Biological Reference Data on CD(SD) IGS Rats - 1998

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PREFACE

In the fall of 1995, Mr. S. Takura and Mr.K. Kamiya of Charles River Japan, Inc. convened persons who were concerned with animal experiments in our company to explain about the CD (SD)IGS strain of rats. Meanwhile I was attending the ICH-2 meeting, Orlando, and could not hear the explanation. Our company decided to assess the usefulness of IGS rats in reproductive toxicity studies. Therefore, we planned to invite personnel of Charles River Japan, Inc. to get more information on how they intended to promote this strain of rats. About one month later, Mr. Takura (Director) and Dr.M. Ichimura (Managing Director) visited us from Charles River Japan, Inc. (Later Mr. Takura was installed as the Secretary-General of the CD(SD)IGS Rat Study Group) Dr. M. Fujiwara and I joined the meeting, where I made two proposals on the promotion of IGS rats. One was to organize a Study Group for the users. The collection of IGS rat background data from the users should confer benefit not only on the supplier, Charles River Japan, Inc., but also on these users themselves, and for this purpose, the research society should be established internationally. In respect to these ideas, I asked them to call for the approval of the President of Charles River Japan, Inc. as well as of the U. S. head company. Mr. Takura made a great effort to accomplish this. The other proposal I made was to them to persuade Dr. H. Inoue, Director of Biosafety Research Center, Foods, Drugs and Pesticides, to head the Study Group as Chairman. Dr. Inoue has been contributing a great amount of data from his research to the Japan Fischer Rat Study Group which was established about twenty years ago.

According to Mr. Takura, the negotiation to get the consent of Dr. Inoue to be Chairman of the Study Group did not go smoothly. There was even a time when I was asked to take that position instead. I recall that I suggested to Mr. Takura on the phone to again request Dr. Inoue to reconsider it. Later on, Mr. A. Nakanishi, President of Charles River Japan, Inc., Dr. H. Inoue, Mr.S. Takura and I had the first meeting at a coffee shop in the Marunouchi Hotel,Tokyo on January 16, 1997, about one year after our attempt to organize the Study Group.

In the meeting we discussed a basic framework such as main activities, a schedule and an election of officers. I also emphasized that the Study Group should collect more data from other colonies, not only from Charles River, Inc., in order to be recognized internationally. Mr. Nakanishi understood this. For the same reason, we also decided to ask members from overseas to occupy some of the officer's seats. Then Mr. Takura and Dr. Morimura (Deputy Director) began a strenuous race. In March 1997, our first official board meeting was held, where the main objectives of the society were explained and the recruitment of members was announced. In July 1997, at the Tokyo Toranomon Pastoral, the Study Group was inaugurated with the presence of 150 members from Japan and North America. At the meeting I made a request to the members to contribute data in a research paper form to the Study Group so that a data file could be prepared one year later. We also entrusted the Food and Drug Safety Center, one of the research laboratories which had provided their data at that first general meeting, with the task of editing their data as a research paper. This paper was distributed as a sample to the prospective contributors in March of this year.

In the fall of 1998

Toshiaki Matsuzawa, Ph. D., Vice-Chairman, Chief Editor

PREFACE

In Japan, CD(SD) rats are commonly used in repeated dose toxicity studies, while Fischer (F344) rats have been commonly used in carcinogenicity studies. I myself have conducted more than 60 carcinogenicity studies using F344 rats, with only a few such studies conducted using CD(SD) rats. One concern related to using CD rats in carcinogenicity studies is their survival rate, but the rate did not go below 50% in 2-year studies I conducted. However, in the U.S.A. where CD rats have been used quite frequently in carcinogenicity studies, the life span of CD rats was considered a serious matter, and discussed as a major topic in the Toxicology Forum at the 1990 Annual Summer Meeting in Colorado. According to the information I have obtained, the FDA suggested using 60-70 animals/sex/group at the initiation of the study, so that more than 25 animals/sex/group could remain at the time of the final necropsy. The FDA also referred to restriction of feed and calories.

Recently in Japan, the number of carcinogenicity studies using oral gavage administration has increased as toxicokinetics studies were included in drug safety evaluation studies, which resulted in an increased number of carcinogenesity studies using CD rats in view of utilizing repeated dose toxicity study data.

In line with this trend, Charles River Japan, Inc., began to produce CD rats by the Golden Standard Method, and I was also informed of this. (Later, this strain of CD rats was designated as “CD IGS rat”.) Although production of the new strain of CD rats was very timely in relation to ICH, it was rather unfavorably received since it required us to collect background data from the beginning. Nevertheless, it is evident that the life span of CD rats has also become shorter in Japan, which may give rise to various problems in future. Therefore, we decided to collect background data on IGS rats.

Meanwhile at the end of 1995, Mr. Takura, Director of Charles River Japan, Inc., contacted me to inform me of the establishment of the CD(SD)IGS Rat Study Group and the intention of Dr. Matsuzawa of Yamanouchi Pharmaceutical Co., Ltd. that I would be Chairman of the Study Group. For about one year I firmly declined the post, since I occupied several roles besides that at the Biosafety Research Center, Foods, Drugs and Pesticides. Dr. Matsuzawa proposed to undertake practical jobs for me; so I finally accepted the offer for fear that my unwillingness might cause the delay of the establishment of this Study Group.

The process after that has already been mentioned by Dr. Matsuzawa in his Preface.

In the CD(SD)IGS Rat Study Group, there are three sections of general toxicity, reproductive and developmental toxicity and carcinogenicity, with each of them having a section leader. Dr. Matsuzawa supervises the general toxicity and reproductive and developmental toxicity sections and I supervise the carcinogenicity section. I foresee that it will take a few years before carcinogenicity background data is generated, and I will take the responsibility for this group until background data is arranged. There are some differences in this Study Group from the Fischer Rat Study Group and I would like to ask for your continuous cooperation and support.

In the fall of 1998

Hiroyuki Inoue, Ph.D. Chairman

PREFACE

Charles River, known as the largest-scale breeder in the world, has recently changed its system to provide animals from uniform parent animal colonies in their eight breeding centers existing in Japan, Europe and North America. The CD(SD)IGS Rat Study Group, consisting of the users, intends to investigate differences between CD(SD)IGS rats and original CD rats produced by individual colonies from the viewpoint of background data and to discuss what are the superior points of the former to the latter. It is also necessary to monitor the phenotype of this strain continuously. These must also be great concern for the supplier. Rather than discussing the problems based on data collected during 10 or 20 years in the future, we think it is necessary for both the animal suppliers and users to clarify the characteristics of this strain in a shorter span of 3 or 5 years.

This publication presents background data mainly generated from short-term repeated dose sections and reproductive/teratology studies in research paper form. Our next or later publications shall focus on data from long-term repeated dose toxicity and carcinogenicity studies. We would also like to report on data indicating differences in responses to drug administration in genetic toxicity, immunotoxicity or safety pharmacology studies. We would appreciate it if you send your papers on these topics to our society office as well as your ideas concerning possible joint research under a standardized protocol.

Some of you may be thinking that you cannot use CD(SD) IGS rats because of no available background data. After reading this publication, we would like to hear your opinions and continue to seek your support and cooperation.

Since the Study Group consists of general toxicity, reproductive toxicity and carcinogenicity sections, the title of this publication was initially “Toxicological Reference Data”. However, the intention of the publication is the promotion of CD(SD)IGS rats and collection of related information, so we named it “Biological Reference Data” instead. This single publication will not be revised. New content and authors will enter every independent volume. We are planning to publish at least three volumes. We would like to promote our work so that our papers can be presented in the Charles River Laboratories Reference Paper in the 21th century.

At the beginning of organizing the Study Group, there was an idea that an editing committee would meet for discussion on the data and proofread the papers. However, editing this publication with this initial idea would have demanded too much man: power, time, and cost, which we could not afford. The editing work was kept to a minimum and I would like to seek your understanding on this point of concern.

Toshiaki Matsuzawa and Hiroyuki Inoue

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We would like to acknowledge the cooperation of the following steering committee members of the CD(SD)IGS Rat Study Group : Dr. M. Hashimoto (Fujisawa Pharmaceutical), Dr.T. Unno (Nihon Schering), Mr.Y. Maeda (Japan Tobacco), Mr.K. Yagi (Taisho Pharmaceutical), Dr. S. Okazaki (Bozo Research Center), and Mr. I. Naeshiro (Takeda Chemical) of the general toxicity section; Dr. M. Fujiwara (Yamanouchi Pharmaceutical), Mr.T. Inoue (Taisho Pharmaceutical), Dr.T. Furuhashi (Nihon Bioresearch), Dr.K. Sanbuissho (Sankyo) and Mr.T.Shibano (Ina Research) of the reproductive toxicity section; and Dr. K. Iwata (Biosafety Research Center, Foods, Drugs and Pesticides), Dr. K. Shibuya (Japan Biological Scientific Research Laboratory) and Dr.M. Takechi (Mitsubishi Chemical Safety Institute) of carcinogenicity section. Most of these members contributed their papers to the first publication. Although background data of carcinogenicity studies was not available for the first publication, it is also worth mentioning that the members are actively continuing their studies.

We were indebted to Ms. A.Tanaka and her colleagues of Food and Drug Safety Center for the preparation of a sample paper.

We also thank Dr. R. Harling of Huntingdon Life Sciences Ltd., U.K. Dr. J. L. Schardein of WIL Research Laboratories, Inc., and Dr. C. Banks of ClinTrial BioResearch, Canada, for their valuable advice for the steering committee as well as their committed contributions to this publication. We were encouraged by the supportive messages from overseas members who could not contribute to the first publication due to their inaccessibility to breeding colonies, but are willing to give their support to the Study Group. We received intermediate help for our communication with European and American members by their agencies or branch offices (liaisons) in Japan. They also helped communication with non-members in Europe and U.S.A. (CROs and pharmaceutical companies).

This publication was realized thanks to the courtesy and support of both Charles River, Inc. in U.S.A. and Japan. We are especially grateful to Mr. Nakanishi, President of Charles River Japan, Inc., for his deep understanding and strong support for the organization of this society. Mr. S. Takura and Dr. E. Morimura took charge of the actual running, especially the promotion of the Study Group, communication and management of the committee. We also acknowledge the efforts made by Dr. Lee who intermediated between Charles River U.S.A. and Charles River Japan.

We thank Ms. M. Kimishima of U-STAFF, Inc., for acquiring a copyright and registration of a book number (ISBN) for the publication and Mr. Y. Tsutome, Manager of Best Printing Inc., for printing.

We would like to express our appreciation for the members and the personnel from the companies concerned for leding their understanding and support to the Study Group's activities.

Lastly we wish you, readers and others concerned, excellent health and prosperity.

Toshiaki Matsuzawa and Hiroyuki Inoue

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CHAPTER 1

Introduction

Consideration of Various Factors Relating to Biological Reference Data on Albino Laboratory Rats

Toshiaki MATSUZAWA and Hiroyuki INOUE*

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ABSTRACT. Biological reference data values change with various factors, which appear as interlaboratory differences. We have reviewed the history of albino laboratory rats, endocrinology, pharmacokinetics, clinical pathology, immunotoxicology, mutagenicity, reproductive toxicology and teratology, behavior and neurotoxicology, nutrition and age-related lesions, and survival and tumors by referring to the literature, with strain differences and various factors as the main focus. In order to maintain high quality of studies with rats, we have made suggestions on the matters of genetic monitoring and technical global standards. It is our hope that this review will be useful for accumulating and analyzing the biological reference data of CD(SD)IGS rats in coming years. —Key words: biological reference data, strain difference, albino laboratory rat

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INTRODUCTION

It is well known that values of biological reference data fluctuations are due to various factors, including animal factors such as age, sex, and strain differences; environmental conditions such as temperature, relative humidity and lighting in animal rooms; housing conditions such as cage size, number of animals per cage, feed and drinking water; handling, restraining and anesthetizing animals; sampling of blood, urine, tissues, and organs; fixation and storage of samples; and methods and techniques for examinations, observations, and measurements. These variations are generally regarded as "interlaboratory differences."

In this publication, Chapter 2 reports on body weight, food consumption, clinical pathology examinations and other matters concerning general toxicity studies. Chapter 3 mainly introduces data from reproductive toxicity studies with respect to body weight and food consumption of pregnant animals, and fetal weight and morphology (external, visceral, and skeletal anomalies). There are interlaboratory differences in biological reference data described in the papers of this publication, since the studies were conducted in various facilities according to their respective standard operation procedures (SOPs) and methods under individual environmental conditions. Papers directly concerned with carcinogenicity, immunotoxicity, genetic toxicity, and behavior/neurotoxicity are not included in this publication. In order for our prospective writers to utilize information for as coming publications, we refer to differences in strains, ages, feed, and laboratories based on the literature.

It need hardly be said that accumulation and analysis of data for CD(SD)IGS rats are required so that this strain of rat is accepted internationally.

LITERATURE REVIEW

History of Albino Laboratory Rats: The origin of laboratory rats which are presently used worldwide dates back approximately to 1930 [47, 48, 83]. Around 1950, several laboratories distributed their rats to commercial breeders and breeding colonies. In the 1960's, Specific Pathogen-Free (SPF) rats produced by pathogenic bacterial control began to be on the market [23, 65, 87]. In Japan, SPF Sprague-Dawley(SD) rats began to come used for toxicity studies in 1995, and death caused by infection from mycoplasma pulmonis, commonly seen in ordinary rats, had ceased. Charles River U.S.A., Inc. began to breed SPF SD rats by the Caesarian-derived system (CD) and brought them into the market. During that time, many background data on comparison between SPF (including gnotobiote) and conventional rats were published [64 80]. For example, there are data on comparisons among Charles River-CD, Carworth Farm-Nelson, and Carworth Farm-Wistar rats.

Since the supply of SPF rats became available, long-term toxicity studies and carcinogenicity studies began to be conducted largely using them. At present, Wistar, F344 and SD strains of albino rats are widely used in toxicity studies of drugs, agro-chemicals, chemical compounds or environmental pollutants. In the selection of strains of rats, there are regional differences in Japan, Europe and North America, since accumulation of background data and constant supply are regarded as important. For example, SD rats are mainly used in the U.S.A. [41]. F344 rats are commonly used in the US-National Toxicology Program and NCI's carcinogenicity studies[36]. In Europe, Wistar rats are mainly used [47, 73]. In Japan, SD rats are commonly used in general toxicity studies [56] and reproductive toxicity studies [63], while F344 rats are widely used in carcinogenicity studies [33, 35, 52, 53]. These regional differences originated in the 1960's.

In the 1970's, laboratory rats were bred, supplied and maintained not only for non-clinical toxicity studies, but also for pharmacological studies of drugs and pathophysiological research of diseases such as hypertension as a human disease model [20, 74], and for genetic and congenital anomaly research. The duration of carcinogenicity studies was set at 2 years, and at that time the survival rates apparently exceeded 50%. Their life-span was also extended and was longer than that at present.

In the 1980's, the accumulated background data of ten years demonstrated the decline of their survival rates for 2 years and shortened life-span. In the report by Rao *et al*. [70], the body size of rats in 1981 was greater than that in 1971. The survival rates for 2 years were not less than 80% in 1971, but in 1981 the rates declined to 63% in males and to 71% in females. This problem has been reported by quite a number of nutritionists such as McCay *et al*. [59], and Berg and Simms [6]. To deal with this problem, several types of feed were put on the market. However, toxicologists and toxicological pathology specialists did not consider the feed factor, since a control group was always included in each repeated dose toxicity study. For the last twenty years we have obtained numerous data indicating that the incidence and degree of lesions, survival rates and life-span are affected by changing nutritional ingredients and amount of feed supply. It has become common understanding among toxicologists and pathologists that there are differences in these effects among the strains and breeders [72].

Between 1977 and 1983, there were some episodes of corona virus or sialodacryoadenitis virus infection in rats used in studies of NCI-NTP in the U.S.A. [69]. Consequently, interlaboratory variability was recognized for the occurrence of leukemia and pituitary tumors.

In 1990, the survival rates of SD rats in 2-year carcinogenicity studies declined to lower than 50% due to obesity [9, 66]. In particular, the SD(CD) rats produced by Charles River U.S.A., Inc. had a tendency to become over-weight more rapidly, and their survival rates for 2 years were lower than those in other colonies and breeders, which became a problem [54, 68]. For this reason this company prepared a production and supply system for IGS rats in their eight colonies existing throughout the world. The development and history of CD(SD)IGS rats will be described later by Dr. William of Charles River U.S.A., Inc. in this publication.

Endocrinology: It is known that changes in the endocrine glands affect the onset of age-related lesions and neoplastic lesions as well as fertility. It has been reported that plasma thyroidal hormone (T3 and T4) concentrations are significantly decreased by very short-term food deprivation [15]. Badger *et al.* [2] have reported that LH is affected by fasting. It is well known that blood gonadotropin concentrations vary according to the strain, age or sex of rats [74, 75]. We are expecting reports describing data for changes in the endocrine glands and hormonal levels in long- and short-term housing of CD(SD)IGS rats.

Pharmacokinetics: Colvin *et al.* [14] investigated fluctuations not only in the blood warfarin concentrations but also in prothrombin times, plasma protein fractions, and hematocrit by changing protein content in feed given to SD rats. It is known that the expressions of drug-metabolizing enzymes (UDP-glucuronosyltransferase, cytochochrome p450 (CPY)-CYP2C11, CYP2E1, 7 α-hydroxylation, 7-ethoxyresorufin-deethylase activities) in the liver and testes vary due to different rat strains and nutritional ingredients [46]. We are expecting papers on pharmacokinetic studies of CD(SD)IGS rats.

Clinical Pathology: Clinical pathology is one of the parameters in general toxicity studies. Numerous clinical pathology values are presented in this publication. Electrophoretic patterns of serum proteins in Buffalo, Fischer, Lewis, SD and Wistar rat strains [94] have been investigated [90, 94]. These values and patterns vary with feed, environment, inflammation or infection, and age. Total protein or albumin levels are compared between the original CD rats and CD(SD)IGS rats in a paper in this publication.

There may be considerable artifacts due to differences in conditions prior to measurements including sampling conditions, such as strain, age, anesthesia, restricted feed, fasting, sampling site and anticoagulants, in blood chemistry values [1, 10, 11, 27, 28, 38, 45, 50, 56, 57, 71, 78, 84, 88, 91, 92, 93, 98, 99]. Papers presented in this publication show comparisons of clinical pathology data between the original CD rats and CD(SD)IGS rats. Interlaboratory differences are also seen in clinical pathology data on CD(SD)IGS rats. Coleman *et al*. [13] have reported on the relations among age-related increases in the severity of renal diseases, decreases in total protein and albumin, and increases in α-globulin and cholesterol.

It should be considered that artifacts are sometimes included in biological background data. To eliminate such artifacts, studies should be conducted in accordance with the standard protocols, SOPs and standard analytical methods by personnel who have standardized levels of skill, experience and diligence. Control surveillance indicated that interlaboratory differences are also derived from methods and equipment of measurements [58]. It has been reported that loading of swimming and treadmill exercise of more than 30 minutes to animals increased plasma AST, CPK and LDH levels [49], suggesting a problem related to stress and handling of animals.

There is also a paper investigating enzyme inheritance in clinical pathology genetically [38].

Immunotoxicology: Serum interleukin-6 and corticosterone levels were measured in 4 strains of rats after footshock exposure, which resulted in differences among the strains. It is well known that genetically different strains of rats show significant interstrain differences in immunological and neuroendocrine features [44, 89]. Dhabhar *et al.* [19] have reported differences in corticosterone secretion and activation of adrenal steroid receptors among SD, F344 and Lewis rat strains. We are also expecting a study of comparison with CD(SD)IGS rats. Derijk and Sternberg [18] have reported that there are interstrain differences in neuroendocrine-immune interactions by investigating ACTH and corticosterone responses in SD and F344 rats. Immunotoxicology studies in CD(SD) IGS rats will be expected.

Mutagenicity: Howard [30] and Hungerford and Nowell [32] have investigated differences in chromosome marker systems and sex chromosome polymorphism, respectively, among 3 strains of Wistar rats. It is unclear what the differences mean [39]. Study results of chromosomes and genetic toxicity with CD(SD)IGS rats are expected.

Reproduction Toxicology and Teratology: Some background data on teratogenicity have been published in Japan, Europe and the U.S.A. There are differences among strains and colonies [3, 24]. In this publication, although data on external, visceral and

skeletal anomalies based on reproductive toxicity studies are shown, there is no report on reproductive physiology parameters such as litter size and intrauterine position, fetal weight, placental weight and length of gestation [4, 5].

In Japan, surveys of background data on spontaneous anomalies in rat fetuses have been conducted at least 3 times during these past twenty years [40, 62, 63]. From the results of these surveys, differences in external and skeletal anomalies among strains or breeding colonies are unclear due to their low incidences. There are large differences in the incidence of visceral anomalies and skeletal variations, probably due to differences in criteria and detectability among laboratories. Particularly in visceral anomalies, there are large interlaboratory differences in the incidence of thymic remnant in the neck and dilatation of renal pelves and ureters. In the survey conducted in 1987, the incidence of thymic remnant in the neck was higher in Slc:SD rats than in Jcl:SD and Crj:SD rats when a comparison was made among breeders of SD rats.

Comparison among breeders of Wistar rats revealed a higher incidence of this anomaly in Slc:Wistar rats than those in Jcl:Wistar and Wistar-Imamichi rats. In the survey conducted in 1987, the incidence of thymic remnant in the neck was slightly lower in Jcl:SD rats among SD rats. With respect to the 14th rib, a skeletal variation, the incidence was higher in SD rats than in Wistar rats in 1980, and was higher in Jcl:SD rats than in Crj:SD and Slc:SD rats in 1987 and 1997. In Japan, SD rats, especially Crj:SD rats, are used for reproductive toxicity studies in most testing facilities.

Effects of feed, age in weeks and so forth on the skeletal morphology were investigated in 7 strains of rats, i.e., Osborne Mendel, SD, Hoppert, Wistar-Lewis, Hooded, MSU Gray and S5B/P1 [61]. It is expected that data for effects on CD(SD)IGS rats when administered at different embryonic stages [60] will be obtained as further reproductive toxicity studies.

Neurotoxicology and Behavior: Strain differences in conditioned avoidance responses in the open-field behavior test have been reported by Holland and Gupta using 2 strains of rats [29] and by Harrington using 12 inbred strains such as ACI, F344, IR, MNR, TSI, WAG and others [26]. Strain differences in responses to shock have also been investigated using the shuttle-box test [96]. Not only genetic and environmental correlation, but also human factors may affect the responses.

