)	11.44 ± 0.96	9.95 ± 0.86	**	22.17 ± 3.45	14.67 ± 1.98	**
Kidneys (g)	2.67 ± 0.14	2.47 ± 0.14	**	4.13 ± 0.32	3.43 ± 0.37	**
Spleen (g)	0.69 ± 0.08	0.65 ± 0.09		1.00 ± 0.16	0.82 ± 0.12	**
Testes (g)	3.09 ± 0.15	2.78 ± 0.18	**	3.61 ± 0.72	3.56 ± 0.47	
Prostate (g)	0.40 ± 0.10	0.40 ± 0.08		0.50 ± 0.14	0.61 ± 0.21	
Submaxillary glands (g)	0.58 ± 0.06	0.55 ± 0.05		0.78 ± 0.08	0.72 ± 0.08	*
Pituitary gland (mg)	11.6 ± 0.9	9.7 ± 1.1	**	14.1 ± 2.3	12.1 ± 1.6	**
Thyroid gland (mg)	16.4 ± 2.1	13.8 ± 2.9	*	29.5 ± 4.8	23.2 ± 5.8	**
Adrenal gland (mg)	51.8 ± 5.1	44.5 ± 5.5	**	58.2 ± 8.0	51.6 ± 9.3	*
Relative (mg/g)						
Brain	5.48 ± 0.36	5.87 ± 0.25	*	2.74 ± 0.29	3.52 ± 0.39	**
Thymus	1.80 ± 0.34	1.51 ± 0.22	*	0.21 ± 0.10	0.24 ± 0.09	
Heart	3.31 ± 0.19	3.31 ± 0.12		2.36 ± 0.16	2.51 ± 0.19	*
Lunge	3.32 ± 0.26	3.37 ± 0.19		1.98 ± 0.19	2.40 ± 0.15	**
Liver	32.57 ± 2.23	30.79 ± 1.84		28.58 ± 2.86	24.42 ± 1.22	**
Kidneys	7.61 ± 0.48	7.65 ± 0.48		5.60 ± 0.42	5.76 ± 0.66	
Spleen	1.98 ± 0.23	2.02 ± 0.23		1.29 ± 0.18	1.36 ± 0.18	
Testes	8.82 ± 0.64	8.64 ± 0.87		4.63 ± 0.82	6.00 ± 0.94	**
Prostate	1.12 ± 0.24	1.23 ± 0.23		0.65 ± 0.21	1.02 ± 0.36	**
Submaxillary glands	1.66 ± 0.14	1.71 ± 0.21		1.02 ± 0.13	1.21 ± 0.11	**
Pituitary gland	0.033± 0.003	0.030± 0.004		0.018± 0.003	0.020± 0.002	*
Thyroid gland	0.047± 0.007	0.043± 0.009		0.038± 0.006	0.039± 0.009	
Adrenal gland	0.147± 0.013	0.138± 0.017		0.076± 0.012	0.086± 0.012	*

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * $p<0.05$ and ** $p<0.01$ (by Student's t-test)

Table 6. Organ weight in females

	4-week study			26-week study		
	CD(SD) n=10	CD(SD)IGS n=10		CD(SD) n=20	CD(SD)IGS n=20	
Final body weight (g)	229.2 ±30.0	193.6 ±21.3	**	396.8 ±84.4	314.7 ±36.0	**
Absolute						
Brain (g)	1.78 ± 0.06	1.71 ± 0.08	*	1.91 ± 0.08	1.90 ± 0.07	
Thymus (g)	0.48 ± 0.09	0.41 ± 0.08		0.15 ± 0.03	0.13 ± 0.04	
Heart (g)	0.82 ± 0.09	0.66 ± 0.08	**	1.15 ± 0.17	0.91 ± 0.07	**
Lunge (g)	0.88 ± 0.07	0.82 ± 0.05		1.11 ± 0.10	0.99 ± 0.08	**
Liver (g)	6.98 ± 1.12	5.66 ± 0.63	**	9.83 ± 2.70	7.16 ± 0.85	**
Kidneys (g)	1.72 ± 0.23	1.52 ± 0.17	*	2.39 ± 0.16	1.94 ± 0.18	**
Spleen (g)	0.50 ± 0.12	0.41 ± 0.05	**	0.64 ± 0.12	0.51 ± 0.08	**
Uterus (g)	0.42 ± 0.18	0.38 ± 0.12		0.69 ± 0.35	0.60 ± 0.18	
Submaxillary glands (g)	0.40 ± 0.06	0.37 ± 0.04		0.49 ± 0.06	0.43 ± 0.04	**
Pituitary gland (mg)	11.8 ± 1.3	11.3 ± 1.8		17.8 ± 6.0	15.6 ± 2.8	
Thyroid gland (mg)	12.9 ± 2.4	11.9 ± 1.2		19.7 ± 4.3	14.3 ± 3.2	**
Adrenal gland (mg)	63.0 ±11.2	58.5 ± 7.1		74.8 ±13.2	62.1 ±10.9	**
Ovaries (mg)	86.8 ±15.7	78.3 ± 9.7		72.2 ±20.1	65.8 ±12.7	
Relative (mg/g)						
Brain	7.87 ± 0.98	8.92 ± 0.84	*	5.01 ± 0.99	6.13 ± 0.76	**
Thymus	2.11 ± 0.34	2.14 ± 0.33		0.38 ± 0.11	0.41 ± 0.11	
Heart	3.58 ± 0.20	3.44 ± 0.21		2.96 ± 0.35	2.91 ± 0.22	
Lunge	3.86 ± 0.29	4.28 ± 0.33	**	2.86 ± 0.42	3.18 ± 0.29	**
Liver	30.4 ± 1.64	29.29 ± 1.82		24.77 ± 3.62	22.8 ± 1.63	*
Kidneys	7.52 ± 0.60	7.89 ± 0.53		5.99 ± 0.87	6.04 ± 0.43	
Spleen	2.20 ± 0.42	2.14 ± 0.23		1.63 ± 0.22	1.64 ± 0.22	
Uterus	1.83 ± 0.77	1.97 ± 0.66		1.76 ± 0.76	1.97 ± 0.71	
Submaxillary glands	1.73 ± 0.14	1.92 ± 0.24	*	1.26 ± 0.22	1.37 ± 0.19	
Pituitary gland	0.052± 0.007	0.058± 0.008		0.045± 0.011	0.050± 0.010	
Thyroid gland	0.057± 0.013	0.062± 0.007		0.051± 0.011	0.046± 0.012	
Adrenal gland	0.275± 0.033	0.306± 0.052		0.192± 0.028	0.199± 0.037	
Ovaries	0.379± 0.057	0.407± 0.054		0.186± 0.048	0.211± 0.044	

Each value represents the Mean±S.D. (Unit: grams)
Significant difference from CD(SD), * $p<0.05$ and ** $p<0.01$ (by Student's t-test)

Table 7-1. Histopathological findings in males

	4-week study											
	CD(SD) (n=10)						CD(SD)IGS (n=10)					
	−	±	+	++	+++	Pos.	−	±	+	++	+++	Pos.
Lung												
Accumulation, foam cell	10	0	0	0	0	0	7	3	0	0	0	3
Hemorrhage	9	1	0	0	0	1	9	1	0	0	0	1
Metaplasia, osseous	10	0	0	0	0	0	8	0	2	0	0	2
Pancreas												
Proliferation, ductule	9	1	0	0	0	1	10	0	0	0	0	0
Hemorrhage, pancreatic islet	10	0	0	0	0	0	9	0	1	0	0	1
Liver												
Fatty change, periportal	0	3	7	0	0	10	0	1	9	0	0	10
Fatty change, focal	10	0	0	0	0	0	9	0	1	0	0	1
Heart												
Myocardial degeneration	7	2	1	0	0	3	8	2	0	0	0	2
Kidney												
Basophilic tubule, cortex	3	7	0	0	0	7	3	7	0	0	0	7
Cyst	10	0	0	0	0	0	9	0	1	0	0	1
Spleen												
Deposit, pigment, brown	10	0	0	0	0	0	10	0	0	0	0	0
Hematopoiesis, extramedullary	0	1	9	0	0	10	0	6	4	0	0	10
Testis												
No remarkable change												

−, Negative; ±, Very slight; +, Slight; ++, Moderate; +++, Severe
Pos., Total of Positive grade

Table 7-2. Histopathological findings in males

	26-week study											
	CD(SD) (n=19)						CD(SD)IGS (n=20)					
	−	±	+	++	+++	Pos.	−	±	+	++	+++	Pos.
Lung												
Accumulation, foam cell	10	9	0	0	0	9	2	8	8	2	0	18
Hemorrhage	10	6	3	0	0	9	17	2	1	0	0	3
Cellular infiltration, neutrophil, in hemorrhagic focus	19	0	0	0	0	0	19	1	0	0	0	1
Deposit, pigment, brown, in alveolar macrophage	17	1	1	0	0	2	20	0	0	0	0	0
Fibrosis, pleura, focal	18	0	1	0	0	1	20	0	0	0	0	0
Metaplasia, osseous	16	0	3	0	0	3	18	0	2	0	0	2
Pancreas												
Hemorrhage, pancreatic islet	18	0	1	0	0	1	17	3	0	0	0	3
Fibrosis, pancreatic islet	3	1	13	2	0	16	12	1	5	2	0	8
Granulation tissue, pancreatic islets	18	0	1	0	0	1	19	0	1	0	0	1
Deposit, pigment, brown	2	2	15	0	0	17	10	3	7	0	0	10
Focus, basophilic acinar cell	18	0	1	0	0	1	18	0	2	0	0	2
Proliferation, ductule	19	0	0	0	0	0	18	1	0	1	0	2
Cellular infiltration, lymphocyte	18	0	1	0	0	1	20	0	0	0	0	0
Liver												
Fatty change, periportal	0	2	12	5	0	19	0	9	9	2	0	20
Fatty change, focal	14	0	5	0	0	5	14	0	6	0	0	6
Focus of cellular alteration	18	0	1	0	0	1	20	0	0	0	0	0
Necrosis, focal	17	1	1	0	0	2	19	1	0	0	0	1
Fibrosis, focal	19	0	0	0	0	0	18	1	1	0	0	2
Deposit, pigment, brown, fibrotic area	19	0	0	0	0	0	19	0	1	0	0	1
Proliferation, bile duct	14	4	1	0	0	5	18	2	0	0	0	2
Heart												
Myocardial degeneration	3	8	8	0	0	16	12	5	3	0	0	8
Myocardial fibrosis	6	4	7	2	0	13	14	3	3	0	0	6
Kidney												
Basophilic tubule, cortex	1	8	7	3	0	18	2	15	2	1	0	18
Deposit, pigment, brown, proximal tubule	1	11	7	0	0	18	5	11	4	0	0	15
Eosinophilic body	11	4	4	0	0	8	10	7	3	0	0	10
Cast	7	4	8	0	0	12	11	5	4	0	0	9
Hyaline droplet, tubular epithelium	14	2	3	0	0	5	19	1	0	0	0	1
Mineralization, cortico-medullary junction	15	4	0	0	0	4	14	6	0	0	0	6
Cyst	18	0	1	0	0	1	20	0	0	0	0	0
Spleen												
Deposit, pigment, brown	0	0	0	18	1	19	0	0	2	17	1	20
Hematopoiesis, extramedullary	0	2	16	1	0	19	0	16	4	0	0	20
Testis												
Atrophy, seminiferous tubule, focal	18	1	0	0	0	1	19	1	0	0	0	1
Atrophy, seminiferous tubule diffuse, bilateral	18	0	0	0	1	1	20	0	0	0	0	0

−, Negative; ±, Very slight; +, Slight; ++, Moderate; +++, Severe
Pos., Total of Positive grade

Table 8-1. Histopathological findings in females

	4-week study											
	CD(SD) (n=10)						CD(SD)IGS (n=10)					
	−	±	+	++	+++	Pos.	−	±	+	++	+++	Pos.
Lung												
Accumulation, foam cell	8	2	0	0	0	2	9	1	0	0	0	1
Hemorrhage	9	0	1	0	0	1	10	0	0	0	0	0
Pancreas												
Proliferation, ductule	10	0	0	0	0	0	9	1	0	0	0	1
Microgranuloma	9	1	0	0	0	1	10	0	0	0	0	0
Liver										0	0	10
Fautty change, periportal	0	5	5	0	0	10	0	4	6			
Fatty change, focal	10	0	0	0	0	0	8	2	0	0	0	2
Heart												
No remarkable change												
Kidney												
Basophilic tubule, cortex	1	9	0	0	0	9	6	4	0	0	0	4
Mineralization, cortico-medullary junction	7	2	1	0	0	3	8	2	0	0	0	2
Spleen												
Deposit, pigment, brown	0	10	0	0	0	10	1	9	0	0	0	9
Hematopoiesis, extramedullary	0	10	0	0	0	10	2	8	0	0	0	8
Ovary												
No remarkable change												

−, Negative; ±, Very slight; +, Slight; ++, Moderate; +++, Severe
Pos., Total of Positive grade

Table 8-2. Histopathological findings in females

	26-week study											
	CD(SD) (n=19)						CD(SD)IGS (n=20)					
	−	±	+	++	+++	Pos.	−	±	+	++	+++	Pos.
Lung												
Accumulation, foam cell	11	9	0	0	0	9	7	8	5	0	0	13
Fibrosis, pleura, focal	20	0	0	0	0	0	19	0	1	0	0	1
Pancreas												
Fibrosis, pancreatic islet	19	0	1	0	0	1	20	0	0	0	0	0
Deposit, pigment, brown	17	2	1	0	0	3	19	1	0	0	0	1
Focus, basophilic acinar cell	19	0	1	0	0	1	20	0	0	0	0	0
Proliferation, ductule	19	1	0	0	0	1	16	4	0	0	0	4
Cellular infiltration, lymphocyte	18	2	0	0	0	2	19	1	0	0	0	1
Liver												
Fatty change, periportal	6	9	5	0	0	14	3	13	4	0	0	17
Fatty change, focal	12	2	6	0	0	8	16	1	3	0	0	4
Focus of cellular alteration	18	1	1	0	0	2	20	0	0	0	0	0
Proliferation, bile duct	19	1	0	0	0	1	19	1	0	0	0	1
Hematopoiesis, extramedullary	19	1	0	0	0	1	20	0	0	0	0	0
Heart												
Myocardial degeneration	16	4	0	0	0	4	17	3	0	0	0	3
Myocardial fibrosis	17	3	0	0	0	3	19	1	0	0	0	1
Kidney												
Basophilic tubule, cortex	12	5	3	0	0	8	17	3	0	0	0	3
Cast	14	3	3	0	0	6	20	0	0	0	0	0
Hyaline droplet, tubular epithelium	16	3	1	0	0	4	20	0	0	0	0	0
Cellular infiltration, lymphocyte, cortex	18	0	2	0	0	2	19	1	0	0	0	1
Mineralization, cortico-medullary junction	10	3	7	0	0	10	15	4	1	0	0	5
Hyperplasia, pelvic epithelium	19	1	0	0	0	1	20	0	0	0	0	0
Mineralization, pelvic mucosa	19	1	0	0	0	1	20	0	0	0	0	0
Cellular infiltration, lymphocyte, pelvic mucosa	20	0	0	0	0	0	18	0	2	0	0	2
Pyelitis	19	1	0	0	0	1	19	1	0	0	0	1
Spleen												
Deposit, pigment, brown	0	0	0	16	4	20	0	0	0	18	2	20
Hematopoiesis, extramedullary	0	3	16	1	0	20	0	17	3	0	0	20
Ovary												
Atrophy	8	3	2	7	0	12	13	0	3	4	0	7
Cyst, follicular	18	0	2	0	0	2	19	0	1	0	0	1
Cyst, corpus luteum	19	0	1	0	0	1	18	0	2	0	0	2

−, Negative; ±, Very slight; +, Slight; ++, Moderate; +++, Severe
Pos., Total of Positive grade

Table 9. Hematology in males

Item	4W			26W		
	CD(SD)IGS		CD(SD)	CD(SD)IGS		CD(SD)
	n=10		n=10	n=20		n=19
General						
RBC (×10⁴/mm³)	713 ±28	*	674 ± 39	842 ±36	**	799 ±32
Hb (g/dl)	14.6± 0.7		14.3± 0.6	14.8± 0.6	*	14.4± 0.6
Ht (%)	43.0± 1.9		42.1± 1.7	43.7± 1.9	*	42.4± 1.7
MCV (μm³)	60.4± 1.7	*	62.5± 1.7	51.9± 1.9		53.1± 2.0
MCH (pg)	20.5± 0.7	*	21.2± 0.6	17.6± 0.6		18.0± 0.8
MCHC (%)	34.0± 0.4		33.9± 0.2	34.0± 0.6		33.9± 0.6
Retc.(%)	3.0± 0.8		3.3± 0.5	1.9± 0.6	**	2.5± 0.8
WBC (×100/mm³)	54 ±14		65 ± 24	58 ±19	*	74 ±22
Band neutrophil (%)	0 ± 0		0 ± 0	0 ± 0		0 ± 0
Segmented neutrophil (%)	11 ± 9		8 ± 6	15 ± 6		13 ± 4
Eosinophil (%)	0 ± 0		1 ± 1	1 ± 1		1 ± 1
Basophil (%)	0 ± 0		0 ± 0	0 ± 0		0 ± 0
Monocyte (%)	1 ± 1		0 ± 1	1 ± 1		1 ± 1
Lymphocyte (%)	88 ± 9		90 ± 7	84 ± 6		85 ± 4
PLT (×10⁴/mm³)	92.8± 9.5	*	103.2± 9.1	90.3± 7.3	**	103.3±12
Coagulation						
PT (sec)	30.9±10		24.2± 6.1	35.4± 9.3		26.3± 4.5
APTT (sec)	25.4± 2.5		26.0± 3.4	26.0± 3.9		24.3± 2.7
Fibrinogen (mg/dl)	227 ±12		225 ±114	205 ±23		225 ±17

Each value represents the Mean±S.D.
Significant difference from CD(SD), *p<0.05 and **p<0.01 (by Student's t-test).

Table 10. Hematology in females

Item	4W			26W		
	CD(SD)IGS		CD(SD)	CD(SD)IGS		CD(SD)
	n=10		n=10	n=20		n=19
General						
RBC (×10⁴/mm³)	715 ±24		709 ±54	751 ±39	**	722 ±28
Hb (g/dl)	14.5± 0.5		14.7± 0.8	14.1± 0.5		14.1± 0.4
Ht (%)	42.7± 1.3		43.2± 2.6	42.2± 2.0		41.7± 1.6
MCV (μm³)	59.7± 1.4		61.0± 1.6	56.2± 1.8	**	57.7± 1.6
MCH (pg)	20.2± 0.5	*	20.7± 0.6	18.8± 0.7	**	19.5± 0.5
MCHC (%)	33.9± 0.4		34.0± 0.5	33.4± 0.8		33.8± 0.7
Retc. (%)	1.7± 0.7		2.3± 0.7	1.7± 0.5	**	2.6± 0.7
WBC (×100/mm³)	44 ± 8	*	59 ±20	22 ± 7	**	29 ± 7
Band neutrophil (%)	0 ± 0		0 ± 0	0 ± 0		0 ± 0
Segmented neutrophil (%)	8 ± 3		7 ± 5	20 ± 7		18 ± 8
Eosinophil (%)	0 ± 0		0 ± 0	2 ± 2		2 ± 1
Basophil (%)	0 ± 0		0 ± 0	0 ± 0		0 ± 0
Monocyte (%)	1 ± 1		0 ± 1	1 ± 2		1 ± 1
Lymphocyte (%)	91 ± 3		92 ± 6	77 ± 7		79 ± 8
PLT (×10⁴/mm³)	94.5± 5.8		100.0± 7.5	81.8±13		88.3±14
Coagulation						
PT (sec)	15.4± 0.9		16.1± 0.9	19.2± 1.4		19.1± 2.1
APTT (sec)	20.2± 1.9		21.1± 1.5	25.7± 3.4		28.0± 5.2
Fibrinogen (mg/dl)	176 ±19		196 ±31	147 ±14		163 ±18

Each value represents the Mean±S.D.
Significant difference from CD(SD), *p<0.05 and **p<0.01 (by Student's t-test).

Table 11. Blood biochemistry in males

Item	4W		26W	
	CD(SD)IGS	CD(SD)	CD(SD)IGS	CD(SD)
	n=10	n=10	n=20	n=19
General				
TP (g/dL)	5.0 ± 0.2	5.0 ± 0.1	5.6 ± 0.3 **	5.9 ± 0.2
ALB (g/dL)	2.9 ± 0.2	2.8 ± 0.1	2.9 ± 0.2	2.9 ± 0.2
GOT (U/L)	70 ± 9	68 ± 8	91 ± 28 *	70 ± 24
GPT (U/L)	29 ± 5	28 ± 4	46 ± 18	41 ± 13
ALP (U/L)	416 ± 66	346 ± 51	136 ± 18	148 ± 49
LDH (U/L)	125 ± 36	105 ± 16	253 ± 98	315 ±203
γ-GTP (U/L)	0.3 ± 0.5	0 ± 0	0.4 ± 0.5	0.1 ± 0.5
CPK (U/L)	521 ±228	381 ±132	688 ±482	579 ±368
CHE (U/L)	280 ± 55	321 ± 56	366 ±129 **	518 ±153
LAP (U/L)	36 ± 5	34 ± 3	30 ± 3 **	25 ± 2
T. BIL (mg/dL)	0.08± 0.02	0.07± 0.03	0.08± 0.03	0.08± 0.03
GLU (mg/dL)	141 ± 14	132 ± 15	163 ± 20 **	196 ± 27
BUN (mg/dL)	19 ± 2 **	15 ± 2	16 ± 4 *	13 ± 5
CRE (mg/dL)	0.7 ± 0.1 **	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.2
CA (mg/dL)	8.8 ± 0.3	8.9 ± 0.3	8.6 ± 0.3 **	9.0 ± 0.3
IP (mg/dL)	7.5 ± 0.8	7.4 ± 0.2	5.0 ± 0.5	4.9 ± 0.7
T. CHO (mg/dL)	33 ± 5 **	43 ± 9	56 ± 16 **	83 ± 23
PL (mg/dL)	65 ± 8 **	82 ± 10	89 ± 18 **	136 ± 31
TG (mg/dL)	40 ± 14 **	67 ± 11	60 ± 28 **	143 ± 72
F. CHO (mg/dL)	9 ± 1 *	12 ± 3	17 ± 5 **	27 ± 7
HDL. CHO (mg/dL)	21 ± 3	24 ± 4	35 ± 10 **	47 ± 11
NEFA (mEq/L)	0.46± 0.12	0.53± 0.11	0.42± 0.13	0.19± 0.15
Electrolytes (mEq/L)				
Na	144.6 ± 1.3	144.0 ± 0.8	145.0 ± 0.8 *	144.4 ± 0.9
K	3.99± 0.39	3.82± 0.23	4.22± 0.26 *	4.07± 0.2
Cl	108.5 ± 1.3	107.7 ± 1.1	107.8 ± 1.5 **	106.2 ± 1.7
Serum protein fraction				
ALB (%)	52.1 ± 1.9	51.3 ± 1.9	45.6 ± 1.6 **	42.6 ± 2.2
α_1-globulin (%)	21.4 ± 2.3	22.9 ± 2	24.5 ± 1.3 **	26.7 ± 2.4
α_2-globulin (%)	7.9 ± 0.8	7.7 ± 0.7	8.2 ± 0.7	8.5 ± 0.6
β-globulin (%)	16.0 ± 0.8	15.8 ± 1.3	17.2 ± 1.2 **	18.6 ± 1.3
γ-globulin (%)	2.6 ± 0.4 *	2.2 ± 0.3	4.5 ± 0.7 **	3.6 ± 0.5

Each value represents the Mean±S.D.
Significant difference from CD(SD), *p<0.05 and **p<0.01 (by Student's t-test).

Table 12. Blood biochemistry in female

Item	4W		26W	
	CD(SD)IGS	CD(SD)	CD(SD)IGS	CD(SD)
	n=10	n=10	n=20	n=19
General				
TP (g/dL)	5.2 ± 0.2	5.3 ± 0.2	5.6 ± 0.4 **	6.1 ± 0.4
ALB (g/dL)	3.1 ± 0.1	3.1 ± 0.2	3.3 ± 0.3 **	3.7 ± 0.3
GOT (U/L)	62 ± 7 *	76 ± 17	80 ± 16	91 ± 47
GPT (U/L)	20 ± 4	28 ± 12	39 ± 9 *	61 ± 44
ALP (U/L)	244 ± 59	214 ± 42	55 ± 18	69 ± 26
LDH (U/L)	115 ± 36	131 ± 33	203 ± 72	239 ±130
γ -GTP (U/L)	0.6 ± 0.7	0.5 ± 0.5	0.7 ± 0.7	0.6 ± 0.5
CPK (U/L)	222 ±103	283 ±100	800 ±561	723 ±370
CHE (U/L)	1250 ±257	1389 ±325	2450 ±620 *	2918 ±640
LAP (U/L)	30 ± 3	31 ± 3	23 ± 2 **	21 ± 2
T. BIL (mg/dL)	0.09± 0.01 *	0.12± 0.03	0.1 ± 0.02	0.1 ± 0.03
GLU (mg/dL)	116 ± 9	108 ± 11	127 ± 13 **	143 ± 20
BUN (mg/dL)	26 ± 7	22 ± 5	18 ± 4	17 ± 5
CRE (mg/dL)	0.8 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
CA (mg/dL)	8.7 ± 0.3 **	9.1 ± 0.2	8.2 ± 0.4 *	8.4 ± 0.4
IP (mg/dL)	6.9 ± 1.0	6.5 ± 0.5	4.6 ± 0.7 **	4.0 ± 0.7
T. CHO (mg/dL)	39 ± 10 **	54 ± 12	57 ± 12 **	75 ± 18
PL (mg/dL)	75 ± 16 **	106 ± 24	111 ± 20 **	159 ± 35
TG (mg/dL)	21 ± 5	40 ± 26	40 ± 10 **	119 ± 80
F. CHO (mg/dL)	13 ± 3 **	19 ± 3	19 ± 4 **	26 ± 6
HDL. CHO (mg/dL)	27 ± 6 *	34 ± 7	39 ± 8 **	48 ± 9
NEFA (mEq/L)	0.42± 0.12 **	0.68± 0.13	0.46± 0.15 **	0.62± 0.14
Electrolytes (mEq/L)				
NA	145.6 ± 1.0	144.8 ± 1.0	146 ± 1.1	145.3 ± 1.2
K	3.58± 0.41	3.58± 0.27	4.92± 0.93 *	4.28± 0.55
Cl	110.8 ± 1.8	109.7 ± 2.0	110.9 ± 1.1 *	109.5 ± 2.0
Serum protein fraction				
ALB (%)	56.3 ± 1.7 *	54.2 ± 2.5	54.3 ± 1.6 *	53.1 ± 2.0
α_1-globulin (%)	18.5 ± 1.5 **	20.1 ± 0.9	17.5 ± 1.3 **	19.3 ± 1.9
α_2-globulin (%)	6.6 ± 0.7	7.4 ± 1.1	7.3 ± 0.7	7.3 ± 0.9
β -globulin (%)	15.1 ± 1.0 **	15.7 ± 1.4	15.1 ± 1.0	15.6 ± 1.3
γ -globulin (%)	3.6 ± 0.8 **	2.6 ± 0.5	5.8 ± 0.9	4.7 ± 0.6

Each value represents the Mean±S.D.
Significant difference from CD(SD), *p<0.05 and **p<0.01 (by Student's t-test).

Histopathological Study on Spontaneous Cardiac and Hepatic Lesions in Crj:CD(SD)IGS Rats Derived from Three Different Breeding Colonies

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ABSTRACT. To elucidate the interlaboratories heterogeneity of spontaneous lesions in Crj:CD(SD)IGS (IGS) rats, histopathological observations of the heart and liver were performed in the rats derived from three different breeding colonies (Atsugi, Hino, and Tsukuba Breeding Centers), designated the Atsugi, Hino, and Tsukuba IGS groups, respectively, and the results were compared with those in Crj:CD(SD) rats derived from the Hino Breeding Center, designated the Hino CD group. Incidences of cardiac granuloma in males of the Atsugi, Hino, and Tsukuba IGS groups were significantly higher in that of the Hino CD gourp, but there were no siginificant differences of the lesion in males and females among those three groups of IGS rats. Cardiac granuloma was observed as small foci of histiocytic and lymphoid cell accumulation with or without minimal myocardial degeneration. There were no significant differences in the incidences of other cardiac lesions in male and females among all the IGS groups and the CD group. In the liver, granuloma, i.e. microgranuloma, occurred with a high incidence in males and females of all the IGS groups and the CD group. However, there were no significant differences in the incidences of the granuloma and other hepatic lesions in males and females among all the IGS groups and the CD group. Thus, it is likely that there are no interlaboratories heterogeneity of spontaneous cardiac and hepatic lesions in IGS rats bred in the three breeding colonies in Japan. — Key words: Cardiac lesions, Crj:CD(SD)IGS, Hepatic lesions, Rat.

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INTRODUCTION

Crj:CD(SD)IGS rats have been created by the international genetic standard system, which was organized to supply experimental animals with homogeneous characteristics, minimizing genetic diversification in Charles River, Inc. The animals are expected to meet internationalization of research and development of new drugs. In regard of the breeding system, we examine histopathologically spontaneous cardiac and hepatic lesions in Crj:CD(SD)IGS rats derived from different breeding colonies to elucidate the interlaboratories heterogeneity.

MATERIALS AND METHODS

Thirty each of male and female Crj:CD(SD)IGS rats were bred in Atsugi Breeding Center (Charles River Japan, Inc., Kanagawa: the Atsugi IGS group), in Hino Breeding Center (Shiga: the Hino IGS group), and in Tsukuba Breeding Center (Ibaraki: the Tsukuba IGS group). The same numbers of Crj:CD(SD) rats were bred in Hino Breeding Center (the Hino CD group). These rats were purchased at 9, 8, 9, and 9 weeks of age, respectively and examined in the Nippon Institute for Biological Science (Tokyo). The animals were housed individually in a wire-mesh cage (21 ×35 ×20 cm) and were maintained in a barrier-sustained room controlled at 20-25℃ and 30-70% relative humidity, and ventilated 10 times per hr, with a 12-hr light-dark cycle. The animals had free access to a commercial standard diet for rats (CRF-1, Oriental Yeast Co., Tokyo) and tap water. They cared for and were treated humanely in accordance with the *Guidelines for Animal Experimentation*, published by the Japanese Association for Laboratory Animal Science (Exp. Anim. 36: 285-288, 1987).

All rats were observed for clinical signs and mortality twice daily and weighed twice in the 7-day-acclimatization period (day-1 and day-7). Following the acclimatization, these rats were euthanatized by ether inhalation and subjected to a complete necropsy. The heart, liver, and tissues with gross lesions were fixed in 10% neutral buffered formalin. Two parts of tissues were trimmed from the outer left lobe and the inner right lobe of the liver, and a longitudinal section was trimmed from the heart. These tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined histopathologically. The degree of granuloma in the liver was graded as follows: score 0, no granulomas; score +, 1 to 5 foci of granulomas; score ++, 6 to 10 foci of granulomas; and score +++, more than 11 foci of granulomas. The degree of other lesions observed was graded as follows: score 0, no lesion; score +, slight lesion and score ++, moderate lesion. The numbers of granuloma lesions were calculated by observing all areas of the liver tissues in each animal.

The statistical significance was assessed by Fisher's exact test for differences in the incidences of all lesions and by Student's t-test for differences in the number of hepatic granuloma among the Astugi IGS, Hino IGS, Tsukuba IGS, and Hino CD groups.

RESULTS

During the acclimatization period, no animals showed any abnormalities in clinical signs. Body weights on the day before necropsy (day-7) were increased in all rats as compared with those at receipt (day-1).

Grossly, pelvic dilatation of the kidney was observed in one male of the Atsugi IGS group and one female of the Tsukuba IGS group. A supernumerary lobe of the liver was observed in one female of the Tsukuba IGS group. No abnormal findings were found in any animals of the Hino CD and Hino IGS groups.

Histopathologically, cardiac granuloma was detected in males and females of all the IGS groups and the CD group (Table 1). The granuloma was observed as small foci of inflammatory cell aggregations consisting of macrophages, histiocytic and lymphoid cells, and occasionally granulocytes, with or without minimal degeneration of myocardial fibers. The total incidences of the granulomas in the Hino CD, Atsugi IGS, Hino IGS, and Tsukuba IGS groups were 3.3%, 23.3%, 23.3%, and 36.7% in males, and 6.7%, 13.3%, 10.0%, and 10.0% in females, respectively. These incidences in males of all the IGS groups were significantly ($p<0.05$ in the Atsugi IGS and Hino IGS groups and $p<0.01$ in the Tsukuba IGS group) higher than that of the Hino CD group. The

Table 1. Histopathology of the heart and liver in rats

			Group	Hino CD	Atsugi IGS	Hino IGS	Tsukuba IGS
Sex	Organs	Findings	No. of rats	30	30	30	30
Male	Heart	Granuloma +		1 (3.3)	6 (20.0)	6 (20.0)	11 (36.7) **
		Granuloma ++		0 (0.0)	1 (3.3)	1 (3.3)	0 (0.0)
		Granuloma, total		1 (3.3)	7 (23.3) *	7 (23.3) *	11 (36.7) **
		Myocardial degeneration+		1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)
		Epicardial proliferation +		1 (3.3)	0 (0.0)	0 (0.0)	0 (0.0)
		Medial proliferation of artery +		0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
	Liver	Focal necrosis +		0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)
		Cell infiltration +		0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)
		Granuloma +		23 (76.7)	20 (66.7)	21 (70.0)	20 (66.7)
		Granuloma ++		2 (6.7)	2 (6.7)	1 (3.3)	4 (13.3)
		Granuloma +++		0 (0.0)	0 (0.0)	0 (0.0)	2 (6.7)
		Granuloma, total		25 (83.3)	22 (73.3)	22 (73.3)	26 (86.7)
		Altered cell foci +		0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
Female	Heart	Cell infiltration +		0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
		Granuloma +		2 (6.7)	4 (13.3)	3 (10.0)	2 (6.7)
		Granuloma ++		0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)
		Granuloma, total		2 (6.7)	4 (13.3)	3 (10.0)	3 (10.0)
		Myocardial degeneration +		0 (0.0)	1 (3.3)	0 (0.0)	1 (3.3)
	Liver	Hemorrhage +		0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
		Focal necrosis +		0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
		Cell infiltration +		0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)
		Granuloma +		19 (63.3)	25 (83.3)	19 (63.3)	23 (76.7)
		Granuloma ++		7 (23.3)	2 (6.7)	6 (20.0)	6 (20.0)
		Granuloma +++		3 (10.0)	0 (0.0)	3 (10.0)	1 (3.3)
		Granuloma, total		29 (96.7)	27 (90.0)	28 (93.3)	30 (100.0)

*: Significantly different from the Hino CD group at $p<0.05$.
**: Significantly different from the Hino CD group at $p<0.01$.
+: Slight. ++: Moderate. +++: Severe.

incidences of slight (+) lesions in the Hino CD, Atsugi IGS, Hino IGS, and Tsukuba IGS groups were 3.3%, 20.0%, 20.0%, and 36.7% in males and 6.7%, 13.3%, 10.0%, and 6.7% in females, respectively. This incidence in males of the Tsukuba IGS group was significantly ($p<0.01$) higher than that of the Hino CD group. Moderate (++) lesions were found in one male (3.3%) each of the Atsugi IGS and Hino IGS groups and one female (3.3%) of the Tsukuba IGS group. There were no significant differences of cardiac granulomas in males and females among all the IGS groups. The lesions occurred solitary or multiple in the right and left ventricles and the septum. Myocardial degeneration was found in one male (3.3%) each of all groups and one female (3.3%) each of the Atsugi and Tsukuba IGS groups, and many of them were complicated with granulomatous lesions. Focal proliferation of the epicardium and medial proliferation of the coronary artery in the right ventricle were detected in one male (3.3%) each of the Hino CD and Atsugi IGS groups, respectively. A focus of inflammatory cell infiltration in the myocardium was observed in one female (3.3%) of the Atsugi IGS group.

In the liver, granuloma was observed with high incidences in males and females of all the IGS groups and the CD group (Table 1). The granulomas were observed as small scattered foci consisting of lymphoid cells, macrophages, and histiocytic cells and contained occasionally a few degenerated hepatocytes. The total incidences of the granulomas in the Hino CD, Atsugi IGS, Hino IGS, and Tsukuba IGS groups were 83.3%, 73.3%, 73.3%, and 86.7% in males, and 96.7%, 90.0%, 93.3%, and 100.0% in females, respectively. The incidences of slight (+) lesions in the Hino CD, Atsugi IGS, Hino IGS, and Tsukuba IGS groups were 76.6%, 66.7%, 70.0%, and 66.7% in males, and 63.3%, 83.3%, 63.3%, and 76.7% in females, respectively. The incidences of moderate (++) lesions in the Hino CD, Atsugi IGS, Hino IGS, and Tsukuba IGS groups were 6.7%, 6.7%, 3.3%, and 13.3% in males, and 23.3%, 6.7%, 20.0%, and 20.0% in females, respectively. Severe (+++) lesion was observed in 2 (6.7%) males of the Tsukuba IGS group and 3 (10.0%), 3 (10.0%), and one (3.3%) female of the Hino CD, Hino IGS, and Tsukuba IGS groups, respectively. There were no significant differences in total incidences or incidences of each severity for the granulomas among all the IGS groups and the Hino CD group. There were no significant differences of the numbers of granulomas in the liver of males between the all IGS groups and the Hino CD group, whereas those in the Hino IGS group was significantly ($p<0.05$) smaller than those in the Tsukuba IGS group in males (Table 2). The numbers of granulomas in females of the Atsugi IGS group was significantly ($p<0.01$) smaller than those in the Hino CD group and also significantly ($p<0.05$) smaller than those in the Hino IGS group. In the other IGS groups any statistical significant differences were not detected in the numbers of granulomas as compared with those of the Hino CD group. Focal hepatocellular necrosis and inflam-

Table 2. Number of granuloma in the liver of rats

Sex	Group	No. of rats	No. of granuloma
Male	Hino CD	30	2.0±1.7 a
	Atsugi IGS	30	2.0±2.2
	Hino IGS	30	1.7±1.5 †
	Tsukuba IGS	30	3.6±4.4
Female	Hino CD	30	5.5±4.3
	Atsugi IGS	30	2.8±1.7 **, ‡
	Hino IGS	30	4.2±3.4
	Tsukuba IGS	30	4.1±3.2

a: Mean±SD.
**: Significantly different from the Hino CD group at $p<0.01$.
† : Significantly different from the Tsukuba IGS group at $p<0.05$.
‡ : Significantly different from the Hino IGS group at $p<0.05$.

matory cell infiltration were observed in one male of the Hino IGS group, hemorrhage, focal hepatocellular necrosis and inflammatory cell infiltration in one female of Atsugi IGS group, and altered cell foci in one female of the Tsukuba IGS group.

In the kidney, pelvic dilatation observed grossly in one male of the Atsugi IGS group and in one female of the Tsukuba IGS group was confirmed histologically. Basophilic tubular epithelium was detected in this male rat of the Atsugi IGS group.

DISCUSSION

Pelvic dilatation of the kidney was observed in two IGS rats, and supernumerary lobes of the liver in an IGS rat. Pelvic dilatation (hydronephrosis) of the kidney has been reported to develop spontaneously in young rats with the incidence being 10% in both male and female CD(SD) rats, and considered to be one of the congenital or developmental anomaly [3]. Supernumerary lobes of the liver is frequently accompanied with diaphragmatic hernia and also called hepatodiaphragmatic nodule of the liver [1]. The hepatic lesion has been considered to be a developmental anomaly involving both the liver and diaphragm [1]. It has been reported that the incidence of the lesion was 2.5% in Sprague-Dawley (SD) rats at 13 weeks of age [2].

Cardiac granuloma is one of the spontaneous lesions observed frequently in rats regardless of the strains [1,4]. Histopathological appearance of cardiac granuloma is varied depend on the examination time and the early lesion is comprised by myocardial degeneration and reactive cellular infiltration [2,4]. Local ischemia and nutritional defect may be a possible cause of this lesion, though there is no reliable evidence provided [2,4]. It has been known that the granuloma had no specific site in occurrence in the heart and would occur in rats as young as 10 weeks of age [4], coincided with our results. Cardiac granuloma has been shown an age-related increase in incidence, which was higher in males than females under the same conditions [4]. In the present study, cardiac granuloma was observed more frequently in males than females. Total incidences of cardiac granuloma in males of all the IGS groups were significantly higher than that of the Hino CD group, and the incidence of slight (+) lesions was significantly higher in males of the Tsukuba IGS group than the Hino CD group. However, the granulomas detected in all the IGS groups were histologically similar to those in the Hino group, and there were no significant differences in the incidences of the lesions among the IGS groups derived from three different breeding colonies. Accurate causes of higher incidences of cardiac granulomas in the IGS rats remained to be elucidated. Myocardial degeneration, which was occasionally observed within granulomatous lesions, is mentioned as cardiomyopathy and the etiology is unknown [1].

Hepatic granuloma is one of the spontaneous lesions observed frequently in several strains of rats including the SD strain and is generally more prevalent in females than males [1, 2, 4]. In the present study, hepatic granulomas were observed in most of both CD(SD) and IGS rats and the incidences of the lesions in females were slightly higher than those in males. However, the majority of hepatic granulomas in both CD(SD) and IGS rats showed a slight lesion score. There were no significant differences in the incidences and lesion degrees of hepatic granulomas among all the IGS groups and the Hino CD group. Though the numbers of the granulomas in the male Hino IGS group and in the female Atsugi IGS group were significantly smaller than those in the male Tsukuba IGS group and in the female Hino CD and IGS groups, respectively, there were no significant differences in those among the other IGS groups and the Hino CD group. Histopathological characteristics of hepatic granuloma observed in these IGS rats were essentially identical to those in CD(SD) rats. These results suggest that the degree of granulomatous lesions of the liver detected in IGS rats was not substantially different from that in CD(SD) rats. Hence the incidences, degrees, and numbers of hepatic granulomas seem similar among the IGS groups bred in three different breeding colonies. The other lesions detected in the liver of IGS and CD(SD) rats are generally considered as spontaneous lesions of rats.

From the results mentioned above, it is likely that there are no interlaboratories heterogeneity of spontaneous cardiac and hepatic lesions in IGS rats bred in the three breeding colonies in Japan.

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REFERENCES

1. Boorman, G. A., Eustis, S. L., Elwell, M. R., Montgomery, C. A. and MacKenzie, W. F. 1990. Pathology of the Fischer rat. Academic Press, Inc., San Diego.
2. Enomoto, M. and Akazaki K. 1987. Color Atlas of Toxicologic Pathology. Soft Science Piblications, Tokyo (in Japanese).
3. Glaister, J R. 1986. Principles of toxicological pathology. Taylor & Francis, London
4. Maekawa, A. and Hayashi, Y. 1992. Toxicologic Pathology. Chijin-shokan Co., Ltd., Tokyo (in Japanese).

Effects of Twenty Percent Dietary Restriction on Body Weight, Hematology, Blood Chemistry and Organ Weight in Crj:CD (SD) IGS Rats

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ABSTRACT. This study was performed to compare rats with and without dietary restriction and to obtain the background data essential for the 13-week preliminary study of carcinogenicity studies.
Body weight of the 20%-restricted diet (DR) group changed at a level approximately 20% below the *ad libitum* (AL) group in both males and females. No differences were observed in the hematological examination between the groups. In the blood chemical examination, a lower urea nitrogen value was observed in both males and females in the DR group, which was considered to be a change due to the decreased intake of protein and, at the same time, to be due to decreased strain on the kidneys. In respect to organ weight, lower values were observed in many organs, accompanied by lower values in body weight, in the DR group, with higher ratio to body weight in many organs.
Based on the ratio of organ weight to brain weight, the heart, liver and kidneys showed substantially lower values.
In the necropsy findings, no differences were observed in males or females in either group. －Key words: food restriction, body weight, organ weight.
CD(SD)IGS-1999: 141-147

INTRODUCTION

In Japan, F344 rats are being used most commonly in carcinogenicity studies. However, oral administration by gavage is required recently in the carcinogenicity studies, studies using SD rats tend to be increasing in number. Because of the short life span of SD rats, it is recommended that carcinogenicity studies be performed under dietary restricted conditions.

In this study, we had IGS(CD)SD rats under unrestricted and 20%-restricted dietary conditions and collected data on body weight, food consumption, hematology, blood chemistry, organ weights and macroscopic observation. The purpose of this study was to obtain data on the effects of dietary restriction in the 13-week preliminary study to prepare for carcinogenicity studies using IGS(CD)SD rats under dietary restricted conditions.

MATERIALS AND METHODS

Animals and housing conditions:

Twenty-five male and 25 female Crj:CD(SD)IGS rats were purchased from Charles River Japan Inc. at 4 weeks of age and, after one week of acclimation to the housing environments, were assigned to the AL and DR groups, 10 males and 10 females each, and were bred for 13 weeks starting from 5 weeks of age.

The animals were housed in an animal room maintained at 23 ± 3℃ and 55 ± 20% relative humidity. The room air was ventilated 20 times/hour and illuminated 12 hours/day. Animals were housed individually in the aluminum cage with stainless mesh at the front and the floor (W 20.0 × D 28.2 × H 18.0 cm) and had free access to tap water. Animals in the AL group had free access to the commercial diet, Modified NIH Open Formula Rat Ration (Oriental Yeast Co., Ltd., Tokyo) sterilized with γ-ray irradiation and animals in the DR group were provided 80% of the amount consumed daily calculated based on the background food consumption data for the rats of the same strain.

Observations and examinations:

The general condition was observed twice daily, in the morning and afternoon. Body weight and food consumption were measured once weekly for the AL group and once daily for the DR group. Animals were fasted at the end of the testing period for 16 hours and blood was collected under ether anesthesia via abdominal aorta. EDTA-2K-added blood was analyzed for hematocrit values (HCT: calculated from RBC, MCV), hemoglobin values (HGB: cyanmethemoglobin method), red blood cell counts (dark-field disk method), mean corpuscular volume (MCV: dark-field disk method), mean corpuscular hemoglobin (MCH: calculated from HGB, RBC), mean corpuscular hemoglobin concentration (MCHC: calculated from HGB, HCT), platelet counts (PLT: dark-field disk method), white blood cell counts (WBC: flow cytometory method) and differential leukocyte counts (flow cytometory method) with THMS H・1E (Miles Laboratories, USA). Furthermore, blood plasma obtained by centrifuging blood containing 3.13% sodium citrate was measured for prothrombin time (PT: quick 1 step method) and activated partial thromboplastin time (APTT: clot method) with the blood clotting time automatic analyzer KC-40 (Amelung Co., Germany).

Furthermore, serum was analyzed for total protein (T-protein: Biuret method), albumin (BCG method), A/G (A/G: calculated from TP and Alb), glucose (HK-G-6PDH method), triglyceride (GK-GPO glycerate oxidase method), total cholesterol (T.cholesterol: cholesterol oxidase method and HDAOS method), urea nitrogen (BUN: urease and GLDH method), creatinine (Creatinine: enzyme method), total bilirubin (T.bilirubin: vanadic acid oxidase method), glutamic oxalacetic transaminase (GOT: enzyme-UV method), glutamic pyruvic transaminase (GPT: enzyme-UV method), alkaline phosphatase (ALP: p-nitrophenylphosphate substrate method), calcium (MXB method) and inorganic phosphorus (I. phosphorus: PNP-XOD method) with an automatic chemical analyzer HITACHI 7170 (Hitachi Ltd., Tokyo) and for sodium (electrode method), chloride (electrode method) and potassium (electrode method) with the electrolyte analyzer EAO6R (A&T Corporation, Tokyo). Furthermore, protein electrophoretic examinations were performed on the above serum with the automatic electrophoretic apparatus, Epalyzer, and TITAN Ⅲ cellulose acetic membranes (Helena Laboratories, Saitama, Japan).

Animals euthanized by blood collection were subjected to macroscopic observation. Brain, livers, kidneys, spleens, adrenal glands, testes, ovaries and mandibular gland (including sublingual gland) were weighed and organ weight to body weight ratios were calculated.

Body weight, food consumption, hematological values, blood chemical values, organ weight and organ weight to body weight ratios were subjected to F test. Significance of differences between the two groups was analyzed with Student's *t*-test when the differences were not significant and with the Aspin-Welch *t*-test when the differences were significant.

RESULTS

1. Mortality

No dead animals were found in either males or females in either group.

2. Observations of general conditions

No abnormal animals were found in either males or females in either group.

3. Body weight (Table 1 and Fig. 1)

Body weight gain was suppressed from Week 1 of study in both males and females in the DR group in comparison with the AL group. As shown in Fig. 2, rates of decrease of the mean body weight in the DR group versus the AL group at each week of study fluctuated above and below 20% after Week 4 in males, after Week 3 in females. The mean body weight in the DR group at Week 13 was lower than that in the AL group by 21.4% for males and 23.8% for females.

4. Food consumption (Table 2)

Food consumption at every week in the DR group which was provided daily with a diet equivalent to 80% of the back-

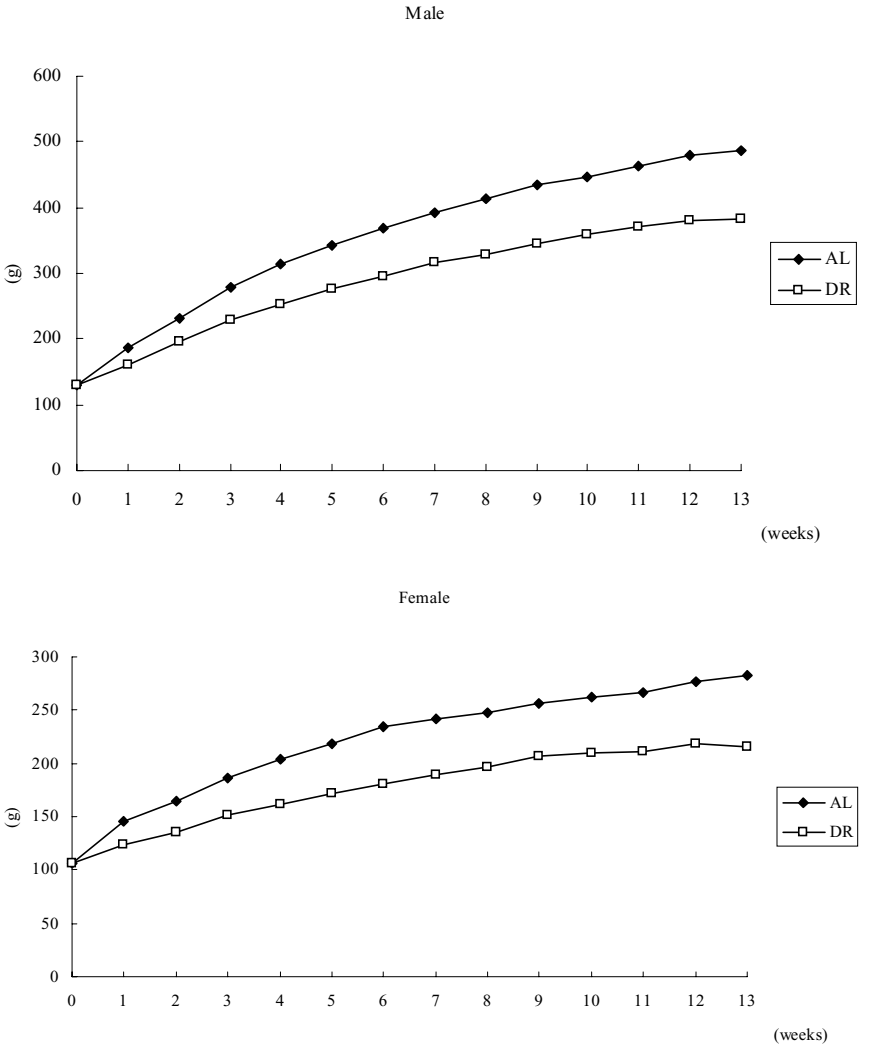

Figure 1. Body weight of CD(SD)IGS rats fed ad libitum (AL) or 20% dietary restricted (DR)

Table 1. Body weight of CD(SD)IGS rats fed *ad libitum* (AL) or 20% dietary restricted (DR)

Male (unit: g)

Weeks	AL	DR
0	129±6	129±6
1	187±9	160±6***
2	232±10	196±8***
3	278±14	230±8***
4	313±20	253±10***
5	343±27	277±14***
6	368±32	295±15***
7	391±35	316±14***
8	413±36	328±16***
9	434±41	346±19***
10	447±43	359±18***
11	462±44	378±18***
12	480±48	381±20***
13	486±53	382±21***

Female (unit: g)

Week	AL	DR
0	107±5	107±5
1	145±16	124±4**
2	165±18	136±4***
3	187±20	151±6***
4	204±24	162±5***
5	219±25	172±8***
6	234±31	181±8***
7	242±39	190±8**
8	247±34	197±7**
9	256±33	207±7**
10	262±34	209±8***
11	267±34	211±8***
12	277±37	218±8***
13	282±41	215±7***

Values are expressed as Mean±S.D.
Significant difference from AL group;
*: P≦0.05 **: P≦0.01 ***: P≦0.001

Table 2. Food consumption of CD(SD)IGS rats fed *ad limitum* (AL) or 20% dietary restricted (DR)

Male (unit: g/week)

Weeks	AL	DR
1	148±10	119±0
2	160±5	126±0
3	166±9	126±0
4	170±11	126±0
5	173±15	126±0
6	172±14	133±0
7	173±19	133±0
8	178±18	133±0
9	173±16	133±0
10	177±17	126±0
11	180±15	126±0
12	174±17	126±0
13	179±17	126±0

Female (unit: g/week)

Week	AL	DR
1	117±13	91±0
2	118±12	91±0
3	121±12	91±0
4	128±16	97±2
5	124±13	98±0
6	127±21	98±0
7	125±27	97±1
8	119±13	98±0
9	118±13	98±0
10	119±13	91±0
11	121±10	91±0
12	114±11	91±0
13	124±19	84±0

Values are expressed as Mean±S.D.

ground consumption of rats of the same strain and same age was 70.0～80.4% (average restriction 25.2%) for males and 67.7 ～ 83.1% (average restriction 22.6%) for females as compared to the AL group.

5. Hematology (Table 3)

In males, slightly lower values in red blood cell counts and slightly higher values in MCV and MCH were observed in the DR group versus the AL group. Furthermore, slightly shortened PT and APTT were observed in the DR group in the blood clotting examinations. In females, no differences were observed between the groups in any items of examination.

6. Blood chemistry (Table 4)

In males, slightly lower values were observed in glucose, urea nitrogen, GOT and GPT in the DR group versus the AL group.

In females, slightly higher values in A/G, lower values in urea nitrogen and slightly lower values in potassium and inorganic phosphorus were observed in the DR group versus the AL group.

In the serum protein electrophoretic examination, slightly lower values were observed only in α1-globulin fraction ratios in females in the DR group versus the AL group.

7. Organ weight (Table 5)

In males, lower values in absolute weight of heart, liver, kidneys and spleen and higher values in relative weight of brain, testes and mandibular gland were observed in the DR group versus the AL group.

In females, lower values in absolute weight of heart, liver, kidneys, adrenal glands and ovaries and higher values in relative weight of brain, liver, kidneys, spleen and mandibular gland were observed in the DR group versus the AL group.

8. Macroscopic observation

In males, no abnormal animals were found in either the DR group or the AL group.

In females, a white node (1×2 mm) in the liver in 1 ani-

Table 3. Hematology of CD(SD)IGS rats fed *ad libitum* (AL) or 20% dietary restricted (DR)

Male

Item		AL	DR
No. of animals		10	10
HCT	(%)	45.0±1.8	45.2±1.4
HGB	(g/dL)	16.0±0.6	16.0±0.5
RBC	($\times 10^6$/mm^3)	9.20±0.32	8.90±0.28*
MCV	(μm^3)	48.9±1.8	50.8±1.2*
MCH	(pg)	17.4±0.7	18.0±0.5*
MCHC	(%)	35.5±0.4	35.4±0.3
PLT	($\times 10^3$/mm^3)	1047±99	1102±66
WBC	($\times 10^3$/mm^3)	9.6±3.0	7.1±2.2*
Differential leukocyte counts (%)			
NEUT		12±2	13±4
LYMPH		82±2	82±4
MONO		3±2	2±1
EOSN		1±1	2±1
BASO		0±0	0±0
LUC		2±1	1±0*
PT	(sec.)	15.8±0.9	15.1±0.3*
APTT	(sec.)	27.1±1.3	25.1±2.3*

Female

Item		AL	DR
No. of animals		10	10
HCT	(%)	42.3±1.4	43.0±0.8
HGB	(g/dL)	15.1±0.5	15.4±0.4
RBC	($\times 10^6$/mm^3)	8.12±0.28	8.14±0.28
MCV	(μm^3)	52.2±1.2	52.8±1.3
MCH	(pg)	18.6±0.4	18.9±0.5
MCHC	(%)	35.7±0.4	35.8±0.3
PLT	($\times 10^3$/mm^3)	1050±113	1056±126
WBC	($\times 10^3$/mm^3)	5.2±2.2	4.5±1.5
Differential leukocyte counts (%)			
NEUT		16±5	17±6
LYMPH		78±5	78±7
MONO		3±1	2±1
EOSN		2±1	2±1
BASO		0±0	0±0
LUC		1±1	1±0
PT	(sec.)	15.3±0.4	15.3±0.6
APTT	(sec.)	21.7±1.6	20.5±1.1

NEUT:Neutrophil LYMPH:Lymphocyte MONO:Monocyte EOSN:Eosinophil
BASO:Basophil LUC:Large unstained cells
Values are expressed as Mean±S.D.
Significant difference from AL group; *: P≦0.05 **: P≦0.01 ***: P≦0.001

Table 4. Blood chemistry of CD(SD)IGS rats fed *ad libitum* (AL) or 20% dietary restricted (DR)

Male

Item		AL	DR
No. of animals		10	10
Glucose	(mg/dL)	161±20	145±14*
T.cholesterol	(mg/dL)	51±17	55±16
Triglyceride	(mg/dL)	60.2±57.4	49.5±35.1
BUN	(mg/dL)	15.2±1.8	13.4±1.9*
Creatinine	(mg/dL)	0.28±0.05	0.27±0.04
T.bilirubin	(mg/dL)	0.04±0.01	0.05±0.01
T.protein	(g/dL)	5.92±0.22	5.81±0.19
Albumin	(g/dL)	3.26±0.12	3.29±0.11
A/G		1.24±0.11	1.31±0.08
Sodium	(mmol/L)	142.9±1.7	143.3±1.0
Potassium	(mmol/L)	4.44±0.44	4.62±0.22
Chloride	(mmol/L)	106.3±2.2	107.8±1.0
Calcium	(mg/dL)	9.68±0.27	9.56±0.23
I.phoshorus	(mg/dL)	6.08±0.28	5.91±0.46
GOT	(U/L)	72±9	62±11*
GPT	(U/L)	28±4	24±4*
ALP	(U/L)	360±84	330±55
Albumin	(%)	46.9±2.2	48.4±1.8
Alpha-1	(%)	23.5±2.3	22.3±2.2
Alpha-2	(%)	8.7±0.5	8.7±0.6
Beta	(%)	16.2±1.3	15.7±0.7
Gamma	(%)	4.7±0.9	4.9±0.8
A/G		0.89±0.08	0.94±0.07

Values are expressed as Mean±S.D.
Significant difference from AL group; *: P≦0.05 **: P≦0.01 ***: P≦0.001

Table 4. -continued Blood chemistry of CD(SD)IGS rats fed a*d libitum* (AL) or 20% dietary restricted (DR)

Female

Item		AL	DR
No. of animals		10	10
Glucose	(mg/dL)	147±19	136±17
T.cholesterol	(mg/dL)	73±12	63±10
Triglyceride	(mg/dL)	19.9±7.6	23.0±7.9
BUN	(mg/dL)	17.6±2.8	14.2±1.5**
Creatinine	(mg/dL)	0.34±0.04	0.31±0.04
T.bilirubin	(mg/dL)	0.05±0.02	0.06±0.01
T.protein	(g/dL)	6.35±0.30	6.28±0.37
Albumin	(g/dL)	3.75±0.25	3.84±0.30
A/G		1.44±0.12	1.58±0.13*
Sodium	(mmol/L)	142.9±1.8	143.7±0.9
Potassium	(mmol/L)	4.25±0.26	4.00±0.16*
Chloride	(mmol/L)	109.3±1.7	110.8±1.5
Calcium	(mg/dL)	9.77±0.33	9.71±0.30
I.phoshorus	(mg/dL)	4.57±0.43	3.94±0.66*
GOT	(U/L)	88±33	66±7
GPT	(U/L)	38±24	25±4
ALP	(U/L)	199±73	247±62
Albumin	(%)	51.8±2.2	52.9±1.4
Alpha-1	(%)	19.6±1.6	18.2±1.3*
Alpha-2	(%)	7.5±0.7	7.8±0.6
Beta	(%)	14.4±0.8	14.7±0.6
Gamma	(%)	6.7±1.4	6.5±1.3
A/G		1.08±0.10	1.12±0.06

Values are expressed as Mean±S.D.
Significant difference from AL group; *: P≦0.05 **: P≦0.01 ***: P≦0.001

Table 5. Absolute and relative organ weights of CD(SD)IGS rats fed *ad limitum* (AL) or 20% dietary restricted (DR)

Male

Item		AL	DR
No. of animals		10	10
Body weight (g)		486±53	382±21***
Absolute organ weight			
Brain	(g)	2.18±0.11	2.14±0.06
Heart	(g)	1.37±0.08	1.12±0.08***
Liver	(g)	12.15±1.93	9.36±0.77**
Kidneys	(g)	3.23±0.37	2.66±0.15***
Spleen	(g)	0.72±0.06	0.62±0.10*
Adrenals	(mg)	58±7	53±5
Testes	(g)	3.23±0.16	3.31±0.29
Mandibular gland	(mg)	739±52	726±59
Relative organ weight			
Brain	(%)	0.454±0.046	0.561±0.040***
Heart	(%)	0.284±0.020	0.294±0.011
Liver	(%)	2.491±0.173	2.449±0.167
Kidneys	(%)	0.668±0.060	0.696±0.035
Spleen	(%)	0.148±0.013	0.162±0.021
Adrenals	(%)	0.012±0.003	0.014±0.002
Testes	(%)	0.672±0.075	0.867±0.072***
Mandibular gland	(%)	0.154±0.022	0.190±0.017***

Mean±S.D.
Significant difference from AL group; *: P≦0.05 **: P≦0.01 ***: P≦0.001

Table 5. -continued Absolute and relative organ weights of CD(SD)IGS rats fed *ad limitum* (AL) or 20% dietary restricted (DR)

Female

Item		AL	DR
No. of animals		10	10
Body weight (g)		282±41	215±7***
Absolute organ weight			
Brain	(g)	2.02±0.08	1.96±0.07
Heart	(g)	0.84±0.11	0.71±0.06**
Liver	(g)	6.63±1.04	5.63±0.51*
Kidneys	(g)	1.82±0.21	1.60±0.13**
Spleen	(g)	0.49±0.11	0.42±0.04
Adrenals	(mg)	72±10	61±8*
Ovaries	(mg)	87±15	75±8*
Mandibular gland	(mg)	497±36	510±54
Relative organ weight			
Brain	(%)	0.726±0.082	0.909±0.036***
Heart	(%)	0.301±0.037	0.330±0.026
Liver	(%)	2.352±0.125	2.613±0.218**
Kidneys	(%)	0.653±0.066	0.741±0.051**
Spleen	(%)	0.173±0.026	0.197±0.020*
Adrenals	(%)	0.026±0.004	0.028±0.003
Ovaries	(%)	0.031±0.005	0.035±0.003
Mandibular gland	(%)	0.179±0.019	0.237±0.026***

Mean±S.D.
Significant difference from AL group; *: P≦0.05 **: P≦0.01 ***: P≦0.001

mal, malformed node (9 × 11 mm, adhered to diaphragm) in the liver in 1 animal and dilated lumen of uterus in 1 animal were observed in DR group.

DISCUSSION

In 1969, "Principles for the Testing and Evaluation of Drugs for Carcinogenicity" [1] was issued by WHO. Since then, various guidelines for carcinogenicity studies for chemical substances including pharmaceuticals have been made based on these principles in many countries and carcinogenicity studies have been performed pursuant to such guidelines.

All these guidelines require life span tests to be performed in two species of rodents. On the relation between calorie intake and life span in the long-term studies, Berg *et al.* [2] raised the question in 1961 that they correlated inversely but all the guidelines were without dietary restriction.

In the early 1990's, Imai *et al.* [3] and Boutwell (1992) [4] proved that dietary restriction caused delay in development of spontaneous tumors. In U.S.A., where SD rats were used frequently in the carcinogenicity studies, the life span of the rats became considerably shorter to such levels which were unable to fulfill the requirements of the guidelines and for this reason, Keenan *et al.* (1992) [5] proposed that dietary restriction be considered in the carcinogenicity studies.

In Japan, F344 rats have been used in the carcinogenicity studies but since oral administration by gavage has been required recently in the carcinogenicity studies, studies using SD rats have been on the increase, inevitably requiring dietary restriction issues to be addressed.

When carcinogenicity studies are performed under restricted dietary conditions, preliminary 13-week studies are also required to be performed under the same restricted dietary conditions and in view of this, this study was performed in the rats had under 20% restricted dietary conditions for 13 weeks to obtain data on body weights, food consumption, hematology, blood chemistry, organ weights and gross observation.

Many dietary restriction studies have been performed more or less using a 40%-restricted diet because it definitely prolongs the life span of the animals. However, high degrees of restriction may cause reduced susceptibility of animals to carcinogenicity due to the reduction in cell division and inhibition of metabolic activation. Based on the results of the comparative study between the moderate restriction (75 ∼ 80% of AL) and marked restriction (50% of AL), *Keenan et al.* [6] reported that a moderate dietary restriction (75 ∼ 80% of AL) was most appropriate as a model for carcinogenicity evaluation.

Taking such background into consideration, a 20% diet restriction was selected in this study. The actual average percentage of restriction slightly exceeded the theoretical value, 25.2% for males and 22.6% for females, but was considered satisfactory.

In respect to body weight of both males and females in the DR group, roughly 20% suppression was observed nearly in correlation to the dietary restriction.

In respect to the hematological and blood chemical examination, statistically significant differences were observed in some of the items examined, but they were not considered as important except for urea nitrogen.

In the long-term studies in rats, chronic nephropathy occurs very frequently, partly causing reduction in mortality. Gumprecht [7] and Keenan *et al.* [8] proved that there were considerable differences in chronic nephropathy development between animals on restricted and unrestricted dietary conditions and that the dietary restriction prevented at an early stage the glomerular hypertrophy which triggers chronic nephropathy.

Lower values of urea nitrogen in the blood serum in the DR group observed in this study is considered due to the decrease in protein intake as a direct cause but in the longer term, lower values in urea nitrogen are considered to reduce the load on the kidneys, delaying development of chronic nephropathy.

In respect to organ weight, lower values in absolute weight and higher values in organ weight to body weight ratios were observed in many organs of both males and females in the DR group accompanied by lower body weight versus the AL group. Since brain, liver and kidneys showed lower values in the organ weight to body weight ratios in both males and females, these three organs were considered to show substantially lower values.

In the gross observation, no marked changes were observed in either males or females.

REFERENCES

1. World Healthy Organization, 1969. Principles for the testing and evaluation of drugs for carcinogenicity. Technical Report Series No. 426. WHO, Geneva.
2. Berg, B.N., Simms, S., 1961. Nutrition and longevity in rat. J. Nutr 74: 23-32.
3. Imai, K., Yoshimura, S., Hashimoto, K., Boorman, G.A., 1991. Effect of dietary restriction on age-associated pathological changes in Fischer 344 rats. In Fishben L (ed), Biological effects of dietary restriction. ILSI Monographs. Springer-Verlag, New York, pp 87-98.
4. Boutwell, R.K., 1992. Caloric intake, dietary fat level and experimental carcinogenesis. In Jacos, M.M. (ed), Exercise, calories, fat and cancer. Plenum Press, New York, pp 95-101.
5. Keenan, K.P., Smith, P., Ballam, G., *et al.*, 1992. The effects of diet and dietary optimization (caloric restriction) on survival in carcinogenicity studies – an industry viewpoint. In McAuslane, J.A.N., Lumeley, C.E., Walker, S.R. (eds), The carcinogenicity debate. CMR Workshop Series. Quay Publishing, Lancaster, UK, pp 77-102.
6. Keenan, K.P., *et al.*, 1998. The effects of ad libitum (AL) overfeeding and moderate or marked dietary restriction (DR) on body weight and consumption, survival, and causes of death in Sprague-Dawley (SD) rats. Conference: The role of diet and caloric intake in aging, obesity, and cancer. October 26-28, 1998, Reston, VA.
7. Gumprecht, L.A., 1993. The early effects of dietary restriction on the pathogenesis of chronic reanal disease in Sprague-Dawley rats at 12 months. Toxicol. Pathol. 21 (6): 528-537.
8. Keenan, K.P., Soper, K.A., Hertzog, P.R., *et al.*, 1995. Diet, overfeeding and moderate dietary restriction in control Sprague-Dawley rats, II: Effects on age-related proliferative and degenerative lesions. Toxicol. Pathol. 23(3): 287-302.

CHAPTER 3

Reproduction Toxicology Related To

Comparison of Sperm Characteristics between Crj:CD (SD) rats and Crj:CD (SD) IGS rats

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ABSTRACT. In order to clarify sperm characteristics in Crj:CD(SD)IGS rats, we compared background data such as sperm count, sperm motility, sperm viability and incidence of sperm with abnormalities in 9 to 11 fertility studies using Crj:CD(SD)IGS rats conducted from November 1995 to December 1998 and in 18 to 25 fertility studies using Crj:CD(SD) rats conducted from August 1994 to December 1998. Although significantly high sperm count and significantly low sperm motility were noted in individual data of Crj:CD(SD)IGS rats, no significant differences between Crj:CD(SD)IGS rats and Crj:CD(SD) rats were noted in the study data and it was considered that these parameters in Crj:CD(SD)IGS rats were almost the same as in Crj:CD (SD) rats. However, the incidence of sperm with abnormalities in Crj:CD (SD) IGS rats were higher than in Crj:CD (SD) rats.

Key words: Crj:CD(SD)IGS rats, Sperm characteristics

CD(SD)IGS-1999: 149-152

INTRODUCTION

We compared reproductive and developmental background data on Crj:CD(SD) rats and Crj:CD(SD)IGS rats in a previous report [1]. We have accumulated background data from the sperm examinations of the control groups in fertility studies using Crj:CD (SD)IGS rats (hereafter referred to as IGS rats) conducted from November 1995 to December 1998. In this report, we compared data on sperm characteristics of IGS rats with those from fertility studies using Crj:CD(SD) (hereafter referred to as CD rats) rats conducted from August 1994 to December 1998.

MATERIALS AND METHODS

We compared background data regarding the sperm characteristics such as sperm count, sperm motility, sperm viability and incidence of sperm with abnormalities from 203 to 223 males in 9 to 11 fertility studies using IGS rats with those from 349 to 448 males in 18 to 25 fertility studies using CD rats.

Animals were obtained from Charles River Japan Inc. (Atsugi Breeding Center) in all studies. The animals were housed in animal rooms which were maintained at a temperature of 23 ± 3℃ and a relative humidity of 50 ± 20%, air ventilation at 10 - 15 times per hour, and 12-hour light cycle. The animals were housed individually in wire mesh cages, and pellet diet (NMF: Oriental Yeast, Co., Ltd.) and tap water were provided *ad libitum*.

Sperm examination was done at about 17 to 21 weeks of age. The animals were sacrificed by exsanguination under ether anesthesia. The epididymides were removed and the right epididymis was used for the sperm examination. After measurement of the weight, small cuts were made on the caudal epididymis in Hanks' solution including 0.5% concentration of Bovine Serum Albumin and sperm suspensions were made at 37℃.