There are strain differences in audiotoxicity of kanamycin [55].

Nutrition, Age-related Lesions, Survival and Tumors: There are strain differences among albino rats in body weight, food consumption, types and incidences of age-related lesions, affected organs or incidences of tumors and life-span. Even if the same strain are used, there is also differences among breeding colonies (producers).

In F344 rats, mononuclear cell leukemia, chronic nephropathy [25], corneal dystrophy [7], testicular interstitial cell tumors, pituitary neoplasms, etc. are observed at high incidences [79]. However, the incidences of mononuclear cell leukemia and corneal dystrophy are extremely low in F344 rats produced in Japan. The incidences of mammary tumors and pancreatic adenoma are high in SD rats. It has been reported that the pathogenesis of chronic renal diseases in SD rats [25] is different from that in F344 rats.

It was already mentioned that the incidence of corneal dystrophy is high in F344 rats in Europe and North Ameria. Focal linear retinopathy and coloboma are the most common findings in SD rats, but there are differences in the incidence among colonies [31, 43]. Lesions such as optic nerve aplasia are known to occur predominantly in Wistar rats [81].

In a 104-week study in SD rats, testicular atrophy or inflammatory lesions of testicular blood vessels were observed, but spontaneous testicular neoplasia is not an important feature of the SD rat [38]. The incidence of interstitial cell tumors of the testes is extremely high in F344 rats, while it is low in SD rats.

Cornwell *et al.* [16] have reported that collagen deposition in the left ventricle was increased in an age-related manner in their study concerning myocardial fibrosis in aging in germ-free and conventional Lobund-Wistar rats. Berg and Simms [6] reported that feed restriction by 46% resulted in alleviation of renal, vascular, myocardial and skeletal muscle lesions and mammary fibroadenoma in females when compared to *ad libitum* feeding. It was also reported that life expectancy was extended by 25% in males and by 39% in females. Burek *et al.* [8] reported on degenerative melopathy in three strains of aging rats.

Roe [73] and Schardein *et al.* [76] reported historical control data and background data of tumors in Holtzman rats, respectively. Mackenzie and Garner [51] compared the incidences of tumors in SD rats among 6 colonies of 4 commercial breeders such as Sprague-Dawley Inc., Charles River Inc. and Haltzman Inc. Generally, predominant tumors in SD rats included thyroid and pituitary tumors in females and adrenal medullary and islet cell tumors of the pancreas in males, but there are interlaboratory differences in their incidences. Pituitary tumors and mammary tumors spontaneously develop in female F344 and SD rats, however, but there are differences in the onset time between these 2 strains [12, 83, 86].

It is well known that rich-nutrition diets with high protein and high calorie are deeply related to obesity and longevity of animals. Such diets affect the duration, scale or evaluation of carcinogenicity studies [22]. It has been reported that in long-term toxicity/carcinogenicity studies in Crl:CDBR(CD) and Hsd:Sprague Dawley rats, increased body weight and food consumption, but decreased survival rates were observed in the former when compared to the latter [54, 68]. This fact suggests the shortening of longevity due to obesity. A high-fat diet tended to induce dietary obesity in 7 strains of rats such as Wistar and SD [77]. Dietary factors such as overnutrition (overfeeding), moderate dietary restriction and malnutrition (low protein, severe dietary restriction) are discussed not only for SD rats [41, 42] and F344 rats [33, 34, 82, 100] but also for Wistar rats [73]. The survival rate was apparently increased with 80% feed restriction when compared to high-protein feed given *ad libitum* [73]. This was due to decreased incidences of lesions indicative of nephropathy and chronic myocarditis. Longevity was extended in F344 rats with 60% food restriction. This feeding method is supposed to inhibit or delay the development of lesions such as interstitial cell tumors of the

testes, bile duct hyperplasia, myocardial fibrosis, and myocardial degeneration [33, 34, 82, 100].

In order to solve the dietary problem, Keenan *et al.* [42] attempted to evaluate phenobarbital and clofibrate, HMG CoA reductase inhibitors, and cyclosporin, a dopamine agonist, using a moderate dietary condition (Purina Chow, 21.4% protein, 5.7% fat, 65% diet restriction), and consequently obtained satisfactory results.

In this publication, comparison data between CD(SD)IGS rats and original CD rats when provided with a high-protein diet (24%) and a low-protein diet (15%) for 3 months or 6 months are presented. Data on development of age-related lesions and longevity in longer-term studies will be presented in the next publication.

In carcinogenicity studies, variable biological data were obtained even if the same strain of rats from the same colony was used. It has been pointed out that there are some human factors such as differences in education/training and experience of personnel (pathologists and so) or in SOPs or other factors such as environmental conditions [73] These matters will be mentioned in the next article.

SCOPE

Monitoring and Global Standards: Maintenance of rat strains is quite important. The origins, number of generations, grade of bacterial control and genetic control should be established to maintain the strains. In the Japan Central Institute for Experimental Animals, more than twenty strains including ACI/N, BN/SsN, F344/N and LEW/SsN are accurately maintained. Attached to this laboratory, the ICLAS Monitoring Center, the only one in the world, was established, where bacterial and genetic monitoring as well as frozen storage of fertilized ova are contracted. This sort of project is considered very important for animal experimentalists.

It has been repeatedly mentioned that phenotypes differ according to breeding colonies though the strain is the same. This has become a serious problem in evaluation of drugs or determination of reproducibility of results in pre-clinical studies including pharmacological, metabolism and toxicity studies. Especially in carcinogenicity studies, changes in the untreated control groups occasionally caused false positive and/or false negative results. Reluctantly, historical control data are sometimes quoted from the literature. Nutritional ingredients and amounts of feed are quite important as extrinsic factors in toxicity studies of drugs, since these factors greatly affect absorption of drugs and the incidence and degree of lesions. Therefore, global standards for nutritional ingredients and feed amount are necessary and important for reduction of the use of animals and feed resources.

Guidelines for toxicity studies of pharmaceutical products have been standardized globally for several years. Improvement in quality and efficiency of toxicity studies is related to suitable materials and location. The world's largest animal supplier, Charles River Inc., has been struggling with global standardization and constant supply of CD rats, which is also a great concern for users.

The necessity of global standardization of strains, housing conditions, genetic control, or microbiological control of rats is a great concern for the authors of this publication and many other users, and they also have many demands for realizing standardization. In order to closely monitor rat phenotypes, it is necessary to establish high-quality global standards on a scientific basis for techniques and methods of animal handling, housing conditions, sampling conditions, animal protection/welfare [17], statistical methods, pathological terms, teratological terms [97], clinical pathology examinations [95], neurotoxicological observation methods [21, 67], *etc*. With this aspect in mind, some international conferences have already been held and publications produced. It is important that the global standards will be maintained and improved upon with the progress of science.

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The Development and Maintenance of the Crl:CD®(SD)IGS BR Rat Breeding System

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ABSTRACT. Systems for genetically standardizing inbred strains of rodents have been well documented. With the internationalization of biomedical research, it has become necessary for multi-national laboratory animal suppliers to globally standardize more commonly used non-inbred toxicology models such as the Sprague Dawley and Hanover Wistar rat stocks. This paper describes one such breeding system, the International Genetic Standard (IGS®) system to produce the Crl:CD®(SD) IGS BR and Crl:WI(Glx/BRL/Han) IGS BR rats for the international biomedical research community. —Key words: Outbred, breeder, breeding system, International Genetic Standard, IGS rats, SD rats, reproduction, inbred, random system

CD (SD) IGS-1998: 8-14

BACKGROUND

The CD rat has a long history of use in toxicology research. This stock of rats has been maintained for research purposes for over 50 years and traces its origins back to a stock developed by Robert S. Dawley in the 1920s. The original Sprague-Dawley stock was developed from Wistar stock and a hybrid stock produced from laboratory and wild populations. Founder animals were obtained from Sprague-Dawley in the 1950s and caesarean rederived by Charles River Laboratories (CRL) to achieve an improved microbiological status. This stock was maintained using a random mating system and by the early 1990s was produced by the company in 23 separate production colonies in 8 different countries.

Late in the 1980s a disturbing trend towards a decrease in longevity was detected in the CD rat as well as other stocks of rats produced commercially including some inbred strains. Even though the cause of these changes still remains unknown, a genetic component resulting from unconscious selection pressures associated with the use of a random mating system may have resulted in inadvertent loss of heterozygosity that contributed to these changes. While other avenues have been sought to address problems associated with decreased longevity, Charles River Laboratories decided to reexamine its breeding practices and to take steps to minimize selection pressures on this heterogeneous population by instituting a comprehensive outbreeding system (for reviews of longevity related issues see Reference 1, 4, 5). At the same time, with the globalization of biomedical research, it became clear that steps also need to be taken to harmonize the breeding populations of CD rats throughout the world in order to minimize the degree of variation associated with genetic drift between these populations. Hence, in February of 1992, the company launched a comprehensive restructuring of its breeding programs for outbred stocks including the CD rat. This program resulted in the repopulation of all CRL production colonies of CD rats with a genetically harmonized stock of animals referred to as Crl:CD®(SD)IGS BR rats. Conversion of production colonies to CRL:CD®(SD)IGS BR colonies was gradual with the first animals available for commercial sale in 1994 with complete conversion and world-wide availability achieved by the beginning of 1998.

GENETIC BASIS FOR THE INTERNATIONAL GENETIC STANDARD BREEDING PROGRAM

In general, there are three broad genetic classifications of laboratory rodents commonly used in research. These are inbreds, F1 hybrids, and non-inbreds. Transgenic animals which are becoming increasingly more common in research can be created on any of these backgrounds (for reviews of genetic management concepts see Reference 2, 3).

Inbred animals are the result of 20 generations of brother-sister mating resulting in over 98% genetic uniformity (homozygosity). In order to maintain this level of homozygosity, a rigorous program of brother-sister mating must be maintained using a very structured colony set up (see Figure 1).

A gnotobiotic foundation colony is usually maintained by commercial breeders in isolators and is extensively monitored genetically and microbiologically. This colony serves as the source of stock for individual pedigreed nucleus colonies at various production sites. Since spontaneous mutations can occur and may become fixed in a population that is separated from another, it is necessary every 5 to 10 generations (an arbitrary figure) to restart the pedigreed nucleus colony in each production room with founder animals from the gnotobiotic foundation colony. This assures that the inbred animals produced do not differ significantly from the foundation stock.

Hybrid animals are the product of the crossing of two inbred strains. These animals are heterozygous at all gene loci at which their parental strains differ. F1 hybrids are not self-perpetuating; therefore, it is necessary to maintain colonies of both parental inbred strains. F1 hybrids do provide advantages in terms of uniformity while not being homozygous as are their parental strains. The consistency of F1 hybrids is largely dependent upon (1) the breeding program for the inbred parental stains and (2) a carefully structured breeding program for the hybrids that avoids mismatings.

Non-inbred animals which include the designations random bred and outbred are animals derived from mating unrelated individuals. Presumably, they are desirable to the biomedical research community because of their great degree of individual

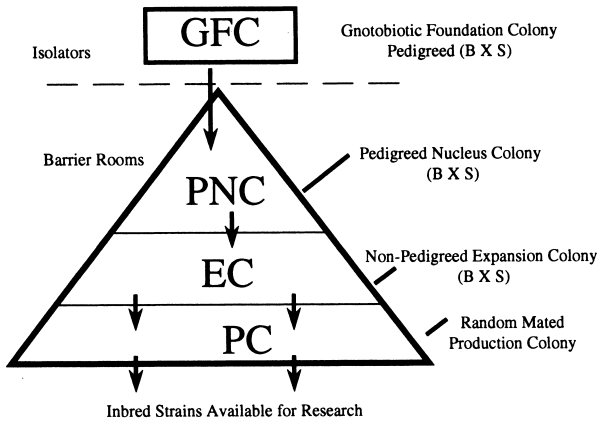

Fig. 1. International Standard Breeding Program for Inbred Strains

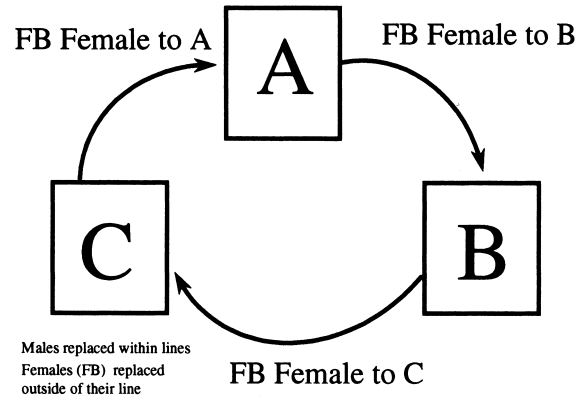

Fig. 2. Fixed 3-Line Outbreeding System - Single Sex Rotation

diversity. Like man, significant sample sizes must be used in order to acquire a representation of the entire population. It is also critical that concurrent and historical controls be acquired when conducting studies in order to characterize the population both at the time of the study as well as changes in the population overtime.

In order to produce non-inbred animals, inbreeding needs to be avoided. While the goal of any breeding program producing these animals is genetically heterogeneity, absolute heterogeneity is never achieved since there is always some unconscious selection imposed on the population which results in a tendency towards inbreeding. Some of these selection pressures are unavoidable if a constant supply of animals are to be produced by the colony.

Random breeding systems have been used to produce non-inbred animals in the past but depend upon a number of assumptions including (1) an infinite population size, (2) that every reproductively fit animals has an equal chance of participating in the breeding program, and (3) that there is no structured breeding program or selection criteria that would inhibit randomization. Unfortunately, these criteria are probably never met even in wild populations. In production colonies, a significant proportion of the colony is used for research and does not have an opportunity to participate in a breeding program. Limits on the numbers of animals to be produced, as well as on the sex of animals required for research, coupled with the physical limitations for housing the colony prohibit true random breeding from occurring. The fact that animals must be housed in cages and in groups automatically divides the population based on criteria that may not be completely random. In addition, unconscious selection criteria, as well as some conscious selection criteria for factors such as large litter size, fecundity, ability to bring a litter to term, aggressiveness, and perhaps even morphologic characteristics, may result in a tendency toward inbreeding. Failure of replacement breeders to accurately reflect the genetic make up of the existing colony as a result of such selection pressures or sampling error can result in long term colony alterations in genotype and phenotype which may compound similar changes resulting from spontaneous mutations that become fixed in the population.

In order to overcome some of the difficulties associated with random mating, purposeful mating systems that attempt to maximize heterozygosity (individual variation), referred to as outbreeding schemes, have been developed. These systems minimize the chance of inbreeding, ensure that a large percent of the population can participate in the breeding system, and reduce selection criteria, therefore, decreasing tendency towards inbreeding.

Generally, outbreeding systems divide the population into a series of groups that are referred to as families or lines. Replacement breeding pairs within lines are selected partially or in total from outside of that line using a fixed system of crosses (Figure 2). As an alternative, when the population is small enough, kinship (relatedness) can be calculated mathematically provided that all animals are pedigreed and new pairs can be constructed based upon mating least related animals.

As a general rule, the greater the number of lines and the greater the numbers of individuals per line, the less inbreeding that will occur. From a practical standpoint, however, the more lines and the more complex the migration patterns used to construct new pairs for replacement breeders, the more logistically difficult the system, the more costly and space intensive the system, and the more prone the system will be to error. Maintaining pedigree information on populations over 1000 breeders is impractical. For example, production colonies in academic, government, and pharmaceutical companies usually number no more than several 1000 individuals of any given species and stock whereas individual production colonies maintained at commercially breeders usually range between 60,000 and 300,000 individuals.

In addition to minimizing kinship, outbreeding systems must seek to reduce breeder selection criteria to an absolute minimum number of factors; and they must resist the temptation to utilize unjustified phenotypic characteristics for selection. Hence, while large litter size might be desirable from a commercial standpoint, the mature body size of breeders required to maintain such large litters may also select for obesity and decreased life span. On the other hand, very small litters may not be commercially viable and may have undesirable research

consequences. The number of siblings or related animals in the population used for breeding should be minimized, and steps such as selecting one pup per litter and selecting males and females from litters born on separate days of the week for replacement breeders ensures that inadvertent brother-sister matings do not occur. Similarly, selecting replacement breeders from the third through fifth litters of rodent breeders in a polygamous system, eliminates the possibility that male replacement breeders could be sexually mature at the same time that their mothers were still reproductively active members of the breeding colony.

Even though a purposeful outbreeding system can minimize inbreeding within a single non-inbred population, the random genetic drift that occurs both over time and geographically between different colonies can still occur if steps are not taken to somehow genetically link the colonies. There are three interrelated causes that can result in genetic divergence and loss of heterozygosity. The first of these is referred to as a genetic bottleneck or the founder effect. This occurs when only a small number of animals are used to start a non-inbred colony. This frequently results from the need to improve the health status of animals through a process called rederivation which can be done by caesarean section or embryo transfer of animals to produce founder animals of the appropriate microbiological profile for a new colony. As can be seen in Figure 3, the number of breeding pairs of unrelated animals greatly influences the rate of inbreeding produced by random mating as evidenced by a measure of kinship such as the coefficient of inbreeding. Clearly, the larger the number of founder animals, the slower the lost of heterozygosity and the greater the likelihood that the degree of heterozygosity will reflect the parent population.

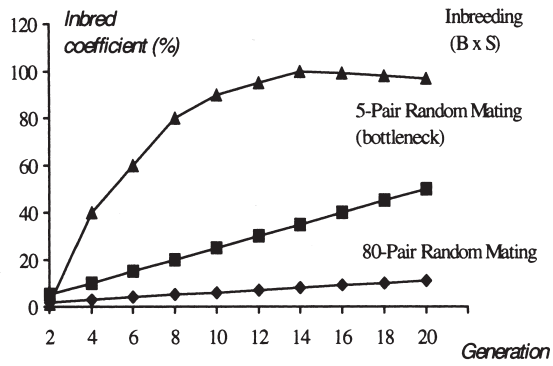

Fig. 3. Coefficient of Inbreeding with Different Colony Size and Mating Systems

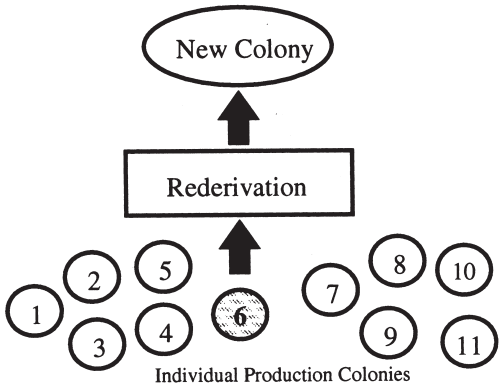

Fig. 4. Historical Process for New Colony Set-up

The second cause of genetic divergence or loss of heterozygosity is sampling error. This occurs when the breeders chosen to start a new colony are not an exact genetic representation of the colony from which they were derived. This can be magnified by the process of genetic bottlenecking which was previously described but can also occur in a broader sense if only one colony out of a series of several colonies is used to start a new colony. As illustrated in Figure 4, if only a single colony is chosen, the genotypic frequency of the rest of the population maintained in the other colonies is lost. The only way to overcome this is to take a large enough number of breeders from every population to start a new colony so that the frequency of genotypes of all the colonies taken as a whole is represented in the new one.

The third process that results in genetic divergence and lost of heterozygosity is mutation. As mutations occur within a colony, they may either become fixed or may not persist in the colony especially if the mutation occurs in an animal that is not a breeder. Some mutations are not advantageous and may be selected against such as a mutation that causes infertility.

The prevalence of various phenotypes/genotypes within a non-inbred population is constantly changing due to random assortment and mutation. For example, as illustrated in Table 1, a non-inbred population of CD-1 mice was established at four separate locations simultaneously using the same numbers of breeders for each population. Other parameters including colony size, breeding scheme and environmental conditions were similar between colonies. Three years after initial colony set up, the populations were surveyed by sampling 100 animals selected at random and assaying for a number of isoenzymes in blood and tissues that are known to be polymorphic (existing in more than one form).

Table 1. Distribution of Allelic Forms of Biochemical Markers Between Various Colonies of CD-1 Mice

Biochemical Marker (allele)	A	B	C	D
Hbb (d)	23	14	8	11
Hbb (sd)	60	42	40	50
Hbb (s)	17	44	52	39
Gpi-1 (a)	70	46	34	46
Gpi-1 (ab)	26	46	58	49
GPi-1 (b)	4	8	8	5
Gpd-1 (a)	3	12	34	28
Gdp-1 (ab)	46	38	34	47
Gpd-1 (b)	51	50	32	25
Pgm-1 (a)	27	38	34	31
Pgm-1 (ab)	50	46	52	50
Pgm-1 (b)	23	16	14	19
Mod-1 (a)	7	10	2	1
Mod-1 (ab)	21	14	10	6
Mod-1 (b)	72	76	88	93

As can be seen in Table 1, the distribution of allelic forms of certain isoenzymes was very similar between two or more of the colonies. However, with other isoenzymes, genetic divergence had clearly occurred between the populations suggesting that genotypic and hence phenotypic manifestations of this divergence might be able to be demonstrated depending upon the types of studies in which animals were used. Undoubtedly, many of the differences seen between non-inbred colonies sampled contemporaneously for use in similar studies, as well as those conducted on different populations or even the same population over the course of several years, can be attributed to this phenomenon.

In developing the IGS system, the challenge was to develop a mechanism to minimize the divergence that occurs between geographically separate colonies. One way to do this, is to migrate animals between colonies. Migration can be viewed as a form of genetic glue that holds colonies together and sets a limit on the amount of genetic divergence that occurs. Migration of animals is not without its difficulties since other factors such as the potential for microbiological contamination of existing colonies must be considered in the migration process. By trading breed stock between colonies or as with inbreds by forward migrating breed stock from a central foundation colony, the replacement of a portion of the breed stock in each production colony introduces a representative sampling of the genetics of other production or foundation colonies into each production colony. The size of the infusion and the frequency of this migration of breed stock determine how quickly and completely the genetic divergence within the production population is altered to more closely resemble the foundation colony as well as other colonies in the production program. Large or frequent infusions cause rapid corrections and potentially major shifts in the allele frequency of the production population. Smaller or infrequent migrations may make smaller or less significant changes in the populations. While the appropriate size and frequency of migrations are clearly a matter of professional judgment, measures of the heterogeneity of the population prior to migration using population genetics parameters calculated from assays of allele frequency can assist in making such determinations.

Backward migration of animals from each production colony (transfer of animals to the foundation colony) on a regular basis ensures that new phenotypes as well as representation of predominant genotypes within an given production colony is represented in the foundation colony. Given the microbiological risk associated with this process, rederivation is required. No single colony should unduly influence a production colony especially if newly formed genotypes resulting from mutations may have undesirable effects. Bringing them back to the foundation colony does not guarantee the fixation of these genotypes within the foundation colony but does allow that possibility to occur. Since the need for refocusing of the foundation colony by such infusions is much less than the need for forward migrations to production colonies, the interval for backward migrations can be greater than for forward migrations. Given the small size of the foundation colony relative to the production colonies and the relatively large number of production colonies, replacement of 1% of the foundation breeders with each backward migration from each production colony is an arbitrary but an appropriate level of replacement. Both the forward and backward migration processes favor gradual change in the colonies over time.

THE IGS SYSTEM

Given the large number of CD rat production colonies that existed in 1992, migration of breed stock between production colonies in order to minimize the effects of genetic divergence would have been a very risky, time consuming and cumbersome task. In order to effect such an exchange process, regular forward and backward migration from each colony would be required as depicted in Figure 5. New colony start-up would require contributions from all existing colonies as depicted in Figure 6. As an alternative, a reference colony developed from existing genetic material in the various production colonies could be used as a means for maintaining a genetically diverse population that could be used for forward migration purposes as well as start-ups of new colonies as depicted in Figure 7. Since the size of the foundation colony can be kept within manageable limits, more complex outbreeding schemes could be applied in order to ensure that the tendency towards inbreeding is greatly reduced.

In selecting the CD rat breeding population that comprised the Crl:CD®(SD)IGS BR foundation colony, it was necessary to balance two competing concerns. The first was to select as large a number of breeders as possible from all of the colonies in order to accurately represent the genetic diversity of the entire production population. On the other hand, colonies that had recently been set up from other existing colonies either by rederivation or direct transfer of animals between colonies had not developed significant genetic drift from the parent population as compared to that which might be obtained by colonies that had been in production with no transfer of animals for at least five to ten years.

In reviewing all of the existing colonies in 1992, a total of 8 colonies (lines) were found that had been separated for a

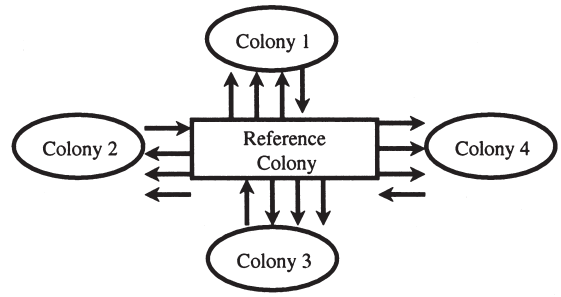

Fig. 5. Regular Forward and Backward Migration Using a Reference Colony System

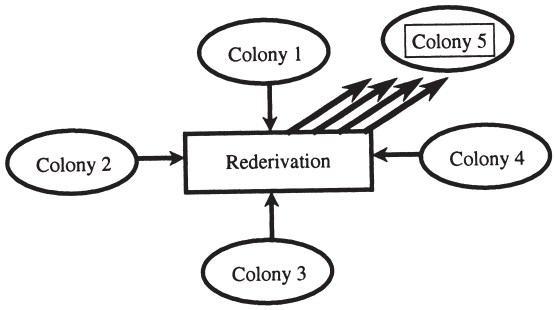

Fig. 6. New Colony Start-Up Using Stock Migration from Production Colonies

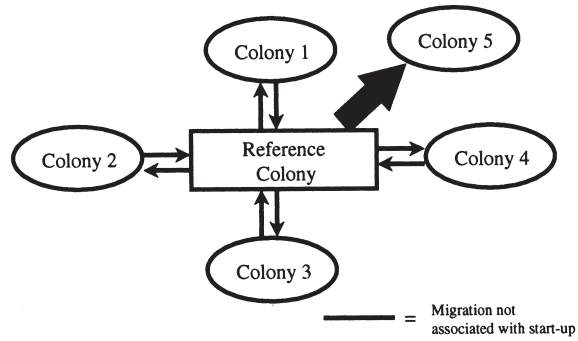

Fig. 7. New Colony Start Up Using the Reference Colony System

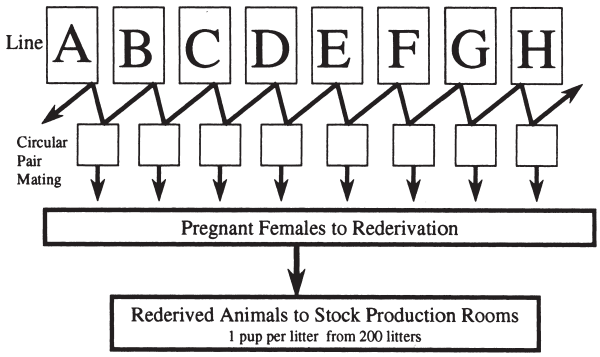

Fig. 8. Crl:CD,(SD)IGS BR Foundation ColonySystem for Producing Stock for Forward Migration

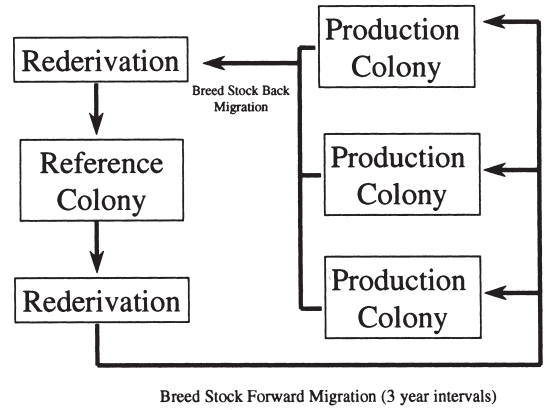

Fig. 9. Crl:CD,(SD)IGS BR Rat Forward and Backward Migration System

minimum of 12 years. A total of 100 breeding pairs were selected at random from each of these colonies and used to form a reference colony maintained within a barrier room at Charles River Laboratories' corporate headquarters in Wilmington, Massachusetts, USA. Initially, the foundation colony was maintained using a circular paired-mating system whereby the 8 lines were crossed in a systematic fashion to develop mating pairs that produced pregnant females for rederivation purposes. Once the pregnant females had undergone caesarean section and their pups transferred to foster mothers maintained in flora-defined isolators under the appropriate microbiological conditions, one pup per litter was used with a stocking number of 200 animals to be sent to start new Crl:CD®(SD)IGS BR colonies in existing barrier rooms (see Figure 8). The first Crl:CD®(SD)IGS BR colony to be stocked was in Hollister, California, USA in 1993. It was followed in short order by colonies in Raleigh, North Carolina, USA; several countries in Europe; and Japan.

The migration scheme utilizing the reference colony is depicted in Figure 9 At three year intervals, forward migration of breed stock to each production colony is made from the foundation colony. Since a polygamous mating system is used for rat production in order to efficiently produce large numbers of animals, the most effective method for incorporation of migrated breed stock is the transfer of males. To that end, sufficient male animals representing one pup per litter from foundation colony breeding pairs replace 25% of the male breeding population in each production colony.

At five year intervals, each production colony selects at random sufficient breeders to replace 1% of the foundation colony. These animals undergo rederivation such that one pup per litter is selected as a replacement breeder. Animals are introduced into the foundation colony only after their health status has been assured. Assignment of replacements by this process in the foundation colony is done using a table of random numbers. Breeding pairs so replaced cannot be replaced again with backward migrated animals from production colonies for at least six months. Backward migrations are staggered so these infusions do not occur all at the same time. Similarly, forward migrations are spread out over the three-year period so as not to unduly represent any given time period in the foundation colony's existence.

While this initial foundation colony set-up proved workable for several years, a number of disadvantages become evident.

The first of these was the inherent risk associated with trying to maintain a defined microbiological profile suitable for animal transfer to many colonies throughout the Charles River Laboratories system while housing animals in a barrier production room. Barrier production rooms provide a reasonable means for excluding rodent specific pathogens; however, the regular need for rederivation of animals from this large breeding population in the foundation colony in order to allow forward migrations introduces substantial risk of microbiological contamination through mishaps in the rederivation process. Moreover, such problems could have significant impact on the whole Crl:CD (SD)IGS BR production system if they went undetected for a very prolonged period of time.

Second, the relatively large colony size of 800 breeding pairs limits the ability to use more precise kinship mating systems effectively. Ideally, if each animal within the colony were pedigreed (breeders as well as replacement breeders), then all breeding could be done based upon mating least related animals using the coefficient of inbreeding as a basis for making such comparisons. This has the effect of making each breeding pair its own line thereby increasing the number of lines by hundreds as compared to the existing eight. This would provide a substantial improvement in the maintenance of individual heterozygosity.

Finally, since the entire population was maintained within a single barrier room, a disastrous incident affecting that barrier room such as the introduction of a pathogenic organism or a breach in the barrier caused by some natural or manmade disaster would result in the entire loss of the foundation colony. While reconstruction of such a colony would be possible using existing production colonies, the risk was deemed unacceptable.

Hence, starting in the fall of 1997 and completed in 1998, the Crl:CD(SD)IGS BR foundation colony was rederived and placed in 20 large semirigid isolators each holding 27 cages of animals. The foundation colony size was reduced to 250 breeding pairs based upon a change from a line breeding system to a computer assisted coefficient of inbreeding system. Prior to starting the rederivation process, kinship relationships and pedigrees were maintained manually in preparation for setting up the computer assisted program.

All breeding pairs are individually identified with ear tags as are replacement breeders from each pair. One male and one female is selected from the progeny of each breeding pair and is maintained as individually identified animals for future breed replacement. These animals are regularly replaced in the future breed section to ensure that animals that are young enough for breeding purposes are always available. The remainder of the animals produced from the matings are available for migration purposes but are not maintained beyond 4 weeks of age.

In the initial line breeding system for the foundation colony within the barrier room, breeders within a line were replaced with breed stock generated by males selected within the line and females selected from another line. In the case of the isolator maintained foundation colony, animals are retired from the breeding program at six months after being set up as a breeding pair or when replaced by backward migration. When replacing a breeding pair, the male future breeder from that breeding pair is set up with a new female selected by the computer based upon least relatedness. That female may exist within that isolator or within another isolator. A female in another isolator is transferred into the appropriate isolator to set up the new breeding pair using aseptic transfer techniques.

All materials introduced into the isolators are sterilized or suitably decontaminated. The microbiological status of the isolators is monitored by environmental bacteriological culturing and regular whole animal health monitoring conducted on each isolator. In addition, prior to any forward migration or new colony set-up, additional health monitoring and environmental culturing is done to ensure the microbiological status of each isolator.

The foundation colony is surveyed for a number of polymorphic microsatellite markers using a large sampling of animals distributed over all of the isolators in the foundation colony. Similarly, prior to each forward migration, the production colonies are also sampled for polymorphic markers. The results of this sampling are compiled and the distribution of markers is used to calculate a number of population genetic statistics. These serve as a guide to analyzing the degree of heterozygosity present in each population and to compare the amount of genetic divergence between the foundation and individual production colonies. An IGS advisory panel that includes population geneticists and veterinarians meets on a regular basis to review these findings as well as the details of the genetic monitoring, health and production programs. At three-year intervals, embryos from the foundation colony will be cryopreserved in order to guard against disastrous loss of the colony and to allow the possibility of genetic infusions reflective of the distribution of genotypes in past foundation colony samplings should that be warranted.

Within the individual production colonies, a purposeful outbreeding scheme has been put in place. Each production colony of Crl:CD®(SD)IGS BR animals utilizes a line breeding system with three lines. Each breeder male and female is ear punched to identify it as to which line it belongs to. A rotational system is used to set up replacement breeders in each line as illustrated in Figure 2. Males are replaced within their own line whereas females are rotated between lines in a fixed pattern. More complex biorotational systems were not considered necessary given the forward migration process. Certain basic selection criteria have been imposed on the line breeding system within each production room. These included the use of only one pup per litter for replacement breeders; the selection of male and female replacement breeders on separate days of birth; the selection of males only from the mothers' third through fifth litters; and selection criteria on litter sizes designed to reduce the tendency to select for very large litters.

A cap was placed on litter size for future breed selection such that future breeders could only be selected from animals having between 4 and 16 pups. Given the average litter size of CD rats, a lower cap of four pups was arbitrary but deemed appropriate given practical and economic production concerns. The use of litters with less than four pups would make production economically difficult. The cap on the upper end of 16 pups

was designed to reverse the trend which was presumed to have occurred in years past of unconsciously selecting replacement breeders from large litters. Animals produced from each line not only are used for replacement breeders, but also are commingled in stock cages to be used for sale in biomedical research (Figure 10).

SUMMARY

The system of mating used to manage the Crl:CD(SD)IGS BR rat colonies has been purposely designed to produce non-inbred animals that possess a great degree of individual heterozygosity while harmonizing the range and distribution of genotypes/phenotypes within production colonies throughout the world. This has been accomplished utilizing well-established genetic management techniques. The same principals have been applied to the management of certain other non-inbred strains produced by Charles River Laboratories and bear some correlation to well-established concepts for linking geographically separated inbred colonies.

The Crl:CD®(SD)IGS BR rat is not a new stock. No foreign genetic material has been added. No selection for specific traits has been conducted. Rather, a process that retrieved the full range of diversity found within the existing CD populations was employed. The resulting foundation colony on which all production of Crl:CD®(SD)IGS BR animals is based reflects this diversity and, through the forward migration process, regularly links all production colonies to it in a way that the range of variation in phenotypes within individual production colonies becomes similar and is directly related to the degree of variation within the foundation colony. Loss of heterozygosity through inbreeding has been minimized hence the fixation of detrimental phenotypes within the population should be less likely. Overall, the global biomedical research community now has available to it a fully harmonized animal model that should react in a similar fashion no matter where in the world it is obtained.

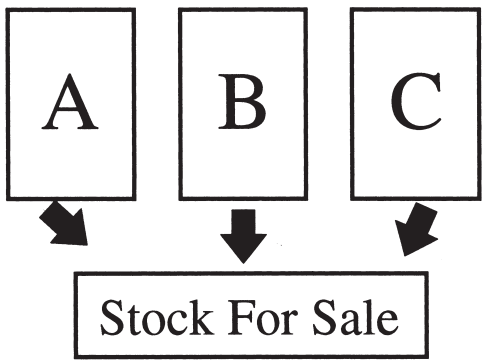

Fig. 10. Production of Crl:CD,(SD)IGS BR Rats for Sale Using a 3-Line Outbreeding System

TERMS

Crl:CD®(SD)IGS BR - Stock designation of CD rats produced by Charles River Laboratories that have been produced using the IGS genetic management system.

Crl:CD®(SD) BR - Stock designation of CD rats produced by Charles River Laboratories that were not produced using the IGS genetic management system.

International Genetic Standard (IGS) - A globally integrated genetic management system using pedigreed gnotobiotic foundation colonies, a program of regular breed stock migration, and in the case of non-inbreds an avoidance of inbreeding production system.

Gold Standard - A term formerly used to describe an intensively managed gnotobiotic foundation colony used to standardize geographically separated production colonies by breed stock migrations.

CD Rat - A non-inbred stock of rats acquired by official transfer of breed stock from Sprague Dawley Inc. in 1950 often referred to as Sprague Dawley (SD) rats. The stock was caesarean derived ("CD") in 1955 from original Charles River SD(tm) colonies to form the nucleus of the current CD stock. The term is a contraction of the official designation Crl:CD®(SD)IGS BR or Crl:CD®(SD) BR.

New Generation or International Standard - Terms replaced by the term International Genetic Standard.

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Body Weight, Hematology, Blood Chemistry and Reproduction Data in Our Facility

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ABSTRACT. Changes in body weight (3-10 weeks old, every 7 days), weight of various organs (12 weeks old), i. e. ,brain, thymus, heart, lungs, liver, spleen, kidneys, adrenal glands, testes or ovaries, hematology(12 weeks old), i. e. , WBC, RBC, Hb, Ht, MCV, MCH, MCHC, PLT and blood chemistry(12 weeks old), i. e. , GOT, GPT, BUN, CRE, GLU, TP, ALB, T-CHO, TG,T-BIL, IP, Ca, CPK, ALP, LAP were examined to obtain the basic data of CD (SD) IGS rats.
Furthermore, we have performed reproductive study to get the delivery and nursing data of these animals. —Key words: Body weight, hematology, blood chemistry, reproduction, IGS rat.

CD (SD) IGS-1998: 15-18

INTRODUCTION

We introduced CD (SD) IGS rats from 1994 to 1995 into Tsukuba Breeding Center. In order to obtain the basic data of CD (SD) IGS rats, we have performed some investigations about these animals under daily work in the breeding room and our laboratory room.
We show these data which were collected in 1997 or 1998 as follows.

METHODS

1. Animal husbandry
(A)Room and environment

The animals were raised in breeding room of Tsukuba Breeding Center. The room was kept at a temparature of 21-25°C, with a relative humidity at 50-65%, and ventilation of 15-18cycle/hour and 12-hour light-dark cycle.
(B)Cage and housing

We change cage size and animal number per cage according to the stage of animals.

After birth to weaning, pups were nursed with their litter mates, dam, and sire. They were housed in a L-cage which is made of polycarbonate and has 540mm long × 340mm wide × 160mm high. Newborns were farmed out or eliminated at the next day of birth, adjusting number per litter to 14. At 21 day of birth, pups were weaned.

After weaning, animals were devided by their sex and housed in J-cage which size is 640mm long × 380mm wide × 200mm high. When just weaned, animals were housed 20 per cage. The number of animals per cage were reduced according to aging. When 6 weeks old, males per cage were changed into 15, at 7 weeks into 13, and in 8-10 weeks 12 males were housed in each cage. In the case of female, 20 animals were housed in 3-8 weeks old, and the number were reduced to 15 in 8-10 weeks.
(C)Diet

CRF-1 pellet diet(Oriental Yeast Co.,) was given *ad libitum*. The diet was sterilized before supplying by autoclave.
(E)Water

Tap Water was provided *ad libitum* via an automatic water supply system. Before supplying, we added sodium hypochlorite into water, and controlled the chlornde density in 1.5-2.0ppm.
(F)Bedding

Beech chips or White Flakes were used for bedding. We changed them once or twice a week.
2. Body weight
(A)Weanlings

There are so many animals in the breeding room, and to minimize sampling error, we mesured body weight of all weaning animals at one day. We calculated mean, standard deviation and distribution.
(B)Time series from 3 to 10 weeks

After measuring weanlings' body weight, we decided the range of starting weight, and selected 40 animals from each sex. They are divided 20 animals per cage ,measure body weights every week. They were reduced according to the number of housing.
3. Reproduction data
(A)Delivery data

We examined delivery data of females which were mated during one week. We recorded the date of delivery and caliculated duration from set up to deliver. We regarded the females which did not reveal symptom of pregnancy after 25 days from the start of mating as sterility.
(B)Nursing Data

We examined nursing data of dams and pups which are deliverd during one week. We recorded number of newborn, number of loss from the next day of birth to weaning. If litter size was smaller than 8, the litter were eliminated from nursing. When mother killed their pups by eating we recorded it as LDB.
4. Laboratory data

We selected 10 male and 10 female animals from breeding room. When they were in 12 weeks old, after overnight starvation, they were anethtized by pentobarbital sodium i.p. After collecting blood for haematology and blood chemistry from caudal vein, the animals were sacrificed and removed organs for weight measurment.
(A)Hematology

The samples were collected into EDTA as anticoagullant. They were examined with the microcell counter(NIHON KODEN) for white blood cells(WBC), red blood cells(RBC), hemoglobin(Hb), hematocrit(Ht), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC) and

platelets(PLT).

(B)Blood chemstry

Blood samples were centrifuged(2,800rpm, 10minutes) and serum samples were collected to examine GOT, GPT, BUN, CRE, GLU, TP, ALB, TCHO, TG, TBIL, IP, Ca, CPK, ALP, LAP with DRI-CHEM 3000V(Fuji film).

(C)Organ weight

Brain, thymus, heart, lungs, liver, spleen, kidneys, adrenal grands,testes and ovaries were removed from each animal and weighed(absolute weights). And the organ/body weight ratio(relative weights) calculated from the terminal body weights.

RESULTS

1. Body Weight

The body weight of weaning animals are shown in TABLE 1. From this result, we decided starting range of time series weighing as 43g-47g for male and 41g-45g for female. The body weight of each age is shown in TABLE 2.

2. Reproduction data

Delivery data and nursing data are shown in Table 3 and Table 4.

3. Laboratory data

Hematology and blood chemistry are shown in Table 5 and Table 6. Organ weight is shown in Table 7.