For the sperm count, the sperm suspensions were diluted 40 times by volume with 0.5% formalin saline solution. The total number of sperm in a small volume of the diluted sample was counted using a Thoma's hemocytometer. For sperm motility, the sperm suspensions were diluted 40 times by volume with Hanks' solution at 37℃. The number of nonmotile sperm in a small volume of the diluted sample was counted using a phase-contrast microscope. For sperm viability, a small volume of the sperm suspensions was put into 0.1% Eosin Y solution and stained at 37℃. Then, the stained sperm samples were smeared on glass slides, dried and treated with 1% acetic acid solution in order to remove excessive staining. The dead sperm (stained) and live sperm (not stained) were observed from the smear specimen using a microscope. For sperm abnormalities, the number of abnormal sperm was counted in 200 sperm from the smear specimen used for the viability index of sperm. The sperm count per 1g caudal epididymis, sperm motility, sperm viability and incidence of sperm with abnormalities were calculated by the following formulas:

Sperm count per 1g caudal epididymis =
Mean No. of sperm × 40 × 2.5 × 10^4 × 1/ Caudal epididymis weight (g)

Sperm motility (%) =
[(Total No. of sperm - Total No. of nonmotile sperm) / Total No.of sperm] × 100

Viability index of sperm (%) =
[(No. of sperm observed - No. of stained sperm) / No. of sperm observed] × 100

The incidence of sperm with abnormalities (%) =
(No. of sperm with abnormalities / 200) × 100

For statistical analyses, parametric data were analyzed by Student's t-test or Aspin-Welch's t-test. Non-parametric data were analyzed by the Wilcoxon rank sum method. Statistical analyses were made between IGS and CD rats at two-tailed 5 and 1% levels of significance.

RESULTS AND DISCUSSION

Sperm count

Mean sperm count in IGS rats tended to be high in comparison with that of CD rats in both study data and individual data and significant difference from CD rats was noted in the individual data of IGS rats (Table 1). Regarding minimum and maximum values, they were almost the same in CD rats and IGS rats in the study data. However, a wide range of values in both CD rats and IGS rats were noted among individual data. They ranged from 18.1 × 10^6/g caudal epididymis to 771.4 × 10^6/g caudal epididymis in CD rats and from 113.4 × 10^6/g caudal epididymis to 751.3 × 10^6/g caudal epididymis in IGS rats (Table 1). In the histogram of the data, the sperm count in about 64% of studies ranged from 400 × 10^6/g caudal epididymis to 499 × 10^6/g cau-

dal epididymis in both rats in the study data and the distribution curves were almost the same in CD rats and IGS rats (Fig. 1). The distribution curves in the individual data were also almost the same in CD rats and IGS rats but animals in which sperm count showed 150× 10^6/g caudal epididymis or less tended to be more numerous in CD rats than in IGS rats (Fig. 2).

Table 1 Comparison of Characteristics of Sperm from Caudal Epidydimis between CD Rats and IGS Rats

Items		Study Data		Individual Data	
		CD Rats	IGS Rats	CD Rats	IGS Rats
Sperm count (×10^6/g caudal epididymis)					
No.		25	11	448	223
Mean±S.D.		457.5±55.4	476.7±53.8	458.5±94.3	478.0±88.8**
Range	Min.	310.1	336.2	18.1	113.4
	Max.	589.8	608.5	771.4	751.3
Sperm motility (%)					
No.		23	11	425	223
Mean±S.D.		78.4±3.7	75.6±4.1	78.6±7.1	75.6±7.5**
Range	Min.	65.0	61.3	0.0	0.0
	Max.	85.3	83.1	92.2	88.1
Viability index (%)					
No.		18	10	349	203
Mean±S.D.		79.2±3.0	79.0±3.1	79.1±7.7	78.9±7.3
Range	Min.	70.5	62.3	3.2	18.2
	Max.	85.1	85.7	90.2	89.3
Incidence of sperm with abnormalities (%)					
No.		19	9	377	203
Mean±S.D.		0.00±0.00	1.08±1.89	0.37±0.54	1.81±0.50**
Range	Min.	1.15	0.03	0.00	0.00
	Max.	0.38	17.83	4.00	62.00

**: P<0.01, Significant difference from CD Rats

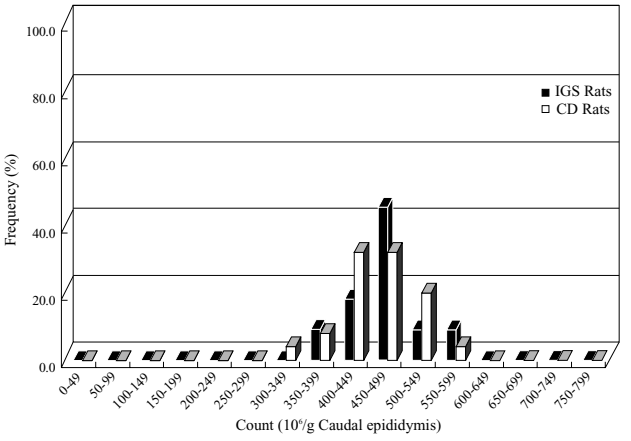

Fig. 1 Histogram of Sperm Count in IGS Rats and CD Rats (Study Data)

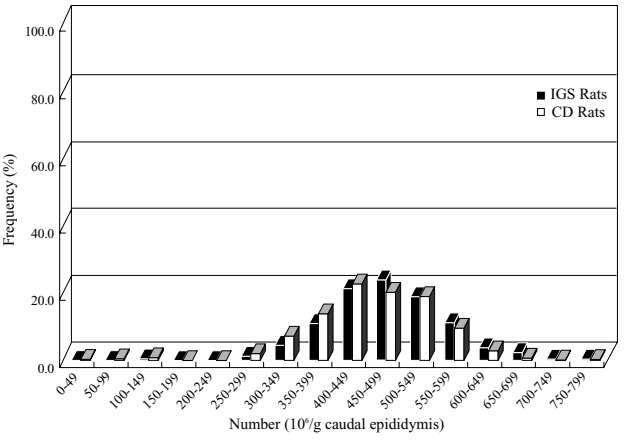

Fig. 2 Histogram of Sperm Count in IGS Rats and CD Rats (Individual Data)

Sperm motility

Mean sperm motility in IGS rats tended to be low in comparison with that of CD rats in both study data and individual data and significant difference from CD rats was noted in the individual data of IGS rats (Table 1). Regarding minimum and maximum values, they were almost the same in CD rats and IGS rats in both study data and individual data. In the histogram of the data among studies (Fig. 3), the range of highest frequency was from 75% to 79% in CD rats but it was from 70% to 74% in IGS rats. However, about 60% of studies in both CD rats and IGS rats were included in the range from 70% to 79%. In the histogram of the data among individuals (Fig. 4), the range of highest frequency was from 80% to 84% in CD rats. On the other hand, it was from 75% to 79% in IGS rats and the distribution curve in IGS rats was lower than in CD rats.

Viability of sperm

Mean viability index of sperm and minimum and maximum values in both CD rats and IGS rats were almost the same in both study data and individual data (Table 1). The distribution curves in the study data and individual data were also almost the same in CD rats and IGS rats (Figs. 5 and 6).

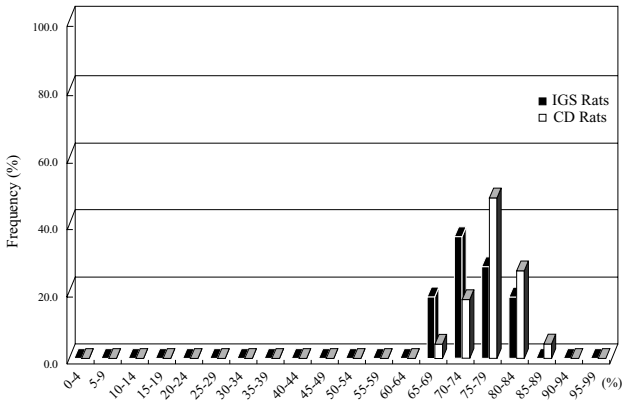

Fig. 3 Histogram of Sperm Motility in IGS Rats and CD Rats (Study Data)

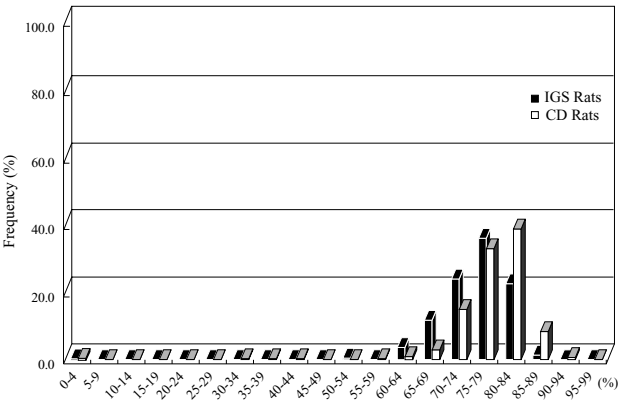

Fig. 4 Histogram of Sperm Motility in IGS Rats and CD Rats (Individual Data)

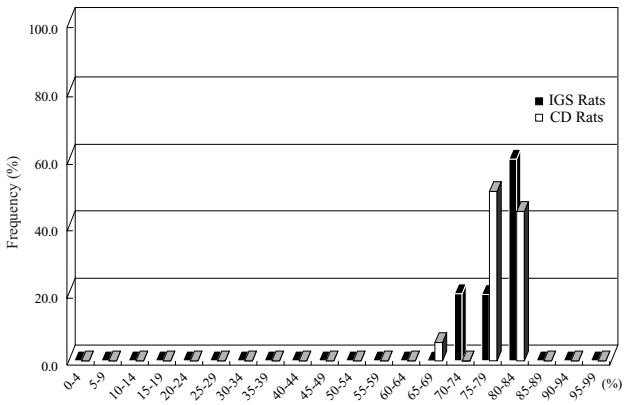

Fig. 5 Histogram of Viability of Sperm in IGS Rats and CD Rats (Study Data)

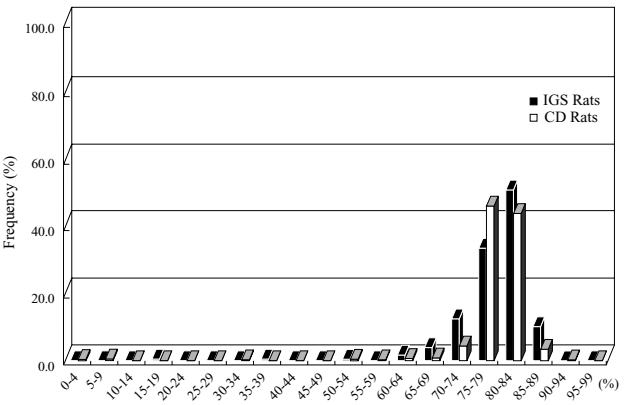

Fig. 6 Histogram of Viability of Sperm in IGS Rats and CD Rats (Individual Data)

Abnormalities of sperm

Mean incidence of sperm with abnormalities and maximum values in IGS rats showed high values in comparison with those of CD rats in both study data and individual data, and significant difference from CD rats was noted in the individual data of IGS rats (Table 1). In the histogram of the data among studies (Fig. 7), the range of highest frequency was from 0.1% to 0.4% in both CD rats and IGS rats. However, the studies in IGS rats which were included in this range were a minority (about 33% of studies) as against about 90% of studies in CD rats. Moreover, although all studies in CD rats were included in the range from 0.1% to 1.9%, about 33% of studies in IGS rats exceeded this range and were included in the range from 2.0% to 9.9%. In the histogram of the data among individuals (Fig. 8), the range of highest frequency was from 0.1% to 0.4% in IGS rats as against 0% in CD rats. About 50% of individuals showed no abnormalities in CD rats, but individuals with no abnormalities were a minority (about 24% of individuals) in IGS rats. Moreover, individuals included in the range of 20% or more were noted in the frequency of 2.4% in IGS rats. The most common abnormality observed in IGS rats was no head.

From above results, although significant differences between IGS rats and CD rats were noted in sperm count and sperm motility in individual data, no significant differences between IGS rats and CD rats were noted in the study data and it was concluded that these parameters in IGS rats were almost the same as those from CD rats. However, the incidence of sperm with abnormalities in IGS rats was higher than in CD rats.

ACKOWLEDGMENTS

We thank the members of the reproduction section at the Gotemba Laboratory of Bozo Research Center Inc.

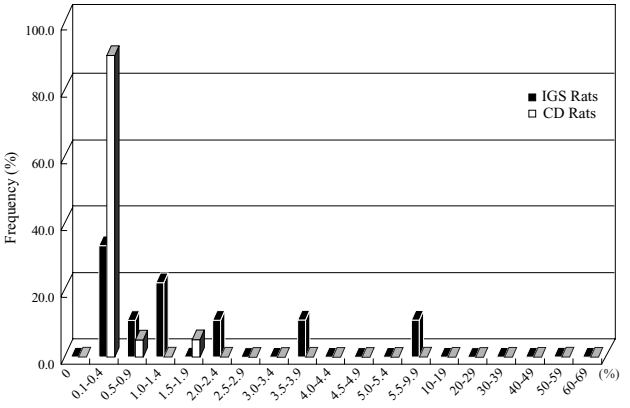

Fig. 7 Histogram of Incidence of Sperm with Abnormalities in IGS Rats and CD Rats (Study Data)

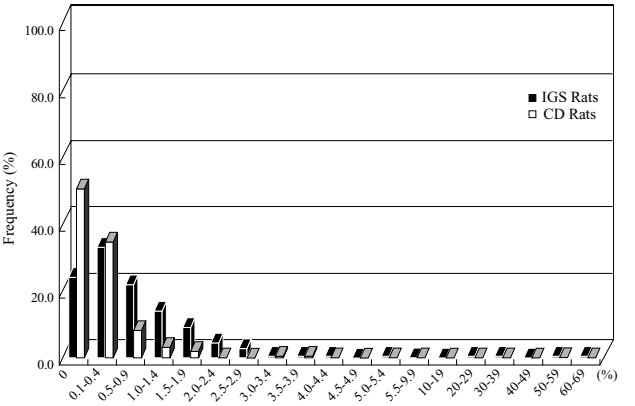

Fig. 8 Histogram of Incidence of Sperm with Abnormalities in IGS Rats and CD Rats (Individual Data)

REFERENCES

1. Umemura, T., Ishida, S. and Katsumata, Y. 1998. Comparison of reproductive and developmental parameters between Crj:CD(SD) rats and Crj:CD(SD)IGS rats, pp. 206 - 213 In “Biological Reference Data on CD(SD)IGS Rats” edited by CD(SD)IGS Study Group

Reproductive and Developmental Data in Crj:CD (SD) IGS Rats

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ABSTRACT. In order to collect background data on reproductive and developmental parameters using Crj:CD(SD)IGS rats which were developed by Charles River, Inc., We performed a same examination usually employed in reproductive and developmental toxicity studies. We found no clear differences between the Crj:CD(SD)IGS rats and the Crj:CD(SD) rats in the reproductive and developmental parameters. It is considered that Crj:CD (SD)IGS rats can be used in our reproductive and developmental toxicity studies.— Key words: CD(SD)IGS rats, Reproduction, Development

CD(SD)IGS-1999: 153-156

INTRODUCTION

The gold standard system, a new animal breeding system, has been developed by Charles River, Inc. to meet the demands for internationalization of research and development of new drugs by supplying, as much as possible, uniform experimental animals through minimizing genetic ramifications. We performed a same examinations usually employed in reproductive and developmental toxicity studies to obtain background data of Crj:CD(SD)IGS rats, and compared with our Crj:CD(SD) rat's background data.

MATERIALS AND METHODS

Crj:CD(SD)IGS male and female rats were supplied from the Tsukuba Breeding Center, Charles River Japan Inc. at 10 weeks of age and acclimated and quarantined for 1 week or more. Healthy animals that had no abnormalities in appearance and showed normal weight gain were used. The animals were housed in a room maintained at constant temperature 22 ± 2 ℃ and relative humidity 55±15%. Room air was ventilated 10 - 15 times per hour and a 12-hour light-dark cycle (lighting 8:00 - 20:00) was imposed. The animals were given a pellet diet (CRF-1, Oriental Yeast Co., Ltd.) and tap water ad libitum. At 11 weeks of age, females were paired with 11-week-old males of the same strain. The day on which the presence of a vaginal plug and sperm in the vaginal smear was confirmed, was designated as day 0 of gestation. Throughout the experimental period, dams were observed for clinical signs once daily. All dams were weighed on days 0, 4 and 7 - 20 of gestation and those of the natural delivery group were weighed on days l, 4, 7,14, and 21 of lactation. Food consumption per day was determined on the same days on which animals were weighed except day 0 of gestation.

Dams in the cesarean section group were necropsied on day 20 of gestation. The ovaries and uterus were examined for number of corpora lutea, implantations, live/dead fetuses and resorptions. The resorptions were typed as early or late. In addition, the visceral organs of dams were observed grossly. After measurement of fetal body weights and placenta weight, live fetuses were sexed and observed for external anomalies including the oral cavity. Approximately half of the fetuses in each litter were separated for preparation of skeletal specimens. They were stained by Alizarin-red S [1], and observed for abnormalities, variations and the state of ossification. The remaining fetuses were fixed in Bouin's solution for visceral examination by Wilson's method [2] and modified Nishimura's method [3].

All dams in the natural delivery group were allowed to deliver. They were observed for external abnormalities in delivery and the duration of gestation was determined. After delivery, they were examined for nursing behavior and lactation. Offspring were examined for mortality, sex and external anomalies. On day 4 of lactation, the litter size was adjusted to 4 males and 4 females. Male and female offspring were weighed individually on a litter basis on days 0, 4, 7, l4 and 21 of lactation. As a postnatal developmental test, separation of ear auricula, appearance of abdominal hair, eruption of lower incisors and separation of eyelids were examined until weaning. The stillborn index, birth index, survival index on days 4 and 21 of lactation were determined. On day 22 of lactation, dams were necropsied and implantation sites were counted.

On day 21 of lactation, 2 males and 2 females from each litter were selected for examinations after weaning and the remaining offspring were necropsied and their visceral organs were observed grossly. After weaning, as a reflex function test, visual placing reflex, pain response, Preyer's reflex, corneal reflex, pinna reflex, righting reflex and Ipsilateral flexor reflex were examined at 4 weeks of age. After weaning, the growth of offspring was examined by observing of development of U-type penis and opening of vagina, and body weight of offspring were measured once a week until the age of 8 weeks. At 8 weeks of age, male and female from each litter were necropsied and their visceral organs were observed grossly. The present results were compared to our Crj:CD (SD) rat's background data (range of means per tests).

RESULTS AND DISCUSSION

No abnormal clinical signs were observed in dams during gestation and lactation periods. Body weight and food consumption of dams are shown in Table 1 and 2, respectively. Body weight and food consumption were within the range of our background data. Findings at cesarean section and the result of visceral examination of fetuses are shown in Table 3. The number of corpora lutea, implantations, live fetuses, incidence of dead fetuses, sex ratio, fetal body weight and placental weight were within the range of our background data. No external malformations were noted. In visceral examination, thymic reminant in the neck, left umbilical artery, ventricular septal defect and testicular hypopla-

sia were observed. However, the incidence of total visceral anomalies was within the range of our background data. Findings at skeletal examination of fetuses are shown in Table 4. No skeletal malformations were noted. As skeletal variations, splitting of thoracic vertebral body, asymmetry of sternebra, shortness of 13th rib and lumbar rib were observed. However, there were no characteristic changes in these skeletal variations. The states of ossification were similar to our background data.

Findings at spontaneous delivery and lactation of dams are shown in Table 5. There were no abnormalities in delivery and nursing behavior in dams. The gestation index, duration of gestation, the number of implantation and live newborns, incidence of stillborn or birth index were normal and these parameters were within the ranges of our background data. The viability index on day 4, weaning index and sex ratio in newborns seemed to be normal. As external malformations, three incidence of abnormal skin (wavy skin) were seen. Postnatal differentiation of offspring is shown in Table 6. Separation of ear auricula on day 4, appearance of abdominal hair on day 12, separation of eyelids on day 12 and the time of opening of vagina were seemed to be normal. The incidence of eruption of lower incisors was out of the ranges of our background data. However, the difference seemed to be small. As the test of male sex maturation, development of U - type penis observed first in our laboratory. The change of W-type penis to U-type penis occurred between day 38 and 44 after birth and it was easy to distinguish. All of reflex function test at 4 weeks of age was normal. Body weight of offspring during the lactation period and the period after weaning is shown Table 7. All of body weight in male and female offspring was within the range of our background data.

In conclusion, the present study indicate that, there were no rmarkable differences between Crj:CD(SD)IGS rat's data and our Crj:CD(SD) rat's background data. Therefor, we consider that Crj:CD(SD)IGS rats can be used in our reproductive and developmental toxicity studies.

Table 1. Body weight (g) of dams during gestation and lactation periods

Strain		IGS	SD (background data)
Gestation period			
No. of animals		48	236
Days of gestation	0	261.5±2.2	238.9-293.4
	4	287.8±2.2	261.1-322.2
	7	299.0±2.2	271.5-335.9
	14	333.0±2.6	297.7-371.4
	20	411.3±3.8	362.6-448.0
Lactation period			
No. of animals		24	148
Days of lactation	1	308.3±4.3	285.5-342.6
	4	334.5±3.6	312.4-359.6
	7	341.7±3.4	318.0-368.9
	14	354.2±3.2	330.7-377.0
	20	329.9±3.3	307.3-345.1

Values represent mean±S.E.

Table 2. Food consumption (g) of dams during gestation and lactation periods

Strain		IGS	SD (background data)
Gestation period			
No. of animals		48	236
Days of gestation	0	20.3±0.3	17.2-24.6
	4	23.6±0.4	21.9-28.9
	7	23.5±0.3	21.7-28.7
	14	23.3±0.3	21.3-29.4
	20	25.1±0.5	22.0-28.4
Lactation period			
No. of animals		24	148
Days of lactation	1	15.4±1.7	9.7-21.5
	4	44.8±1.0	39.8-46.8
	7	45.8±0.8	42.9-48.4
	14	60.1±1.0	54.3-64.4
	20	73.9±1.0	73.3-86.1

Values represent mean±S.E.

Table 3. Observations at cesarean section of dams

Strain	IGS	SD (background data)
No. of dams	24	184
No. of corpora lutea per litter	17.3±0.4	14.8-19.1
No. of implantations per litter	16.1±0.5	14.0-17.0
No. of dead fetuses		
Early (%)	20 (4.9)	(1.9-13.2)
Late (%)	1 (0.3)	(0.0-0.3)
No. of live fetuses per litter		
Male	8.4±0.5	6.2-8.5
Female	6.8±0.5	6.3-9.2
Sex ratio (%)[a)]	55.1	41.6-57.0
Fetal body weight per litter		
Male (g)	3.7±0.1	3.3-3.9
Female (g)	3.5±0.0	3.1-3.8
Placental weight per litter (g)	0.44±0.01	0.43-0.53
Total no. of fetuses with external malformations (%)	0 (0.0)	(0.0)
Total no. of fetuses with visceral anomalies (%)	12 (5.8)[b)]	(0.0-11.9)

Values represent mean±S.E.

a) : (Total no. of male fetuses / Total no. of live fetuses)×100

b) : Thymic reminant in the neck(6), Left umbilical artery(1), Thymic reminant in the neck and left umbilical artery(1), Dilatation of renal pelvis(1), Ventricular septal defect(2), Testicular hypoplasia(1)

Table 4. Skeletal examination of fetuses

Strain	IGS	SD (background data)
No. of litters	24	183
No. of fetuses examined	176	1679
No. of fetuses with malformations	0	(0.0-5.6)
Misarranged lumbar vertebral arches	0	(0.0-0.7)
Wavy rib	0	(0.0-5.6)
Accessory rib	0	(0.0-0.4)
Atlant - occipital assimilation	0	(0.0-1.4)
No. of fetuses with variations	21 (11.3)	(2.1-19.9)
Cervical rib	0	(0.0-1.1)
Splitting of thoracic vertebral body	1 (0.5)	(0.0-5.3)
Asymmetry of sternebra	2 (1.0)	(0.0-2.1)
Shortness of 13th rib	1 (0.6)	(0.0-2.9)
Lumbar rib	17 (9.2)	(0.0-14.8)
14th rib	0	(0.0-3.3)
States of ossification		
No. of ossified sternebrae	5.6±0.1	5.4-5.8
No. of ossified metacarpus	7.2±0.1	6.3-7.7
No. of ossified metatarsus	8.0±0.0	8.0-8.0
No. of ossified sacral and caudal vertebrae	8.0±0.1	7.1-8.1

Values represent mean±S.E.
Values in parentheses indicate average percentage to one litter

Table 5. Observations made at the time of spontaneous delivery and lactation of dams

Strain	IGS	SD (background data)
No. of dams	24	162
No. of dams with live newborns	24	160
Gestation index (%)[a)]	100.0	95.8-100.0
Duration of gestation (day)	21.8±0.1	21.6-22.8
No. of implantations per litter	16.3±0.2	14.2-17.6
No. of stillborns (%)	2 (0.6)	(0.0-6.0)
No. of live newborns per litter	15.4±0.4	13.1-15.8
Birth index (%)[b)]	94.5	89.5-96.6
Viability index on day 4 (%)[c)]	97.5	96.3-100.0
Weaning index (%)[d)]	100.0	96.2-100.0
Sex ratio (%)[e)]	48.4±3.2	42.3-57.4
Total no. of newborns with external malformations (%)	3 (0.8)[f)]	(0.0-1.3)

Values represent mean±S.E.
a) : (No. of dams with live newborn / No. of dams)×100
b) : (No. of live newborns / No. of implantations)×100
c) : (No. of young alive on day 4 after birth / No. of live newborns)×100
d) : (No. of live weaning / No. of young alive on day 4 after birth or immediately after elimination)×100
e) : (Total no. of male newborns / Total no. of live newborns)×100
f) : Abnormal skin

Table 6. Postnatal differentiation of offspring

Strain	IGS	SD (background data)
No. of litters	24	155
Separation of ear auricula on day 4 (%)[a)]	100.0±0.0	100.0
Appearance of abdominal hair on day 8 (%)	100.0±0.0	100.0
Eruption of lower incisors on day 12 (%)	70.3±5.6	84.2-100.0
Separation of eyelids on day 12 (%)	100.0±0.0	100.0
No. of males	48	−
Development of U-type penis (day)	40.2±0.2	−
No. of females	48	283
Opening of vagina (day)	33.4±0.3	30.4-34.0

Values represent mean±S.E.
a) : (No. of offspring developed / No. of offspring examined)×100

Table 7. Body weight (g) of offspring

Strain		IGS	SD (background data)
Male			
Days after birth	0	6.1±0.1	6.1-7.0
	4	10.0±0.2	9.9-11.3
	7	17.0±0.3	16.8-18.6
	14	36.6±0.5	35.7-39.1
	21	59.7±0.6	57.6-66.6
	28	102.7±0.9	100.9-128.3
	35	167.4±1.6	160.3-195.6
	42	236.3±2.3	224.6-260.3
	49	301.9±2.9	285.6-319.1
	56	363.8±3.5	342.1-383.4
Female			
Days after birth	0	5.8±0.1	5.7-6.6
	4	9.5±0.2	9.9-10.9
	7	16.0±0.3	15.8-17.8
	14	35.1±0.6	33.8-37.8
	21	56.6±0.8	54.7-64.1
	28	92.8±1.0	92.7-109.8
	35	139.3±1.7	134.7-154.9
	42	174.1±2.3	169.0-192.0
	49	200.7±2.8	193.5-220.7
	56	226.7±3.2	217.9-249.5

Values represent mean±S.E.

REFERENCES

1. Kimmel, C. A. and Trammell, C. 1981. A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain Tecnol., 56, 271
2. Wilson, J.G. 1965. Methods for administering agents and detecting malformations in experimental animals, PP, 262-227. In: “Teratology: Principles and Techniques” (Wilson. J.G. and J. Warkany, J. eds), Univ. of Chicago Press, Chicago, Illinois.
3. Nishimura, K. 1974. A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. Cong. Anom., 14: 23-40.

Comparison of Developmental Indices between Crj:CD(SD)IGS Rats and Crj:CD(SD) Rats

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ABSTRACT. The Crj:CD(SD)IGS (hereafter referred to as "IGS") and Crj:CD(SD) (hereafter referred to as "SD") rats used in this study were supplied by Atsugi Breeding Center of Charles River Japan Inc. One female rat detected the optimum day for mating (proestrous stage) paired with one male rat of the same strain, and all presumed-pregnant female rats were allowed to litter normally and nurtured the offspring. The F_1 rats were examined for their growth, physical development, behavioral ontogeny, emotionality, learning ability and memory, and motor activity.
Body weight gains and food consumption were lower in IGS F_0 dams than in SD F_0 dams during the gestation and lactation periods. Though the number of implants and live newborns at birth were decreased in IGS rats as compared with SD rats, there were no differences regarding the gestation period as well as the gestation and birth indices. The viability index on postnatal day 4 and weaning index in IGS F_1 rats were comparable those in SD F_1 rats. Body weight gains and food consumption tended to reduce in IGS F_1 rats of both sexes compared with SD F_1 rats. The physical development of eyelid opening and vaginal opening were delayed in IGS F_1 rats. The latency time of surface righting was shorter in IGS F_1 rats than in SD F_1 rats on postnatal day 5. Further, the latency time of olfactory orientation were longer in IGS F_1 rats than in SD F_1 rats on postnatal day 10, 11 and 12. However, there were no differences between IGS and SD F_1 rats with respect to the emotionality, learning ability and memory, and spontaneous motor activity. ─ Key words: Crj:CD(SD)IGS Rat, F_1 Offspring, Physical Development, Behavioral Ontogeny, Emotionality, Learning Ability and Memory, Motor Activity

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INTRODUCTION

In the reproductive and developmental toxicity studies of new drugs, the effects of new drugs on developmental indices have been usually evaluated in F_1 generation. In our laboratory, developmental indices such as body weight gains, physical development, behavioral ontogeny, emotionality, learning ability and memory, and spontaneous motor activity have been examined in order to assess the effects of new drugs on the development in F_1 offspring. The authors have already reported the results on shuttle box avoidance test in Crj:CD(SD)IGS and Crj:CD(SD) F_1 rats [1]. The present study was conducted to obtain the developmental background data in Crj:CD(SD)IGS rats, and these data were compared to those in Crj:CD(SD) rats.

MATERIALS AND METHODS

Animals:

Ten-week-old male and female Crj:CD(SD)IGS (hereafter referred to as "IGS") rats and Crj:CD(SD) (hereafter referred to as "SD") rats were supplied by Atsugi Breeding Center of Charles River Japan Inc. These animals were housed individually in stainless steel wire bottom cages (W240 × D200 × H180 mm) in an air-conditioned room which was maintained at a room temperature of 21-25℃ and a relative humidity of 40-60%, ventilated 10-20 times/hour and provided with light for 12 hours a day (6:00 to 18:00). They were allowed free access to laboratory chow (CRF-1, Oriental Yeast Co., Ltd.) and sterilized tap water *ad libitum*. Male and female rats of both strains were used for the study after one-week acclimation.

Measurements and observations:

Copulation in F_0 dams: The female rats were detected the optimum day for mating (proestrous stage) by means of measuring the electrical impedance in the vagina by the impedance checker (MK-10, Muromachi Kikai Co., Ltd.) from 13:30 to 15:00 [2-5]. One male rat paired with one female rat detected the optimum day for mating overnight in the cage of male rat. Females with spermatozoa observed in smears of vaginal contents were considered on day 0 of gestation, and they were returned to their original cages. On day 20 of gestation, all pregnant female rats were housed singly in the breeding cages (W220 × D380 × H200 mm).

Parturition in F_0 dams: All pregnant dams were allowed to litter normally and nurtured the offspring. The day of parturition was considered on postnatal day 0 (day PN0). On day PN0, all litters were examined for gross external malformations, sexed, weighed, and any dead pups noted. Stillborn pups were differentiated from pups dying soon after birth by removing the lungs and immersing them in water. Pups with lungs, which sank, were considered stillbirths; whereas pups with lungs, which floated, were considered to have died shortly after birth. On day PN0, one male and one female pups per litter were selected randomly and subsequently evaluated for the growth, physical development, behavioral ontogeny, emotionality, learning ability and memory, and spontaneous motor activity. On day PN4, litters were reduced to 8 pups/litter with equal numbers per sex when possible, and remaining pups were euthanatized by intraperitoneal injection of excess of pentobarbital sodium (Nembutal, Dainippon Pharmaceutical Co., Ltd.) after they were examined grossly. Each litter was examined daily for dead pups (F_1) at the time when the dam (F_0) was evaluated. On day PN21, the pups, which were selected on day PN0, were weaned and remaining pups were euthanatized by inhalation of carbon dioxide. On postpartum day 21, all dams were euthanatized by inhalation of carbon dioxide and examined for the number of implants.

Body weight and food consumption in F_0 dams: Body weights and food consumption in F_0 dams were recorded on days 0, 3, 7, 10, 14, 17 and 20 of gestation, as well as on postpartum days 0, 4, 7, 10, 14, 17 and 21.

Physical development in F_1 rats: One male and one female F_1 rats per litter, which were selected on day PN0, were observed for following indices of physical development including pubertal development.

Pinnae unfolding: The pups were observed daily from day PN2. When one or both pinnae had separated from head, pinnae un-

folding was recorded as having occurred.

Emergence of hair: The pups were observed daily from day PN6 for development of hair and results were recorded.

Incisor eruption: The pups were observed daily from day PN8. When either the upper or lower incisors had perforated the skin, incisor eruption was recorded.

Eyelid opening: The pups were observed daily from day PN11. If either eyelid had any degree of opening, the eyelids were considered open and recorded.

Vaginal opening: The female pups were observed daily from day PN25. When the membranous sheath, covering the orifice, had completely ruptured, the vagina was considered open and recorded.

Preputial separation: The male pups were observed daily from day PN25. When the prepuce had separated from the glans penis, preputial separation was recorded as having occurred [6].

Behavioral ontogeny in F_1 rats: One male and one female F_1 rats per litter, which were selected on day PN0, were observed for following indices of behavioral ontogeny.

Surface righting: The pup was tested once daily from days PN3 to PN11 for a positive response, which the pup was able to right itself successfully within 30 seconds. Each pup was placed on its back on a flat surface, which was made of plywood (300• × 300 mm), and held in this position momentarily using the thumb and forefinger. The pup was released and the latency time it was allowed to right itself to a fully prone position (all limbs extended outward) was measured with a stopwatch and recorded. The maximum allotted time for each trial was 30 seconds per pup. If a pup did not respond within 30 seconds, it was given a score of 30.0 (30 seconds) at that day.

Negative geotaxis: Each pup was tested once daily from days PN5 to PN11 for a positive response, which the pup was able to rotate 180° on an inclined plane within 30 seconds. Each pup was placed on an inclined plane with 25° angle, which was made of plywood (300 × 300 mm), in a headdown position. The latency time it took each pup to turn 180° was measured with a stopwatch and recorded. The maximum allotted time for each trial was 30 seconds per pup. If the pup fell off shortly after it was placed on an inclined plane (maximum three times), it was placed back at the starting position and the stopwatch was restarted. If a pup did not respond within 30 seconds, it was given a score of 30.0 (30 seconds) at that day.

Olfactory orientation: Each pup was tested once a day from days PN8 to PN14 for a positive response, which the pup was able to enter the positive bin of the testing apparatus within 90 seconds. The testing apparatus was made of clear thick plexiglass, with attachable bins (120 × 120 × 100 mm) on either side. Each bin had an opaque screen that separated the bin from the main box (300 × 120 × 100 mm). Each screen contained a pathway (50 × 60 mm) at the bottom for the pup to go through or poke its head through. Home cage bedding (approximately 10g) was deposited into a petri dish and the dish was placed in the positive bin. Uncontaminated bedding (approximately 10g) was deposited into a petri dish and the dish was placed in the negative bin. The pup being tested was placed in the middle of the main box. When the pup was released, the stopwatch was started. The test in each day was completed when the pup forcibly tried to enter the positive or negative bin or after 90 seconds had elapsed. The latency time in each day when the pup tried to enter the positive bin was recorded. If a pup failed to enter the positive bin or tried to enter the negative bin within the allotted 90 seconds, it was a score of 90.0 (90 seconds) at that day.

Auditory startle: Each pup was tested daily from days PN9 to PN14 for a positive response which the pup displayed a clear startle response to an auditory stimulus (e.g., sudden backward motion of the pinna). The auditory stimulus was obtained from the audiometer (PA-1, Nagashima Medical Instruments Co., Ltd.), set at 12000 Hz. The pup was subjected to three stimuli from the audiometer at 2 seconds intervals and the response was recorded. The animals were scored as responsive or non-responsive to the three stimuli in each testing day. The positive response rate was calculated as follows:

The positive response rate = (number of animals shown the positive response/number of animals tested) × 100.

Air righting: Each pup was tested three times daily from days PN 12 to PN20 for a positive response. The pup was held in an inverted position, dorsal surface facing downward, and approximately 300 mm above a shock absorbent material (Memory Foam, Sanko Yugyo Co., Ltd.). The pup was held momentarily in this position before being dropped. If the pup turned in mid-air and landed in a fully prone position (all legs extended outward) on three tests in each testing day, this was considered a positive response and recorded. The positive response rate was calculated as follows:

The positive response rate = (number of animals shown the positive response/number of animals tested) × 100.

Body weight and food consumption in F_1 rats: Body weights were recorded on days PN0, 4, 7, 10, 17 and 21 in male and female F_1 pups before weaning. Body weights and food consumption were recorded once a week from weaning to 11-week old in F_1 rats of both sexes.

Open-field test in F_1 rats: The open-field test was performed at 4 and 5 weeks of age in male and female F_1 rats, respectively, in order to examine the emotionality. The open-field apparatus (ANAC-50, Okazaki Sangyo Co., Ltd.; 800 mm diameter with 300 mm height) consisted of 25 divisions. Each animal was subjected to the open-field trial for 3 minutes a day. This test was continued for 3 days. The latency time required for an animal to go out of central division, as well as frequencies of ambulation, rearing, grooming, defecation and urination were recorded [7].

Shuttle box avoidance test in F_1 rats: The shuttle box avoidance test was conducted at 6 and 7 weeks of age in male and female F_1 rats, respectively, in order to examine the learning ability. This test was repeated at 8 and 9 weeks of age in male or female F_1 rats, respectively, to examine the memory. The shuttle box apparatus (MSB-001, Toyo Sangyo Co., Ltd.) consisted two separate components and each component was equipped with grids for foot shock. Each animal was tested for 50 trials per day over 3 days at 6 or 7 weeks of age and again once at 8 or 9 weeks of age. Each trial consisted of the following sequence of events. The animal was given a conditioned stimulus (light and pure tone) for 10 seconds. During the last 5 seconds of the conditioned stimulus, the animal was given 5 seconds of unconditioned stimulus (1400 V, 0.7 mA of electric current) with the conditioned stimu-

lus simultaneously. There was a 20-second interval between each trial (intertrial interval). The numbers of avoidance, escape, errors and intertrial interval responses for each animal was recorded automatically. The latency time of avoidance and escape for each animal was also recorded automatically. The avoidance and escape rates were calculated as follows:

Avoidance rate = (number of avoidance responses/number of trials per day) × 100.

Escape rate = (number of escape responses/number of trials per day) × 100.

Spontaneous motor activity in F_1 rats: Spontaneous motor activity was measured at 9 and 10 weeks of age in male and female F_1 rats, respectively. Each animal was housed singly in a plastic cage (W100 × D110 × H400 mm), which was placed on the animal movement analyzing system (Scanet SV-10, Toyo Sangyo Co., Ltd.) from 9:30 to 16:00. The frequencies of locomotor activity and rearing for each animal were recorded automatically for 6 hours (10:00 to 16:00).

Statistical analysis:

When the data were analyzed statistically, litter means were used as unit of measures regarding body weights in F_1 pups during the lactation period, physical development and behavioral ontogeny. Statistical analyses of data were performed using following methods. Fisher's exact test [8] was used to analyze the incidence data. The numerical data were analyzed using Student's t-test [8] or Aspin-Welch test [8]. Statistical analyses were made between IGS rats and SD rats at two tailed 5% level of significance.

RESULTS AND DISCUSSION

Body weight gains and food consumption in F_0 dams: Body weight gains and food consumption in F_0 dams during the gestation and lactation periods are shown in Fig. 1.

Body weight gains were suppressed in IGS rats from day 14 of gestation to postpartum day 17 as compared with SD rats. Further, food consumption was lower in IGS rats than in SD rats during the gestation and lactation periods excluding day 20 of gestation.

Delivery status in F_0 dams and postnatal viability in F_1 offspring: The delivery status in F_0 dams and the postnatal viability in F_1 offspring are presented in Table 1.

Abortion, premature birth or dystocia were not observed for both IGS and SD strains. The number of implants and pups born alive were decreased in IGS rats in comparison with SD rats. These results differ from our previous report [1] that the number of implants and live neonates at birth in IGS rats were similar to those in SD rats. However, it has been reported that the number of implants and live newborns were significantly decreased in IGS rats compared with SD rats [9]. Therefore, it is considered that the number of implants and live pups tend to be lower in IGS strain than those in SD strain. On the other hand, there were no differences between IGS and SD rats with regard to the gestation period and sex ratio of live offspring as well as the gestation and birth indices. Furthermore, no significant differences of body weights at birth were noted between IGS and SD rats of both sexes.

The viability index on postnatal day 4 and weaning index in IGS pups were comparable to those in SD pups. Further, no progeny with external malformations were observed for both IGS and SD strains.

Body weight changes and food consumption in F_1 rats: Body weight changes and food consumption in male and female F_1 rats are shown in Fig. 2.

Body weight gains were suppressed in IGS rats of both sexes as compared with SD rats from postnatal weeks 2 to 11. Further, food consumption was lower in IGS rats of both sexes than in SD rats from postnatal weeks 4 to 11. The authors have been previously reported that the suppression of body weight gains were noted in male IGS rats in comparison with SD rats [1]. Matsumoto *et al.* [10, 11] have been reported that body weight gains in both male and female F_1 rats were lower in IGS strain than in SD strain. Thus, these findings indicate that there are differences between IGS and SD strains regarding the growth of F_1 offspring.

Physical development in F_1 rats: The results of physical development including pubertal development in F_1 rats are presented in Table 2.

The days required eyelid opening and vaginal opening were delayed in IGS rats compared to SD rats. However, there were no differences between both strains with regard to the days required pinnae unfolding, emergence of hair, incisor eruption and preputial separation. It has been reported that significantly low values were observed for opening of the vagina on day PN35 in IGS rats compared with SD rats [12], and the completion rate for separation of eyelids on days PN14 and PN15 was higher in SD rats than in IGS rats [9]. Consequently, it is considered that the days required eyelid opening and vaginal opening are retarded in IGS rats in comparison with SD rats.

Behavioral ontogeny in F_1 rats: The results of behavioral ontogeny in F_1 rats are shown in Fig. 3.

Although the latency time of surface righting was shorter in IGS rats than in SD rats on day PN5, there were no differences between IGS and SD rats on the other days. Further, the latency times of olfactory orientation were longer in IGS rats than in SD rats on days PN 10, PN11 and PN12, but no differences were observed between two strains on the other days. On the other hand, there were no differences between IGS rats and SD rats with respect to the latency time of negative geotaxis as well as the positive response rates of auditory startle and air righting.

Open-field test in F_1 rats: The results of open-field test in F_1 rats are shown in Table 3.

There were no differences between IGS rats and SD rats of both sexes regarding the latency time as well as the numbers of ambulation, rearing, grooming, defecation and urination.

Shuttle box avoidance test in F_1 rats: The results of shuttle box avoidance test in F_1 rats are presented in Table 4.

There were no differences between IGS rats and SD rats of both sexes with regard to the avoidance time, avoidance rate, escape time and escape rate as well as the numbers of errors and intertrial interval responses. The authors have been reported that the avoidance rates and numbers of intertrial interval responses were increased and the escape rates, avoidance times and escape times were decreased in IGS rats as compared with SD rats [1] , and those findings are not consistent with the present results.

Although we had been used SD rats which were bred in Hino Breeding Center of Charles River Japan Inc. for the previous reproductive and developmental toxicity studies, SD rats employed in this study were supplied by Atsugi Breeding Center of Charles River Japan Inc. This may be one of causes, which the results of the present study differ from the previous study.

Spontaneous motor activity in F_1 rats: The results of spontaneous motor activity in F_1 rats are shown in Table 5.

There were no differences between IGS rats and SD rats of both sexes with respect to the number of locomotor activity and rearing for 6 hours.

In conclusion, there were differences between IGS and SD rats regarding the growth, physical development and behavioral ontogeny; however, no differences were observed for emotionality, learning ability and memory, and motor activity.

REFERENCES

1. Kai, S., Kohmura, H. and Koizumi S. 1998: Comparison between Crj:CD(SD)IGS and Crj:CD(SD) rats in behavioral observation. pp.174-177. *In*: Biological Reference Data on CD(SD)IGS Rats - 1998 (Matsuzawa, T. and Inoue, H. eds.). CD(SD)IGS Study Group, Yokohama.
2. Bartos, L. 1977: Vaginal impedance measurement used for mating in the rat. Laboratory Animals. 11, 53-55.
3. Koto, M., Miwa, M., Tsuji, K., Okamoto, M. and Adachi J. 1987: Change in the electrical impedance caused by cornification of the epithelial cell layer of the vaginal mucosa in the rat. Exp. Anim. 36, 151-156 (in Japanese).
4. Koto, M., Miwa, M., Togashi, M., Tsuji, K., Okamoto, M. and Adachi J. 1987: A method for detecting the optimum day for mating during 4-day estrous cycle in the rat; Measuring the value of electrical impedance of vagina. Exp. Anim. 36, 195-198 (in Japanese).
5. Sunahori, S., Kitajima, H., Yokoyama, A. and Takizawa, H. 1992: Preparation for pregnant SD rats in reproductive and developmental toxicological studies (RDTS) measuring the value of electrical impedance of vagina (EIV). Cong. Anom. 32, 250 (Abstract).
6. Korenbrot, C.C., Huhtaniemi, I.T. and Weiner, R.I. 1977: Preputial separation as an external sign of pubertal development in the male rat. Biol. Reprod., 17, 298-303.
7. Hall, C.S. 1934: Drive and emotionality; Factors associated with adjustment in the rat. J. Comp. Psychol., 17, 89-108.
8. Gad, S.C. and Weil, C.S. 1982: Statistics for toxicologists. pp. 273-320. *In*: Principles and Methods of Toxicology (Hays, A.E. ed.). Raven Press, New York.
9. Tanaka, R., Fujiwara, C., Ito, K., Sakamoto, C., Yamada, R., Ogawa, T. and Inoue, H. 1998: Comparison of reproductive and developmental parameters in Crj:CD(SD)IGS rats with the historical control data of Crj:CD(SD) rats. pp. 248-260. *In*: Biological Reference Data on CD(SD) IGS Rats - 1998 (Matsuzawa, T. and Inoue, H. eds.). CD(SD)IGS Study Group, Yokohama.
10. Matsumoto, A., Ohta, R., Sato, M., Kojima, K., Seki, T., Azegami, J. and Nagao, T. 1997: Background control data of reproductive and developmental toxicity study in Crj:CD(SD)IGS rats. Annual Report of Hatano Research Institute, 20, 40-53 (in Japanese)
11. Matsumoto, A., Ohta, R., Sato, M., Kojima, K., Seki, T., Azegami, J. and Nagao, T. 1998: Comparison between Crj:CD(SD)IGS and Crj:CD(SD) rats in reproductive and developmental parameters. pp. 155-163. *In*: Biological Reference Data on CD(SD)IGS Rats - 1998 (Matsuzawa, T. and Inoue, H. eds.). CD(SD)IGS Study Group, Yokohama.
12. Umemura, T., Ishida, S. and Katsumata, Y. 1998: Comparison of reproductive and developmental parameters between Crj:CD(SD) rats and Crj:CD(SD)IGS rats. pp. 206-213. *In*: Biological Reference Data on CD(SD)IGS Rats - 1998 (Matsuzawa, T. and Inoue, H. eds.). CD(SD)IGS Study Group, Yokohama.

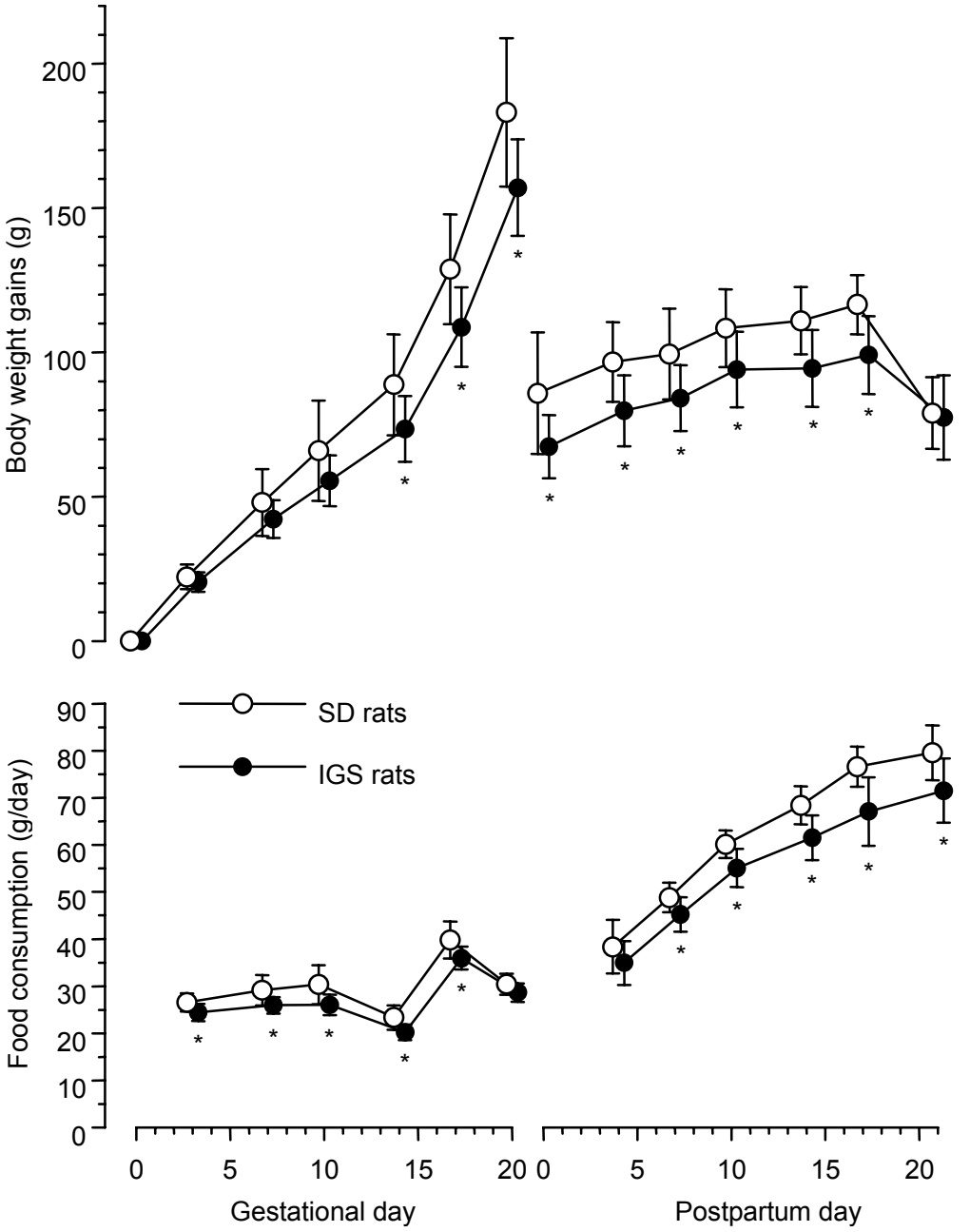

Fig. 1. Body weight gains and food consumption in F_0 rats during the gestation and lactation periods.
Each value shows mean±S.D.
* $p<0.05$: Significant difference from SD rats (Student's t-test or Aspin-Welch test).

Table 1. Delivery status in F_0 dams, and postnatal viability in F_1 offspring.

Groups			SD rats	IGS rats
No. of pregnant dams			10	11
No. of dams with live offspring			10	11
Gestation index (%)[a)]			100.00	100.00
Gestation period (day)		Mean±S.D.	22.30±0.48	22.09±0.30
No. of implants		Total	165	158
		Mean±S.D.	16.50±2.59	14.36±1.86*
No. of offspring born alive		Total	155	146
		Mean±S.D.	15.50±2.37	13.27±2.05*
Body weights at birth	Males (g)	Mean±S.D.	6.98±0.75	6.92±0.56
	Females (g)	Mean±S.D.	6.65±0.66	6.48±0.54
Birth index (%)[b)]		Mean±S.D.	94.10±3.85	92.18±6.50
Sex ratio[c)]		Mean±S.D.	0.43±0.18	0.45±0.18
No. of offspring alive on postnatal day 4			155	145
Viability index on postnatal day 4 (%)[d)]		Mean±S.D.	99.33±2.11	99.30±2.32
No. of offspring alive immediately after culling			80	80
No. of live weanlings			80	80
Weaning index (%)[e)]		Mean±S.D.	100.00±0.00	100.00±0.00
External malformations:				
No. of offspring with external malformations (%)			0 (0.00)	0 (0.00)

a): (No. of dams with live offspring/No. of pregnant dams)×100.
b): (No. of offspring born alive/No. of implants)×100.
c): (No. of male offspring/No. of offspring born alive).
d): (No. of offspring alive on postnatal day 4/No. of offspring born alive)×100.
e): (No. of live weanlings/No. of offspring alive immediately after culling)×100.
* $p<0.05$: Significant difference from SD rats (Student's t-test).

Table 2. Physical development in F_1 rats.

Groups	SD rats	IGS rats
No. of litters examined	10	11
Pinnae unfolding	2.50±0.47	2.23±0.41
Emergence of hair	9.35±0.67	9.09±0.30
Incisor eruption	10.60±0.88	10.64±0.81
Eyelid opening	13.10±0.66	13.64±0.39*
Vaginal opening	29.90±1.79	32.36±1.12*
Preputial separation	33.30±0.82	32.82±1.54

Each value shows mean±S.D. (day).
* $p<0.05$: Significant difference from SD rats (Student's t-test).

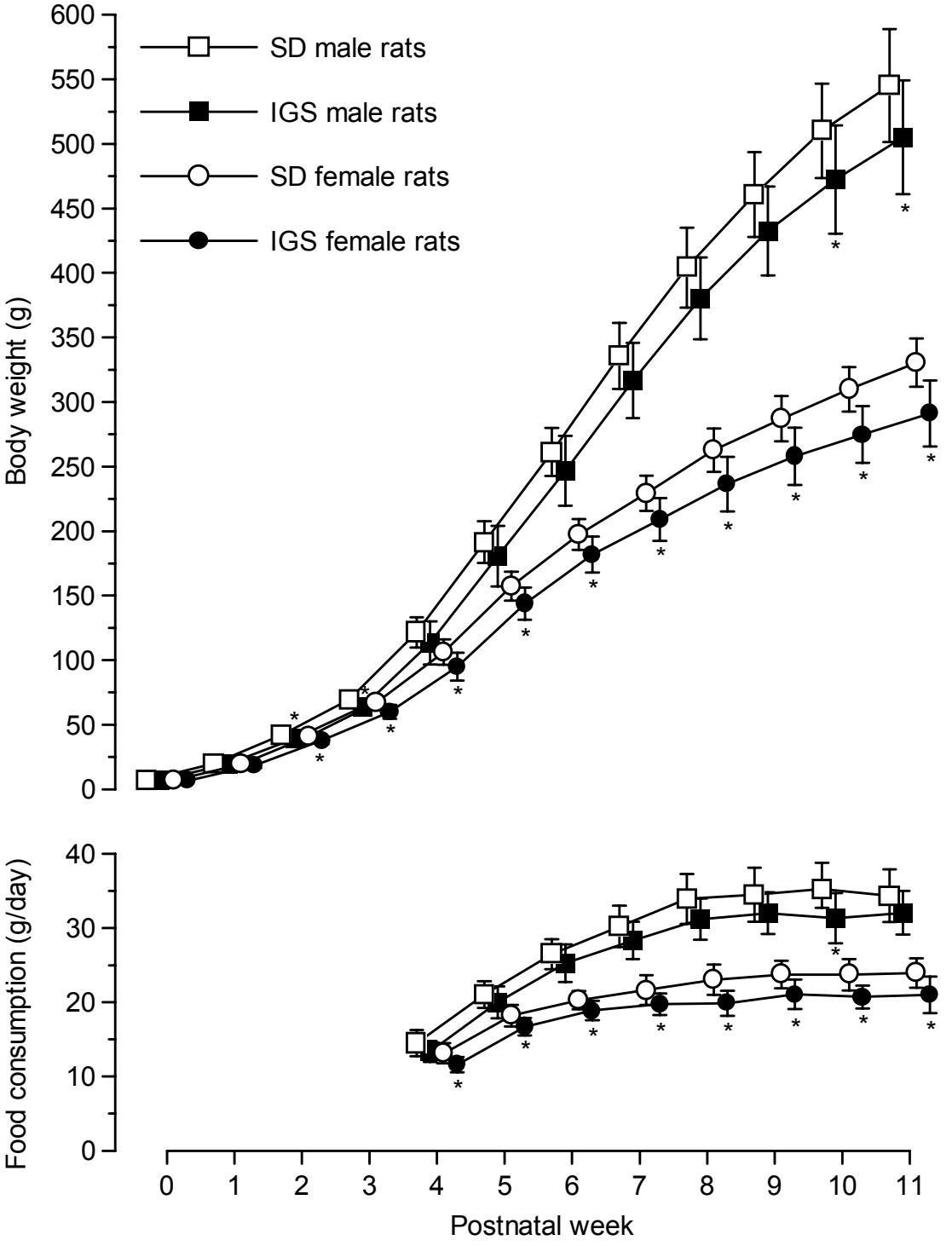

Fig. 2. Body weight changes and food consumption in F_1 rats.
Each value shows mean±S.D.
* $p<0.05$: Significant difference from SD rats (Student's t-test or Aspin-Welch test).

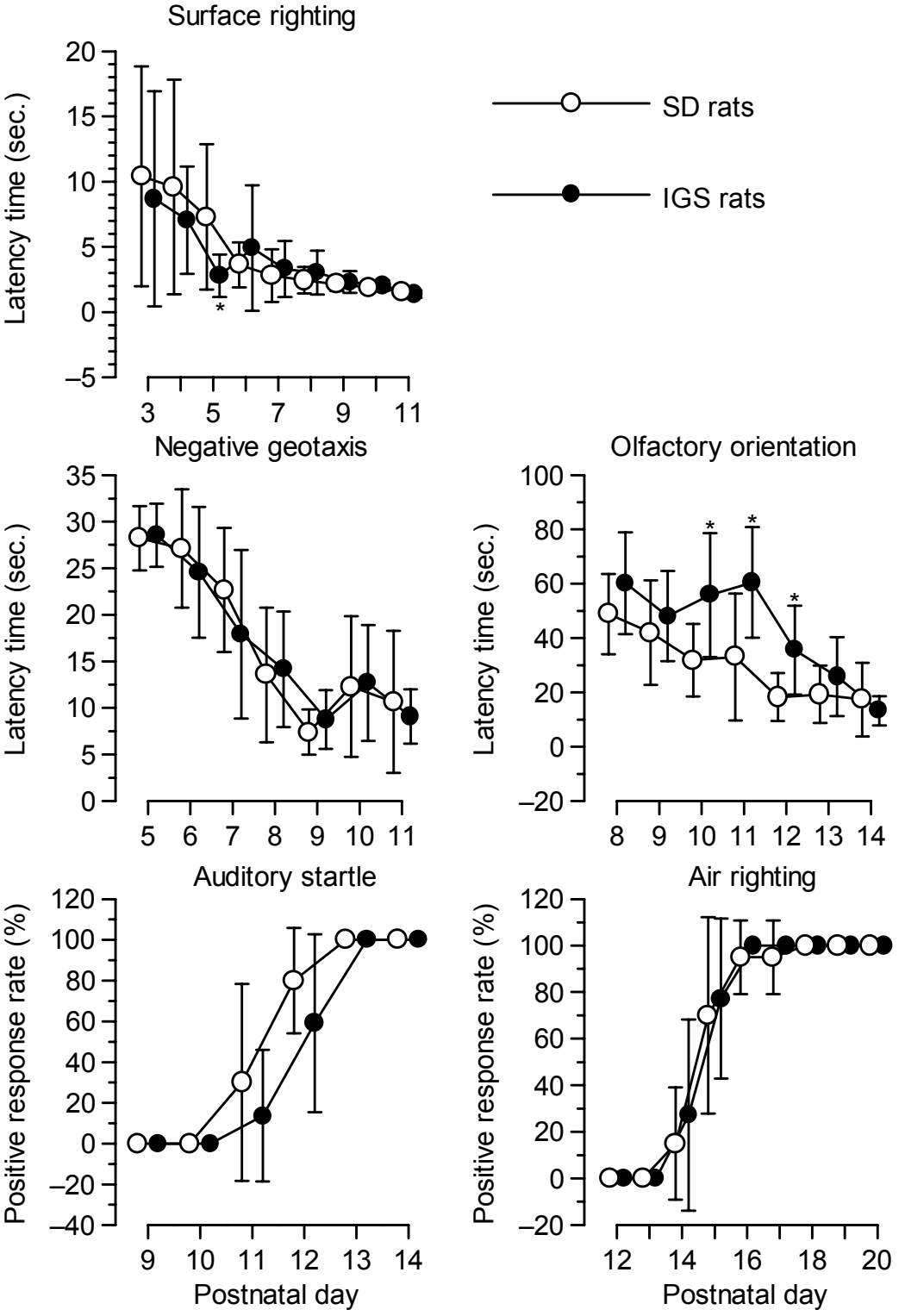

Fig. 3. Behavioral ontogeny in F_1 rats.
Each value shows mean ± S.D.
* $p<0.05$: Significant difference from SD rats (Student's t-test or Aspin-Welch test).

Table 3. Open-field test in F_1 rats.

	Groups		SD rats	IGS rats
Males	No. of F_1 rats examined		10	11
	Latency time (sec.)	Day 1	28.70±44.49	24.19±10.74
		Day 2	25.26±24.67	17.32±10.18
		Day 3	9.79±3.61	9.04±3.93
	Ambulation	Day 1	38.90±26.48	30.73±15.28
		Day 2	57.80±37.04	57.36±19.22
		Day 3	98.90±72.67	83.18±48.12
	Rearing	Day 1	8.30±5.93	5.27±3.52
		Day 2	8.40±5.56	5.82±3.60
		Day 3	10.20±9.50	6.73±4.65
	Grooming	Day 1	2.20±1.99	1.45±1.57
		Day 2	1.30±0.95	1.45±1.04
		Day 3	1.20±1.14	1.55±1.13
	Defecation	Day 1	1.00±1.25	1.09±0.94
		Day 2	1.20±1.23	1.09±1.38
		Day 3	0.90±1.37	1.00±1.41
	Urination	Day 1	0.40±0.52	0.55±0.93
		Day 2	0.20±0.42	0.09±0.30
		Day 3	0.10±0.32	0.00±0.00
Females	No. of F_1 rats examined		10	11
	Latency time (sec.)	Day 1	28.33±19.25	23.41±15.52
		Day 2	11.48±5.99	13.86±7.67
		Day 3	6.17±2.34	7.53±3.43
	Ambulation	Day 1	55.50±16.04	59.64±16.91
		Day 2	89.90±31.10	79.27±16.83
		Day 3	85.70±10.22	69.00±25.20
	Rearing	Day 1	12.20±6.89	9.18±5.15
		Day 2	14.00±6.78	11.09±6.28
		Day 3	11.70±4.50	8.18±4.90
	Grooming	Day 1	1.70±1.34	0.82±0.75
		Day 2	1.00±1.25	1.45±1.13
		Day 3	1.00±1.49	1.27±0.90
	Defecation	Day 1	0.40±1.26	0.09±0.30
		Day 2	0.30±0.67	0.27±0.90
		Day 3	0.20±0.63	0.45±0.82
	Urination	Day 1	1.10±0.88	0.64±0.67
		Day 2	0.00±0.00	0.00±0.00
		Day 3	0.50±0.71	0.36±0.67

Each value shows mean±S.D.

Table 4. Shuttle box avoidance test in F_1 rats

	Groups			SD rats	IGS rats
Males	No. of F_1 rats examined			10	11
	Avoidance time (sec.)	Day	1	2.45±0.67	2.37±0.49
			2	1.98±0.72	1.98±0.45
			3	2.05±0.44	1.97±0.64
			15	2.30±0.75	2.12±0.50
	Avoidance rate (%)	Day	1	43.40±26.80	51.82±23.52
			2	73.40±32.61	83.45±21.30
			3	78.40±30.94	88.91±13.06
			15	74.20±31.26	84.73±17.92
	Escape time (sec.)	Day	1	0.79±0.36	0.76±0.32
			2	0.47±0.19	0.44±0.24
			3	0.42±0.23	0.52±0.36
			15	0.42±0.25	0.43±0.29
	Escape rate (%)	Day	1	55.00±24.68	47.82±23.25
			2	26.60±32.61	16.55±21.30
			3	21.20±30.13	11.09±13.06
			15	25.80±31.26	15.09±17.60
	No. of errors	Day	1	0.80±1.93	0.18±0.40
			2	0.00±0.00	0.00±0.00
			3	0.20±0.42	0.00±0.00
			15	0.00±0.00	0.09±0.30
	No. of ITI responses[a)]	Day	1	11.70±7.15	12.64±8.31
			2	23.60±21.96	12.64±11.46
			3	24.60±27.76	13.00±9.94
			15	18.00±16.20	11.09±9.29
Females	No. of F_1 rats examined			10	11
	Avoidance time (sec.)	Day	1	2.05±0.90	2.46±0.92
			2	1.94±0.90	2.11±0.77
			3	1.68±0.81	1.91±0.37
			15	1.85±0.47	1.94±0.50
	Avoidance rate (%)	Day	1	57.20±32.93	42.73±34.32
			2	73.60±38.36	73.45±26.44
			3	73.20±38.63	83.64±22.82
			15	78.00±33.12	89.82±10.22
	Escape time (sec.)	Day	1	0.71±0.28	0.76±0.38
			2	0.39±0.19	0.58±0.31
			3	0.33±0.26	0.36±0.25
			15	0.27±0.23	0.34±0.20
	Escape rate (%)	Day	1	41.60±31.11	52.55±31.74
			2	26.20±37.96	26.36±26.42
			3	26.60±38.49	16.36±22.82
			15	22.00±33.12	10.18±10.22
	No. of errors	Day	1	0.60±1.90	2.36±3.91
			2	0.10±0.32	0.09±0.30
			3	0.10±0.32	0.00±0.00
			15	0.00±0.00	0.00±0.00
	No. of ITI responses[a)]	Day	1	18.70±18.82	11.55±9.94
			2	32.90±27.77	19.27±17.67
			3	37.20±31.51	25.55±16.77
			15	30.90±20.02	27.82±11.69

Each value shows mean±S.D.
a) No. of intertrial interval responses.

Table 5. Spontaneous motor activity in F_1 rats.

	Groups	SD rats	IGS rats
	No. of F_1 rats examined	10	11
	Locomotor activity		
	0- 30 minutes	2524.10±1037.34	2244.91±650.94
	30- 60 minutes	2117.10±675.46	1853.27±552.37
	60- 90 minutes	1407.00±784.68	1408.27±998.31
	90-120 minutes	963.20±1093.64	1009.64±914.18
	120-150 minutes	568.20±717.63	545.45±672.81
	150-180 minutes	302.80±488.78	165.82±151.03
	180-210 minutes	164.80±67.64	130.09±49.44
	210-240 minutes	308.10±391.72	134.82±81.66
	240-270 minutes	310.80±584.03	269.45±310.62
	270-300 minutes	326.60±286.46	338.64±418.19
	300-330 minutes	224.20±149.25	133.18±79.16
Males	330-360 minutes	559.10±706.16	155.45±130.45
	Rearing		
	0- 30 minutes	27.80±14.53	22.82±12.43
	30- 60 minutes	26.00±16.92	21.09±10.32
	60- 90 minutes	16.60±15.32	14.55±12.52
	90-120 minutes	10.60±15.40	6.45±10.24
	120-150 minutes	5.00±10.12	2.36±3.85
	150-180 minutes	1.50±4.74	0.55±1.29
	180-210 minutes	0.10±0.32	0.00±0.00
	210-240 minutes	1.00±3.16	0.00±0.00
	240-270 minutes	1.90±6.01	0.73±1.62
	270-300 minutes	1.00±2.00	2.00±6.63
	300-330 minutes	0.70±1.64	0.18±0.60
	330-360 minutes	7.00±13.86	0.00±0.00
	No. of F_1 rats examined	10	11
	Locomotor activity		
	0- 30 minutes	2836.40±632.84	2605.36±687.04
	30- 60 minutes	2141.60±803.52	2037.09±649.60
	60- 90 minutes	1744.90±633.65	1653.18±902.56
	90-120 minutes	1110.50±776.57	1179.64±941.40
	120-150 minutes	714.70±865.59	437.73±445.44
	150-180 minutes	632.60±751.43	493.27±282.38
	180-210 minutes	602.50±494.91	229.82±273.94
	210-240 minutes	316.70±399.99	224.09±309.07
	240-270 minutes	468.30±760.79	574.45±473.16
	270-300 minutes	204.10±268.35	188.82±227.18
	300-330 minutes	427.00±396.33	501.91±687.11
Females	330-360 minutes	373.80±303.80	311.91±134.34
	Rearing		
	0- 30 minutes	37.80±13.97	36.64±24.39
	30- 60 minutes	26.00±14.29	26.00±22.38
	60- 90 minutes	18.40±10.53	21.91±25.38
	90-120 minutes	10.70±11.97	7.91±7.83
	120-150 minutes	7.60±13.42	3.55±6.56
	150-180 minutes	6.90±10.68	3.09±4.61
	180-210 minutes	5.80±7.19	0.82±2.40
	210-240 minutes	2.40±5.08	0.82±2.40
	240-270 minutes	3.00±7.89	3.27±3.55
	270-300 minutes	0.70±2.21	0.27±0.90
	300-330 minutes	3.10±6.98	4.18±7.86
	330-360 minutes	3.40±5.52	1.64±2.69

Each value shows mean±S.D.
a) No.of intertrial interval responses.

Comparison between Crj:CD(SD)IGS and Slc:SD Rats in Reproductive Parameters: Fertility and Early Embryonic Development

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ABSTRACT. A fertility study with Crj:CD(SD)IGS rats, which have recently become commercially available from Charles River Inc., was conducted. The data from Crj:CD(SD)IGS rats were compared with those from Slc:SD rats, our standard strain used in reproductive toxicology studies. Behavioral data of animals were also collected to know the sensitivity of the two strains about handling.

When compared with Slc:SD rats, male and female Crj:CD(SD)IGS rats showed greater body weight. Also, Crj:CD(SD)IGS females showed larger food consumption before mating and during gestation. Mean absolute and relative testes weights of Crj:CD(SD)IGS rats were lower than Slc:SD rats. For prostate and seminal vesicles, mean absolute and relative weights of Crj:CD(SD)IGS rats were greater than Slc:SD rats. Sperm motility, sperm count, estrus cycle, copulation and pregnancy indices were similar between the strains. The numbers of corpora lutea, implantation sites and live fetuses of Crj:CD(SD)IGS rats were larger than Slc:SD rats. The size of corpora lutea of Crj:CD(SD)IGS rats was irregular when compared with Slc:SD rats and therefore more difficult to count. In the behavioral tests for ease of handling, Slc:SD males were more sensitive than Crj:CD(SD)IGS males. On the other hand, Crj:CD(SD)IGS females were more sensitive than Slc:SD females.

These results suggest that there were minor differences in reproductive and behavioral parameters in Crj:CD(SD)IGS rats compared with Slc:SD rats.

— Key words : Crj:CD(SD)IGS rat, Fertility, Background data, Handling

CD(SD)IGS-1999: 168-175

INTRODUCTION

The gold standard system, a new laboratory animal breeding system, was recently established by Charles River Inc. The fertility study was conducted to obtain the background data of Crj:CD(SD)IGS rats produced by the above system. The data were compared with the data from Slc:SD rats.

Crj:CD(SD)IGS rats seemed to react much more sensitively than Slc:SD rats when they were touched. Therefore, to compare the sensitivity of each strain of rats, the tests for ease of handling were performed using the FOB method.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats and Slc:SD rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan) and Japan SLC Inc. (Shizuoka, Japan), respectively. Ten week-old 20 males and 20 females of each strain were used in this study.

Animals had free access to tap water and to a pelleted commercial laboratory animal chow (CE-2, CLEA Japan Inc.). Animal room temperature and relative humidity controls were set at 23 ± 2℃ and 55 ± 5%, respectively. Lighting was controlled to give a light (6 a.m. to 6 p.m.) and dark cycle. The animals were housed individually in suspended stainless steel wire cages except for the mating period, during which one male and one female were placed together.

The aqueous solution of 0.5% methylcellulose was administered orally (esophageal intubation) using metal catheters in a volume of 10ml/kg. Males were dosed beginning 28 days prior to mating for a total of 63 or 64 days and females were dosed for 14 days prior to mating, during the cohabitation period and through gestation day 7. All animals were observed daily for clinical signs. Body weights and food consumption were determined at 3 day intervals for males and daily for females. Food consumption was not recorded during the period of cohabitation.

The estrous cycle was recorded for 14 days prior to mating and during mating until a sperm positive vaginal smear was found. The females that were not cycling prior to mating were not used in the study. Each male was mated with one female. During the 14 day cohabitation period, the females were inspected each morning for their vaginal smear. The day on which sperm was detected in the vaginal smear was designated as gestation day 0. When copulation was confirmed the females were removed from the males' cages and were individually housed.

All sperm-positive females were sacrificed on gestation day 14 by cervical dislocation. The uteri and ovaries were removed and a brief gross examination of the dam was performed. The numbers of corpora lutea, implantation sites, viable fetuses and resorptions were recorded. All males were weighed and then sacrificed by exsanguination from the abdominal aorta under anesthesia by an intraperitoneal injection of pentobarbital sodium beginning on study day 63. Each male rat underwent a gross necropsy. The weights of the testes (right, left), epididymides (right, left), prostate and seminal vesicles from all males were recorded and organ to body weight ratios calculated.

Sperm motility examinations were performed using sperm collected from the left cauda epididymis. The cauda epididymis was held with forceps and three stabs were made with a scalpel blade. Then it was put in with the Medium 199 with Hank's salts and L-glutamine (Gibco, Grand Island, NY), containing bovine serum albumin. That had been adjusted to pH of 7.4 and warmed to approximately 37℃. Sperm motility was examined using the HTM-IVOS Motility Analyzer (Hamilton Thorne Research, Beverly, MA). The right cauda epididymis was homogenized and placed into a stain reaction vial (Supra Vital IDENT Stain Kit, Hamilton Thorne Research, Beverly, MA) and sperm count analysis was performed using the HTM-IDENT option of the IVOS analyzer.

The "ease of removing animal from cage" and the "reactivity to being handled" of the rats were scored using the functional observational battery (FOB) [1] methods to compare the sensitivity of each strain of rats (Table 1).

Table 1. Scoring Criteria of Handling Reactivity

Ease of removing animal from cage

Score 1)	very easy (sits quietly, allows observer to pick it up)
Score 2)	easy (vocalizations, picked up with little or no resistance)
Score 3)	moderately difficult (rears, often following observer's hand)
Score 4)	animal flinches (with or without vocalizations)
Score 5)	difficult (runs around cage, is hard to grab, with or without vocalizations)
Score 6)	very difficult (tail and throat rattles, attempts to bite, with or without vocalizations)

Reactivity to being handled

Score 1)	low (no resistance, is easy to handle)
Score 2)	moderately low (slight resistance, with or without vocalizations)
Score 3)	moderately high (may freeze, be tense, or rigid in hand, with or without vocalizations)
Score 4)	high (squirming, twisting, or attempting to bite, with or without vocalizations)

Statistical analyses of data were performed using the following methods. The copulation and fertility indices and behavioral parameters were analyzed using a chi-square test. The other data were analyzed using the F test for homogeneity of variance followed by the Student's t test or the Aspin & Welch t test. Statistical analyses were made between Slc:SD and Crj:CD(SD)IGS strains at two-tailed 5% and 1% levels of significance.

RESULTS AND DISCCUSSION

All of the data from Slc:SD rats in this study were comparable to our historical data [2].

One Crj:CD(SD)IGS male presented hair loss. During the study, there were no other clinical signs in either strain.

When compared to Slc:SD rats, male and female Crj:CD(SD)IGS rats showed significantly greater body weight and food consumption (Figure 1, 2, 3 and 4, Table 2).

Terminal body weights of Crj:CD(SD)IGS rats were significantly higher than that of Slc:SD rats (Table 3). Mean absolute and relative testes weights of Crj:CD(SD)IGS rats were significantly lower than that of Slc:SD rats. On the other hand, mean absolute and relative prostate and seminal vesicle weights of Crj:CD(SD)IGS rats were significantly higher than that of Slc:SD rats. Mean absolute epididymides weights of Crj:CD(SD)IGS rats were significantly higher than that of Slc:SD rats. Sperm motility was comparable between the two strains (Table 4). Epididymal sperm count of Crj:CD(SD)IGS rats was slightly low when compared with that of Slc:SD rats but there was no statistically significant difference between the two strains. Estrus cycle, copulation and pregnancy indices were similar between the two strains (Table 5, 6). The numbers of corpora lutea, implantation sites and live fetuses of Crj:CD(SD)IGS rats were significantly larger than that of Slc:SD rats. The size of corpora lutea of Crj:CD(SD)IGS rats was irregular when compared with that of Slc:SD rats and therefore more difficult to count (Figure 5). This observation was similar to the result of the teratology study [3].

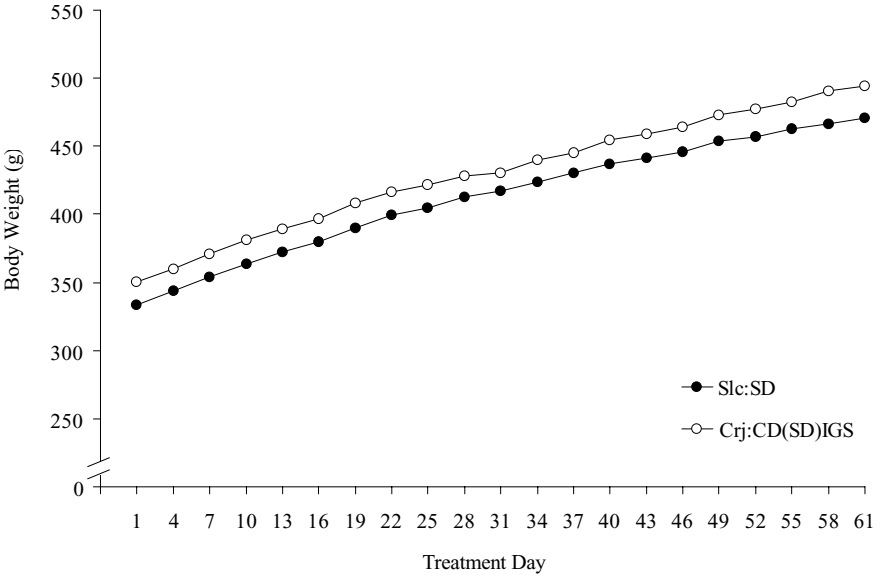


Figure 1. Mean Body Weights of Males

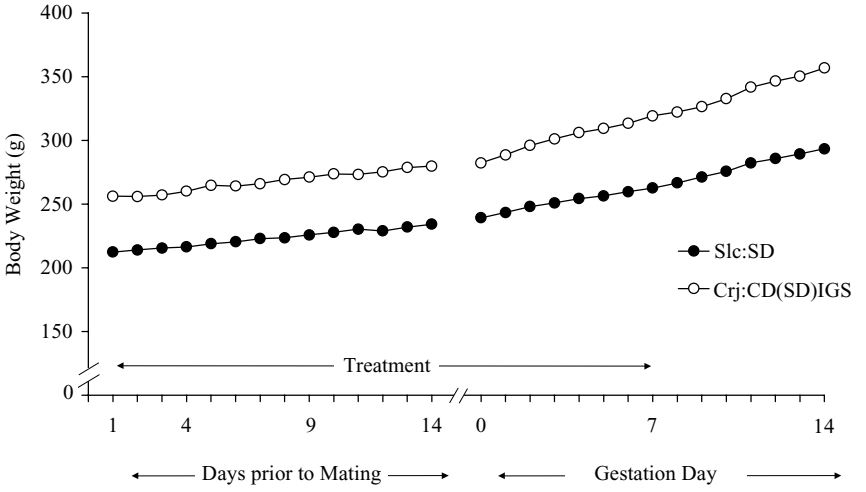


Figure 2. Mean Body Weights of Females

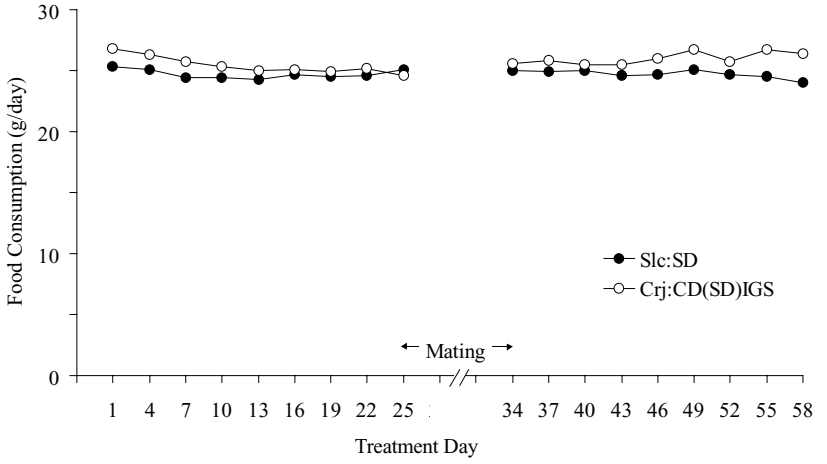


Figure 3. Mean Food Consumption of Males

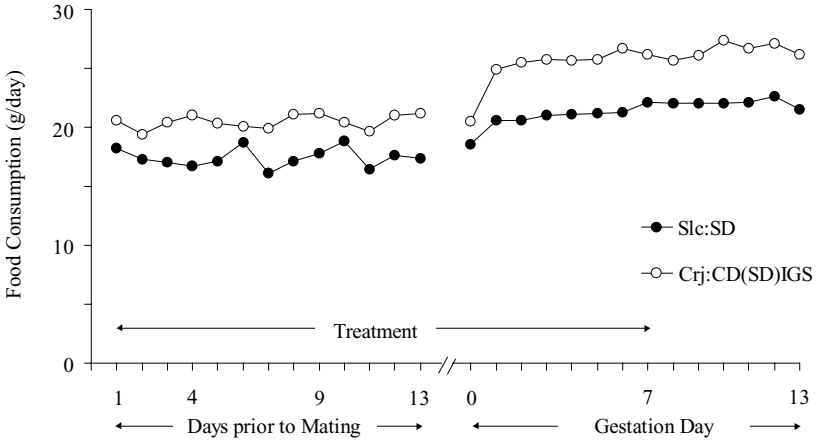
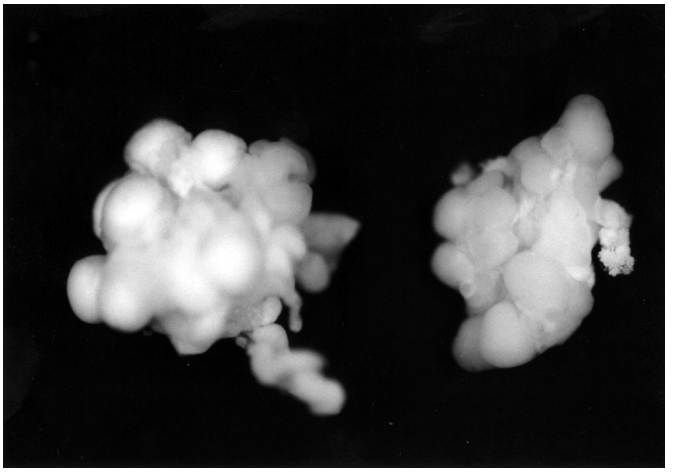


Figure 4. Mean Food Consumption of Females



Slc:SD Crj:CD(SD)IGS

Figure 5. The corpora lutea of Slc:SD rat (left) and Crj:CD(SD)IGS rat (right)

Table 2. Mean Body Weight Gains and Food Consumption of Females

BODY WEIGHT GAIN (G)

Treatment	Slc:SD		Crj:CD(SD)IGS		
Days					
1- 8	11.2±5.2	(20)	13.2±7.3	(20)[a)]	
8-14	10.6±4.9	(20)	10.5±7.9	(20)	
1-14	21.8±5.0	(20)	23.8±11.3	(20)	
Gestation					
Days					
0- 8	27.5±7.6	(18)	39.9±8.1	(20)	**
8-14	26.7±5.5	(18)	34.5±5.3	(20)	**
0-14	54.2±10.6	(18)	74.4±9.6	(20)	**

FOOD CONSUMPTION (G/INTERVAL)

Treatment	Slc:SD		Crj:CD(SD)IGS		
Days					
1- 7	121.1±11.2	(20)	141.7±13.3	(20)	**
8-13	105.2±10.1	(20)	124.5±10.9	(20)	**
1-13	226.3±20.8	(20)	266.3±21.4	(20)	**
Gestation					
Days					
0- 7	166.4±19.7	(18)	201.1±13.0	(20)	**
8-13	132.2±16.7	(18)	159.2±12.6	(20)	**
0-13	298.6±35.6	(18)	360.3±24.0	(20)	**

Each value represents the mean and standard deviation.
a) Numbers of animals used for calculation
** $P<0.01$

Table 3. Summary of Male Reproductive Organ Weights

		Slc:SD	Crj:CD(SD)IGS
No. of males		20	20
Terminal body weights(g)		474.2±35.1	500.5±36.9*
Absolute organ weights(g)			
Testis	Right	1.84±0.10	1.72±0.11**
	Left	1.89±0.10	1.72±0.10**
Epididymis	Right	0.66±0.04	0.73±0.03**
	Left	0.66±0.03	0.69±0.04**
Prostate		1.34±0.19	1.73±0.28**
Seminal vesicle		1.31±0.15	1.53±0.24**
Relative organ weights(g%)			
Testis	Right	0.39±0.03	0.35±0.03**
	Left	0.40±0.03	0.34±0.03**
Epididymis	Right	0.14±0.01	0.15±0.01
	Left	0.14±0.01	0.14±0.01
Prostate		0.29±0.04	0.35±0.06**
Seminal vesicle		0.28±0.04	0.31±0.05*

Each value represents the mean and standard deviation.
* P<0.05, ** P<0.01

Table 4. Summary of Epididymal Sperm Analysis

	Slc:SD	Crj:CD(SD)IGS
No. of males	20	20
Motility (%)	79.5±6.0	82.5±6.1
Progressive Motility (%)	39.2±9.2	33.7±14.1
VAP (μ m/s)	150.7±7.4	144.5±12.3
VSL (μ m/s)	103.5±6.5	98.7±11.2
VCL (μ m/s)	351.5±15.2	326.4±29.8**
ALH (μ m)	20.9±0.8	20.8±1.0
BCF (Hz)	25.1±1.4	25.5±1.7
STR (%)	68.0±2.6	67.3±3.8
LIN (%)	29.1±1.4	29.8±1.7
Count (x10^6 sperm/gram)	799.5±100.8	737.4±112.5

Each value represents the mean and standard deviation.
** P<0.01

Table 5. Fertility and Reproductive Data on Gestation Day 14

	Slc:SD	Crj:CD(SD)IGS
No. of females mated	18	20
No. of females copulated, (%)	18(100.0)	20(100.0)
No. of females pregnant, (%)	18(100.0)	20(100.0)
No. of corpora lutea		
Total	252	341
Mean±S.D.	14.0±1.6	17.1±1.6**
No. of implantation sites		
Total	231	332
Mean±S.D.	12.8±3.0	16.6±1.0**
Embryomortality, %		
Mean±S.D.	9.4±15.6	7.0±11.1
No. of resorption		
Total	18	23
Mean±S.D.	1.0±1.8	1.2±1.8
No. of live fetuses		
Total	213	309
Mean±S.D.	11.8±3.7	15.5±2.1**

** P<0.01

Table 6. Pre-Mating Estrous Cycle of Female Rats

	Slc:SD	Crj:CD(SD)IGS
No. of animals examined	18	20
Estrous cycle length (days)		
Mean±S.D.	4.1±0.3	4.2±1.0
Total occurrence of estrus	63	60
Estrus (%) with each duration		
1 day	59(93.7)	55(91.7)
2 days	4(6.3)	5(8.3)
3 days or more	0(0.0)	0(0.0)
Cohabitation period (days)		
Mean±S.D.	2.7±1.2	2.0±1.1

In the behavioral tests, the scores of "ease of removing animal from cage" and "reactivity to being handled" of Crj:CD(SD)IGS males were slightly lower than Slc:SD males (Figure 6, 7). Conversely, the scores of "ease of removing animal from cage" and "reactivity to being handled" of Crj:CD(SD)IGS females were slightly higher than Slc:SD females (Figure 8, 9).

These results suggest that there were minor differences in reproductive and behavioral parameters in Crj:CD(SD)IGS rats compared with Slc:SD rats.

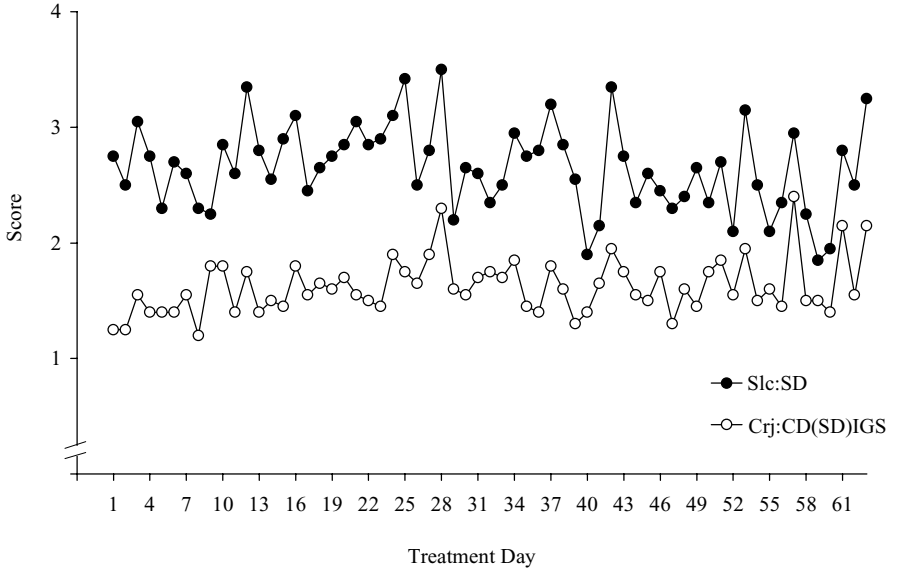


Figure 6. Handling Reactivity on Males : Ease of Removing Animal from Cage

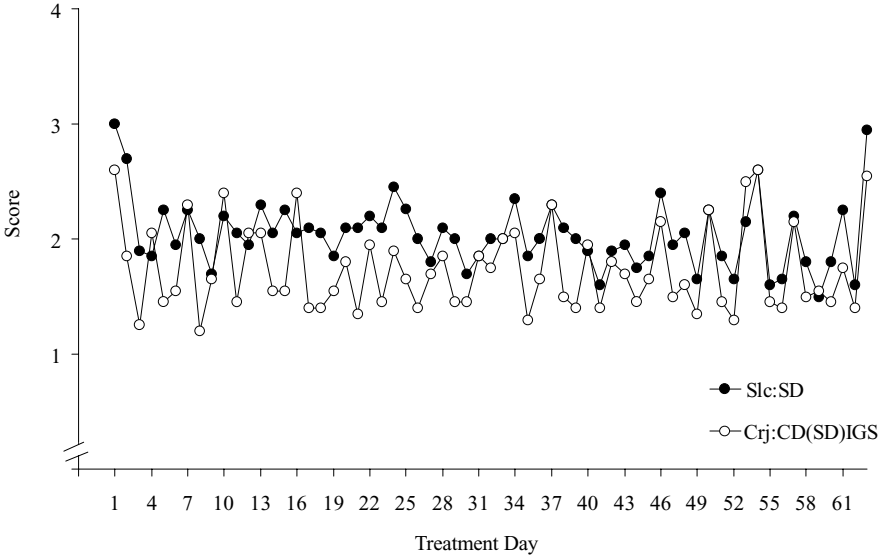


Figure 7. Handling Reactivity on Males : Reactivity to Being Handled

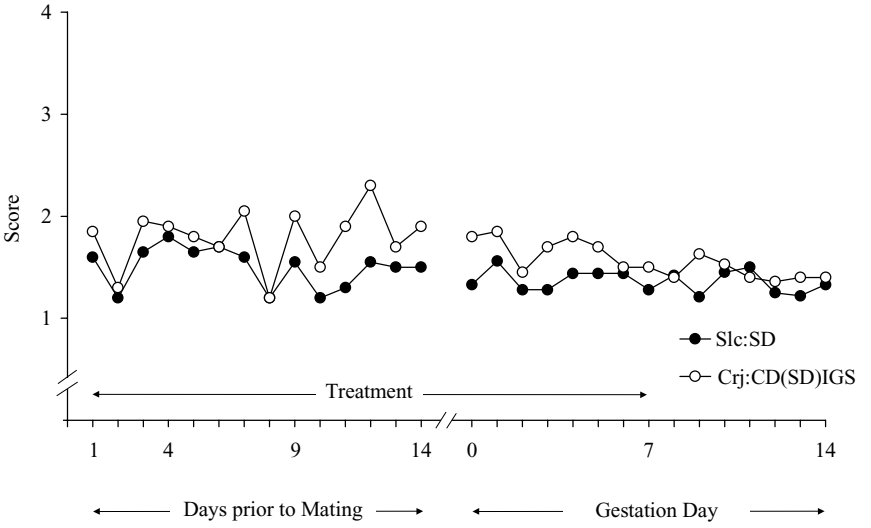


Figure 8. Handling Reactivity on Females : Ease of Removing Animal from Cage

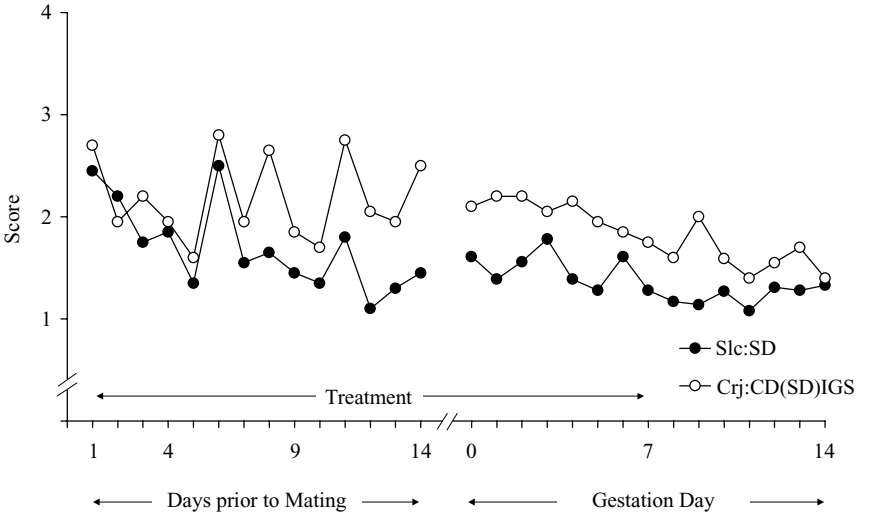


Figure 9. Handling Reactivity on Females : Reactivity to Being Handled

REFERENCES

1. Moser, V.C., McCormick, J.P., Creason, J.P., MacPhail, R.C. 1988. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund. Appl. Toxicol., 11, 189-206.
2. Nakatsuka, T., Horimoto, M., Ito, M., Matsubara, Y., Akaike, M. and Ariyuki, F. 1997. Japan Pharmaceutical Manufactures Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Cong. Anom., 37, 47-138.
3. Sakimura, M., Hisada, T., Zhou, Y., Ohiwa, K. and Horimoto, M. 1999. Comparison between Crj:CD(SD)IGS and Slc:SD rats in reproductive parameters: Effects on embryo - fetal development. Biological Reference Data on CD(SD)IGS-1999, 176-181.

Comparison between Crj:CD(SD)IGS and Slc:SD Rats in Reproductive Parameters: Embryo - Fetal Development

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ABSTRACT. A teratology study with Crj:CD(SD)IGS rats, which have recently become commercially available from Charles River Inc., was conducted. The data from Crj:CD(SD)IGS rats were compared with those from Slc:SD rats, our standard strain used in reproductive toxicology studies. Behavioral data of dams were also collected to know the sensitivity of the two strains about handling.

The mean maternal body weight, body weight gain, food consumption, number of corpora lutea, implantation sites and viable fetuses from Crj:CD(SD)IGS rats were slightly higher than the data from Slc:SD rats. The size of corpora lutea of Crj:CD(SD)IGS rats was irregular when compared with Slc:SD rats and therefore more difficult to count. No fetuses with external abnormalities were observed in either strain. There was a very low incidence of visceral and skeletal abnormalities, but the patterns and numbers of abnormalities were similar between the strains. In the skeletal examination, the mean numbers of ossified metatarsals and sacral/caudal vertebrae were slightly lower in Crj:CD(SD)IGS rats compared to Slc:SD rats. In the behavioral tests for ease of handling, Crj:CD(SD)IGS rats were slightly more sensitive than Slc:SD rats.

These results suggest that there were minor differences in reproductive and behavioral parameters in Crj:CD(SD)IGS rats compared with Slc:SD rats.

— Key words: Crj:CD(SD)IGS rat, Teratology study, Background data, Handling

CD(SD)IGS-1999: 176-181

INTRODUCTION

The gold standard system, a new laboratory animal breeding system, was recently established by Charles River Inc. The teratology study was conducted to obtain the background data of Crj:CD(SD)IGS rats produced by the above system. The data were compared with the data from Slc:SD rats.

Crj:CD(SD)IGS rats seemed to react much more sensitively than Slc:SD rats when they were touched. Therefore, to compare the sensitivity of each strain of rats, the tests for ease of handling were performed using the FOB method.

MATERIALS AND METHODS

Sexually mature Crj:CD(SD)IGS rats and Slc:SD rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan) and Japan SLC Inc. (Shizuoka, Japan), respectively. At 10 - 11 weeks of age, each female was caged with one male of the same strain until there were 25 females/strain with evidence of a positive mating. The day on which sperm was detected in the vaginal smear was designated as gestation day 0.

Animals had free access to tap water and to a pelleted commercial laboratory animal chow (CE-2, CLEA Japan Inc.). Animal room temperature and relative humidity controls were set at 23 ± 2℃ and 55 ± 5%, respectively. Lighting was controlled to give a light (6 a.m. to 6 p.m.) and dark cycle. The animals were housed individually in suspended stainless steel wire cages.

The aqueous solution of 0.5% methylcellulose was administered orally (esophageal intubation) using metal catheters from gestation day 6 to 17 in a volume of 10 ml/kg. Dosage volumes were calculated based upon the daily body weight. All animals were observed daily for clinical signs. Body weights and food consumption were recorded daily. Cesarean sections were performed on gestation day 21. Animals were sacrificed by cervical dislocation. The uteri and ovaries were removed and a brief gross examination of the dam was performed. The numbers of corpora lutea, implantation sites, late and early resorptions, viable and dead fetuses were recorded for each dam. Any dam found non-gravid had its uterus stained with 2% sodium hydroxide to visualize implantation sites and to confirm its non-gravid status [1]. The viable fetuses were weighed individually, and examined for external anomalies. Individual placental weights were also recorded. Approximately half of the fetuses were fixed in Bouin's solution and examined for visceral anomalies. Odd numbered fetuses were eviscerated, skinned and then ethanol fixed for skeletal examinations.

Visceral examinations of the pelvis, abdomen and head were performed following a modified method of the Wilson sectioning technique [2]. Visceral thorax examinations were performed following the Nishimura procedures [3]. Thorough examinations of the cranial, pelvis, abdomen and thorax regions were done and any anomalies were recorded.

Fetal skeletons were visualized by staining with alcian blue and alizarin red using the Sakura Skeletal Processor. The stained skeletons were then examined under a dissecting microscope for degree of ossification and for any anomalies or variations that may have occurred.

The "ease of removing animal from cage" and the "reactivity to being handled" of the rats were scored using the functional observational battery (FOB) [4] methods to compare the sensitivity of each strain of rats (Table 1).

Statistical analyses of data were performed using the following methods. The pregnancy rate and behavioral parameters were analyzed using a chi-square test. The other data were analyzed using the F test for homogeneity of variance followed by the Student's t test or the Aspin & Welch t test. Statistical analyses were made between Slc:SD and Crj:CD(SD)IGS strains at two-tailed 5% and 1% levels of significance.

RESULTS AND DISCCUSSION

All of the data from Slc:SD rats in this study were comparable to our historical data [5].

During the study, there were no deaths or clinical signs in either strain.

Table 1. Scoring Criteria of Handling Reactivity

Ease of removing animal from cage
Score 1) very easy (sits quietly, allows observer to pick it up)
Score 2) easy (vocalizations, picked up with little or no resistance)
Score 3) moderately difficult (rears, often following observer's hand)
Score 4) animal flinches (with or without vocalizations)
Score 5) difficult (runs around cage, is hard to grab, with or without vocalizations)
Score 6) very difficult (tail and throat rattles, attempts to bite, with or without vocalizations)
Reactivity to being handled
Score 1) low (no resistance, is easy to handle)
Score 2) moderately low (slight resistance, with or without vocalizations)
Score 3) moderately high (may freeze, be tense, or rigid in hand, with or without vocalizations)
Score 4) high (squirming, twisting, or attempting to bite, with or without vocalizations)

Mean body weight and mean body weight gains of Crj:CD(SD)IGS rats were higher than those of Slc:SD rats (Figure 1 and Table 2). Mean food consumption of Crj:CD(SD)IGS rats was 17 % higher than that of Slc:SD rats (Figure 2 and Table 2).

The numbers of corpora lutea, implantation sites and viable fetuses from Crj:CD(SD)IGS rats were slightly higher than the data from Slc:SD rats. The fetal body weights and placental weights for Crj:CD(SD)IGS rats were similar to those for Slc:SD rats (Table 3). The size of corpora lutea of Crj:CD(SD)IGS rats was irregular when compared with Slc:SD rats and therefore more difficult to count. This observation was similar to the result of the fertility study [6].

No fetuses with external abnormalities were observed in either strain. There was a very low incidence of visceral and skeletal abnormalities, but the patterns and numbers of abnormalities were similar between the strains (Table 3). In the skeletal examination, the mean numbers of ossified metatarsals and sacral/caudal vertebrae were slightly lower in Crj:CD(SD)IGS rats compared to Slc:SD rats (Table 4).

Table 2. Mean Body Weight Gains and Food Consumption of Pregnant Females

Gestation	Body Weight Gain (g)	
Days	Slc:SD	Crj:CD(SD)IGS
0-6	29.1±4.7(25)	36.5±6.6**(23)[a)]
6-12	29.8±7.1(25)	31.0±6.3(23)
12-18	50.7±15.4(25)	60.1±9.1*(23)
18-21	38.6±10.5(25)	41.5±11.2(23)
Gestation	**Food Consumption (g/interval)**	
Days	Slc:SD	Crj:CD(SD)IGS
0-5	122.2±6.3(25)	143.8±11.1**(23)
6-11	128.6±10.9(25)	154.5±15.0**(23)
12-17	141.1±17.6(25)	165.4±13.7**(23)
18-20	68.0±10.3(25)	76.1±9.5**(23)

Each value represents the mean and standard deviation.
a) Numbers of animals used for calculation
*:p<0.05, **:p<0.01

Figure 1. Mean Body Weights of Pregnant Females

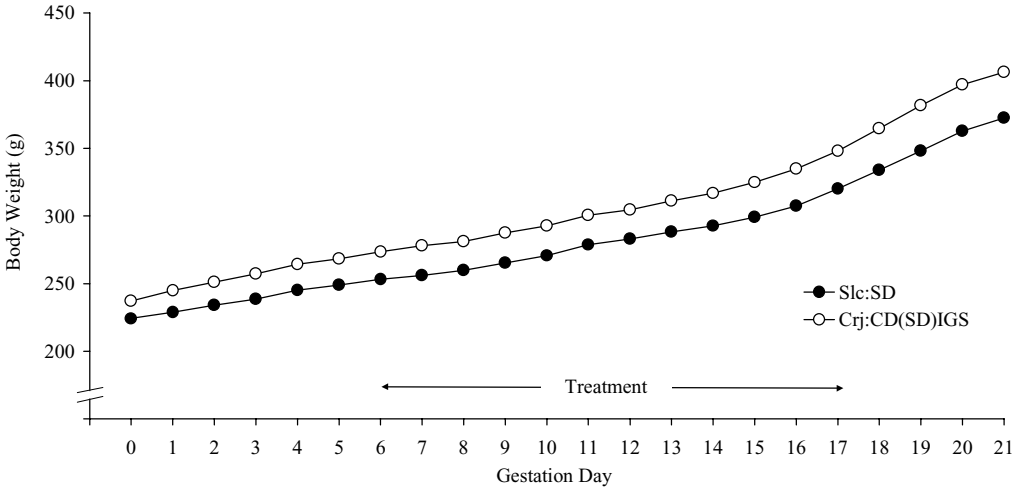


Figure 2. Mean Food Consumption of Pregnant Females

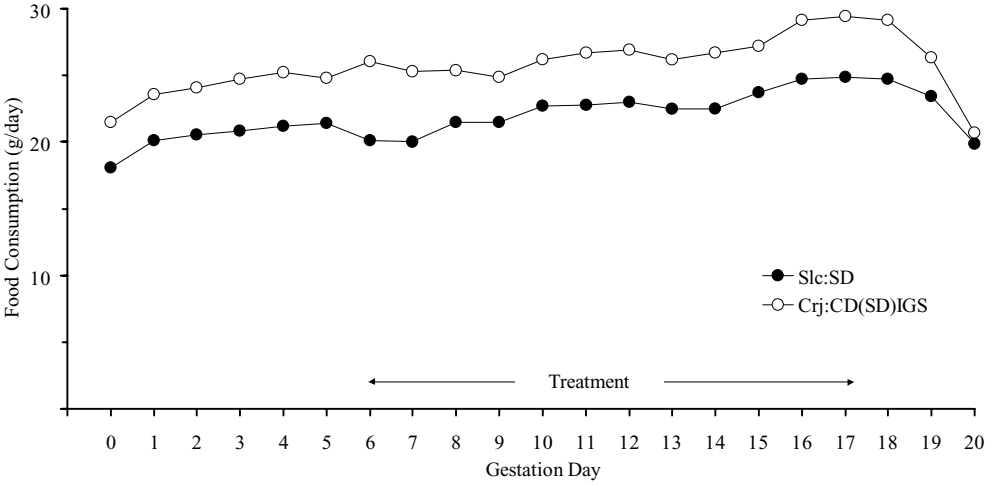


Table 3. Mean Reproductive and Fetal Data

	Slc:SD	Crj:CD(SD)IGS
No. of sperm-positive females	25	25
No. of dead females	0	0
No. of non-gravid females	0	2
No. of females with viable fetuses	25	23
No. of females with entire dead implants	0	0
Pregnancy rate, %	100.0	92.0
No. of corpora lutea		
Mean±S.D.	15.0±1.6	16.2±2.2*
No. of implantation sites		
Mean±S.D.	14.4±2.4	15.3±3.0
Embryomortality, %		
Mean±S.D.	6.7±16.3	2.4±3.5
No.of early resorptions	25	9
late resorptions	0	0
dead fetuses	0	0
No. of viable fetuses		
Total (Males/Females)	334 (184/150)	344 (176/168)
Mean±S.D.	13.4±3.2	15.0±2.9
Fetal body weight, g		
Males Mean±S.D.	5.39±0.22	5.51±0.35
Females Mean±S.D.	5.11±0.22	5.17±0.26
Placental weight, g		
Mean±S.D.	0.43±0.04	0.48±0.06**
No. of fetuses with		
external anomalies	0	0
visceral anomalies		
diaphragmatic hernia	0	1
dilated renal pelvis	0	2
thymic remnant in the neck	1	0
skeletal anomalies		
cervical rib	3	1

*:$p<0.05$, **:$p<0.01$

Table 4. Skeletal Examination of Fetuses : Degree of Ossification

	Slc:SD	Crj:CD(SD)IGS
Total No. of fetuses (Litters)	172 (25)	178 (23)
Sternebrae		
No. of fetuses examined	172	178
Mean±S.D.	6.00±0.00	5.99±0.05
Metacarpals		
No. of fetuses R/L	172/172	178/178
Mean±S.D. R	4.00±0.00	4.00±0.00
Mean±S.D. L	4.00±0.00	4.00±0.00
Metatarsals		
No. of fetuses R/L	172/172	178/178
Mean±S.D. R	5.00±0.00	4.82±0.20**
Mean±S.D. L	5.00±0.00	4.85±0.20**
Thoracic/Lumbar vertebrae #	172	178
Mean±S.D.	19.0±0.0	19.0±0.0
Sacral/Caudal vertebrae #	172	178
Mean±S.D.	12.4±0.7	10.2±0.6**

**:p<0.01

In the behavioral test, the scores of "ease of removing animal from cage" of Crj:CD(SD)IGS rats were significantly lower than Slc:SD rats (Figure 3). On the other hand, the scores of "reactivity to being handled" in Crj:CD(SD)IGS rats during the treatment period were significantly higher than those of Slc:SD rats (Figure 4). These data suggest that Crj:CD(SD)IGS rats were calmer than Slc:SD rats when they were given mild stimulus. However, when they were given strong retention, Crj:CD(SD)IGS rats were more sensitive than Slc:SD rats.

These results suggest that there were minor differences in reproductive and behavioral parameters in Crj:CD(SD)IGS rats compared with Slc:SD rats.

Figure 3. Handling Reactivity : Ease of Removing Animal from Cage

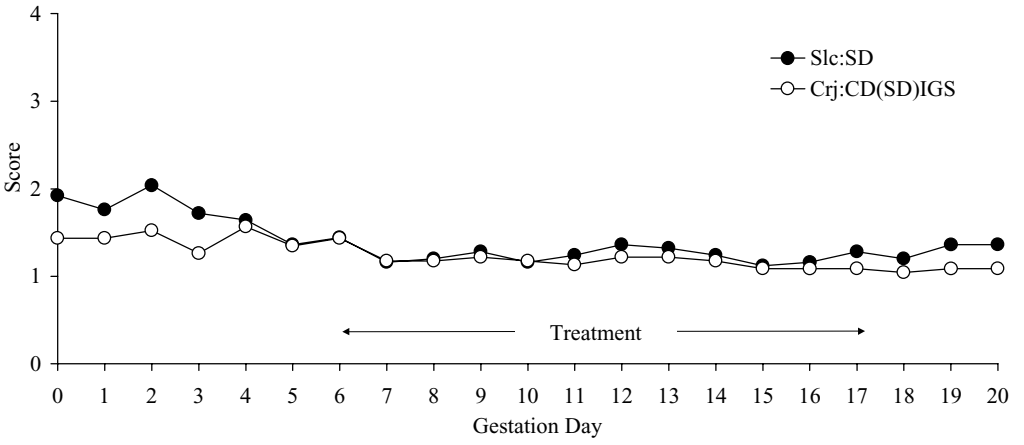
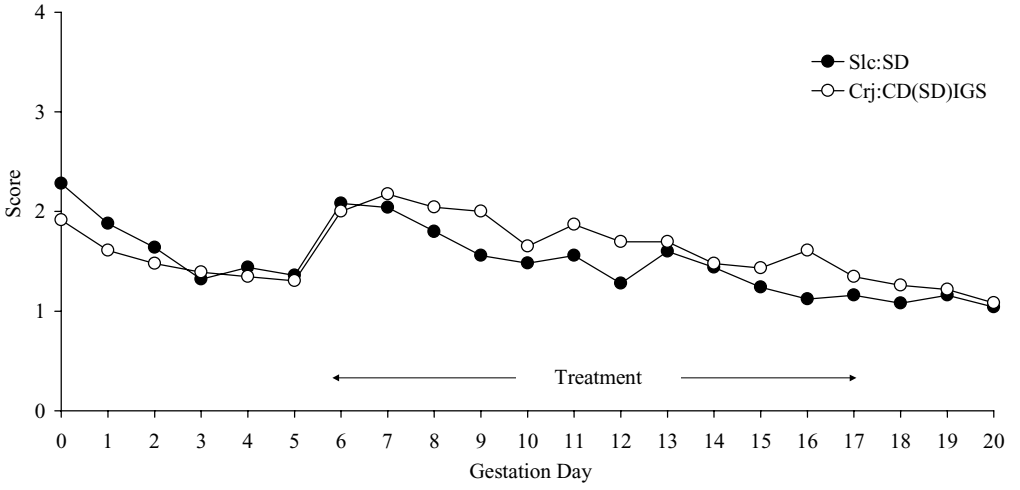


Figure 4. Handling Reactivity : Reactivity to being Handled



REFERENCES

1. Yamada, T., Ohsawa, K. and Ohno, H. 1988. The usefulness of alkaline solutions for clearing the uterus and staining implantation sites in rats. Exp. Anim., 37, 325-331.
2. Wilson, J.G., Warkany, J. 1965. IN: Teratology: Principles and Techniques, 1st ed. Univ. of Chicago Press, Chicago IL., 262-77.
3. Nishimura, K. 1974. A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. Cong. Anom., 14, 23-40.
4. Moser, V.C., McCormick, J.P., Creason, J.P. and MacPhail, R.C. 1988. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund Appl. Toxicol., 11, 189-206.
5. Nakatsuka, T., Horimoto, M., Ito, M., Matsubara, Y., Akaike, M. and Ariyuki, F. 1997. Japan Pharmaceutical Manufacturers Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Cong. Anom., 37, 47-138.
6. Isobe, Y., Mako, T., Matsui, J. and Horimoto, M. 1999. Comparison between Crj:CD(SD)IGS and Slc:SD rats in reproductive parameters: Fertility and early embryonic development. Biological Reference Data on CD(SD)IGS Rats – 1999, 168-175.

Accumulation of Background Data in Crj:CD(SD)IGS Rats on Reproductive and Developmental Toxicity Study

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ABSTRACT. Crj:CD(SD)IGS rats developed by Charles River Inc. is an experimental animal having a uniform quality worldwide. We intend to use Crj:CD(SD)IGS rats for reproductive and developmental toxicity study of new drug, we therefore obtained the back ground data of Crj:CD(SD)IGS rats regarding delivery and lactation of dams, and growth of offspring including the physical and functional development, emotionality, learning ability and reproductivity. And these data were compared with that of Crj:CD(SD) rats or Slc:SD rats which we have used.
As the result, there were no significant differences in the either findings between Crj:CD(SD)IGS rats and Crj:CD(SD) rats or Slc:SD rats.
Key words: Crj:CD(SD)IGS rat, Crj:CD(SD) rat, Slc:SD rat, Background data, Reproduction, Development

CD(SD)IGS-1999: 182-189

INTRODUCTION

Crj:CD(SD)IGS rat developed by Charles River Inc. is an experimental animal having a uniform quality worldwide. It was developed in order to internationalize the scientific research and development of new drug. We intend to use Crj:CD(SD)IGS rats for reproductive and developmental toxicity study of new drug, we therefore obtained the background data of Crj:CD(SD)IGS rats regarding delivery and lactation of dams and growth of offspring including the physical and functional development, emotionality, learning ability and reproductivity. And these data were compared with that of Crj:CD(SD) rats or Slc:SD rats which we have used.

MATERIALS AND METHODS

Ten male, and 40 female Crj:CD(SD)IGS rats, ten-week-old, respectively, arrived from Charles River Japan, Inc., Hino Breeding Center on October 30, 1997 (with males weighing from 312-334 g, and females from 201-234 g). After a 7-day quarantine period, they were acclimated for 5 days till initiation of mating, and those that showed favorable weight gains and general conditions were used for the experiments. The females weighed 243-290 g on day 0 of pregnancy. Out of 28 females that were confirmed to have copulated, 22 were chosen for evaluation in sequence of delivering days.

The rats were housed in an animal room maintained in the temperature range from 20-26℃ and relative humidities of 30-70%, illuminated on a 12-hour light-dark cycle (with the light turned on at 6 A.M. and turned off at 6 P.M.) and with ventilation of not less than 10 air changes per hour.

The rats were given a pellet feed (CA-1, Japan CLEA, Inc.) and tap water via an automatic water supplier *ad libitum*.

Each rat was identified by attaching an ear tag punched with individual number on arrival of F_0 and on weaning of F_1; and F_1 neonates were tattooed by the limbs after adjusting the litter size. Further, a cage label bearing the study number, animal species (strain) and animal number, etc. was posted on each cage.

Pregnant rats were administered with 0.5% w/v HPMC (hydroxypropylmethylcellulose, Lot no. 608439, Shinestu Chemical Co. Ltd) which is usually used at our laboratories as the vehicle of insoluble substance from day 7 of pregnancy till weaning day(21 days postpartum). The HPMC was administered in a dosing volume of 1 mL/100 g body weight, using the body weight on day 7 of pregnancy during the pregnancy period, and using the latest body weight after parturition.

The 0.5% w/v HPMC solution was prepared with distilled water, in a frequency of not less than once every 7 days.

The F_0 dams were observed for clinical signs, weighed and measured for food consumption.

The F_0 dams that delivered between day 21 and 23 of pregnancy were observed for the status of parturition, with the duration of pregnancy counted. They were also observed for the status of lactation till the day of weaning.

The F_0 dams with total litter loss during lactation period or at weaning on day 21 postpartum were sacrificed by exsanguination under ether anesthesia for autopsy. At autopsy on day 21 postpartum, thymus, lungs, heart, liver, spleen, kidneys and adrenal glands were weighed, and according to the body weight on the day of autopsy, their relative weights to 100 g of body weight were calculated.

At birth, the number of live neonates and dead neonates were counted; the live neonates were weighed, observed for external abnormalities and sex.

Dams with 9 neonates or more per litter on day 4 postpartum were adjusted to 8 neonates, i.e., 4 males and 4 females, to the extent possible. The surplus neonates were euphorized with carbon dioxide gas.

Two males and 2 females were weaned from each dam on day 21 postpartum; one pair of male and female were reared for examination of learning ability, and another pair for examination of reproductivity. Surplus neonates were sacrificed by exsanguination under ether anesthesia for autopsy.

Of the physical development test, pinna unfolding (unfolding of bilateral pinnae) was observed in neonates from day 4 postpartum till day of complete unfolding, dorsal hair growth (growth of hair in the dorsal region) from day 7 postpartum till day of complete growth, incisor eruption (upper incisor eruption) from day 11 postpartum till day of complete eruption, eye opening (complete opening of bilateral eyelids) from day 15 postpartum till day of complete opening, testicular descent (descent of bilateral testis) from day 23 postpartum till day of complete descent, cleav-

age of balanopreputial gland (cleavage of the balanopreputial gland to a crater-like form) from day 33 postpartum till day of complete formation, and vaginal opening (opening of the vagina) on day 37 postpartum. The first-day finding was taken as the observation of each item when observation was conducted over multiple days.

Of the functional development tests, the surface righting reflex test (if the rat returned from the dorsal position to the prone position within 30 seconds) was conducted in all rats on days 7 and 8 postpartum; the sniffing orienting response test (if the rat returned to the home cage litter within 90 seconds), on days 10 and 12 postpartum; negative geotaxis test (if the rat rotated 180° within 30 seconds), on days 11-13 postpartum; mid-air righting reflex test (if the rat was capable of landing on the limbs), on days 13-15 postpartum; swimming test (if the rat was capable of swimming by keeping the face above the water surface), on day 14 postpartum; auditory startle test (Galton whistle Preyer reflex; the Galton whistle was whistled 3 times above each ear to see bilateral pinna reflexes) on day 14 postpartum; pain response test (if the rat responded to gentle pinching of the tail), on day 18 postpartum; and visual placing reflex test (if the rat stretched the forelimbs before their wisker attached to the bar), on day 18 postpartum.

Emotionality test was conducted by the open field test for 2 days from day 35 postpartum. The test was conducted for 3 minutes a day, measuring the latency time, ambulation number, rearing frequency, defecation and urination frequency, and grooming time.

The learning ability test was performed using a water multiple T-maze apparatus from day 42 postpartum by 4 trials of a straight water course a day and 4 trials of a water maze course a day for 3 consecutive days, measuring the elapsed time to goal and/or counting the errors made.

The F_1 rats used for the emotional and learning ability tests were sacrificed by exsanguination under ether anesthesia at 10 weeks of age; the thymus, lungs, heart, liver, spleen, kidneys, adrenals, testes, epididymides, ovaries and uterus were weighed, and according to the body weight on the day of autopsy, their relative weights to 100g of the body weight were calculated.

The F_1 female rats that were reared for reproductivity test were observed for estrus cycle by vaginal smear test during the 7-day period on days 71-77 postpartum.

For reproductivity test, the F_1 rats were cohabited 1 to 1 from 11 weeks of age for a maximum of 14 days until copulation was confirmed while avoiding sib mating. The F_1 females that were confirmed to have copulated were sacrificed by exsanguination under ether anesthesia on day 14 of pregnancy to confirm if they were pregnant. In the F_1 females with confirmed pregnancy, the number of implantations and the number of corpora lutea were counted, and the embryos were checked for life or death.

The results of the above tests and measurements were compared with the findings in Crj:CD(SD) rats or Slc:SD rats available in our laboratory.

RESULTS

F_0 dams

On observation for abnormalities in clinical sign, reddish tears were observed in one dam only. The mean body weight (range) was 291.8 g (265-319 g) on day 4 of pregnancy, 305.0 g (284-339 g) on day 7 of pregnancy, 331.2 g (304-374 g) on day 13 of pregnancy, and 414.4 g (362-466 g) on day 21 of pregnancy. The mean weight gains (range) were 27.1 g (17-44 g) on day 4 of pregnancy, 40.3 g (29-64 g) on day 7, 66.5 g (50-97 g) on day 13, and 149.7 g (100-195 g) on day 21 of pregnancy. The mean food consumption (range) was 23.2 g (20-27 g) on day 4, 23.2 g (18-28 g) on day 7, 24.4 g (20-32 g) on day 13, and 18.8 g (7-31 g) on day 21 of pregnancy.

The mean body weight (range) during the lactation period was 304.3 g (261-355 g) on day 0 postpartum, 327.5 g (287-387 g) on day 4 postpartum, 356.2 g (322-393 g) on day 14 postpartum, and 340.1 g (304-378 g) on day 21 postpartum.

No anomalies were found in either dams on autopsy.

Table 1 shows the mean organ weights. The mean absolute weight was 0.248 g for thymus, 1.264 g for lungs, 1.105 g for heart, 15.206 g for liver, 0.644 g for spleen, 1.191 and 1.168 g for kidney (right and left), 36.33 and 40.10 mg for adrenal gland (right and left).

Table 1. Organ weights of weaning dams in Crj:CD(SD)IGS rats

No. of dams	Terminal Body Weight (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)
22	332.9±19.0	0.248±0.087	1.264±0.086	1.105±0.079	15.206±1.622
—	—	0.074±0.024	0.380±0.027	0.332±0.020	4.566±0.394

Spleen (g)	Kidney(R) (g)	Kidney(L) (g)	Adrenal(R) (mg)	Adrenal(L) (mg)
0.644±0.078	1.191±0.090	1.168±0.096	36.33±4.04	40.10±4.72
0.193±0.019	0.358±0.024	0.351±0.027	10.93±1.20	12.07±1.45

Mean±S.D. Upper:Absolute weight
Lower:Relative weight

Table 2 shows the results of parturition and lactation. The mean pregnancy period was 21.5 days. The gestation index was 95.8%, because no delivery (only implantation site) occurred in one dam. The birth index was 91.7%. The survival rate at 4 day postpartum decreased to 96.8%, because there was one dam that failed to take postpartum treatment and give subsequent lactation, with all its neonates being dead on day 3 postpartum. However, the weaning rate was 100% as no neonatal mortality occurred thereafter.

F_1 rats

No external anomalies were found on examination of neonates at delivery.

The male neonates weighed 6.5 g, and the females, 6.1 g at birth. The male neonates then gained 48.0 g of body weight and the females 46.2 g by weaning. The body weight (range) at 77 days of age were 482.0 g (417-527 g) for males, and 297.0 (247-344 g) for females, then gains (range) from weaning till 77 days of age were 422.5 g (368-472 g) for males and 241.4 g (198-285 g) for females.

Table 3 shows the results of growth differentiation and function tests. The positive rate of pinna unfolding was 93.2% at 4 days of age and 100% at 6 days of age. The positive rate of dorsal hair growth was 10.2% at 7 days of age, 84.7% at 8 days of age, and 100% at 10 days of age. Incisor eruption was positive in 74.7% on 11 days of age, and 100% on 13 days of age. The eye opening rate was 92.5% at 15 days of age, and 100% at 16 days of age. Testicular descent was positive in 93.2% on 23 days of age, and 100% on 24 days of age. Cleavage of the balanopreputial gland was positive in 52.3% on 33 days of age, 84.1% on 34 days of age, and 100% on 36 days of age. Opening of the vagina was positive in 100% on 37 days of age that was the first day of observation.

Equilibrium test (righting reflex) was complete 100% on 8 days of age. Negative geotaxis was complete in 97.2% on 13 days of age. Olfactory test (sniffing orienting response) was complete in 82.9% on 12 days of age. Swimming test was complete in 100% on 14 days of age. Auditory test was complete in 100% on 14 days of age. Mid-air righting reflex was complete in 61.6% on 15 days of age. Visual test was 100% complete at 18 days of age. Pain test was 100% complete at 18 days of age.

Table 4 shows the results of open field test. The mean latency time was 18.6 seconds on day 1, and 12.9 seconds on day 2 for males, and 23.4 seconds on day 1, and 21.0 seconds on day 2 for females. Males made 51.6 ambulations on day 1, and 53.6 ambulations on day 2; and females made 51.6 ambulations on day 1, and 51.2 ambulations on day 2. The mean frequency of rearing was 13.0 times for males on day 1, and 12.5 times on day 2; and 13.5 times and 13.9 times for females, respectively. The mean grooming time was 0.9 seconds for males on day 1, and 3.5 seconds on day 2; and 1.7 seconds for females on day 1, and 5.3 seconds on day 2. The mean number of feces defecated was 1.4 for males on day 1, and 2.1 on day 2; and 1.4 and 1.5 for females, respectively. The mean urinating site was 0.5 on day 1 and 0.8 on day 2 for males, and 1.3 on day 1 and 0.9 on day 2 for females.

Table 2. Delivery and viability data of neonates

Item	Crj:CD(SD)IGS	Crj:CD(SD)	Slc:SD
No. of pregnant dams	24	12	22
No. of delivered dams	23	12	22
No. of dams with live neonates	23	12	22
Pregnant period(day)	21.5±0.5	22.0±0.0	22.0±0.2
No. of implantations(Mean±SD)	15.2±3.4	17.4±1.6	13.8±2.0
Gestation index	95.8	100.0	100.0
Birth index	91.7±11.3	—	—
No. of neonates(male/female)			
Day 0	152 / 171	74 / 97	152 / 129
4	141 / 161	—	—
(4)	85 / 86	—	—
7	85 / 86	40 / 37	88 / 78
14	85 / 86	40 / 37	88 / 78
21	85 / 86	40 / 37	88 / 78
Viability index (Day 0-4)	96.8±7.3	74.7±39.4	94.0±21.2
(Day 4-7)	100.0±0.0	—	—
Weaning index (Day 4-21)	100.0±0.0	100.0±0.0	100.0±0.0
No. of neonates with anomalies	0	0	0

(4):After culling
Gestation index:No. of dams with live neonates/No. of pregnant dams×100
Birth index:No. of live neonates/No. of implantations×100
Viability index:No. of neonates on day 4 or 7/No. of neonates on day 0 or 4×100
Weaning index:No. of neonates on day 21/No. of neonates on day 4 after culling

Table 3. Physical and functional development findings of offspring

Item			Crj:CD(SD)IGS	Crj:CD(SD)	Slc:SD
Pinna unfolding	Day	4	93.2±15.8	100.0	100.0
		5	99.4±2.7		
		6	100.0±0.0		
Hair growth	Day	7	10.2±29.5		
		8	84.7±31.8		
		9	99.4±2.7		
		10	100.0±0.0		
		11		100.0	100.0
Incisor eruption	Day	11	74.7±32.8		
		12	96.4±10.0	97.5	97.6
		13	100.0±0.0		
Eye opening	Day	15	92.5±15.3		
		16	100.0±0.0	100.0	97.0
Testis descent	Day	23	93.2±23.4		
		24	100.0±0.0		
		28		100.0	100.0
Cleavage of balanopreputial gland	Day	33	52.3±47.5		
		34	84.1±35.8		
		35	97.7±10.7		
		36	100.0±0.0		
Vaginal opening	Day	35		100.0	76.2
		37	100.0±0.0		
Surface righting reflex	Day	7	98.3±5.8		
		8	100.0±0.0		
		21		100.0	100.0
Negative geotaxis	Day	11	94.9±9.2		
		12	98.3±4.4		
		13	97.2±6.6		
Sniffing orienting response	Day	10	74.9±20.0		
		11	79.0±20.9		
		12	82.9±17.9		
Swimming test	Day	14	100.0±0.0	100.0	100.0
Auditory startle response	Day	14	100.0±0.0		
		21		100.0	100.0
Mid-air righting reflex	Day	13	26.3±20.7		
		14	26.9±27.8		
		15	61.6±28.1		
Visual placing response	Day	18	100.0±0.0		
		21		100.0	100.0
Pain response	Day	18	100.0±0.0		
		21		100.0	100.0

Mean±S.D. Data represents percentage of positive findings

Table 4. Open field test findings of offspring

	Item		Crj:CD(SD)IGS	Crj:CD(SD)	Slc:SD
Male	1st Day		(22)	(10)	(21)
	Latency time	(sec.)	18.6±9.6	—	—
	Ambulations	(no.)	51.6±21.4	72.5±27.9	64.7±22.9
	Rearings	(no.)	13.0±6.9	12.4±5.7	15.3±7.9
	Grooming time	(sec.)	0.9±2.0	4.3±5.9	1.2±2.1
	Defecations	(no.)	1.4±2.1	2.0±1.5	2.0±1.7
	Urinations	(no.)	0.5±1.0		
	2nd Day				
	Latency time	(sec.)	12.9±6.2		
	Ambulations	(no.)	53.6±21.7		
	Rearings	(no.)	12.5±6.1	—	—
	Grooming time	(sec.)	3.5±4.5		
	Defecations	(no.)	2.1±2.1		
	Urinations	(no.)	0.8±0.9		
Female	1st Day		(22)	(10)	(21)
	Latency time	(sec.)	23.4±13.2	—	—
	Ambulations	(no.)	51.6±26.4	82.6±25.4	77.7±28.5
	Rearings	(no.)	13.5±8.5	14.0±4.4	18.6±6.8
	Grooming time	(sec.)	1.7±3.4	1.8±2.9	1.8±3.5
	Defecations	(no.)	1.4±1.4	0.8±1.2	0.3±0.7
	Urinations	(no.)	1.3±0.9		
	2nd Day				
	Latency time	(sec.)	21.0±13.8		
	Ambulations	(no.)	51.2±30.5		
	Rearings	(no.)	13.9±10.0	—	—
	Grooming time	(sec.)	5.3±10.8		
	Defecations	(no.)	1.5±1.5		
	Urinations	(no.)	0.9±1.2		

Mean±S.D.

Table 5 shows the results of the water T-maze test. The elapsed time to reach the goal of maze was 46.0 seconds for males on day 1, 22.7 seconds on day 2, and 19.2 seconds on day 3, and 45.0 seconds for females on day 1, 20.9 seconds on day 2 and 17.4 seconds on day 3. On the other hand, the mean daily errors were 5.3 for males on day 1, 1.3 on day 2, and 0.7 on day 3, and 5.2 for females on day 1, 1.4 on day 2, and 0.6 on day 3: both the elapsed time to the goal and the number of errors decreased with the repetition of trials in both males and females.

On autopsy at 10 weeks of age were found no abnormalities in either rats. Table 6 shows the mean organ weights. The mean absolute organ weights were such as 0.625 g of thymus, 1.538 of lungs, 1.350 g of heart, 19.105 g of liver, 0.814 g of spleen, 1.585 and 1.578g of kidney (right and left), 32.76 and 34.51mg of adrenal gland (right and left), 1.631 and 1.685g of testis (right and left), 0.488 and 0.462g of epididymis (right and left) for males, and 0.544 g of thymus, 1.169 g of lung, 0.939 g of heart, 11.097 g of liver, 0.621 g of spleen, 1.004 and 0.990g of kidney (right and left), 37.55 and 40.05mg of adrenal gland (right and left), 57.86 and 57.57 mg of ovary (right and left) and 0.454 g of uterus for females.

On the estrus cycle test, 3 of the 22 F_1 females were not found estrous.

Table 7 shows the results of reproductivity test. The copulation index was 90.5%, and fertility index, 84.2%. The 3 F_1 females that were not estrous on the estrus cycle test became estrous during the cohabitation period; and they copulated and became pregnant. The females that did not copulate were mated with males that had proved fertile copulated and became pregnant. The males that were not confirmed to have copulated or was fertile and those without mating females were mated with untreated females: all were found to have copulated, but one of them was infertile.

Table 8 shows the results of cesarean section of the F_1 dams. The mean number of corpora lutea was 17.8, and mean number of implantations was 17.1, with an implantation index of 96.0%. The postimplantation loss was 8.5%, and there were a mean of 15.6 live embryos available.

Table 5. Multiple T-maze test findings of offspring

Item				Crj:CD(SD)IGS		Crj:CD(SD)		Slc:SD	
Training day				Male(21)	Female(22)	Male(10)	Female(9)	Male(21)	Female(20)
	Trial 1	Time	(sec.)	18.7±10.2	18.0±8.1				
	2	Time	(sec.)	6.5±1.7	7.5±3.6				
	3	Time	(sec.)	5.8±2.3	5.5±1.5	—		—	
	4	Time	(sec.)	5.9±2.4	5.3±1.8				
	Total	Time	(sec.)	9.2±2.8	9.1±2.2				
1st day									
	Trial 1	Time	(sec.)	59.9±36.8	58.0±38.2	106.8±72.5	55.1±14.6	63.3±38.2	76.5±52.2
		Error	(no.)	8.0±3.9	8.2±7.0	12.3±9.0	7.3±3.3	8.4±5.4	10.9±8.2
	2	Time	(sec.)	49.7±27.7	58.4±50.0	62.4±32.4	49.1±19.8	61.1±38.0	57.7±33.8
		Error	(no.)	6.1±4.4	6.3±7.5	6.4±4.0	6.4±2.1	7.4±5.0	7.2±4.8
	3	Time	(sec.)	35.2±18.6	38.5±17.6	50.8±31.7	45.7±23.7	32.6±23.2	43.2±32.4
		Error	(no.)	3.5±3.2	4.5±3.6	4.9±2.5	5.3±4.1	2.9±3.5	4.9±5.3
	4	Time	(sec.)	39.3±32.7	25.3±14.2	36.8±31.1	34.6±18.5	30.6±18.0	25.0±16.6
		Error	(no.)	3.7±4.1	2.0±2.3	1.9±2.4	3.1±2.4	2.8±2.8	2.4±3.1
	Total	Time	(sec.)	46.0±13.4	45.0±15.7	—		—	
		Error	(no.)	5.3±1.8	5.2±2.9				
2nd day									
	Trial 1	Time	(sec.)	25.6±12.1	27.9±17.0	31.9±21.1	28.6±18.8	24.0±13.1	33.7±27.1
		Error	(no.)	1.8±2.2	3.2±3.6	2.4±2.5	3.1±3.9	2.6±2.9	4.5±5.3
	2	Time	(sec.)	25.1±11.8	19.1±8.6	24.6±12.3	37.6±23.4	17.6±9.1	18.4±6.5
		Error	(no.)	2.0±2.6	1.0±1.6	1.6±1.6	4.6±3.6	1.2±1.8	1.6±2.0
	3	Time	(sec.)	21.5±8.9	18.6±8.0	32.9±22.7	16.4±5.0	18.1±10.9	16.8±8.4
		Error	(no.)	1.0±1.5	1.0±1.4	2.2±3.4	0.4±0.7	1.4±3.3	1.3±1.9
	4	Time	(sec.)	18.5±5.6	18.0±7.2	27.9±11.8	17.1±9.5	19.4±11.7	14.8±7.1
		Error	(no.)	0.3±0.7	0.6±1.0	1.2±1.5	0.6±1.3	1.1±1.6	0.9±1.9
	Total	Time	(sec.)	22.7±6.8	20.9±7.7	—		—	
		Error	(no.)	1.3±1.0	1.4±1.4				
3rd day									
	Trial 1	Time	(sec.)	20.3±8.7	19.5±8.6				
		Error	(no.)	1.0±2.0	1.1±1.9				
	2	Time	(sec.)	18.9±8.5	19.7±11.7				
		Error	(no.)	0.6±1.4	0.7±1.2				
	3	Time	(sec.)	20.0±11.2	15.8±7.7	—		—	
		Error	(no.)	0.7±1.6	0.5±0.9				
	4	Time	(sec.)	17.4±4.9	14.8±4.8				
		Error	(no.)	0.4±1.0	0.2±0.4				
	Total	Time	(sec.)	19.2±5.9	17.4±6.1				
		Error	(no.)	0.7±1.1	0.6±0.7				

Mean±S.D.

Table 6. Organ weights of 10 weeks aged offspring

Item		Crj:CD(SD)IGS		Crj:CD(SD)	
No. of offspring		22	22	10	9
		Male	Female	Male	Female
Terminal Body Weight (g)		444.4±29.2	287.1±32.8	451.8±52.4	251.4±29.5
Thymus	(g)	0.625±0.081	0.544±0.119	0.56±0.124	0.40±0.047
	(g%)	0.141±0.016	0.189±0.033	0.123±0.022	0.161±0.018
Lung	(g)	1.538±0.124	1.169±0.131	1.31±0.135	1.02±0.078
	(g%)	0.347±0.030	0.409±0.040	0.290±0.026	0.406±0.028
Heart	(g)	1.350±0.126	0.939±0.092	1.32±0.147	0.85±0.093
	(g%)	0.304±0.024	0.328±0.023	0.293±0.014	0.342±0.036
Liver	(g)	19.105±2.213	11.097±1.757	18.82±2.961	9.36±1.401
	(g%)	4.291±0.288	3.851±0.247	4.160±0.364	3.712±0.223
Spleen	(g)	0.814±0.104	0.621±0.074	0.80±0.186	0.59±0.097
	(g%)	0.183±0.022	0.217±0.020	0.178±0.032	0.233±0.034
Kidney (R)	(g)	1.585±0.184	1.004±0.140	1.51±0.086	0.90±0.060
	(g%)	0.356±0.028	0.350±0.029	0.338±0.032	0.362±0.030
Kidney (L)	(g)	1.578±0.193	0.990±0.134	1.49±0.100	0.88±0.064
	(g%)	0.355±0.029	0.345±0.026	0.333±0.029	0.352±0.028
Adrenal (R)	(mg)	32.76±4.18	37.55±7.59	32.8±4.09	36.4±3.82
	(mg%)	7.37±0.77	13.19±2.92	7.3±1.1	14.6±1.9
Adrenal (L)	(mg)	34.51±4.71	40.05±9.38	34.3±4.34	39.2±3.25
	(mg%)	7.77±0.91	14.03±3.18	7.7±1.1	15.7±1.9
Testis (R)	(g)	1.631±0.129	—	1.61±0.081	—
	(g%)	0.368±0.029		0.358±0.030	
Testis (L)	(g)	1.685±0.233	—	1.61±0.094	—
	(g%)	0.380±0.053		0.359±0.030	
Epididymis (R)	(g)	0.488±0.049	—	0.49±0.038	—
	(g%)	0.110±0.013		0.108±0.011	
Epididymis (L)	(g)	0.462±0.037	—	0.47±0.055	—
	(g%)	0.104±0.009		0.104±0.010	
Ovary (R)	(mg)	—	57.86±16.14	—	51.5±8.54
	(mg%)		20.10±4.67		20.7±4.1
Ovary (L)	(mg)	—	57.57±9.36	—	47.0±7.61
	(mg%)		20.19±3.43		18.9±3.4
Uterus	(g)	—	0.454±0.125	—	0.45±0.114
	(g%)		0.160±0.050		0.183±0.056

Mean±S.D. Upper:Absolute weight
Lower:Relative weight

Table 7. Reproductive performance of F1 offspring

Item	Crj:CD(SD)IGS	Crj:CD(SD)	Slc:SD
No. of cohabitated pairs	21	10	21
No. of copulated pairs (%)	19(90.5)	10(100.0)	20(95.0)
No. of pregnant pairs (%)	16(84.2)	9(90.0)	20(100.0)

Table 8. Caesarean section data of Crj:CD(SD)IGS F1 offspring

Item	Crj:CD(SD)IGS
No. of dams examined	17
No. of corpora lutea	302
Mean±SD	17.8±1.7
No. of implantation sites	290
Mean±SD	17.7±1.9
Implantation rate(%) (Mean±SD)	96.0±5.9
Post implantation loss(%) (Mean±SD)	8.5±5.2
No. of dead or resorbed fetuses	24
Metrial gland	10
Placental remnant	4
Early	0
Late	0
Macerate	0
Dead	10
No. of live fetuses	266
Mean±SD	15.6±2.2

CONCLUSION AND DISCUSSION

Data became available from the tests on the delivery and lactation of dams, and afterbirth development of offspring of Crj:CD(SD)IGS rats in our laboratory. The pregnant dams were administered orally by gavage with 0.5% w/v HPMC which is in frequent use as a vehicle of hardly soluble drugs at our laboratory from day 7 of pregnancy till day 21 postpartum.

The reddish tears that were observed in the F_0 dams had also occasionally been empirically observed in the control group in toxicity study using Crj:CD(SD) rats at our laboratory. The F_0 dams that failed to do postpartum treatment and lactation behavior, resulting in total litter loss, have also been occasionally experienced with the control groups in toxicity study using Crj:CD(SD) rats or Slc:SD rats at our laboratory, and no difference was noted in the incidence of such findings.

No distinct difference was noted between Crj:CD(SD)IGS rats including the results of delivery and lactation of F_0 dams, those of physical differentiation and growth tests, sensory function tests, emotionality tests, organ weights, learning ability tests and reproductivity tests, and those of Crj:CD(SD) rats or Slc:SD rats in our laboratory.

Background Control Data of Reproductive and Developmental Toxicity Study in Crj:CD(SD)IGS Rats -1999.

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ABSTRACT. The background control data in reproductive and developmental toxicity study were investigated using Crj:CD(SD)IGS rats under the inhalation testing condition through last one year. These include body weights and food consumption of female rats for pregnant and lactation periods, fertility, reproductive and fetal parameters obtained by cesarean section, findings of fetal morphological observations, findings of delivery in dams and observations on pups, body weight of pups, findings of observations for behavioral and physical development in pups, and results of open field test and water-filled multiple T-maze test of F_1 rats. These background data will contribute to evaluate reproductive and developmental toxicity studies of chemical compounds in Crj:CD(SD)IGS rats.
Key words: CD(SD)IGS Rat, Development, Reproduction

CD(SD)IGS-1999: 190-195

INTRODUCTION

Crj:CD(SD)IGS rats are being recently used, therefore, adequate historical control data are very important. Because the inhalation toxicity studies have been mainly conducted in our laboratory, we investigated the background control data of reproductive and developmental toxicity study of rats kept in the inhalation chamber for definite periods, as the continuation of our previous paper [5].