1. Body Weight

Table 1. Body weight of weanlings(3 weeks old) unit:g

Male		
N	564	
mean	45.3	
s.d.	6	
distribution		
range	Number	Frequency
22(min.)-32	5	0.9%
33-36	19	3.4%
37-40	71	12.6%
41-44	184	32.6%
45-48	150	26.6%
49-52	91	16.1%
53-56	26	4.6%
57-60	9	1.6%
61-64	2	0.4%
65-77(max.)	7	1.2%

Female		
N	272	
mean	44.6	
s.d.	6.8	
distribution		
range	Number	Frequency
27(min.)-32	2	0.7%
33-36	10	3.7%
37-40	52	19.1%
41-44	99	36.4%
45-48	62	22.8%
49-52	24	8.8%
53-56	7	2.6%
57-60	4	1.5%
61-64	3	1.1%
65-72(max.)	9	3.3%

Table 2. Body weight of time series from 3 to 10 weeks old unit:g

Male								
Age(week)	3	4	5	6	7	8	9	10
N	40	40	40	40	30	26	24	24
mean	45.0	77.4	125.0	167.8	219.0	264.7	304.6	334.5
s.d.	0.7	3.3	6.6	8.6	7.1	9.0	14.2	18.6

Female								
Age(week)	3	4	5	6	7	8	9	10
N	40	40	40	40	40	40	30	30
mean	43.3	73.0	113.1	148.1	171.6	193.3	215.2	230.9
s.d.	0.8	3.8	6.5	8.7	10.2	11.7	10.2	11.2

2. Reproduction data

Table 3. Delivery data

Pregnant rate	99.0%	
Delivering duration(days)		
mean	24.7	
s.d.	1.6	
distribution		
range	Number	Frequency
22	6	5.9%
23	17	16.8%
24	29	28.7%
25	17	16.8%
26	17	16.8%
27	12	11.9%
28	2	2.0%
29	0	0.0%
30(max.)	1	1.0%

Table 4. Nursing data

No. of newborns per litter		
Delivering duration(days)		
male	6.9	
female	6.5	
s.d.	1.6	
total	13.4	
distribution		
range	Number	Frequency
4(min.)	1	0.7%
5- 6	4	2.9%
7- 8	5	3.6%
9-10	12	8.7%
11-12	24	17.4%
13-14	38	27.5%
15-16	34	24.6%
17-18	16	11.6%
19-20	3	2.2%
21-23(max.)	1	0.7%
Rate of LDB	0.0%	
Rate of wening		
male	98.8%	
female	98.8%	
total	98.8%	

3. Laboratory data

Table 5. Haematology

Male	WBC	RBC	Hb	Ht	MCV	MCH	MCHC	PLT
	×100/ μl	*×10000/ μl*	*g/dl*	*%*	*fl*	*pg*	*g/dl*	*×10000/ μl*
N	10	10	10	10	10	10	10	10
mean	111.4	790.0	15.00	38.93	49.40	18.99	38.55	83.82
s.d.	24.8	31.7	0.51	1.59	1.35	0.51	0.41	6.20

Female	WBC	RBC	Hb	Ht	MCV	MCH	MCHC	PLT
	×100/ μl	*×10000/ μl*	*g/dl*	*%*	*fl*	*pg*	*g/dl*	*×10000/ μl*
N	10	10	10	10	10	10	10	10
mean	68.6	741.6	14.45	38.71	52.40	19.50	37.31	78.23
s.d.	22.0	53.1	1.03	2.67	2.12	0.72	0.53	8.87

Table 6. Blood Chemistry

Male	GOT	GPT	BUN	CRE	GLU	TP	ALB	TCHO
	(U/l)	(U/l)	(mg/dl)	(mg/dl)	(mg/dl)	(g/dl)	(g/dl)	(mg/dl)
N	10	10	10	10	10	10	10	10
mean	84.1	22.1	16.2	0.4	103.4	5.2	3.4	53.5
s.d.	8.4	2.4	2.8	0.1	6.8	0.3	0.2	7.0
	TG	TBIL	IP	Ca	CPK	ALP	LAP	
	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(U/l)	(ug/dl)	(U/l)	
N	54.7	0.3	8.8	8.9	479.6	452.5	53.9	
mean	16.8	0.1	0.4	0.4	131.0	72.1	6.4	
s.d.	8.4	2.4	2.8	0.1	6.8	0.3	0.2	

Female	GOT	GPT	BUN	CRE	GLU	TP	ALB	TCHO
	(U/l)	(U/l)	(mg/dl)	(mg/dl)	(mg/dl)	(g/dl)	(g/dl)	(mg/dl)
N	10	10	10	10	10	10	10	10
mean	82.8	17.7	16.7	0.5	106.8	5.5	3.8	66.2
s.d.	33.3	8.0	1.8	0.1	4.8	0.4	0.5	16.4
	TG	TBIL	IP	Ca	CPK	ALP	LAP	
	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(U/l)	(ug/dl)	(U/l)	
N	10	10	10	9	10	10	10	
mean	54.0	1.0	7.0	8.8	437.2	245.2	48.2	
s.d.	11.2	1.9	0.4	0.4	167.9	121.3	6.5	

Table 7. Organ Weight

Absolute											unit:mg	
Male	Brain	Thymus	Heart	Lungs	Liver	Spleen	Kidneys		Adrenal		Testes	
							Right	Left	Right	Left	Right	Left
N	10	10	10	10	10	10	10	10	10	10	10	10
mean	2023.6	344.3	1159.2	1221.0	9287.6	615.9	1279.0	1280.9	22.8	27.2	1589.9	1606.2
s.d.	73.4	64.2	70.2	66.9	591.2	69.4	93.4	85.1	5.1	3.1	103.7	98.6

Female	Brain	Thymus	Heart	Lungs	Liver	Spleen	Kidneys		Adrenal		Ovaries	
							Right	Left	Right	Left	Right	Left
N	10	10	10	10	10	10	10	10	10	10	10	10
mean	1942.4	386.9	829.7	1051.7	6165.2	528.6	822.0	809.8	25.7	27.7	37.1	35.0
s.d.	69.2	59.5	71.2	52.1	517.0	64.5	64.1	61.1	5.8	7.5	5.0	5.6

Relative											unit:mg/100gBW	
Male	Brain	Thymus	Heart	Lungs	Liver	Spleen	Kidneys		Adrenal		Testes	
							Right	Left	Right	Left	Right	Left
N	10	10	10	10	10	10	10	10	10	10	10	10
mean	547.5	93.2	313.6	330.2	2510.5	166.7	345.8	346.4	6.2	7.3	429.4	434.0
s.d.	28.9	17.8	21.9	18.9	150.4	20.1	25.3	24.3	1.4	0.8	18.8	21.2

Female	Brain	Thymus	Heart	Lungs	Liver	Spleen	Kidneys		Adrenal		Ovaries	
							Right	Left	Right	Left	Right	Left
N	10	10	10	10	10	10	10	10	10	10	10	10
mean	806.3	160.2	343.5	436.5	2554.9	219.0	340.9	336.0	10.6	11.5	15.4	14.5
s.d.	46.6	22.7	19.6	28.3	189.0	25.1	26.9	27.5	2.2	3.0	1.9	1.9

CHAPTER 2

General Toxicology

Response to Commercial Low Protein Diet in Crj:CD(SD)IGS Rats

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ABSTRACT. The effects of diet protein contents (about 18% and 25%) for physiological and pathological findings were studied using Crj:CD(SD)IGS rats for 13 or 26 weeks from 5 weeks of age. Body weight gain was reduced and alleviation of spontaneous lesions such as hepatic fatty change and myocardial degeneration in males fed on 18% protein diet were observed. In females fed 18% protein, the diet showed no growth retardation, but the estrous cycles of some animals changed slightly. Food intake was increased and changes in erythrocytic parameters were found in both sexes. However, the 18% protein diet did not effect the general condition of rats. —Key words: Crj:CD(SD)IGS, Rats, Low protein diet

CD (SD) IGS-1998: 19-30

INTRODUCTION

Diets for experimental animals are manufactured and distributed by many food suppliers in accordance with the nutrient requirements for animal food recommended by the National Research Council in the United States. Recently, it has been suggested that this nutritional standard has resulted in hypernutrition, which in turn has mainly adverse affects on the health condition of animals. On the other hand, it has been reported that on 18% protein diet causes no nutritional problem in rats, such as reduction and retardation of spontaneous lesions, and is effective in prolonging life span.

We used a 25% protein diet for 13- or 26-week general toxicity studies in rats. In this study, the 18% protein diet assessed in rats was expected to reduce lesions caused by hypernutrition. This study was also designed to collect general toxicity background data in Crj:CD(SD)IGS rats, a strain of SD rats. This strain was produced by the gold standard system, a new breeding system developed by Charles River Inc. to make diet uniform experimental animals with minimized genetic ramifications as well as internationalization of research and development of new drugs.

MATERIALS AND METHODS

Animals : Male and female Crj:CD(SD)IGS rats were purchased from Charles River Japan Inc. (Tsukuba, Japan) at 4 weeks of age and acclimated for one week. Healthy animals that had no abnormalities in appearance and showed normal weight gain were selected and used at 5 weeks of age. The animals were housed in an animal room maintained at 24±1°C and 50-65% relative humidity. The room air was ventilated 15 times per hr and a 12 hr/12hr light-dark cycle (lighting 7:00-19:00) was imposed. The animals were given a commercially available pelleted diet, such as the 25% protein diet, for an acclimation period and a different protein diet for the experimental period, with tap water ad libitum.

Group formation and dietary components : Animals at 5 weeks of age were randomly divided into 2 groups of 30 animals/sex. One group (group name:LP) received on 18% protein diet (CR-LPF, Oriental Yeast Co., Tokyo, Japan); the other group (group name:C) received on 25% protein diet (CE-2, CLEA Japan Inc.). Furthermore, each group had 10 animals/sex for the 13-week study and 20 animals/sex for the 26-week study, respectively. Dietary components were as follows:

Group	Control(C)	Low protein(LP)
Type	CE-2	CR-LPF
Gross energy (kcal/100g)	342	349
Moisture (%)	8.4	8.4
Crude protein (%)	25.6	17.7
Crude fat (%)	4.4	5.4
Crude fiber (%)	4.6	6.4
Crude ashes (%)	6.9	5.0
Nitrogen-free extract (%)	50.1	57.1

Observation and examination:

General signs : Throughout the experimental period, external appearance was observed daily. Body weight and food consumption over 24 hr were measured once weekly, with the first day and week of the experiment designated as day 1 and week 1. Protein intake was calculated as based on food consumption. Water intake was measured for the 26-week study at 1, 13 and 26 weeks during urinalysis.

Urinalysis : The following items were tested for the 26-week study at 1, 13 and 26 weeks using metabolic cages. Fresh urine was collected under the fasting and water supply. Urinary pH, color/turbidity, occult blood, protein, ketones, urobilinogen, bilirubin and sediment were measured using fresh urine. After fresh urine was collected, the diet was supplied. Urinary volume, specific gravity and electrolytes were measured for 24 hr urine collection. Urinary volume was measured by manual means as weight/specific gravity. Color/turbidity were examined by appearance. Specific gravity was measured by the weighing method (AE-200, Mettler). Urinary pH, occult blood, protein, ketones, urobilinogen and bilirubin were examined using test paper (Multistix, Sankyo Co. Ltd., Japan). Sediment was examined by the microscopic method. Electrolytes (sodium, potassium and chloride) were measured by an ion-selective electrode method using an automated analyzer (EA05, A&T).

Ophthalmology : Ophthalmological observations were performed at 1, 13 and 26 weeks for 10 animals in the 26-week

study. The cornea, iris and conjunctiva were examined using a portable ophthalmoscope (Mini-set, Heine) and ocular fundus examined using a fundus camera (Genesis, Kowa Co., Ltd.).

Estrous cycle : Estrous cycles were observed at 6-7, 10-11, 14-15, 18-19 , 22-23 and 25-26 weeks by the method of vaginal smear.

Necropsy : After 13 or 26 weeks, animals were fasted before sacrifice (18hr or more). The blood was drawn from the abdominal caval vein of each animal under pentobarbital anethesia immediately before sacrifice, using syringes as follows: sodium citrate treatment syringe for coagulation test, EDTA treatment syringe for other hematological observations, untreated syringe for serum protein fraction, and heparin treatment syringe for other blood biochemical observations. The dissected animals were examined macroscopically, and then the following organs were removed and weighed: brain, pituitary, heart, lungs, liver, spleen, kidneys, testes, prostate, ovaries, uterus, submaxillary glands (sub.m.gl.), thymus, adrenal glands and thyroids (with parathyroids).

Hematology : Red blood cell counts (RBC), white blood cell counts (WBC), hemoglobin (Hg), mean corpuscular volume (MCV) and platelet counts (PL) were examined using the Coulter Counter Model S-Plus IV (Coulter Electronics). Hematocrit (Ht), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Differential WBC (Wright-Giemsa method) and reticulocyte (Brecher method) counts were measured with a light microscope. Activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen were measured using an automated coagulation analyzer (CA-3000, Toa Medical Electronics).

Blood biochemistry : Glucose, total cholesterol, triglyceride, total protein, albumin, transaminases (GOT, GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca), inorganic phosphorus, total bilirubin and blood urea nitrogen (BUN) were measured using the COBAS-FARA centrifugal analyzer (Roche). A/G was calculated. Sodium (Na), potassium (K) and chloride (Cl) were measured by EA05. Serum protein was analyzed by electrophoresis (Helena). Lipid peroxide level was measured by the TBA method.

Histopathology : The heart, lungs, liver, pancreas, kidneys and testes were processed and stained with hematoxylin-eosin and subjected to histopathological examination.

Statistical analysis : The significance of differences in test data between C and LP were examined by Student's t-test with respect to body weight, food intake, urinary volume, specific gravity and electrolytes, hematological parameters, blood biochemical parameters and organ weights.

RESULTS

No abnormal clinical signs were observed throughout the experimental period. Body weight changes are shown in Table 1. In males of the LP group, there was lower body weight at and after 2 weeks compared to the C group, and this was significantly different from the C group at 2-7 weeks particularly. In females, body weights were no difference in both groups. Food consumption changes are shown in Table 2. There was continuously higher food consumption in both sexes of the LP group throughout the observation period except at 23rd week in males. In the LP group, 1-3 and 6 weeks in males and intermittently 1-25 weeks in females showed statistically significant differences in food consumption compared to the C group. Protein intake is shown in Table 3. Protein intake of each sex in the LP group at 1 week was about 89% in each sex of the C group. However, great differences were observed at and after 2 weeks in the LP group compared to the C group, such as 75% in females and 74% in males, but no sex difference was observed. Water intake showed no differences (data not shown). Estrous cycles are shown in Table 4. Female's incomplete return to estrous was observed in the early period of the study in the LP group compared to the C group. The type of change in the estrous cycle also increased.

The results of urinalysis are shown in Table 5. Urinary volume significantly decreased and specific gravity significantly increased in the LP group of females compared to the C group at 1 week. In males, specific gravity significantly decreased in the LP group at 13 weeks compared to the C group. Furthermore, the sodium excretory volume of both sexes at 1 week and 26 weeks in females, the potassium excretory volume of 1 and 26 weeks in females, the chloride excretory volume of both sexes at 1 week and at 13 and 26 weeks in females with significantly increased in the LP group compared to the C group. The number of animals shown with 9.0 or more of urinary pH tended to increase in the LP group of both sexes from 1 to 26 weeks (26 weeks data not shown).

Ophthalmological abnormality was not observed in any of the animals.

The results of hematological observations are shown in Table 6. Significant decreases of some parameters were found in males of the LP group compared to the C group as follows: RBC and Ht at 13 and 26 weeks and Hb, MCHC and reticulocyte at 26 weeks. Other parameters increased in the males of the LP group compared to the C group as follows: MCH and MCHC at 13 weeks, fibrinogen at 26 weeks. PT was shortened at 13 weeks. In females of the LP group, Ht and MCHC significantly increased at 13 and 26 weeks, MCV significantly decreased at 13 weeks, and reticulocyte and WBC significantly increased at 26 weeks compared to the C group.

The results of blood biochemical observations are shown in Table 7. In males, total protein at 13 weeks and triglyceride and sodium at 26 weeks had significantly decreased, α_2-globulin fraction at 13 weeks as well as total cholesterol and potassium at 26 weeks had significantly increased in the LP group compared to the C group. In females, total cholesterol at 13 and 26 weeks as well as A/G and α_2-globulin fraction, along with triglyceride, lipid peroxide, albumin and calcium at 26 weeks had significantly increased, and α_1-globrin fraction at 13 weeks and chloride at 26 weeks had significantly decreased in the LP group compared to the C group.

Absolute organ weights are shown in Table 8. In males, absolute prostate weight had significantly decreased at 13 weeks in the LP group compared to the C group. In females, absolute and relative weights of ovaries and adrenal glands had

significantly decreased at 26 weeks in the LP group compared to the C group. Necropsy findings are shown in Table 9. There were no marked lesions with the exception of a yellowish discoloration of the liver observed in 1 of 10 males in the C group at 13 weeks and 2 of 20 males in the same group at 26 weeks. Histopathological findings are shown in Tables 10-1 and 10-2. Accumulation of fat in the peripheral hepatocytes was observed in almost all animals. However, the LP group males at 26 weeks were inclined more slightly to fat than the C group. Eosinophilic bodies were observed in the proximal tubular epithelial cells of the kidney in males, but the frequency and severity were lower in the LP group compared to the C group at 13 weeks. Furthermore, fibrosis of the pancreatic islet and pigmentation around the islet, along with myocardial degeneration or fibrosis in the LP group males showed lighter lesions than those in the C group. Frequency or severity of other findings showed no differences between the two groups.

DISCUSSION

Protein is a nutrilite for the life-support of animals, and about 12% of protein content has been presented as the nutrient requirement in rats. At present, the standard content of protein in the commercial diet for experimental rats is about 25%. On the other hand, a low-protein diet has proved that spontaneous lesions and tumors are decreased and life span is prolonged [1]. Vital responses to these different protein contents in diets were investigated in Crj:CD(SD)IGS rats in this study. When an 18% protein diet was supplied for 13 or 26 weeks, there was no problem of life-support in rats and the incidence of spontaneous lesions in the liver, kidneys, heart and pancreas was reduced histopathologically. The same effects were previously reported in a long-term limited feeding study [2, 3]. In this study, it was suggested that the effect of a reduction of lesions would appear in the early stage of the study. However, growth retardation was observed remarkably on a growth phase in the LP group of males even though food consumption had increased. No statistical differences were shown at and after 8 weeks, while a lower body weight continued to the end of this study. In males, an 18% protein content diet could possibly effect normal growth, but this phenomenon is also thought to be an action taken to decrease excessive fat accumulation. Furthermore, prevention of oxidative stress, which reduces spontaneous lesions or shows little decrease in lipid peroxide level was observed in males. As for females, the estrous cycle varied in the LP group, but most animals completed estrus and no abnormalities were observed in the other parameters.

Given these results, it is concluded that the provision of a lower protein diet has a beneficial significance in decreasing interference factors for toxicity evaluation in long-term studies, but further investigation is needed to determine the optimal dietary protein level.

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Table 1. Weekly Mean Body Weight Changes in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Supply weeks	Female		Male	
	C	LP	C	LP
1st	116.6 ± 5.2(30)[a)]	116.5 ± 5.0(30)	136.2 ± 4.6(30)	137.2 ± 5.3(30)
2nd	150.9 ± 8.4(30)	147.9 ± 7.6(30)	200.8 ± 8.1(30)	195.0 ± 8.7(30)*
3rd	178.7 ± 11.4(30)	176.0 ± 10.7(30)	265.5 ± 10.5(30)	256.4 ± 11.3(30)**
4th	201.0 ± 14.7(30)	198.0 ± 12.9(30)	319.1 ± 14.2(30)	306.3 ± 14.9(30)**
5th	218.0 ± 17.6(30)	216.0 ± 15.4(30)	360.9 ± 17.9(30)	347.7 ± 17.5(30)**
6th	235.2 ± 20.3(30)	231.9 ± 17.4(30)	396.2 ± 20.0(30)	383.7 ± 20.6(30)*
7th	244.0 ± 20.6(30)	242.8 ± 16.5(30)	422.7 ± 21.8(30)	410.1 ± 24.5(30)*
8th	254.2 ± 21.9(30)	254.3 ± 17.7(30)	446.5 ± 25.4(30)	435.1 ± 27.1(30)
9th	264.3 ± 23.2(30)	263.5 ± 18.0(30)	466.1 ± 27.4(30)	453.7 ± 28.5(30)
10th	273.3 ± 24.2(30)	272.8 ± 19.4(30)	485.3 ± 29.2(30)	469.9 ± 30.8(30)
11th	278.1 ± 24.7(30)	276.8 ± 20.2(30)	501.5 ± 33.1(30)	487.1 ± 29.8(30)
12th	282.1 ± 24.9(30)	282.1 ± 20.4(30)	515.5 ± 34.8(30)	500.6 ± 32.0(30)
13th	289.1 ± 27.0(30)	289.0 ± 20.5(30)	527.3 ± 36.2(30)	512.0 ± 33.5(30)
14th	290.5 ± 27.1(20)	295.3 ± 25.3(20)	535.8 ± 38.2(20)	520.1 ± 40.5(20)
15th	292.4 ± 27.7(20)	297.1 ± 26.3(20)	546.5 ± 39.3(20)	531.5 ± 41.1(20)
16th	296.9 ± 28.1(20)	302.5 ± 26.9(20)	556.0 ± 42.4(20)	541.7 ± 43.3(20)
17th	300.6 ± 29.4(20)	307.1 ± 28.3(20)	564.8 ± 42.7(20)	547.3 ± 44.1(20)
18th	302.9 ± 30.3(20)	306.7 ± 28.1(20)	573.0 ± 44.6(20)	551.4 ± 44.9(20)
19th	305.8 ± 30.3(20)	310.6 ± 28.6(20)	580.1 ± 46.0(20)	556.1 ± 51.2(20)
20th	309.3 ± 30.5(20)	313.5 ± 28.2(20)	587.4 ± 48.5(20)	566.9 ± 47.6(20)
21st	314.6 ± 32.1(20)	319.0 ± 30.2(20)	595.7 ± 50.1(20)	576.7 ± 48.9(20)
22nd	318.6 ± 31.1(20)	323.0 ± 29.5(20)	603.2 ± 53.7(20)	581.7 ± 49.6(20)
23rd	319.3 ± 32.8(20)	325.0 ± 31.0(20)	609.5 ± 54.2(20)	587.4 ± 52.1(20)
24th	319.1 ± 32.6(20)	327.5 ± 31.4(20)	614.7 ± 55.4(20)	591.0 ± 51.8(20)
25th	323.3 ± 34.2(20)	329.7 ± 32.3(20)	620.6 ± 56.0(20)	598.7 ± 53.1(20)
26th	324.5 ± 35.1(20)	330.5 ± 32.8(20)	622.8 ± 56.3(20)	600.4 ± 53.3(20)

a): mean ± S.D. (N), grams.
*: Significantly different from the C group, $p<0.05$.
**: Significantly different from the C group, $p<0.01$.