MATERIALS AND METHODS

Nine-week-old female and 10-week-old male Crj:CD(SD)IGS rats were purchased from Charles River Japan, Atsugi. A total of 228 females and 228 males in 12 studies performed during 12-month (March 1998 - February 1999) were used in this study. Following quarantine for 1 week, they were kept in the inhalation chamber (1.06m^3). They were individually housed in the suspended stainless-steel wire-mesh cage except for mating and lactation periods. Animals allowed to deliver were removed from the inhalation chamber on day 20 of gestation, and animals were housed in aluminium cages with paper pulp tip as nesting material (ALPHA-dri., Shepherd Specialty Paper, INC., USA). Room temperature and humidity were maintained at 22 ± 2℃, 55 ± 10%, respectively, with a 12-hr light/ dark cycle (08:00-20:00/ 20:00-08:00). The chamber environment was maintained at 23 ± 2℃, 55 ± 10%, and 12 ± 1 times/h ventilation. Tap water and commercial pellet diet (CRF-1, γ -irradiated with 30 KGy, Oriental Yeast, Tokyo) were given *ad libitum*.

The animals were observed for clinical signs once a day. The females were weighed on days 0, 7, 14, 20 of gestation, and on days 0, 4, 7, 14, 21 of lactation. Food consumption was measured during days 0-7, 7-14, 14-20 of the gestation period, and during days 0-4, 4-7, 7-14 of the lactation period. All females were examined daily for estrous cycles using the vaginal smear method from 9 weeks of age. At 10 weeks of age, they were paired 1:1 basis with males of 11 weeks of age for a maximum of 4 days. By the presence of a vaginal plug or sperms in the vaginal smear, day 0 of gestation was determined. The copulation index, fertility index, and days until copulation were determined.

For the cesarean section, 10-13 pregnant females on each study were necropsied on day 20 of gestation. Embryos/fetuses were removed, the number of live fetuses and dead embryos were counted. The ovaries were examined for the number of corpora lutea, and the uterus for the number of implantations using a modified Salewski's methods [6]. The embryo/fetal mortality was determined based on the number of implantations. After measurement of fetal body weights and placental weights, fetuses were observed for sex and external abnormality. Two-third of live fetuses in each litter was assigned for preparation of skeletal specimens by Dawson's methods [2], skeletal abnormality and the number of ossified sacral-caudal vertebrae were examined. The remaining live fetuses were observed for visceral abnormality by Wilson's and Nishimura's methods [4,7]. Terms for these observations mostly quoted from a previous report [4].

Six pregnant females were allowed to deliver for examination of the abnormalities in delivery and the duration of gestation was determined. Pups were examined for external abnormalities on the day of birth (day 0 of lactation), and the birth index and live birth index on day 0 of lactation were determined based on the litter size (total number of live and dead newborn). The number of pups counted daily after birth to determined the viability index on day 4 of lactation and weaning index. On day 4 of lactation, the number of pups per litter was adjusted to 4 animals of each sex. Male and female pups were weighed separately on a litter basis on days 0, 4, 7, 14 and 21 of lactation. They were weighted individually once a week from weaning until the age of 10 weeks. During the lactation period, 2 males and 2 females selected from each litter were examined for surface righting reflex, cliff drop aversion response and negative geotaxis as indices of early behavioral ontogeny and for eruption of upper incisors, ear opening and eyelid opening as indices of physical development until completion of each development. After weaning an open field test and a multiple T-shaped water maze test were performed on 2 animals per sex per litter at the ages of 4-5 weeks and 5-6 weeks, respectively. The open field test was performed in a round field apparatus (75 cm diameter) to examine emotionality using an image analyzer (CompACT VAS, MUROMACHI KIKAI CO., LTD.) once daily (3 minutes) for 3 days. In the T-shaped water maze test, the animals were tested in Biel's [1] water maze box 3 times daily for 3 days and the time required for goal and the number of errors were measured by same analyzer as using by the open field test.

RESULTS AND CONCLUSION

Body weights and food consumption of dams are shown in Table 1 and 2, respectively. Through the examination of estrous cycles (at least 8 days), one or two estrus were observed in all female rats. Fertility in female rats is shown in Table 3. Reproductive and fetal parameters obtained by the cesarean sectioning are shown in Table 4. Morphological findings in fetuses are shown in Table 5. Most frequent skeletal variation was short supernumerary rib, and following dumbbell ossification of thoracic centrum. Most frequent visceral abnormality and variation were ventricular septum defect and thymic remnant in neck, respectively. No remarkable differences were observed in comparison with our previous data [5].

The natural delivery parameter and viability of pups are shown in Table 6, and body weight of pups in Table 7. No abnormal clinical signs were observed in all pups. Observations for behavioral and physical development of pups are shown in Table 8. The results of the open field test and the water-filled multiple T-maze test of F1 rats are shown in Table 9 and 10, respectively.

These data will contribute to evaluate the results in reproductive and developmental toxicity study in Crj:CD(SD)IGS rats.

Table 1. Body Weight of Dams

Gestational period			
No. of dams		212	
Body weight (g)			
	GD 0	248±6	(234-256)[a)]
	7	288±7	(271-297)
	14	325±9	(310-339)
	20	404±9	(388-415)
Lactational period			
No. of dams		72	
Body weight (g)			
	LD 0	305±13	(285-334)
	4	320±11	(303-346)
	7	326±12	(307-346)
	14	342±13	(324-359)
	21	322±12	(304-342)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)
GD: gestational day LD: lactational day

Table 2. Food Consumption of Dams

Gestational period		
No. of dams	212	
Food consumption (g/day)		
GD 0-7	24±1	(23-25) [a)]
7-14	25±1	(24-26)
14-20	27±1	(25-28)
Lactational period		
No. of dams	72	
Food consumption (g/day)		
LD 0-4	31±2	(27-34)
4-7	43±2	(40-46)
7-14	56±3	(51-60)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)
GD: gestational day LD: lactational day

Table 3. Fertility in Female Rats

No. of mated pairs	228	
Copulation index (%)	95.6±5.9	(84.2-100)[a)]
Fertility index (%)	97.3±4.3	(88.9-100)
Days until copulation	2.4±0.3	(1.9-2.8)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)
Copulation index (%)=(No. of animals copulated successfully/no. of mated animals)×100
Fertility index (%)=(No. of pregnant animals/no. of animals copulated successfully)×100

Table 4. Reproductive and Fetal Parameters obtained by Cesarean Section

No. of dams		140	
No. of corpora lutea		16.7±0.7	(15.6-17.5)[a)]
No. of implantation		15.4±0.6	(13.9-16.0)
Implantation rate (%)		94.1±2.3	(91.4-97.7)
Implantation loss (%)		5.4±2.9	(1.1-13.1)
No. of live fetuses	Male	7.3±0.6	(6.5-8.2)
	Female	7.3±0.7	(6.5-8.3)
	Total	14.6±0.6	(13.3-15.7)
Sex ratio		1.3±0.3	(0.9-2.0)
Fetal weight (g)	Male	3.89±0.09	(3.79-4.07)
	Female	3.65±0.14	(3.23-3.79)
Placental weight (g)	Male	0.50±0.02	(0.48-0.54)
	Female	0.48±0.02	(0.45-0.51)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)
Implantation rate (%)=(No. of corpora lutea/no. of implantations)×100
Implantation loss (%)=(No. of intrauterine death/no. of implantations)×100
Sex ratio=No. of male live fetuses/no. of female live fetuses

Table 5. Morphological Observations in Fetuses

	Number	%	(Min.-Max.)[b)]
External observation (1705) [a)]			
External abnormalities	5	0.29	(0-2.05)
Generalized edema	2	0.12	(0-0.54)
Absent eye bulge*	1	0.06	(0-0.54)
Anal atresia	1	0.06	(0-0.54)
Thread-like tail	1	0.06	(0-0.51)
Short tail	1	0.06	(0-0.51)
Absence of tail	1	0.06	(0-0.54)
Ectrodactyly	1	0.06	(0-0.51)
Skeletal observation (1175)			
Skeletal abnormalities	3	0.26	(0-1.01)
Absent thoracic centrum	1	0.09	(0-1.01)
Fused cervical arch	1	0.09	(0-0.79)
Fused rib	1	0.09	(0-0.74)
Skeletal valiations	179	15.23	(7.52-20.79)
Dumbbell ossification of thoracic centrum	37	3.15	(0.75-6.80)
Bipartite ossification of thoracic centrum	20	1.70	(0-4.04)
Short supernumerary rib	121	10.30	(1.56-14.91)
Cervical rib	11	0.94	(0-2.22)
Short rib	3	0.26	(0-2.36)
Incomplete ossification	1	0.09	(0-0.74)
No. of ossified sacral-caudal vertebrae	7.9±0.1[c)]		
Visceral observation (530)			
Visceral abnormalities	14	2.64	(0-5.08)
Atrial septum defect	2	0.38	(0-3.28)
Membranous ventricular septum defect	11	2.08	(0-4.08)
Microphthalmia	1	0.19	(0-1.69)
Small spleen	1	0.19	(0-1.69)
Right-sided aortic arch	1	0.19	(0-1.69)
Malpositioned subclavian branch	1	0.19	(0-1.69)
Situs inversus	1	0.19	(0-1.64)
Abnormal lung lobation	1	0.19	(0-1.64)
Persistent A-V canal	1	0.19	(0-1.64)
Visceral variations			
Persistent left umbilical artery	7	1.32	(0- 3.39)
Thymic remnant in neck	21	3.96	(0-10.17)
Dilated renal pelvis	2	0.38	(0-3.39)
Supernumerary coronary ostium	2	0.38	(0-2.22)
Small lung	1	0.19	(0-1.69)
Transposed azygos	1	0.19	(0-1.69)

( )a) No. of fetuses examined
( )b) Minumum and maximum values in incidence of 10 studies
c) Values represent mean±S.D.
* Microphthalmia in visceral abnormalities

Table 6. Delivery Parameter and Viability of Pups

No. of dams		72	
Gestation length (day)		22.1±0.3	(21.8-22.8)[a)]
No. of implantations		15.2±0.9	(13.8-17.0)
No. of pups delivered		14.2±1.0	(11.8-15.8)
No. of live pups		14.0±0.9	(11.8-15.2)
Birth index (%)		92.2±4.2	(83.8-96.6)
Sex ratio		1.2±0.3	(0.9-1.7)
Viability index (%)	at birth	98.6±3.2	(88.6-100)
	day 4	98.9±1.0	(96.7-100)
	day 21	99.6±0.8	(97.9-100)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)
Birth index (%)=(No. of live pups/no. of implantations)×100
Sex ratio=No. of male live fetuses/no. of female live fetuses

Table 7. Body Weight of Pups

Before weaning				
No. of dams	72			
Body weight (g)	Male		Female	
at birth	6.7±0.3	(6.3-7.4)[a)]	6.4±0.3	(6.1-6.9)
day 4	10.6±0.7	(9.1-11.6)	10.0±0.7	(8.7-10.9)
day 7	17.8±0.9	(16.6-19.1)	17.1±0.8	(15.8-18.3)
day 14	36.3±2.0	(33.1-39.7)	34.7±1.8	(31.5-37.2)
day 21	59.0±2.2	(54.8-61.9)	56.3±2.1	(52.4-59.9)
After weaning				
	Male		Female	
No. of pups	132		129	
Body weight (g)				
week 4	103.1±4.3	(95.6-109.3)	93.9±4.0	(89.2-100.7)
week 5	165.6±6.6	(153.9-172.0)	138.8±5.7	(127.0-147.4)
week 6	231.9±8.3	(217.2-245.7)	174.0±7.1	(157.3-183.8)
week 7	298.4±11.0	(278.4-313.2)	201.5±7.9	(183.9-211.9)
week 8	359.4±12.9	(336.3-376.9)	226.0±9.0	(205.7-237.0)
week 9	411.5±15.6	(386.9-432.7)	248.6±10.8	(223.7-260.2)
week 10	456.8±15.7	(432.6-476.5)	265.3±11.6	(241.3-275.5)

[a)] mean±S.D. (minimum and maximum values in average of 11 studies)
Body weights of pups before weaning were weighed separately on a litter basis

Table 8. Behavial and Physical Development of Pups

Behavial development (Day of achievment)				
	Male		Female	
No. of pups	144		144	
Surface righting	2.9±0.4	(2.0-3.4)[a)]	3.6±0.7	(2.5-4.7)
Cliff aversion	7.7±0.6	(6.8-8.4)	7.8±0.9	(6.5-9.3)
Negative geotaxis	11.2±0.9	(9.8-12.8)	11.2±1.1	(9.1-12.7)
Physical development (Day of achievment)				
	Male		Female	
No. of pups	144		144	
Incisor eruption	10.4±0.5	(9.6-11.2)	10.3±0.4	(9.7-10.8)
Ear opening	12.5±0.5	(11.9-13.4)	12.3±0.5	(11.6-13.3)
Eye opening	14.4±0.2	(13.9-14.8)	14.2±0.2	(13.8-14.5)

[a)] mean±S.D. (minimum and maximum values in average of 12 studies)

Table 9. Open Field Test of F_1 Rats

		Male		Female	
No. of rats		60		57	
Moving distance (cm)	D1	901.4±232.1	(558.3-1101.9)[a)]	1036.9±354.1	(630.6-1316.8)
	D2	999.5±249.4	(738.7-1321.3)	1157.0±286.3	(736.2-1419.2)
	D3	1124.0±240.9	(796.2-1354.4)	1354.1±362.3	(876.4-1704.6)
Moving time (sec)	D1	37.8±6.9	(26.8-43.1)	43.3±12.1	(29.5-54.9)
	D2	39.7±6.6	(32.3-47.4)	44.8±8.9	(33.1-54.4)
	D3	42.2±6.9	(32.0-48.6)	48.9±10.2	(36.2-58.8)
Turn	D1	261.7±55.6	(168.7-304.4)	270.4±67.8	(182.1-349.0)
	D2	244.8±65.4	(146.6-304.8)	249.6±53.4	(191.4-302.1)
	D3	253.9±64.6	(152.3-316.7)	259.3±69.9	(176.0-336.4)
Circling	D1	2.8±0.7	(2.0-3.8)	2.5±0.8	(1.2-3.1)
	D2	2.6±0.9	(1.3-3.7)	2.6±0.7	(1.5-3.1)
	D3	2.7±0.8	(2.0-4.0)	2.3±0.6	(1.7-3.4)
Defecation	D1	0.4±0.3	(0.0-0.8)	0.2±0.3	(0.0-0.6)
	D2	0.6±0.4	(0.2-1.1)	0.2±0.3	(0.0-0.8)
	D3	0.6±0.5	(0.0-1.3)	0.3±0.5	(0.0-1.1)
Urination	D1	0.1±0.2	(0.0-0.3)	0.0±0.1	(0.0-0.2)
	D2	0.1±0.1	(0.0-0.2)	0.0±0.0	(0.0-0.0)
	D3	0.0±0.1	(0.0-0.2)	0.0±0.0	(0.0-0.0)
Grooming	D1	0.2±0.2	(0.0-0.5)	0.1±0.1	(0.0-0.2)
	D2	0.1±0.1	(0.0-0.2)	0.3±0.2	(0.1-0.5)
	D3	0.2±0.2	(0.0-0.5)	0.4±0.4	(0.0-0.8)
Preening	D1	1.0±0.5	(0.4-1.4)	0.7±0.3	(0.4-1.1)
	D2	1.9±0.6	(0.3-2.5)	1.9±0.9	(0.8-3.3)
	D3	2.1±1.3	(1.0-4.1)	1.8±1.1	(0.5-3.4)
Rearing	D1	7.5±1.5	(5.4-9.2)	10.0±1.4	(7.8-11.3)
	D2	7.3±1.5	(5.2-8.8)	8.8±1.7	(7.0-10.6)
	D3	8.2±1.7	(6.4-11.0)	13.1±2.4	(10.7-16.7)

[a)] mean±S.D. (minimum and maximum values in average of 5 studies)

Table 10. Water-Filled Multiple T-Maze Test of F_1 Rats

			Male		Female	
No. of rats			60		57	
Error	D1	1st	8.6±1.4	(6.9-10.7)[a)]	8.4±3.1	(5.0-13.3)
		2nd	9.7±3.3	(6.7-14.9)	9.4±2.6	(5.3-12.5)
		3rd	7.2±2.4	(3.6-10.1)	7.1±2.8	(3.3-9.1)
	D2	1st	5.3±1.7	(3.0-7.6)	5.9±1.7	(3.3-7.8)
		2nd	4.7±1.3	(3.2-6.3)	4.2±1.7	(2.3-6.2)
		3rd	2.2±0.6	(1.3-2.7)	2.4±1.6	(0.9-5.0)
	D3	1st	1.7±0.5	(1.1-2.1)	2.6±1.0	(1.4-4.2)
		2nd	1.5±0.5	(1.0-2.2)	2.2±0.5	(1.5-2.7)
		3rd	1.6±0.3	(1.3-1.9)	1.6±0.3	(1.3-1.9)
Time (sec)	D1	1st	62.8±5.9	(53.4-67.8)	59.3±15.5	(46.3-86.1)
		2nd	71.2±12.8	(54.8-85.3)	67.1±20.5	(41.0-98.3)
		3rd	62.9±22.2	(33.0-93.6)	61.5±24.2	(31.5-89.1)
	D2	1st	50.2±12.9	(32.0-66.7)	50.1±11.1	(32.3-63.0)
		2nd	40.4±8.6	(32.6-54.3)	35.5±11.7	(22.4-48.7)
		3rd	29.0±6.7	(23.2-39.2)	26.5±12.0	(17.3-47.3)
	D3	1st	24.1±4.2	(20.2-30.7)	28.6±5.8	(23.6-38.0)
		2nd	19.0±1.3	(17.7-21.0)	23.9±3.7	(19.7-27.9)
		3rd	19.3±3.3	(17.0-24.9)	19.2±1.9	(16.4-21.2)

[a)] mean±S.D. (minimum and maximum values in average of 5 studies)

REFERENCES

1. Biel, W. C. 1960. Early age differences in maze performance in the albino rat. J. Genet. Psychol., 56: 439-453.
2. Dawson, A.B. 1926. A note on the staining of the skeleton of cleared specimens with alizarin red S. Stain Technol., 1: 123-124.
3. Horimoto, M. 1998. Terminology of developmental abnormalities in common laboratory mammals (Japanese version 1). Cong. Anom., 38: 153-237.
4. Nishimura, K. 1974. A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. Cong.Anom., 14: 23-40.
5. Okuda, H. 1998. Background control data of reproductive and developmental toxicity study in Crj:CD(SD)IGS rats. CD(SD)IGS-1998: 183-187.
6. Salewski, E. 1964. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedeberg's Arch.Exp.Pathol. Pharmakol., 247: 367.
7. Wilson, J.G. 1965. Methods for administering agents and detecting malformations in experimental animals. pp.262-277. In:Teratology Principles and Techniques(Wilson, J. G. and Warkany, J. eds.). Univ.of Chicago Press, Chicago, Illinois.

Evaluation for Fertility under Optimized Diet Restriction in Crj:CD(SD)IGS Male Rats

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ABSTRACT. This study was designed to compare the effects of *ad libitum* feeding and optimized measured feeding on body weight, reproductive performance, reproductive status of mated females, testicular weight and histology, sperm number and sperm motions in Crj:CD(SD)IGS male rats. Males were fed PMI Certified Rodent Chow #5002 *ad libitum* (AL group) or 22.0 g/day (DR group). At 14 weeks of age, all males were cohabited with females of the same strain until confirmation of mating for a maximum of 12 days. During this cohabitation period, all males had free access to the diet. The females were fed the same diet *ad libitum* throughout the study. The day on which mating was confirmed was designated as gestational day (GD) 0. Mated females were cesarean-sectioned on GD 15, and their reproductive status was recorded. Males were necropsied at 18 weeks of age, and the testes were weighed and fixed in Bouin's solution for microscopic examination. The left cauda epididymis was frozen for the subsequent sperm head counting and the left vas deferens was incubated in media for sperm motion analyzed by CellSoft 4000.

The average body weight gain during the entire study period in the DR group was 67% of that of the AL group, and was less variable than that of the AL group. The daily food consumption through the same period was 30.9 g/day and 21.9 g/day in AL and DR groups, respectively, showing that males of the DR group consumed 29% less feed than those in the AL group. Compared to the AL group, there were no significant differences in reproductive performance of males, reproductive status of mated females, testicular weight and microscopic findings, sperm number and sperm motions in the DR group.

In conclusion, the reproductive parameters collected under 22.0 g/day of diet optimization were comparable to those under *ad libitum* feeding, indicating that the present diet restriction regimen will be applicable to fertility studies in male rats. The data from this study can form the basis of historical controls for future male fertility studies under optimized diet restriction in Crj:CD(SD)IGS rats. —Key words: Crj:CD(SD)IGS rat, Diet restriction, Male fertility

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INTRODUCTION

Overfeeding by *ad libitum* food consumption is the most significant, uncontrolled variable affecting the outcome of the current rodent bioassay. The correlation of food consumption, the resultant adult body weight and the 2-year survival in Sprague-Dawley rats is highly significant. Optimized food restriction improved survival and delayed the onset of spontaneous degenerative disease and diet-related tumors [1]. Toxicokinetic studies of several compounds demonstrated steady-state systematic exposures that were equal or higher in rats under optimized diet restriction [2]. This study was conducted whether the optimized diet restriction regimen (22.0 g/day/male) is applicable to fertility studies in male rats. The study was also conducted to form the basis of historical controls for male fertility studies under optimized diet restriction in Crj:CD(SD)IGS rats.

MATERIALS AND METHODS

One hundred Crj:CD(SD)IGS male rats, approximately 4 weeks of age, purchased from Charles River Japan Inc., were kept in an animal room where the temperature (22 ± 2℃), the relative humidity (55±10%) and the light and dark cycle (12 hr each) were controlled. The males were divided into 2 groups [50 males/group: diet restriction (DR) group and *ad libitum* (AL) group]. After 1 week of the quarantine period, males in the DR group were fed 22 g/day of PMI Certified Rodent Chow #5002 from the pretest period through terminal necropsy except for the cohabitation period (Fig. 1). Males in the AL group were allowed to free access to the same diet throughout the study. If food remained in the DR group, the amount of remaining food was measured and the daily food consumption was calculated. Food consumption in the AL group was measured over 3-day or 4-day intervals throughout the study except for the cohabitation period, and was expressed as daily consumption.

After 5 weeks of the pretest period, all males were given 0.5% aqueous methylcellulose orally by gavage during the 4 weeks of pre-cohabitation, 12 days of cohabitation and approximately 2 weeks of post-cohabitation intervals. Body weights in males were recorded once a week during the pretest period, and twice a week thereafter until termination. All males were observed for physical signs during the treatment period, and checked for mortality during the rest of the study.

Each male was cohabited with one untreated female of the same strain at approximately 12 weeks of age until confirmation of mating for a maximum of 12 days. A daily check was made for copulatory plugs on the cage pan and/or in the vagina, and vaginal lavages were examined microscopically for the presence of sperm. The day on which plugs and/or sperm were found was designated as gestational day (GD) 0. Mated females were euthanized on GD 15, and the numbers of corpora lutea and implants were counted. Each conceptus was classified into live fetus, dead fetus or resorption.

All males were euthanized at approximately 18 weeks of age, and the thoracic and abdominal viscera including, testes and epididymides were grossly examined. The testes and right epididymis were fixed in Bouin's solution, dehydrated, embedded in paraffin, sectioned at 3 μm thickness, and stained with hematoxylin and eosin for subsequent histological examination. The left cauda epididymis was weighed and stored at -60℃ for later sperm head counting. Sperm motions were analyzed with the CellSoft-CASA™ (computer-assisted sperm motion analysis) 4000 series system (CRYO Resources, Ltd., NY, USA). Two approximately 0.5 cm sections of the left vas deferens were placed in 10 ml of warmed (37℃) Dulbecco's phosphate buffered saline with calcium and magnesium plus bovine serum albumin (10 mg/ml). The sperm preparation was incubated for 10 min at 37℃, after which a 25 μl aliquot was dropped on the warmed culture dish and a

coverslip was placed over the drop. Immediately thereafter, approximately 20 microscope fields were videotaped and analyzed later using CellSoft. The system settings were as follows: number of frames to analyze, 15; number of frames per second, 30; minimum sampling for motility, 3; minimum sampling for curvilinear velocity (VCL), 5; velocity range, 10 to 1100 μm/s; minimum number of points for amplitude of lateral head displacement (ALH), 7; minimum velocity for ALH, 20 μm/s; minimum linearity for ALH, 0; pixel scales, 3.4 μm/pixel, cell size range, 40 to 200 pixels.

For epididymal sperm head counting, the left cauda epididymis was thawed, minced and homogenized for 4 min in 10 ml of 0.9% NaCl solution containing 0.1% Triton X-100 (Sigma Chemical Co., MO, USA). The homogenate was allowed to settle at 4℃, diluted to 50 ml, and lightly stained with a few drops of 40% eosin solution. After agitation of the stained samples, an aliquot was immediately dropped into a hematocytometer, and homogenization-resistant sperm heads were counted [3].

For statistical analyses between AL and DR groups, Student's t-test or Wilcoxon rank sum test was applied for body weight gain, testicular weight, sperm number, sperm motion parameters and reproductive parameters of mated females. Non-parametric data such as copulation and fecundity indices were analyzed using a Chi-square test. Significant differences were determined at confidence level of $p<0.05$.

RESULTS AND DISSCUSSION

One male in the AL group was found dead on Pretest Day 33. The cause of death was uncertain because the animal did not show any abnormal signs up to the day before death and there were no gross lesions at necropsy. One male in the DR group was euthanized and discarded on Treatment Day 5 due to the fracture of bilateral upper incisors. No other deaths and abnormal physical signs were noted in the AL and DR groups during the study.

Average body weight changes in the AL and DR groups throughout the study are shown in Figure 2. There were significant decreases, compared to the AL group, in the average body weight gain of males in the DR group during the pretest, pre-cohabitation and post-cohabitation periods (Table 1). The average body weight gains in DR group were approximately 61%, 73% and 24% of those of the AL group for the pretest, cohabitation and post-cohabitation periods, respectively. During the cohabitation period in which males in the DR group had free access to the diet, the average body weight gain in the DR group were greater than the AL group (41% increase). Average food consumption calculated as one week interval during the pretest, pre-cohabitation and post-cohabitation periods is shown in Figure 2. The average food consumption for the first week of pretest period in the AL group was comparable to that in the DR group, but there were difference in the average food consumption thereafter. The food consumption in the DR group averaged for the pretest, pre-cohabitation and post-cohabitation periods was approximately 72%, 66% and 71% of the AL group, respectively.

Average testicular weights, epididymal sperm numbers and sperm motion parameters in the vas deferens are shown in Table 2. There were no significant differences in absolute testicular weight, sperm number and each sperm motion parameter between the AL and DR groups. Average of relative testicular weight in the DR group was significantly higher than that in the AL group since the final body weight of the AL group was 39% higher than that of the DR group. The group averages for absolute testicular weight were 3.54 and 3.51 g in these groups. The individual sperm numbers ranged from 574×10^6 to 975×10^6 and 636×10^6 to 987×10^6 in the AL and DR groups, respectively, expressed as sperm numbers per gram cauda epididymis. The percent of motile sperm individually ranged from 77.4% to 93.1% and 78.2% to 91.2%, in the AR and DR groups, respectively.

There were no gross lesions in the thoracic and abnormal viscera of males except for one male in the AL group with bilateral testicular atrophy. Histopathologically (Table 3), the male showed moderate seminiferous epithelial degeneration that was associated with a markedly decreased number of sperm in the epididymal tubules. Another male in the DR group showed very slight degeneration in the testis similar to that of the above-mentioned AL animal without decrement of the sperm number in the epididymis. Based on the similarity of the findings between the AL and DR groups, the low incidence of this change is not considered to suggest a biologically significant difference. There was very slight focal cellular infiltration in the perivascular and/or peritubular regions in the epididymides of 21 (43%) and 17 (35%) males in the AL and DR groups, respectively. Each focus was randamly distributed in the epididymis. Based on the identical findings and the distribution between groups, the slight difference in the incidence between groups is not considered to suggest biological significance. The exact cause of the high incidence of focal cellular infiltration in the epididymis is uncertain.

There were no significant differences between the AL and DR groups in reproductive performance of males and reproductive status of mated females (Table 4). The copulation index (%, mated females/females cohabited) was 100% in each group. Fecundity index (%, pregnant females/mated females) was 94% and 92% in the AL and DR groups, respectively. The group averages for the number of corpora lutea, % preimplantation loss, number of implants, % postimplantation loss and number of live fetuses were 15.4 and 15.2, 5.5% and 6.1%, 15.0 and 14.5, 7.3% and 5.6%, and 13.9 and 13.7 in the AL and DR groups, respectively.

In conclusion, there were comparable male reproductive findings between the AL and DR feedings, indicating that the present DR regimen will be applicable to male fertility studies in rats. The data from this study can form the basis of historical controls for future male fertility studies under optimized diet restriction in Crj:CD(SD)IGS rats.

REFERENCES

1. Keenan, K. P., Ballam, G. C., Dixit, R., Soper, K. A., Laroque, P., Mattoson, E. A., Adams, S. P., and Coleman, J. B. 1997: The effects of diet, overfeeding and moderate dietary restriction on Sprague-Dawley rat survival, disease and toxicology. *J. Nutr.* 127: 851S-856S.
2. Dixit, R., Keenan, K. P., Patrick, D., Huber, A. C., Robyak, K., and Zacchei, A. 1995: Effects of moderate dietary restriction (MDR) on toxicity and toxicokinetic (TK) profiles of non-genotoxic pharmaceuticals in rats. *Fund. Appl. Toxicol.* 15: 32.
3. Meistrich, M. L. 1989: Evaluation of reproductive toxicity by testicular sperm head counts. *J. Amer. Coll. Tox.* 8: 551-567.

Table 1. Average body weight gains (g) of males during the pretest, pre-cohabitation, cohabitation and post-cohabitation periods.

Groups	*Ad libitum*	Diet restriction
Number of males examined	49	49
Pretest period	305±28[a]	187±15*
Pre-cohabitation period	94±16	69±11*
Cohabitation period	29±8	41±11*
Post-cohabitation period	29±10	7±6*

[a] Values are mean ± SD.
*Significant difference (P≤0.05) between *ad libitum* and diet restriction groups.

Table 2. Testicular weight, sperm number and sperm motion parameters of males.

Groups	*Ad libitum*	Diet restriction
Number of males examined	49	49
Testicular weight		
Absolute weight (g)	3.54±0.46[a]	3.51±0.27
Relative weight (%, BW)	0.60±0.09	0.81±0.07*
Sperm number ($\times 10^6$)		
/g cauda epididymis	804±80	824±82
Sperm motions		
% motile sperm	86.6±2.7	87.1±3.2
Curvilinear velocity (μm)	448.5±34.0	448.4±31.7
Linearity	2.9±0.2	3.0±0.2
ALH[b] (max, μm)	32.3±3.0	32.1±3.8
ALH[b] (mean, μm)	24.7±2.6	24.6±2.9
BCF[c] (Hz)	7.6±0.4	7.7±0.4

[a] Values are mean ± SD.
[b] Amplitude of lateral head displacement
[c] Beat cross frequency
*Significant difference (P≤0.05) between *ad libitum* and diet restriction groups.

Table 3. Histopathological findings in the testis and epididymis of males.

Groups	*Ad libitum*	Diet restriction
Number of males examined	49	49
Testis		
Not remarkable change	48[a]	48
Seminiferous tubule, degeneration	1	1
Epididymis		
Not remarkable change	27	32
Focal perivascular cellular infiltration	15	16
Focal peritubular cellular infiltration	8	2
Decreased number of sperms	4	0

[a] Number of males observed.

Table 4. Reproductive performance of males and reproductive status of untreated female mated with males.

Groups	*Ad libitum*	Diet restriction
Number of females cohabited	49	49
Number of females mated	49	49
Number of pregnant females	46	45
Copulation index (%)[a]	100	100
Fecundity index (%)[b]	94	92
Number of corpora lutea/litter	15.4±1.7[c]	15.2±2.0
Preimplantation loss (%)	5.5±3.5	6.1±10.0
Number of implants/litter	15.0±2.5	14.5±2.1
Postimplantation loss (%)	7.3±8.5	5.6±7.9
Number of live fetuses/litter	13.9±2.6	13.7±2.2

[a] (No. females mated/no. females cohabited with males)×100
[b] (No. pregnant females/no. females mated with males)×100
[c] Values are mean±SD.

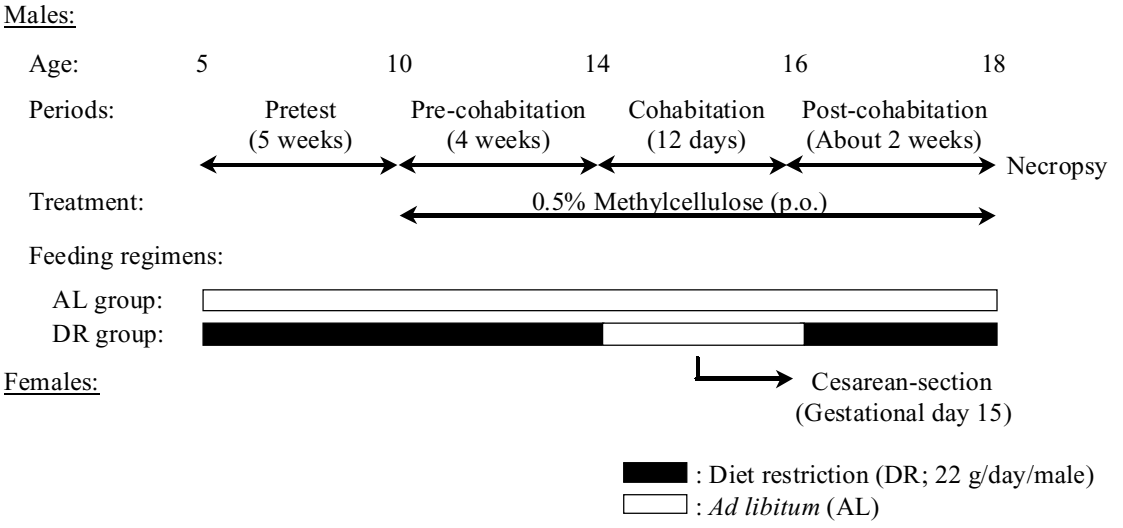

Fig 1. Experimental design

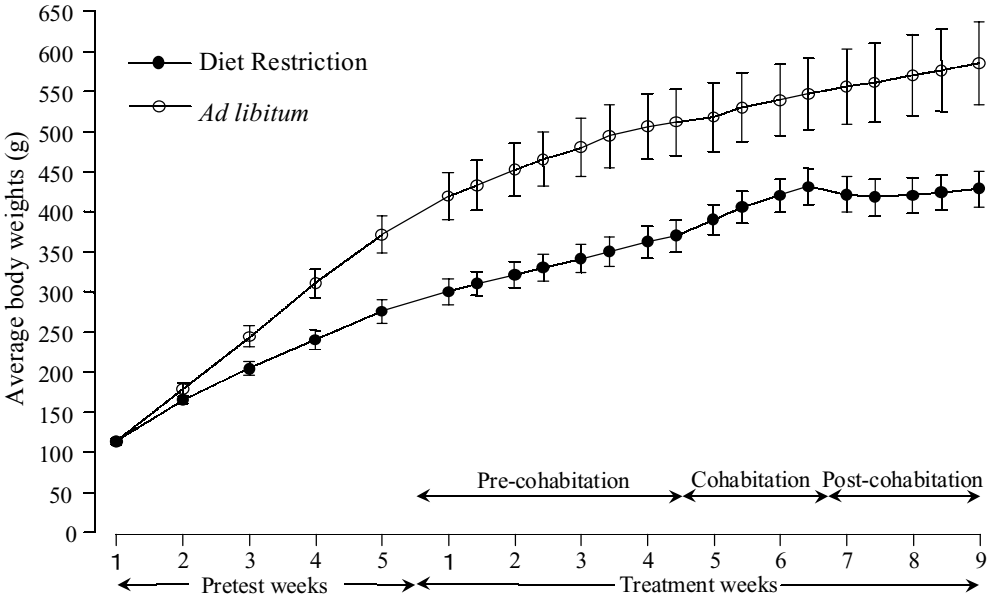

Fig 2. Body weight changes (mean ± SD) of males

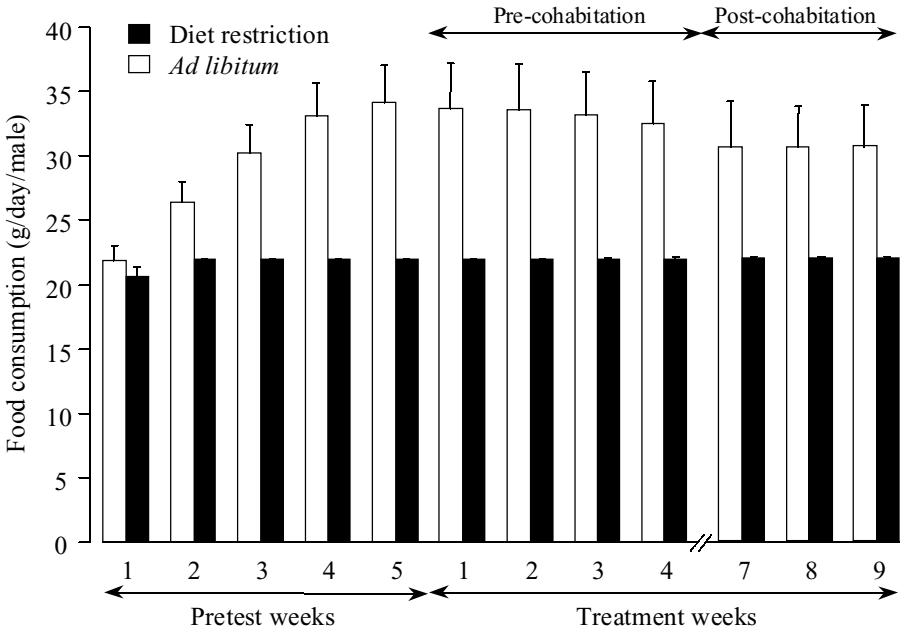

Fig 3. Food consumption (mean ± SD) of males

Sperm Abnormalities and Histopathological Changes in The Testes in Crj:CD(SD)IGS Rats

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ABSTRACT. In this study, morphological examination and computer-assisted sperm analysis (CASA) of epididymal spermatozoa in non-treated Crj:CD(SD)IGS rats were performed, and the relationship between the data obtained and the retention of step 19 spermatids in Stage IX to XI seminiferous tubules was examined.

Retention of step 19 spermatids in Stage IX to XI seminiferous tubules was observed in all 50 untreated males, and the incidence ranged from 3.3% to 100%. Eighteen animals showed a high incidence of retention (74.7 ± 14.2%, HIR for short), and the others showed a low incidence (24.9 ± 11.0%, LIR for short). Although the incidence of retention in Stage X and XI seminiferous tubules was very low in LIR males, it was high in HIR males (1.8 ± 3.0% vs 58.6 ± 23.2%). Morphological abnormalities of sperms in the caudal region of the epididymis, mainly amorphous head and no head, were more frequently observed in HIR males than in LIR males (36.2 ± 28.5% vs 1.8 ± 1.2%). Sperm analysis also revealed some differences between HIR and LIR males: sperm motility in HIR males was severely lower than that in LIR males, and sperm velocity, beat/cross frequency and amplitude of lateral head displacement in HIR males were lower than the corresponding values in LIR males.

In summation, retention of step 19 spermatids frequently occurred in the non-treated Crj:CD(SD)IGS males, and a relationship between the retention of these spermatids and sperm abnormalities, such as morphologically abnormal sperms, low motility and other items revealed by sperm analysis (CASA), was suggested. − Key words: Retention of step 19 spermatids, Sperm morphological abnormalities, Sperm analysis by CASA

CD(SD)IGS-1999: 201-206

INTRODUCTION

Sprague-Dawley rats have been widely used in toxicological studies. However, intrastrain differences in fetal weight and body weight gain after birth have arisen because this strain has been used for such a long time, and high mortality within 2 years has also been noted. An International Gold Standard system has therefore been proposed by the Charles River groups, and Crj:CD(SD)IGS rats have been named as the strain to be used around the world.

Recently, analysis of sperm characteristics such as sperm count, sperm motility and sperm morphology has been recommended as an optional procedure for confirmation or characterization of the effects of compounds on male fertility, and histopathologic examination of the testes and epididymides has also been recommended on case-by-case basis (ICH guideline).

In the present study, to collect historical data on sperm characteristics and histopathology of the testes and to investigate the relationship between sperm abnormalities and histopathologic changes in the testes of Crj:CD(SD)IGS rats, morphological features of sperms collected from the caput, corpus and caudal areas of the epididymides were examined microscopically; a sperm motility analysis was performed by computer-assisted sperm analysis (CASA); and the testes were examined histopathologically.

MATERIALS AND METHODS

Animals: Fifty sexually mature (18-week-old) Crj:CD(SD)IGS male rats (Charles River Japan, Inc.) were used in this study. They were acclimatized to the environmental conditions for about 6 weeks. Animals were housed individually in metal cages in a clean booth which was placed in an animal room with a temperature of 20-26℃, relative humidity of 40-70%, air exchange about 10 times/hr and a 12-hr light/dark cycle. The animals were allowed free access to tap water and a laboratory animal diet (CRF-LPF, Oriental Yeast Co. Ltd. γ-ray irradiated). The animals weighed 474-628 g at necropsy.

Morphological examination of sperms: All males were weighed and were then exsanguinated under ether anesthesia. Their reproductive organs were examined macroscopically. The testes and epididymides were weighed. After weighing, the right epididymis was cut to divide the caput, corpus and caudal regions, and the 3 regions were placed in Petri dishes containing 5 or 10 ml of medium (37℃, M-199 containing 0.5% BSA) and cut with scissors to diffuse the sperms. Sperms were smeared on a slide glass, and sperm smear samples were fixed in 10% neutral buffered formalin and were stained with eosin. Four hundred sperms in each sample were examined microscopically for the following morphological abnormalities: 1) no head, 2) no hook, 3) amorphous head, 4) flection at the cervical region and 5) others.

Sperm analysis: Before the cutting of the right epididymis, the caudal region was pricked with the tip of a surgical knife, and a small amount of the sperm fluid which leaked out was collected using a glass rod. The rod with the sperm fluid was carefully put in medium to diffuse the sperms. These sperm suspensions were examined microscopically, and the movements of the sperms were recorded on video tape. Two hundred or more sperms per each suspension were analyzed by CellSoft-4000 to obtain data on sperm motility, curvilinear velocity, straight line velocity, linearity, beat/cross frequency and amplitude of lateral head displacement (ALH for short).

Histopathologic examination of the testes: The right testes from all males were fixed in Bouin's solution for a few days and then were fixed in 10% neutral buffered formalin. They were embedded in paraffin, sectioned and stained with periodic acid-schiff (PAS). The number of seminiferous tubules which showed retention of step 19 spermatids was counted in about 30 Stage IX to XI seminiferous tubules in each male.

Statistical analysis: The data were tested by the F-test for homogeneity of variance. If the variances were homogeneous, Student's t-test was applied. If the variances were heterogeneous, Aspin-Welch's t-test was applied to compare the LIR (low incidence of retention of step 19 spermatids) group and the HIR (high incidence of retention of step 19 spermatids) group. Both t-tests

Note: This paper is reprinted from original paper (J. of Toxicological Sciences; 24(1), 63-68, 1999)

were conducted at the two-tailed significance levels of 0.05 and 0.01, and the F-test was conducted at the significance level of 0.2.

RESULTS

Sexually mature Crj:CD(SD)IGS males were examined for variations in sperm morphology and motion. Table 1 shows body weight and the testis and epididymis weights. The mean testis weight was 1.72 g for both the right and left side, but the ranges were wide (1.22 to 2.14 g for the right testis and 1.06 to 2.07 g for the left testis). The mean testis weight in the undermentioned HIR group (1.69 g for the right side and 1.71 g for the left side) was comparable to that in the LIR group (1.74 g for both the right and left sides). A similar wide deviation was observed with the epididymides. However, there was no relationship between these deviations and the results of histopathologic analysis of the testes or sperm examination described below.

Figures 1, 3 and 5 depict normal Stage Ⅸ, Ⅹ and Ⅺ seminiferous tubules, respectively. In these tubules, step 19 mature spermatids had been released. Figures 2, 4 and 6 depict abnormal Stage Ⅸ, Ⅹ and Ⅺ seminiferous tubules, respectively. In these tubules, retention of step 19 spermatids was observed. This abnormal finding was frequently observed in Stage Ⅸ seminiferous tubules in this strain but not in Stage Ⅹ or Ⅺ seminiferous tubules.

Table 1. Body weight and the testis and epididymis weights in untreated Crj:CD(SD)IGS males

		Body weight (g)	Testis weight (g)		Epididymis weight (mg)	
			Right	Left	Right	Left
Mean		559	1.72	1.72	595	586
S.D.		31	0.16	0.17	83	59
Range	Max	628	2.14	2.07	794	699
	Minimum	474	1.22	1.06	403	377

Table 2 shows the incidence of retention of step 19 spermatids in Stage Ⅸ, Ⅹ and Ⅺ seminiferous tubules of which approximately 30 were examined for each male. Data from 1 of the 50 males were excluded from this table because this male showed atrophy in almost all seminiferous tubules. The remaining 49 males were divided into two groups according to the incidence of retention of step 19 spermatids in Stage Ⅹ and Ⅺ seminiferous tubules; 32 males showed an incidence of less than 10% and were allocated to the low incidence of retention (LIR for short) group, and the other 17 males, which showed an incidence of 15% or more, were allocated to the high incidence of retention (HIR for short) group. In the LIR group, retention was frequently observed in Stage Ⅸ seminiferous tubules (mean: 60.4%, range: 8.3 to 100%); however, the mean incidence was 3.3% and 0% in Stage Ⅹ and Stage Ⅺ tubules, respectively. On the other hand, all Stage Ⅸ seminiferous tubules showed retention of step 19 spermatids in 12 of the 17 males in the HIR group, and the mean incidence in this group was higher than that in the LIR group (94.6% vs 60.4%). Moreover, the incidence of retention in Stage Ⅹ and Ⅺ seminiferous tubules in the HIR group was severely high when compared with that in the LIR group (58.6% vs 1.8%).

Table 2. Incidence of retention of step 19 spermatids in Stage Ⅸ-Ⅺ seminiferous tubules in untreated Crj:CD(SD)IGS males

	No. of males	Total incidence (%)	Stage Ⅸ (%)	Stage Ⅹ＋Ⅺ (%)
LIR group	32	24.9±11.0	60.4±24.2	1.8±3.0
HIR group	17	74.7±14.2 **	94.6±9.8 **	58.6±23.2 **

LIR: Low incidence of retention of step 19 spermatids
HIR: High incidence of retention of step 19 spermatids
**: Significantly different from the LIR group value ($p<0.01$)
Data are expressed as mean±S.D.

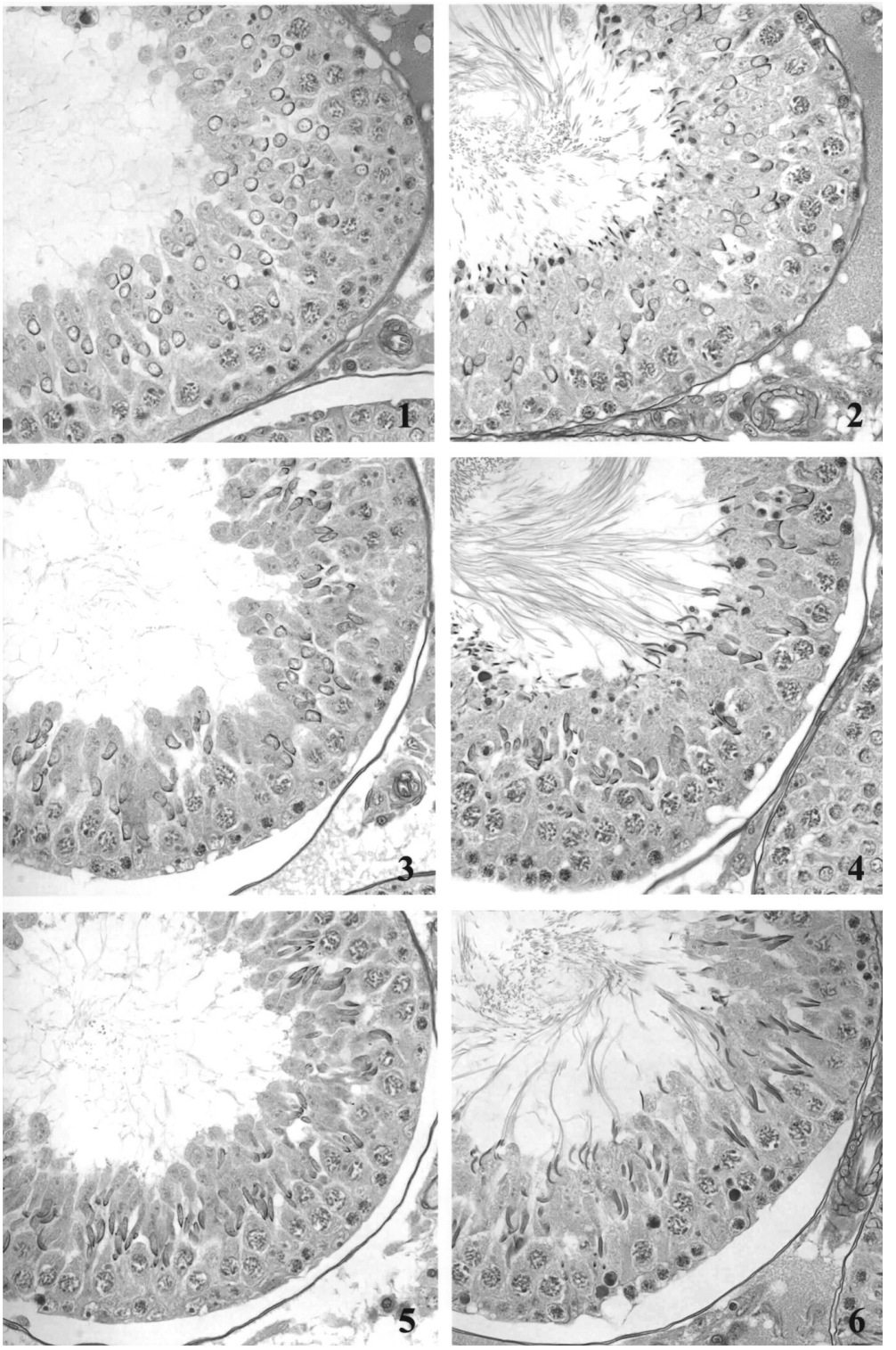

Photo 1 ～ 6. Histopathology of seminiferous tubules in Crj:CD(SD)IGS males. Photos 1 and 2 show Stage Ⅸ of seminiferous tubules, Photo 3 and 4 Stage Ⅹ, and Photo 5 and 6 Stage Ⅺ, respectively. Photo 1, 3 and 5 show normal features and Photo 2, 4 and 6 show retention of step 19 spermatids in Stages Ⅸ, Ⅹ and Ⅺ seminiferous tubules, respectively.

Table 3 showes morphological examination data of sperms from the caput, corpus and caudal regions of the epididymides. The HIR group contained 1 male which showed atrophy of almost all seminiferous tubules. The frequency of total abnormalities in the HIR group was high in all 3 regions when compared with that in the LIR group (11.1% vs 2.2% in caput region, 10.9% vs 1.7% in corpus region and 36.2% vs 1.8% in caudal region). The main type of abnormalities was sperms with an amorphous head in the caput and corpus regions and sperms with no head in the caudal region. Sperms with tail abnormalities were not seen in any region.

Table 3. Morphological abnormalities of sperms from the caput, corpus and caudal regions of the epididymides in Crj:CD(SD)IGS males

		Total abnormalities (%)	No head (%)	No hook (%)	Amorphous (%)	Flection (%)
Caput	LIR	2.2±1.3	0.6±0.4	0.3±0.3	1.2±1.0	0.1±0.2
	HIR	11.1±9.4**	2.6±2.5	1.1±1.4	6.8±6.2	0.6±1.0
Corpus	LIR	1.7±1.0	0.5±0.4	0.2±0.3	0.9±0.6	0.1±0.2
	HIR	10.9±11.0**	3.9±5.0	0.8±1.0	5.5±5.2	0.7±1.0
Caudal	LIR	1.8±1.2	1.0±0.9	0.2±0.3	0.6±0.5	0.0±0.1
	HIR	36.2±28.5**	32.7±27.1	0.4±0.4	2.8±2.5	0.3±0.5

LIR: Low incidence of retention of step 19 spermatids
HIR: High incidence of retention of step 19 spermatids
**: Significantly different from the LIR group value ($p<0.01$)
Data are expressed as mean±S.D.

Table 4 shows data from the computer-assisted sperm analysis (CASA) using sperm suspensions collected from the caudal region of the epididymides. Sperm motility in the HIR group was lower than that in the LIR group (40.6% vs 83.9%). Straight-line velocity in the HIR group was 83 μm/s and was also low when compared with the value in the LIR group (101 μm/s). Beat/cross frequency and ALH (mean and max.) values were slightly low in the HIR group. There were no significant differences in curvilinear velocity or linearity values between the HIR group and the LIR group.

Table 4. Characteristics of sperms from the caudal region of the epididymis in Crj:CD(SD)IGS rats

Item	LIR group	HIR group
Motility (%)	83.9±4.4	40.6±27.0**
Curvilinear velocity (μm/s)	409±37	376±103
Straight line velocity (μm/s)	101±11	83±27*
Linearity	2.6±0.2	2.5±0.6
Beat/Cross frequency (Hz)	10.9±1.8	9.3±2.1**
ALH mean (μm)	24.4±2.7	20.3±6.2*
ALH max (μm)	29.7±2.8	24.9±8.2*

LIR: Low incidence of retention of step 19 spermatids
HIR: High incidence of retention of step 19 spermatids
ALH: Amplitude of lateral head displacement
*, **: Significantly different from the LIR group value ($p<0.05$, $p<0.01$)
Data are expressed as mean±S.D.

DISCUSSION

When male rats are treated with testicular toxicants, necrotic changes in germ cells or exfoliation of these cells into the tubular lumen occurs, and prolonged dosing will almost invariably result in severe germ cell loss and atrophy of the seminiferous tubules [1-3]. The retention of step 19 spermatids in Stage IX to XI seminiferous tubules also occurs when the testes are only mildly affected by treatment with boric acid [4-7], bromoacetic acids [8], sodium dichloroacetate [9] and cadmium [10]. Although the cause of spermatic retention has not been determined, hormonal changes are considered to affect the function of Sertoli cells. Some compounds such as reserpine [11], nefiracetam [12] and bromoacetic acid [13] have been reported to affect production/secretion of testosterone and to induce retention of step 19 spermatids in Stage IX to XI seminiferous tubules in rats. In the present study, 17 out of 50 males (HIR rats) showed severe retention of step 19 spermatids in Stage IX to XI seminiferous tubules, but these males were not treated with any testicular toxicant. Lee, K.-P., *et al.* [14] reported an increased incidence in spermatid retention in control animals used for inhalation toxicity studies when compared with control animals used for oral toxicity studies. The reason for this change has been considered to be the stress associated with immobilization in the restrainer used for nose-only exposure conditions. Lee and his colleagues used Crl:CD^{R}/BR rats, and we used Crj:CD(SD)IGS rats. Since these two strains are genetically similar, the retention of step 19 spermatids observed in the HIR males in this study may be related to hormonal changes caused by some type of stress associated with animal husbandry. However, further investigation will be needed to clarify the exact reason for this change.

Toth, G.P., *et al.* [9] reported that in rats treated with sodium dichloroacetate inhibited spermiation was observed upon histopathological examination of the testes, and sperms with loose heads were also observed upon epididymal sperm examination. Similar changes such as retention of step 19 spermatids and morphological abnormalities of epididymal sperms were seen in rats treated with boric acid [4], dibromoacetic acid [8], methoxychlor [13] and dinitrobenzene [15]. In this study, sperm head abnormalities, amorphous head and no head, were observed in the caput, corpus and caudal regions of the epididymides, and retention of spermatids was also observed in the HIR rats. Therefore, sperm head abnormalities are considered to be closely related to retention of step 19 spermatids in Stage IX to XI seminiferous tubules because release of mature spermatids is a function of the Sertoli cells mediated by specialized cell-to-cell junctions between these cells and the spermatids.

Decreases in motility, straight-line velocity, beat/cross frequency and ALH values were noted in the computer-assisted sperm analysis results in the HIR rats in this study. Similar results in rats treated with sodium dichloroacetate [9] and dibromoacetic acid [8] have been reported, and morphological sperm head abnormalities were also observed in these rats.

However, we reported that these changes in histopathology of the testes, sperm morphology and sperm analyses using the CASA system were not observed in untreated Jcl:Wistar or F344/DuCrj rats [16].

In summation, failure of sperm release in Stage IX seminiferous tubules can easily occur in Crj:CD(SD)IGS rats, and sperm morphological abnormalities and decreases in sperm motility and velocity are considered to be associated with failure of mature sperm release from the Sertoli cells.

REFERENCES

1. Jezek, D., Simunic-Banek, L. and Pezerovic-Panijan, R. 1993: Effects of high doses of testosterone propionate and testosterone enanthate on rat seminiferous tubules - a stereological and cytological study. Arch. Toxicol., 67, 131-140
2. Creasy, D.M. 1997: Evaluation of testicular toxicity in safety evaluation studies: The appropriate use of spermatogenic staging. Toxicol. Pathol., 25, 119-131
3. Hess, R.A., Moore, B.J., Forrer, J., Linder, R.E. and Abuel-Atta, A.A. 1991: The fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules. Fund. Appl. Toxicol., 17, 733-745
4. Linder, R.E., Strader, L.F. and Rehnberg, G.L. 1990: Effect of acute exposure to boric acid on the male reproductive system of the rat. J. Toxicol. Environ. Health, 31, 133-146
5. Ku, W.W., Chapin, R.E., Wine, R.N. and Gladen, B.C. 1993: Testicular toxicity of boric acid (BA): relationship of dose to lesion development and recovery in the F344 rat. Reprod. Toxicol., 7, 305-319
6. Chapin, R.E. and Ku, W.W. 1994: The reproductive toxicity of boric acid. Environ. Health Perspect., 102 (Suppl 7), 87-91
7. Fail, P.A., Chapin, R.E., Price, C.J. and Heindel, J.J. 1998: General, reproductive, developmental, and endocrine toxicity of boronated compounds. Reprod. Toxicol., 12, 1-18
8. Linder, R.E., Klinefelter, G.R., Strader, L.F., Suarez, J.D. and Dyer, C.J. 1994: Acute spermatogenic effects of bromoacetic acids. Fund. Appl. Toxicol., 22, 422-430
9. Toth, G.P., Kelty, K.C., George, E.L., Read, E.J. and Smith M.K. 1992: Adverse male reproductive effects following subchronic exposure of rats to sodium dichloroacetate. Fund. Appl. Toxicol., 19, 57-63
10. Hew, K-W., Ericson, W.A. and Welsh, M.J. 1993: A single low cadmium dose causes failure of spermiation in the rat. Toxicol. Appl. Pharmacol., 121, 15-21
11. Kishimoto, K., Fukuyado, T., Sawamoto, O. and Kurisu, K. 1995: Influence of daily subcutaneous administration of reserpine for 4 weeks or 9 weeks before mating on testis, sperm and male fertility in rats. J. Toxicol. Sci., 20, 367-374
12. Harada, S., Sakaguchi, Y., Shimada, M., Matsuhashi, K., Kakihata, K., Nomura, M. and Takayama, S. 1995: Male reproductive Toxicity study of nefiracetam in rats. J. Toxicol. Sci., 20, 309-317
13. Linder, R.E., Strader, L.F., Slott, V.L. and Suarez, J. D. 1992: Endpoints of spermatotoxicity in the rat after short

duration exposures to fourteen reproductive toxicants. Reprod. Toxicol., 6, 491-505

14. Lee, K.P., Frame, S.R., Sykes, G.P. and Valentine, R. 1993: Testicular degeneration and spermatid retention in young male rats. Toxicol. Pathol., 21, 292-302
15. Hess, R.A., Linder, R.E., Strader, L.F. and Per-reault, S.D. 1988: Acute effects and long-term sequelae of 1,3-dinitrobenzene on male reproduction in the rat. II. Quantitative and qualitative histopathology of the testis. J. Androl., 9, 327-342
16. Matsumoto, K., Matsumoto, S., Samejima, K. and Ooshima, Y. 1998: Strain differences in sperm characteristics in Crj:CD(SD)IGS, Jcl: Wistar and F344/DuCrj rats. J. Toxicol. Sci., 23, 292

Background Control Data of Litter Parameters and Fetal Observations on Crj:CD(SD)IGS Rats

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ABSTRACT. We present background data of Crj:CD(SD)IGS rats from the control groups of five reproductive and developmental toxicity studies performed in our laboratory during 1996-1998.

The numbers of corpora lutea, implantations and live fetuses were 15.5-17.9, 13.6-15.3 and 13.3-14.6 respectively. Rate of Pre-and post-implantation losses were 3.57-12.35% and 2.75-9.22% respectively. Sex ratio were 0.82-1.34, fetal body weight in males were 3.48-3.94g, in females were 3.33-3.71g. Frequencies of external, visceral and skeletal abnormalities were 0%, 0-1.4%, and 0-4.2% respectively. Frequencies of visceral and skeletal variations were 0-1.8%, and 6.8-16.1% respectively. The number of ossified sacral and caudal vertebrae were 7.8-8.3. — Key words: Crj:CD(SD)IGS rats, reproductive and developmental toxicity study.

CD(SD)IGS-1999: 207-209

INTRODUCTION

The international genetic standard system, a new animal breeding system, has been developed by Charles River Inc. for supplying uniform experimental animals with minimizing the genetic variations.

The Crj:CD(SD)IGS rat (IGS rats) was developed by this system. However, background data on IGS rats have yet been fully accumulated. It is important to accumulate background data for accurate study evaluation. Therefore, we present historical control data of litter parameters and fetal observations on IGS rats performed in our laboratory during 1996-1998.

MATERIALS AND METHODS

Virgin female IGS rats and supplier mated IGS rats were obtained from Charles River Japan Inc (Table 1). Virgin female IGS rats were mated overnight with IGS male rats on one-to-one bases. The day on which sperm in the vaginal smear were observed was considered as day 0 of gestation.

The animals were housed in an air-conditioned animal room which maintained the temperature at 20-26℃, relative humidity at 30-70%, air ventilation at 10 times per hour or more, and a 12-hour light cycle.

The animals were housed individually in plastic cages with wood chip or paper chip as bedding and provided a solid food (MF: Oriental Yeast Co., Ltd.) and disinfected water *ad libitum*.

On day 20 of gestation, all dams were sacrificed by exsanguination under ether anesthesia. The numbers of corpora lutea, implantations, live fetuses and dead fetuses were recorded. Dead fetuses were classified as resorptions, early death (brown mass larger than resorptions), middle death (intermediate size between early and late stage with unclear head and limbs), and late death (with intact head and limbs). The live fetuses were examined for external abnormalities including those in the oral cavity and were weighted after sex determination. Approximately one-half of live fetuses from each litter were fixed in Bouin's solution. The remaining live fetuses from each litter were eviscerated and fixed in the 70% ethanol. The fetuses fixed in Bouin's solution were examined for visceral abnormalities and variations according to the free-hand razor method of Wilson [1] and microdissection method of Nishimura [2]. The fetuses in the 70% ethanol were processed alizarin red-S staining skeletal specimen according to Dawson's method [3] and examined for skeletal abnormalities, variations and degree of ossification. Visceral and skeletal observations were conducted under stereoscopic microscope.

RESULT AND DISCUSSION

Findings of litter parameters and fatal observations at cesarean section are shown in Table 1.

The numbers of corpora lutea, implantations and live fetuses were 15.5-17.9, 13.6-15.3 and 13.3-14.6 respectively. Rate of Pre-and post-implantation losses were 3.57-12.35% and 2.75-9.22% respectively. Sex ratio were 0.82-1.34, fetal body weight in males were 3.48-3.94g, in females were 3.33-3.71g. No fetus with external abnormality was observed.

Fetal body weight was slightly higher than those of in CD rats (breeding center: Atsugi) in our laboratory (male; 3.25-3.87g, female; 3.09-3.72g) [4].

Table 1. Detail of IGS rats obtained from Charles River Japan

Study No.	96IGS(1)	97IGS(1)	98IGS(1)	98IGS(2)	98IGS(3)
Birth date	95/12/20-95/12/22	97/07/01-97/07/03	98/06/30-98/07/02	98/07/28-98/07/30	unknown
Mating	in-house mated	in-house mated	in-house mated	in-house mated	suppier mated
Lot No.	T21-0053-1457	T21-0032-1459	T21-0003-1448	T21-0009-1448	T21-0006-0940
Breeding center	Tukuba	Tukuba	Tukuba	Tukuba	Tukuba

Table 2. Findings in dams (F_0) and fetuses (F_1) on 20 day of gestation

Study No.	96IGS(1)	97IGS(1)	98IGS(1)	98IGS(2)	98IGS(3)
No. of dams	20	8	8	11	8
No. of corpora lutea	17.0±2.3	15.9±0.9	17.0±2.0	17.9±3.4	15.5±1.7
No. of implantation sites	15.0±2.3	15.3±1.3	15.1±2.5	15.3±2.1	13.6±1.3
Pre-implantation loss (%)[a)]	41(12.06)	5(3.57)	15(11.03)	23(12.35)	15(11.58)
Post-implantation loss (%)[b)]	19(6.36)	9(6.14)	11(9.22)	7(5.09)	3(2.75)
Resorption	18(6.08)	8(5.40)	11(9.22)	6(4.40)	3(2.75)
Early death	1(0.28)	1(0.74)	0(0.00)	1(0.69)	0(0.00)
Middle death	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Late death	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
No. of live fetuses	14.0±2.6	14.3±0.9	13.8±2.9	14.6±2.1	13.3±1.5
Sex ratio(M/F)	0.82	1.26	1.34	0.90	0.89
Fetal weight(g)	3.94±0.34	3.68±0.23	3.56±0.19	3.48±0.46	3.78±0.14
	3.71±0.26	3.44±0.28	3.39±0.26	3.33±0.42	3.59±0.15
No. of external anomalies (%)[c)]	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
No. of placental anomalies (%)[c)]	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

a): ((No. of corpora lutea - No. of implantation sites)/ No. of corpora lutea)×100
b): (No. of fetuses resorbed or dead / No. of implantation sites)×100
c): (Total fetuses with external anomalies or placental anomalies / No. of fetuses observed)×100

Visceral examinations in fetuses are shown in Table 3.

Frequencies of visceral abnormalities and variations were 0-1.4 and 0-1.8% respectively. Visceral abnormalities observed were persistent atrioventricular canal and malpositioned right carotid branch. Visceral variations observed were left umbilical artery and thymic remnant in neck.

In visceral examination, no remarkable change is observed in IGS rats.

Findings of skeletal examination in fetuses are shown in Table 4.

Frequencies of skeletal abnormalities and variations were 0-4.2% and 6.8-16.1% respectively.

A skeletal abnormality observed was absent ribs. Skeletal variations observed were splitting of vertebral bodies, dumbbell shape of vertebral bodies, cervical ribs, lumbar ribs, short ribs, 5 lumbar vertebrae and sacralization of the lumbar vertebra.

In the progress of ossification, the number of ossified sacral and caudal vertebrae were 7.8-8.3.

In skeletal examination, incidences of lumbar ribs was slightly higher than those of in CD rats in our laboratory (0-4.8%) [4].

The IGS rat is usually used in some studies of our laboratories in order to promote international development of new drugs. Therefore, it is suggested that we need to continuously accumulate background data for accurate evaluation of reproductive and developmental toxicity studies on IGS rats.

Table 3. Visceral observations of fetuses (F_1)

Study No.	96IGS(1)	97IGS(1)	98IGS(3)
No. of dams	20	8	8
No. of fetuses examined	142	50	50
Abnormalities			
Persistent atrioventricular canal	1(0.7)	0(0.0)	0(0.0)
Malpositioned right carotid branch	1(0.7)	0(0.0)	0(0.0)
Total(%)[a)]	2(1.4)	0(0.0)	0(0.0)
Variations			
Thymic remnant in neck	0(0.0)	0(0.0)	1(1.8)
Left umbilical artery	1(0.7)	0(0.0)	0(0.0)
Total(%)[b)]	1(0.7)	0(0.0)	1(1.8)

Mean±S.D.
a): (Total fetuses with abnormalities / No. of fetuses observed)×100
b): (Total fetuses with variations / No. of fetuses observed)×100

Table 4. Skeletal observations of fetuses (F_1)

Study No.	96IGS(1)	97IGS(1)	98IGS(3)
No. of dams	20	9	8
No. of fetuses examined	137	66	54
Abnormalities			
Absent ribs	0(0.0)	3(4.2)	0(0.0)
Total(%)[a)]	0(0.0)	3(4.2)	0(0.0)
Variations			
Splitting of vertebral bodies	2(1.5)	0(0.0)	0(0.0)
Dumbbell shape of vertebral bodies	3(2.2)	1(1.6)	0(0.0)
Cervical ribs	2(1.1)	5(6.9)	0(0.0)
Lumbar ribs	3(2.2)	3(4.8)	5(10.4)
Short ribs	0(0.0)	7(9.7)	0(0.0)
5 lumbar vertebrae	0(0.0)	7(9.7)	0(0.0)
Sacralization	0(0.0)	0(0.0)	0(0.0)
Total(%)[b)]	9(6.8)	11(16.1)	5(10.4)
Ossification			
No. of sacral and caudal vertebrae	8.3±0.41	7.8±0.44	8.0±0.43

Mean±S.D.
a): (Total fetuses with abnormalities / No. of fetuses observed)×100
b): (Total fetuses with variations / No. of fetuses observed)×100

REFERENCES

1. Wilson, J. G. 1965: Methods for administering agents and detecting malformations in experimental animals. "Teratology: Principles and Techniques" edited by Wilson, J. G. and Warkany, J., Univ. Chicago Press, Chicago, pp. 262-277.
2. Nishimura, K. 1974: A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. Congenital Anomalies. 14: 23-40.
3. Dawson, A. B. 1926: A note on the staining of the skeleton of cleared specimens with alizarin red S. Stain Technol. 1: 123-124.
4. Nakatsuka, T. et al 1997: Japan pharmaceutical manufactures association (JPMA) survey on background control data of developmental and reproductive toxicity in rats, rabbits and mice. Congenital Anomalies. 37: 47-138.

Findings in Fetuses from Rat Dams [Crj:CD(SD)IGS] Supplied with CRF-1 (Protein Content: 23.1%) or CR-LPF (Protein Content: 18.4%)

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ABSTRACT. Dams were supplied with either of 2 kinds of feed [CRF-1 (protein content: 23.1%) and CR-LPF (protein content: 18.4%)], and effects of the feed on fetuses were examined.

At caesarean section of the dams, no differences were seen in the number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, or pre-implantation loss rate between the CRF-1 group and the CR-LPF group.

In the fetuses, no differences were seen in the number of deaths or resorptions, death or resorption rate, number of live fetuses, fetal body weight of either sex, incidence of external anomalies, incidence of skeletal anomalies, incidence of skeletal variations, degree of ossification, or incidence of visceral anomalies between the CRF-1 group and the CR-LPF group.

From the above results, it can be said that the low-protein feed CR-LPF has no effect on fetuses in rats. —Key words: Rats, Crj:CD(SD)IGS, Reproduction, Low protein.

CD(SD)IGS-1999: 210-213

INTRODUCTION

The feed whose protein content is about 23% is commonly used in reproductive studies using rats. A lower protein feed, whose protein content is about 18%, was given to dams, and effects of the difference in protein content on fetuses were examined.

METHODS

Test Animals and Housing Conditions: Male and female Sprague-Dawley strain rats [Crj:CD(SD)IGS, SPF, Charles River Japan, Inc.] were used for the present study.