Table 2. Weekly Changes of Daily Mean Food Consumption in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Supply weeks	Female		Male	
	C	LP	C	LP
1st	15.3 ± 1.7(30)[a)]	18.7 ± 1.0(30)**	18.1 ± 0.9(30)	22.2 ± 1.4(30)**
2nd	17.4 ± 2.2(30)	18.5 ± 1.7(30)*	24.6 ± 1.7(30)	25.5 ± 1.6(30)*
3rd	18.6 ± 2.4(30)	19.5 ± 1.6(30)	25.8 ± 2.0(30)	27.1 ± 2.7(30)*
4th	19.1 ± 1.9(30)	20.1 ± 2.7(30)	28.0 ± 2.3(30)	29.6 ± 2.3(30)
5th	19.0 ± 2.3(30)	19.8 ± 2.4(30)	27.8 ± 2.1(30)	28.8 ± 2.0(30)
6th	19.2 ± 2.5(30)	20.4 ± 2.3(30)	27.6 ± 2.1(30)	29.4 ± 2.6(30)**
7th	19.0 ± 2.5(30)	20.5 ± 1.8(30)*	28.6 ± 2.9(30)	29.5 ± 2.3(30)
8th	19.1 ± 2.3(30)	19.6 ± 2.9(30)	27.4 ± 2.4(30)	27.2 ± 2.5(30)
9th	17.9 ± 2.6(30)	19.4 ± 2.5(30)*	27.5 ± 2.7(30)	27.9 ± 2.1(30)
10th	17.6 ± 2.5(30)	18.5 ± 2.6(30)	26.1 ± 2.6(30)	27.0 ± 3.0(30)
11th	17.2 ± 2.4(30)	18.6 ± 1.9(30)*	27.0 ± 2.9(30)	28.1 ± 3.0(30)
12th	16.7 ± 2.6(30)	17.3 ± 2.8(30)	26.4 ± 2.4(30)	27.5 ± 2.3(30)
13th	16.2 ± 2.6(30)	17.5 ± 2.1(30)*	25.9 ± 3.0(30)	26.4 ± 2.5(30)
14th	16.3 ± 2.6(20)	18.3 ± 2.3(20)*	24.8 ± 2.7(20)	26.4 ± 2.4(20)
15th	16.3 ± 2.7(20)	18.7 ± 2.2(20)**	25.8 ± 2.6(20)	27.3 ± 2.4(20)
16th	16.3 ± 2.6(20)	17.3 ± 2.8(20)	25.7 ± 3.1(20)	25.7 ± 2.6(20)
17th	15.8 ± 2.7(20)	17.2 ± 2.2(20)	25.2 ± 2.8(20)	25.7 ± 2.9(20)
18th	16.2 ± 2.1(20)	17.9 ± 2.2(20)*	25.0 ± 2.7(20)	25.6 ± 1.8(20)
19th	16.0 ± 2.1(20)	17.5 ± 1.7(20)*	24.6 ± 3.2(20)	25.6 ± 3.5(20)
20th	16.1 ± 2.5(20)	17.1 ± 2.7(20)	24.5 ± 2.8(20)	26.0 ± 2.4(20)
21st	15.3 ± 2.5(20)	18.3 ± 2.9(20)**	25.7 ± 3.0(20)	26.3 ± 2.7(20)
22nd	15.8 ± 2.5(20)	17.9 ± 2.3(20)**	24.6 ± 2.2(20)	25.7 ± 2.8(20)
23rd	16.0 ± 2.6(20)	18.7 ± 2.2(20)**	25.0 ± 2.4(20)	24.0 ± 5.2(20)
24th	15.7 ± 3.2(20)	17.1 ± 2.6(20)	24.5 ± 2.7(20)	25.9 ± 2.8(20)
25th	15.8 ± 2.4(20)	17.9 ± 3.1(20)*	25.4 ± 2.7(20)	26.1 ± 3.0(20)
26th	16.3 ± 3.6(20)	18.3 ± 3.2(20)	25.2 ± 2.6(20)	25.4 ± 2.4(20)

a): mean ± S.D. (N), grams.
*: Significantly different from the C group, $p<0.05$.
**: Significantly different from the C group, $p<0.01$.

Table 3. Weekly Changes of Daily Protein Intake (g) in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Supply	Female			Male		
weeks	C	LP	LP/C(%)	C	LP	LP/C(%)
1st	3.8	3.4	89.5	4.5	4.0	88.9
2nd	4.4	3.3	75.0	6.2	4.6	74.2
3rd	4.7	3.5	74.5	6.5	4.9	75.4
4th	4.8	3.6	75.0	7.0	5.3	75.7
5th	4.8	3.6	75.0	7.0	5.2	74.3
6th	4.8	3.7	77.1	6.9	5.3	76.8
7th	4.8	3.7	77.1	7.2	5.3	73.6
8th	4.8	3.5	72.9	6.9	4.9	71.0
9th	4.5	3.5	77.8	6.9	5.0	72.5
10th	4.4	3.3	75.0	6.5	4.9	75.4
11th	4.3	3.3	76.7	6.8	5.1	75.0
12th	4.2	3.1	73.8	6.6	5.0	75.8
13th	4.1	3.2	78.0	6.5	4.8	73.8
14th	4.1	3.3	80.5	6.2	4.8	77.4
15th	4.1	3.4	82.9	6.5	4.9	75.4
16th	4.1	3.1	75.6	6.4	4.6	71.9
17th	4.0	3.1	77.5	6.3	4.6	73.0
18th	4.1	3.2	78.0	6.3	4.6	73.0
19th	4.0	3.2	80.0	6.2	4.6	74.2
20th	4.0	3.1	77.5	6.1	4.7	77.0
21st	3.8	3.3	86.8	6.4	4.7	73.4
22nd	4.0	3.2	80.0	6.2	4.6	74.2
23rd	4.0	3.4	85.0	6.3	4.3	68.3
24th	3.9	3.1	79.5	6.1	4.7	77.0
25th	4.0	3.2	80.0	6.4	4.7	73.4
26th	4.1	3.3	80.5	6.3	4.6	73.0

Table 4. Summary of Estrous Cycles in Rats Supplied with C or LP Diets for 26 Weeks

Supply weeks	6-7th		10-11th		14-15th		18-19th		22-23th		25-26th	
	C	LP	C	LP	C	LP	C	LP	C	LP	C	LP
Observed	20	20	20	20	20	20	20	20	20	20	20	20
Complete return to estrus												
Return type:												
Regularly 4-day	20	19	20	19	20	18	20	13	15	12	17	13
Regularly 5-day	0	1	0	1	0	0	0	0	0	1	0	2
Regularly 4 or 5-day	0	0	0	0	0	1	0	6	5	4	2	0
Irregularly:												
shorter than 4 days	0	0	0	0	0	0	0	0	0	0	0	2
longer than 5 days	0	0	0	0	0	1	0	0	0	1	0	0
Incomplete return to estrus												
Uncycler	0	0	0	0	0	0	0	1	0	1	0	1
Anestrus	0	0	0	0	0	0	0	0	0	1	1	2
Unchanged from the last preceding week			20	20	20	18	20	14	15	6	17	12
Changed from the last preceding week												
Complete to complete			0	0	0	2	0	5	5	11	2	6
Complete to incomplete			0	0	0	0	0	1	0	1	1	1
Incomplete to complete			0	0	0	0	0	0	0	0	0	0
Incomplete to incomplete			0	0	0	0	0	0	0	1	0	1

Table 5. Urinalysis in Rats Supplied with C or LP Diets for 1 or 13 Weeks

Week	1				13			
Sex	Female		Male		Female		Male	
Group	C	LP	C	LP	C	LP	C	LP
Number of animals observed	10	10	10	10	10	10	10	10
Volume[a)] (ml/24hr)	10.1	7.0	9.3	9.9	14.1	12.2	12.8	14.0
	3.9	1.5	1.7	3.2	5.0	6.4	2.8	3.0
Specific gravity[a)]	1.048	1.061*	1.051	1.047	1.044	1.049	1.058	1.050*
	0.016	0.010	0.013	0.013	0.018	0.014	0.009	0.007
Color:light yellow	8	9	8	7	7	8	7	8
yellow	2	1	2	3	3	2	3	2
Turbidity:negative	6	3	7	1	9	5	10	10
slight	4	7	3	9	1	5	0	0
pH: 6.0	0	0	1	0	0	0	0	0
6.5	1	0	2	0	2	1	1	0
7.0	2	0	3	0	3	1	2	0
7.5	1	0	1	0	1	1	2	1
8.0	1	0	0	0	0	0	0	0
8.5	1	0	1	0	0	2	2	1
9.0 and more	4	10	2	10	2	5	1	7
Protein: negative	10	8	2	7	10	6	0	0
trace	0	2	5	2	0	1	0	0
30 mg/dl	0	0	3	1	0	3	7	8
100 mg/dl	0	0	0	0	0	0	3	2
Glucose: negative	10	10	10	10	10	10	10	10
Ketone:negative	10	8	5	8	9	7	2	1
trace	0	2	5	2	1	3	3	2
slight	0	0	0	0	0	0	5	6
moderate	0	0	0	0	0	0	0	1
Bilirubin:negative	10	10	9	10	10	10	10	10
slight	0	0	1	0	0	0	0	0
Occult blood: negative	10	10	10	10	10	10	10	10
Urobilinogen: 0.1 EU/dl	9	10	9	10	10	10	10	10
1.0 EU/dl	1	0	1	0	0	0	0	0
Micro scopic examination of urinary sediment								
RBC: not observed	10	10	10	10	10	10	10	10
Crystal: not observed	0	7	3	4	0	6	0	6
a few	9	3	7	6	10	4	7	4
abundunt	1	0	0	0	0	0	3	0
Cast: not observed	10	10	10	10	10	10	10	10
WBC:not observed	10	10	9	10	10	10	10	10
1-9 / 3 visual fields	0	0	1	0	0	0	0	0
Epithelial cell:								
not observed	1	1	6	2	4	2	4	2
a few	9	9	4	8	6	8	6	8
Electrolytes[a)]:								
Na (mEq/24hr)	0.65	0.98	0.86	1.09	0.88	1.04	1.26	1.48
	0.26	0.26	0.09	0.26	0.33	0.33	0.45	0.52
K (mEq/24hr)	1.73	2.35*	2.19	2.62*	2.03	2.49	3.37	3.44
	0.36	0.43	0.26	0.59	0.86	0.68	0.89	0.83
Cl (mEq/24hr)	0.86	1.43**	1.14	1.59	0.96	1.51*	1.51	1.98
	0.29	0.37	0.16	0.45	0.44	0.54	0.46	0.75
Na/K	0.37	0.41**	0.39	0.42	0.45	0.41	0.37	0.42*
	0.10	0.03	0.09	0.06	0.10	0.03	0.09	0.06

a): upper=mean, lower= ± S.D.
*: Significantly different from the C group, $p<0.05$.
**: Significantly different from the C group, $p<0.01$.

Table 6. Hematological Findings in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Items (unit)	Female		Male	
	C	LP	C	LP
13 weeks				
General items				
Number of animals observed	10	10	10	10
RBC (x10⁴/mm³)	767 ± 28	753 ± 37	876 ± 42	812 ± 46**
Hb (g/dl)	14.9 ± 0.7	14.5 ± 0.7	15.7 ± 0.7	15.0 ± 0.8
Ht (%)	41.9 ± 2.1	39.8 ± 1.9*	45.0 ± 2.2	42.1 ± 2.1**
MCV (μm³)	54.6 ± 1.5	52.9 ± 1.0**	51.4 ± 2.2	51.9 ± 1.0
MCH (pg)	19.4 ± 0.5	19.3 ± 0.5	17.9 ± 0.7	18.5 ± 0.4*
MCHC (%)	35.5 ± 0.8	36.4 ± 0.5**	34.9 ± 0.4	35.7 ± 0.4**
Reticulocyte (%)	1.6 ± 0.6	1.6 ± 0.5	1.5 ± 0.8	2.0 ± 0.8
WBC (x100/mm³)	56 ± 28	60 ± 20	53 ± 12	66 ± 13
Band neutrophil (%)	NG	NG	0 ± 0	NG
Segmented (%)	14 ± 9	9 ± 7	16 ± 5	18 ± 9
Eosinophil (%)	2 ± 2	1 ± 2	1 ± 1	1 ± 1
Basophil (%)	0 ± 0	NG	NG	NG
Monocyte (%)	2 ± 1	2 ± 1	4 ± 2	3 ± 2
Lymphocyte (%)	82 ± 9	88 ± 7	79 ± 6	78 ± 11
PL (x10⁴/mm³)	103 ± 13	96 ± 9	108 ± 13	97 ± 7
Coagulation items				
Number of animals observed	5	5	5	5
APTT (sec)	23.2 ± 0.9	22.9 ± 1.4	27.5 ± 2.9	25.4 ± 1.2
PT (sec)	11.2 ± 0.3	10.8 ± 0.3	15.0 ± 1.9	12.5 ± 1.0*
Fibrinogen (mg/dl)	211.0 ± 96.0	168.6 ± 15.8	232.8 ± 16.2	224.8 ± 26.4
26 weeks				
General items				
Number of animals observed	20	20	20	20
RBC (x10⁴/mm³)	758 ± 46	735 ± 42	871 ± 48	832 ± 37**
Hb (g/dl)	14.7 ± 0.8	14.2 ± 0.6	15.6 ± 0.4	15.0 ± 0.6**
Ht (%)	41.6 ± 2.3	39.6 ± 1.8**	45.1 ± 1.6	42.7 ± 2.2**
MCV (μm³)	55.0 ± 1.8	54.0 ± 1.8	51.9 ± 2.3	51.4 ± 1.7
MCH (pg)	19.4 ± 0.6	19.4 ± 0.6	17.9 ± 0.9	18.0 ± 0.5
MCHC (%)	35.3 ± 0.6	36.0 ± 0.7**	34.5 ± 0.6	35.1 ± 0.8**
Reticulocyte (%)	1.6 ± 0.6	2.2 ± 0.6**	1.6 ± 0.7	2.3 ± 0.8**
WBC (x100/mm³)	37 ± 8	53 ± 15**	54 ± 13	60 ± 14
Band neutrophil (%)	0 ± 0	NG	0 ± 0	NG
Segmented (%)	18 ± 9	14 ± 4	23 ± 7	25 ± 11
Eosinophil (%)	2 ± 2	2 ± 1	2 ± 2	1 ± 1
Basophil (%)	0 ± 0	NG	0 ± 0	NG
Monocyte (%)	3 ± 2	2 ± 2*	6 ± 3	5 ± 3
Lymphocyte (%)	77 ± 9	83 ± 5*	70 ± 9	69 ± 13
PL (x10⁴/mm³)	99 ± 8	94 ± 9	102 ± 9	108 ± 14
Coagulation items				
Number of animals observed	5	5	5	5
APTT (sec)	21.6 ± 1.6	21.3 ± 0.9	28.1 ± 3.2	25.7 ± 2.1
PT (sec)	10.9 ± 0.3	10.8 ± 0.1	13.4 ± 0.7	12.7 ± 0.7
Fibrinogen (mg/dl)	167.4 ± 12.5	162.8 ± 11.9	220.2 ± 11.7	241.8 ± 13.6*

*: Significantly different from the C group, $p<0.05$.
**: Significantly different from the C group, $p<0.01$.

Table 7. Blood Chemical Observations in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Items (unit)	Female		Male	
	C	LP	C	LP
13 weeks				
General items				
Number of animals observed	10	10	10	10
Glucose (mg/dl)	143.8 ± 21.8	142.0 ± 22.0	134.6 ± 7.6	135.7 ± 18.9
Cholesterol (mg/dl)	50.9 ± 8.4	61.3 ± 8.7*	44.0 ± 17.2	55.2 ± 14.6
Triglyceride (mg/kg)	46.0 ± 13.7	47.6 ± 14.4	50.3 ± 12.7	45.8 ± 10.7
Lipid peroxide (nm/ml)	7.9 ± 1.2	8.6 ± 1.2	7.9 ± 2.4	7.2 ± 1.3
Total protein (g/dl)	6.3 ± 0.2	6.1 ± 0.3	5.8 ± 0.5	5.4 ± 0.2*
Albumin (g/dl)	3.4 ± 0.3	3.5 ± 0.2	3.0 ± 0.2	2.9 ± 0.2
GOT (IU/l)	54 ± 7	55 ± 8	63 ± 16	57 ± 6
GPT (IU/l)	25 ± 4	24 ± 5	29 ± 4	26 ± 3
ALP (IU/l)	82 ± 21	79 ± 20	184 ± 37	169 ± 36
LDH (IU/l)	131 ± 32	155 ± 50	159 ± 41	138 ± 31
A/G	1.19 ± 0.18	1.35 ± 0.11*	1.09 ± 0.10	1.10 ± 0.10
Na (mEq/l)	144.0 ± 1.1	143.4 ± 0.6	145.2 ± 1.0	144.7 ± 0.7
K (mEq/l)	4.13 ± 0.39	4.30 ± 0.19	4.04 ± 0.18	4.11 ± 0.22
Cl (mEq/l)	108.7 ± 1.7	107.5 ± 1.8	108.6 ± 1.8	108.7 ± 1.2
Ca (mg/dl)	9.1 ± 0.2	9.2 ± 0.2	8.8 ± 0.4	8.7 ± 0.2
Inorganic phosporus (mg/dl)	4.4 ± 0.6	4.8 ± 0.8	5.4 ± 0.4	5.5 ± 0.3
Total bilirubin (mg/dl)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
BUN (mg/dl)	19.6 ± 2.8	17.6 ± 1.6	17.1 ± 1.4	18.0 ± 2.6
Serum protein fraction items				
Number of animals observed	5	5	5	5
Albumin (%)	54.2 ± 1.1	53.9 ± 1.2	46.3 ± 2.1	47.5 ± 0.9
α_1-globulin (%)	18.8 ± 0.8	16.9 ± 0.7**	25.0 ± 1.7	23.3 ± 1.1
α_2-globulin (%)	6.4 ± 0.5	7.5 ± 0.4**	8.0 ± 0.7	8.9 ± 0.5*
β-globulin (%)	14.5 ± 0.6	15.4 ± 0.8	16.3 ± 0.7	16.7 ± 0.6
γ-globulin (%)	6.0 ± 0.6	6.4 ± 0.7	4.5 ± 0.6	3.6 ± 1.2
26 weeks				
General items				
Number of animals observed	20	20	20	20
Glucose (mg/dl)	133.9 ± 13.6	134.6 ± 13.1	137.1 ± 14.3	140.6 ± 16.1
Cholesterol (mg/dl)	55.1 ± 9.9	83.3 ± 14.0**	51.0 ± 15.6	61.9 ± 14.4*
Triglyceride (mg/kg)	50.8 ± 12.8	64.2 ± 20.8*	69.2 ± 26.4	50.0 ± 9.8**
Lipid peroxide (nm/ml)	13.8 ± 2.3	16.0 ± 2.9**	17.4 ± 4.8	15.4 ± 2.6
Total protein (g/dl)	6.3 ± 0.4	6.6 ± 0.5	5.9 ± 0.3	5.8 ± 0.4
Albumin (g/dl)	3.6 ± 0.3	3.9 ± 0.4*	2.9 ± 0.2	2.9 ± 0.2
GOT (IU/l)	88 ± 73	147 ± 157	61 ± 19	69 ± 30
GPT (IU/l)	40 ± 37	59 ± 65	32 ± 14	32 ± 16
ALP (IU/l)	51 ± 10	45 ± 12	128 ± 24	130 ± 22
LDH (IU/l)	174 ± 81	205 ± 144	186 ± 122	172 ± 92
A/G	1.33 ± 0.18	1.41 ± 0.14	1.01 ± 0.12	0.99 ± 0.12
Na (mEq/l)	144.3 ± 1.1	144.0 ± 0.9	145.9 ± 0.8	145.3 ± 0.7*
K (mEq/l)	4.01 ± 0.60	3.99 ± 0.40	4.02 ± 0.16	4.29 ± 0.21**
Cl (mEq/l)	108.1 ± 1.3	107.3 ± 1.1*	108.5 ± 1.5	108.7 ± 1.1
Ca (mg/dl)	8.9 ± 0.3	9.2 ± 0.3**	8.8 ± 0.2	8.7 ± 0.3
Inorganic phosphorus (mg/dl)	3.6 ± 0.7	3.8 ± 0.7	4.7 ± 0.4	4.6 ± 0.5
Total bilirubin (mg/dl)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
BUN (mg/dl)	16.3 ± 2.0	16.2 ± 3.0	14.5 ± 2.5	14.9 ± 2.0
Serum protein fraction items				
Number of animals observed	5	5	5	5
Albumin (%)	53.4 ± 2.9	53.2 ± 5.5	46.0 ± 1.7	48.6 ± 2.4
α_1-globulin (%)	16.4 ± 0.9	15.4 ± 1.9	22.3 ± 2.4	22.0 ± 1.3
α_2-globulin (%)	6.6 ± 0.7	7.7 ± 1.2	8.9 ± 0.9	7.8 ± 2.1
β-globulin (%)	15.9 ± 1.3	16.8 ± 4.0	17.6 ± 0.3	16.9 ± 2.0
γ-globulin (%)	7.7 ± 1.7	6.9 ± 1.4	5.2 ± 0.9	4.8 ± 0.5

*:Significantly different from the C group, $p<0.05$.
**:Significantly different from the C group, $p<0.01$.

Table 8. Absolute Organ Weights in Rats Supplied with C or LP Diets for 13 or 26 Weeks

Items (unit)		Female		Male	
		C	LP	C	LP
13 weeks					
Number of animals observed		10	10	10	10
Body weight	(g)	278.0 ± 27.6	266.5 ± 11.5	507.3 ± 40.3	492.4 ± 26.3
Brain	(g)	1.828 ± 0.071	1.837 ± 0.086	1.969 ± 0.101	2.002 ± 0.057
Thymus	(g)	0.282 ± 0.074	0.249 ± 0.058	0.334 ± 0.079	0.301 ± 0.080
Heart	(g)	0.843 ± 0.075	0.812 ± 0.044	1.394 ± 0.171	1.309 ± 0.096
Lung	(g)	0.936 ± 0.073	0.952 ± 0.050	1.301 ± 0.092	1.277 ± 0.067
Liver	(g)	6.57 ± 0.53	6.19 ± 0.49	12.91 ± 2.04	12.12 ± 1.55
Kidney	(g)	1.682 ± 0.154	1.562 ± 0.121	2.997 ± 0.190	2.876 ± 0.226
Spleen	(g)	0.527 ± 0.093	0.457 ± 0.054	0.738 ± 0.139	0.765 ± 0.118
Testis	(g)			3.116 ± 0.211	3.088 ± 0.229
Uterus	(g)	0.500 ± 0.127	0.474 ± 0.186		
Prostate	(mg)			0.705 ± 0.113	0.580 ± 0.104*
Pituitary	(mg)	14.0 ± 2.4	13.3 ± 1.7	12.1 ± 0.9	12.9 ± 1.0
Thyroid	(mg)	14.4 ± 3.7	12.0 ± 1.6	17.6 ± 3.3	16.2 ± 2.6
Adrenal	(mg)	64.4 ± 10.4	57.2 ± 8.9	48.8 ± 6.0	49.2 ± 7.0
Ovary	(mg)	78.1 ± 8.2	72.4 ± 9.5		
Sub.m.gl.	(g)	0.400 ± 0.049	0.415 ± 0.050	0.669 ± 0.081	0.666 ± 0.067
26 weeks					
Number of animals observed		20	20	20	20
Body weight	(g)	303.4 ± 32.8	308.7 ± 32.2	597.8 ± 56.1	573.2 ± 54.4
Brain	(g)	1.873 ± 0.101	1.843 ± 0.086	2.049 ± 0.064	2.051 ± 0.088
Thymus	(g)	0.148 ± 0.051	0.152 ± 0.040	0.160 ± 0.039	0.148 ± 0.038
Heart	(g)	0.897 ± 0.091	0.882 ± 0.096	1.482 ± 0.152	1.454 ± 0.129
Lung	(g)	0.989 ± 0.095	0.997 ± 0.064	1.377 ± 0.070	1.377 ± 0.091
Liver	(g)	6.83 ± 0.75	7.12 ± 0.73	14.12 ± 1.78	13.16 ± 1.21
Kidney	(g)	1.788 ± 0.187	1.738 ± 0.165	3.182 ± 0.264	3.046 ± 0.233
Spleen	(g)	0.508 ± 0.093	0.471 ± 0.041	0.842 ± 0.140	0.872 ± 0.176
Testis	(g)			3.367 ± 0.272	3.414 ± 0.247
Uterus	(g)	0.564 ± 0.115	0.614 ± 0.180		
Prostate	(mg)			0.671 ± 0.160	0.622 ± 0.161
Pituitary	(mg)	15.6 ± 3.3	16.3 ± 3.8	12.4 ± 2.3	12.7 ± 1.6
Thyroid	(mg)	14.6 ± 4.3	14.9 ± 2.9	21.4 ± 3.9	20.6 ± 4.7
Adrenal	(mg)	65.9 ± 10.0	58.6 ± 9.0*	51.9 ± 6.0	49.3 ± 7.3
Ovary	(mg)	77.3 ± 14.1	65.9 ± 12.0**		
Sub.m.gl.	(g)	0.433 ± 0.061	0.435 ± 0.054	0.668 ± 0.041	0.688 ± 0.069

*:Significantly different from the C group, $p<0.05$.
**:Significantly different from the C group, $p<0.01$.