The animals were kept in an animal room with a 12-hour light and dark cycle (lighting: 6:00 a.m. - 6:00 p.m.), a temperature range of 20 - 24℃, a relative humidity range of 40-70%, and filtered fresh air changes of 12 times per hour.

Dams were housed individually in stainless steel cages.

The animals in the CRF-1 group were given free access to CRF-1 (containing crude protein: 23.1%, water: 7.7%, crude fat: 5.9%, crude ash: 6.5%, and crude fiber: 3.3%; Oriental Yeast Co., Ltd.), and those in the CR-LPF group were given free access to CR-LPF (containing crude protein: 18.4%, water: 7.5%, crude fat: 4.8%, crude ash: 6.3%, and crude fiber: 5.0%; Oriental Yeast Co., Ltd.). All animals were given free access to tap water.

Mating of Parental Animals: Male and female rats which had been supplied with CRF-1 or CR-LPF after weaning until they reached the age of 12 weeks were paired on a one-to-one basis from the evening to the next morning. Copulation was checked at nearly the same time every morning. Females which had a vaginal plug or sperm in the vaginal smear were regarded as having copulated. The day when the vaginal plug or sperm was found was defined as Day 0 of pregnancy.

Observation and Examinations:

(1) Dams

The dams were observed for mortality and general signs once daily.

Females which had copulated were sacrificed on Day 20 of pregnancy by exsanguination from the abdominal aorta under ether anesthesia and necropsied. Then the number of corpora lutea and number of implantation sites were counted.

(2) Fetuses

The numbers of dead and resorbed fetuses (embryos) and live fetuses were counted for each dam.

Live fetuses were observed for external abnormalities including those in the oral cavity, sexed, and weighed.

For each dam, about two-thirds of the live fetuses were selected at random. After their thoracic and abdominal organs were removed, these fetuses were fixed in alcohol. The remaining fetuses were fixed in Bouin's solution.

Cleared and stained skeletal specimens of the fetuses fixed in alcohol were prepared by Dawson's method [1] and examined for skeletal anomalies and variations and degree of ossification (sternebrae, metacarpal bones, forelimb proximal phalanges, forelimb middle phalanges, metatarsal bones, hindlimb proximal phalanges, hindlimb middle phalanges, and sacrococcygeal vertebral bodies). Regarding the fetuses fixed in Bouin's solution, the thoracic organs were examined by Nishimura's method [2], and other visceral organs were examined by Barrow's method [3].

Statistical Methods: For the dams' body weight, food consumption, number of corpora lutea, number of implantation sites, number of pre-implantation losses, number of deaths or resorptions, number of live fetuses, sex ratio, fetal body weight of both sexes, and degree of ossification, Student's t test was used when group variance was found to be homogeneous by F test. On the other hand, Aspin-Welch's t test was used when group variance was found to be inhomogeneous.

For the implantation rate, pre-implantation loss rate, death or resorption rate, incidence of external anomalies, incidence of abnormal placentae, incidence of skeletal anomalies, incidence of skeletal variations, and incidence of visceral anomalies, Wilcoxon's rank test was used.

RESULTS

Dams: Neither dead animals nor moribund animals were noted in either group. No abnormal general signs were noted in either

group.

At necropsy, no abnormalities were noted in either group.

Observation of Dams at Caesarean Section:

Results of the observation of the dams at caesarean section are shown in Table 1.

No significant differences were seen in the number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, or pre-implantation loss rate between the CRF-1 group and the CR-LPF group.

Table 1. Observation of fetuses

Food	CRF-1	CR-LPF
Number of dams	43	38
Corpora lutea		
Mean±S.D. per dam	16.6±1.7	15.9±2.9
Implantation sites		
Mean±S.D. per dam	15.3±1.9	14.7±3.1
Implantation rate		
Mean%±S.D. per dam[a)]	92.1±9.1	92.8±11.8
Pre-implantation losses[b)]		
Mean±S.D. per dam	1.3±1.5	1.2±1.9
Pre-implantation loss rate		
Mean%±S.D. per dam[c)]	8.0±9.2	7.2±11.8
Deaths or resorptions		
Mean±S.D. per dam	0.8±0.9	0.6±0.7
Mean%±S.D. per dam[d)]	5.3±5.8	3.9±5.4
Live fetuses		
Mean±S.D. per dam	14.5±1.9	14.1±3.2
Sex ratio		
Mean±S.D. per dam[e)]	0.472±0.117	0.454±0.133
Mean fetal body weight (g)		
Male (Mean±S.D. per dam)	3.650±0.220	3.703±0.262 (37)
Female (Mean±S.D. per dam)	3.476±0.211	3.490±0.265
External anomalies		
Mean%±S.D. per dam [f)]	0.2±1.1	2.8±16.2
Maxillary micrognathia		
Mean%±S.D. per dam	0.0±0.0	2.6±16.2
Gastroschisis		
Mean%±S.D. per dam	0.0±0.0	0.2±1.1
Brachyury		
Mean%±S.D. per dam	0.2±1.1	0.0±0.0

Figures in parentheses indicate number of dams.
a): (Number of implantation sites/number of corpora lutea)×100.
b): Number of corpora lutea - number of implantation sites.
c): (Number of pre-implantation losses/number of corpora lutea)×100.
d): (Number of deaths or resorptions/number of implantation sites)×100.
e): Number of males/number of live fetuses.
f): (Number of fetuses with external anomalies/number of live fetuses)×100.

Effects on Fetuses:

(1) Observation at Caesarean Section

Results of the observation of the fetuses at caesarean section are shown in Table 1.

No significant differences were seen in the number of deaths or resorptions, death or resorption rate, number of live fetuses, or fetal body weight of either sex between the CRF-1 group and the CR-LPF group.

Regarding external anomalies, brachyury was noted in the CRF-1 group, and maxillary micrognathia and gastroschis were noted in the CR-LPF group. However, no differences were seen between the CRF-1 group and the CR-LPF group.

(2) Skeletal Examination

Results of the skeletal examination of the fetuses are shown in Table 2.

Skeletal anomalies were noted as follows: in the CRF-1 group, absence of sacrococcygeal vertebra, fusion of rib, and nodulation of the spina seapula; and in the CR-LPF group, deformity of the skull, fusion of the thoracic vertebral arch, absence of the 13th rib, fusion of rib, fusion of sternebra, and nodulation of the spina seapula. However, no differences were seen between the CRF-1 group and the CR-LPF group.

Skeletal variations were noted as follows: variation in the number of presacral vertebrae, which was significantly greater in the CR-LPF group than in the CRF-1 group. In addition to

this change, the following skeletal variations were noted in both groups sporadically: splitting of the cervical vertebral arch, shortening of the thoracic vertebral arch, splitting of the thoracic vertebral body, cervical rib, shortening of the 13th rib, the 14 rib, and splitting of sternebra. However, no differences were seen between the CRF-1 group and the CR-LPF group.

Regarding the degree of ossification, no differences were seen between the CRF-1 group and the CR-LPF group.

Table 2. Skeletal examination of fetuses

Food	CRF-1	CR-LPF
Number of dams	43	38
Number of fetuses examined	415	362
Anomalies		
Number of fetuses with anomalies[a)]	1.2±5.4	3.7±16.4
Deformity of skull[a)]	0.0±0.0	2.6±16.2
Fusion of thoracic vertebral arch[a)]	0.0±0.0	0.3±1.6
Absence of sacrococcygeal vertebra[a)]	0.2±1.5	0.0±0.0
Absence of 13th rib[a)]	0.0±0.0	0.3±1.6
Fusion of rib[a)]	0.2±1.4	0.5±2.2
Fusion of sternebra[a)]	0.0±0.0	0.6±2.6
Nodulation of spina seapula[a)]	0.8±5.0	0.3±1.6
Variations		
Number of fetuses with variations[a)]	14.1±16.3	15.9±15.9
Splitting of cervical vertebral arch[a)]	0.3±1.7	2.2±6.7
Shortening of thoracic vertebral arch[a)]	0.0±0.0	0.3±1.6
Splitting of thoracic vertebral body[a)]	1.4±3.7	1.6±3.8
Variation of number of presacral vertebrae[a)]	0.2±1.5	1.9±4.5*
Cervical rib[a)]	0.5±2.2	0.3±1.6
Shorting of 13th rib[a)]	0.5±2.2	0.5±2.2
14th rib[a)]	12.1±16.6	12.1±14.6
Splitting of sternebra[a)]	0.0±0.0	0.3±1.6
Ossifications		
Sternebrae[b)]	5.32±0.52	5.43±0.62
Metacarpals of forepaw[b)]	6.93±0.59	6.97±0.74
Proximal phalanges of forepaw[b)]	0.17±0.41	0.16±0.51
Middle phalanges of forepaw[b)]	0.00±0.00	0.00±0.00
Metatarsals of hindpaw[b)]	8.00±0.03	7.96±0.14
Proximal phalanges of hindpaw[b)]	0.00±0.00	0.00±0.00
Middle phalanges of hindpaw[b)]	0.00±0.00	0.00±0.00
Sacrococcygeal vertebral bodies[b)]	7.79±0.50	7.83±0.43

a): Each value shows mean%±S.D. per dam.
b): Each value shows mean±S.D. per dam
Significantly different from CRF-1 control (*: P<0.05).

(3) Visceral Examination

Results of the visceral examination are shown in Table 3.

Visceral anomalies were noted as follows: in the CRF-1 group, thymic remnant in the neck, ventricular septal defect, riding aorta, and left umbilical artery; and in the CR-LPF group, dilatation of cerebral ventricle, thymic remnant in the neck, ventricular septal defect, persistent truncus arteriosus, abnormal origin of the right subclavian artery, and left umbilical artery. However, no differences were seen between the CRF-1 group and the CR-LPF group.

DISCUSSION

Dams were supplied with either of 2 kinds of feed [CRF-1 (Protein Content: 23.1%) and CR-LPF (Protein Content: 18.4%)], and effects of the feed on fetuses were examined.

According to observation at caesarean section of the dams, no differences were seen in the number of corpora lutea, number of implantation sites, implantation rate, number of pre-implantation losses, or pre-implantation loss rate between the CRF-1 group and the CR-LPF group.

Regarding the fetuses, no differences were seen in the number of deaths or resorptions, death or resorption rate, number of live fetuses, fetal body weight of either sex, incidence of external anomalies, incidence of skeletal anomalies, incidence of skeletal variations, degree of ossification, or incidence of visceral anomalies between the CRF-1 group and the CR-LPF group.

Compared with the CRF-1 group, a significantly higher incidence of variation in the number of presacral vertebrae was noted in the CR-LPF group. This finding is considered to be incidental change, since no difference was seen in the incidence of fetuses with skeletal variations between these 2 groups. The external anomalies, skeletal anomalies and variations, and visceral anomalies which were noted in the present study are known to occur spontaneously in Crj: CD (SD) strain rats [4], [5].

From the above results, it can be said that the low-protein feed CR-LPF has no effect on fetuses in rats.

Table 3. Visceral examination of fetuses

Food	CRF-1	CR-LPF
Number of dams	43	37
Number of fetuses examined	207	175
Anomalies		
Number of fetuses with anomalies	2.5±7.0	3.8±8.1
Dilatation of cerebral ventricle	0.0±0.0	0.5±3.3
Thymic remnant in neck	0.6±3.8	0.5±3.3
Ventricular septal defect	1.4±5.4	1.1±4.6
Riding aorta	0.5±3.0	0.0±0.0
Persistent truncus arteriosus	0.0±0.0	0.5±3.3
Abnormal origin of right subclavian artery	0.0±0.0	0.5±3.3
Left umbilical artery	0.5±3.0	1.1±4.9

Each value shows mean%±S.D. per dam.

REFERENCES

1. Dawson, A.B. 1926: A note on the staining of the skeleton of cleared specimens with alizarin red S. *Stain Technol.*, 1, 123-124
2. Nishimura, K. 1947: A microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses. *Cong. Anom.*, 14, 23-40
3. Barrow, M.V. and Taylor, W.J. 1969: A rapid method for detecting malformations in rat fetuses. *J. Morph.*, 127; 291-306
4. Morita, H. *et al.* 1987: Spontaneous malformations in laboratory animals: Frequency of external, internal and skeletal malformations in rats, rabbits and mice. *Cong. Anom.*, 27, 147-206
5. Nakatsuka, T. *et al.* 1997: Japan Pharmaceutical Manufacturers Association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. *Cong. Anom.*, 37, 47-138

Developmental Parameters as Assessed in Embryo-Fetal Studies in CD(SD)IGS Rats Compared to CD(SD) Rats

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ABSTRACT. Parameters from CD(SD)IGS rats from 10 recently conducted developmental toxicity studies were compared to a large historical control database comprising 158 studies of CD(SD) rats. This, the largest data set yet published, demonstrated with one exception, no significant differences between the two groups of rats in any developmental parameter. There was an increased incidence in 14th rib variations in IGS rats, the significance of which is not readily apparent. — Key words: CD(SD)IGS Rat, Developmental Parameters

CD(SD)IGS-1999: 214-216

INTRODUCTION

The Charles River CD, Sprague-Dawley (S-D) derived rat was redesigned genetically by the manufacturer in 1992 to enhance genetic stability. This was done purposefully due to observations by researchers that long term survival rates for the CD(SD) rat were decreasing over time [10]. Reproductive toxicologists also observed that fertility rates were not as consistently high as noted earlier and litter mortality was greater than formerly.

The new animal, termed "International Genetic Standard" (IGS) or CD(SD)IGS, is said to be uniform regardless of source, and that no significant differences exist between the CD(SD) / IGS and the CD(SD) rat with respect to developmental toxicology evaluations.

Cursory examination of data published on the new animal however, revealed some possible biologically significant differences between the two animals in developmental toxicity studies conducted in a number of laboratories [8]. Specifically, reduced numbers of corpora lutea, increased fetal body weights, and increased incidence of several developmental variations were reported in the CD(SD)IGS rat in comparison to the CD(SD) rat. In addition, the CD(SD)IGS dams in studies were found to have reduced body weights, less food consumption, and reduced weight of some internal organs. These values were also different when compared to historical values in one large database, the MARTA/MTA database [5].

It was these observations that prompted us to examine the developmental parameters of control CD(SD)IGS animals utilized in studies performed in WIL Research Laboratories over a recent interval compared to those of our historical control database, and to those performed in Japanese, Canadian and European laboratories as published in 1998 in a reference text [8] addressing these issues.

MATERIALS AND METHODS

The data reported in this paper are from studies conducted in support of regulatory submissions of new materials. The studies were conducted in accordance with appropriate guidelines for investigation of effects on embryo-fetal development including those of ICH, OECD, USA EPA and JMAFF. To ensure 20 pregnant dams per group, 25 females were allocated to each group; the majority of studies consisted of a control and 3 test groups. All animals originated from Charles River Breeding Laboratories, Kalamazoo, MI or Kingston, NY, USA. At WIL Research Laboratories, animals were housed in a controlled room temperature of 72° ± 4° F and relative humidity between 30 and 70%. Artificial light gave 12 hours light and 12 hours darkness. All animals were given free access to tap water and to Purina Certified Rodent Chow #5002 which were analyzed on a regular basis for nutrients, contaminants and microorganisms. The day of mating was considered as Day 0 of pregnancy, judged by the vaginal smear or presence of a vaginal plug. Animals were maintained throughout according to GLP standards under the supervision of veterinary staff. During gestation, clinical signs, maternal body weight, and food consumption were recorded according to protocol requirements.

Adult virgin female rats were subjected to 10 days quarantine before being mated with stock males of the same strain; at study commencement dams were at a minimum weight of 220 g and were 80 to 120 days of age. On group allocation they were assigned a number identified by a metal eartag. Dams were housed individually during the respective study.

On Day 20 of gestation, dams were euthanized by CO_2 asphyxiation and examined for macroscopic pathological changes in maternal organs and congenital abnormalities. The number of corpora lutea was recorded. The uterus and ovaries were removed and examined in detail; embryo-fetal deaths were classified as "early" or "late." Placental weight was not recorded. Uteri of apparently non-pregnant dams were examined for evidence of implantation sites using a modified Salewski technique [12]. Live young were examined externally, weighed and individually identified. The fetuses in each litter were examined by fresh dissection [13]; the heads were removed from approximately one-half of each litter and placed in Bouin's solution for subsequent sectioning and examination [16]. Fetuses for skeletal examination were macroscopically viscerally examined, sexed, and fixed in ethyl alcohol for subsequent skeletal examination.

Data shown here are taken from control group animals (Table 1). Statistical analysis was performed on individual studies making up the comparison but not as a pairwise comparison between IGS and SD rats.

Table 1. Comparison of Developmental Toxicity Parameters in CD(SD) and CD(SD)IGS Rats in WIL Research Laboratories

	CD(SD)IGS	CD(SD)
Number studies	10	158
Number dams	244	3926
Number dams that died	1	1
Number gravid	224	3585
% gravid	91.8	91.3
Number with resorptions only	0	11
% resorptions only	0	0.3
Mean number viable fetuses/dam	14.9	14.2
% viable fetuses/litter	95.1	94.3
Mean number postimplantation loss/dam	0.8	0.8
% postimplantation loss/litter	4.9	5.7
Mean number corpora lutea/dam	17.5	16.8
Mean number implantations/dam	15.6	15.0
Mean number preimplantation loss/dam	1.9	1.8
% preimplantation loss/litter	9.9	10.5
Sex ratio (% M/F)	50.3/49.7	49.7/50.3
Mean g fetal body weight (combined sexes)	3.6	3.5

RESULTS AND DISCUSSION

A total of 10 developmental toxicity studies conducted for commercial clients in the interval April, 1998 through January, 1999 were used for data comparisons. These studies comprised 224 litters and 3,336 fetuses from CD(SD)IGS rats. These were compared to 3,585 litters and 50,858 fetuses collected over the past years from CD(SD) rats. Developmental parameters are compared in Table 1.

Although no statistical comparisons were conducted, there were no obvious biological differences in the two populations in any parameter. The reduced number of corpora lutea reported in IGS rats by others [2, 4, 6, 14] was not observed in our comparison; in fact, we found a slight, but probably nonsignificant increase in ovarian corpora lutea in IGS rats compared to CD(SD) rats in our laboratory.

Similarly, the increased body weights recorded for IGS rat fetuses by other investigators [2, 3, 4, 6, 7, 10, 14, 15] were not evident in our series, the difference of 0.1g mean value not considered biologically relevant.

All other parameters, including pre-and postimplantation loss, pregnancy rates, whole litter resorption rate, implantations, and sex ratios were essentially equivalent between CD(SD)IGS and CD(SD) rats in our experience.

Of the developmental variations encountered, fetuses with 14 (lumbar) ribs were increased among IGS litters (37.5%) compared to CD(SD) litters (24.4%) in our laboratory. This finding was also reported by many investigators in multiple studies in a recent publication [8]. In contrast, we found no increase in ossification of sacro-caudal vertebrae in IGS rat fetuses as has been reported in a number of published studies [2, 3, 6, 7, 15]. Several other reported findings in IGS rats reported by others, including increased hydronephrosis and dilated ureter [4, 10], ossification of the 5th sternebrae and metatarsi [15], increased ventricular septal defect [3, 14], presence of the left umbilical artery [14], skewed sex distribution in litters [9] and increased preimplantation loss [14] were not observed in our series, and must be considered incidental findings in those studies.

In summary, in our evaluation of data from the largest number of CD(SD)IGS rats yet published on, we found no significant differences, with one exception, in any developmental parameter, between CD(SD) and CD(SD)IGS rats. We did note an increased incidence in the skeletal developmental variation 14th ribs in IGS rats, and it is not readily apparent why this is so. Further comparison of developmental parameters in IGS rats is warranted, although the strain appears to be satisfactory for developmental studies.

REFERENCES

1. Colley, M., Coulby, D.S., Drain, M.R., Critchell, K.A., and Wilby, O.K. 1998. A comparison of Caesarian litter parameters and fetal observations from developmental toxicity studies on Crl:CD(SD)IGS and Crl:CD(SD) rats using in-house and supplier mated animals. In: "Biological Reference Data on CD(SD)IGS Rats-1998. "CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 200-205.
2. Kamijima, M., Kinoshita, K., Koike, A., Sasaki, M., Sato, S., Tabata, K., Onotogi, M., Shimojima, A. and Shibano, T. 1998. Historical control data for reproductive and developmental toxicity studies in Crj:CD(SD)IGS rats-Teratology study In: "Biological Reference Data on CD(SD) IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 238-239.
3. Kato, Y., Fujita, K., Uchiyama, J., and Sanbuissho, A. 1998. A comparison study for embryo-fetal development of the Crj:CD(SD)IGS and Crj:CD(SD) rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 219-222.

4. Kizaki, H., Shibuya, K., Saitoh, T., Shibuya, N., Ihara, M., Itabashi, M., Kudow, S., Nunoya, T., and Tajima, M. 1998. Background data for reproductive and developmental toxicity studies in Crj:CD(SD)IGS rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 240-243.
5. MARTA and MTA, 1995. Historical control data (1992-1994) for developmental and reproductive toxicity studies using the Crl:CD BR rat. Charles River Laboratories.
6. Matsumoto, A., Ohta, R., Sato, M., Kojima, K., Seki, T., Azegami, J., and Nagao, T. 1998. Comparison between Crj:CD(SD)IGS and Crj:CD(SD) rats in reproductive and developmental parameters. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, p. 155-163.
7. Matsumoto, H., Fukumura, M., Yoshihara, K., Sasa, H., and Tanaka, C. 1998. Characteristics of Crj:CD(SD)IGS rats compared with Crj:CD(SD) rats based on a study for effects on embryo-fetal development. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 191-193.
8. Matsuzawa, T. and Inoue, H. (eds.) 1998. Biological Reference Data on CD(SD)IGS Rats-1998. CD(SD)IGS Study Group, Yokohama, Best Printing Co., Ltd., Tokyo.
9. Okada, A., Yahagi, K., Fujiwara, M., and Mastuzawa, T. 1998. Reference data of reproductive and developmental toxicity studies in CD(SD)IGS rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 188-190.
10. Okuda, Y., Noda, Y., Okada, F., Matsubara, Y., and Sagami, F. 1998. Reproductive and developmental data in Crj:CD(SD)IGS rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 167-169.
11. Reference Paper, Charles River Laboratories, 1999. Charles River Laboratories' International Genetic Standard (IGS®). 11 (1).
12. Salewski, V.E. 1964. Farbemethode zum makroskopischen Nachweis von Implantationstellen am Uterus der Ratte. Naunyn-Schm. Archiv. Für Exper. Pathol. Pharm. 247:367.
13. Stuckhardt, J.L. and Poppe, S.M. 1984. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogenesis, Carcinogenesis, Mutagenesis 4:181-188.
14. Tanaka, R., Fujiwara, C., Ito, K., Sakamoto, C., Yamada, R., Ogawa, T., and Inoue, H. 1998. Comparison of reproductive and developmental parameters in Crj:CD(SD)IGS rats with the historical control data of Crj:CD(SD) rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue, eds., Best Printing Co., Ltd., Tokyo 1998, pp. 248-260.
15. Umemura, T., Ishida, S., and Katsumata, Y. 1998. Comparison of reproductive and developmental parameters between Crj:CD(SD) rat and Crj:CD(SD) rats. In: "Biological Reference Data on CD(SD)IGS Rats-1998." CD(SD)IGS Study Group, Yokohama. T. Matsuzawa and H. Inoue. eds., Best Printing Co., Ltd., Tokyo, 1998, pp. 206-213.
16. Wilson, J.G. 1965. Embryological considerations in teratology. In:Teratology-Principles Techniques. (Wilson, J. G. and Warkany, J., eds.) The University of Chicago Press, Chicago, IL, pp. 251-277.

Comparisons of Testicular Histopathology and Sperm Evaluation between Crj:CD(SD) and Crj:CD(SD)IGS Rats

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ABSTRACT. The aim of this study was to examine whether there exist the strain differences in testicular histopathology and sperm parameters between Crj:CD(SD) and Crj:CD(SD)IGS rats at the age of 8, 12, 16 and 20 weeks. There were no differences between Crj:CD(SD) and Crj:CD(SD)IGS rats in sex organ weights or gross findings at necropsy. The two strains did not differ in the motility, counts, and morphology of caudal epididymal sperm. At 8 weeks old, the percentage of the motile sperm and the sperm count in both strains were much lower compared with those for the other ages. At this age, morphologically abnormal sperm were frequently observed in both strains. Although histopathological examination of the testis revealed no strain-specific lesion, an increased incidence of very slight vacuolation in the Sertoli cells was observed in 8-week-old rats.
From these results, Crj:CD(SD) and Crj:CD(SD)IGS rats do not differ in spermatogenesis or sperm maturation, and the time when stable sperm production is observed is at the age of 12 weeks in both strains. − Key words: sperm examination, testicular histopathology, CASA system, IGS rats
CD(SD)IGS-1999: 217-220

INTRODUCTION

The International Genetic Standard system (IGS system), a breeding system, was developed by the Charles River Laboratories in order to produce non-inbred animals and to harmonize the range and the variations of phenotypes [1]. The Crj:CD(SD)IGS rats had been established from the original Crj:CD(SD) rats by use of this breeding system.

Recently, it is recommended that the male reproductive parameters such as the sperm motility, counts or morphology are examined in the reproductive and developmental toxicity studies. In addition to these sperm indices, histopathological examinations of the testis are required to evaluate the male reproduction.

In this study, we examined the histopathology of the testis and sperm parameters in the new strains of the Crj:CD(SD)IGS rats, and compared with their originated strains, the Crj:CD(SD) rats.

MATERIALS AND METHODS

Ten males each from Crj:CD(SD) rats (hereafter referred to as SD rats) and Crj:CD(SD)IGS rats (hereafter referred to as IGS rats) at the age of 8, 12, 16 and 20 weeks respectively, were received from Charles River Japan Inc. (Tsukuba or Hino Breeding Center, Japan). All rats were killed by an ether overdose after having been weighed on the day of arrival, and then necropsied. At necropsy, the reproductive organs were mainly observed with the naked eye, and the following organs were weighed: testis, epididymis, ventral prostate, seminal vesicle.

For sperm examination, the right caudal epididymis was used except when abnormality was observed in the right testis or epididymis. Sperm samples from the caudal epididymis were collected at necropsy and were used for sperm motility and sperm morphology. The distal tubule in caudal epididymis was punctured with needles, and flooding luminal fluid was suspended in Medium 199 with Hank's salts (Gibco BRL) containing 0.5% bovine serum albumin (Intergen). After collecting caudal fluid, the remaining caudal epididymis was stored at -20℃ until sperm counts. The percentage of motile sperm was analyzed using the computer-assisted sperm motion analysis (CASA) system (HTM-IVOS, Hamilton-Thorne Research). The caudal sperm suspensions used for the motility were subsequently smeared by the drop over the slide for evaluation of sperm morphology. After dried, the smears fixed with methanol and stained with anilineblue and eosin. A total of 200 sperm were evaluated per animal and the percentage of abnormal sperm was calculated. For determination of sperm counts, the stored caudal epididymis were homogenized for 2 min with sonication, and the sperm heads in the homogenized epididymis were counted using Ident-Stain kit (HTM-IVOS, Hamilton-Thorne Research) by the CASA system.

The testes were fixed in Bouin's solution. Hematoxylin and eosin-stained paraffin sections of the testes from each rat were prepared and examined under a light microscope.

The statistical analysis of the data was performed using Student's *t*-test or Aspin-Welch *t*-test. A p value of less than 0.05 or 0.01 was chosen as the level of statistical significance.

RESULTS AND DISCUSSION

The general condition of all animals was normal before necropsy.

In the animals sacrificed at 12 weeks of age, one SD rat had a yellowish nodule on the epididymis, and one IGS rat had a large right testis, a small left testis and small epididymides. In the animals sacrificed at 20 weeks of age, one SD rats had a small testis. At 8 and 16 weeks of age, there was no abnormal observation in both strains of rats. From these gross findings, there is no strain-specific abnormality in the male reproductive organs in both strains.

Results of absolute and relative organ weights are summarized in Table 1. There were some statistically significant changes in organ weights except at 12 weeks old. These changes in the organ weights, however, did not suggest strain differences, because there were no tendencies toward strain-specific differences between SD and IGS rats throughout all ages. Moreover, the weights of reproductive organs continued to increase with age, but the relative organ weights did not increase after 12 weeks of age.

Table 2 presents the mean percentage of motile sperm and the mean epididymal sperm counts. There were no significant differences between the two strains at any age. For 8-week-old ani-

Table 1. Absolute and relative organ weights

Strain	Crj:CD(SD)			
Age (weeks)	8	12	16	20
No. of animals examined	10	10	10	10
Final body weight (g)	275±8	411±9	469±10	521±10
Absolute organ weight (g)				
Testis (right)	1.34±0.06	1.61±0.11	1.64±0.12	1.61±0.36
Testis (left)	1.36±0.09	1.58±0.11	1.64±0.09	1.71±0.19
Testes (both)	2.70±0.13	3.19±0.21	3.27±0.21	3.31±0.44
Epididymides	0.55±0.07	0.99±0.07	1.11±0.09	1.23±0.13
Ventral prostate	0.32±0.08	0.64±0.07	0.71±0.14	0.73±0.13
Seminal vesicle	0.61±0.11	1.58±0.23	1.81±0.16	2.04±0.39
Relative organ weight (g/100g BW)				
Testes	0.98±0.05	0.78±0.05	0.70±0.05	0.64±0.09
Epididymides	0.20±0.03	0.24±0.02	0.24±0.02	0.24±0.03
Ventral prostate	0.11±0.03	0.16±0.02	0.15±0.03	0.14±0.02
Seminal vesicle	0.22±0.04	0.38±0.05	0.39±0.04	0.39±0.08
Strain	Crj:CD(SD)IGS			
Age (weeks)	8	12	16	20
No. of animals examined	10	10	10	10
Final body weight (g)	302±7**	410±6	531±15**	562±13**
Absolute organ weight (g)				
Testis (right)	1.37±0.13	1.58±0.22	1.73±0.11	1.84±0.16
Testis (left)	1.38±0.13	1.74±0.35	1.73±0.10	1.87±0.18
Testes (both)	2.75±0.26	3.32±0.24	3.46±0.20	3.71±0.34*
Epididymides	0.53±0.04	0.96±0.15	1.21±0.06*	1.36±0.12*
Ventral prostate	0.32±0.03	0.61±0.07	0.74±0.14	0.71±0.11
Seminal vesicle	0.71±0.09*	1.47±0.15	2.02±0.29	2.02±0.24
Relative organ weight (g/100g BW)				
Testes	0.91±0.08*	0.81±0.05	0.65±0.04*	0.66±0.05
Epididymides	0.17±0.01*	0.23±0.04	0.23±0.01	0.24±0.02
Ventral prostate	0.10±0.01	0.15±0.02	0.14±0.03	0.13±0.02
Seminal vesicle	0.24±0.03	0.36±0.04	0.38±0.06	0.36±0.04

Parameters presented as the mean±S.D.
*: Significantly different between Crj:CD(SD) and Crj:CD(SD)IGS, $p<0.05$.
**: Significantly different between Crj:CD(SD) and Crj:CD(SD)IGS, $p<0.01$.

mals, because of the scarcity of the sperm in three SD rats and two IGS rats, the sperm motility of these rats could not be examined. At this age, the inter-animal variation of the percent of motile sperm was very large (SD rats, 0.5-88.5%; IGS rats, 0.0-64.5%) in both strains of rats whose sperm could be examined. In 12-week-old rats, the percentage of motile sperm was more than 80% in both strains, except in the one IGS rat that showed testicular and epididymal abnormalities macroscopically. The sperm counts per caudal weight consistently increased with age up to 16 weeks; especially from 8 to 12 weeks of age, the sperm concentration increased about 3-fold. The sperm concentration was nearly the same at 16 and 20 weeks.

Table 3 shows the mean percentages of morphologically abnormal sperm at the various ages. No significant difference in the mean percentage of abnormal sperm between SD rats and IGS rats was found at any age examined. At 8 weeks of age abnormal sperm were frequently observed in both strains, but the ratio of abnormalities decreased as the animals aged, except for the one

Table 2. Epididymal sperm findings

Strain	Crj:CD(SD)	Crj:CD(SD)IGS
Age (weeks)	8	8
Percent of motile sperm (%)	27.5±40.1 (7)	19.0±25.7 (8)
Sperm counts/ caudal epididymis ($\times 10^6$)	19.0±11.0 (10)	16.6±11.3 (10)
Sperm counts/ caudal weight ($\times 10^6$/g)	209.2±108.9(10)	173.3±98.4 (10)
Age (weeks)	12	12
Percent of motile sperm (%)	93.7±4.5 (10)	83.0±29.1 (10)
Sperm counts/ caudal epididymis ($\times 10^6$)	122.5±27.2 (10)	109.7±39.4 (10)
Sperm counts/ caudal weight ($\times 10^6$/g)	603.5±101.5(10)	541.2±155.0(10)
Age (weeks)	16	16
Percent of motile sperm (%)	95.5±2.8 (10)	95.5±2.8 (10)
Sperm counts/ caudal epididymis ($\times 10^6$)	174.1±28.3 (10)	189.1±35.0 (10)
Sperm counts/ caudal weight ($\times 10^6$/g)	739.2±104.3(10)	728.2±131.4(10)
Age (weeks)	20	20
Percent of motile sperm (%)	95.1±2.7 (10)	95.6±2.5 (10)
Sperm counts/ caudal epididymis ($\times 10^6$)	213.2±52.6 (10)	237.6±73.1 (10)
Sperm counts/ caudal weight ($\times 10^6$/g)	733.3±128.9(10)	773.6±166.1(10)

Parameters presented as the mean±S.D. (N).

IGS rat that showed testicular and epididymal abnormalities macroscopically. Irrespective of age or strain, the most observed abnormal sperm type was isolated head. In addition to this, in 8-week-old rats an amorphous head was observed in more than 10% of the sperm in both strains.

Histopathological evaluations of the testis are shown in Table 4. There was no strain difference in the severity or the incidence of testicular lesions. At 8 weeks of age, very slight vacuolation in the Sertoli cells was observed in three SD rats and in four IGS rats. In 12-week-old rats, very slight vacuolation in the Sertoli cells was observed in only one SD rat, and severe atrophy in the seminiferous tubules associated with germ cell loss and the formation of multinucleate giant cells were observed in one IGS rats. Since 16 weeks old, no rat had testicular abnormality except for one SD rats with macroscopically abnormal testis. That is, severe atrophy of the seminiferous tubules associated with germ cell loss and the slight hyperplasia in the Leydig cells was found in one SD rats.

In conclusion, there appear to be no strain differences in spermatogenesis or sperm maturation between Crj:CD(SD) and Crj:CD(SD)IGS rats. Moreover, the large variances of each sperm examination and the high incidences of testicular abnormality were found at 8 weeks of age. These results suggest that male rats in both strains may not complete sexual maturation at 8 weeks old.

REFERENCES

1. White, W. J., and Lee, C. S. 1998. The development and maintenance of the Crj:CD(SD)IGS BR rat breeding system. Biological Reference Data on CD(SD)IGS rats-1998. pp. 8-14.

Table 3. Epididymal sperm morphology

Strain	Crj:CD(SD)	Crj:CD(SD)IGS
Age (weeks)	8	8
No. of sperm examined	2000	1975
No. of abnormal sperm	1268	1367
Abnormality (%)	63.4±28.3[a)]	69.3±20.4
Age (weeks)	12	12
No. of sperm examined	2000	2000
No. of abnormal sperm	79	252
Abnormality (%)	4.0±2.1	12.6±26.2
Age (weeks)	16	16
No. of sperm examined	2000	2000
No. of abnormal sperm	70	59
Abnormality (%)	3.5±2.2	3.0±1.5
Age (weeks)	20	20
No. of sperm examined	2000	2000
No. of abnormal sperm	80	30
Abnormality (%)	4.0±3.5	1.5±0.9

a): Parameters presented as the mean±S.D.

Table 4. Summary of histpathological findings on the testis

Strain	Crj:CD(SD)																			
Age (weeks)	8					12					16					20				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
	[10]					[10]					[10]					[10]				
Vacuolation, Sertoli cell	7	3	0	0	0	9	1	0	0	0	10	0	0	0	0	10	0	0	0	0
Atrophy, seminiferous tubule	9	1	0	0	0	10	0	0	0	0	10	0	0	0	0	9	0	0	0	1
Hyperplasia, Leydig cell	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0	9	0	1	0	0

Strain	Crj:CD(SD)IGS																			
Age (weeks)	8					12					16					20				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
	[10]					[10]					[10]					[10]				
Vacuolation, Sertoli cell	6	4	0	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Atrophy, seminiferous tubule	10	0	0	0	0	9	0	0	0	1	10	0	0	0	0	10	0	0	0	0
Hyperplasia, Leydig cell	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10	0	0	0	0

−, negative; ±, very slight; +, slight; ++, moderate; +++, severe
[ ], number of animals examined

Comparison of Variant Patterns Induced by Retinoic Acid between Crj:CD(SD)IGS and Crj:CD (SD) Rat Fetuses

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Abstract: In order to compare the susceptibility to teratogenic substance between Crj:CD(SD)IGS and Crj:CD(SD) rats, all-trans retinoic acid was given to pregnant rats on day 10 and 13 of gestation at doses of 10 and 40 mg/kg, respectively. Fetuses were delivered by cesarean section on day 21 of gestation and examined for minor variations which are suggested as indicators of teratogenicity. Half of the fetuses were fixed in Bouin's solution and examined for variant patterns of palatal rugae, and further examined for anomalies of digits and foot pads. The remaining half of fetuses were stained by cartilage and bone double staining method and examined for 14th ribs classified into "rib type" and "process type".

In the examination of palatal rugae, supernumerary ruga in zone Ⅰ, supernumerary ruga of extra type, division and aberrant median continuity in zone Ⅱ, median discontinuity, division and extra peak in zone Ⅲ were significantly increased in the fetuses from dams treated with retinoic acid on day 13 of gestation. The increasing pattern of these variants compared very well between Crj:CD(SD)IGS and Crj:CD(SD) rats. Digital anomalies, such as oligodactyly and syndactyly were observed in 26 % of the fetuses, and foot pads were abnormal in about 95 % of the fetuses from dams treated on day 13 of gestation equally in both strains of rats. Also in both strains of rats, "rib type" 14th rib was significantly increased and pre-sacral vertebral number was mostly 27 in the fetuses from dams treated on day 10 of gestation. In the fetuses from dams treated on day 13, "process type" 14th rib was significantly increased and pre-sacral vertebral number was 26 as same as controls in both strains. From the results of this study, it can be said that sensitivity to retinoic acid is same in Crj:CD(SD)IGS and Crj:CD(SD) rats. Key words: IGS rat, variant pattern, retinoic acid, palatal ruga, foot pad, 14th rib

CD(SD)IGS-1999: 221-228

INTRODUCTION

Crj:CD(SD)IGS rats (IGS rats) has been developed by Charles River Inc.'s Gold standard system to provide internationally uniform experimental animals. Aiming to use IGS rats in reproductive and developmental toxicity studies, background control data have been collected by CD(SD)IGS study group. However, collection of data on the sensitivities of IGS rats to test substances is also considered to be necessary. The purpose of this study was to examine susceptibility of fetuses of IGS rats to teratogenic substance, comparing with Crj:CD(SD) rats (SD rats) which we have been used in reproduction studies until now. In the field of developmental toxicology, some minor variations, like as 14th rib, anomalous pattern of platal rugae and foot pads, have been suggested to be indicators of teratogenicity of the substances in rodents [1-6]. These are considered to be more sensitive to teratogen, because they are induced at lower doses than teratogenic doses, or in higher rates than malformation rates. All-trans retinoic acid (RA) was used as a teratogen in this study, since it has been well investigated for these variants and repeatedly reported [6-11].

MATERIALS AND METHODS

Animals:Thirty females (10 weeks old) and fifteen males (12 weeks old) each of Crj:CD(SD)IGS and Crj:CD(SD) rats were purchased from Charles River Japan Inc. After a quarantine and acclimatization period for one week, the females were mated overnight with males of the same strain in the ratio 1:1. On the following morning, the vaginal smears were prepared and examined for the presence of sperm. When sperm were presented the day was designated as day 0 of pregnancy. The inseminated females of each strain were assigned to the series to the three groups so that the mean weight of each group was similar. 7 inseminated females were used per group. The rats were individually identified by an ear tattoo number. The study number, animal number with color code were displayed on a white plastic card attached to each cage.

Accomodation: The animal room was fully air-conditioned and maintained at 22 (19-24)℃ and at 55 (40-75) % relative humidity. There were more than 15 changes of air each hour and the lighting schedule of 12hr light (from 6:00 to 18:00) and 12 hr darkness. Rats were housed individually in standard polycarbonate plastic cages type CL-0105-1 (floor area: 777cm^2), filled with Omega dry® bedding (Oriental Yeast Co., Ltd., Japan). Cage and bedding were changed once weekly or more frequently depending on necessity. Standardized dry pellet diet CRF-1 (γ-ray irradiated diet, Oriental Yeast Co., Ltd., Japan) and municipal tap water from drinking bottles were available *ad libitum.*

Test substance: All-trans retinoic acid (R2625, Sigma-Aldrich Co.) was stored at −20℃ in the freezer until preparation. RA was dissolved in corn oil (C8267, Sigma-Aldrich Co.) just before administration and given orally at a dosing volume of 1 ml/100 g body weight.

Study design: Six groups were settled in this study. Strain of rats, number of animals, dosages and gestation day of treatment at each group were as follows:

Group	Strain of rats	Number of dams	Dose (mg/kg)	Day of treatment (gestation day)
0	Crj:CD(SD)	7	0 (Control)	10, 13
1	Crj:CD(SD)	7	10	10*
2	Crj:CD(SD)	7	40	13*
3	Crj:CD(SD)IGS	7	0 (Control)	10, 13
4	Crj:CD(SD)IGS	7	10	10*
5	Crj:CD(SD)IGS	7	40	3*

*Only vehicle was given on day 13 in group 1 and 4, and on day 10 in group 2 and 5 as same as on day 10 and 13 in control groups.

Ikemi et al. [3, 8], reported that incidence of variant patterns of palatal rugae in fetuses from SD rat treated with RA at dosage of 40 mg/kg on gestation day 13 and 14 dramatically increased with concurrent induction of cleft palate in low incidence. Higuchi et al. [11] reported that different types of 14th ribs were increased in SD rat fetuses when dams were treated with RA at 10 mg/kg on gestation day 10 and at 50 mg/kg on day 13, respectively. From these knowledges, treatment with 10 mg/kg on gestation day 10 and 40 mg/kg on day 13 were selected in this study.

Observation of dams and cesarean section: During gestation dams were checked for general conditions and the body weight was measured daily. On day 21 of gestation, dams were killed by exsanguination under ether anesthesia. Immediately after uterine incision, the number of fetuses, their placement in the uterine horns, the number of live fetuses and fetal loss (of early and late resorption, and dead fetuses) were determined per dam. The corpora lutea was counted after fixation of ovaries with 10% phosphate buffered formalin. Live fetuses were given an identity number, sexed, weighed and examined for external abnormalities. Half of the live fetuses were identified by a label hanged attached on the lower jaw by skin stapler (Precise® DS-25,3M) and fixed in 99.5% ethanol for bone and cartilage double staining. Since it was later found that the plastic label used for identification was not tolerated in acetone for double staining, the fetuses of each litter were tied with a thread in a row for identification. The remaining fetuses were individually marked on the back by felt pen and fixed in Bouin's solution.

Examination of palatal rugae: After fixation in Bouin's solution, the rugae in the palatal region of fetuses were observed under a dissecting microscope by dissecting away the lower jaw and tongue. Rugae showing features different from normal patterns were carefully identified. According to Ikemi's method [3, 8], the palate was divided into anterior (zone Ⅰ, rugae 1-3), intermediate (zone Ⅱ, rugae 4-6), and posterior (zone Ⅲ, rugae, 7-8) regions, and variant patterns were identified and recorded as follows (Fig. 1):

1. Supernumerary ruga: Small ruga or localized protrusion of the palatal mucous membrane in addition to the eight rugae. This variant was classified into rudiment and extra (longer than half the palatal shelf) types.
2. Division: Ruga separated into two parts.
3. Median discontinuity: Ruga attached incompletely to the midline.
4. Discordance: Ruga coming in contact with unmatched points of attachment on the midline.
5. Aberrant median continuity: Lateral rugae corresponding with each other irregularly mismatched.
6. Peakless: Ruga with a waveless regular curve in zone Ⅱ
7. Extra peaks: Ruga having more than two peaks in zone Ⅱ or having a peak in zone Ⅲ
8. Fusion: Two adjacent rugae fused at the lateral or medial end.
9. Shortness: Ruga with length less than half the usual size.
10. Absence: Less than eight rugae.

*Examination of digits and foot pad*s: Fore and hind limbs of the fetuses fixed in Bouin's solution were cut from the trunk and carefully examined for anomalies of foot pads as well as digital and nail anomalies under a dissecting microscope .

Examination of 14th ribs: The fetuses were eviscerated, immersed in water bath heated 70℃ for about 10 seconds and skinned. After fixation with 99.5 % ethanol, they were placed in acetone for two days, stained with alcian blue and arizerin red S (0.2% alcian blue 8GS/70% ethanol : 0.1% arizarin red S/99.5% ethanol : acetic acid : 70% ethanol = 1:1:1:17) for one week, washed in water, immersed in 1% KOH solution for one day, and cleared through 50 and 70 % glycerin [12-14]. Cleared specimens were examined for 14th rib under a dissecting microscope. According to Higuchi [11], 14th ribs were classified into "rib type" which is separated from vertebra and "process type" which is connected to vertebra. Further, these types of 14th rib were classified into rudiment type and extra type (longer than half the 13th rib). Number of pre-sacral vertebrae were counted. When unilateral lumbarization occurred, number of pre-sacral vertebrae were recorded like as 26/27.

Statistical analysis: Values of treated groups were compared with those of the control group of the same strain of rats. Student t-test was applied to the parameters represented as mean and standard deviation. Rank-sum test was applied to the mean percentage values of litter parameters. Fisher's exact test was applied to the frequency values. For comparison between strains, two-way analysis of variance was applied to the parameters represented as mean values and Mantel-Haenszel test or Fisher's exact test was applied to the values represented as frequencies.

RESULTS

Effects on dams: There were no notable changes in general conditions of control and treated dams during the entire gestation period. Mean body weight gains of dams are shown in Table 1. Within each strain, there were no significant differences in the initial body weight and the body weight gains during gestation between the groups except slight but significant differences before treatment. If compared between strains, the initial body weight of IGS rats was significantly lower than that of SD rats. The body weight gains during gestation did not differ between strains.

Effects on litter parameters and gross anomalies: Litter parameters were shown in Table 2. Within each strain there were no significant differences in all litter parameters between the groups. In comparison between strains, there were no significant differences. But, there may be a tendency that number of corpora lutea, implantation and live fetuses are slightly fewer and weights of fetuses are slightly higher in IGS rats than those in SD rats.

In the external examination at cesarean section, only one fetus from a dam of IGS strain treated with 40 mg/kg of RA on day 13 of gestation showed multi-complex malformations (short trunk, anury, umbilical hernia, adactyly and monodactyly). There were no other gross malformations other than digital anomalies and cleft palate which were confirmed under dissecting microscope after fixation.

Variant patterns of palatal rugae: Results of palatal examination are shown in Table 3. In the control group of both strains, 75 % of fetuses showed some variant patterns of palatal rugae. Supernumerary ruga of rudiment type anterior to ruga 5 (zone Ⅱ), peakless of ruga 6 (zone Ⅱ) and median discontinuity of ruga 7

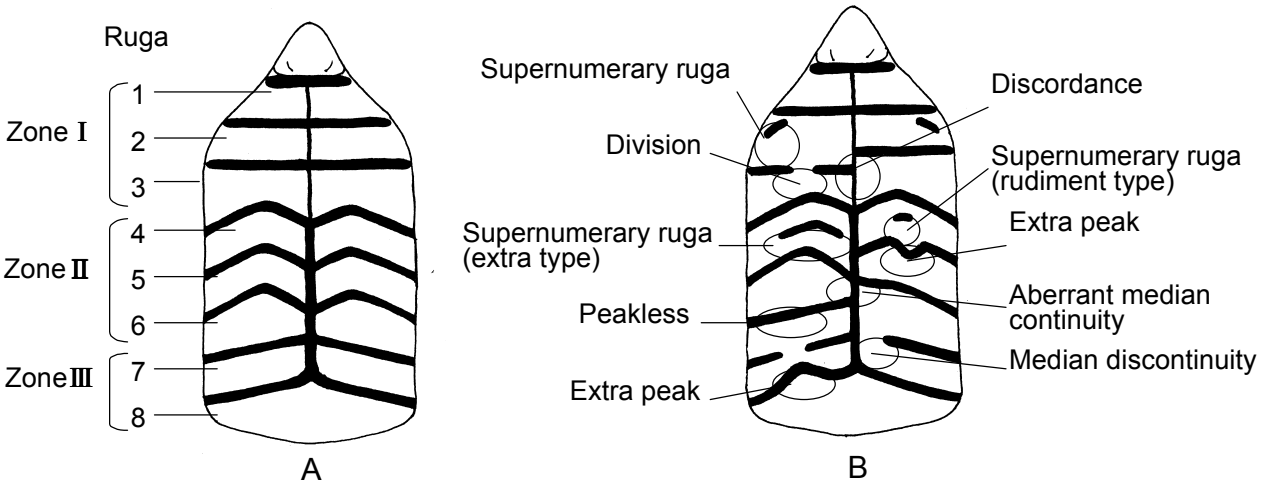

Fig. 1 Schematic drawing of palatal rugae in rat fetus (referred to Ikemi's method).
A: Normal pattern B: Variant patterns

Table 1. Body weight gain of pregnant Crj:CD(SD) and Crj:CD(SD)IGS rats treated with all-trans retinoic acid

Mean±S.D.

Strain		Crj:CD(SD)			Crj:CD(SD)IGS			Comparison between strains p=
Group		0	1	2	3	4	5	
Treatment		Control	Day 10 10 mg/kg	Day 13 40 mg/kg	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	
Number of dams		7	7	7	7	7	7	
Initial body weight(g)		253±10	250±12	254±7	228±10	233±11	229±4	0.000**
Body weight gain (g) on day of gestation	4	19.7±4.7	24.4±4.8	25.4±4.4*	20.3±3.4	18.6±4.1	25.1±6.1	0.203
	7	27.9±8.3	34.0±7.5	34.6±7.6	31.3±5.1	30.3±5.8	34.9±7.1	1.000
	10	41.0±7.2	47.6±9.4	51.1±8.6*	43.1±7.3	44.0±4.5	47.3±9.9	0.481
	11	44.3±8.3	47.7±10.1	53.4±11.7	48.1±6.4	47.1±4.5	51.4±9.1	0.874
	13	56.1±10.0	59.0±9.0	66.4±11.9	57.4±8.2	60.0±5.0	63.6±10.7	0.948
	14	60.6±12.8	62.3±6.9	70.6±13.6	61.9±7.9	62.7±5.9	68.9±11.0	1.000
	17	90.6±17.5	93.1±11.7	99.1±15.3	91.1±10.0	92.1±10.8	99.1±11.1	0.972
	19	120±25	120±14	133±20	121±12	122±13	129±13	0.993
	21	154±29	155±22	164±23	152±13	155±14	166±16	0.946

t-test was applied for comparison between treated groups and control group of each strain.
Two way analysis of variance was applied for comparison between strains. *: $p<0.05$, **: $p<0.01$

Table 2. Litter parameter from Crj:CD(SD) rats and Crj:CD(SD)IGS rats treated with all-trans retinoic acid

Mean±S.D.

Strain of rats	Crj:CD(SD)			Crj:CD(SD)IGS			Comparison between strains p=
Group	0	1	2	3	4	5	
Day of treatment Dose	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	
Maternal animals							
Females employed	7	7	7	7	7	7	
Pregnant females	7	7	7	7	7	7	
Mortality	0	0	0	0	0	0	
Pregnant females at term	7	7	7	7	7	7	
Dams with live fetuses	7	7	7	7	7	7	
Total number							
Corpora lutea	125	117	120	107	122	108	
Pre-implantation loss	16	7	6	6	10	3	
Implantation sites	109	110	114	101	112	105	
Post-implantation loss	5	6	7	5	9	4	
Early resorptions	5	6	7	5	7	1	
Late resorptions	0	0	0	0	2	3	
Dead fetuses	0	0	0	0	0	0	
Live fetuses	104	104	107	96	103	101	
Sex ratio(M:F)	46:58	55:49	48:59	46:50	51:52	49:52	
Average per litter							
Corpora lutea	17.9±2.6	16.7±2.6	17.1±1.8	15.3±1.6	17.4±3.0	15.4±1.5	0.095
Pre-implantation loss(%)	11.7	6.3	4.8	5.2	7.5	2.5	0.436
Implantation sites	15.6±3.6	15.7±3.0	16.3±1.8	14.4±1.0	16.0±2.2	15.0±1.2	0.326
Post-implantation loss(%)	4.3	5.1	6.3	5.2	7.9	4.2	0.797
Live fetuses	14.9±3.4	14.9±2.8	15.3±2.1	13.7±1.6	14.7±2.4	14.4±1.9	0.348
Litter weight(g)	74.3±18.2	75.1±14.2	77.3±11.4	72.8±9.1	75.1±12.7	72.8±12.5	0.635
Fetal weight(g) Males	5.14±0.38	5.18±0.19	5.20±0.27	5.42±0.29	5.18±0.31	5.21±0.31	0.295
Females	4.89±0.29	4.92±0.21	4.94±0.37	5.18±0.25	5.02±0.38	4.91±0.47	0.108

Fisher's exact test, t-test or rank-sum tests were applied for comparison between treated groups and control group of each strain.
Two way analysis of variance was applied for comparison between strains.

(zone Ⅲ) were relatively frequent. Supernumerary ruga, division and median discontinuity in zone Ⅰ, and division, aberrant median discontinuity, discordance, extra peak in zone Ⅱ or Ⅲ were observed in low frequencies. In the fetuses from dams treated with 10 mg/kg of RA on day 10 of gestation, the patterns and frequencies of variants were almost same as those in the control fetuses. When the dams were treated with 40 mg/kg of RA on day 13 of gestation, cleft palate was observed in one among 54 fetuses of SD strain and in four among 50 fetuses of IGS strain. In the same groups, 100% of fetuses showed anomalous palatal rugae and the variant patterns were dramatically changed in both strains. In zone Ⅰ, supernumerary ruga posterior to ruga 2 was markedly increased, while no other variants were seen. In zone Ⅱ, supernumerary ruga of extra type anterior to ruga 5, which was rare at the control and day 10 treated groups, markedly increased. Also in Zone Ⅱ, division of ruga 5, peakless of ruga 6 and aberrant median continuity were significantly increased compared with controls. In zone Ⅲ median discontinuity and division of ruga 7 and extra peak of ruga 8 were significantly increased. The increasing pattern with treatment was surprisingly same in both strains and the statistical comparisons of frequencies of theses variants showed almost no differences between SD and IGS rats.

Anomalies of digits and foot pads: Results of examination of digits and foot pads are shown in Table 4. In both strains, the control fetuses showed no abnormalities in digits and foot pads, as well as the fetuses from dams treated with RA on day 10. When dams were treated with RA on day 13 of gestation, 26% of fetuses had digital anomalies and almost all fetuses showed anomalies in foot pads in both strains. Digital anomalies observed were mainly olygodactyly, syndactyly and brachydactyly. Among them incidence of oligodactyly (four digits) in hind limbs was the highest in both strains. In fore paws, brachydactyly or nail anomalies were seen only in IGS rats, while syndactyly was seen only in SD rats. One fetus of IGS strain showed severe anomalies, adactyly in right fore paw and monodactyly in left fore paw and olygodactyly (three digits) in hind paws. But, their incidences were low and did not differ significantly between strains. Fetuses with abnormal foot pad was 96.3% and 94.0% in SD and IGS rats, respectively. In fore paws, fusion of 4th interdigital pad and hypothener pad was frequently observed, and the incidence was 72.2% and 74.0%, respectively in each strain. In hind paws, fusion of 1st interdigital pad and medial tarsal pad were frequently observed, and the incidence was 68.5% and 74.0%, respectively in each strain. Accompanied with olygodactyly or syndactyly, reduced number of interdigital pads or hypoplasia of interdigital

Table 3. Variant patterns of palatal rugae of fetuses from Crj:CD(SD) and Crj:CD(SD)IGS rats treated with all-trans retinoic acid

( ) : %

Strain of rats		Crj:CD(SD)			Crj:CD(SD)IGS			Comparison between strains
Group		0	1	2	3	4	5	
Day of treatment Dose		Control	Day 10 10 mg/kg	Day 13 40 mg/kg	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	p=
Litters examined		7	7	7	7	7	7	
Fetuses examined		52	53	53	48	51	45	
Fetuses with findings		39 (75.0)	41 (77.4)	53 (100)**	36 (75.0)	34 (66.7)	45 (100) **	0.471
Zone Ⅰ	Supernumerary ruga	2 (3.8)	1 (1.9)	43 (81.1)**	5 (10.4)	4 (7.8)	35 (77.8) **	0.474
	Division	3 (5.8)	5 (9.4)	0	1 (2.1)	4 (7.8)	0	0.633
	Median discontinuity	2 (3.8)	3 (5.7)	0	1 (2.1)	1 (2.0)	0	0.490
	Discordance	1 (1.9)	3 (5.7)	0	0	1 (2.0)	0	0.394
Zone Ⅱ	Supernumerary ruga (extra)	0	0	46 (86.8)**	1 (2.1)	0	37 (82.2) **	0.960
	Supernumerary ruga (rudiment)	9 (17.3)	13 (24.5)	6 (11.3)	15 (31.3)	8 (15.7)	7 (15.6)	0.616
	Division	5 (9.6)	14 (26.4)	38 (71.7)**	4 (8.3)	8 (15.7)	33 (73.3) **	0.512
	Aberrant median continuity	5 (9.6)	6 (11.3)	14 (26.4)*	1 (2.1)	1 (2.0)	12 (26.7) **	0.181
	Discordance	5 (9.6)	13 (24.5)	5 (9.4)	8 (16.7)	10 (19.6)	2 (4.4)	0.938
	Fusion	0	0	0	0	0	3 (6.7)	0.189
	Median discontinuity	3 (5.8)	4 (7.5)	8 (15.1)	1 (2.1)	0	8 (17.8) *	0.453
	Peakless	15 (28.8)	13 (24.5)	28 (52.8)*	17 (35.4)	20 (39.2)	23 (51.1)	0.275
	Extra peak	6 (11.5)	4 (7.5)	8 (15.1)	3 (6.3)	3 (5.9)	9 (20.0)	0.983
	Absence	0	0	0	1 (2.1)	0	0	0.968
	Shortness	0	0	0	0	0	2 (4.4)	0.407
Zone Ⅲ	Median discontinuity	22 (42.3)	17 (32.1)	38 (71.7)**	12 (25.0)	16 (31.4)	39 (86.7) **	0.927
	Division	6 (11.5)	0 *	18 (34.0)**	2 (4.2)	3 (5.9)	10 (22.2) *	0.333
	Extra peak	8 (15.4)	0 **	40 (75.5)**	2 (4.2)	3 (5.9)	24 (53.3) **	0.035*
	Aberrant median continuity	4 (7.7)	4 (7.5)	4 (7.5)	2 (4.2)	0	8 (17.8) *	0.947
	Shortness	1 (1.9)	0	0	0	0	0	0.968
	Fusion	0	0	3 (5.7)	0	0	6 (13.3) *	0.340
	Discordance	0	0	0	0	1 (2.0)	0	0.985
	Supernumerary ruga	0	0	0	0	0	1 (2.2)	0.935

Fisher's exact test was applied for comparison between treated groups and control group of each strain.
Mantel-Haenszel test was applied for comparison between strains. *:P<0.05, **:P<0.01

pad were observed. There were no differences in incidences of each pattern of anomalous foot pads between strains.

14th rib and number of pre-sacral vertebrae: Results of examination of 14th ribs are shown in Table 5. In the control groups, only two fetuses of SD strain and only one fetus of IGS strain showed 14th rib. These three cases of 14th rib was rudimental "process type" only. All of totally 99 control fetuses had 26 pre-sacral vertebrae except one fetus of SD strain with 27 pre-sacral vertebrae. In all treated groups, fetuses with 14th ribs were significantly increased. The fetuses from dams treated with RA on day 10 of gestation, "rib type" 14th rib was significantly increased in both strains. Among "rib type" 14th ribs, incidence of rudiment type was equal in both strains. Extra type was observed in both strains with significant difference in SD strain, but without significant defference between strains. Process type 14th rib was also increased in these groups, while significant difference was observed only within IGS strain. In the groups with treatment on day 10, fetuses with 26 pre-sacral vertebrae was significantly decreased to about 15 % instead that fetuses with 27 pre-sacral vertebrae was significantly increased to about 80 % in both strains. On the other hand, in the fetuses from dams treated with RA on day 13 of gestation "process type" 14th rib was markedly increased to 60.4% in SD strain and to 84.3% in IGS strain, and most of them were rudiment in both strains. "Rib type" 14th rib was seen only in two fetuses of IGS strain. In all fetuses of day 13 treated groups, number of pre-sacral vertebrae was 26. By statistical comparison between strains, significant differences were shown in number of fetuses with process type rudiment 14th ribs, resulted in significant differences in total number of fetuses with 14th ribs.

DISCUSSION

In this study, we aimed to examine susceptibility of Crj:CD (SD)IGS rats to teratogenic substance comparing with Crj:CD(SD) rats. All-trans retinoic acid, a well-known teratogen was given to pregnant rats at a dose of 10 mg/kg on day 10 or 40 mg/kg on day 13 of gestation. Minor variations which are known to be indicators of teratogenicity were examined in their fetuses.

The body weight of IGS rats was significantly lower than that of SD rats, like as repeatedly shown by CD(SD)IGS Study Group

Table 4. Anomalies of digits and foot pads in fetuses from Crj:CD(SD) and Crj:CD(SD)IGS rats treated with all trans-retinoic acid

( ) : %

Strain of rats	Crj:CD(SD)			Crj:CD(SD)IGS		
Group	0	1	2	3	4	5
Treatment	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	Control	Day 10 10 mg/kg	Day 13 40 mg/kg
Litters examined	7	7	7	7	7	7
Fetuses examined	52	53	54	48	51	50
Digits						
Fetuses with digital anomaly	0	0	14 (25.9)**	0	0	13 (26.0)**
Fore paw						
Adactyly and monodactyly	0	0	0	0	0	1 (2.0)
Oligodactyly (four digits)	0	0	2 (3.7)	0	0	1 (2.0)
Syndactyly (3rd and 4th digits)	0	0	2 (3.7)	0	0	0
Syndactyly (4th and 5th digits)	0	0	1 (1.9)	0	0	0
Brachydactyly with flat nail (2nd digit)	0	0	0	0	0	4 (8.0)
Brachydactyly without nail (2nd digit)	0	0	0	0	0	0
Brachydactyly without nail (3rd and 4th digits)	0	0	0	0	0	1 (2.0)
Aplasia of nail (3rd digit)	0	0	0	0	0	2 (4.0)
Hypoplasia of nail (4th digit)	0	0	0	0	0	1 (2.0)
Hind paw						
Oligodactyly (three digits)	0	0	0	0	0	1 (2.0)
Oligodactyly (four digits)	0	0	9 (16.7)**	0	0	7 (14.0)*
Syndactyly (1st and 2nd digits)	0	0	0	0	0	1 (2.0)
Syndactyly (2nd and 3rd digits)	0	0	4 (7.4)	0	0	2 (4.0)
Syndactyly (3rd and 4th digits)	0	0	0	0	0	1 (2.0)
Foot pads						
Fetuses with abnormal foot pad	0	0	52 (96.3)**	0	0	47 (94.0)**
Fore paw						
Fusion (4th interdigital and hypothener pads)	0	0	39 (72.2)**	0	0	37 (74.0)**
Fusion (3rd and 4th interdigital pads)	0	0	1 (1.9)	0	0	2 (4.0)
Bifid (2nd interdigital pad)	0	0	1 (1.9)	0	0	1 (2.0)
Reduced number of interdigital pads	0	0	4 (7.4)	0	0	2 (4.0)
Hypoplasia (2nd interdigital pad)	0	0	1 (1.9)	0	0	1 (2.0)
Hypoplasia (3rd interdigital pad)	0	0	1 (1.9)	0	0	0
Hind paw						
Fusion (1st interdigital and medial tarsal pads)	0	0	37 (68.5)**	0	0	37 (74.0)**
Fusion (4th interdigital and lateral tarsal pads)	0	0	6 (11.1)*	0	0	3 (6.0)
Fusion (2nd and 3rd interdigital pads)	0	0	5 (9.3)	0	0	1 (2.0)
Fusion (medial and lateral tarsal pads)	0	0	1 (1.9)	0	0	0
Bifid (2nd interdigital pad)	0	0	4 (7.4)	0	0	0
Reduced number of interdigital pads	0	0	4 (7.4)	0	0	4 (8.0)
Hypoplasia (2nd interdigital pad)	0	0	3 (5.6)	0	0	3 (6.0)
Hypoplasia (3rd interdigital pad)	0	0	1 (1.9)	0	0	5 (10.0)

Fisher's exact test was applied for comparison between treated group and control group of each strain. *:$p<0.05$, **$p<0.01$
For comparison between strain Fisher's exact test was applied, and no significant differences were observed.

[15, 16]. On the other hand, the body weight gain of pregnant dams during gestation did not differ between SD and IGS rats. In comparison of body weight gain between control and treated groups within each strain, no significant differences were observed except before treatment. Therefore, treatment with RA did not influence maternal body weight in both strains.

There were no differences in litter parameters between the groups within each strain. Therefore, 10/40 mg/kg RA given to pregnant rats on day 10/13, respectively, did not show lethal or toxic effects on fetuses. Also in comparison between strains, there were no significant differences. But, there may be very slight tendencies that number of corpora lutea, implantation and viable fetuses are slightly fewer and weights of fetuses are slightly higher in IGS rats than those of SD rats, as mentioned in several reports of CD(SD)IGS Study Group [16, 17] .

In the examination of palatal rugae, supernumerary ruga of rudimentary type and peakless in zone Ⅱ and median discontinuity in zone Ⅲ were mainly observed in the control fetuses. Including them the frequencies of variant patterns observed in control fetuses agreed well between SD and IGS rats. In the fetuses from dams treated on day 10, variant patterns were almost same as controls in both strains. These results indicate that there are no differences in patterns of palatal rugae between the strains at spontaneous condition and that the treatment with 10 mg/kg of RA on

Table 5. Type of 14th rib and number of pre-sacral vertebrae in fetuses from Crj:CD(SD) and Crj:CD(SD)IGS ratstreated with all-trans retinoic acid

( ) : %

Strain of rats	Crj:CD(SD)			Crj:CD(SD)IGS			Comparison between strains p=
Group	0	1	2	3	4	5	
Day of treatment Dose	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	Control	Day 10 10 mg/kg	Day 13 40 mg/kg	
Litters examined	7	7	7	7	7	7	
Fetuses examined	51	51	53	48	52	51	
Fetuses with 14th rib	2 (3.9)	20 (39.2)**	32 (60.4)**	1 (2.1)	25 (48.1)**	43 (84.3)**	0.032*
"Rib type "	0	15 (29.4)**	0	0	12 (23.1)**	2 (3.9)	0.962
rudiment	0	10 (19.6)**	0	0	10 (19.2)**	1 (2.0)	0.966
extra	0	9 (17.6)**	0	0	2 (3.8)	1 (2.0)	0.124
"Process type"	2 (3.9)	6 (11.8)	32 (60.4)**	1 (2.1)	15 (28.8)**	43 (84.3)**	0.003**
rudiment	2 (3.9)	6 (11.8)	31 (58.5)**	1 (2.1)	15 (28.8)**	41 (80.4)**	0.005**
extra	0	0	2 (3.8)	0	1 (1.9)	5 (9.8)	0.257
Pre-sacral vertebral number							
26	50 (98.0)	8 (15.7)**	53 (100)	48 (100)	8 (15.4)**	51 (100)	0.961
26/27	0	4 (7.8)	0	0	1 (1.9)	0	0.350
27	1 (2.0)	39 (76.5)**	0	0	43 (82.7)**	0	0.770

Fisher's exact test was applied for comparison between treated groups and control group of each strain.
Mantel-Haenszel test was applied for comparison between strains. *:p<0.05, **:p<0.01

day 10 of gestation had no influence on formation of palatal rugae. When dams were treated with 40 mg/kg of RA on day 13, cleft palate was observed in low frequencies, while all fetuses showed anomalous variant patterns of palatal rugae. In the fetuses of these groups supernumerary ruga in zone Ⅰ and supernumerary ruga of extra type in zone Ⅱ and extra peak in zone Ⅲ were markedly increased, in contrast to that they were rare in the control and day 10 treated groups. Division, aberrant median continuity and peakless in zone Ⅱ, median discontinuity and division in zone Ⅲ were significantly increased compared with the controls. The increasing pattern agreed very well between SD and IGS rats. Therefore, regarding variant patterns of palatal rugae, sensitivity of SD and IGS rats to RA was considered to be same.

Digital and foot pad anomalies were also observed only in day 13 treated groups. Digital anomalies were observed in 26% of fetuses equally in both strains. In fore paws syndactyly was seen only in a few fetuses of SD strain and brachydactyly with nail anomalies was seen only in a few fetuses of IGS strain. However, type of digital anomalies and their incidences were not different overall between strains. Anomalous foot pads were observed in about 95% of fetuses also equally in both strains. The abnormal patterns and their frequencies were compared well between the strains. These results also showed same sensitivity of SD and IGS rats to RA.

In this study, incidence of 14th rib was low in the control group of both strains, Cnamely 3.9% in SD strain and 2.1% in IGS strain. Almost all control fetuses of both strains had 26 pre-sacral vertebrae. In the reports presented in Biological Reference Data on CD(SD)IGS Rats, incidences of 14th ribs in IGS rats have large variation from 1.1% to 16.2% and were relatively high, in contrast to that SD rats showed low values of 0 - 2.9% [16-24]. When dams were treated on day 10 of gestation, fetuses with "rib type" 14th rib were significantly increased accompanied with increase of pre-sacral vertebral number to 27. When dams were treated on day 13 of gestation, fetuses with "process type" 14th rib were markedly increased without increase of pre-sacral vertebral number. These results were well agreed with Higuchi's reports [11], and equally seen in both strains in this study. The difference between strains observed in this study was only higher rate of "process type" 14th rib in treated groups of IGS strain. Probably it may be not due to a difference of reaction of animals to RA but due to that IGS is a strain with relatively high frequency of 14th rib at spontaneous condition, in spite of occasionally low incidence in the controls of this study.

From these results in this study, it can be concluded that there are no differences in susceptibility of fetuses to all-trans retinoic acid between Crj:CD(SD)IGS and Crj:CD)SD rats.

REFFERENCES

1. Yasuda, M. and Maeda, H. 1973. Significance of the lumbar rib as an indicator in teratogenicity tests. Cong. Anom., 13: 25-29.
2. Yasuda, M. Ohya R., Sato, T. and Inoue N. 1992. Variations in the palatal rugae in the mouse as an indicator for detection of teratogenicity. J. Toxicol. Sci., 17: 348
3. Ikemi, N., Kawata, M., Goto, T. and Yasuda, M. 1994. Variations in palatal rugae in near term fetuses from Crj:CD rats. Cong. Anom., 34: 268.
4. Yasuda, M. Ohya R. and Sato, T. 1994. Variations in palatal rugae in near term fetuses from untreated Jcl: ICR mice. Cong. Anom., 34: 71-75.
5. Yasuda, M., Horie, S., Matsui, K., Takagi, T. and Yamashita, K. 1998. Variant patterns of palatal rugae induced by chemicals in mouse fetuses. Cong. Anom., 38: 87-95.

6. Tsugane, M. and Yasuda, M. 1993. Dermatoglyphics on mouse palm and sole: Abnormal patterns induced by all-trans retinoic acid. Cong. Anom., 33: 302-303.
7. Horie, S. and Yasuda, M. 1994. Variations in palatal rugae in mouse fetuses from dams treated with retinoic acid (2nd report). Cong. Anom., 34: 274.
8. Ikemi, N., Kawata, M. and Yasuda, M. 1995. All-trans-retinoic acid-induced variant patterns of palatal rugae in Crj:SD rats fetuses and their potential as indicators for teratogenicity. Reproductive Toxicology, 9: 369-377.
9. Yasuda, M., Ohya R., Sato, T and Tsugane, M. 1993. Variations in the mouse palatal rugae induced by retinoic acid. Cong. Anom., 33: 281.
10. Higuchi, H., Kawamura, T., Kato, T. and Shiota, K. 1993. Effects of retinoic acid on development of axial skeleton of rat fetus. Cong. Anom., 33: 301-302.
11. Higuchi, H., Kawamura, S., Matsuo, M. and Shiota, K. 1994. Two types of 14th rib in the rat fetus induced by retinoic acid. Cong. Anom., 34: 274.
12. Inouye, M. 1976. Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S. Cong. Anom., 16: 171-173.
13. Kimmel, C. and Trammell, C. 1981. A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain Technology, 56: 271-273.
14. Shinomiya, M., Yoshida, T., Itoh, K., Nishimura, T., Terasaka, M., Yahagi, K., Igarashi, E. and Yasuda, M. 1998. Double staining methods for fetal rat and rabbit skeletons in reproductive and developmental toxicity studies. Cong. Anom., 38: 338
15. Yamamoto, T., Kakamu, S., Suzuki, K., Sugiyama, Y., Mukai, D., Ohba, K., Yamakawa, S., Iwata, H. and Inoue, H. 1998. Mortality, body weight, food consumption, clinical laboratory test and pathological examination in Crj:CD (SD)IGS rats in subchronic and chronic toxicity studies. 1998. CD(SD)IGS-1998: 39-42.
16. Matsumoto, A., Ohta, R., Sato, M. Kojima, K., Seki, T., Azegami, J. and Nagao, T. 1998. Comparison between Crj:CD(SD)IGS and Crj:CD(SD) rats in reproductive and developmental parameters. CD(SD)IGS-1998: 155-163.
17. Tanaka, R., Fujiwara, C., Ito, K., Sakamoto, C., Yamada, R., Ogawa, T. and Inoue, H. 1998. Comparison of reproductive and developmental parameters in Crj:CD(SD)IGS rats with the historical control data of Crj:CD(SD) rats. CD(SD)IGS-1998: 248-260.
18. Okada, A., Yahagi, K., Fujiwara, M. and Matsuzawa, T. 1998. Reference data of reproductive and developmental toxicity studies in CD(SD)IGS rats. CD(SD)IGS-1998: 188-190.
19. Matsumoto, H., Fukumura, M. Yashihara, K., Sasa, H. and Tanaka, C. 1988. Characteristics of Crj:CD(SD)IGS rats compared with Crj:CD(SD) rats based on a study for effects on embryo-fetal development. CD(SD)IGS-1998: 191-193.
20. Umemura, T., Ishida, S. and Katsumata, Y. 1998. Comparison of reproductive and developmental parameters between Crj:CD(SD) rat and Crj:CD(SD)IGS rats. CD (SD)IGS-1998: 206-213.
21. Matsumoto, K., Fuji, J., Sugitani, T., and Ooshima, Y. 1998. Fertility and general reproductive performance of Crj:CD (SD)IGS rats fed a low protein diet. CD(SD)IGS-1998: 223-232.
22. Matsumoto, K., Kawamura, Y., Nakatsu, T. and Ooshima, Y. 1998. Historical control data for Crj:CD(SD)IGS rats used in teratology studies. CD(SD)IGS-1998: 233-235.
23. Kamijima, M., Kinoshita, K., Koike, A., Sasaki, M., Sato, S., Tabata, K., Onotogi, M., Shimojima, A. and Shibano, T. 1998. Historical control data for reproductive and developmental toxicity studies in Crj:CD(SD)IGS rats - Teratology study -. CD(SD)IGS-1998: 238-239.
24. Kizaki, H., Shibuya, K., Saitoh, T., Shibuya, N., Ihara, M., Itabashi, M., Kudow, S., Nunoya, T. and Tajima, M. 1998. Background data for reproductive and developmental toxicity studies in Crj:CD(SD)IGS rats. CD(SD)IGS-1998: 240-243.

CHAPTER 4

Carcinogenicity Related To

A Comparison of the In-Life Parameters and Preliminary Tumour Data in Ten Gang-Housed Dietary Tumorigenicity Studies Using the Charles River International Genetic Standard or Original Strain Designations of Sprague-Dawley Rat.

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ABSTRACT. At Huntingdon Life Sciences, data obtained from the Charles River International Genetic Standard (IGS) strain of Sprague-Dawley rat have been closely monitored since the introduction of this new strain designation in 1996. In-life and preliminary tumour data from the control groups of ten gang-housed dietary tumorigenicity studies have been assessed, with five IGS rat studies (completed 1998-99) compared against five studies (completed 1994-97) using the original strain of Sprague-Dawley rat. Low protein maintenance diet was used in all these studies. These comparisons have shown that the IGS rat is showing a similarly high mortality pattern to that seen in the original strain studies. The bodyweight growth pattern, bodyweight gain and food consumption data analysed over the first year have only shown minor differences between the IGS rat and the original strain of rat. Preliminary assessment of the tumour profile has not shown any major differences between the two groups. From the results currently available, it can be concluded that the IGS rat is not remarkably different from the original strain of rat, showing the same high mortality pattern particularly in female rats. — Key words: Bodyweight, Crl:CD® BR (VAF) IGS rat, food consumption, mortality, tumour profile.

CD(SD)IGS-1999: 229-235

INTRODUCTION

The Charles River International Genetic Standard (IGS) strain designation of Sprague-Dawley rat Crl:CD® BR (VAF) superseded the original strain designation of rat from 1996. At these laboratories, the data obtained from the IGS rat have been closely monitored and compared with data obtained from the original strain designation of Sprague-Dawley rat obtained from Charles River UK or USA [1, 2]. A comparison of data obtained from the first 13 weeks of gang housed dietary studies has shown that there were no remarkable differences in the in-life, laboratory and organ weight parameters examined between the IGS and original strain of Sprague-Dawley rat [3, 4]. The subject of this review is the assessment of the data obtained from ten gang-housed dietary tumorigenicity studies. Five IGS rat studies (completed 1998-99) were compared with five studies (completed 1994-97) using the original strain designation of Sprague-Dawley rat. The mortality pattern, bodyweight and food consumption data, and preliminary tumour profile have been assessed. This has been first opportunity, at these laboratories, to establish if there have been any major shifts in the tumour profile of the Charles River Sprague Dawley rat. As some of the IGS studies have only recently been completed, it is acknowledged that the data analysis is not complete. Further analysis, to include factors contributory to death and the number of tumour-bearing animals, will be performed and presented in future communications.

MATERIALS AND METHODS

Animals:

Male and female Sprague-Dawley Crl:CD® BR (VAF) rats obtained from Charles River breeding laboratories in the UK or USA and maintained as control rats for tumorigenicity studies at Huntingdon Life Sciences' Huntingdon facility. The rats were approximately 6 weeks of age at start of study, housed 5 rats/cage and maintained under standard laboratory conditions, with target ranges of 19-23℃ for temperature and 40-70% for relative humidity. A 12 hour light and 12 hour dark cycle was maintained. The animals were given a low protein rodent maintenance diet (Special Diets Services Rat and Mouse No. 1, typically 14.5% protein, 3% fat, 4% fibre) *ad libitum* throughout the study and tap water was also supplied *ad libitum* to the animals via water bottles.

Study Design:

The studies reviewed were restricted to dietary administered tumorigenicity studies that terminated between 1994 and 1999. The control groups reviewed were 5 completed studies for the original strain and 5 completed studies for the IGS strain designation. There were at least 50 males and 50 females in each control group.

Histopathological procedures:

In the comparison studies, the full list of tissues required by the regulatory authorities were taken at the necropsy, both at termination and from animals killed *in extremis* or found dead during the study. The tissues were fixed in 10% formalin (4% formaldehyde). Subsequently, the tissues were embedded in paraffin wax and sections, approximately 4 micrometer thick, were cut, processed routinely and stained with haematoxylin and eosin. The microscopic slides were read by a Pathologist and subjected to a peer review.

Data presentation and analysis:

Mortality:

The mortality pattern is presented in Figures 1 and 2 for the period of Weeks 52 to 104 only, as mortality in the first year is very low. The IGS rat studies completed in 1998-99 are compared with the original strain designation studies completed 1994-97. The mean terminal (Week 104) percentage mortality values are presented in Table 1 and a comparison based on a 't' distribution was made following analysis of variance to compare differences between the comparison groups.

Bodyweight:

The bodyweight growth pattern over the 104-week treatment

period is presented in Figure 3. The IGS rat studies are compared with the original strain studies. The mean bodyweight gain values over Weeks 0 to 52 (the period of maximal growth) are also compared over the same periods and presented graphically in Figure 4 and tabulated in Table 2. A comparison based on a 't' distribution was made following analysis of variance to compare differences.

Food consumption:

The mean weekly food consumption (g/rat/week) values are compared over the period of Weeks 1 to 52, graphically in Figure 5, with the IGS rat studies compared against the original strain studies, and tabulated in Table 2. A comparison based on a 't' distribution was made following analysis of variance to compare differences.

Tumour assessment:

From the histopathological data obtained from the comparison studies, a preliminary assessment of the tumours present has been performed and the results are presented in Table 4. Differences in percentage incidence of tumours of 5% or greater were found in male rats only and are presented graphically in Figure 6.

RESULTS AND DISCUSSION

The mortality pattern over the period of Weeks 52 to 104 is presented in Figures 1 (male rats) and 2 (female rats) and it is apparent that the pattern for the IGS rat dietary studies is similar to that of studies using the original strain of Sprague-Dawley rat.

Figure 1. Mortality over Weeks 52 to 104 in dietary studies - males

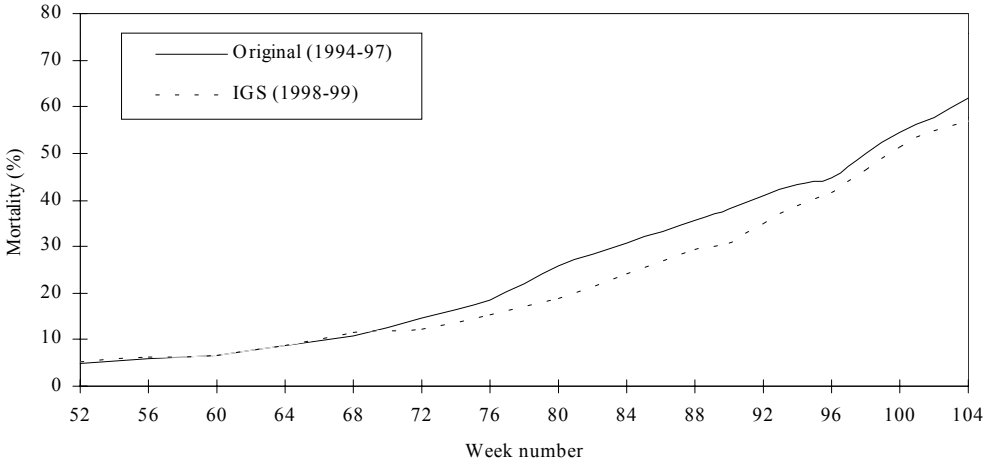
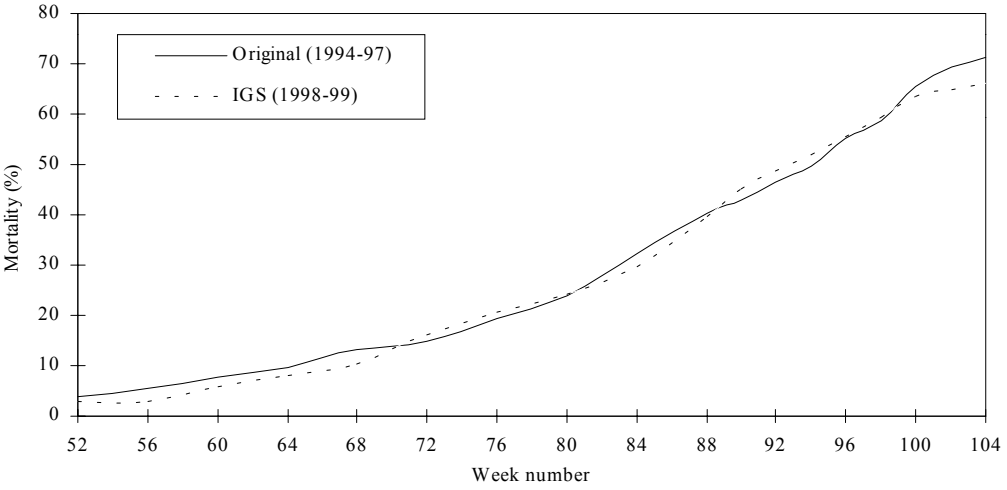


Figure 2. Mortality over Weeks 52 to 104 in dietary studies - females



The mean mortality at Week 104, together with the Standard deviation and number of studies, are detailed in Table 1. Although the terminal mortality values for the IGS rat studies were slightly lower than the values for the original rat studies, statistical significance was not demonstrated.

Table 1. Mortality (%) at Week 104 for dietary studies

Parameter	Week	Sex	Original/1994-97			IGS/1998-99			% difference	Significance
			Mean	SD	n	Mean	SD	n		
Mortality (%)	104	Males	62	7.3	5	57	6.2	5	- 8.1	ns
		Females	71	1.8	5	66	5.5	5	- 7.0	ns

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of studies.

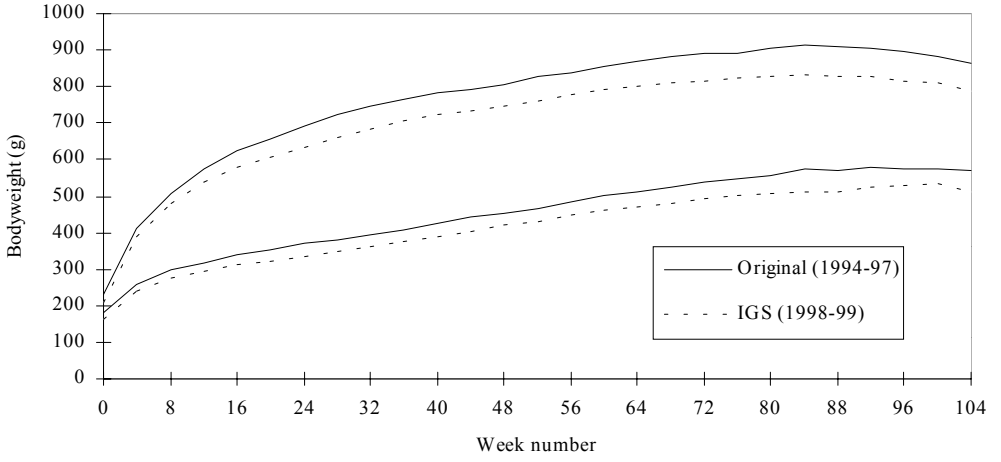
The bodyweight growth pattern over the 104-week study period for male and female IGS rats (Figure 3) was marginally lower than that recorded for the original strain of rat. Similarly, the bodyweight gain over Weeks 0 to 52 in male and female IGS rats (Figure 4) was marginally lower than the original strain comparison group, but statistical difference was not demonstrated (Table 2).

Table 2. Bodyweight gain (g) and mean food consumption values (g/rat/week) for dietary studies

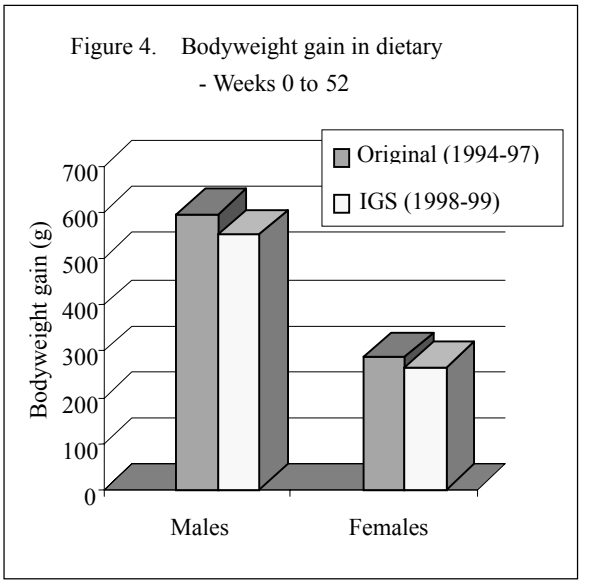
Parameter	Weeks	Sex	Original/1994-97			IGS/1998-1999			% difference	Significance
			Mean	SD	n	Mean	SD	n		
Bodyweight gain (g)	0 to 52	Males	597	42.1	5	551	18.8	5	- 7.7	ns
		Females	286	32.9	5	265	15.1	5	- 7.3	ns
Food consumption (g/rat/week)	1 to 52	Males	216	2.1	5	203	1.6	5	- 6.0	P<0.001
		Females	167	5.7	5	152	2.8	5	- 9.0	P<0.01

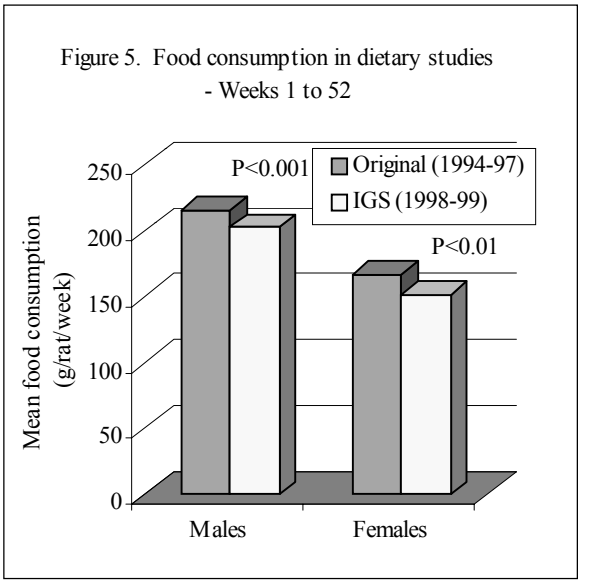
ns Not statistically significant (P>0.05), SD Standard deviation, n Number of studies.

Figure 3. Bodyweight growth pattern in dietary studies - Weeks 0 to 104



The mean weekly food consumption over the period of Weeks 1 to 52 (Figure 5 and Table 2) showed slightly lower values for the IGS rat studies in comparison to the original rat studies, with statistical significance attained for both males (P<0.001) and females (P<0.01). However, the magnitude of the difference was small and comparable to that seen for the bodyweight gain over this period.





No major changes in the tumour incidence (Table 3) have been noted in this first assessment. Differences in percentage incidence of 5% or greater were only observed for a small number of tumours in male rats only (Figure 6). In the light of the other information available in relation to bodyweight changes and survival, this is perhaps not a surprising finding.

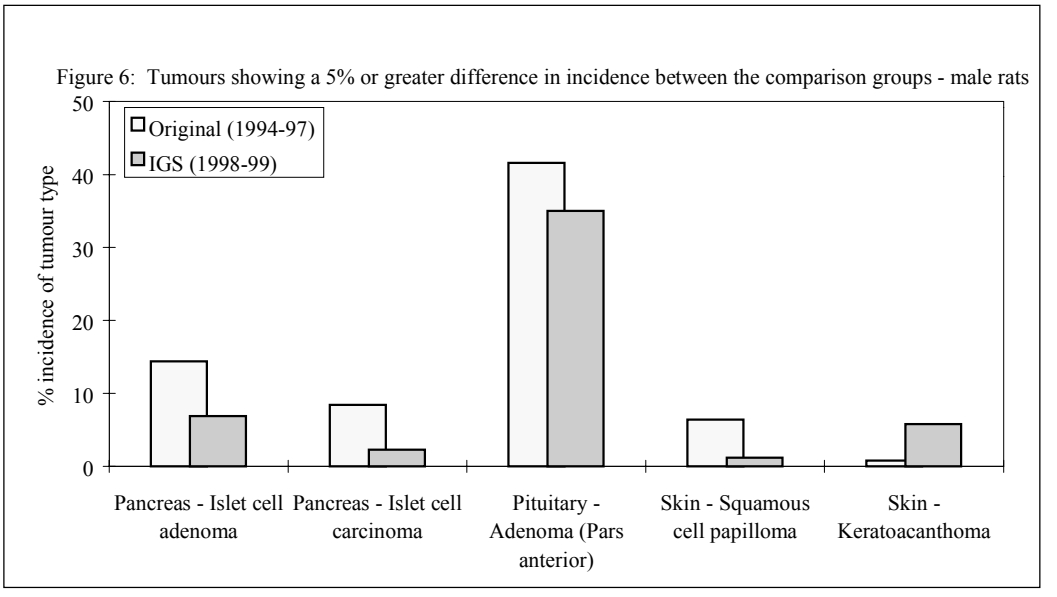


Table 3. Tumour incidence (%) in dietary studies

Tissue	Tumour type	Sex	Original/1994-97			IGS/1998-99		
			D	T	C	D	T	C
	No. of animals	M	156	94	250	147	113	260
		F	178	72	250	171	89	260
Lymphoid/ multicentric	Malignant lymphoma	M	3.2	0	2.0	2.0	0.9	1.5
		F	1.1	1.4	1.2	1.2	0	0.8
	Myeloid leukaemia	M	1.3	2.1	1.6	0.7	0	0.4
		F	0	0	0	0	0	0
	Histiocytic sarcoma	M	3.8	1.1	2.8	4.1	0	2.3
		F	3.4	0	2.4	2.3	0	1.5
Lungs	Bronchiolar alveolar adenoma	M	0	1.1	0.4	0.7	0	0.4
		F	0	0	0	0	0	0
	Bronchiolar alveolar carcinoma	M	0	1.1	0.4	0	0	0
		F	0	0	0	0	0	0
	Squamous cell carcinoma	M	0	0	0	0.7	0	0.4
		F	0	0	0	0	0	0
Liver	Hepatocellular adenoma	M	0.6	4.3	2.0	2.0	5.3	3.5
		F	0.6	4.2	1.6	0	0	0
	Hepatocellular carcinoma	M	0.6	0	0.4	0	1.8	0.8
		F	0	0	0	0	1.1	0.4
Pancreas	Exocrine adenoma	M	4.5	14.9	8.4	2.0	5.3	3.5
		F	1.7	0	1.2	0	1.1	0.4
	Islet cell adenoma	M	13.5	16.0	14.4	4.8	9.7	6.9
		F	4.0	6.9	4.8	2.3	4.5	3.1
	Islet cell carcinoma	M	3.9	16.0	8.4	2.0	2.7	2.3
		F	1.1	0	0.8	0	0	0
Kidney	Renal tubule adenoma	M	0	1.1	0.4	0	0	0
		F	0	0	0	0	0	0
	Renal tubule carcinoma	M	0	1.1	0.4	0.7	0	0.4
		F	0	0	0	0	0	0
	Pelvic transitional cell carcinoma	M	0	0	0	0.7	0	0.4
		F	0	0	0	0	0	0
Testis	Interstitial cell tumour	M	2.6	9.6	5.2	1.4	4.4	2.3
Prostate	Adenoma	M	0	0	0	0.7	0	0.4
	Adenocarcinoma	M	1.3	0	0.8	1.4	0	0.8
Ovary	Tubular adenoma	F	0.6	0	0.4	0	0	0
	Thecal cell tumour	F	0	1.4	0.4	0	0	0
	Tubulostromal adenoma	F	0	2.8	0.8	1.2	6.7	3.1
Uterus	Polyp	F	7.3	11.1	8.4	5.3	10.1	6.9
	Squamous cell carcinoma	F	0.6	0	0.4	0.6	0	0.4
Cervix	Fibroma	F	0.6	0	0.4	0.6	0	0.4
	Polyp	F	1.1	0	0.8	1.8	2.2	1.9
Thyroid	Follicular adenoma	M	2.6	1.1	2.0	4.8	7.1	5.8
		F	0	1.4	0.4	0.6	2.2	1.2
	Follicular carcinoma	M	2.6	2.1	2.4	0	2.7	1.2
		F	0	1.4	0.4	0	0	0
	C cell adenoma	M	5.8	9.6	7.2	2.0	11.5	6.2
		F	4.0	11.1	6.0	6.4	6.7	6.5
	C cell carcinoma	M	2.6	1.1	2.0	2.7	8.8	5.4
		F	1.7	2.8	2.0	1.8	3.4	2.3
	Ganglioneuroma	M	0.6	0	0.4	0	0	0
		F	0	0	0	0	0	0
Parathyroid	Adenoma	M	1.3	1.1	1.2	0	0.9	0.4
		F	0	0	0	0	0	0

M Males, F Female, D Decedent incidence (%), T Terminal incidence (%), C Combined incidence (%)

Table 3. Tumour incidence (%) in dietary studies - continued

Tissue	Tumour type	Sex	Original/1994-97			IGS/1998-99		
			D	T	C	D	T	C
Adrenal	Phaeochromocytoma	M	12.2	12.8	12.4	12.2	19.5	15.4
		F	2.8	1.4	2.4	3.5	4.5	3.8
	Malignant phaeochromocytoma	M	0.6	3.2	1.6	1.4	4.4	2.7
		F	1.1	0	0.8	0	0	0
	Cortical adenoma	M	0.6	1.1	0.8	1.4	0.9	1.2
		F	0.6	2.8	1.2	1.8	1.1	1.5
	Cortical carcinoma	M	0	0	0	0	0.9	0.4
		F	1.7	1.4	1.6	0	0	0
Pituitary	Adenoma pars anterior	M	41.6	42.6	41.6	32.7	38.1	35.0
		F	67.2	60.6	64.8	64.9	61.8	63.8
	Adenocarcinoma pars anterior	M	1.3	1.1	1.2	2.7	0	1.5
		F	12.4	7.0	10.8	11.7	6.7	10.0
	Adenoma pars intermedia	M	0.6	0	0.4	0.7	0	0.4
		F	0	0	0	0	0	0
Skin	Squamous cell papilloma	M	5.1	8.5	6.4	1.4	0.9	1.2
		F	0.6	0	0.4	0	2.2	0.8
	Sebaceous adenoma	M	0	1.1	0.4	0	0.9	0.4
		F	0	0	0	0	0	0
	Basal cell adenoma	M	1.3	1.1	1.2	1.4	1.8	1.5
		F	0	0	0	0	0	0
	Basal cell carcinoma	M	0	0	0	0.7	0	0.4
		F	0	0	0	0	0	0
	Keratoacanthoma	M	0.6	1.1	0.8	1.4	11.5	5.8
		F	0	0	0	0	0	0
Subcutis	Fibroma	M	15.4	17.0	16.0	10.2	14.2	11.9
		F	5.6	8.3	6.4	7.6	4.5	6.5
	Fibrosarcoma	M	1.9	1.1	1.6	3.4	0	1.9
		F	2.8	1.4	2.4	1.2	0	0.8
	Lipoma	M	4.5	10.6	6.8	5.4	9.7	7.3
		F	4.5	4.2	4.4	2.9	3.4	3.1
	Haemangiosarcoma	M	0.6	0	0.4	0	0	0
		F	0	0	0	0	0	0
	Anaplastic sarcoma	M	0.6	0	0.4	0	0	0
		F	0	0	0	0	0	0
	Osteosarcoma	M	0	0	0	1.4	0	0.8
		F	0	0	0	0	0	0
Mammary gland	Mammary adenoma	M	0	0	0	0	0	0
		F	3.4	4.2	3.6	1.8	0	1.2
	Mammary fibroadenoma	M	1.9	2.1	2.0	1.4	2.7	1.9
		F	63.5	75.0	66.8	55.0	80.0	63.5
	Mammary adenocarcinoma	M	0	2.1	0.8	1.4	0.9	1.2
		F	15.7	30.6	20.0	24.0	25.8	24.6
	Mammary fibroma	M	0	0	0	0	0	0
		F	2.2	8.3	4.0	1.2	3.4	1.9
Head	Squamous cell carcinoma (Zymbal)	M	0.6	0	0.4	2.0	0.9	1.5
		F	0	0	0	1.8	1.1	1.5

M Males, F Female, D Decedent incidence (%), T Terminal incidence (%), C Combined incidence (%)

In conclusion, the results from the data analysed from gang-housed dietary tumorigenicity studies, for mortality pattern, bodyweight pattern and food consumption, indicate that the IGS rat is not remarkably different from recent studies using the original strain of Charles River Sprague-Dawley rat. However, the longevity of the IGS rat does not appear, at this stage, to be an improvement over the original strain of rat, particularly in female rats. Additionally, the preliminary assessment of the tumour profile has shown no major differences between the two strain designations, which is not surprising in view of the similarity of the in-life parameters and longevity.

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REFERENCES

1. Hooks, W.N. and Saunders, M.D. 1998. A review of mortality in tumorigenicity studies, conducted using Charles River Sprague-Dawley rats, completed over the period 1987 to 1998. Toxicology Letters 1/85: 184.
2. Hooks, W.N. An ongoing review of the mortality, bodyweight and food consumption of Charles River Sprague-Dawley International Genetic Standard rat in comparison with the original strain designation in long term studies. Biological Reference Data on CD(SD)IGS Rats – 1998, edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.
3. Hooks, W.N. and Harris, B. 1998. A comparison between the original and International Genetic Standard Charles River (UK) designations of Sprague-Dawley rat, at the 13-week time point. The Toxicologist, 42: 59.
4. Hooks, W.N. A comparison between the original and International Genetic Standard Charles River (UK) designations of Sprague-Dawley rat, at the 13-week timepoint. Biological Reference Data on CD(SD)IGS Rats – 1998), edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.

An Ongoing Review of the Mortality, Bodyweight and Food Consumption of Charles River Sprague-Dawley International Genetic Standard Rats in Comparison with the Original Strain Designation in Long Term Studies – 1999 Update.

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ABSTRACT. At Huntingdon Life Sciences, data obtained from the Charles River International Genetic Standard (IGS) strain of Sprague-Dawley rat have been closely monitored since the introduction of this new strain designation in 1996. In this 1999 update, data from the control groups of ongoing and completed IGS rat tumorigenicity studies have been compared with the data from up to 70 studies using the original strain of Sprague-Dawley rat. Low protein maintenance diet was used in all these studies. These comparisons of dietary and oral gavage studies have shown that the IGS rat is showing a similarly high mortality pattern to that seen in the original strain studies completed during 1996-97, but an increased pattern when compared with studies completed during 1993-95. Review of the terminal mortality against time has demonstrated an increasing trend towards higher values over the period of 1987 to 1997, particularly in females. The 11 completed IGS rat studies show a similar trend to the original strain designation. The bodyweight growth pattern, bodyweight gain and food consumption data analysed over the first year have only shown minor differences between the IGS rat and the original strain of rat. From the results currently available, it can be concluded that the IGS rat is not remarkably different from the original strain of rat, showing the same high mortality pattern, particularly in female rats. — Key words: Bodyweight, Crl:CD® BR (VAF) IGS rat, food consumption, mortality, tumorigenicity studies.

CD(SD)IGS-1999: 236-242

INTRODUCTION

The Charles River International Genetic Standard (IGS) strain designation of Sprague-Dawley rat Crl:CD® BR (VAF) superseded the original strain designation of rat from 1996. At these laboratories, the data obtained from the IGS rat have been closely monitored and compared with data obtained from the original strain designation of Sprague-Dawley rat obtained from Charles River UK or USA [1, 2]. A comparison of data obtained from the first 13 weeks of gang housed dietary studies has shown that there were no remarkable differences in the in-life, laboratory and organ weight parameters examined between the IGS and original strain of Sprague-Dawley rat [3, 4]. The subject of this ongoing review, first published in this journal in 1998 [2], is the assessment of the performance of the IGS rat in dietary and oral gavage tumorigenicity studies in comparison with the original strain designation of Sprague-Dawley rat. The mortality pattern together with bodyweight and food consumption data have been assessed for ongoing and completed IGS studies and compared with data obtained over recent years (1993 to 1997) from the original strain designation of rat. As the longevity of the Sprague-Dawley rat continues to cause concern in laboratories throughout the world, a review of the mortality pattern has been conducted for studies completed over the period of 1987 to 1999. Previous reviews of the longevity of the Sprague-Dawley rat at these laboratories have been published for studies completed during 1985 to 1992 [5], which showed that changing from a high protein breeding diet to a lower protein maintenance diet resulted in lower mortality, mainly as a result of reduced incidence of death due to progressive glomerulonephrosis [6].

At these laboratories, a total of 11 IGS rat studies have completed 104 weeks and 13 studies have completed 52 weeks. This increasing IGS rat database will be subject to an ongoing review. As the data becomes available, an assessment of the histopathological profile of the IGS rat will be performed and presented in a subsequent publication.

MATERIALS AND METHODS

Animals:

Male and female Sprague-Dawley Crl:CD®BR (VAF) rats obtained from Charles River breeding laboratories in the UK or USA and maintained as control rats for tumorigenicity studies at Huntingdon Life Sciences' Huntingdon facility. The rats were approximately 6 weeks of age at start of study, mainly housed 5 rats/cage (singly housed studies are indicated, where appropriate) and maintained under standard laboratory conditions, with target ranges of 19-23°C for temperature and 40-70% for relative humidity. A 12 hour light and 12 hour dark cycle was maintained. The animals were given a low protein rodent maintenance diet (Special Diets Services Rat and Mouse No. 1, typically 14.5% protein, 3% fat, 4% fibre) *ad libitum* throughout the study and tap water was also supplied *ad libitum* to the animals via water bottles.

StudyDesign:

The studies reviewed were dietary or oral gavage administered tumorigenicity studies that terminated between 1987 and 1999. The numbers of control groups reviewed were 70 completed studies for the original strain and 11 studies for the IGS strain designation (the IGS data included studies ongoing in addition to the completed studies). There were at least 50 males and 50 females in each control group.

Data presentation and analysis:

Mortality:

The mortality pattern is presented in Figures 1 and 2 for the period of Weeks 52 to 104 only, as mortality in the first year is very low. The IGS rat studies, ongoing or completed in 1998-99 are compared with the original strain designation studies (1993-

95 and 1996-97). The ongoing IGS rat studies comprised 13 studies at Week 52 reducing to 11 studies at Week 104. Mean terminal (Week 104) percentage mortality values are presented over selected time periods for studies completed from 1987 to 1999, both graphically (Figure 3, all studies) and in Table 1 (detailing the route of administration and housing conditions). The distribution of the percentage mortality for each control group at study Week 104 is presented chronologically between 1987 and 1999 (Figures 4 and 5). A regression analysis of mortality against time [7] was performed followed by a two-tailed t-test of the slope; P-values and the direction of slope are presented for studies using the original strain designation of rat and completed during 1987 to 1997. Similar comparisons were also performed over the same period for gang-housed dietary studies only and over 1993 to 1997 for all studies.

Bodyweight:

The bodyweight growth pattern over the 104-week treatment period is presented in Figure 6. The IGS rat studies, ongoing or completed in 1998-99, are compared with the original strain studies (1993-95 and 1996-97). The mean bodyweight gain values over Weeks 0 to 52 (the period of maximal growth) are also compared over the same periods, graphically in Figure 7 for all studies; a comparison based on a 't' distribution was made following analysis of variance to compare intergroup differences. For comparative purposes, the bodyweight gain (Weeks 0 to 52) values are detailed in Table 2 for dietary and oral gavage administered studies over selected time periods.

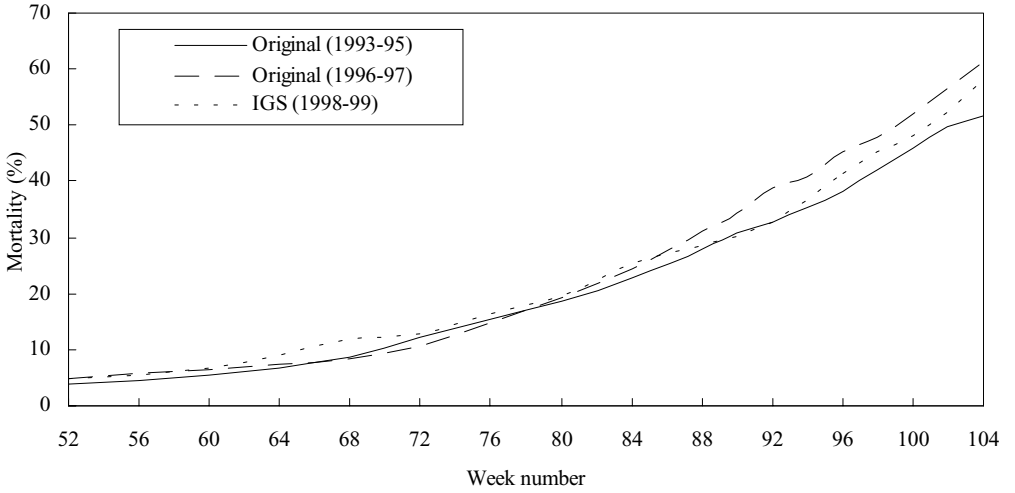
Food consumption:

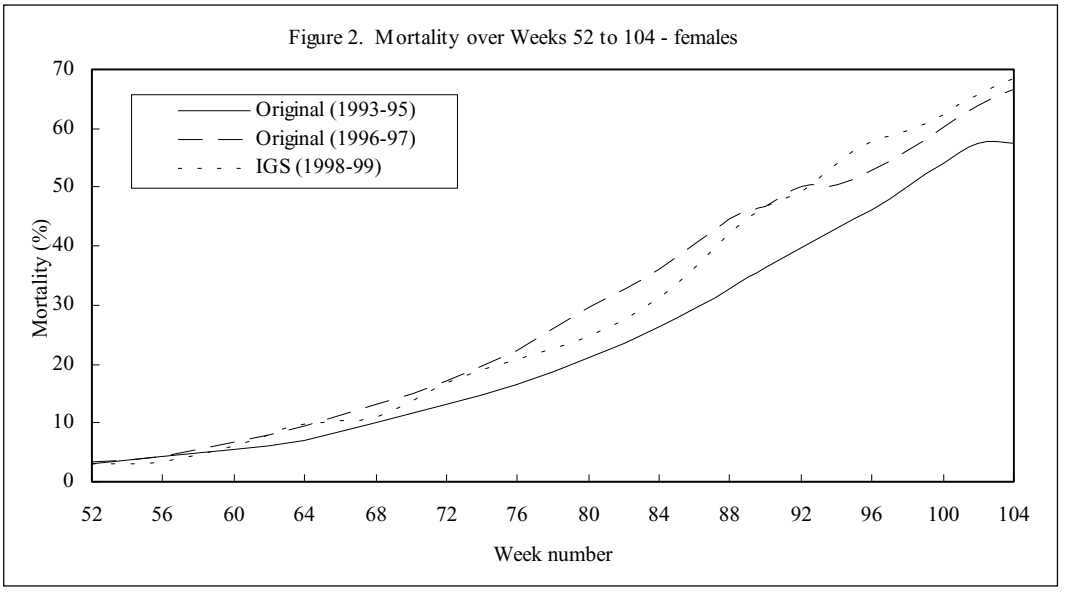
The mean weekly food consumption (g/rat/week) values are compared over the period of Weeks 1 to 52, graphically in Figure 8 for all studies. The IGS rat studies, ongoing or completed in 1998-99, are compared with the original strain studies (1993-95 and 1996-97); a comparison based on a 't' distribution was made following analysis of variance to compare intergroup differences. For comparative purposes, the food consumption (Weeks 1 to 52) values are detailed in Table 3 for dietary and oral gavage administered studies over selected time periods.

RESULTS AND DISCUSSION

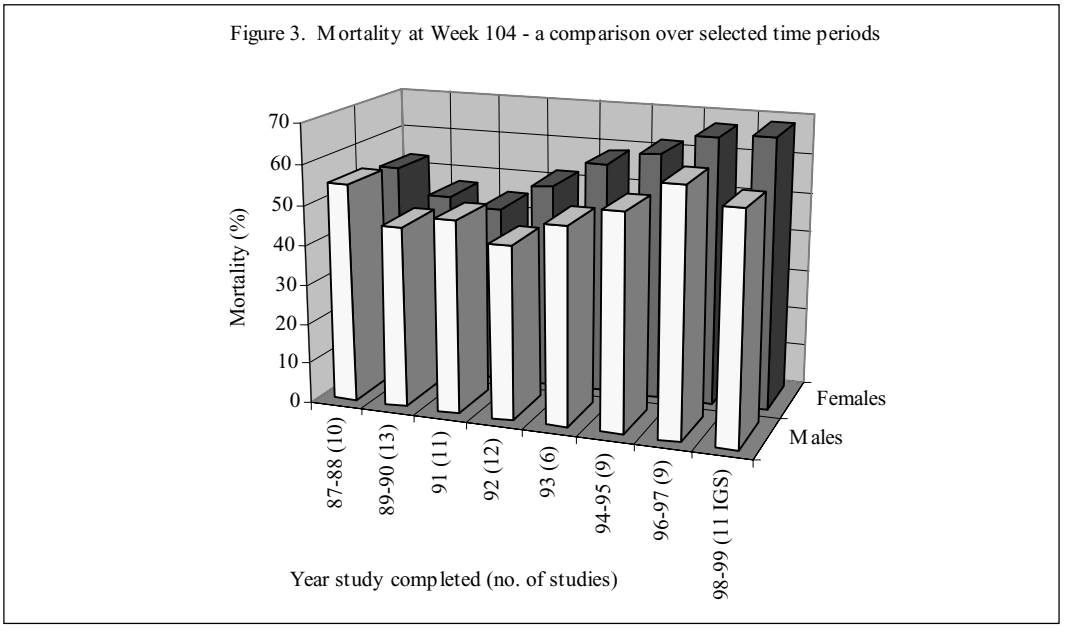
The mortality pattern over the period of Weeks 52 to 104 is presented in Figures 1 (male rats) and 2 (female rats) and it is apparent that the pattern for the IGS rat studies (including ongoing studies) is similar to that of studies completed over 1996-97, but increased when compared with studies completed over 1993-1995.

Figure 1. Mortality over Weeks 52 to 104 - males





In order to place the Sprague-Dawley rat mortality values in historical perspective, the terminal mortality values are presented in Figure 3, over selected time periods from 1987. The trend towards increasing mortality is apparent, particularly from 1993 in female rats.



In order to test the apparent trend statistically, the individual terminal mortality values were plotted against time (Figures 4 and 5) and a regression analysis performed. The statistical analysis of the trend line for all studies completed over the period of 1987 to 1997 has shown a positive trend in both male and female rats (P=0.0520 for males and P=0.0001 for females) and the trend line is presented in Figures 4 and 5. Similar findings were apparent when gang housed dietary studies only were compared over this period (P=0.0433 for males and P=0.0008 for females) and when all studies were compared over 1993 to 1997 (P=0.0274 for males and P=0.0391 for females). The 11 IGS rat studies completed in 1998-99 were not included in the statistical analysis, but the trend towards higher terminal mortality in these studies is similar to that of the original strain designation of rat.

Figure 4. Mortality distribution at Week 104 - males

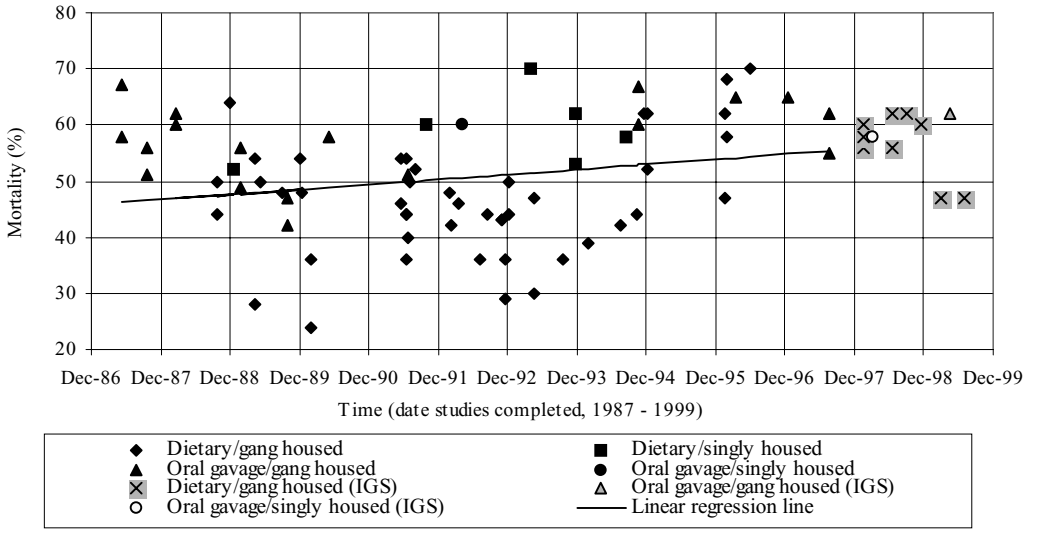
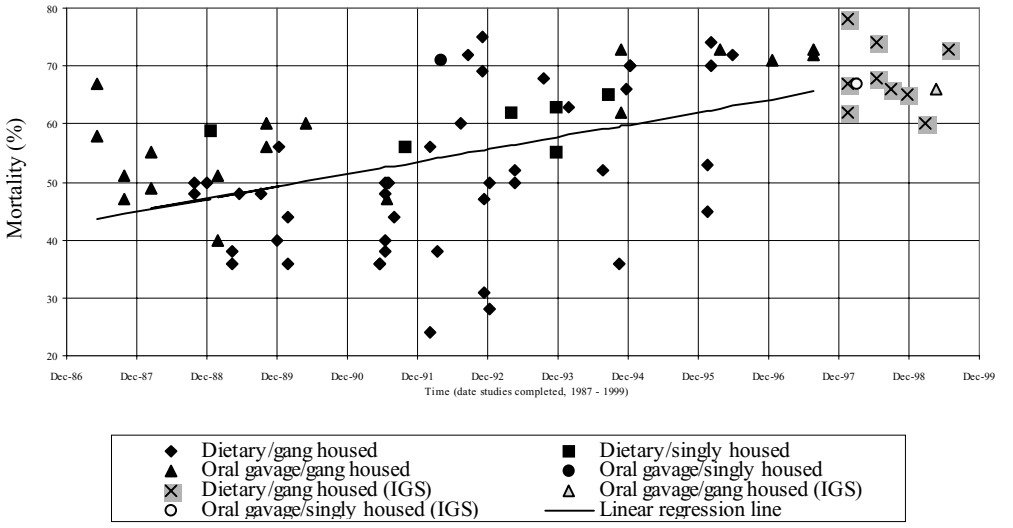


Figure 5. Mortality distribution at Week 104 - females



For comparative purposes, the mean mortality, together with the Standard deviation and number of studies, are detailed in Table 1 for the different study types over selected time periods.

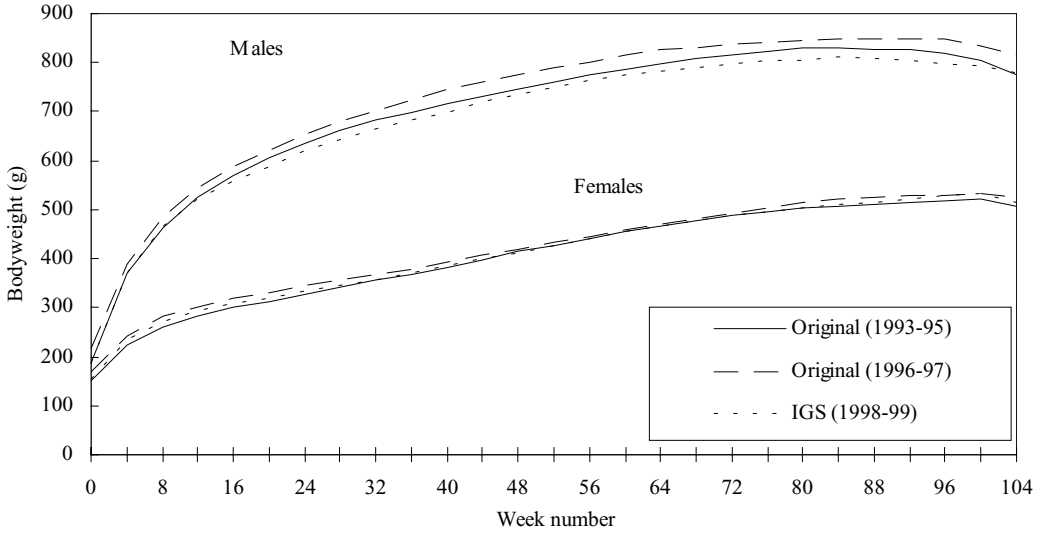
Table 1. Mortality (%) at Week 104 - dietary and oral gavage studies

Studies completed/ Strain designation		Dietary/ gang housed		Dietary/ singly housed		Oral gavage/ gang housed		Oral gavage/ singly housed		All Studies	
		M	F	M	F	M	F	M	F	M	F
1987-92/ Original	Mean	45	46	56	58	54	53	60	71	48	49
	SD	8.6	11.9	5.7	2.1	5.8	7.4	—	—	9.0	11.5
	n	31	31	2	2	12	12	1	1	46	46
1993-95/ Original	Mean	46	59	61	61	64	68			52	60
	SD	11.0	11.7	7.2	4.3	4.9	7.8			12.1	9.8
	n	9	9	4	4	2	2	0	0	15	15
1996-97/ Original	Mean	61	63			62	72			61	67
	SD	9.2	13.0			4.7	1.0			7.1	10.5
	n	5	5	0	0	4	4	0	0	9	9
1998-99/ IGS	Mean	56	68			62	66	58	67	57	68
	SD	5.8	5.9			—	—	—	—	5.4	5.3
	n	9	9	0	0	1	1	1	1	11	11

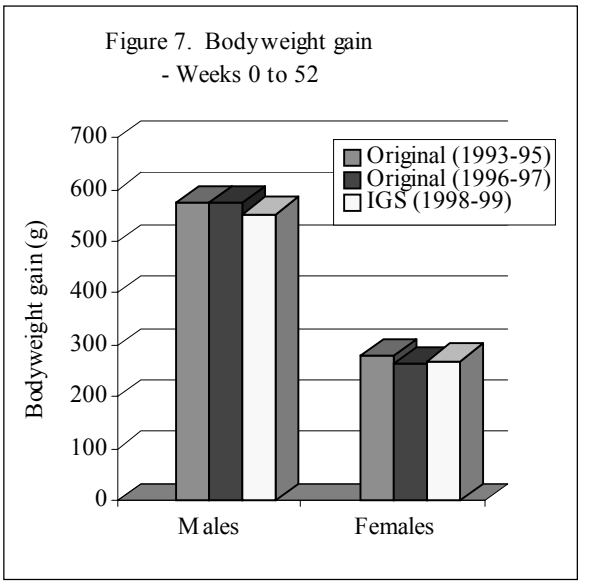
M Male rats, F Female rats, SD Standard deviation, n Number of studies

The bodyweight growth pattern over the 104-week study period for male IGS rats (Figure 6) was similar to that seen for the original strain of rat over 1993-95, but marginally lower than that recorded for the 1996-97 group.

Figure 6. Bodyweight growth pattern - Weeks 0 to 104



Although the bodyweight gain over Weeks 0 to 52 in male rats (Figure 7) was marginally lower than the original strain comparison groups, statistical difference was not demonstrated. In female rats, there was no discernible difference between the growth pattern (Figure 6) or statistical difference in bodyweight gain (Figure 7) between the IGS group and both comparison groups of original strain of rat. For comparative purposes, the bodyweight gain (Weeks 0 to 52) values are detailed in Table 2 for the different study types over selected time periods.



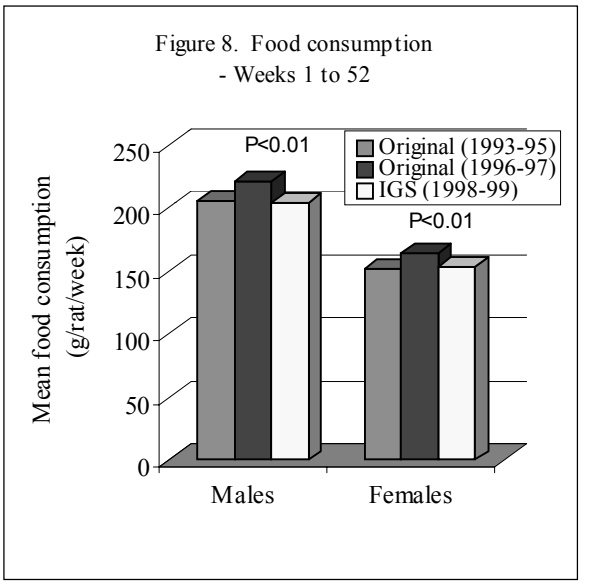


Table 2. Bodyweight gain (g) over Weeks 0 to 52 - dietary and oral gavage studies

Studies completed/ Strain designation		Dietary/ gang housed		Dietary/ singly housed		Oral gavage/ gang housed		Oral gavage/ singly housed		All Studies	
		M	F	M	F	M	F	M	F	M	F
1993-95/ Original	Mean	570	278	574	280	583	273			573	278
	SD	44.7	33.2	80.4	10.7	20.5	2.1			50.8	25.7
	n	9	9	4	4	2	2	0	0	15	15
1996-97/ Original	Mean	564	256			588	274			574	263
	SD	52.2	25.3			35.4	9.6			45.6	21.8
	n	6	6	0	0	4	4	0	0	10	10
1998-99/ IGS	Mean	557	269			577	271	488	268	553	269
	SD	34.7	15.6			—		—	—	37.7	14.3
	n	11	11	0	0	1	1	1	1	13	13

M Male rats, F Female rats, SD Standard deviation, n Number of studies

The mean weekly food consumption over the period of Weeks 1 to 52 (Figure 8) was not statistically different between the IGS male and female rats and original strain of rat 1993-95 comparison group. However, the 1996-97 comparison group did show statistically higher (P<0.01) values than the 1993-95 group and the IGS group. This was considered to be partially due to the influence of the four oral gavage studies in this group, which tend to have slightly higher food consumption values in comparison with dietary studies [5].

For comparative purposes, the mean food consumption values (Weeks 1 to 52) are detailed in Table 3 for the different study types over selected time periods.

Table 3. Mean food consumption (g/rat/week) over Weeks 1 to 52 - dietary and oral gavage studies

Studies completed/ Strain designation		Dietary/ gang housed		Dietary/ singly housed		Oral gavage/ gang housed		Oral gavage/ singly housed		All Studies	
		M	F	M	F	M	F	M	F	M	F
1993-95/ Original	Mean	199	150	212	152	223	158			206	152
	SD	13.2	15.2	8.8	2.4	2.1	0			14.0	11.9
	n	9	9	4	4	2	2	0	0	15	15
1996-97/ Original	Mean	214	160			235	170			222	164
	SD	6.1	8.1			1.6	4.8			11.9	8.3
	n	6	6	0	0	4	4	0	0	10	10
1998-99/ IGS	Mean	201	151			223	160	224	170	204	153
	SD	3.8	4.2			—		—	—	9.2	6.9
	n	11	11	0	0	1	1	1	1	13	13

M Male rats, F Female rats, SD Standard deviation, n Number of studies

In conclusion, the results from the data currently available, for mortality pattern, bodyweight pattern and food consumption from tumorigenicity studies, indicate that the IGS rat is not remarkably different from recent studies using the original strain of Sprague-Dawley rat. However, the longevity of the IGS rat does not appear, at this stage, to be an improvement over the original strain of rat and is possibly, particularly in females, reduced in comparison with the 1993-95 comparison group. Therefore, when the IGS strain designation of rat is used for tumorigenicity studies, strategies must be considered to ensure that the survival incidence at termination of the study is scientifically valid and acceptable to regulatory authorities. It would therefore be advisable to reach a consensus opinion between the toxicology laboratories and the regulatory authorities on how to proceed. One such strategy, when using this strain of rat, could be to terminate when the 50% mortality point is reached (approximately Week 100 in males and Week 92 in females), treating the sexes separately. This strategy would ensure that the studies have reached the lifespan for the strain of rat, ensure that there were sufficient survivors available for tumorigenic evaluation, and decrease the undue influence of geriatric lesions in the pathological examinations.

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REFERENCES

1. Hooks, W.N. and Saunders, M.D. 1998. A review of mortality in tumorigenicity studies, conducted using Charles River Sprague-Dawley rats, completed over the period 1987 to 1998. Toxicology Letters 1/85: 184.
2. Hooks, W.N. An ongoing review of the mortality, bodyweight and food consumption of Charles River Sprague-Dawley International Genetic Standard rat in comparison with the original strain designation in long term studies. Biological Reference Data on CD (SD) IGS Rats – 1998, edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.
3. Hooks, W.N. and Harris, B. 1998. A comparison between the original and International Genetic Standard Charles River (UK) designations of Sprague-Dawley rat, at the 13-week time point. The Toxicologist, 42: 59.
4. Hooks, W.N. A comparison between the original and International Genetic Standard Charles River (UK) designations of Sprague-Dawley rat, at the 13-week timepoint. Biological Reference Data on CD (SD) IGS Rats – 1998), edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.
5. Hooks, W.N., Newton, D.F., Green, O.P., and Harling, R.J. 1993. A review of mortality in tumorigenicity studies conducted on gang-housed Sprague-Dawley rats over the period 1985 to 1992. The Toxicologist, 13, 419.
6. Hooks, W.N., Gopinath, C., Newton, D.F., and Harling, R.J. 1994. Factors affecting mortality in tumorigenicity studies conducted on gang-housed Sprague-Dawley rats. Toxicology Letters 1/74: 37.
7. Snedecor, G.W. and Cochran, W.G. 1989. Statistical Methods 8th Edition, Iowa University Press, U.S.A.

A Control Data of the Mortality, Body Weight, Food Consumption, Hematological Data and Neoplastic Lesions in Long-term Examination in Crj:CD(SD)IGS Rats - Comparison with Data in Crj:CD(SD) Rats -

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ABSTRACT. This study was performed to collect the basic background data for carcinogenicity studies using Crj:CD(SD)IGS rats produced using the International Genetic Standard System. Furthermore, comparative studies were performed with the existing background data for Crj:CD(SD) rats. Comparison of the viability indices at Week 104 of the study revealed a higher viability for Crj:CD(SD)IGS rats than Crj:CD(SD) rats in both males and females. However, the viability indices of Crj:CD(SD)IGS rats still remained lower than those of rats of other strains such as Fischer 344 and Wistar. Body weight and food consumption of Crj:CD(SD)IGS rats were lower than those of Crj:CD(SD) rats in both males and females throughout the study period. In the hematological examination, neutrophil ratios were higher than lymphocyte ratios in differential leukocyte counts of males in Crj:CD(SD) rats, while the former was lower than the latter in Crj:CD(SD)IGS rats. As a result of the pathological examination, pituitary gland adenoma, mammary gland fibroadenoma, mammary gland adenocarcinoma and pancreatic islet adenoma occurred in a high frequency and in addition, neoplastic lesions such as mammary gland adenoma, endometrial stromal polyp of uterus, pituitary gland adenocarcinoma, C-cell adenoma of thyroid gland, cortical adenoma of adrenal gland, pheochromocytoma of adrenal gland and pancreatic islet adenocarcinoma were observed. It was characteristic of Crj:CD(SD)IGS rats that tumors occurred frequently in endocrine organs, as in Crj:CD(SD) rats. —Key words: Crj:CD(SD)IGS rats, background data, carcinogenicity studies.

CD(SD)IGS-1999: 243-251

INTRODUCTION

Data on the viability, body weight, food consumption, hematological values and occurrence rates of spontaneous tumors or age-related lesions which are observed as the background histological changes in the test animals are very useful for the evaluation of chronic toxicity and carcinogenicity studies (1-17). The purpose of this study was to obtain the basic background data for carcinogenicity studies using Crj:CD(SD)IGS rats newly produced by the International Genetic Standard System. At the same time, comparative studies were performed with the existing background data for Crj:CD(SD) rats.

MATERIALS AND METHODS

Fifty male and 50 female animals were provided from Charles River Japan Inc. (Atsugi, Kanagawa, Japan) at 4 weeks of age and, after one week of quarantine and acclimation, were assigned to the study for 2 years (104 weeks). Throughout the study period, animals were housed individually in stainless cages (W 20.0 ×D 28.2×H 18.2 cm). Temperature and relative humidity were maintained at 23 ± 2℃ and 55 ± 10%, respectively, and lights were on 12 hours a day (7:00 a.m. ～ 7:00 p.m.) throughout the study period. The animals had free access to tap water and a commercial diet (Modified NIH Open Formula Rat and Mouse Ration, Oriental Yeast Co., Ltd., Tokyo) which consisted of 5% skim milk powder, 10% fish meal, 15% soybean powder, 4% alfalfa powder, 24.5% corn powder, 23% hard winter wheat, 10% coarse flour, 2% dried beer yeast, 1.5% dried molasses, 2.5% soybean oil, 0.5% salt, 1.25% calcium secondary phosphate, 0.5% limestone and 0.25% premix. In the analysis of contaminants, aflatoxin, γ-BHC, heptachlor, DDT, polychlorobiphenyl, dieldrin and estradiol were not detected.

Body weight was measured once weekly from Week 1 (5 weeks of age) through Week 26 (31 weeks of age) and once every other week from Week 26 until the end of the study (109 weeks of age). Food consumption was calculated once weekly based on the unconsumed diet.

In the hematological examination, hematocrit values, hemoglobin volume, red blood cell counts, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, white blood cell counts and differential leukocyte counts were measured with THMS H・1E (Miles Laboratories, USA).

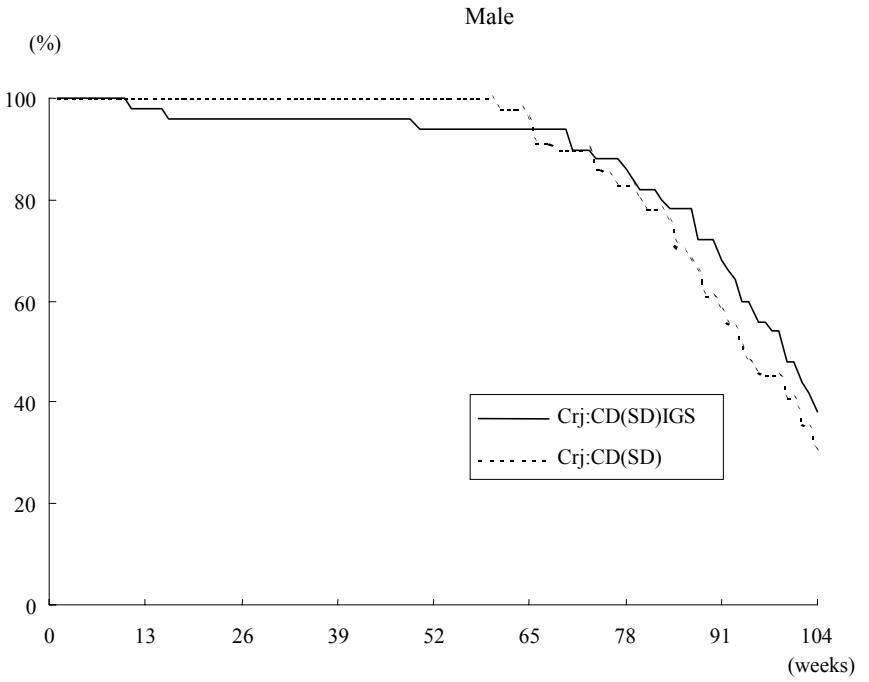
Animals which were killed as scheduled, killed moribund or found dead were subjected to the thorough necropsy examination each time and gross findings were recorded in detail. Skin, mammary gland, lymph nodes, salivary gland, sternum, femur (bone marrow), thymus, trachea, lungs and bronchus, heart, thyroid gland and parathyroid gland, tongue, esophagus, stomach and duodenum, small intestine, large intestine, liver, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicle, prostate gland, testes, ovaries, uterus, vagina, eyes, Harderian gland, brain, pituitary gland, spinal cord and other macroscopic lesions were fixed in 10% neutral buffer formalin, embedded in paraffin according to the standard method, HE stained and examined under a microscope.

The background data for Crj:CD(SD) rats at this institute with which the data obtained in this study were compared in all items of the examination had been obtained under the same study conditions.

RESULTS

Changes in viability are presented in Figure 1.

Dead animals gradually increased in number after Week 78 in



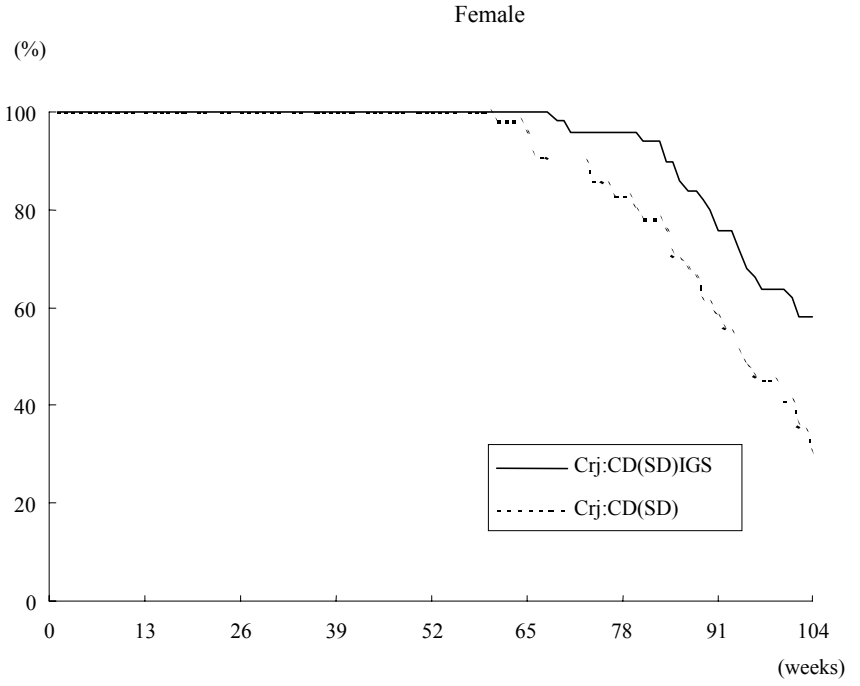


Figure 1. Survival curves of Crj:CD(SD)IGS rats and Crj:CD(SD) rats.

both males and females, the viability indices at Week 104 being, 38% for males (12 killed moribund, 19 dead) and 58% for females (13 killed moribund, 8 dead). Since the viability indices at Week 104 for Crj:CD(SD) rats in the long-term studies at this institute were 30% for males and 25% for females, Crj:CD(SD)IGS rats showed slightly higher viability in comparison with Crj:CD(SD) rats.

Changes in body weight are presented in Figure 2.

Gain in the mean body weight was observed in males until Week 88 and in females until Week 90 but the mean body weight tended

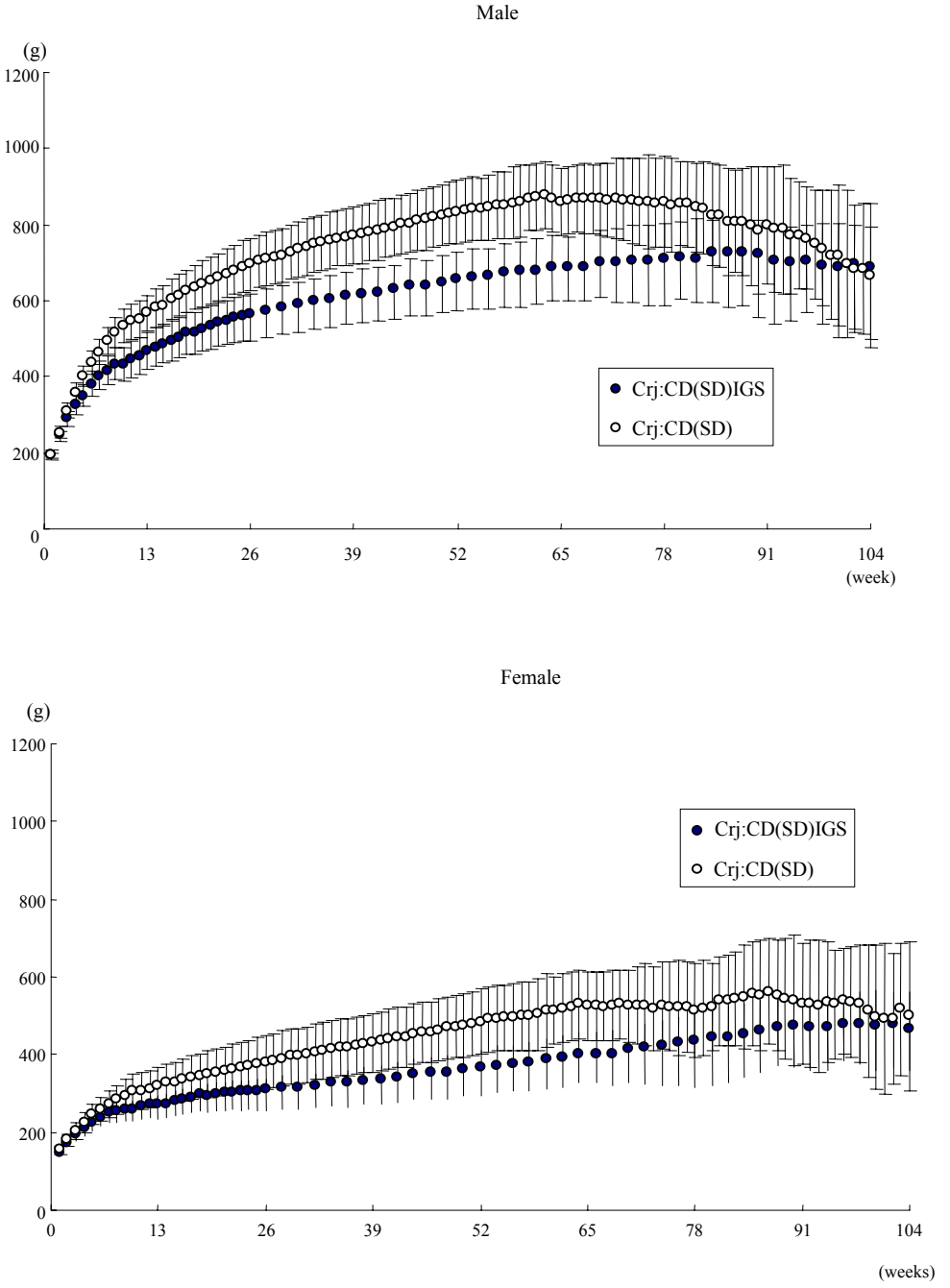


Figure 2. Mean body weight in Crj:CD(SD)IGS rats and Crj:CD(SD) rats.
The vertical bars represent the standard deviations from the mean.

to decrease slightly thereafter. The mean body weight of Crj:CD(SD)IGS rats was similar to that of Crj:CD(SD) rats in both males and females, both at the initiation and termination of the study, but lower during the study period. At Week 52, the mean body weight was 657 ± 72 g for male and 366 ± 45 g for female Crj:CD(SD)IGS rats in contrast to 834 ± 83 g for male and 485 ± 73 g for female Crj:CD(SD) rats.

Changes in food consumption are presented in Figure 3.