Table 9. Summary of Macroscopic Findings in Rats Supplied with C or LP Diets for 13 or 26 weeks

	Female								Male							
	13 weeks				26 weeks				13 weeks				26 weeks			
	C (n=10)		LP (n=10)		C (n=20)		LP (n=20)		C (n=10)		LP (n=10)		C (n=20)		LP (n=20)	
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Abdominal cavity																
Mass	10	0	10	0	19	1	20	0	9	1	10	0	20	0	18	2
Mandibular lymph node																
Spot, dark	10	0	9	1	20	0	20	0	10	0	10	0	20	0	20	0
Enlargement	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Pale	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Thyroid gland																
Small size	9	1	9	1	20	0	20	0	10	0	10	0	20	0	20	0
Enlargement	9	1	10	0	19	1	20	0	9	1	10	0	20	0	18	2
Hard	10	0	10	0	20	0	20	0	10	0	10	0	19	1	20	0
Thymus																
Spot, dark	9	1	8	2	20	0	20	0	10	0	10	0	20	0	20	0
Small size	9	1	10	0	13	7	12	8	10	0	9	1	10	10	10	10
Lung																
Dark	10	0	10	0	20	0	20	0	10	0	10	0	20	0	19	1
Spot, dark	10	0	10	0	20	0	20	0	10	0	9	1	19	1	19	1
Spot, pale	10	0	10	0	15	5	17	3	10	0	10	0	16	4	11	9
Spleen																
Accessory spleen	10	0	10	0	20	0	20	0	9	1	10	0	20	0	20	0
Enlargement	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Accentuated follicle	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Cyst	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Ovary																
Spot, dark	10	0	10	0	20	0	19	1	-	-	-	-	-	-	-	-
Enlargement	10	0	10	0	19	1	20	0	-	-	-	-	-	-	-	-
Uterus																
Swelling	10	0	10	0	19	1	20	0	-	-	-	-	-	-	-	-
Seminal vesicle																
Enlargement	-	-	-	-	-	-	-	-	10	0	10	0	20	0	19	1
Pituitary gland																
Cyst	10	0	10	0	20	0	20	0	10	0	10	0	19	1	20	0
Kidney																
Cyst	10	0	10	0	20	0	19	1	10	0	10	0	20	0	20	0
Liver																
Accetuated lobular pattern	10	0	10	0	20	0	20	0	10	0	9	1	18	2	20	0
Spot, pale	10	0	9	1	18	2	19	1	8	2	9	1	20	0	17	3
Spot, dark	9	1	10	0	20	0	19	1	10	0	10	0	19	1	20	0
Area, pale	9	1	10	0	20	0	20	0	10	0	10	0	20	0	19	1
Yellowish	10	0	10	0	20	0	20	0	9	1	10	0	18	2	20	0
Hair																
Alopecia	9	1	10	0	20	0	20	0	10	0	9	1	20	0	20	0
Skin																
Swelling, pinna	9	1	10	0	20	0	20	0	10	0	10	0	20	0	20	0
Stomach																
Spot, pale	10	0	10	0	19	1	19	1	9	1	10	0	19	1	20	0
Elevated area	10	0	10	0	19	1	20	0	9	1	10	0	20	0	20	0
White cloudy mucosa	10	0	10	0	19	1	20	0	10	0	10	0	19	1	20	0

-: Negative; +: Positive

Table 10-1. Summary of Histopathological Findings in Rats Supplied with C or LP Diets for 13 or 26 weeks (female)

	13 weeks										26 weeks									
	C (n=10)					LP (n=10)					C (n=20)					LP (n=20)				
	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Liver																				
Microgranuloma	0	10	0	0	0	0	10	0	0	0	0	16	4	0	0	0	16	4	0	0
Fatty change, periportal	0	2	7	1	0	0	2	8	0	0	0	4	12	4	0	0	5	13	2	0
Fatty change, focal	10	0	0	0	0	8	1	1	0	0	15	4	1	0	0	18	2	0	0	0
Hemorrhage	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Necrosis, focal	9	1	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Focus of cellular alteration, clear cell	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Kidney																				
Basophilic tubule, cortex	8	2	0	0	0	6	4	0	0	0	14	5	1	0	0	17	3	0	0	0
Mineralization, cortico-medullary junction	9	1	0	0	0	10	0	0	0	0	16	4	0	0	0	19	1	0	0	0
Mineralization, papilla	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	0	0	1	0
Deposit, pigment, brown proximal tubule	10	0	0	0	0	10	0	0	0	0	19	1	0	0	0	20	0	0	0	0
Cellular infiltration, lymphocyte	10	0	0	0	0	9	1	0	0	0	17	3	0	0	0	18	2	0	0	0
Cast	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Cyst	10	0	0	0	0	10	0	0	0	0	18	1	1	0	0	19	0	1	0	0
Pyelitis	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Fibrosis	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Heart																				
Myocardial degeneration	10	0	0	0	0	9	1	0	0	0	20	0	0	0	0	18	2	0	0	0
Myocardial fibrosis	9	1	0	0	0	10	0	0	0	0	18	2	0	0	0	16	4	0	0	0
Hemorrhage	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Lung																				
Cellular infiltration, lymphocyte	10	0	0	0	0	9	1	0	0	0	19	1	0	0	0	19	1	0	0	0
Mineralization, artery	5	5	0	0	0	6	4	0	0	0	13	7	0	0	0	15	5	0	0	0
Osseous metaplasia	10	0	0	0	0	10	0	0	0	0	17	1	2	0	0	19	0	1	0	0
Accumulation, foam cell	8	2	0	0	0	9	1	0	0	0	10	8	2	0	0	14	4	2	0	0
Inflammation, focal	10	0	0	0	0	10	0	0	0	0	19	1	0	0	0	20	0	0	0	0
Pancreas																				
Proliferation, ductule	10	0	0	0	0	8	2	0	0	0	18	2	0	0	0	17	3	0	0	0
Microglanuloma	10	0	0	0	0	9	1	0	0	0	20	0	0	0	0	20	0	0	0	0

-, Negative; ± , Very slight; +, Slight; ++, Moderate; +++, Severe

Table 10-2. Summary of Histopathological Findings in Rats Supplied with C or LP Diets for 13 or 26 weeks (male)

	13 weeks										26 weeks									
	C (n=10)					LP (n=10)					C (n=20)					LP (n=20)				
	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Liver																				
Microgranuloma	0	10	0	0	0	0	10	0	0	0	0	19	1	0	0	0	18	2	0	0
Fatty change, periportal	0	2	7	1	0	0	2	7	1	0	0	2	8	10	0	0	6	11	3	0
Fatty change, focal	9	1	0	0	0	8	2	0	0	0	15	0	5	0	0	19	0	1	0	0
Necrosis, focal	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	16	4	0	0	0
Kidney																				
Basophilic tubule, cortex	0	10	0	0	0	1	9	0	0	0	1	16	3	0	0	0	19	1	0	0
Basophylic tubule, medulla	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Mineralization, cortico-medullary junction	10	0	0	0	0	8	2	0	0	0	12	8	0	0	0	15	5	0	0	0
Eosinophilic body	2	4	1	3	0	5	4	0	1	0	4	11	3	2	0	13	7	0	0	0
Deposit, pigment, brown proximal tubule	10	0	0	0	0	10	0	0	0	0	0	20	0	0	0	2	17	1	0	0
Cellular infiltration, lymphocyte	7	3	0	0	0	8	2	0	0	0	14	6	0	0	0	12	8	0	0	0
Cast	9	0	1	0	0	10	0	0	0	0	14	4	2	0	0	17	3	0	0	0
Cyst	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	18	0	2	0	0
Pyelitis	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Fibrosis	10	0	0	0	0	10	0	0	0	0	20	0	0	0	0	19	1	0	0	0
Heart																				
Myocardial degeneration	3	6	1	0	0	6	4	0	0	0	9	10	1	0	0	9	9	2	0	0
Myocardial fibrosis	6	4	0	0	0	8	2	0	0	0	8	10	2	0	0	13	6	1	0	0
Lung																				
Cellular infiltration, lymphocyte	7	3	0	0	0	9	1	0	0	0	15	5	0	0	0	18	2	0	0	0
Mineralization, artery	5	5	0	0	0	5	5	0	0	0	10	10	0	0	0	6	14	0	0	0
Osseous metaplasia	8	0	2	0	0	9	0	1	0	0	18	0	2	0	0	18	0	2	0	0
Accumulation, foam cell	8	2	0	0	0	5	5	0	0	0	11	9	0	0	0	9	10	1	0	0
Pancreas																				
Deposit, pigment, brown	9	1	0	0	0	10	0	0	0	0	4	14	2	0	0	7	13	0	0	0
Fibrosis, pancreatic islet	8	2	0	0	0	10	0	0	0	0	9	1	10	0	0	13	5	2	0	0
Proliferation, ductule	7	3	0	0	0	10	0	0	0	0	14	6	0	0	0	11	8	1	0	0
Microglanuloma	10	0	0	0	0	10	0	0	0	0	18	0	2	0	0	18	0	2	0	0
Testis																				
Atrophy, seminiferous tubule, focal	10	0	0	0	0	10	0	0	0	0	18	2	0	0	0	17	2	0	1	0

-, Negative; ±, Very slight; +, Slight; ++, Moderate; +++, Severe

Background Data of General Toxicological Parameters in Crj:CD(SD)IGS rats at Three Age Levels

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ABSTRACT. The purpose of this study was to accumulate data of the general toxicological parameters in Crj:CD(SD)IGS rats and to compare the data collected from the animals at 3 different ages. There were age related differences in the following items: pH, protein, and volume in urinalysis, WBC, lymphocytes and neutrophils in hematology, AL-P activity, inorganic phosphate, total protein, TIBC, albumin and iron in blood chemistry, and absolute organ weights except the thymus, adrenals and ovary weights. —Key words: Crj:CD(SD)IGS rats, General toxicological parameter.

CD (SD) IGS-1998: 31-38

INTRODUCTION

Crj:CD(SD)IGS rats were produced by the gold standard system, which is 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 collected data on the parameters commonly evaluated in general toxicity tests by using these animals at 3 different ages levels, and comparing the differences with aging.

MATERIALS AND METHODS

Animals and rearing conditions: One hundred and eighty male and 180 female Crj:CD(SD)IGS strain rats aged 4 weeks were purchased on 3 separate occasions from Charles River Japan Inc.(Hino farm) on May 8, June 5 and July 17 ,1997. After more than 1 week of acclimation including quarantine, the animals were used in groups of 60/sex at the age of 6 weeks. The animals were stratified by body weight and allocated by a computerized randomization process on the day of the start of examination. The animals were given water for injection (Otsuka Pharmaceutical Factory Inc.) orally at 5mL/kg once a day for 4,13 or 26weeks to mimic conditions similar to those in repeated dose oral toxicity studies. The individual dose volumes were calculated from the most recent body weight.

Room temperature and relative humidity of the room were respectively 22.0-23.8°C and 44-69% and ventilation was set at 14-20 changes per hour. The room was lighted automatically from 7:00 a.m. to 7:00 p.m. The animals were allowed free access to standard laboratory food (CRF-1,autoclaved, Oriental Yeast Co., Tokyo Japan) and filtered tap water containing 2 ± 1 ppm chlorine adjusted with sodium hypochlorite from an automatic dispenser. The cages were made of stainless steel, and the animals were housed 2 to a cage. Polyvinyl resin-coated roll paper was spread on the trays beneath the cages and was changed daily except on holidays.

Observation items: General signs, body weight, food consumption, urinalysis, hematology, blood chemistry, gross pathology, and organ weight were investigated.

1) General signs: The animals were observed once a day during the experimental period.
2) Body weight: The body weight of each animal was recorded once a week during the experimental period and group mean body weights and their standard deviation were calculated.
3) Food consumption: The quantities of food offered and left over were recorded for each cage once a week during the experimental period, and the mean food consumption per day per rat was calculated from the total food consumed on each recording day.
4) Urinalysis: Fresh urine was collected from all the animals at ages of 5, 9, 18 and 31 weeks. Each animal was housed in a stainless steel metabolism cage without food or water, and pH, glucose, protein, occult blood, ketone body, urobilinogen, and bilirubin were analyzed with a Clinitek 200 + (Bayer-Sankyo Co., Ltd.) using N-Multistix SG (Bayer-Sankyo Co., Ltd.). Urinary sediments were examined microscopically, and urine color was observed macroscopically. Additionally, urine was collected for about 16 hours (from 5 p.m. to 9 a.m. next day) under free access to food and water, and urine volume was determined with a graduated test tube. Specific gravity was determined with a Clinitek Atlas (Bayer-Sankyo Co., Ltd.) and urine electrolytes with an automated electrolytes analyzer (Model 710, Hitachi, Co., Ltd.).
5) Ophthalmology: A mydriatic (Mydrin P, Santen Pharmaceutical Co., Ltd.) was instilled and examined with a binocular indirect ophthalmoscope (IO- α, Neitz) for the pupil, conjunctiva, cornea, iris, lens, vitreous body and fundus oculi. The fundic oculus was photographed with a fundus camera (K9L29, Kowa).
6) Blood collection: The animals were deprived of food overnight (about 16 hours)on the day before blood collection, and blood was collected from the abdominal aorta under ether anesthesia. As anticoagulants, sodium citrate was used for the parameters of blood coagulation test and EDTA-2K for the other parameters. After a portion of the collected blood was centrifuged, the serum was analyzed for blood chemistry.

7) Hematology: Hematocrit (HCT), hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), platelet count (Platelets), MCV, MCHC and MCH were analyzed using an automated hematology analyzer (Technicon H*1 System, Bayer-Sankyo Co., Ltd.). Differential white blood cell counts were measured by pattern identification method using OMRON MICROX HEG-120NA (Omuron Tateisi Electronics Co., Ltd.). Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen were analyzed using CA-5000 (Toa Medical Electronics Co., Ltd.). Reticulocytes were measured by the Brecher method.
8) Blood chemistry: Total protein (T.P.), albumin (ALB), total bilirubin (T.BIL) , total cholesterol (CHOL), triglyceride (TRIG) , alkaline phosphatase (AL-P), transaminases (GOT,GPT), lactate dehydrogenase (LDH), γ glutamyl transpeptidase (GGTP), glucose (GLU), blood urea nitrogen (BUN), uric acid (U.A) inorganic phosphorus (I.P), calcium (Ca), sodium (Na), potassium (K), chloride (Cl) iron (Fe), unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), creatine phosphotransferase (CPK), phospholipids (PL),and magnesium (Mg) were analyzed by an automated blood chemistry analyzer (Model 7150, Hitachi, Ltd.). ALB/GLB ratio was calculated from total protein and albumin, and BUN/CREA ratio from blood urea nitrogen and creatinine.
9) Gross pathology and organ weight analysis: After blood collection, the animals were killed by bleeding and necropsied immediately, and the heart, spleen, lungs, liver, kidneys, brain, pituitary, thymus, adrenals, thyroids, testes, ovaries, prostate, uterus, seminal vesicle, salivary glands and cecum were weighed. Relative organ weights were also expressed as percentage of body weight on the day of necropsy.

Statistical analysis: The group means and standard deviations were calculated for body weight, food consumption, urine volume, urinary specific gravity, electrolytes, hematology, blood chemistry, absolute and relative organ weights values.

RESULTS

General signs: Hair loss and sparse fur development was observed in 3 males and 6 females from 12 weeks onward, swelling of the auricle in 4 males from 12 weeks onward, subcutaneous mass in 1 male and 1 female from 18 weeks onward, and paleness of the conjunctiva in 1 male from 24 weeks onward.

Body weight (Table 1.) : Body weight gains of the males and females were 281and 163g, respectively, at 10 weeks; 409 and 225g, respectively, at 19 weeks; and 509 and 263g, respectively, at 32 weeks.

Food consumption (Table 2.) : Food consumption was 24-26g in the males and 17-20g in the females, and there were no changes throughout the experimental period.

Urinalysis (Table 3-1 to -2.): Urinary pH was 9.0 in almost

Table 1. Weekly mean body weight changes

Age (weeks)	Male		Female	
3	74.0 ± 2.6	(180)	68.0 ± 2.6	(180)
4	121.0 ± 4.8	(180)	109.0 ± 4.9	(180)
6	198.0 ± 11.0	(180)	158.0 ± 8.0	(180)
7	245.0 ± 15.5	(180)	181.0 ± 10.6	(180)
8	289.0 ± 20.1	(180)	200.0 ± 14.3	(180)
9	327.0 ± 24.2	(180)	218.0 ± 15.4	(180)
10	355.0 ± 26.1	(180)	231.0 ± 16.9	(180)
11	383.0 ± 29.9	(120)	245.0 ± 18.5	(120)
12	405.0 ± 32.8	(120)	254.0 ± 20.3	(120)
13	426.0 ± 36.0	(120)	263.0 ± 20.4	(120)
14	444.0 ± 38.3	(120)	270.0 ± 21.2	(120)
15	459.0 ± 38.9	(120)	276.0 ± 22.6	(120)
16	472.0 ± 41.0	(120)	283.0 ± 23.8	(120)
17	483.0 ± 42.5	(120)	288.0 ± 23.2	(120)
18	494.0 ± 43.7	(120)	293.0 ± 24.1	(120)
19	494.0 ± 45.7	(60)	295.0 ± 23.4	(60)
20	516.0 ± 45.1	(60)	302.0 ± 26.5	(60)
22	531.0 ± 47.7	(60)	309.0 ± 26.0	(60)
24	547.0 ± 48.8	(60)	316.0 ± 27.9	(60)
26	558.0 ± 51.2	(60)	320.0 ± 29.6	(60)
28	570.0 ± 53.1	(60)	327.0 ± 30.7	(60)
30	580.0 ± 56.9	(60)	329.0 ± 29.3	(60)
32	583.0 ± 60.5	(60)	331.0 ± 30.2	(60)

( ) : No. of animals
Values are expressed as Mean ± S. D.

Table 2. Weekly changes of daily mean food consumption

Age (weeks)	Male		Female	
6	24 ± 1.6	(90)	17 ± 1.0	(90)
7	25 ± 1.6	(90)	18 ± 1.3	(90)
8	25 ± 1.7	(90)	19 ± 1.3	(90)
9	25 ± 1.7	(90)	19 ± 1.4	(90)
10	25 ± 1.7	(60)	19 ± 1.3	(60)
11	25 ± 1.9	(60)	19 ± 1.3	(60)
12	26 ± 2.0	(60)	19 ± 1.4	(60)
13	26 ± 1.9	(60)	19 ± 1.5	(60)
14	26 ± 1.6	(60)	19 ± 1.5	(60)
15	25 ± 1.7	(60)	19 ± 1.4	(60)
16	26 ± 1.8	(60)	19 ± 1.6	(60)
17	25 ± 1.6	(60)	19 ± 1.5	(60)
18	24 ± 2.0	(60)	18 ± 1.4	(60)
19	25 ± 1.9	(30)	18 ± 1.4	(30)
20	25 ± 1.7	(30)	20 ± 1.6	(30)
21	25 ± 1.4	(30)	19 ± 1.5	(30)
22	25 ± 1.5	(30)	19 ± 1.4	(30)
23	25 ± 1.5	(30)	19 ± 1.4	(30)
24	25 ± 1.5	(30)	19 ± 1.6	(30)
25	24 ± 1.3	(30)	18 ± 1.6	(30)
26	25 ± 1.5	(30)	19 ± 1.6	(30)
27	25 ± 1.6	(30)	18 ± 1.8	(30)
28	25 ± 2.5	(30)	18 ± 2.4	(30)
29	25 ± 2.0	(30)	18 ± 1.7	(30)
30	25 ± 1.5	(30)	19 ± 1.5	(30)
31	22 ± 1.8	(30)	17 ± 1.6	(30)

( ) : No. of animals
Values are expressed as Mean ± S. D.

Table 3-1. Urinalysis

	Male				Female			
	Age in weeks				Age in weeks			
	5	9	18	31	5	9	18	31
	(60)	(60)	(55)	(55)	(54)	(55)	(56)	(57)
pH								
6.0	0	0	0	2	2	1	4	8
6.5	1	0	0	0	0	2	4	2
7.0	0	0	1	2	3	2	5	6
7.5	1	0	1	5	2	0	4	9
8.0	0	2	3	9	3	3	3	6
8.5	3	5	10	11	3	3	11	8
9.0	55	53	40	26	41	44	25	18
Protein								
–	17	0	5	2	24	5	23	14
±	10	2	6	5	9	12	6	13
+	28	34	24	35	15	20	14	24
++	5	22	16	13	6	18	9	4
+++	0	2	4	0	0	0	4	2
Glucose								
–	60	60	55	55	54	55	56	57
±	0	0	0	0	0	0	0	0
+	0	0	0	0	0	0	0	0
++	0	0	0	0	0	0	0	0
+++	0	0	0	0	0	0	0	0
Ketones								
–	27	11	21	11	28	22	30	24
±	25	15	14	23	13	24	17	24
+	8	31	20	21	13	9	9	9
++	0	3	0	0	0	0	0	0
+++	0	0	0	0	0	0	0	0
Occult blood								
–	55	60	51	54	54	55	53	56
±	2	0	1	0	0	0	2	0
+	1	0	2	1	0	0	1	1
++	0	0	0	0	0	0	0	0
+++	2	0	1	0	0	0	0	0
Bilirubin								
–	40	45	46	38	31	33	35	25
+	20	15	9	17	23	22	21	32
++	0	0	0	0	0	0	0	0
+++	0	0	0	0	0	0	0	0
Urobilinogen								
±	59	60	55	55	53	53	53	57
+	1	0	0	0	1	2	3	0
++	0	0	0	0	0	0	0	0
+++	0	0	0	0	0	0	0	0

( ): No. of animals

all the animals early in the examination times, but lowered with aging. Urinary protein was positive in two-thirds of the males and females at 5weeks, and thereafter, the number of animals with positive protein increased and the degree of positive protein also increased. Ketone body was positive (+) in half of the animals, and there were age related differences between weeks 5 and 9 or later in the males, but not in the females. Urinary bilirubin was positive(+) in 1/3 males and 1/2 females with no difference. Occult blood was negative in almost all the animals. Urobilinogen was positive (±) in almost all the animals. Urine volume was 9.5-11.9mL in the males and 10.6-14.5mL in the females in the early 3 test times, and tended to increase with aging. Specific gravity was 1.038-1.041 in the males and 1.030-1.032 in the females, with no age difference.

In Electrolytes, Na and Cl contents tended to decrease in the males with aging . There was no age difference in K content in the males and females.

Ophthalmology: Abnormal ophthalmological findings were observed in the cornea, lens, anterior chamber, ciliary body, iris, pupil, vitreous body and retina. There were leukoma(1.7-32.8%), roughend surface(0.4-1.7%), injury(0.4-1.7%), and opacity(0.4-0.6%)in the cornea; opacity(0.4%) in the anterior

Table. 3-2 Urinalysis

Male	Age in weeks 9 (60)	18 (60)	31 (58)
Volume (mL/16hr.)	9.5 ± 5.19	11.9 ± 5.78	11.5 ± 5.32
Specific gravity	1.041 ± 0.0135	1.038 ± 0.0121	1.040 ± 0.0137
Electrolytes (mEq/L)			
Na	1.37 ± 0.309	1.27 ± 0.276	0.99 ± 0.261
K	2.33 ± 0.472	2.29 ± 0.419	2.28 ± 0.455
Cl	1.69 ± 0.378	1.44 ± 0.327	1.14 ± 0.308

Female	Age in weeks 9 (60)	18 (60)	31 (57)
Volume (mL/16hr.)	10.6 ± 5.36	13.2 ± 7.16	14.5 ± 7.80
Specific gravity	1.031 ± 0.0126	1.032 ± 0.0140	1.030 ± 0.0105
Electrolytes (mEq/L)			
Na	1.15 ± 0.297	1.29 ± 0.335	1.20 ± 0.271
K	1.88 ± 0.421	2.27 ± 0.546	2.23 ± 0.451
Cl	1.37 ± 0.341	1.52 ± 0.376	1.43 ± 0.374

( ): No. of animals
Values are expressed as Mean ± S. D.

chamber, coloboma(1.7%) in the ciliary body; abnormal vasculature(0.4-1.3%), hemorrhage(1.7%) in the iris; corectopia(0.4%) in the pupil; white focus(1.7-13.3%), nucleus lentis(0.4-2.2%) in the lens, persistence of hyaloid artery(1.3-6.1%), opacity(0.6-1.7%), arteria hemorrhage(0.4-1.7%) in the vitreous body and tortuous vessels(4.7%), arterio-venous crossing(0.6-1.3%), loop(0.4-1.7%), abnormal vasculature(0.4-1.7%), narrow vessels(0.4%), and hemorrhage(0.4-0.6%) in the retina.

Hematology (Table 4.): White blood cell counts tended to decrease in the males and females with aging. In differential white blood cell count, lymphocytes also tended to decrease, but neutrophils (segmented cells) tended to increase in both the males and female with aging. Red blood cell counts tended to increase with aging in the males.

Blood chemistry (Table 5-1 to -2.): AL-P activity and I.P. decreased in both the males and females with aging; TIBC tended to increase in the males and females; and T.P., ALB and Fe increased in the females.

Organ weight(Table 6, Table 7): Relative organ weights decreased with aging of the animals except the seminal vesicle weight, which did not change in 10, 19 or 32 weeks. Absolute organ weights except the thymus, adrenal, and ovary weights increased as the animals aged, and the thymus weight decreased. The organ weights of adrenals and ovaries did not change in 10, 19 or 32 weeks.

Gross pathology: There were bilateral small-sized testes in 1 male and yellowish white foci in the liver in 1 female at 10 weeks, and unilateral small-sized testis in 1 male, white spots in the liver in 1 male, unilateral pelvic dilatation in 2 males, and adhesion of the lungs with rib pleural in 1 female at 19 weeks. There were yellow, white or yellowish white foci in 3 males and 2 females, raised foci in the spleen in 1 male, mass in the subcutis (pinna, inguinal, and rostrum) in 2 males and 2 females, black spots in the pituitary in 1 female, large-sized pituitary in 2 females, white foci in the glandular stomach in 1 male, nodule in the trachea in 1 female, white foci, partial loss of the ciliary body and rough surface of the cornea in the eye in 1 female and hair loss in 1 male at 32 weeks.

DISCUSSION

In Crj:CD(SD)IGS rats, there were age related differences in general toxicological parameters such as urinalysis, hematology, blood chemistry, and organ weights.

In urinalysis, pH lowered and positive urinary protein increased with aging of the males and females, and urine volume increased in the females. In hematology, WBC count decreased along with decreased lymphocyte ratio and increased neutrophil(segment cell) ratio. RBC count increased in the males with aging. In blood chemistry, AL-P, and I.P. decreased in both the males and females with aging, and TIBC increased in these animals. Total protein, albumin and serum iron increased with the aging in the females. In organ weights, thymus decreased with aging of the animals, and some genital or endocrine organs such as ovaries or adrenals did not change from 10 weeks onward.

In other Sprague-Dawley(SD)rats, it is known that urinary protein increased according to age[1] and Weaver et al. reported age related increase in incidence of chronic nephrosis, and as a result, albumin increased and globulins(especially α-globulin)decreased. [2]

As for decreased WBC along with decreased lymphocyte ratio and increased neutrophil ratio, Worlford et al. reported that WBC count was high in young and old rats, and lymphocytes decreased as the animals aged and neutrophils increased from 6 months onward. It is generally known that RBC count is higher in males than in females owing to sex hormones. Additionally, in another report, RBC count was higher in the males than in the females from 10 weeks onward although there was no sex

Table 4. Hematological findings

Male	Age in weeks		
	10 (60)	19 (60)	32 (60)
HCT(%)	42.7 ± 1.69	44.5 ± 1.70	43.7 ± 4.99
Hb(g/dL)	14.9 ± 0.49	15.2 ± 0.44	14.7 ± 1.78
RBC(10^6/ μL)	7.74 ± 0.334	8.68 ± 0.367	8.58 ± 0.969
Retic.cytes(%)	2.2 ± 0.57	1.8 ± 0.38	3.0 ± 10.76
MCV(fL)	55.1 ± 1.61	51.3 ± 1.62	51.0 ± 2.00
MCHC(g/dL)	34.9 ± 0.59	34.1 ± 0.74	33.5 ± 1.55
MCH(pg)	19.2 ± 0.56	17.5 ± 0.64	17.1 ± 1.00
Platelets(10^3/ μL)	1072 ± 93.5	1021 ± 98.5	1034 ± 115.4
Plot.time(sec.)	16.8 ± 1.48	17.0 ± 1.84	15.2 ± 1.29
APTT(sec.)	20.1 ± 1.35	19.7 ± 1.58	18.8 ± 1.45
Fibrinogen(mg/dL)	213.3 ± 16.04	206.5 ± 12.92	211.9 ± 13.22
WBC(10^3/ μL)	9.05 ± 1.267*	8.70 ± 1.290	8.25 ± 1.279
LYMP(%)	88.4 ± 5.22	83.0 ± 6.45	81.9 ± 5.10
A–LYMP(%)	0.1 ± 0.22	0.1 ± 0.22	0.0 ± 0.07
BAND(%)	0.1 ± 0.23	0.2 ± 0.32	0.2 ± 0.35
SEG(%)	8.6 ± 4.62	12.4 ± 5.17	13.1 ± 4.57
EOSI(%)	1.2 ± 0.93	1.7 ± 0.98	1.9 ± 1.05
BASO(%)	0.0 ± 0.00	0.0 ± 0.07	0.0 ± 0.13
MONO(%)	1.7 ± 1.25	2.7 ± 1.60	2.9 ± 1.73

Female	Age in weeks		
	10 (58)	19 (60)	32 (60)
HCT(%)	40.6 ± 1.64	41.3 ± 1.76	41.7 ± 1.92
Hb(g/dL)	14.6 ± 0.59	14.3 ± 0.53	14.2 ± 0.67
RBC(10^6/ μL)	7.49 ± 0.315	7.68 ± 0.362	7.59 ± 0.405
Retic.cytes(%)	1.8 ± 0.53	1.8 ± 0.60	1.6 ± 0.49
MCV(fL)	54.2 ± 1.25	53.9 ± 1.66	55.1 ± 2.21
MCHC(g/dL)	36.1 ± 0.52	34.7 ± 0.74	33.9 ± 0.51
MCH(pg)	19.6 ± 0.44	18.7 ± 0.61	18.7 ± 0.71
Platelets(10^3/ μL)	1116 ±106.2	1080 ±110.2	1022 ± 187.6
Plot.time(sec.)	15.8 ± 0.55	15.5 ± 0.62	13.7 ± 0.41
APTT(sec.)	16.2 ± 0.93	16.5 ± 0.96	16.1 ± 1.01
Fibrinogen(mg/dL)	175.6 ± 11.62	164.2 ± 12.90	158.8 ± 11.82
WBC(10^3/ μL)	6.53 ± 1.313*	5.60 ± 1.308	5.20 ± 1.337
LYMP(%)	90.5 ± 3.56	84.6 ± 6.00	81.5 ± 5.22
A–LYMP(%)	0.0 ± 0.14	0.1 ± 0.25	0.0 ± 0.00
BAND(%)	0.0 ± 0.14	0.2 ± 0.26	0.3 ± 0.36
SEG(%)	6.8 ± 3.21	11.7 ± 5.34	13.5 ± 4.75
EOSI(%)	1.3 ± 0.99	1.6 ± 0.89	2.3 ± 1.24
BASO(%)	0.0 ± 0.00	0.0 ± 0.09	0.0 ± 0.11
MONO(%)	1.3 ± 0.90	1.8 ± 1.28	2.3 ± 1.45

( ): No. of animals

Values are expressed as Mean ± S. D.

*: S. D. was calculated after logarithmic transformation

Table 5-1. Blood chemical findings

Male	Age in weeks		
	10 (59)	19 (60)	32 (60)
T.P.(g/dL)	5.6 ± 0.25	6.0 ± 0.24	6.1 ± 0.29
ALB(g/dL)	3.1 ± 0.13	3.1 ± 0.16	3.1 ± 0.16
A/G	1.2 ± 0.12	1.1 ± 0.11	1.0 ± 0.12
T.BIL(mg/dL)	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
CHOL(mg/dL)	60 ± 1.2*	59 ± 1.3	65 ± 1.3
TRIG(mg/dL)	41 ± 1.5*	45 ± 1.6	65 ± 1.6
AL-P(mU/mL)	447 ± 1.2*	196 ± 1.2	152 ± 1.3
GOT(mU/mL)	73 ± 1.1*	80 ± 1.3	84 ± 1.5
GPT(mU/mL)	27 ± 1.2*	33 ± 1.3	36 ± 1.6
LDH(mU/mL)	217 ± 1.8*	259 ± 2.2	257 ± 2.3
γ-GTP(mU/mL)	1 ± 1.1*	1 ± 1.1	1 ± 1.5
GLU(mg/dL)	125 ± 16.2	145 ± 16.2	153 ± 14.2
BUN(mg/dL)	17 ± 1.1*	18 ± 1.1	18 ± 1.1
CREA(mg/dL)	0.5 ± 0.06	0.5 ± 0.07	0.5 ± 0.07
BUN/CREA	34.5 ± 1.14*	33.0 ± 1.15	36.1 ± 1.18
U. A. (mg/dL)	1.3 ± 1.40*	1.1 ± 1.38	1.0 ± 1.44
I. P. (mg/dL)	8.0 ± 0.62	6.2 ± 0.59	5.5 ± 0.41
CA(mg/dL)	10.0 ± 0.39	9.9 ± 0.34	10.2 ± 0.34
NA(mEq/L)	144 ± 1.5	145 ± 1.9	145 ± 1.4
K(mEq/L)	4.2 ± 0.38	4.2 ± 0.32	4.2 ± 0.32
CL(mEq/L)	107 ± 1.8	107 ± 2.0	107 ± 1.7
FE(μg/dL)	118 ± 1.6*	120 ± 1.2	121 ± 1.6
UIBC(μg/dL)	306 ± 70.5	352 ± 49.8	360 ± 61.9
TIBC(μg/dL)	437 ± 41.4	474 ± 49.0	488 ± 55.2
CPK(mU/mL)	175 ± 56.7	174 ± 142.1	183 ± 253.8
PL(mg/dL)	104 ± 13.5	107 ± 22.6	113 ± 24.2
MG(mg/dL)	2.3 ± 0.25	2.1 ± 0.17	2.0 ± 0.17

( ): No. of animals

Table 5-2. Blood chemical findings

Female	Age in weeks		
	10 (58)	19 (60)	32 (60)
T.P.(g/dL)	6.0 ± 0.34	6.6 ± 0.34	7.0 ± 0.43
ALB(g/dL)	3.5 ± 0.23	3.8 ± 0.28	4.1 ± 0.36
A/G	1.4 ± 0.13	1.4 ± 0.14	1.4 ± 0.19
T.BIL(mg/dL)	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.05
CHOL(mg/dL)	66 ± 1.2*	69 ± 1.2	81 ± 1.2
TRIG(mg/dL)	12 ± 1.6*	16 ± 1.8	25 ± 1.9
AL-P(mU/mL)	234 ± 1.2*	80 ± 1.3	58 ± 1.4
GOT(mU/mL)	68 ± 1.2*	76 ± 1.5	100 ± 1.9
GPT(mU/mL)	22 ± 1.2*	30 ± 1.6	45 ± 2.0
LDH(mU/mL)	221 ± 1.6*	221 ± 1.8	214 ± 1.9
γ-GTP(mU/mL)	1 ± 1.2*	1 ± 1.3	1 ± 1.6
GLU(mg/dL)	108 ± 16.6	128 ± 17.7	135 ± 13.0
BUN(mg/dL)	18 ± 1.1*	19 ± 1.1	19 ± 1.1
CREA(mg/dL)	0.5 ± 0.06	0.6 ± 0.08	0.6 ± 0.07
BUN/CREA	37.0 ± 1.18*	31.7 ± 1.14	34.1 ± 1.15
U. A. (mg/dL)	1.0 ± 1.36*	1.0 ± 1.41	1.0 ± 1.34
I. P. (mg/dL)	7.9 ± 0.71	6.0 ± 0.83	5.3 ± 0.87
CA(mg/dL)	10.3 ± 0.45	10.5 ± 0.41	10.8 ± 0.41
NA(mEq/L)	143 ± 1.3	144 ± 1.7	144 ± 1.5
K(mEq/L)	4.0 ± 0.33	3.9 ± 0.34	3.9 ± 0.35
CL(mEq/L)	109 ± 2.1	108 ± 1.7	109 ± 2.4
FE(μg/dL)	203 ± 1.3*	280 ± 1.2	311 ± 1.2
UIBC(μg/dL)	226 ± 67.4	203 ± 49.6	201 ± 70.7
TIBC(μg/dL)	435 ± 39.8	488 ± 37.1	519 ± 45.1
CPK(mU/mL)	142 ± 58.9	119 ± 61.7	107 ± 64.7
PL(mg/dL)	122 ± 18.0	144 ± 22.6	174 ± 30.7
MG(mg/dL)	2.3 ± 0.20	2.1 ± 0.16	2.2 ± 0.18

( ): No. of animals
Values are expressed as Mean ± S. D.
*: S. D. was calculated after logarithmic transformation

Table. 6 Absolute organ weight

Male	Age in weeks		
	10 (60)	19 (60)	32 (60)
Heart(mg)	1133 ± 99.8	1330 ± 130.0	1433 ± 125.6
Spleen(mg)	597 ± 90.4	678 ± 90.2	717 ± 95.6
Thymus(mg)	421 ± 94.5	285 ± 84.4	174 ± 59.7
Lung(mg)	1243 ± 101.3	1417 ± 128.4	1557 ± 129.9
Liver(mg)	9444 ± 1064.7	11507 ± 1400.7	13163 ± 1864.9
Kidneys(mg)	2325 ± 205.4	2708 ± 270.2	2975 ± 265.8
Brain(mg)	2031 ± 79.9	2143 ± 86.6	2217 ± 80.3
Pituitary(mg)	10 ± 2.5	11 ± 2.1	13 ± 2.4
Adrenals(mg)	48 ± 7.0	48 ± 7.6	49 ± 8.3
Thyroids(mg)	15 ± 4.3	20 ± 5.6	19 ± 4.2
Testes(mg)	2925 ± 393.0	3256 ± 285.4	3351 ± 406.4
Prostate(mg)	888 ± 205.7	1267 ± 227.4	1472 ± 245.6
Seminal vesicle(mg)	1121 ± 190.4	1623 ± 279.8	1822 ± 311.9
Salivary glands(mg)	527 ± 51.7	619 ± 66.7	652 ± 70.3
Cecum without contents(mg)	932 ± 125.3	1083 ± 157.1	1093 ± 165.4
Cecum with contents(mg)	3118 ± 573.3	3674 ± 858.4	4146 ± 1002.8

Female	Age in weeks		
	10 (60)	19 (60)	32 (60)
Heart(mg)	782 ± 70.8	897 ± 74.3	945 ± 86.2
Spleen(mg)	452 ± 72.1	475 ± 58.4	486 ± 72.1
Thymus(mg)	413 ± 90.2	248 ± 54.7	151 ± 40.9
Lung(mg)	994 ± 75.8	1117 ± 78.0	1147 ± 76.5
Liver(mg)	6317 ± 666.3	6965 ± 693.5	7409 ± 1023.7
Kidneys(mg)	1606 ± 146.7	1761 ± 145.5	1812 ± 165.5
Brain(mg)	1916 ± 74.0	1994 ± 64.9	2021 ± 77.0
Pituitary(mg)	13 ± 2.8	16 ± 3.1	16 ± 4.1
Adrenals(mg)	61 ± 8.7	61 ± 7.8	61 ± 10.9
Thyroids(mg)	14 ± 5.4	15 ± 4.4	15 ± 4.1
Ovaries(mg)	80 ± 15.5	80 ± 13.5	69 ± 15.9
Uterus(mg)	470 ± 119.6	590 ± 140.3	630 ± 140.6
Salivary glands(mg)	367 ± 38.4	404 ± 46.7	419 ± 46.7
Cecum without contents(mg)	757 ± 141.3	867 ± 139.3	842 ± 118.5
Cecum with contents(mg)	2550 ± 572.2	2973 ± 558.4	3191 ± 815.9

( ): No. of animals
Values are expressed as Mean ± S. D.

difference until 10 weeks of age [4]. Unakami et al. [5] reported that decreased AL-P resulted from decreased bone-originated AL-P isoenzyme activity and was considered to be a reflection of normal bone growth. It is well known that I.P. decreases according to age.[3] For total protein, there were different reports such as total protein did not change in 80 to 560 days[6] or increased with aging.[7] For albumin and A/G ratio, there were reports[6,8] that these values decreased with aging, and the data were different from those in Crj:CD(SD)IGS rats.

Body weights of Crj:CD(SD)IGS rats increased in almost the same extent as those of other SD strain rats throughout the experimental period.

In conclusion, differences in the accumulated data of general toxicological parameters in the Crj:CD(SD)IGS rats with aging were similar to those of other SD rats except for a few parameters.

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Table. 7 Relative organ weight (%)

Male	Age in weeks		
	10	19	32
	(60)	(60)	(60)
Heart	0.34 ± 0.023	0.28 ± 0.023	0.26 ± 0.027
Spleen	0.18 ± 0.025	0.15 ± 0.018	0.13 ± 0.019
Thymus	0.127 ± 0.0243	0.061 ± 0.0161	0.031 ± 0.0097
Lung	0.38 ± 0.029	0.30 ± 0.024	0.28 ± 0.029
Liver	2.86 ± 0.162	2.45 ± 0.141	2.36 ± 0.208
Kidneys	0.70 ± 0.045	0.58 ± 0.048	0.54 ± 0.046
Brain	0.62 ± 0.043	0.46 ± 0.044	0.40 ± 0.045
Pituitary (10^{-3})	3.10 ± 0.726	2.39 ± 0.442	2.30 ± 0.520
Adrenals (10^{-2})	1.463 ± 0.2110	1.030 ± 0.1668	0.893 ± 0.1961
Thyroids (10^{-3})	4.60 ± 1.386	4.27 ± 1.191	3.43 ± 0.782
Testes	0.890 ± 0.1219	0.699 ± 0.0812	0.607 ± 0.0895
Prostate	0.27 ± 0.067	0.27 ± 0.055	0.27 ± 0.050
Seminal vesicle	0.34 ± 0.057	0.35 ± 0.062	0.33 ± 0.064
Salivary glands	0.160 ± 0.0172	0.132 ± 0.0135	0.118 ± 0.0138
Cecum without contents	0.28 ± 0.031	0.23 ± 0.033	0.20 ± 0.027
Cecum with contents	0.94 ± 0.163	0.78 ± 0.174	0.75 ± 0.186

Female	Age in weeks		
	10	19	32
	(60)	(60)	(60)
Heart	0.36 ± 0.025	0.33 ± 0.027	0.30 ± 0.023
Spleen	0.21 ± 0.029	0.17 ± 0.021	0.16 ± 0.020
Thymus	0.191 ± 0.0377	0.090 ± 0.0203	0.049 ± 0.0137
Lung	0.46 ± 0.025	0.41 ± 0.035	0.37 ± 0.031
Liver	2.93 ± 0.210	2.52 ± 0.169	2.36 ± 0.231
Kidneys	0.74 ± 0.057	0.64 ± 0.050	0.58 ± 0.050
Brain	0.89 ± 0.068	0.73 ± 0.063	0.65 ± 0.062
Pituitary (10^{-3})	6.15 ± 1.268	5.83 ± 1.081	5.19 ± 1.376
Adrenals (10^{-2})	2.832 ± 0.3900	2.230 ± 0.3317	1.934 ± 0.3212
Thyroids (10^{-3})	6.63 ± 2.629	5.39 ± 1.602	4.73 ± 1.234
Ovaries	0.037 ± 0.0064	0.029 ± 0.0049	0.022 ± 0.0053
Uterus	0.22 ± 0.056	0.21 ± 0.054	0.20 ± 0.045
Salivary glands	0.170 ± 0.0153	0.146 ± 0.0161	0.134 ± 0.0145
Cecum without contents	0.35 ± 0.068	0.31 ± 0.051	0.27 ± 0.040
Cecum with contents	1.17 ± 0.235	1.08 ± 0.211	1.02 ± 0.241

( ): No. of animals
Values are expressed as Mean ± S. D.