Except for Weeks 12 and 13 when both males and females showed slightly lower values, food consumption was almost con-

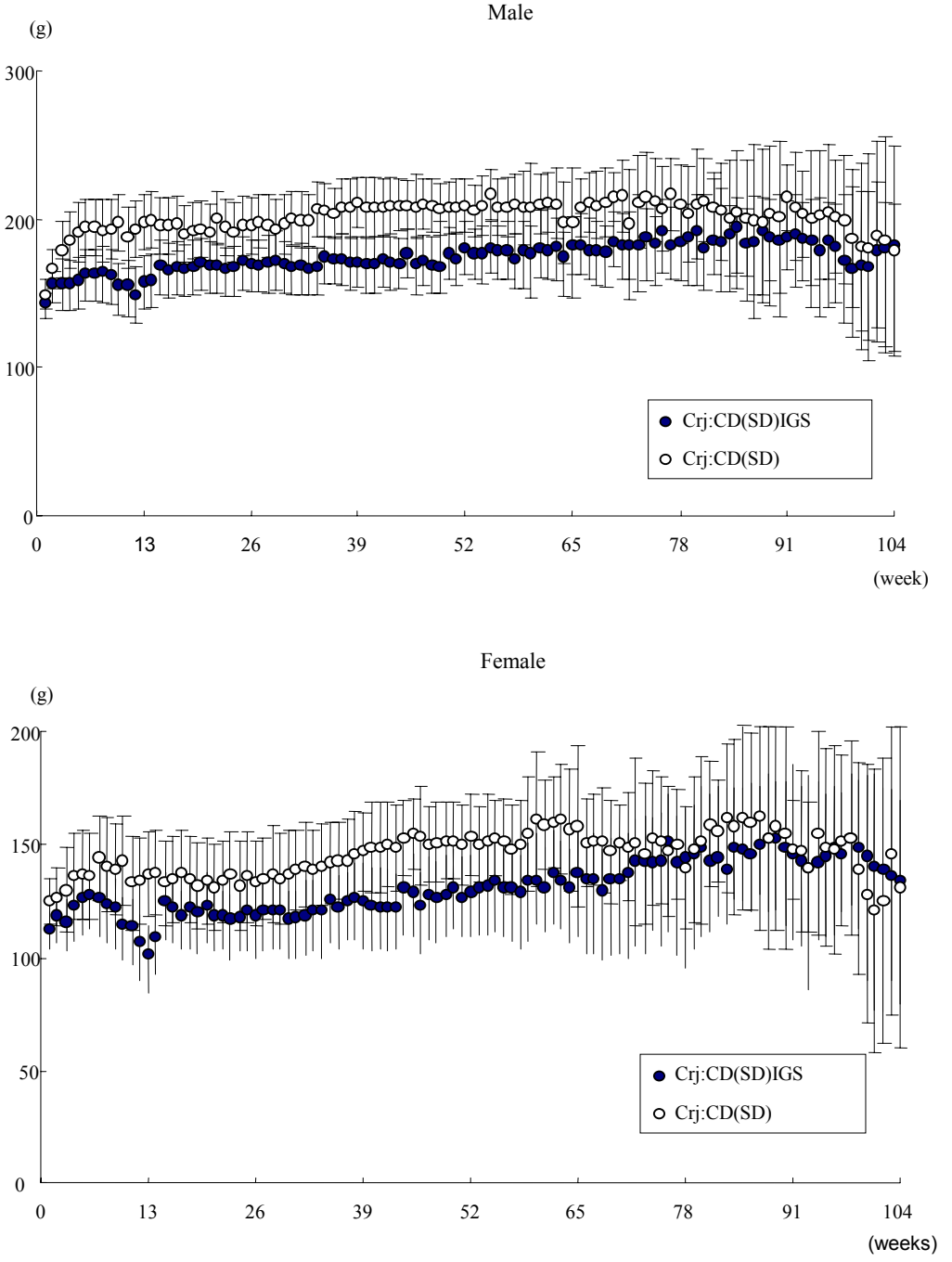


Figure 3. Mean food consumption in Crj:CD(SD)IGS rats and Crj:CD(SD) rats.
The vertical bars represent the standard deviations from the mean.

stant throughout the breeding period. The mean food consumption of Crj:CD(SD)IGS rats was lower than that of Crj:CD(SD) rats throughout the study period in both males and females. At Week 52, the mean food consumption was 181 ± 11 g for male and 129 ± 16 g for female Crj:CD(SD)IGS rats in contrast to 210 ± 18 g for male and 154 ± 20 g for female Crj:CD(SD) rats.

Hematological values are presented in Table 1.

Table1. Hematological data in Crj:CD(SD)IGS rats and Crj:CD(SD) rats.
The vertical bars represent the standard deviations from the mean.

Item	CD(SD)IGS		CD(SD)	
	Male	Female	Male	Female
No.of animals	19	29	18	15
HCT (%)	40.1±6.7	39.8±4.7	34.5±7.2	39.1±3.7N
HGB (g/dl)	13.4±2.6	13.6±2.1	11.5±3.0	13.7±1.5N
RBC ($\times 10^6$/mm^3)	7.24±1.52	6.96±1.26	6.18±1.68	6.85±0.71N
MCV (μm^3)	56.1±4.7	58.5±8.8	57.1±6.3	57.2±3.0N
MCH (pg)	18.6±1.0	19.7±1.6	18.8±1.5	19.9±1.0
MCHC (%)	33.1±1.6	33.9±1.8	33.0±2.4	34.8±0.7
PLT ($\times 10^6$/mm^3)	1351±297	986±200	1665±246	1089±150
WBC ($\times 10^6$/mm^3)	11.5±4.2	8.5±4.7	16.7±4.1	7.0±1.3N
Differential leukocyte counts (%)				
NEUT	39±10	48±14	54±15	43±10
LYMPH	51±10	42±14	37±13	48±10
MONO	6±2	7±2	5±1	6±2
EOSN	1±1	1±1	1±1N	1±0N
BASO	0±0	0±0	0±0	0±0
LUC	2±2	2±1	3±1	2±1

NEUT: Neutrophil, LYMPH: Lymphocyte, MONO: Monocyte, EOSN: Eosinophil, BASO: Basophil, LUC: Large unstained cells
Values are expressed as Mean±S.D.
N: Non parametric analysis

Comparison of the hematological values at Week 104 revealed slightly higher values in hematocrit, hemoglobin and red blood cell counts in male Crj:CD(SD)IGS rats than in male Crj:CD(SD) rats. Furthermore, differential leukocyte counts revealed that male Crj:CD(SD)IGS rats showed lower neutrophil ratios (39 ± 10%) than lymphocyte ratios (51 ± 10%), while Crj:CD(SD) rats showed higher neutrophil ratios (54±15%) than lymphocyte ratios (37 ± 13%).

In females, however, no differences were observed in any hematological values examined between Crj:CD(SD)IGS rats and Crj:CD(SD) rats.

Neoplastic lesions are presented in Table 2 and Figure 4.

Pituitary gland adenoma (males: 62.0%, females: 72.0%), mammary gland fibroadenoma (females: 50.0%), pancreatic islet adenoma (males: 24.0%, females: 12.0%) and mammary gland adenocarcinoma (females: 22.0%) occurred in a high frequency and in addition, neoplastic lesions such as mammary gland adenoma, endometrial stromal polyp of uterus, pituitary gland adenocarcinoma, C-cell adenoma of thyroid gland, cortical adenoma of adrenal gland, pheochromocytoma of adrenal gland, and pancreatic islet adenocarcinoma were observed. Frequently occurring tumors in Crj:CD(SD)IGS rats were the same kind as in Crj:CD(SD) rats.

DISCUSSION

For the analysis of the results of toxicity and carcinogenicity studies of chemical substances, background data on viability indices or body weight and food consumption changes, hematological values or neoplastic lesions which occur with aging are very useful.

This study was performed with the aim of collecting basic background data for carcinogenicity studies using Crj:CD(SD)IGS rats produced using the International Genetic Standard System. Comparative studies with the existing background data for Crj:CD(SD) rats were also performed.

Comparison of the viability indices of Crj:CD(SD)IGS rats with those of Crj:CD(SD) rats at Week 104 (109 weeks of age) revealed higher indices in the former than in the latter in both males and females. However, the viability indices of Crj:CD(SD)IGS rats were still lower than those of the rats of other strains such as Fischer 344 and Wistar.

Body weight and food consumption of Crj:CD(SD)IGS rats showed lower values than those of Crj:CD(SD) rats, both in males and females, throughout the study period.

In the hematological examination, neutrophil ratios were lower than lymphocyte ratios in Crj:CD(SD)IGS rats, while the former was higher than the latter in Crj:CD(SD) rats, in the differential

Table 2. Incidence (Number and Percentage) of Neoplastic Lesions in CD(SD)IGS rats and CD(SD) rats

	Experimental weeks	104							
	Sex	Male				Female			
	Strain	CD(SD)IGS		CD(SD)IGS		CD(SD)IGS		CD(SD)IGS	
Organ	Neoplastic lesions	No.of lesions n=50*	Rate (%)	No.of lesions n=110*	Rate (%)	No.of lesions n=50*	Rate (%)	No.of lesions n=110*	Rate (%)
-CARDIOVASCULAR SYSTEM-									
Heart	schwannoma	0	—	1	0.9	0	—	0	—
-HEMATOPOIETIC SYSTEM-									
Bone marrow	#myelogenous leukemia	1	2.0	1	0.9	0	—	0	—
Spleen	hemangioma	1	2.0	1	0.9	0	—	0	—
	#histiocytic sarcoma	0	—	0	—	0	—	1	0.9
	#LGL leukemia	1	2.0	2	1.8	0	—	0	—
Lymph node	#malignant lymphoma	0	—	0	—	0	—	1	0.9
Thymus	#malignant lymphoma	1	2.0	1	0.9	1	2.0	1	0.9
-DIGESTIVE SYSTEM-									
Esophagus	schwannoma	0	—	1	0.9	0	—	0	—
Stomach	leiomyoma	0	—	0	—	1	2.0	1	0.9
	squamous cell papilloma	0	—	0	—	0	—	1	0.9
Exocrine pancreas	adenoma	2	4.0	5	4.5	0	—	1	0.9
Duodenum	leiomyoma	0	—	0	—	1	2.0	1	0.9
Jejunum	#adenocarcinoma	1	2.0	1	0.9	0	—	0	—
Ileum	#malignant lymphoma	0	—	0	—	1	2.0	1	0.9
Rectum	leiomyoma	0	—	1	0.9	0	—	0	—
Liver	cholangiocellular adenoma	0	—	1	0.9	0	—	1	0.9
	hepatocellular adenoma	4	8.0	4	3.6	0	—	3	2.7
	#hepatocellular carcinoma	2	4.0	4	3.6	0	—	0	—
-URINARY SYSTEM-									
Kidney	adenoma	2	4.0	4	3.6	0	—	1	0.9
	lipoma	0	—	1	0.9	0	—	1	0.9
Urinary bladder	squamous cell papilloma	0	—	0	—	0	—	1	0.9
	transitional cell papilloma	0	—	0	—	0	—	1	0.9
-REPRODUCTIVE SYSTEM-									
Mammary gland	adenoma	0	—	0	—	4	8.0	15	13.6
	fibroadenoma	0	—	2	1.8	25	50.0	45	40.9
	fibroma	0	—	0	—	0	—	1	0.9
	adenolipoma	0	—	1	0.9	0	—	1	0.9
	#adenocarcinoma	0	—	0	—	11	22.0	26	23.6
	#fibrosarcoma	0	—	0	—	0	—	1	0.9
Testis	interstitial cell tumor	0	—	4	3.6	—	—	—	—
Prostate	adenoma	0	—	1	0.9	—	—	—	—
Ovary	Sertoli cell tumor	—	—	—	—	1	2.0	1	0.9
Uterus	adenoma	—	—	—	—	2	4.0	2	1.8
	endometrial stromal polyp	—	—	—	—	5	10.0	6	5.5
Vagina	papilloma	—	—	—	—	1	2.0	1	0.9

*Number of animals examined
#Malignant tumor

Table 2. Incidence (Number and Percentage) of Neoplastic Lesions in CD(SD)IGS rats and CD(SD) rats

	Experimental weeks	104							
	Sex	Male				Female			
	Strain	CD(SD)IGS		CD(SD)IGS		CD(SD)IGS		CD(SD)IGS	
Organ	Neoplastic lesions	No.of lesions n=50*	Rate (%)	No.of lesions n=110*	Rate (%)	No.of lesions n=50*	Rate (%)	No.of lesions n=110*	Rate (%)
-ENDOCRINE SYSTEM-									
Pituitary gland	adenoma	31	62.0	73	66.4	36	72.0	82	74.5
	adenoma pars intermedia	0	—	1	0.9	0	—	0	—
	#adenocarcinoma	1	2.0	4	3.6	4	8.0	15	13.6
Thyroid gland	C-cell adenoma	2	4.0	8	7.3	5	10.0	9	8.2
	follicular cell adenoma	3	6.0	6	5.5	0	—	1	0.9
	#follicular cell carcinoma	1	2.0	3	2.7	0	—	0	—
Adrenal gland	adenoma	2	4.0	3	2.7	5	10.0	11	10.0
	pheochromocytoma	5	10.0	12	10.9	2	4.0	2	1.8
	#adenocarcinoma	0	—	1	0.9	0	—	1	0.9
	#malignant pheochromocytoma	1	2.0	2	1.8	0	—	1	0.9
Pancreatic Islet	adenoma	12	24.0	24	21.8	6	12.0	9	8.2
	#adenocarcinoma	5	10.0	10	9.1	2	4.0	4	3.6
-NERVOUS SYSTEM-									
Brain	meningioma	0	—	0	—	1	2.0	1	0.9
	#astrocytoma	1	2.0	4	3.6	0	—	0	—
	#malignant pinealoma	0	—	0	—	0	—	1	0.9
-SPECIAL SENSE SYSTEM-									
Eye	amelanotic melanoma	0	—	1	0.9	0	—	0	—
Eyelid	amelanotic melanoma	1	2.0	—	—	—	—	—	—
Nose	squamous cell papilloma	1	2.0	—	—	—	—	—	—
Zymbal's gland	#carcinoma	1	2.0	—	—	—	—	—	—
-INTEGUMENTARY SYSTEM-									
Skin	keratoacanthoma	1	2.0	3	2.7	2	4.0	2	—
	squamous cell papilloma	1	2.0	2	1.8	0	—	0	—
	fibroma	1	2.0	1	0.9	0	—	0	—
	#basal cell carcinoma	1	2.0	1	0.9	0	—	0	—
Subcutaneous tissue	epidermal cyst	0	—	1	0.9	0	—	0	—
	fibroma	1	2.0	6	5.5	0	—	0	—
	lipoma	1	2.0	2	1.8	0	—	3	2.7
	#histiocytic sarcoma	0	—	2	1.8	0	—	0	—
	#malignant fibrous histiocytoma	0	—	0	—	1	2.0	1	0.9
	#malignant schwannoma	1	2.0	1	0.9	0	—	0	—
-MUSCULOSKELETAL SYSTEM-									
Bone	osteoma	1	2.0	1	0.9	0	—	0	—

*Number of animals examined
#Malignant tumor

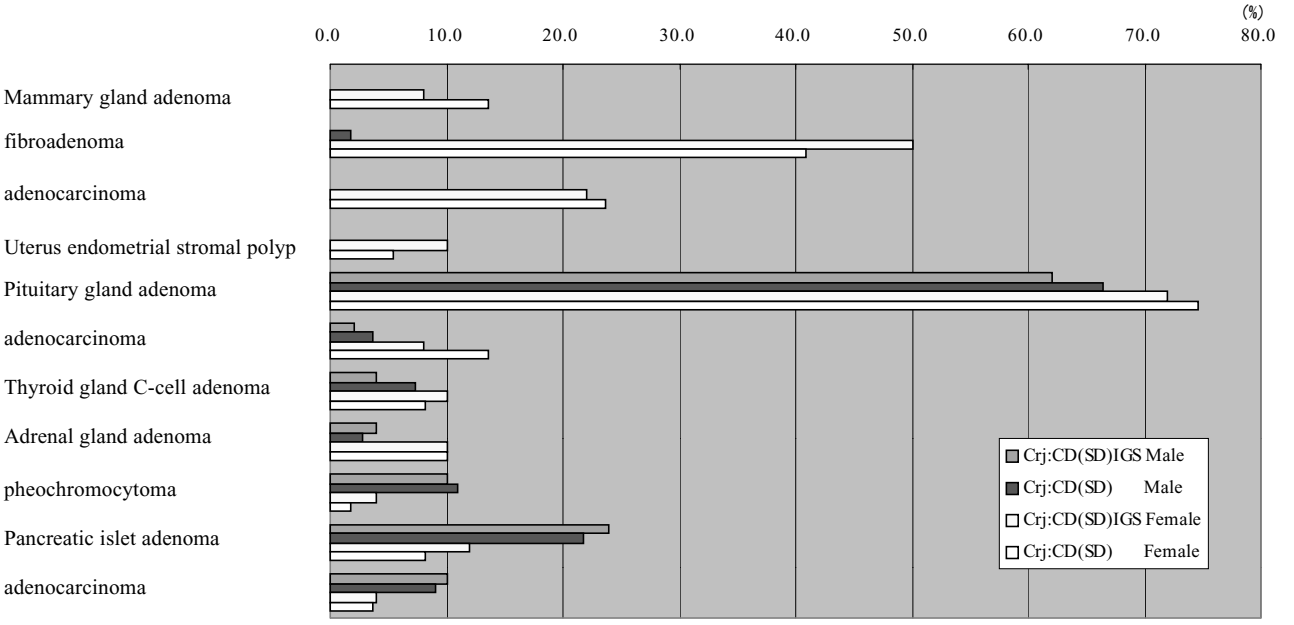


Figure 4. Incidence of Main Neoplastic lesions in Crj:CD(SD)IGS rats and Crj:CD(SD) rats

leukocyte counts in males, but the significance of the difference was uncertain.

In the Pathological examination, pituitary gland adenoma, mammary gland fibroadenoma, mammary gland adenocarcinoma and pancreatic islet adenoma occurred in a high frequency and in addition, neoplastic lesions such as mammary gland adenoma, endometrial stromal polyp of uterus, pituitary gland adenocarcinoma, C-cell adenoma of thyroid gland, cortical adenoma of adrenal gland, pheochromocytoma of adrenal gland and pancreatic islet adenocarcinoma were observed. It was characteristic of Crj:CD(SD)IGS rats that tumors occurred frequently in endocrine organs, as in Crj:CD(SD) rats.

Accelerated secretion of prolactin of pituitary gland due to dopamine depletion in the hypothalamo-hypophyseal system caused with aging is suggested to be associated with the occurrence of pituitary gland adenoma and is also considered to induce mammary gland tumors. It was presumed that such abnormalities in endocrinal environments had taken place in Crj:CD(SD)IGS rats as well. Comparison of the kinds and frequency of occurrence of neoplastic lesions in Crj:CD(SD)IGS rats with Crj:CD(SD) rats revealed no marked differences, but since the number of test animals was limited (50 males and 50 females), the characteristics of the occurrence of rare tumors, the differences among the lots of test animals, and the differences due to diet or breeding environment were not clear. Accumulation of sufficient data in future is expected to shed light on these issues.

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REFERENCES

1. Sass, B, Kabstein, LS, Madison, K, Ninns, RM, Peters, RL and Kelloff, GJ, 1975. Incidence of spontaneous neoplasms in F334 rats throughout the natural life-span. J Natl Cancer Inst 54: 1449-1456.
2. Coleman. GL, Barthold, SW, Osbaldiston, GW, Foster, SJ and Jonas, AM, 1977. Pathological changes during aging in barrier-reared Fischer 344 male rats. J Gerontol 32: 258-278.
3. Goodman, DG, Ward, JM, Squire RA, Chu KC and Linhart MS, 1979. Neoplastic and non-neoplastic lesions in aging F344 rats. Toxicol App 1 Pharmacol 48: 237-248.
4. Sher, SP, Jensen, SD and Bokelman, DL, 1982. Spontaneous tumors in control F344 and Charles River-CD rats and Charles River CD-1 and B6C3F1 mice. Toxicol Letters 11: 103-110.
5. Maekawa, A, Kurokawa, Y, Takahashi, M, Kokubu, T, Ogiu, T, Onodera, H, Tanigawa, H, Ohno, Y, Hurukawa, F and Hayashi, Y, 1983. Spontaneous tumors in F344/DuCrj rats. Gann 74: 365-372.
6. Solleveld HA, Haseman, JK, and McConell, EE, 1984. Natural history of body weight gain, survival, and neoplasia in the F344 rat. J Natl Cancer Inst 72: 929-940.
7. Haseman, JK, Huff, JE, Rao, GN, Armold, JE, Boorman, GA and McConnell, EE, 1984. Neoplasms observed in untreated and corn oil gavage control group of F344/N rats and (C57BL/6N×C3H/Hen)F1(B6C3F1)mice. J Natl Cancer Inst 75: 975-984.

8. Maita, K, Hirano, M, Harada, T, Mitsumori, K, Yoshida, A, Takahashi, K, Nakashima, N, Enomoto, A, Inui, K and Shirasu, Y, 1987. Spontaneous tumors in F344/DuCrj rats from 12 control groups of chronic and oncogenicity studies. J Toxicol Sciences 12: 111-126.
9. Takaki, Y, Kitamura, S, Uekusa, T, Honma, S, Aze, Y, Wakabayashi, K, Kuwabara, N and Fukuda, Y, 1989. Spontaneous tumors in F344/Jcl rats. J Toxicol Sciences 14: 181-195.
10. Iwata, H, Hagiwara, T, Ooba, Y, Kato, M, Oribe, S, Koike, Y, Hirouchi, Y, Yamamoto, T, Kobayashi, K, Inoue, H and Enomoto, M, 1993. Gross findings and organ weight of F344/DuCrj rats. Japan J Exper Animals: 42: 383-396.
11. Ward, JM, 1983. Background data and variations in tumor rate of control rats and mice. Prog exp Tumor Res 26: 241-258.
12. Tarone, RE, Chu, KC and Ward, JM, 1981. Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N × C3H/HeN)F1, (B6C3F1)mice. J Natl Cancer Inst 66: 11-75.
13. Rao, GN, Haseman, JK, Grumbein, S, Crawford, DD and Eustis, SL, 1990. Growth, body weight, survival and tumor trends in F344/N rats during an eleven-year period. Toxicol. Path. 18: 61-70.
14. Iwata, H, Hirouchi, Y, Koike, Y, Yamakawa, S, Kobayashi, K, Yamamoto, T, Inoue, H and Enomoto, M, 1991. Historical control data of non-neoplastic and neoplastic lesions in F344/DuCrj rats. Japanese J. Toxicol. Pathol., Vol. 4, No. 4: 1-24.
15. Maekawa, A, Onodera, H, Tanigawa, H, Furuta, K, Kodama, U, Horiuchi, S and Hayashi, Y, 1983. Neoplastic and non neoplastic lesions in aging Slc:Wistar rats. J. Toxicological Science, Vol. 8: 279-290.
16. Nishibe T, 1976. Pathological studies of age-related changes in rats. J. Nara Med. Ass. 27: 1-26.
17. Bomhard, E and Rinke M, 1994. Frequency of spontaneous tumors in Wistar rats in 2-year studies. Exp. Toxic. Pathol, 24: 17-29.

Mortality, Body Weight and Food Consumption in Crj:CD(SD)IGS Rats Fed Low Protein Commercial Diet

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ABSTRACT. To clarify the biological characteristics of Crj:CD(SD)IGS rats (IGS rats), 100 IGS rats of each sex were reared for 104 weeks fed low protein commercial diet (CR-LPF, protein content 18%). In the present paper, mortality, body weight and food consumption are shown and these data were compared with historical in-house data of F344/Du Crj (Fischer) rats. Survival rate of IGS rats at the end of the 104-week observation period was 54% in males and 49% in females and was lower than that of F344 rats (81% in males and 82% in females). The most dominant cause of death in IGS rats estimated from the clinical signs and gross pathology, was pituitary tumor for both sexes (of the dead animals, 29.8% of males and 76.5% of females died of pituitary tumor), and it was different from F344 rats (leukemia was dominant). The terminal mean body weight was 716g in males and 446g in females and was approximately 1.5-times that of F344 rats. Daily food consumption was approximately 27g in males and 20g in females, and similar to the body weight, food consumption in IGS rats was 1.5-times that of F344 rats. — Key words: Mortality, Crj:CD(SD)IGS rats, low protein diet, 104-week observation

CD(SD)IGS-1999: 252-254

INTRODUCTION

Crj:CD(SD)IGS rats have been newly produced by Charles River Inc. and are originally derived from Crj:CD(SD) rats. Prior to introducing this strain into carcinogenicity studies, it is necessary to know their biological characteristics. Therefore, male and female IGS rats were reared for 104 weeks and the data were compared to those of F344 rats, that are frequently used in A carcinogenicity studies in Japan. The largest problem in using SD rats in carcinogenicity studies is their low survival rate, and one of the life-threatening factors is said to be a high content of protein in the feed as well as hyperneutrition due to overfeeding [1, 2]. CRF-1 is one of the most widely distributed commercial diets in Japan as a rodent chow and contains approximately 23% crude protein; but recently, CR-LPF with approximately 18% crude protein began to be available as a new rodent chow. In this situation, low protein diet CR-LPF was used in the present study instead of CRF-1.

MATERIALS AND METHODS

Animals: Fifty (50) male and 50 female Crj:CD(SD)IGS rats, at 4 weeks of age, were obtained twice on different dates (Lot 1: delivered on October 2, 1996, Lot 2: delivered on October 9, 1996) from Charles River Japan Inc. (Hino, Japan). Therefore, 100 males and 100 females were used in the present study. For each Lot, the animals were acclimatized for 2 weeks and healthy animals were used at 6 weeks of age and were housed in an animal room under the following conditions: temperature at 23 ± 3℃, relative humidity at 50±20%, air ventilation at 10 to 15 times per hour and 12-hour illumination (07:00 to 19:00). These animals were housed individually in hanging stainless-steel wire mesh cages and commercial low protein feed (approximately 18% crude protein), CR-LPF (Oriental Yeast Co., Ltd.), and tap water were provided *ad libitum*.

In-house data on F344 rats: The data from the control animals in the carcinogenicity studies carried out in our laboratory were used. F344 rats were fed CRF-1 (approximately 23% crude protein).

Observations and examinations:

General condition: The general condition of the animals was observed daily.

Body weight and food consumption: The body weight was recorded weekly. One day's food consumption was calculated based on the 7 day's cumulative consumption determined weekly.

Necropsy: At the end of the 104-week observation period, all survivors were necropsied after exsanguination under ether anesthesia. The animals found dead were necropsied as soon as possible after discovery.

RESULTS AND DISCUSSION

Mortality and survival rate are summerized in Table 1. The death of animals began to occur twenty-six weeks after the start of observation for both sexes. The survival rate at the termination of the observation period was 58% for Lot 1 and 50% for Lot 2 in males and 50% for Lot 1 and 48% for Lot 2 in females, and no apparent difference in the survival rate was noted between Lot 1 and Lot 2. Combined survival rate of the two lots was 54% in males and 49% in females. When the survival rate of IGS rats is compared to that of F344 rats, the survival rate of IGS rats was remarkably lower than that of F344 rats (81% in males and 82% infemales) for both sexes.

Abnormalities of the general condition observed at a high incidence in males that died were as follows: abnormal breathing (60.0%), unkempt fur (51.1%), decreased spontaneous movement (36.2%), hypothermia (29.8%) and prone/lateral position (25.5%). For females that died, the abnormalities of the general condition observed at a high incidence were as follows: abnormal breathing (72.5%), unkempt fur (66.7%), decreased spontaneous movement (62.7%), torticollis (27.5%), hypothermia (23.5%) and abnormal gait (23.5%). Almost all signs indicate an exhausted state, but torticollis and abnormal gait in females might be closely related to the situation that a majority of deaths was caused by pituitary tumor.

Table 1. Summary of mortality and survival rate in IGS rats fed CR-LPF

Sex	Male		Female	
Lot	Lot 1	Lot 2	Lot 1	Lot 2
Initial number of animals	50	50	50	50
Week of experiment				
1-26	0[a)]	0	0	0
27-52	1	0	1	1
53-78	8	5	8	7
79-104	21[b)]	25	25	26
Survival rate (%)				
for each Lot	58	50	50	48
combined	54		49	

a): Figure represents cumulative number of deaths.

Provisional causes of death judged from the clinical signs and necropsy findings (histopathology is now undergoing) are summerized in Table 2.

In both sexes, pituitary tumor was the dominant cause of death in IGS rats. In the cace of F344 rats, the main cause of death was leukemia for both sexes and difference were noted between IGS rats and F344 rats. Histopathological examination is now ongoing and the findings will be shown in the near future.

Table 2. # Provisional main causes of death in IGS rats fed CR-LPF

	Sex	Male			Female		
	Lot	Lot 1	Lot 2	Total	Lot 1	Lot 2	Total
	Initial No. of animals	50	50	100	50	50	100
# Provisional cause of death	Number of deaths	22	25	47	25	26	51
Tumor							
pituitary tumor		6[a)]	8	4(29.8)[b)]	18	21	39 (76.5)
skin/subcutis tumor		2	3	5 (10.6)	3	3	6 (11.8)
leukemia		1	2	3 (6.4)	0	0	0 (0)
renal tumor		1	0	1 (2.1)	0	0	0 (0)
prostate tumor		1	0	1 (2.1)	—	—	—
tumor in oral cavity		1	0	1 (2.1)	0	0	0 (0)
tumor in abdominal cavity		0	1	1 (2.1)	0	0	0 (0)
thymus tumor		0	0	0 (0)	0	1	1 (2.0)
tumor in cephalic cavity		0	0	0 (0)	1	0	1 (2.0)
total		12	14	26 (55.3)	22	25	47 (92.2)
Non-tumor							
hemorrhage (anemia)		0	2	2 (4.3)	2	0	2 (3.9)
liver injury		2	0	2 (4.3)	0	0	0 (0)
urination disturbance		1	1	2 (4.3)	0	0	0 (0)
renal injury		0	1	1 (2.1)	0	0	0 (0)
cyst in abdominal cavity		0	1	1 (2.1)	0	0	0 (0)
circulatory disturbance		0	0	0 (0)	0	1	1 (2.0)
fractured incisors		0	0	0 (0)	1	0	1 (2.0)
total		3	5	8 (17.0)	3	1	4 (7.8)
Unclear		7	6	13 (27.7)	0	0	0 (0)

a): Figure represents number of animals.
b): Number in parenthesis represents the percentage against all deaths.

Body weights are summerized in Table 3.

The mean terminal body weight of males was 705g for Lot 1 and 728g for Lot 2 and that of females was 450g for Lot 1 and 441g for Lot 2. Mean body weight of both lots was 716g for males and 446g for females, and was approximately 1.5-times that of F344 rats (440g in males and 293g in females).

Table 3. Mean body weight in IGS rats fed CR-LPF

	Male			Female		
	Lot 1	Lot 2	Total	Lot 1	Lot 2	Total
Initial number of animals	50	50	100	50	50	100
Week of experiment						
initial	184±7	182±8	183±7	142±6	139±7	141±7
26	582±55	588±59	585±57	314±33	299±32	306±33
52	667±74	673±83	669±79	371±44	351±48	361±47
78	730±92	730±107	730±99	433±67	406±61	419±65
104	705±101	728±121	716±110	450±101	441±74	446±88

Value represents Mean±S.D (unit: g).

Food consumption is summerized in Table 4.

Mean food consumption per animal per day in IGS rats ranged from 25.3 to 27.4g for males and 18.5 to 20.6g for females. Mean food consumption of both lots was 26.6g for males and 19.9g for females, and was nearly 1.5-times that of F344 rats (approximately 17g for males and 13g for females). The difference in food consumption between IGS rats and F344 rats was comparable to that of body weight, and therefore, it was suspected that a good appetite in the IGS rats resulted in obesity in this strain as compared to F344 rats.

Table 4. Mean Food consumption in IGS rats fed CR-LPF

	Male			Female		
	Lot 1	Lot 2	Total	Lot 1	Lot 2	Total
Initial number of animals	50	50	100	50	50	100
Week of experiment						
1 to 26	25.9	26.5	262	20.0	18.5	19.5
27 to 52	25.3	27.0	26.2	19.4	19.1	19.3
53 to 78	26.1	27.4	26.8	20.4	19.8	20.1
79 to 104	27.1	27.4	27.3	20.4	20.6	20.5
over-all			26.6			19.9

Valeu represents Mean±S.D (unit: g/rat/day).

REFERENCES

1. Keeman, K. P. et al. 1995. Diet overfeeding and moderate dietary restriction in control Sprague-Dawley rats: Ⅰ. Effects on spontaneoun neoplasms. Toxicol, Pathol., 23, 269-286.
2. Keeman, K. P. et al. 1995. Diet overfeeding and moderate dietary restriction in control Sprague-Dawley rats: Ⅱ. Effects on age-related proliferative and degenerative lesions. Toxicol, Pathol., 23, 287-302.

Background Pathology Data From Carcinogenicity Studies Comparing Crl:CD(SD)IGS and Crl:CD Rats – (1) Neoplastic Lesions

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ABSTRACT. Presented is a comparison of neoplastic findings from a sample of rat carcinogenicity studies conducted at Inveresk Research over a period from the mid 1990's through to 1999. Seven studies were selected for comparison based on their closeness in time, 4 used the older (CD) strain and 3 used the newer (IGS) strain. All were conducted over a 2 year treatment period and housed in very similar conditions, the only notable variable was the route of administration of the test material. — Key words: Carcinogenicity, Neoplastic, Pre-neoplastic

CD(SD)IGS-1999: 255-266

INTRODUCTION

During the late 1980's and into the 1990's much concern was expressed over the apparent decrease in survival performance of the Charles River strain of Sprague Dawley rat (CD®Rats; Crl: CD®BR). As this is one of the most common strains for use on lifetime carcinogenicity studies this was seen as a problem for ensuring acceptability with the regulatory authorities. In the mid 1990's Charles River undertook to rederive their stock to produce a strain of Sprague Dawley rat (the IGS International Genetic Standard-(IGS)CD®Rats; Crl: CD®BR) in an effort to lengthen its longevity under toxicology laboratory conditions.

Inveresk Research (Tranent, Scotland, UK) has used Sprague Dawley rats from Charles River (UK) Ltd for the majority of repeat dose toxicity studies conducted, including carcinogenicity studies, throughout the 1980's and 1990's. Throughout this period Inveresk Research did not experience any problem with survival but due to the change in supply started to use the IGS strain in 1996.

Presented are histopathological data from six carcinogenicity studies, selected for comparison based on their closeness in time, conducted at Inveresk Research over a period from the mid 1990's through to 1999. Four used the older (CD) strain and 2 used the newer (IGS) strain. The standard protocol for conducting carcinogenicity studies at Inveresk Research was used for all studies compared, the only notable variable was the route of administration of the test material. All were run with either single or double control groups, starting with 50 or 60 rats of each sex. This protocol employs a system of gang housing (5 per cage) in large plastic cages with wire grid bottoms and the use of a low calorie/low protein maintenance diet (*ca* 14% protein). Over the period of the comparison there has been very little change in terms of building design, air handling and other environmental conditions to complicate the evaluation of the data compiled.

On completion of 104 weeks of dosing, all surviving animals were killed and a detailed post mortem performed with a list of tissues preserved in a fixative. Histological examination of a comprehensive list of tissues was performed on all animals including premature decedents.

MATERIALS AND METHODS

Animals - Sprague-Dawley ((IGS)CD®Rats; Crl: CD®BR: Strain) rats were obtained, Charles River Limited, Margate, Kent, England. They were ordered to be *ca* 4 weeks old on despatch (typical weights being males – *ca* 80-90 g and females – *ca* 55-65 g).

All the animals were clinically examined on arrival for signs of abnormality or disease. The animals were acclimatised in the Inveresk animal room for approximately 2 weeks prior to commencement of treatment.

Room Environment and Sanitation - All studies was conducted in the rodent toxicology accommodation at the Elphinstone Research Centre of Inveresk Research. There was automatic control of light cycle (0700-1900 h), temperature and humidity (target ranges of 20℃±2℃ and 50%±15% respectively). There were 15-20 air changes per hour.

Each day on completion of all other work, the floor was swept and then mopped with 0.5% solution of Tego®2000 (Th. Goldschmidt, Victoria Road, Ruislip, Middlesex, England), an amphoteric biocide/cleanser. The room was washed with this solution once weekly.

Caging and Cage Sanitation - Generally the animals were housed 5 per cage by sex and dose group in polypropylene cages (*ca* 58 × 38.5 × 20 cm) with stainless steel grid tops,. Beneath the cage was suspended a tray containing absorbent paper. A stainless steel food hopper and a Durethan™ water bottle were provided for each cage. The cages were suspended on racks, each full rack containing 6 rows of 4 cages. Trays and water bottles were changed once weekly. Cages and hoppers were changed at least once every two weeks.

Diet and Water - Rat and Mouse Diet No. 1 SQC was supplied by Special Diet Services (SDS) Limited, Stepfield, Witham, Essex, England, and was available to the animals *ad libitum.* The diet was supplied with a batch analysis for nutritive constituents and a range of significant contaminants. The animals had access to domestic water *ad libitum.* The supply is analysed regularly for dissolved materials, heavy metals, pesticide residues. PH, nitrates and nitrites The water used by the Inveresk laboratories was analysed at 6 monthly intervals. None of the contaminants revealed by the analyses of food and water were considered to have been present in sufficient quantity to have affected the outcome of the study.

Animal Identification and Allocation to Treatment Group - On the day of arrival animals were distributed into cages and treatment groups. Each cage was ascribed a treatment group by the use of computer generated random cage allocation. After alloca-

tion to treatment group each rat received a subcutaneously implanted electronic chip supplied by Biomedic International which identified it individually within the study and corresponded to that animals number. In the event of a failure of the subcutaneously implanted electronic chip to function the affected animals had a replacement electronic chip implanted. The animals were ascribed a cage card which was colour coded for treatment group and marked with the project, cage and animal numbers, sex and the relevant treatment group. Extra animals were monitored during pretrial in case of the need for replacement prior to commencement of dosing. All spare animals were killed following commencement of dosing.

Treatment Regime - The animals were dosed for 104 consecutive weeks as follows:

Study	Strain	Number of Control Rats	Route of Administration
CD-A	CD	100	Gavage
CD-B	CD	100	Gavage
CD-C	CD	50	Diet
CD-D	CD	50	Diet
IGS-A	IGS	100	Implantation
IGS-B	IGS	100	Diet
IGS-C	IGS	120	Gavage

Clinical Observations - All the animals were checked for viability early morning and again as late as practical on each day. Any animals that showed signs of severe debility or intoxication or self-mutilation were killed. The nature, onset, intensity and duration of any signs were recorded. Once each week all animals received a detailed clinical examination and palpation. The clinical examination included appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. The size, appearance, position and duration of any masses detected was recorded.

Body Weight - Body weights were recorded at least once each week for the first 13 weeks and at least once every 4 weeks thereafter up until the end of the study.

Food and Water Consumption - The quantity of food consumed by each cage of animals was measured and recorded for the first 13 weeks of the study and over one week in every 4 thereafter until the end of the study. Water consumption was monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Terminal Studies - After 104 weeks of treatment all surviving animals were killed. All animals, including dead or moribund animals, were subject to a detailed necropsy under the guidance of a veterinary pathologist. The necropsy consisted of an internal and external examination. All gross lesions were recorded in terms of location, size, shape, colour, consistency and number. Selected organs were removed from all animals, cleaned of fat and connective tissue and representative samples of tissues fixed in 10% neutral buffered formalin. Typically the list of organs/tissues included - Adrenals, Aortic Arch, Blood Smear, Brain, Epididymides, Eyes, Femur, Gastro-intestinal Tract (Stomach, Duodenum, Jejunum, Ileum, Caecum, Colon, Rectum), Heart, Kidneys, Lachrymal Gland, Liver, Lung, Mammary Gland, Mesenteric Lymph Node, Nasal Cavity, Oesophagus, Optic Nerves, Ovaries, Pancreas, Pituitary, Prostate, Rib, Sciatic Nerve, Seminal Vesicles, Skin, Spinal Cord, Spleen, Sternum, Submandibular Lymph Node, Submaxillary (Mandibular) Salivary Gland, Testes, Thigh Muscle, Thymus, Thyroids (with Parathyroids), Tongue, Trachea, Urinary Bladder, Uterus, Vagina. Carcasses were discarded following sampling of these tissues. Tissues were processed and sections were cut 4-6 μm thick, stained with haematoxylin and eosin (H & E) and evaluated by a pathologist.

RESULTS

The neoplastic and pre-neoplastic histological findings are presented for both sexes of the two strains of Charles River rat in the tables (Tables 1 and 2). These show that the tumour profiles are essentially the same in both strains. Any variations seen were generally no bigger than that seen with inter-study variation within the same strain.

DISCUSSION

After treatment for 104 weeks (ie ca 110 weeks of age) the tumour profiles with both the CD and IGS strains were essentially the same. These data show that there has been no essential difference in tumour distribution of control rats between the two derivations of the Sprague Dawley rats. This apparent lack of effect of rederiving the strain has not had any noticeable effect on the conduct of carcinogenicity studies at Inveresk Research.

Table 1. Selected Neoplastic and Pre-neoplastic Lesions – Males (1)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
ABDOMINAL CAVITY												
Number examined	0	0	1	1	0	1	1	1	0	0	2	0
GIANT CELL SARCOMA	0	0	0	1	0	0	0	0	0	0	0	0
Peritoneal SARCOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
Total Malignant	0	0	1	0	0	0	0	0	0	0	0	0
Study Total		0		1		0		0		0		0
ADRENAL GLAND												
Number examined	49	34	49	26	46	49	50	49	50	50	60	59
Study Total		83		75		49		99		100		119
Focal medullary hyperplasia	8	4	1	1	14	8	26	24	8	13	12	12
Study Total		12		2	14	8		50		21		24
PHAEOCHROMOCYTOMA [B]	3	4	10	2	8	7	8	9	6	9	2	5
Study Total		7		12	8	7		17		15		7
PHAEOCHROMOCYTOMA [M]	1	2	0	0	0	0	0	1	0	1	0	0
Study Total		3		0	0	0		1		1		0
Focal cortical cell hyperplasia	13	5	4	0	21	8	16	14	6	10	14	11
Study Total		18		4	21	8		30		16		25
CORTICAL ADENOMA [B]	1	0	0	0	1	0	1	2	0	1	2	0
Study Total		1		0	1	0		3		1		2
CORTICAL CARCINOMA [M]	0	0	0	0	1	1	0	1	0	0	2	0
Study Total		0		0	1	1		1		0		2
BONE												
Number examined	0	0	3	0	0	0	50	51	0	1	62	59
Study Total		0		3	0	0		101		1		121
OSTEOSARCOMA [M]	0	0	2	0	0	0	0	1	0	1	2	0
Study Total		0		2	0	0		1		1		2
BRAIN												
Number examined	48	34	49	25	50	50	50	50	50	50	60	60
Study Total		82		74	50	50		100		100		120
MENINGIOMA [B]	0	0	0	0	0	0	0	1	1	0	0	0
GRANULAR CELL TUMOUR [B]	0	2	0	0	1	0	0	0	0	0	0	0
Total Benign	0	2	0	0	1	0	0	1	1	0	0	0
Study Total		2		0	1	0		1		1		0
MENINGEAL SARCOMA [M]	0	0	0	0	0	0	0	0	0	0	1	0
ASTROCYTOMA [M]	1	1	1	0	0	0	0	0	0	2	2	2
Total Malignant	1	1	1	0	0	0	0	0	0	2	3	2
Study Total		2		1	0	0		0		2		5
FOOT/LEG												
Number examined	0	0	0	0	6	12	9	10	12	11	9	9
Study Total		0		0	6	12		19		23		18
FIBROSARCOMA [M]	0	0	0	0	0	0	1	0	0	0	0	0
Study Total		0		0	0	0		1		0		0
HEART												
Number examined	50	34	50	26	50	50	50	50	50	50	60	60
Study Total		84		76	0	50		100		100		120
SCHWANNOMA [B]	0	0	0	0	0	0	0	0	0	0	2	0
MESOTHELIOMA [M]	0	1	0	0	0	0	1	0	1	0	0	0
Study Total		1		0	0	0		1		1		0
KIDNEY												
Number examined	50	49	50	50	44	45	49	44	47	46	60	60
Study Total		99		100	44	45		93		93		120
CARCINOMA [M]	0	0	0	1	0	0	0	0	0	0	0	0
Focal cortical tubular hyperplasia	1	2	0	0	0	0	0	0	0	0	0	0
Study Total		3		0	0	0		0		0		0

Table 1. Selected Neoplastic and Pre-neoplastic Lesions – Males (2)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
LIVER												
Number examined	50	34	50	26	48	49	50	49	48	49	60	59
Study Total		84		76	48	49		99		97		119
HEPATOCELLULAR ADENOMA [B]	0	0	2	0	2	0	1	2	2	2	0	3
Study Total		0		2	2	0		3		4		3
HEPATOCELLULAR CARCINOMA [M]	0	1	0	0	0	1	3	1	0	1	0	1
Study Total		1		0	0	1		4		1		1
Eosinophilic cell focus	8	5	8	6	12	13	21	19	23	20	4	9
Clear cell focus	20	3	8	1	15	19	6	6	18	11	22	25
Total	28	8	16	7	27	32	27	25	41	31	26	34
Study Total		36		23	27	32		52		72		60
Basophilic cell focus	13	4	14	0	12	2	20	21	3	8	12	12
Study Total		17		14	12	2		41		11		24
Amphophilic cell focus	0	0	0	0	0	0	0	0	0	0	1	8
Study Total		0		0	0	0		0		0		9
LUNG												
Number examined	50	34	48	26	44	45	50	49	46	47	60	59
Study Total		84		74		45		99		93		119
Bronchiolo-alveolar hyperplasia, focal	0	0	0	0	0	0	0	0	0	1	2	3
Total hyperplasia	0	0	0	0	0	0	0	0	0	1	2	3
Study Total		0		0	0	0		0		1		5
BRONCHIAL ADENOMA [B], papillary	0	0	0	0	0	0	0	0	1	0	0	0
ADENOMA [B]	0	0	1	0	0	0	0	0	0	0	0	0
BRONCHIOLO-ALVEOLAR ADENOMA [B]	1	0	0	0	1	0	0	0	0	2	0	0
Total benign	1	0	1	0	1	0	0	0	1	2	0	0
Study Total		1		1	1	0		0		3		0
LYMPHORETICULAR/HAEMOPOIETIC SYSTEM												
Number examined	50	34	50	26	50	50	50	50	50	50	60	60
Study Total		84		76	50	50		100		100		120
LYMPHOMA, LYMPHOCYTIC [M], leukaemic	0	0	0	0	0	0	0	0	0	0	1	0
LYMPHOMA, LYMPHOCYTIC [M], lymphoblastic, leukaemic	0	0	0	0	0	0	0	0	0	0	0	1
LYMPHOMA, LYMPHOCYTIC [M]	0	0	0	0	0	0	0	0	0	0	0	1
LYMPHOMA [M]	3	2	2	2	0	1	0	0	0	0	0	0
Total malignant	3	2	2	2	0	1	0	0	0	0	1	2
Study Total		5		4	0	1		0		0		3
LEUKAEMIA [M]	0	0	0	0	3	1	0	0	0	0	0	0
LEUKAEMIA, GRANULOCYTIC [M]	2	0	0	0	0	0	0	1	1	1	0	1
LEUKAEMIA, MONOCYTIC [M]	0	2	0	0	0	0	0	0	0	0	0	0
Total malignant	2	2	0	0	3	1	0	1	1	1	0	1
Study Total		4		0	3	1		1		2		1
HISTIOCYTIC SARCOMA [M]	2	1	1	0	2	4	0	0	1	2	3	1
Study Total		3		1	2	4		0		3		4
MAMMARY GLAND												
Number examined	43	31	49	26	50	46	50	46	38	43	60	60
Study Total		74		75	50	46		96		81		120
ADENOMA [B]	0	0	1	0	0	0	0	0	0	0	0	0
FIBROADENOMA [B]	1	0	0	0	1	2	1	0	1	0	0	1
Total benign	1	0	1	0	1	2	1	0	1	0	0	1
Study Total		1		1	1	2		1		1		1
CARCINOMA [M]	0	0	1	0	0	0	0	0	1	0	0	0
Study Total		0		1	0	0		0		1		0
MESENTERY												
Number examined	0	2	0	0	0	0	1	1	0	0	0	0
MESOTHELIOMA [M]	0	1	0	0	0	0	0	0	0	0	0	0

Table 1. Selected Neoplastic and Pre-neoplastic Lesions – Males (3)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
ORAL CAVITY												
Number examined	0	0	0	0	0	0	0	1	0	0	0	0
OSTEOSARCOMA [M]	0	0	0	0	0	0	0	1	0	0	0	0
Study Total		0		0	0	0		1		0		0
PANCREAS (ENDOCRINE)												
Number examined	47	34	49	26	48	48	50	48	50	50	60	60
Study Total		81		75	48	48		98		100		120
ISLET CELL ADENOMA [B]	1	1	4	1	3	2	5	3	2	3	3	0
Study Total		2		5	3	2		8		5		3
ISLET CELL CARCINOMA [M]	2	1	0	0	0	0	0	0	0	0	1	0
Study Total		3		0	0	0		0		0		1
Islet cell hyperplasia, focal	4	2	0	0	0	1	0	0	0	0	2	2
Study Total		6		0	0	1		0		0		4
PANCREAS (EXOCRINE)												
Number examined	47	34	49	26	48	48	50	48	50	47	60	60
Study Total		81		75	48	48		98		97		120
ACINAR CELL ADENOMA [B]	0	1	0	0	0	1	0	0	0	0	0	0
Study Total		1		0	0	1		0		0		0
Focal acinar cell hyperplasia	2	2	1	1	0	0	3	2	0	1	0	0
Hyperplasia, focal	0	0	0	0	0	1	0	0	0	0	0	0
Total hyperplasia	2	2	1	1	0	1	3	2	0	1	0	0
Study Total		4		2	0	1		5		1		0
PARATHYROID GLAND												
Number examined	43	25	48	24	48	46	50	47	42	42	56	56
Study Total		68		72	48	46		97		84		112
ADENOMA [B]	0	1	0	0	0	1	0	1	0	0	1	1
Study Total		1		0	0	1		1		0		2
Focal hyperplasia, multiple, unilateral	0	0	0	0	0	0	1	1	0	0	0	0
Focal hyperplasia	3	2	0	0	0	0	5	3	0	1	2	4
Focal hyperplasia, bilateral	0	0	0	0	0	0	0	0	0	1	0	2
Hyperplasia, unilateral, focal	0	0	0	0	0	0	0	0	1	0	0	0
Total hyperplasia	3	2	0	0	0	0	6	4	1	2	2	6
Study Total		5		0	0	0		10		3		8
PITUITARY GLAND												
Number examined	49	33	49	26	48	48	50	49	49	49	60	60
Study Total		82		75	48	48		99		98		120
ADENOMA, ANTERIOR LOBE [B]	19	13	23	12	21	19	24	19	16	13	22	23
Study Total		32		35	21	19		43		29		45
CARCINOMA [M]	1	0	0	0	0	0	0	0	0	0	0	0
Study Total		1		0	0	0		0		0		0
Focal hyperplasia, anterior lobe	0	0	4	4	8	12	13	14	2	8	10	13
Study Total		0		8	8	12		27		10		23
ADENOMA, INTERMEDIATE LOBE [B]	1	0	0	0	0	1	0	0	0	0	0	2
Study Total		1		0	0	1		0		0		2
Focal hyperplasia, intermediate lobe	0	0	0	0	1	0	0	0	0	0	2	1
Study Total		0		0	1	0		0		0		3
PREPUTIAL GLAND:												
Number examined	0	0	0	0	1	0	0	0	0	0	0	0
ADENOMA [B]	0	0	0	0	1	0	0	0	0	0	0	0
Study Total		0		0	1	0		0		0		0
PROSTATE												
Number examined	48	34	49	26	50	49	50	50	49	49	60	60
Study Total		82		75	50	49		100		98		120
ADENOMA [B]	0	0	0	0	0	0	0	0	0	1	0	0
Study Total		0		0	0	0		0		1		0
ADENOCARCINOMA [M]	0	0	0	0	1	0	1	0	0	0	0	0
Study Total		0		0	1	0		1		0		0
Focal hyperplasia	1	1	0	0	0	0	0	1	0	0	2	1
Study Total		2		0	0	0		1		0		3

Table 1. Selected Neoplastic and Pre-neoplastic Lesions – Males (4)

	CD Strain						IGS Strain					
Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
SEMINAL VESICLE												
Number examined	48	34	50	26	49	48	50	50	49	49	60	60
Study Total		82		76	49	48		100		98		120
ADENOMA [B]	0	0	0	0	0	1	0	0	0	0	0	0
Study Total		0		0	0	1		0		0		0
SKIN AND SUBCUTIS												
Number examined	49	34	49	26	50	50	50	50	50	50	60	60
Study Total		83		75	50	50		100		100		120
SQUAMOUS-CELL PAPILLOMA [B]	0	2	0	0	0	0	2	0	0	0	0	0
PAPILLOMA [B]	0	0	4	0	0	0	0	0	0	2	0	0
KERATOACANTHOMA [B]	1	1	0	0	2	0	2	3	0	3	2	4
BASAL CELL ADENOMA [B]	0	0	6	4	0	0	1	0	0	0	1	0
SEBACEOUS CELL ADENOMA [B]	0	0	0	0	0	0	0	2	1	0	0	1
Total Benign, epidermal	1	3	10	4	2	0	5	5	1	5	3	5
Study Total		4		14	2	0		10		6		8
BASAL CELL CARCINOMA [M]	0	0	0	0	0	0	0	0	0	1	1	0
SQUAMOUS-CELL CARCINOMA [M]	0	0	0	0	1	1	0	0	1	0	0	0
Total Malignant, epidermal	0	0	0	0	1	1	0	0	1	1	1	0
Study Total		0		0	1	1		0		2		1
Hyperplasia, basal cell, focal	0	0	0	0	0	0	0	0	0	1	0	0
FIBROUS HISTIOCYTOMA [B]	0	0	0	0	0	1	0	0	0	0	0	0
FIBROLIPOMA [B]	0	0	0	0	0	0	1	0	0	0	0	0
FIBROMA [B]	8	4	6	3	9	11	4	5	2	7	3	6
LIPOMA [B]	1	4	0	0	2	1	2	1	1	2	1	5
DERMAL FIBROMA [B]	0	0	0	0	0	0	3	4	0	0	2	4
Total Benign	9	8	6	3	11	13	10	10	3	9	6	15
Study Total		17		9	11	13		20		12		21
SARCOMA (not otherwise specified) [M]	0	0	2	0	1	2	0	0	2	2	0	0
FIBROSARCOMA [M]	0	0	0	0	0	3	2	2	2	0	2	3
Total Malignant	0	0	2	0	1	5	2	2	4	2	2	3
Study Total		0		2	1	5		4		6		5
SPINAL CORD												
Number examined	48	34	49	26	50	50	50	50	50	50	60	60
Study Total		82		75	0	50		100		100		120
MALIGNANT ASTROCYTOMA [M]	0	0	0	0	1	0	0	0	0	0	0	1
Study Total		0		0	1	0		0		0		1
SPLEEN												
Number examined	50	34	49	26	48	49	50	49	49	48	60	60
Study Total		84		75	48	49		99		97		120
SARCOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
Study Total		0		1	0	0		0		0		0
TESTIS												
Number examined	50	34	49	26	50	49	50	50	49	50	60	60
Study Total		84		75	50	49		100		99		120
SERTOLI CELL TUMOUR [B]	0	0	0	0	0	0	1	0	0	0	0	0
INTERSTITIAL CELL ADENOMA [B]	7	3	4	0	5	2	4	1	1	1	1	1
MESOTHELIOMA [B]	0	0	0	1	0	0	0	0	0	0	0	0
Total Benign	7	3	4	1	5	2	5	1	1	1	1	1
Study Total		10		5	5	2		6		2		2
Focal interstitial cell hyperplasia	11	2	0	0	6	3	11	1	2	0	0	1
Study Total		13		0	6	3		12		2		1

Table 1. Selected Neoplastic and Pre-neoplastic Lesions – Males (5)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
THYMUS												
Number examined	46	31	44	26	41	48	47	50	44	30	60	57
Study Total		77		70	41	48		97		74		117
THYMOMA [B]	2	0	0	0	0	2	0	0	0	0	0	0
Study Total		2		0	0	2		0		0		0
CARCINOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
THYMOMA [M]	0	1	0	0	0	0	0	0	0	0	0	0
Total Malignant	0	1	1	0	0	0	0	0	0	0	0	0
Study Total		1		1	0	0		0		0		0
THYROID GLAND												
Number examined	47	28	48	26	43	48	49	44	49	48	58	59
Study Total		75		74	43	48		93		97		117
C-CELL ADENOMA [B]	4	0	3	0	5	3	6	6	3	7	5	8
Study Total		4		3	5	3		12		10		13
C-CELL CARCINOMA [M]	0	1	0	0	0	0	0	0	1	0	0	0
Study Total		1		0	0	0		0		1		0
Focal C-cell hyperplasia	6	3	0	0	2	1	3	3	2	2	6	5
Study Total		9		0	2	1		6		4		11
FOLLICULAR CELL ADENOMA [B]	1	0	0	0	0	1	1	0	0	0	1	0
Study Total		1		0	0	1		1		0		1
FOLLICULAR CELL CARCINOMA [M]	1	0	0	0	0	0	1	0	0	0	1	0
Study Total		1		0	0	0		1		0		1
Focal follicular cell hyperplasia	1	2	0	0	1	0	0	4	0	0	0	1
Study Total		3		0	1	0		4		0		1
VASCULAR SYSTEM												
Number examined	50	34	50	26	50	50	50	50	50	50	60	60
Study Total		84		76	50	50		100		100		120
HAEMANGIOMA [B]	0	0	1	0	0	0	0	1	0	1	1	0
Study Total		0		1	0	0		1		1		1
HAEMANGIOSARCOMA [M]	1	3	0	0	0	1	0	1	0	0	0	0
Study Total		4		0	0	1		1		0		0

Table 2. Selected Neoplastic and Pre-neoplastic Lesions – Females (1)

	CD Strain						IGS Strain					
Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
ADRENAL GLAND												
Number examined	50	28	50	27	50	49	50	50	50	50	60	60
Study Total		78		77	50	49		100		100		120
Focal medullary hyperplasia	10	3	0	0	3	3	8	7	3	3	2	4
Study Total		13		0	3	3		15		6		6
PHAEOCHROMOCYTOMA [B]	1	0	2	1	2	1	2	0	2	1	2	0
Study Total		1		3	2	1		2		3		2
PHAEOCHROMOCYTOMA [M]	1	0	2	4	0	0	2	0	0	0	0	1
Study Total		1		6	0	0		2		0		1
Focal cortical cell hyperplasia	16	4	2	1	28	10	25	37	4	4	2	5
Study Total		20		3	28	10		62		8		7
CORTICAL ADENOMA [B]	2	1	1	0	1	1	0	0	1	1	0	2
Study Total		3		1	1	1		0		2		2
CORTICAL CARCINOMA [M]	0	0	0	0	0	0	0	0	0	1	0	1
Study Total		0		0	0	0		0		1		1
BONE												
Number examined	0	1	0	0	0	1	50	50	0	0	59	62
Study Total		1		0	0	1		100		0		121
OSTEOSARCOMA [M]		0	0	0	0	1	0	0	0	0	0	2
Study Total		0		0	0	1		0		0		2
OSTEOMA [B]	0	1	0	0	0	0	0	0	0	0	0	0
Study Total		1		0	0	0		0		0		0
BRAIN												
Number examined	50	28	50	27	-50	50	50	50	50	50	60	60
Study Total		78		77	50	50		100		100		120
EPENDYMOMA [B]	0	0	0	0	0	0	0	0	1	0	0	0
GRANULAR CELL TUMOUR [B]	0	0	0	0	0	0	0	1	0	0	0	0
Total Benign	0	0	0	0	0	0	0	1	1	0	0	0
Study Total		0		0	0	0		1		1		0
ASTROCYTOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
Study Total		0		1	0	0		0		0		0
CERVIX												
Number examined	50	28	50	26	0	0	2	0	3	6	3	4
Study Total		78		76	0	0		2		9		7
POLYP [B]	0	0	0	0	0	0	0	0	0	1	0	0
STROMAL POLYP [B],	1	0	0	0	0	0	0	0	0	0	0	0
LEIOMYOMA [B]	0	0	0	0	0	0	0	0	0	1	0	0
Total Benign	1	0	0	0	0	0	0	0	0	2	0	0
Study Total		1		0	0	0		0		2		0
CLITORAL GLAND												
Number examined	0	0	0	0	0	0	0	0	0	1	0	0
CARCINOMA [M]	0	0	0	0	0	0	0	0	0	1	0	0
Study Total		0		0	0	0		0		1		0
DUODENUM												
Number examined	50	27	48	24	48	47	46	48	50	49	55	60
LEIOMYOMA [B]	0	0	0	0	0	0	0	0	0	1	0	0
Study Total		0		0	0	0		0		1		0
LEIOMYOSARCOMA [M]	0	0	0	0	0	0	1	0	0	0	0	1
Study Total		0		0	0	0		1		0		1
EAR												
Number examined	1	0	0	0	0	0	1	2	2	2	1	1
Study Total		1		0	0	0		3		4		2
SQUAMOUS-CELL CARCINOMA [M]	0	0	0	0	0	0	1	0	0	0	0	0
Study Total		0		0	0	0		1		0		0

Table 2. Selected Neoplastic and Pre-neoplastic Lesions – Females (2)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
FOOT/LEG												
Number examined	0	1	0	0	0	1	6	4	8	7	5	7
Study Total		1		0	0	1		10		15		12
FIBROSARCOMA [M]	0	0	0	0	0	0	1	0	0	0	0	0
Study Total		0		0	0	0		1		0		0
HEART												
Number examined	50	28	50	27	50	50	50	50	50	50	58	60
Study Total		78		77	50	50		100		100		118
SCHWANNOMA [B]	0	0	0	0	0	0	0	0	0	0	0	1
Study Total		0		0	0	0		0		0		1
KIDNEY												
Number examined	50	50	50	50	47	47	48	50	49	49	60	60
Study Total		100		100	47	47		98		98		120
TRANSITIONAL CELL PAPILLOMA [B]	0	0	0	0	0	0	0	1	0	0	0	0
TUBULAR CELL ADENOMA [B]	0	0	0	0	0	0	0	1	0	0	0	0
Total Benign	0	0	0	0	0	0	0	2	0	0	0	0
Study Total		0		0	0	0		2		0		0
TRANSITIONAL CELL CARCINOMA	0	0	1	0	0	0	0	0	0	0	0	0
Study Total		0		1	0	0		0		0		0
RENAL: LIPOMA [B]	1	0	0	0	0	0	0	0	0	0	0	0
RENAL: LIPOSARCOMA [M]	0	1	0	0	0	0	0	0	0	0	0	0
Total Tumours	1	1	0	0	0	0	0	0	0	0	0	0
Study Total		2		0	0	0		0		0		0
LIVER												
Number examined	50	28	50	27	48	50	50	50	50	50	60	60
Study Total		78		77	48	50		100		100		120
HEPATOCELLULAR ADENOMA [B]	0	0	1	0	1	0	0	0	0	0	0	1
Study Total		0		1	1	0		0		0		1
HEPATOCELLULAR CARCINOMA [M]	1	0	0	0	0	0	0	0	0	0	0	0
TUMOUR(S) [M]	0	0	0	0	1	0	0	0	0	0	0	0
Total Malignant	1	0	0	0	1	0	0	0	0	0	0	0
Study Total		1		0	1	0		0		0		0
Eosinophilic cell focus	11	1	9	0	10	1	4	6	4	8	3	3
Clear cell focus	16	2	6	1	2	8	8	8	3	5	9	10
Group Total	27	3	15	1	12	9	12	14	7	13	12	13
Study Total		30		16	12	9		26		20		25
Basophilic cell focus	29	7	18	7	22	13	38	37	23	27	39	44
Study Total		36		25	22	13		75		50		83
Amphophilic cell focus	0	0	0	0	0	0	0	0	0	0	2	5
Study Total		0		0	0	0		0		0		7
LUNG												
Number examined	50	28	50	27	47	47	50	50	49	49	58	60
Study Total		78		77	47	47		100		98		118
Bronchiolo-alveolar hyperplasia, focal	0	0	0	0	0	0	0	0	0	1	0	1
Study Total		0		0	0	0		0		1		1
ADENOMA [B]	0	0	1	0	0	0	0	0	0	0	0	0
BRONCHIOLO-ALVEOLAR ADENOMA [B]	1	0	0	0	0	0	1	0	1	0	0	0
Total Benign	1	0	1	0	0	0	1	0	1	0	0	0
Study Total		1		1	0	0		1		1		0
LYMPHORETICULAR/HAEMOPOIETIC SYSTEM												
Number examined	0	0	0	0	0	50	50	50	50	50	60	60
Study Total		0		0	0	50		100		100		120
LYMPHOMA, LYMPHOCYTIC [M]	0	0	0	0	0	0	0	0	1	0	0	0
LYMPHOMA, LYMPHOCYTIC [M], lymphoblastic	0	0	0	0	0	0	0	0	0	0	1	0

Table 2. Selected Neoplastic and Pre-neoplastic Lesions – Females (3)

	CD Strain						IGS Strain					
Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
LYMPHOMA, FOLLICULAR CENTRE CELL [M]	0	0	0	0	0	0	0	0	1	1	0	0
LYMPHOMA [M]			0	1		1		0	0	0	0	0
Total Malignant	0	0	0	1	0	1	0	0	2	1	1	0
Study Total		0		1	0	1		0		3		1
LEUKAEMIA, GRANULOCYTIC [M]	0	0	0	0	0	0	0	0	2	0	0	0
Study Total		0		0	0	0		0		2		0
HISTIOCYTIC SARCOMA [M]	2	1	1	0	3	3	0	2	0	0	1	2
Study Total		3		1	3	3		2		0		3
MAMMARY GLAND												
Number examined	50	28	50	27	50	50	50	50	50	50	60	60
Study Total		78		77	50	50		100		100		120
Lobular hyperplasia, focal	0	0	0	0	0	0	3	5	2	1	2	2
Focal hyperplasia	7	4	0	0	0	0	0	1	0	0	3	8
Group Total	7	4	0	0	0	0	3	6	2	1	5	10
Study Total		11		0	0	0		9		3		15
ADENOMA [B]	0	0	24	16	2	0	4	6	2	1	5	4
LIPOMA [B]	0	0	0	0	0	0	1	0	0	0	0	0
FIBROMA [B]	0	0	0	0	0	0	1	0	0	0	0	0
FIBROADENOMA [B]	0	0	0	0	26	24	19	23	20	24	20	34
Total Benign	0	0	24	16	28	24	25	29	22	25	25	38
Study Total		0		40	28	24		54		47		63
CARCINOMA [M]	0	0	2	1	10	8	7	10	13	10	5	7
CARCINOSARCOMA [M]	0	0	0	0	0	0	0	0	0	0	0	1
Total Malignant	0	0	2	1	10	8	7	10	13	10	5	8
Study Total		0		3	10	8		17		23		13
ORAL CAVITY												
Number examined	0	0	0	0	0	0	0	2	0	0	0	0
Study Total		0		0	0	0		2		0		0
SQUAMOUS-CELL CARCINOMA [M]	0	0	0	0	0	0	0	2	0	0	0	0
Study Total		0		0	0	0		2		0		0
OVARY:												
Number examined	50	28	0	0	50	50	50	50	50	50	60	60
Study Total		78		0	50	50		100		100		120
STROMAL POLYP [B]	0	0	0	0	0	0	0	1	0	0	0	0
THECAL CELL TUMOUR [B]	0	0	0	0	0	0	0	0	1	0	0	0
GRANULOSA CELL TUMOUR [B]	0	0	0	0	0	1	0	1	0	0	0	0
Total Benign	0	0	0	0	0	1	0	1	1	0	0	0
Study Total		0		0	0	1		1		1		0
STROMAL SARCOMA [M], undifferentiated	0	0	0	0	0	0	0	0	1	0	0	0
CARCINOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
Total malignant	0	0	1	0	0	0	0	0	1	0	0	0
Study Total		0		1	0	0		0		1		0
OVIDUCT												
Number examined	0	0	0	0	0	0	0	0	0	0	56	58
Study Total		0		0	0	0		0		0		114
LEIOMYOMA [B]	0	0	0	0	0	0	0	0	0	0	0	1
Study Total		0		0	0	0		0		0		1
PANCREAS (ENDOCRINE)												
Number examined	50	26	49	27	49	48	50	49	50	50	60	60
Study Total		76		76	49	48		99		100		120
ISLET CELL ADENOMA [B]	0	0	1	0	0	0	0	1	0	0	1	2
Study Total		0		1	0	0		1		0		3
Islet cell hyperplasia, focal	1	0	0	0	0	0	0	0	0	0	1	1
Study Total		1		0	0	0		0		0		2

Table 2. Selected Neoplastic and Pre-neoplastic Lesions – Females (4)

Study	CD Strain						IGS Strain					
	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
PANCREAS (EXOCRINE)												
Number examined	50	26	49	27	49	48	50	49	50	49	60	60
Study Total		76		76	49	48		99		99		120
ACINAR CELL ADENOMA [B]	0	0	0	0	0	1	0	0	0	0	0	0
Study Total		0		0	0	1		0		0		0
PARATHYROID GLAND												
Number examined	46	28	45	23	40	45	45	48	46	43	49	49
Study Total		74		68	40	45		93		89		98
ADENOMA [B]	0	1	0	0	0	0	0	0	0	0	0	0
Study Total		1		0	0	0		0		0		0
Focal hyperplasia	4	2	0	0	0	0	5	2	2	2	0	3
Study Total		6		0	0	0		7		4		3
PITUITARY GLAND												
Number examined	49	28	50	25	47	49	48	49	49	48	57	59
Study Total		77		75	47	49		97		97		116
ADENOMA, ANTERIOR LOBE [B]	33	23	39	22	0	34	35	25	35	27	28	35
Study Total		56		61	0	34		60		62		63
CARCINOMA [M]	1	2	0	0	3	2	0	3	3	3	1	1
Study Total		3		0	3	2		3		6		2
Focal hyperplasia, anterior lobe	9	1	2	0	10	0	0	0	0	0	0	0
Study Total		10		2	10	0		0		0		0
ADENOMA, INTERMEDIATE LOBE [B]	0	0	0	0	0	0	1	1	2	0	0	1
Study Total		0		0	0	0		2		2		1
Focal hyperplasia, intermediate lobe	0	1	1	0		0	0	0	0	1	0	0
Study Total		1		1	0	0		0		1		0
SKIN AND SUBCUTIS												
Number examined	50	28	50	27	50	50	50	50	50	50	60	60
Study Total		78		77	50	50		100		100		120
SQUAMOUS-CELL PAPILLOMA [B]	1	0	0	0	0	0	0	0	0	0	0	0
KERATOACANTHOMA [B]	0	0	0	0	0	0	1	1	0	1	0	1
BASAL CELL ADENOMA [B]	0	0	1	0	0	0	0	0	0	0	0	0
Total Benign	1	0	1	0	0	0	1	1	0	1	0	1
Study Total		1		1	0	0		2		1		1
BASAL CELL CARCINOMA [M]	0	0	0	0	0	0	0	1	0	0	0	0
CARCINOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
Total malignant	0	0	1	0	0	0	0	1	0	0	0	0
Study Total		0		1	0	0		1		0		0
Hyperplasia, basal cell, focal	0	0	1	0	0	0	0	0	0	0	0	0
Study Total		0		1	0	0		0		0		0
FIBROUS HISTIOCYTOMA [B]	0	0	0	0	0	0	0	1	1	0	0	0
FIBROLIPOMA [B]	0	0	0	0	0	0	0	1	0	0	0	0
FIBROMA [B]	3	1	3	0	2	1	0	1	2	2	5	2
LIPOMA [B]	0	0	0	0	1	0	0	1	4	3	0	0
DERMAL FIBROMA [B]	0	0	0	0	0	0	0	0	0	0	0	1
Total Benign	3	1	3	0	3	1	0	4	7	5	5	3
Study Total		4		3	3	1		4		12		8
SARCOMA (Not Otherwise Specified) [M]	0	0	1	1	0	0	0	0	0	0	0	2
LIPOSARCOMA [M]	0	0	0	0	0	0	1	0	0	0	0	0
FIBROSARCOMA [M]	0	1	0	0	0	0	0	0	2	1	1	2
Total Malignant	0	1	1	1	0	0	1	0	2	1	1	4
Study Total		1		2	0	0		1		3		5
Infiltration by lymphoma cells	0	0	0	0	0	0	0	0	0	1	0	0
Infiltration by histiocytic sarcoma	1	0	0	0	3	0	0	0	0	0	0	0
SPINAL CORD												
Number examined	50	28	50	27	50	50	50	50	50	50	59	60
Study Total		78		77	50	50		100		100		119
MALIGNANT ASTROCYTOMA [M]	0	0	0	0	0	0	1	0	0	0	0	0
Study Total		0		0	0	0		1		0		0

Table 2. Selected Neoplastic and Pre-neoplastic Lesions – Females (5)

	CD Strain						IGS Strain					
Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
Number per group	50	50	50	50	50	50	50	50	50	50	60	60
THYMUS												
Number examined	47	27	49	25	48	48	49	49	44	44	57	59
Study Total		74		74	48	48		98		88		116
THYMOMA [B]	0	0	1	1	0	1	0	0	0	0	0	0
Study Total		0		2	0	1		0		0		0
CARCINOMA [M]	0	0	1	0	0	0	0	0	0	0	0	0
THYMOMA [M]	0	0	0	0	0	0	0	0	0	0	0	2
Total Malignant	0	0	1	0	0	0	0	0	0	0	0	2
Study Total		0		1	0	0		0		0		2
Hyperplasia, epithelial, focal	0	0	0	0	0	0	0	1	0	0	0	0
Tubular cystic hyperplasia, focal	0	0	0	0	0	0	2	11	0	0	0	0
Group Total	0	0	0	0	0	0	2	12	0	0	0	0
Study Total		0		0	0	0		14		0		0
THYROID GLAND												
Number examined	49	28	50	26	48	48	46	48	49	49	57	57
Study Total		77		76	48	48		94		98		114
C-CELL ADENOMA [B]	3	3	1	0	6	4	5	6	2	2	2	5
Study Total		6		1	6	4		11		4		7
C-CELL CARCINOMA [M]	0	2	0	0	1	0	0	0	0	0	0	0
Study Total		2		0	1	0		0		0		0
Focal C-cell hyperplasia	9	5	0	0	4	4	7	4	1	2	3	5
Study Total		14		0	4	4		11		3		8
FOLLICULAR CELL ADENOMA [B]	1	1	1	1	1	0	0	0	1	0	1	0
Study Total		2		2	1	0		0		1		1
Focal follicular cell hyperplasia	0	0	2	3		1	0	1	2	0	0	0
Study Total		0		5	0	1		1		2		0
URINARY BLADDER												
Number examined	49	28	50	27	48	48	50	50	50	49	59	60
Study Total		77		77	48	48		100		99		119
Transitional cell hyperplasia, focal, simple	0	0	0	0	0	0	0	0	1	0	0	0
Study Total		0		0	0	0		0		1		0
UTERUS												
Number examined	50	28	50	27	50	50	50	50	50	50	60	60
Study Total		78		77	50	50		100		100		120
POLYP [B]	0	0	0	0	1	0	0	0	3	3	0	0
STROMAL POLYP [B],	2	1	0	0	0	2	3	5	0	0	5	2
ADENOMA [B]	0	0	0	0	0	0	0	0	0	0	0	1
Total Benign	2	1	0	0	1	2	3	5	3	3	5	3
Study Total		3		0	1	2		8		6		8
ADENOCARCINOMA [M]	0	1	0	0	0	0	0	0	0	0	0	0
CARCINOMA [M]	0	0	0	0	0	0	0	0	0	0	1	0
Total Malignant	0	1	0	0	0	0	0	0	0	0	1	0
Study Total		1		0	0	0		0		0		1
LEIOMYOMA [B]	0	0	2	1	0	0	1	0	0	0	0	0
Study Total		0		3	0	0		1		0		0
STROMAL SARCOMA [M]	0	0	0	0	0	0	0	0	0	0	1	2
Study Total		0		0	0	0		0		0		3
VAGINA												
Number examined	50	27	49	27	50	50	50	50	50	49	0	0
Study Total		77		76	50	50		100		99		0
STROMAL POLYP [B]	0	0	0	0	0	0	1	0	0	1	0	0
Study Total		0		0	0	0		1		1		0
VASCULAR SYSTEM												
Number examined	50	28	50	27	50	50	50	50	50	50	58	60
Study Total		78		77	50	50		100		100		118
HAEMANGIOSARCOMA [M]	0	0	0	0	0	0	0	0	0	1	0	0
Study Total		0		0	0	0		0		1		0

Background Pathology Data From Carcinogenicity Studies Comparing Crl:CD(SD)IGS and Crl:CD Rats – (2) Non-neoplastic Lesions

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ABSTRACT. Presented is a comparison of selected non-neoplastic findings from a sample of rat carcinogenicity studies conducted at Inveresk Research over a period from the mid 1990's through to 1999. Seven studies were selected for comparison based on their closeness in time, 4 used the older (CD) strain and 3 used the newer (IGS) strain. All were conducted over a 2 year treatment period and housed in very similar conditions, the only notable variable was the route of administration of the test material. – Key words: Carcinogenicity, Non-neoplastic, Rat

CD(SD)IGS-1999: 267-283

INTRODUCTION

During the late 1980's and into the 1990's much concern was expressed over the apparent decrease in survival performance of the Charles River strain of Sprague Dawley rat (CD®Rats; Crl: CD®BR). As this is one of the most common strains for use on lifetime carcinogenicity studies this was seen as a problem for ensuring acceptability with the regulatory authorities. In the mid 1990's Charles River undertook to rederive their stock to produce a strain of Sprague Dawley rat (the IGS International Genetic Standard - (IGS)CD®Rats; Crl: CD®BR) in an effort to lengthen its longevity under toxicology laboratory conditions.

Inveresk Research (Tranent, Scotland, UK) has used Sprague Dawley rats from Charles River (UK) Ltd for the majority of repeat dose toxicity studies conducted, including carcinogenicity studies, throughout the 1980's and 1990's. Throughout this period Inveresk Research did not experience any problem with survival but due to the change in supply started to use the IGS strain in 1996.

Presented are histopathological data from six carcinogenicity studies, selected for comparison based on their closeness in time, conducted at Inveresk Research over a period from the mid 1990's through to 1999. Four used the older (CD) strain and 2 used the newer (IGS) strain. The standard protocol for conducting carcinogenicity studies at Inveresk Research was used for all studies compared, the only notable variable was the route of administration of the test material. All were run with either single or double control groups, starting with 50 or 60 rats of each sex. This protocol employs a system of gang housing (5 per cage) in large plastic cages with wire grid bottoms and the use of a low calorie/low protein maintenance diet (*ca* 14% protein). Over the period of the comparison there has been very little change in terms of building design, air handling and other environmental conditions to complicate the evaluation of the data compiled.

On completion of 104 weeks of dosing, all surviving animals were killed and a detailed post mortem performed with a list of tissues preserved in a fixative. Histological examination of a comprehensive list of tissues was performed on all animals including premature decedents.

MATERIALS AND METHODS

Animals - Sprague-Dawley ((IGS)CD®Rats; Crl: CD®BR: Strain) rats were obtained, Charles River Limited, Margate, Kent, England. They were ordered to be *ca* 4 weeks old on despatch (typical weights being males – *ca* 80-90 g and females – *ca* 55-65 g).

All the animals were clinically examined on arrival for signs of abnormality or disease. The animals were acclimatised in the Inveresk animal room for approximately 2 weeks prior to commencement of treatment.

Room Environment and Sanitation - All studies was conducted in the rodent toxicology accommodation at the Elphinstone Research Centre of Inveresk Research. There was automatic control of light cycle (0700-1900 h), temperature and humidity (target ranges of 20℃±2℃ and 50%±15% respectively). There were 15-20 air changes per hour.

Each day on completion of all other work, the floor was swept and then mopped with 0.5% solution of Tego®2000 (Th. Goldschmidt, Victoria Road, Ruislip, Middlesex, England), an amphoteric biocide/cleanser. The room was washed with this solution once weekly.

Caging and Cage Sanitation - Generally the animals were housed 5 per cage by sex and dose group in polypropylene cages (*ca* 58 × 38.5 × 20 cm) with stainless steel grid tops,. Beneath the cage was suspended a tray containing absorbent paper. A stainless steel food hopper and a Durethan™ water bottle were provided for each cage. The cages were suspended on racks, each full rack containing 6 rows of 4 cages. Trays and water bottles were changed once weekly. Cages and hoppers were changed at least once every two weeks.

Diet and Water - Rat and Mouse Diet No. 1 SQC was supplied by Special Diet Services (SDS) Limited, Stepfield, Witham, Essex, England, and was available to the animals *ad libitum.* The diet was supplied with a batch analysis for nutritive constituents and a range of significant contaminants. The animals had access to domestic water *ad libitum.* The supply is analysed regularly for dissolved materials, heavy metals, pesticide residues. PH, nitrates and nitrites The water used by the Inveresk laboratories was analysed at 6 monthly intervals. None of the contaminants revealed by the analyses of food and water were considered to have been present in sufficient quantity to have affected the outcome of the study.

Animal Identification and Allocation to Treatment Group - On the day of arrival animals were distributed into cages and treatment groups. Each cage was ascribed a treatment group by the use of computer generated random cage allocation. After allocation to treatment group each rat received a subcutaneously implanted electronic chip supplied by Biomedic International which

identified it individually within the study and corresponded to that animals number. In the event of a failure of the subcutaneously implanted electronic chip to function the affected animals had a replacement electronic chip implanted. The animals were ascribed a cage card which was colour coded for treatment group and marked with the project, cage and animal numbers, sex and the relevant treatment group. Extra animals were monitored during pretrial in case of the need for replacement prior to commencement of dosing. All spare animals were killed following commencement of dosing.

Treatment Regime - The animals were dosed for 104 consecutive weeks as follows:

Study	Strain	Number of Control Rats	Route of Administration
CD-A	CD	100	Gavage
CD-B	CD	100	Gavage
CD-C	CD	50	Diet
CD-D	CD	50	Diet
IGS-A	IGS	100	Implantation
IGS-B	IGS	100	Diet
IGS-C	IGS	120	Gavage

Clinical Observations - All the animals were checked for viability early morning and again as late as practical on each day. Any animals that showed signs of severe debility or intoxication or self-mutilation were killed. The nature, onset, intensity and duration of any signs were recorded. Once each week all animals received a detailed clinical examination and palpation. The clinical examination included appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. The size, appearance, position and duration of any masses detected was recorded.

Body Weight - Body weights were recorded at least once each week for the first 13 weeks and at least once every 4 weeks thereafter up until the end of the study.

Food and Water Consumption - The quantity of food consumed by each cage of animals was measured and recorded for the first 13 weeks of the study and over one week in every 4 thereafter until the end of the study. Water consumption was monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Terminal Studies - After 104 weeks of treatment all surviving animals were killed. All animals, including dead or moribund animals, were subject to a detailed necropsy under the guidance of a veterinary pathologist. The necropsy consisted of an internal and external examination. All gross lesions were recorded in terms of location, size, shape, colour, consistency and number. Selected organs were removed from all animals, cleaned of fat and connective tissue and representative samples of tissues fixed in 10% neutral buffered formalin. Typically the list of organs/tissues included - Adrenals, Aortic Arch, Blood Smear, Brain, Epididymides, Eyes, Femur, Gastro-intestinal Tract (Stomach, Duodenum, Jejunum, Ileum, Caecum, Colon, Rectum), Heart, Kidneys, Lachrymal Gland, Liver, Lung, Mammary Gland, Mesenteric Lymph Node, Nasal Cavity, Oesophagus, Optic Nerves, Ovaries, Pancreas, Pituitary, Prostate, Rib, Sciatic Nerve, Seminal Vesicles, Skin, Spinal Cord, Spleen, Sternum, Submandibular Lymph Node, Submaxillary (Mandibular) Salivary Gland, Testes, Thigh Muscle, Thymus, Thyroids (with Parathyroids), Tongue, Trachea, Urinary Bladder, Uterus, Vagina. Carcasses were discarded following sampling of these tissues. Tissues were processed and sections were cut 4-6 μm thick, stained with haematoxylin and eosin (H & E) and evaluated by a pathologist.

RESULTS

The non-neoplastic histological findings are presented for both sexes of the two strains of Charles River rat in the tables (Tables 1 and 2). These show that the range, type and number of lesions observed in selected organs are essentially the same in both strains. Any variations seen were generally no bigger than that seen with inter-study variation within the same strain.

DISCUSSION

After treatment for 104 weeks (ie ca 110 weeks of age) the profile of non-neoplastic lesions with both the CD and IGS strains were essentially the same. These data show that there has been no essential difference in distribution in control rats between the two derivations of the Sprague Dawley rats. This apparent lack of effect of rederiving the strain has not had any noticeable effect on the conduct of carcinogenicity studies at Inveresk Research.

Table 1. Selected Non-Neoplastic Lesions – Males (1)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
ADRENAL GLAND														
		Number examined	49	34	49	26	46	49	50	49	50	50	60	59
	Congenital	Accessory cortical nodule	1	1	0	0	1	1	1	1	0	0	0	0
	Degenerative	Cystic degeneration	3	4	0	0	4	0	1	2	3	5	6	12
		Clear cell focus	0	0	0	0	0	0	0	0	2	1	7	1
		Cortical vacuolated cell focus	11	11	0	0	3	1	12	16	4	2	10	7
		Diffuse cortical cell vacuolation	0	1	0	0	2	1	0	0	0	0	0	0
		Necrosis, cortical	2	2	0	0	0	0	0	0	0	0	0	1
		Atrophy	0	0	3	0	1	0	0	0	0	0	2	12
		Mineralisation	0	0	0	0	0	0	1	0	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse medullary cell hyperplasia	0	0	3	1	3	4	0	0	0	0	1	2
		Focal cortical cell hypertrophy	10	11	0	0	0	15	17	23	18	18	11	21
		Diffuse cortical cell hypertrophy	0	0	0	0	0	1	0	1	0	0	4	0
	Inflammatory	Focal lymphocytic infiltration	0	0	0	0	1	0	0	0	0	0	0	0
	Vascular	Sinusoidal dilation	0	0	1	0	0	0	2	2	0	1	1	0
		Congestion	0	0	0	0	0	0	0	0	0	0	0	1
	Other	Medullary cyst	0	0	0	0	1	0	0	0	0	0	0	0
		Extramedullary haemopoiesis	0	1	1	0	0	0	0	0	0	0	0	0
		Basophilic focus	0	0	0	0	5	0	0	0	0	0	0	0
BRAIN														
		Number examined	48	34	49	25	50	50	50	50	50	50	60	60
	Compression	Ventricular dilation	0	0	0	0	0	2	7	3	2	0	1	1
		Hydrocephalus	0	0	3	3	0	0	0	0	0	1	0	0
		Ventral compression	0	0	5	3	0	0	0	0	0	0	0	0
		Compression by pituitary tumour	6	6	0	0	0	5	8	4	0	0	2	2
	Degenerative	Vacuolation	0	0	0	0	0	2	0	4	1	0	0	0
		Focal malacia	0	1	0	0	0	0	0	0	0	0	0	0
	Vascular	Thrombus	0	0	0	0	0	0	0	0	0	0	1	1
		Haemorrhage	0	0	0	0	0	0	0	1	0	0	0	0
	Inflammatory	Gliosis	0	0	0	0	0	2	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Granular cell hyperplasia	0	1	0	0	0	0	0	0	0	0	0	0
EPIDIDYMIS														
		Number examined	7	5	0	0	8	49	50	50	50	49	60	60
	Degenerative	Increased immature sperm	0	0	0	0	0	0	0	0	0	1	0	0
		Atrophy	0	0	0	0	1	0	0	0	0	0	0	0
		Spermatozoal reduction	0	0	0	0	7	0	0	0	0	0	0	0
		Degenerating spermatogenic cells	0	0	0	0	0	0	2	0	0	0	0	0
		Oligospermia	0	0	0	0	0	0	9	4	1	2	1	3
		Vacuolation	0	0	0	0	0	0	0	0	0	0	0	9
	Inflammatory	Inflammatory cell infiltration	5	3	0	0	0	0	0	1	0	0	0	0
		Perivasculitis, focal	0	0	0	0	0	0	2	2	0	0	0	0
		Sperm granuloma	0	0	0	0	1	1	2	1	0	0	0	0
		Arteritis	1	0	0	0	0	0	0	0	0	0	0	0
		Adhesion	0	0	0	0	1	0	0	0	0	0	0	0
		Necrosis (coagulative)	1	0	0	0	0	0	0	0	0	0	0	0
EYE														
		Number examined	39	25	45	25	43	46	49	44	47	46	60	58
	Degenerative	Retinal atrophy	0	0	0	0	0	0	2	0	0	0	0	1
		Cataract	0	0	0	0	0	0	0	1	1	0	1	0
		Retinopathy	3	0	0	0	0	0	0	0	0	0	0	0
		Retinal rosette	0	0	0	0	0	0	1	0	0	0	0	0
	Inflammatory	Iridocyclitis	6	0	0	0	0	0	0	0	0	0	0	1
		Focal keratitis	3	1	0	0	0	0	0	0	0	0	0	0
	Vascular	Haemorrhage, retrobulbar	0	0	0	0	0	0	0	0	1	0	0	0
		Haemorrhage, retinal	0	1	0	0	0	0	0	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (2)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
HEART														
		Number examined	50	34	50	26	50	50	50	50	50	50	60	60
	Inflammatory	Myocarditis	42	31	40	15	0	4	0	0	3	0	2	1
		Valvular endocarditis	0	0	0	0	0	0	0	0	0	0	1	0
		Vasculitis/perivasculitis	0	1	1	0	0	0	0	1	0	0	0	0
		Pericarditis	4	1	0	0	0	0	0	0	0	0	0	0
		Inflammation, pericardial	0	0	0	0	0	0	0	0	0	0	0	0
		Diffuse pericardial fibrosis	1	0	0	0	0	0	0	0	0	0	0	0
		Lymphocytic infiltration	0	0	0	1	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	0	0	1	1	0	0	0	0
	Degenerative	Mineralisation	2	1	0	0	0	0	0	0	0	0	0	0
		Valvular endocardial degeneration	0	1	0	0	0	0	0	0	0	0	0	0
		Atrial myocardial degeneration	0	1	1	0	0	0	0	0	0	0	0	0
		Progressive cardiomyopathy	0	0	0	0	37	40	38	46	35	40	26	33
		Osseous metaplasia	0	1	0	0	0	0	1	0	1	0	0	0
	Vascular	Thrombus	1	0	0	4	0	0	0	1	1	0	0	0
	Disturbances of cell growth/differentiation	Endocardial hyperplasia	0	0	0	0	0	0	0	0	0	0	1	0
KIDNEY														
		Number examined	50	49	50	50	44	45	49	44	47	46	60	60
	Degenerative	Chronic progressive nephropathy	41	39	34	39	28	31	41	43	30	27	27	28
		Basophilic tubules	0	0	0	0	4	5	6	3	1	0	0	0
		Cortical tubular degeneration	0	1	0	0	0	0	0	0	0	0	0	0
		Tubular dilation	0	0	0	1	2	2	0	1	0	1	0	0
		Tubular casts	0	0	0	0	0	0	1	2	0	0	0	0
		Glomerular lipidosis	0	0	0	1	0	0	0	0	0	0	0	0
		Bowman's capsule epithelialisation	0	0	0	0	1	0	0	0	0	0	0	0
		Focal fibrosis	0	0	0	0	1	0	0	0	0	0	0	0
		Pigmentation	7	3	2	2	0	0	0	0	0	0	0	0
		Mineralisation	17	15	1	0	2	4	6	2	3	5	2	1
	Inflammatory	Pyelitis	0	0	3	2	1	5	7	3	5	6	8	9
		Pyelonephritis	0	0	1	1	0	0	0	0	0	0	0	0
		Papillitis	0	0	0	0	0	0	0	0	0	1	0	0
		Pelvic inflammation	4	1	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	3	0	1	1	0	0	0	0
	Congenital	Hydronephrosis	2	2	0	3	3	3	2	3	1	0	3	1
		Cyst	1	0	0	0	0	0	2	3	2	2	1	3
	Disturbances of cell growth/differentiation	Transitional cell hyperplasia	12	7	4	4	14	9	23	18	15	18	15	14
		Papillary hyperplasia, unilateral	0	0	0	0	0	1	0	0	0	0	0	0
		Cortical tubular nuclear enlargement		0	0	0	0	0	0	0	0	0	0	0
	Vascular	Haemorrhage	0	0	0	0	0	0	1	0	0	1	0	0
		Infarction	0	0	1	0	0	0	0	0	0	0	0	0
	Necrosis	Necrosis	0	1	0	0	0	0	1	0	0	0	1	0
		Papillary necrosis	2	0	1	1	0	0	0	0	0	0	0	0
	Other	Hyaline droplets	0	0	0	0	1	3	0	0	0	2	3	0
		Tubular eosinophilia	0	0	1	0	0	0	0	0	0	0	0	0
		Cortical scar (s)	5	4	0	0	0	0	0	0	0	0	0	0
LIVER														
		Number examined	50	34	50	26	48	49	50	49	48	49	60	59
	Degenerative	Vacuolation	12	7	0	0	3	5	6	5	4	6	5	3
		Tension lipidosis	9	10	0	0	11	6	8	9	4	5	11	12
		Cystic degeneration	18	10	0	0	15	12	2	11	6	10	5	9
		Rarefaction	0	0	0	0	0	0	0	0	1	0	0	0
		Sclerosis, bile duct	0	0	0	0	10	13	1	0	10	19	5	1
		Portal tract sclerosis	26	18	0	0	0	0	0	0	0	0	0	0
		Centrilobular degeneration	1	0	0	0	0	0	0	0	0	0	0	0
		Kupffer-cell pigment deposit (s)	1	1	0	0	0	0	0	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (3)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
	Congenital	Ectopic tissue	0	0	0	0	0	0	0	0	0	0	1	0
	Disturbances of cell growth/differentiation	Bile duct hyperplasia	31	23	11	2	15	10	27	23	18	20	16	4
		Oval-cell proliferation	0	0	1	0	0	0	0	0	0	0	0	0
		Pleomorphism, increased, focal	0	0	0	0	0	0	0	0	1	0	0	0
		Hypertrophy	0	0	2	0	0	0	1	1	0	1	2	0
		Reparative hyperplasia	0	1	0	0	0	0	0	0	0	0	0	0
	Inflammatory	Inflammation	0	0	0	0	3	0	5	9	0	0	1	0
		Lymphocytic infiltration	0	0	1	0	0	0	0	0	0	0	1	0
		Inflammatory cells	16	5	0	0	0	0	0	1	0	0	0	0
	Vascular	Angiectasis	2	1	8	5	0	3	2	0	0	2	2	4
		Congestion	0	0	3	0	0	0	0	1	0	0	0	0
		Lobar infarction	0	0	1	1	0	0	0	0	0	0	0	0
		Thrombus	0	1	0	0	0	0	0	0	0	0	0	0
		Haematocyst	0	0	0	0	0	0	2	0	0	0	0	0
		Sinusoidal dilation	2	0	0	0	0	0	0	4	0	0	1	4
	Necrosis	Necrosis, focal	8	4	4	0	1	0	3	2	1	2	2	1
		Necrosis, centrilobular	2	2	0	0	0	0	0	1	0	0	0	1
		Periacinar necrosis	0	0	1	0	0	0	0	0	0	0	0	0
		Lobar necrosis	0	0	0	0	0	0	0	0	0	1	0	0
	Other	Biliary cyst	1	0	0	5	1	0	0	0	2	1	0	0
		Haemopoiesis	1	3	0	0	0	0	2	0	0	0	0	0
LUNG														
		Number examined	50	34	48	26	44	45	50	49	46	47	60	59
	Degenerative	Pigmented macrophages	3	3	0	0	3	2	0	1	0	0	0	0
		Alveolar macrophage accumulation	19	17	0	0	15	25	25	24	24	25	31	22
		Cholesterol clefts	0	0	0	0	3	0	0	0	0	1	0	0
		Mineral deposits, alveolar	0	0	0	0	0	0	1	0	0	0	0	0
	Inflammatory	Fibrosis	0	0	1	0	0	1	0	0	0	0	0	0
		Pleuritis	7	2	1	0	0	0	0	0	0	0	2	4
		Pneumonitis	13	4	6	0	0	0	0	1	0	0	0	0
		Pneumonia	3	0	1	0	0	0	0	0	0	0	0	0
		Alveolitis	0	0	1	0	3	3	5	6	6	7	7	11
		Lymphocytic cuffing	0	0	0	1	0	0	0	0	0	0	3	1
		Multinucleate giant-cell (s)	0	0	3	1	0	0	0	0	0	0	0	0
		Inflammatory cells	20	10	0	0	3	2	0	0	0	0	0	0
		Inflammation, granulomatous	4	1	0	0	0	0	2	5	1	1	3	1
		Adhesion	0	0	0	0	0	0	0	0	0	1	0	0
	Disturbances of cell growth/differentiation	Regenerative hyperplasia	0	0	0	0	0	0	0	0	4	0	0	0
		Bronchiolo-alveolar hyperplasia	0	0	0	0	0	0	0	0	1	0	0	0
		Focal bronchiolar epithelial metaplasia	0	1	0	0	0	0	0	0	0	0	0	0
	Vascular	Myopathy, pulmonary, venous	0	0	0	0	0	0	1	0	0	0	0	0
		Thrombosis	0	0	1	0	0	0	0	2	0	0	0	0
		Vascular mineralisation	0	0	0	0	4	0	0	0	0	0	0	0
		Haemorrhage	0	2	0	0	0	0	0	0	0	0	0	0
		Oedema	6	7	0	1	1	0	1	0	0	0	0	3
		Congestion	15	14	1	0	0	0	0	2	0	0	0	0
	Necrosis	Necrosis, alveolar, focal	0	0	0	0	0	0	0	1	0	0	0	0
	Other	Alveolar bone	0	0	1	0	0	0	0	0	0	0	0	0
		Brown induration	0	0	0	2	0	0	0	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (4)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
LYMPH NODE														
		Number examined	107	68	116	64	105	122	121	124	113	121	134	138
	Disturbances of cell growth/differentiation	Lymphoid hyperplasia	0	0	0	0	30	0	0	0	0	0	0	1
		Plasmacytosis	1	3	32	24	0	0	10	16	1	2	4	1
		Histiocytosis	0	0	31	15	28	7	2	0	0	0	0	0
		Reactive hyperplasia	5	2	0	0	11	1	0	2	1	1	4	3
	Degenerative	Pigment deposits	0	0	0	0	0	0	1	0	0	0	0	1
		Erythrophagocytosis	2	3	0	6	9	2	11	8	8	5	6	4
		Pigmented macrophages	0	0	0	0	4	0	2	0	0	0	0	0
		Dilation, cystic	0	0	0	0	0	0	8	13	0	0	0	0
		Cystic	5	2	0	2	1	0	0	1	2	6	3	3
		Fibroplasia	0	0	0	0	0	0	0	2	0	0	4	2
	Vascular	Proliferation,endothelial	0	0	0	0	0	0	0	2	0	0	0	0
		Congestion	0	1	0	0	2	0	0	0	0	0	0	1
		Sinusoidal dilation	0	0	0	0	2	0	0	0	0	0	0	1
		Oedema	3	1	0	0	0	0	0	0	0	0	0	0
	Inflammatory	Inflammation	0	0	0	0	0	0	2	1	0	0	0	1
		Perivasculitis	0	0	0	0	0	0	0	2	0	0	0	0
		Lymphadenitis	1	0	0	0	0	0	0	1	0	0	0	0
		Neutrophils, sinusoidal	1	0	0	0	0	0	0	0	0	0	0	0
		Abscess	0	0	0	0	0	0	0	0	0	0	0	1
	Other	Erythrocytosis	0	0	0	0	0	0	0	0	1	0	0	0
		Lipid deposition	0	0	0	0	1	0	0	0	0	0	0	0
MAMMARY GLAND														
		Number examined	43	31	49	26	50	46	50	46	38	43	60	60
	Degenerative	Dilated/cystic ducts	0	0	0	0	5	0	0	0	1	1	0	0
		Dilation, alveolar	0	0	0	0	0	0	2	3	0	0	0	0
		Galactocoele	0	0	2	0	0	0	1	0	0	0	0	1
		Lobular cysts	0	0	0	0	0	3	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Lobular hyperplasia, focal	0	0	0	0	0	0	2	2	0	0	0	0
		Acinar hyperplasia	0	0	8	0	0	0	0	0	0	0	0	0
		Cystic hyperplasia	0	0	0	0	0	0	0	0	0	0	2	0
	Inflammatory	Mastitis	0	0	0	0	0	0	1	1	0	0	0	0
		Focus, macrophage	0	0	0	0	0	0	2	0	0	0	0	0
	Other	Alveolar development	0	2	0	0	1	1	0	0	0	0	0	0
		Secretion present	12	9	0	0	0	0	0	0	0	0	0	0
PANCREAS (ENDOCRINE)														
		Number examined	47	34	49	26	48	48	50	48	50	50	60	60
	Degenerative	Islet fibrosis	0	0	0	0	0	0	1	0	2	0	0	0
		Atrophy	0	0	0	0	0	0	0	0	0	2	0	0
	Inflammatory	Inflammatory cell infiltration, focal	0	0	0	0	0	1	0	0	0	0	0	0
		Inflammatory cell infiltration	0	0	0	0	0	0	0	1	0	0	0	0
		Inflammation, islet cell	0	0	0	0	0	0	0	0	0	0	0	1
	Disturbances of cell growth/differentiation	Islet cell hyperplasia	0	0	3	1	26	1	1	1	7	2	0	0
PANCREAS (EXOCRINE)														
		Number examined	47	34	49	26	48	48	50	48	50	47	60	60
	Degenerative	Lobular atrophy	0	0	0	0	2	0	6	4	0	0	0	0
		Acinar cell atrophy	15	7	8	4	21	0	9	5	0	0	10	14
		Atrophy	0	0	0	0	0	16	0	0	22	21	0	0
		Pigment deposit (s)	0	0	0	0	1	0	0	0	0	0	0	0
		Duct ectasia	1	0	0	0	0	0	0	0	0	0	0	0
		Eosinophilic focus (i)	0	1	0	0	0	0	0	0	0	0	0	0
		Basophilic cytoplasmic change	2	1	0	0	2	0	0	0	0	0	0	0
		Lipomatosis	0	0	0	0	27	0	0	0	2	1	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (5)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
	Inflammatory	Inflammation	0	1	1	0	0	0	1	2	0	0	0	0
		Peritonitis	2	0	0	0	0	0	0	0	0	0	0	0
		Arteritis	1	1	0	0	0	0	0	0	0	0	0	0
		Granuloma	0	0	0	0	0	0	1	0	0	0	0	0
		Vasculitis/perivasculitis	0	0	2	1	0	1	0	4	0	1	2	1
		Focal haemorrhage	0	0	0	0	1	0	0	0	0	0	0	0
		Oedema	0	1	0	0	0	0	0	0	0	0	0	0
		Sclerosis, perivascular	0	0	0	0	0	0	0	1	0	0	0	0
	Disturbances of cell growth/differentiation	Hypertrophy	0	0	0	0	0	0	0	0	0	1	2	3
		Hyperplasia, ductular, focal	0	0	0	0	0	0	0	1	0	0	0	0
	Other	Dilated/cystic glands	0	0	0	0	0	0	0	0	0	1	0	0
		Cyst	0	0	0	0	1	0	0	0	0	0	0	0
PITUITARY GLAND														
		Number examined	49	33	49	26	48	48	50	49	49	49	60	60
	Congenital	Tubular remnants, posterior lobe	0	0	0	0	0	0	0	0	0	0	1	0
	Degenerative	Pigmented foamy cells, focal	0	0	0	0	0	0	0	2	0	0	0	0
		Focal vacuolated cell (s)	0	1	0	0	0	0	0	1	0	0	0	0
		Cyst	1	1	0	1	2	4	3	1	1	2	0	0
	Inflammatory	Inflammatory cell infiltration, focal	0	0	0	0	0	0	1	1	0	0	0	0
		Gliosis, posterior lobe, focal	0	0	0	0	0	0	1	0	0	0	0	0
	Disturbances of cell growth/differentiation	Focal hypertrophy	2	0	0	0	0	14	9	12	3	0	2	0
		Pars intermedia proliferation	0	0	1	0	0	0	0	0	0	0	0	0
	Vascular	Angiectasis	0	0	0	0	0	0	0	0	0	1	0	0
PROSTATE														
		Number examined	48	34	49	26	50	49	50	50	49	49	60	60
	Degenerative	Atrophy	0	0	1	1	0	1	0	0	0	0	0	0
	Inflammatory	Adenitis, coagulating gland	0	0	0	0	0	0	0	2	0	0	0	0
		Inflammation	0	0	6	3	2	0	0	0	0	0	1	0
		Prostatitis	6	0	0	0	0	3	2	6	2	5	4	4
		Perivascular inflammatory cells	3	0	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse hyperplasia	0	0	0	0	0	0	0	0	0	0	1	0
	Other	Contraction	0	0	0	0	0	0	0	1	0	0	1	0
SALIVARY GLAND (SUBMAXILLARY)														
		Number examined	45	28	49	25	48	50	49	47	50	49	59	60
	Degenerative	Lobular atrophy	0	0	0	0	0	0	0	0	0	1	0	0
	Inflammatory	Oedema	0	0	0	0	0	0	0	0	0	0	1	0
		Inflammation	0	1	0	0	0	0	0	0	0	1	0	0
	Disturbances of cell growth/differentiation	Hypertrophy, acinar cell	0	0	0	0	0	0	0	2	0	0	0	0
	Other	Acinar cell degranulation	0	0	0	0	0	0	0	0	0	0	1	0
SCIATIC NERVE														
		Number examined	48	34	49	26	50	50	50	50	50	50	60	60
	Degenerative	Neuronal degeneration	0	0	1	0	0	1	0	0	0	0	0	0
		Neuropathy	0	0	0	0	1	0	1	1	1	1	0	0
		Axonal degeneration	15	9	0	0	0	0	0	0	0	0	0	0
		Fibrosis	0	0	1	0	0	0	0	0	0	0	0	0
	Inflammatory	Lymphocytic infiltration	0	0	0	1	0	0	0	0	0	0	0	0
		Cholesterol granuloma	0	0	1	0	0	0	0	0	0	0	0	0
		Inflammatory cells	1	0	0	0	0	0	0	0	0	0	0	0
		Arteritis	0	0	0	1	0	0	0	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (6)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
SEMINAL VESICLE														
		Number examined	48	34	50	26	49	48	50	50	49	49	60	60
	Degenerative	Small	0	0	0	0	0	0	0	0	0	1	0	0
		Reduced secretion	3	5	0	0	0	0	0	0	0	0	0	0
		Atrophy	0	0	1	1	3	0	0	0	0	0	0	0
	Inflammatory	Inflammation	0	0	1	0	0	0	0	0	1	1	0	0
		Adhesion	0	0	0	0	1	0	0	0	0	0	0	0
		Abscess	0	0	0	1	0	0	0	0	0	0	0	0
		Peritonitis	2	1	0	0	0	0	0	0	0	0	0	0
		Vesiculitis	1	1	0	0	0	0	0	0	0	0	2	1
	Other	Dilation	0	1	6	0	0	0	0	0	1	0	0	0
		Cyst	0	0	0	0	0	0	0	1	0	0	0	0
		Secretion increased	0	0	0	0	0	0	0	0	0	0	1	0
		Contraction	0	0	0	0	0	0	1	4	0	0	2	2
SKELETAL MUSCLE														
		Number examined	49	34	50	26	50	49	50	50	50	50	60	60
	Degenerative	Myofibre degeneration	0	1	10	6	0	0	0	1	0	0	0	0
		Myopathy	0	0	0	0	5	0	5	7	0	2	0	0
		Atrophy	9	7	0	0	0	0	0	0	0	0	0	0
	Inflammatory	Myositis	0	0	0	0	0	0	0	0	0	0	6	5
		Granulomatous inflammation	0	1	0	0	0	0	0	0	0	0	0	0
		Acute inflammation	1	0	0	0	0	0	0	0	0	0	0	0
	Other	Focal regeneration	7	3	0	0	0	0	0	0	0	0	0	0
SKIN AND SUBCUTIS														
		Number examined	49	34	49	26	50	50	50	50	50	50	60	60
	Degenerative	Adnexal atrophy	0	0	1	0	0	25	61	30	62	62	2	1
	Inflammatory	Inflammation	1	1	1	1	0	5	21	21	3	5	10	8
		Dermatitis	0	2	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	0	0	1	1	1	1	0	0
		Granuloma	2	0	0	0	0	0	0	0	0	0	0	0
		Folliculitis	0	1	0	0	0	0	0	0	0	0	0	0
		Abscess	2	0	0	0	1	0	0	0	0	0	0	0
		Ulcer	2	2	0	1	0	0	0	0	0	0	0	0
		Necrotic debris	0	0	0	0	0	5	1	1	2	0	0	0
		Osseous metaplasia	0	0	0	0	0	0	1	0	0	0	0	0
	Disturbances of cell growth/differentiation	Epidermal cyst	5	3	1	0	3	8	3	1	1	6	2	3
		Sebaceous cyst	0	0	1	0	0	0	0	1	2	0	0	0
		Hyperplasia, epidermal	0	0	0	0	1	0	0	2	0	1	0	0
		Hyperkeratosis	0	0	0	0	1	0	0	0	0	0	0	1
	Vascular	Haemorrhage	0	0	0	0	0	0	0	1	0	0	1	0
		Oedema	0	0	0	1	0	0	0	0	0	0	0	0
		Haematoma	0	0	0	1	0	0	0	0	0	0	0	0
	Other	Fat depot	0	0	0	0	0	0	1	0	0	0	0	0
		Subcutaneous calcinosis	0	1	0	0	0	0	0	0	0	0	0	0
		Dermal scar (s)	7	3	0	0	0	0	0	0	0	0	0	0
SPINAL CORD														
		Number examined	48	34	49	26	50	50	50	50	50	50	60	60
	Degenerative	White matter vacuolation	0	0	8	0	0	1	0	0	2	1	0	0
		Myelomalacia	0	0	1	2	0	0	0	0	0	0	0	0
		Radiculoneuropathy	16	8	0	0	0	0	22	29	0	3	7	2
	Inflammatory	Lymphocytic infiltration	0	0	1	0	0	0	0	0	0	0	0	0
	Vascular	Haemorrhage	0	0	1	0	0	0	0	1	0	0	1	0
	Other	Cyst	0	0	0	0	0	1	0	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (7)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
SPLEEN														
		Number examined	50	34	49	26	48	49	50	49	49	48	60	60
	Congenital	Deformity	0	0	0	0	0	0	1	0	0	0	0	0
	Degenerative	White pulp depletion	0	0	0	0	1	0	0	0	0	0	0	0
		Fibrosis	0	0	0	0	0	1	0	0	0	0	0	0
		Increased haemosiderin	0	0	2	1	11	7	11	11	0	1	3	5
		Necrosis, localised	2	0	0	0	0	0	0	1	0	0	0	0
	Inflammatory	Inflammatory cells	0	0	0	0	0	0	0	1	0	0	0	0
		Inflammation	0	0	0	0	0	0	0	0	0	0	0	1
		Focal peritonitis	0	1	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Increased haemopoiesis	19	9	6	2	16	0	3	5	5	3	9	10
		Capsular proliferation	0	0	1	0	0	0	0	0	0	0	0	0
		Diffuse stromal proliferation	0	1	0	0	0	0	0	0	0	0	0	0
	Vascular	Congestion	1	0	3	0	3	0	0	0	0	0	0	0
	Other	Cyst, capsular	0	0	0	0	0	0	0	0	1	0	0	0
STERNUM														
		Number examined	48	34	49	26	50	50	50	50	50	50	60	60
	Disturbances of cell growth/differentiation	Marrow hyperplasia	0	0	0	0	0	6	0	0	1	1	7	4
		Myelopoiesis	0	0	0	1	0	0	0	0	0	0	0	0
		New bone formation	0	0	0	0	1	0	0	0	0	0	0	0
		Osseous proliferation	0	0	1	0	0	0	0	0	0	0	0	0
	Degenerative	Myelofibrosis	0	0	0	0	0	0	0	0	1	1	1	0
		Fatty atrophy, marrow	0	0	0	0	0	0	2	1	0	0	0	0
		Fatty marrow	0	0	0	0	19	0	0	0	0	0	0	0
		Foam cell, focal	0	0	0	0	0	0	1	0	0	0	0	0
	Degenerative	Chondropathy	0	0	4	2	6	1	5	2	9	6	6	8
		Focal fibrous osteodystrophy	4	1	0	0	0	0	0	0	0	0	0	0
STOMACH														
		Number examined	48	30	47	26	47	48	50	49	49	48	60	57
	Congenital	Cyst	0	0	0	0	0	0	2	0	0	0	0	0
	Degenerative	Dilated glands	16	9	4	1	28	0	2	0	1	0	0	0
		Focal erosions	1	1	0	0	2	0	0	0	0	0	0	0
		Mineralisation	1	0	3	0	0	0	0	0	0	0	0	0
		Necrosis, non-glandular stomach	0	0	0	0	0	1	0	0	0	0	0	0
		Mucosal fibrosis	0	1	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Intestinal metaplasia	0	0	0	0	0	0	0	1	0	0	0	0
		Squamous epithelium	0	0	0	0	1	0	0	0	0	0	0	0
		Hyperkeratosis	0	0	0	0	0	2	0	0	1	0	0	0
		Hyperplasia	2	2	2	1	1	0	1	0	1	1	0	0
	Inflammatory	Gastritis	0	0	0	0	0	0	5	11	0	0	6	2
		Focal peritonitis	0	1	0	0	0	0	0	0	0	0	0	0
		Lymphocytic infiltration	0	0	0	0	0	0	1	0	0	0	0	0
		Inflammatory cells	1	0	0	0	3	0	0	0	0	0	0	0
		Ulcer	1	4	0	1	0	1	1	2	1	0	0	0
		Inflammation	1	3	2	1	0	0	0	0	1	0	0	0
		Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
	Vascular	Oedema	2	3	0	0	0	0	1	1	0	0	0	0
TESTIS														
		Number examined	50	34	49	26	50	49	50	50	49	50	60	60
	Congenital	Ectopic tissue, lymphoid	0	0	0	0	0	0	1	0	0	0	0	0

Table 1. Selected Non-Neoplastic Lesions – Males (8)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS-A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	50	50
	Degenerative	Mineralisation	6	2	0	0	9	0	1	3	2	2	1	0
		Vacuolation	0	0	0	0	0	0	1	0	0	0	0	0
		Spermatozoa, absent	0	0	0	0	0	0	1	0	0	0	0	0
		Tubular dilation	0	0	0	0	0	0	0	1	0	0	0	0
		Degeneration	15	8	0	0	0	0	1	0	0	1	0	0
		Necrosis (coagulative)	2	0	0	0	0	0	0	0	0	0	0	0
		Atrophy	0	0	5	2	0	0	0	0	7	11	0	0
		Tubular atrophy	0	0	0	0	18	9	12	6	0	0	2	8
	Inflammatory	Lymphocytic infiltration, focal	0	0	0	0	0	0	0	0	0	0	0	1
		Granulomatous peritonitis	0	0	1	0	0	0	0	0	0	0	0	0
		Spermatocoele	1	0	0	0	0	0	1	0	0	0	0	0
		Focal interstitial inflammatory cell	5	3	0	0	0	0	0	0	0	0	0	0
		Arteritis	2	1	3	2	0	0	0	0	0	0	0	0
		Perivasculitis, focal	0	0	0	0	0	0	3	4	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse interstitial cell hyperplasia	0	0	0	0	0	0	0	0	0	1	0	0
	Vascular	Congestion	1	0	0	0	0	0	0	0	0	0	0	0
THYMUS														
		Number examined	46	31	44	26	41	48	47	50	44	30	60	57
	Congenital	Epithelial cyst	0	0	1	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Hyperplasia	0	0	0	0	8	2	1	1	3	0	0	0
	Degenerative	Involution	1	0	0	0	36	42	0	0	35	24	0	0
		Lymphoid depletion	0	0	20	9	0	0	0	0	0	0	0	0
	Inflammatory	Inflammation	6	2	0	0	0	0	0	0	0	0	0	0
		Arteritis	1	1	0	0	0	0	0	0	0	0	0	0
	Vascular	Congestion	0	1	0	0	0	0	0	0	0	0	0	0
		Haemorrhage	0	0	2	0	0	0	0	0	0	0	0	0
THYROID GLAND														
		Number examined	47	28	48	26	43	48	49	44	49	48	58	59
	Degenerative	Dilated/cystic follicles	0	0	1	0	0	0	0	1	1	1	0	0
	Inflammatory	Thyroiditis	0	0	0	0	0	0	0	0	0	0	1	0
		Inflammation	4	1	0	0	0	0	0	0	0	0	0	0
		Arteritis	0	1	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	0	0	1	0	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse C-cell hyperplasia	9	7	0	0	3	4	0	1	0	0	1	0
		Parafollicular cell hyperplasia	0	0	1	0	0	0	0	0	0	0	0	0
TRACHEA														
		Number examined	48	34	49	26	50	49	50	49	50	50	60	60
	Degenerative	Cystic submucosal glands	0	0	0	0	0	0	0	0	0	0	1	0
		Intraluminal inflammatory exudate	0	1	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	0	0	1	1	0	0	0	0
		Tracheitis	1	1	0	0	0	0	0	0	0	0	0	0
		Inflammation	6	2	0	0	0	0	0	0	0	0	2	0
URINARY BLADDER														
		Number examined	49	34	49	26	48	50	50	47	49	50	60	59
	Disturbances of cell growth/differentiation	Transitional cell hyperplasia	2	0	1	2	3	0	0	1	0	0	0	1
	Inflammatory	Inflammation, serosal	0	0	0	0	0	0	2	1	0	0	0	0
		Granuloma	0	0	0	0	0	0	0	1	0	1	0	0
		Cystitis	2	1	0	0	0	2	0	2	0	0	3	2
		Lymphocytic infiltration	0	0	0	0	0	0	0	0	0	0	0	0
		Mural necrosis	0	0	1	0	0	0	0	0	0	0	0	0
		Focal peritonitis	1	0	0	0	0	0	0	0	0	0	0	0
		Arteritis	0	1	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	1	0	0	0	0	0	0	0
	Vascular	Haemorrhage	0	1	1	0	0	0	0	0	1	0	0	0
	Other	Proteinaceous content	0	0	1	0	0	7	0	0	5	0	0	0
		Calculus (i)	0	0	0	0	3	0	0	0	0	0	0	0
		Distension	6	8	1	0	2	0	0	0	0	0	0	0

Table 2. Selected Non-Neoplastic Lesions – Females (1)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
ADRENAL GLAND														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
		No abnormality detected	3	3	9	10	1	7	8	4	10	5	12	5
	Congenital	Accessory cortical nodule	0	0	0	0	0	0	0	1	0	0	0	0
	Degenerative	Cystic degeneration	44	28	0	0	45	18	2	13	41	33	0	2
		Cortical vacuolated cell focus	10	3	0	0	0	1	4	4	0	0	1	1
		Diffuse cortical cell vacuolation	0	0	2	2	2	0	0	0	0	1	0	0
		Focal fibrosis	8	2	0	0	1	0	0	0	0	0	0	0
		Necrosis, cortical	1	1	0	0	0	0	0	0	0	0	0	0
		Atrophy	1	0	0	0	0	2	0	0	1	2	0	1
		Pigment	0	0	0	0	0	0	0	0	1	0	0	0
	Disturbances of cell growth/differentiation	Diffuse medullary cell hyperplasia	0	0	4	0	0	14	0	0	0	0	0	0
		Diffuse zona reticularis hyperplasia	1	0	0	0	0	0	0	0	0	0	0	0
		Subcapsular cell hyperplasia	0	0	0	0	0	0	0	0	0	0	0	1
		Focal cortical cell hypertrophy	17	3	0	0	0	0	25	25	23	14	9	4
	Vascular	Sinusoidal dilation	0	0	35	16	0	0	7	1	1	1	0	0
		Congestion	0	1	0	0	0	0	0	0	0	0	0	0
	Other	Extramedullary haemopoiesis	4	5	0	1	2	0	0	0	0	0	1	0
		Basophilic focus	0	0	0	0	6	0	0	0	0	0	0	0
BRAIN														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Compression	Ventricular dilation	0	0	0	0	0	0	14	12	1	3	11	9
		Hydrocephalus	0	0	10	8	0	0	0	0	0	0	0	0
		Ventral compression	0	0	9	11	0	0	0	0	0	0	0	0
		Compression by pituitary tumour	14	13	0	0	19	25	24	18	0	2	10	9
	Degenerative	Vacuolation	0	0	0	0	0	0	1	0	0	0	0	0
		Mineralisation	0	0	0	0	0	1	0	0	0	0	0	0
	Vascular	Haematoma	0	0	0	0	1	0	0	0	0	0	0	0
		Haemorrhage	0	0	0	0	0	1	0	0	0	0	0	1
	Inflammatory	Arteritis/periarteritis	0	0	0	0	0	0	0	0	2	0	0	0
		Inflammatory cell infiltration	0	0	0	0	0	0	0	0	0	1	0	0
EYE														
		Number examined	45	27	49	27	46	47	46	49	49	48	56	58
	Degenerative	Retinal atrophy	0	0	3	0	0	0	0	0	0	0	0	0
		Cataract	0	0	0	0	0	0	0	2	0	0	0	0
		Corneal mineralisation	0	0	0	0	0	1	0	0	0	0	0	0
		Retinal degeneration	1	0	0	0	0	0	0	0	0	0	0	0
		Retinopathy	0	0	0	0	0	0	0	1	0	0	0	0
	Inflammatory	Blepharitis	1	0	0	0	0	0	0	0	0	0	0	0
		Unilateral chorioretinitis	0	1	0	0	0	0	0	0	0	0	0	0
		Panophthalmitis	0	0	0	0	0	0	0	1	0	0	0	0
		Uveitis, unilateral	0	0	0	0	0	0	0	0	0	0	0	1
	Vascular	Haemorrhage, retrobulbar	0	0	0	0	0	1	0	0	1	1	0	0
	Disturbances of cell growth/differentiation	Eyelid sebaceous hyperplasia	0	0	1	0	0	0	0	0	0	0	0	0
HEART														
		Number examined	50	28	50	27	50	50	50	50	50	50	58	60
	Inflammatory	Valvular endocarditis	1	0	0	0	0	6	1	0	2	4	1	1
		Vasculitis/perivasculitis	0	0	0	0	0	1	0	0	0	1	0	0
		Pericarditis	1	0	0	0	0	0	0	0	0	0	0	0
		Inflammation, pericardial	0	0	0	0	0	0	0	0	0	0	1	0
		Lymphocytic infiltration	0	0	0	0	0	0	0	0	0	0	0	1
		Inflammatory cells	0	0	0	0	0	0	0	1	0	0	0	0
	Degenerative	Mineralisation	0	0	0	0	0	1	0	0	0	0	0	0
		Progressive cardiomyopathy	35	19	0	0	32	21	42	43	30	36	13	16
	Other	Infiltration, cellular, focal	0	0	0	0	0	0	0	0	0	1	0	0

Table 2.Selected Non-Neoplastic Lesions – Females (2)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
KIDNEY														
		Number examined	50	50	50	50	47	47	48	50	49	49	60	60
	Degenerative	Chronic progressive nephropathy	25	25	11	14	29	27	21	17	18	18	11	12
		Basophilic tubules	0	0	0	0	16	5	4	10	1	0	0	0
		Cortical tubular degeneration	1	0	0	0	0	0	0	0	0	0	0	0
		Nephrosis	0	0	0	0	0	0	0	0	1	0	0	0
		Tubular dilation	0	0	0	0	0	2	2	1	0	0	0	0
		Tubular casts	0	0	0	0	0	0	3	5	0	0	0	0
		Necrosis	0	0	0	0	4	1	0	0	0	0	0	0
		Papillary necrosis	0	0	0	0	0	1	0	0	0	0	0	0
		Focal fibrosis	0	0	0	0	0	0	0	0	0	1	0	0
		Pigmentation	43	42	2	0	26	26	51	51	47	55	7	14
	Inflammatory	Pyelitis	0	0	0	1	4	1	4	6	6	5	3	4
		Nephritis	0	1	0	0	0	0	2	0	0	0	1	1
		Inflammation	0	0	0	0	0	0	0	0	0	0	1	0
		Inflammatory cells	0	0	0	0	0	0	5	2	0	0	0	0
	Congenital	Hydronephrosis	0	0	1	1	0	0	3	2	2	1	1	1
		Cyst	1	0	0	0	0	0	1	0	1	0	1	0
	Disturbances of cell growth/differentiation	Transitional cell hyperplasia	9	12	14	11	35	13	20	23	22	32	23	28
	Vascular	Haemorrhage	0	0	0	0	0	0	0	0	0	3	0	0
		Thrombosis	0	0	0	1	0	0	0	0	0	0	0	0
		Infarction	0	0	0	0	1	0	0	0	0	0	0	0
	Other	Hyaline droplets	0	0	0	0	2	3	0	3	0	0	1	0
		Tubular eosinophilia	0	0	1	0	0	0	0	0	0	0	0	0
		Distortion, pelvic	0	0	0	0	0	1	0	0	0	0	0	0
		Cortical scar (s)	4	2	0	0	0	0	0	0	0	0	0	0
LIVER														
		Number examined	50	28	50	27	48	50	50	50	50	50	60	60
	Degenerative	Vacuolation	22	24	0	0	10	0	0	0	0	0	0	0
		Tension lipidosis	5	6	0	0	8	6	15	13	11	11	13	13
		Cystic degeneration	0	1	0	0	2	0	0	0	0	1	0	1
		Rarefaction	0	0	0	0	0	0	0	1	0	1	0	0
		Sclerosis, bile duct	6	4	0	0	5	11	2	1	0	2	0	0
		Centrilobular degeneration	0	0	0	0	0	0	0	0	0	1	0	0
		Pigmented Kupffer cells	0	0	0	0	0	0	0	0	1	0	0	0
	Congenital	Congenital anomaly	0	0	0	0	0	0	0	0	0	0	0	1
	Disturbances of cell growth/differentiation	Bile duct hyperplasia	12	5	7	3	11	7	13	9	15	13	4	1
		Mitotic increase	1	0	0	0	0	0	0	0	0	0	0	0
		Multinucleate hepatocytes	0	0	0	0	0	0	0	0	0	0	1	0
		Karyomegaly	0	0	0	0	0	0	0	2	0	0	0	0
		Oval-cell proliferation	0	0	0	0	0	0	0	0	1	0	0	0
		Pleomorphism, increased	0	0	0	0	0	1	0	0	1	2	1	0
		Hypertrophy	0	0	0	0	0	3	0	0	0	0	0	2
	Inflammatory	Inflammation	0	0	0	0	0	0	3	3	0	0	0	0
		Inflammatory cells	13	3	0	0	0	1	0	0	1	1	0	0
	Vascular	Angiectasis	2	0	1	1	0	0	2	1	1	1	5	1
		Haemorrhage, periportal	0	0	0	0	0	0	0	0	1	0	0	0
		Sinusoidal dilation	4	2	0	0	4	0	5	4	1	0	6	5
	Necrosis	Necrosis	5	1	1	1	1	0	0	0	0	0	1	1
		Necrosis, centrilobular	2	5	0	0	0	0	0	0	0	1	0	0
	Other	Biliary cyst	1	0	0	0	0	0	0	0	1	1	1	0
		Cyst	0	0	0	0	0	0	1	0	0	0	0	0
		Haemopoiesis	4	7	1	0	0	0	1	0	0	1	0	0

Table 2.Selected Non-Neoplastic Lesions – Females (3)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
LUNG														
		Number examined	50	28	50	27	47	47	50	50	49	49	58	60
	Degenerative	Pigmented macrophages	1	2	1	0	0	0	0	0	0	0	0	0
		Alveolar macrophage accumulation	17	15	4	2	12	12	11	14	6	10	5	10
	Inflammatory	Pleuritis	1	2	1	0	0	0	0	0	0	0	2	0
		Pneumonitis	12	9	4	1	0	0	0	0	0	0	0	0
		Pneumonia	0	0	0	0	0	0	0	0	0	0	2	0
		Alveolitis	0	0	0	0	3	0	0	0	0	0	0	0
		Lymphocytic cuffing	0	0	0	0	0	0	0	0	0	0	4	3
		Multinucleate giant-cell (s)	0	0	0	2	0	0	0	0	0	0	0	0
		Inflammatory cells	22	4	0	0	4	0	0	0	1	0	0	0
		Granuloma	2	0	1	0	0	1	1	4	1	0	0	1
	Disturbances of cell growth/differentiation	Bronchiolo-alveolar hyperplasia	0	1	0	0	0	0	1	0	0	0	0	0
	Vascular	Thrombosis	0	0	0	0	1	0	0	0	0	0	0	0
		Vascular mineralisation	0	0	0	0	10	1	0	0	0	0	0	0
		Haemorrhage	2	0	0	0	0	0	0	0	0	0	0	0
		Oedema	7	2	1	0	0	0	0	0	0	0	0	0
		Congestion	11	4	0	0	0	0	0	0	0	0	0	0
LYMPH NODE														
		Number examined	109	64	124	69	89	122	123	130	125	132	145	160
	Disturbances of cell growth/differentiation	Reactive hyperplasia	6	3	0	0	35	2	1	1	1	2	2	4
	Degenerative	Pigment deposits	0	0	1	0	0	0	0	0	0	0	0	0
		Erythrophagocytosis	4	1	5	1	6	2	6	6	9	6	8	7
		Pigmented macrophages	0	0	0	0	6	2	4	3	0	0	0	0
		Dilation, cystic	1	0	0	2	0	0	4	2	2	0	6	0
	Vascular	Congestion	1	0	0	0	2	0	0	0	0	0	0	0
		Sinusoidal dilation	0	0	0	0	0	0	1	0	0	0	0	0
		Oedema	0	1	0	0	2	0	0	0	0	0	0	0
	Inflammatory	Inflammation	0	0	0	0	0	0	1	0	0	0	1	0
		Plasmacytosis	9	6	50	26	9	1	37	31	4	3	6	10
		Histiocytosis	0	0	27	13	34	0	1	2	0	1	0	0
		Lymphadenitis	1	1	0	0	0	0	0	0	0	0	0	0
MAMMARY GLAND														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Degenerative	Dilated/cystic ducts	19	17	0	0	43	0	10	14	0	0	0	0
		Dilation, alveolar	0	0	0	0	0	0	3	10	0	0	0	0
		Galactocoele	0	0	5	2	0	1	1	3	0	1	1	8
		Degeneration, lobular, focal	0	0	0	0	0	0	0	0	1	0	0	0
		Lobular cysts	0	0	0	0	0	30	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Lobular hyperplasia	0	0	0	0	0	0	18	0	1	0	10	9
		Acinar hyperplasia	0	0	4	0	0	0	0	0	0	0	0	0
		Cystic hyperplasia	0	0	0	0	0	0	0	0	0	0	7	11
	Inflammatory	Mastitis	1	0	0	0	0	0	1	4	0	0	1	0
		Inflammation	0	0	0	0	0	0	3	0	0	0	0	0
		Inflammatory cells	1	0	0	0	0	0	0	0	1	0	0	0
	Other	Alveolar development	31	22	0	0	0	0	0	0	0	0	0	0
		Secretion present	46	26	0	0	0	0	0	0	0	0	0	0

Table 2.Selected Non-Neoplastic Lesions – Females (4)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
OVARY:														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Degenerative	Bursal cyst	0	1	0	0	0	0	0	0	0	0	3	0
		Cystic follicle (s)	0	0	0	0	0	0	0	0	0	1	1	0
		Atrophy	0	0	22	13	24	0	0	0	0	0	0	0
		Paraovarian cyst	1	0	0	0	0	0	0	0	0	0	0	0
		Luteal cyst	1	0	0	0	0	0	1	5	0	0	1	4
		Corpora lutea absent	0	0	0	0	0	31	0	0	34	29	29	21
		Pigmented macrophages	0	0	0	0	0	0	1	2	0	0	0	0
		Cyst (s)	0	0	3	2	10	13	14	13	7	7	2	11
	Disturbances of cell growth/differentiation	Sertoliform tubular hyperplasia	26	16	0	0	0	0	0	0	0	0	0	0
		Hyperplasia, stromal	0	0	0	0	0	2	1	1	0	0	6	5
		Hyperplasia, granulosa cell	0	0	0	0	0	1	0	0	0	0	0	0
		Interstitial hyperplasia	0	0	7	3	1	0	5	6	3	1	0	0
	Inflammatory	Oedematous granulation tissue	1	0	0	0	0	0	0	0	0	0	0	0
		Oophoritis, unilateral	0	0	0	0	0	0	0	0	0	0	1	0
	Vascular	Congestion	0	0	0	0	0	0	1	3	0	0	0	0
		Haemorrhage	0	0	0	0	1	0	0	0	0	1	0	0
PANCREAS (ENDOCRINE)														
		Number examined	50	26	49	27	49	48	50	49	50	50	60	60
	Disturbances of cell growth/differentiation	Islet cell hyperplasia	0	0	1	1	10	1	0	0	1	1	0	0
PANCREAS (EXOCRINE)														
		Number examined	50	26	49	27	49	48	50	49	50	49	60	60
		No abnormality detected	33	15	0	0	33	32	40	37	40	43	55	53
	Degenerative	Lobular atrophy	0	0	0	0	1	0	2	8	0	0	0	0
		Acinar cell atrophy	10	4	10	5	5	0	5	4	0	0	5	7
		Atrophy	0	0	0	0	0	13	0	0	6	4	0	0
		Degeneration, focal	0	0	0	0	0	0	0	0	1	0	0	0
		Duct ectasia	1	0	0	0	0	0	0	0	0	0	0	0
		Eosinophilic focus (i)	1	1	0	0	0	0	0	0	0	0	0	0
		Basophilic cytoplasmic change	2	1	0	0	0	1	0	0	0	0	0	0
	Inflammatory	Inflammation	1	2	0	0	0	0	2	0	0	0	0	0
		Peritonitis	1	0	0	0	0	0	0	0	0	0	0	0
		Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
		Vasculitis/perivasculitis	1	0	1	0	0	0	1	0	2	0	0	1
		Inflammatory cells	0	0	0	0	1	0	0	1	0	0	0	0
	Disturbances of cell growth/differentiation	Hyperplasia	0	0	0	0	0	1	0	0	0	0	0	0
PITUITARY GLAND														
		Number examined	49	28	50	25	47	49	48	49	49	48	57	59
	Congenital	Tubular remnants, posterior lobe	0	0	0	0	0	0	1	2	0	0	0	0
	Degenerative	Pigmented foamy cells	0	0	0	0	0	0	0	1	0	0	0	0
		Focal anterior lobe cellular change	0	0	0	0	1	0	0	0	0	0	0	0
		Cyst/dilation	1	0	0	0	4	1	6	4	1	0	0	0
	Disturbances of cell growth/differentiation	Focal hypertrophy	0	0	0	0	0	3	6	14	5	3	0	0
	Vascular	Angiectasis	0	0	0	0	0	0	2	0	0	2	0	0
SALIVARY GLAND (SUBMAXILLARY)														
		Number examined	49	28	50	26	50	48	48	49	50	50	57	59
	Degenerative	Degeneration	0	0	0	0	0	0	1	0	0	0	0	0
		SUBLINGUAL: mineralisation	0	0	0	0	1	0	0	0	0	0	0	0
	Inflammatory	Inflammatory cells	1	0	0	0	0	0	0	0	0	0	0	0
		Inflammation	1	0	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Hypertrophy, acinar cell	0	0	0	0	0	0	1	0	0	0	0	0

Table 2.Selected Non-Neoplastic Lesions – Females (5)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
SCIATIC NERVE														
		Number examined	50	28	50	27	48	49	50	50	50	50	59	60
	Degenerative	Axonal degeneration	11	2	0	0	0	0	0	0	0	0	0	0
	Inflammatory	Lymphocytic infiltration	1	0	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	1	0	0	0	0	0	0	0	0	0	0	0
SKELETAL MUSCLE														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Inflammatory	Myositis	1	0	0	0	0	0	0	0	0	0	1	0
		Inflammatory cells	0	0	0	0	1	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Focal regeneration	1	0	0	0	0	0	0	0	0	0	0	0
SKIN AND SUBCUTIS														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Degenerative	Adnexal atrophy	0	0	0	2	0	0	0	0	0	0	1	7
	Inflammatory	Inflammation	0	0	1	0	0	0	0	0	0	0	0	1
		Dermatitis	0	1	0	0	0	1	6	3	0	0	4	0
		Focal lymphocytic infiltration	0	0	0	1	0	0	0	0	0	0	0	0
		Granuloma	0	0	1	0	0	0	0	0	0	0	0	0
		Panniculitis	0	0	1	0	0	0	0	0	0	0	0	0
		Abscess	1	0	0	1	0	0	2	0	1	0	4	1
		Ulcer	1	3	1	0	0	0	3	1	0	0	0	0
		Necrotic debris	1	1	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Epidermal cyst	1	0	0	0	0	2	0	0	0	2	2	0
		Hyperplasia, epidermal	0	0	0	0	4	0	0	2	0	1	0	0
		Hyperkeratosis	0	0	0	0	0	0	0	0	0	0	0	1
SPINAL CORD														
		Number examined	50	28	50	27	50	50	50	50	50	50	59	60
	Degenerative	White matter vacuolation	0	0	0	0	0	0	0	1	0	2	0	0
		Myelomalacia	0	1	1	1	0	0	0	0	0	0	0	0
		Axonal degeneration	4	4	0	0	0	0	10	9	0	0	3	1
SPLEEN														
		Number examined	50	21	50	27	49	49	50	50	50	49	60	60
	Congenital	Deformity	0	0	0	0	0	0	1	0	0	0	0	0
	Degenerative	Lymphoid atrophy	0	0	0	0	0	0	0	0	0	0	1	0
		Fibrosis	0	0	0	0	1	0	0	0	0	0	0	0
		Red pulp depletion	0	0	0	0	0	0	0	0	0	1	0	0
		Vacuolation	0	0	0	0	0	0	0	1	0	0	0	0
		Increased haemosiderin	0	0	8	1	39	18	36	33	14	9	3	5
		Cyst	0	1	0	0	0	0	0	0	0	0	0	0
		Necrosis, localised	0	0	0	0	0	0	0	1	0	0	0	0
	Inflammatory	White pulp hyperplasia	0	0	0	0	1	0	0	0	0	0	0	0
		Adhesion	0	0	0	0	0	1	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Increased haemopoiesis	36	18	5	4	31	17	11	11	11	10	14	14
	Vascular	Focal angiectasis	1	0	0	0	0	0	0	0	0	0	0	0
		Congestion	0	0	0	0	1	0	0	0	0	0	0	0
	Other	Small	0	0	0	0	0	1	0	0	0	0	0	0
		Contraction	0	0	0	0	0	0	0	1	0	0	0	0
STERNUM														
		Number examined	50	28	50	26	50	50	50	50	50	49	59	60
	Disturbances of cell growth/differentiation	Marrow hyperplasia	0	0	0	0	0	0	0	1	0	0	5	6
		Haemopoiesis, increased	0	0	0	0	0	0	0	0	0	1	0	0
	Degenerative	Myelofibrosis	0	0	0	0	0	0	0	1	0	0	0	0
		Fatty atrophy, marrow	0	0	0	0	21	0	3	2	0	0	0	0
		Chondropathy	0	0	0	1	14	0	3	3	12	11	0	1

Table 2.Selected Non-Neoplastic Lesions – Females (6)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
STOMACH														
		Number examined	50	27	50	26	50	49	49	50	48	49	59	60
	Congenital	Cyst	0	0	0	0	1	0	0	0	0	0	0	0
	Degenerative	Dilated glands	19	10	4	0	26	0	1	2	1	2	0	0
		Focal erosions	3	0	0	0	0	0	0	0	0	0	0	0
		Squamous epithelial vacuolation	0	0	0	0	1	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Hyperkeratosis	0	0	0	0	1	9	0	0	1	2	0	0
		Hyperplasia	2	3	3	3	0	1	0	0	0	0	0	0
	Inflammatory	Gastritis	0	0	0	0	0	0	5	2	1	1	0	0
		Focal peritonitis	1	0	0	0	0	0	0	0	0	0	0	0
		Lymphocytic infiltration	0	0	0	0	0	0	0	0	0	0	0	1
		Inflammatory cells	0	0	0	0	0	1	0	0	1	0	0	0
		Ulcer	1	2	3	3	0	2	0	1	1	0	0	0
		Inflammation	0	2	1	4	0	4	0	0	0	0	0	0
	Vascular	Congestion	0	0	0	0	0	0	1	0	0	0	0	0
		Oedema	1	3	0	0	0	0	0	0	0	0	0	0
THYMUS														
		Number examined	47	27	49	25	48	48	49	49	44	44	57	59
	Congenital	Cyst	0	0	14	5	1	0	0	0	0	0	0	1
	Disturbances of cell growth/differentiation	Hyperplasia	1	0	0	0	11	10	2	0	7	12	2	5
	Degenerative	Involution		1			40	29	0	0	32	38	0	0
		Lymphoid depletion	0	0	11	5	0	0	0	0	0	0	0	0
	Inflammatory	Inflammation	2	1	0	0	0	0	0	0	0	0	1	0
		Lymphoid hyperplasia	0	0	0	0	1	0	1	1	0	0	0	0
	Vascular	Congestion	2	0	0	0	0	0	0	0	0	0	0	0
		Haemorrhage	0	0	1	1	0	0	0	0	0	0	0	0
THYROID GLAND														
		Number examined	49	28	50	26	48	48	46	48	49	49	57	57
	Congenital	Ultimobranchial cyst	0	0	0	0	0	1	0	0	0	0	0	0
	Degenerative	Dilated/cystic follicles	0	1	0	0	2	0	0	0	0	0	0	0
	Inflammatory	Thyroiditis	0	0	0	0	0	0	0	0	0	0	1	0
		Inflammation	1	0	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	0	0	1	1	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse C-cell hyperplasia	19	12	0	0	7	1	4	6	5	3	5	3
		Parafollicular cell hyperplasia	0	0	5	0	0	0	0	0	0	0	0	0
TRACHEA														
		Number examined	50	28	50	27	50	50	50	50	50	50	58	60
	Inflammatory	Tracheitis	2	1	0	0	0	0	0	0	0	0	0	0
		Inflammation	1	1	0	0	0	0	0	0	0	0	0	0
	Disturbances of cell growth/differentiation	Diffuse epithelial hyperplasia	1	0	0	0	0	0	0	0	0	0	0	0
URINARY BLADDER														
		Number examined	49	28	50	27	48	48	50	50	50	49	59	60
	Disturbances of cell growth/differentiation	Transitional cell hyperplasia	0	1	1	0	1	1	0	0	1	0	0	3
	Inflammatory	Cystitis	0	1	0	0	0	0	0	0	0	0	1	0
		Lymphocytic infiltration	0	0	1	0	0	0	0	0	0	0	0	0
		Focal peritonitis	1	0	0	0	0	0	0	0	0	0	0	0
		Inflammatory cells	0	0	0	0	1	0	0	0	0	0	0	0
	Other	Distension	0	1	0	0	0	0	0	0	0	1	0	0

Table 2.Selected Non-Neoplastic Lesions – Females (7)

			CD Strain						IGS Strain					
		Study	CD-A		CD-B		CD-C	CD-D	IGS- A		IGS-B		IGS-C	
		Number per group	50	50	50	50	50	50	50	50	50	50	60	60
UTERUS:														
		Number examined	50	28	50	27	50	50	50	50	50	50	60	60
	Disturbances of cell growth/differentiation	Cystic endometrial hyperplasia	7	3	0	0	4	0	0	0	3	1	2	6
		Squamous metaplasia	3	0	0	0	4	4	0	0	1	0	0	2
		Cervical hypertrophy	0	0	0	0	0	0	1	3	0	0	0	0
		Intraluminal haemorrhage	1	0	0	0	0	0	0	0	0	0	0	0
	Degenerative	Dilation	3	0	4	3	5	1	1	0	4	4	4	9
		Cyst	0	0	0	0	0	0	3	2	0	0	0	0
		Hydrometra	0	0	0	0	0	0	3	1	0	0	0	0
	Inflammatory	Endometritis, in both horns	0	0	0	0	0	0	0	0	0	0	0	1
VAGINA:														
		Number examined	50	27	49	27	50	50	50	50	50	49	0	0
	Inflammatory	Vaginitis	0	1	0	0	0	4	0	0	2	6	0	0
	Degenerative	Atrophied	0	0	0	0	2	0	0	0	0	0	0	0