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Mortality, Body Weight, Food Consumption, Clinical Laboratory Test and Pathological Examination in Crj:CD(SD) IGS Rats in Subchronic and Chronic Toxicity Studies

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ABSTRACT. The Crj:CD (SD)IGS (IGS hereafter) rat strain was developed by Charles River Inc. for the global supply of animals with a uniform genetic character, as a part of an internationalization program. Animals were approximately 5 weeks of age at the start of the study and were assigned to three groups, each consisting of 20 male and 20 female IGS rats, and reared for 4, 13 and 26 weeks. The clinical signs, body weight and food consumption were recorded during the rearing period. At the end of each rearing period, clinical laboratory tests (hematology and blood chemistry) and pathological examinations were performed. —Key words: CD(SD)IGS, rat, historical control data

CD (SD) IGS-1998: 39-42

Sixty male and 60 female 4-week-old IGS rats were supplied by Charles River Japan Inc. (Atsugi, Kanagawa, Japan) and reared for 26 weeks from 5 weeks of age after a 1 week acclimatization period. Rats were maintained in a hygienic barrier system under controlled environmental conditions: air filtration and hyperbaric pressure, sterile cage and equipment, at a temperature of 23 ± 2 °C and humidity of 55 ± 10% under a 12-hr light/12-hr dark cycle. The rats were housed individually in stainless steel wire mesh cages (W 20.0 cm x D 28.2 cm x H 18.0 cm). The animals had free access to a commercial feed (modified NIH open formula rat and mouse ration sterilized by radiation, Oriental Yeast Co., Ltd., Tokyo, Japan) and had free access to tap water via an automatic watering nozzle. Body weight and food consumption were measured weekly. The rats were observed twice daily (morning and afternoon) for clinical signs and were palpated to detect any masses at the time of body weight measurements. Clinical laboratory tests (hematology, blood chemistry and urinalysis), ophthalmological examinations and pathological examinations were conducted on all rats at Weeks 4, 13 and 26.

The overall survival ratio in IGS was 100% and no abnormal findings were obtained during the rearing period. The mean body weight (Figure 1 and 2) and mean food consumption (Table 1) of both the male and female IGS rats were lower than those of the corresponding Crj:CD(SD) rats during the rearing period. Tables 2,3, 4 and 5 show the hematological values, blood chemistry values, absolute organ weights and relative organ weights, respectively. As gross findings, atrophy of the thymus was observed in 5 males and dilated lumen of the uterus was observed in 3 females at 31 weeks of age. As microscopic findings, epidermoid cyst of the thymus was observed as a malignant finding in one male. As a non-neoplastic lesion, pigment deposition in the spleen was increased by aging in IGS rats, and it was observed in all males and all females up to 31 and 18 weeks of age, respectively. Other lesions that increased with aging were cellular infiltration in the heart, vacuolic change of the pancreatic acinus observed in both males and females, appearance of foamy cells in the lungs, eosinophilic body of kidneys and cell debris in the lumen of the prostate observed in males and hyperplasia of epithelium of the thymus observed in females. There were more eosinophilic bodies in the males than in the females, and hyperplasia of epithelium of the thymus was seen more frequently in the females than males. A sex difference was observed in the occurrence of these lesions.

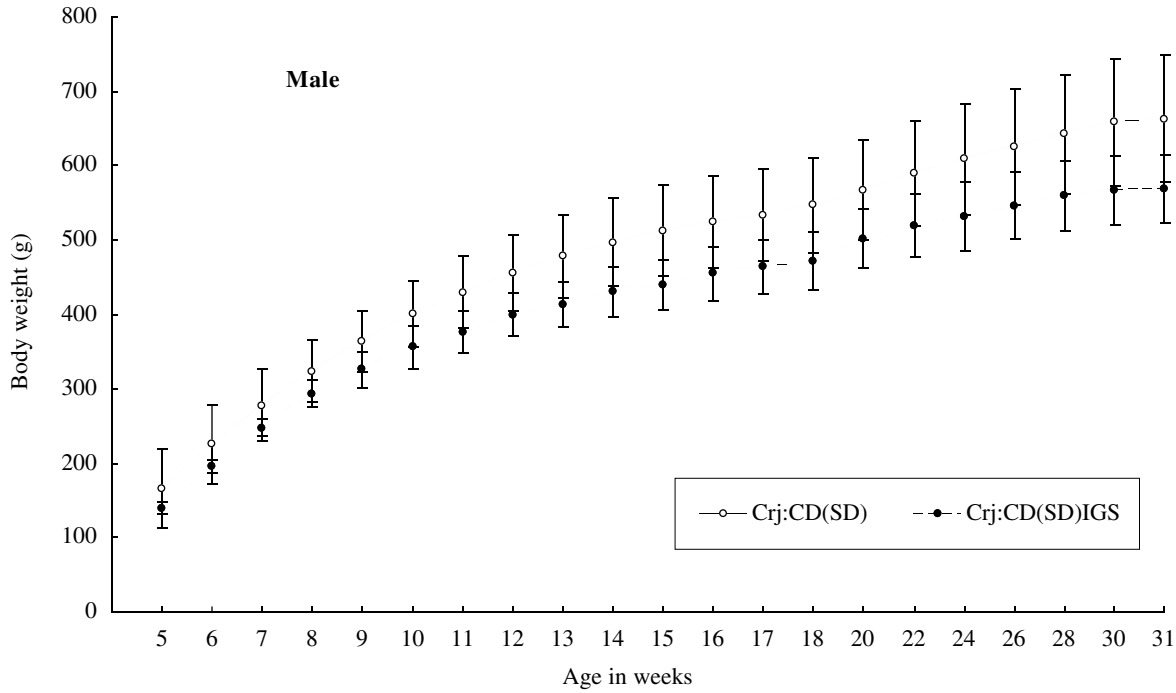

Fig. 1. Mean body weight in Sprague-Dawley rats. The vertical bars represent the standard deviations from the mean.

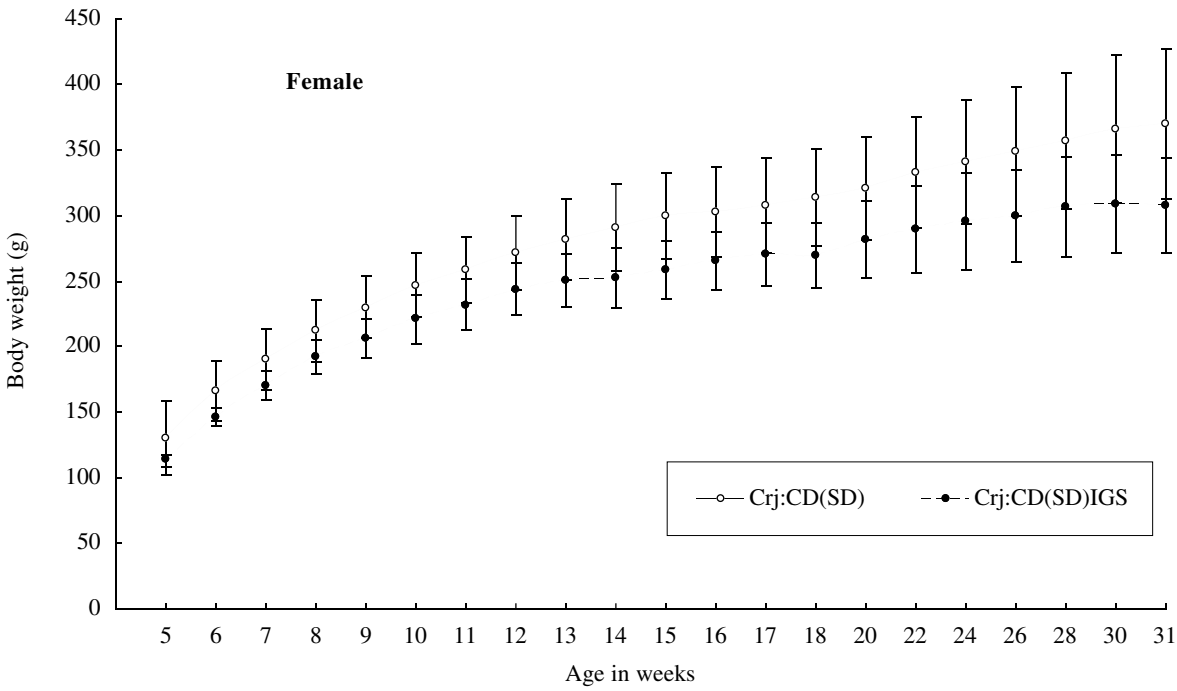

Fig. 2. Mean body weight in Sprague-Dawley rats. The vertical bars represent the standard deviations from the mean.

Table 1. Historical control data of food consumption in Sprague-Dawley rats

Unit: g/week

Age (weeks)	Crj:CD(SD)IGS				Crj:CD(SD)			
	Male		Female		Male		Female	
	Number of animals	Mean±S.D.	Number of animals	Mean±S.D.	Number of animals	Mean±S.D.	Number of animals	Mean±S.D.
6	60	145 ± 8	60	107 ± 9	70	148 ±10	70	124 ± 9
9	60	159 ±17	60	118 ±11	70	181 ±24	70	134 ±16
13	40	161 ±14	40	119 ±12	70	187 ±24	70	138 ±19
18	40	157 ±21	40	107 ±15	70	191 ±26	70	133 ±20
24	20	170 ±16	20	119 ±15	70	186 ±25	70	129 ±19
31	20	158 ±11	20	109 ±13	70	189 ±26	70	128 ±21

Table 2. Historical control data of hematology and coagulation in Crj:CD(SD)IGS rats

ItemUnit		Age (weeks)					
		9		18		31	
		Male	Female	Male	Female	Male	Female
		n=20	n=20	n=20	n=20	n=20	n=20
HCT	(%)	42.3 ± 1.9a)	40.7 ± 1.3	41.7 ± 1.3	39.8 ± 1.4	45.4 ± 1.9	42.4 ± 2.2
HGB	(g/dl)	15.1 ± 0.6	14.8 ± 0.4	15.0 ± 0.6	14.4 ± 0.5	15.5 ± 0.5	14.9 ± 0.6
RBC	(x10^6/mm^3)	7.48 ± 0.39	7.31 ± 0.23	8.49 ± 0.39	7.58 ± 0.33	9.11 ± 0.39	7.99 ± 0.37
MCV	(μm^3)	56.6 ± 1.3	55.6 ± 1.2	49.2 ± 1.8	52.5 ± 1.5	49.8 ± 1.6	53.1 ± 1.6
MCH	(pg)	20.2 ± 0.5	20.2 ± 0.5	17.6 ± 0.6	19.0 ± 0.5	17.1 ± 0.5	18.7 ± 0.5
MCHC	(%)	35.6 ± 0.6	36.3 ± 0.4	35.8 ± 0.6	36.2 ± 0.4	34.3 ± 0.6	35.1 ± 0.7
PLT	(x10^3/mm^3)	1069 ±89	1146 ±165	948 ± 65	1024 ±171	945 ±142	1002 ±102
WBC	(x10^3/mm^3)	9.3 ± 2.9	5.6 ± 1.7	8.0 ± 2.6	4.6 ± 2.3	9.5 ± 2.9	4.4 ± 0.7
NEU	(%)	8 ± 2	10 ± 4	12 ± 7	17 ±13	14 ± 5	16 ± 5
LYM	(%)	89 ± 2	86 ± 5	83 ± 7	78 ±13	80 ± 6	76 ± 6
RC	(‰)	31 ± 8	24 ± 5	23 ± 5	22 ± 6	23 ± 4	25 ± 6
PT	(sec.)	13.7 ± 0.7	13.3 ± 0.4	13.6 ± 0.6	13.3 ± 0.7	14.0 ± 0.5	14.3 ± 0.7
APTT	(sec.)	25.3 ± 1.9	20.5 ± 1.1	24.0 ± 1.2	19.7 ± 1.1	23.0 ± 1.4	18.8 ± 1.0
FIB	(mg/dl)	267 ±18	222 ±12	254 ± 19	188 ±39	253 ± 23	174 ± 16

a) Values represent mean ± S.D.

Table 3. Historical control data of blood chemistry in Crj:CD(SD)IGS rats

ItemUnit		Age (weeks)					
		9		18		31	
		Male	Female	Male	Female	Male	Female
		n=20	n=20	n=20	n=20	n=20	n=20
Glu	(mg/dl)	130.9 ± 16.0a)	115.7 ± 9.6	141.0 ±14.9	124.5 ±12.1	149.3 ± 16.5	118.8 ± 8.1
BUN	(mg/dl)	11.2 ± 1.7	12.2 ± 1.7	11.9 ± 1.4	12.5 ± 1.6	12.2 ± 1.6	13.9 ± 2.1
GOT	(U/l)	46 ± 7	50 ± 8	43 ± 6	57 ±20	47 ± 10	59 ± 18
GPT	(U/l)	13 ± 2	14 ± 6	14 ± 2	21 ±13	17 ± 5	24 ± 12
ALP	(U/l)	193 ± 35	119 ± 23	97 ±22	42 ± 8	69 ± 14	30 ± 7
Cre	(mg/dl)	0.62± 0.12	0.60 ± 0.09	0.60 ± 0.05	0.66 ± 0.06	0.61 ± 0.07	0.67 ± 0.05
T-Cho	(mg/dl)	38 ± 8	43 ± 9	41 ± 9	55 ±13	58 ± 14	64 ± 11
TP	(g/dl)	5.32± 0.16	5.55 ± 0.27	5.76 ± 0.20	6.32 ± 0.48	6.13 ± 0.34	6.44 ± 0.28
T-Bil	(mg/dl)	0.38± 0.01	0.40 ± 0.01	0.29 ± 0.11	0.33 ± 0.09	0.18 ± 0.03	0.23 ± 0.03
Ca	(mg/dl)	10.01± 0.30	10.14 ± 0.29	9.62 ± 0.26	9.89 ± 0.24	10.34 ± 0.33	10.38 ± 0.37
Alb	(g/dl)	2.97± 0.11	3.17 ± 0.14	3.14 ± 0.10	3.65 ± 0.34	3.29 ± 0.22	3.75 ± 0.25
IP	(mg/dl)	7.95± 0.63	6.82 ± 0.42	6.38 ± 0.49	4.72 ± 0.76	6.09 ± 0.38	4.58 ± 0.64
TG	(mg/dl)	33 ± 8	25 ± 4	38 ±10	32 ± 5	68 ± 33	38 ± 11
g-GTP	(U/l)	0.39± 0.41	0.52 ± 0.51	0.31 ± 0.32	0.42 ± 0.28	0.70 ± 0.39	0.59 ± 0.44
A/G		1.27± 0.04	1.33 ± 0.06	1.20 ± 0.06	1.38 ± 0.13	1.16 ± 0.07	1.40 ± 0.11
Na	(mmol/l)	145 ± 1	144 ± 1	146 ± 1	146 ± 1	146 ± 2	145 ± 2
K	(mmol/l)	4.82± 0.34	4.83 ± 0.23	4.51 ± 0.25	4.40 ± 0.33	4.78 ± 0.28	4.44 ± 0.21
Cl	(mmol/l)	109 ± 2	111 ± 1	110 ± 1	113 ± 2	109 ± 2	113 ± 3

a) Values represent mean ± S.D.

Table 4. Absolute organ weight in Crj:CD(SD)IGS rats

Strain	Age (weeks)		Body weight(g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)	Spleen (g)	Adrenals (mg)	Testes (g)	Ovaries (g)	Thyroid (mg)	Pituitary (mg)	Thymus (mg)	Prostate (mg)
Male	9	Mean	326	2.02	1.07	1.10	9.36	2.44	0.54	53	3.01	-	22.2	10.7	516	596
		±S.D.	24	0.07	0.11	0.12	1.21	0.23	0.08	7	0.12	-	2.8	1.7	122	142
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
	18	Mean	472	2.18	1.33	1.28	11.20	3.03	0.62	59	3.29	-	23.2	12.0	274	988
		±S.D.	39	0.08	0.14	0.16	1.44	0.21	0.10	8	0.30	-	3.8	1.6	76	197
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
	31	Mean	568	2.27	1.51	1.52	14.51	3.44	0.71	56	3.51	-	32.6	14.6	165	1063
		±S.D.	46	0.10	0.15	0.12	1.78	0.37	0.11	9	0.27	-	4.8	2.3	68	214
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
Female	9	Mean	206	1.87	0.71	0.89	5.74	1.62	0.39	64	-	81	19	12	457	-
		±S.D.	15	0.05	0.07	0.10	0.54	0.12	0.06	8	-	10	2	2	94	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	-
	18	Mean	270	1.98	0.84	1.02	6.61	1.83	0.44	68	-	75	20	17	249	-
		±S.D.	25	0.07	0.08	0.07	0.56	0.22	0.06	9	-	14	4	3	61	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	-
	31	Mean	308	2.05	0.92	1.13	7.05	1.99	0.43	70	-	77	25	20	155	-
		±S.D.	36	0.07	0.09	0.08	0.91	0.19	0.10	8	-	12	5	5	33	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	

Table 5. Relative organ weight in Crj:CD(SD)IGS rats

Strain	Age (weeks)		Body weight(g)	Brain	Heart	Lungs	Liver	Kidneys	Spleen	Adrenals	Testes	Ovaries	Thyroid	Pituitary	Thymus	Prostate
Male	9	Mean	326	0.633	0.333	0.344	2.908	0.762	0.167	0.017	0.942	-	0.007	0.003	0.160	0.186
		±S.D.	24	0.056	0.020	0.020	0.185	0.050	0.020	0.002	0.082	-	0.001	0.001	0.032	0.044
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
	18	Mean	472	0.477	0.288	0.279	2.431	0.661	0.135	0.013	0.720	-	0.005	0.003	0.060	0.216
		±S.D.	39	0.035	0.020	0.029	0.172	0.028	0.019	0.002	0.080	-	0.001	0.000	0.015	0.044
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
	31	Mean	568	0.402	0.267	0.268	2.550	0.607	0.125	0.010	0.620	-	0.006	0.003	0.029	0.188
		±S.D.	46	0.036	0.022	0.018	0.183	0.061	0.019	0.001	0.061	-	0.001	0.000	0.010	0.038
		No. of animals	20	20	20	20	20	20	20	20	20	-	20	20	20	20
Female	9	Mean	206	0.927	0.349	0.438	2.835	0.802	0.190	0.032	-	0.040	0.009	0.006	0.226	-
		±S.D.	15	0.073	0.020	0.033	0.164	0.049	0.023	0.004	-	0.005	0.001	0.001	0.044	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	-
	18	Mean	270	0.741	0.315	0.381	2.467	0.683	0.163	0.025	-	0.028	0.007	0.006	0.093	-
		±S.D.	25	0.044	0.023	0.026	0.172	0.059	0.023	0.003	-	0.006	0.002	0.001	0.022	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	-
	31	Mean	308	0.673	0.300	0.371	2.286	0.650	0.140	0.023	-	0.025	0.008	0.007	0.050	-
		±S.D.	36	0.082	0.023	0.036	0.123	0.048	0.033	0.003	-	0.005	0.002	0.002	0.009	-
		No. of animals	20	20	20	20	20	20	20	20	-	20	20	20	20	-

Changes in Body Weight, Food Intake and Blood Biochemical Parameters in Crj:CD(SD)IGS Rats

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ABSTRACT. The general toxicity background data were collected from Crj:CD(SD)IGS rats supplied different protein contents in diets (about 18% and 20%) for 26 weeks. Decreased body weight gain was observed in both sexes fed on a lower protein diet, whereas increased food intake was observed in both sexes of the same group. In addition, decrease in glucose, triglyceride, β-lipoprotein and total bilirubin, increase in A/G ratio and inorganic phosphate, and decreased liver weight were also observed in both sexes fed on a lower protein diet. However, the low protein diet did not affect the general condition of rats. —Key words: Crj:CD(SD)IGS Rat, General toxicity background data, Low protein diet

CD (SD) IGS-1998: 43-49

INTRODUCTION

Crj:CD(SD)IGS rats were produced by the gold standard system, a new animal breeding system developed by Charles River Inc. for internationalization of scientific research and development of new drugs. In the present study, the general toxicity background data were collected from Crj:CD(SD)IGS rats supplied different protein contents in diets (about 18% and 20%) for 26 weeks.

MATERIALS AND METHODS

Animals and housing conditions

Four-weeks old Crj:CD(SD)IGS rats, each 83 males and 83 females, were purchased from Charles River Japan Inc. (Hino Breeding Center, Shiga, Japan) on June 11, 1996. No abnormalities were observed in the animals at the arrival as well as during the acclimatization period for one week. Each 80 males and females selected from those having shown smooth increase in the body weight were entered into the present study at the age of 5-weeks old. The body weight at the beginning to the treatment ranged from 115.9 to 157.6 g in males and from 107.7 to 130.8 g in females. The animals were housed individually in a wire mesh-bottomed metal cage for rats (W 240 mm × D 380 mm × H 200 mm) in the animal room under the conditions of room temperature of 21-25˚C, relative humidity of 45-65%, lighting of 12-hour/day (from 07:00 to 19:00), and ventilation of 15-25 times/hour. The animals were fed a commercially available solid diet, such as F-2 (Funabashi Farm, Chiba, Japan) for an acclimation period, and CR-LPF (Oriental Yeast Co., Tokyo, Japan) or F-2 for the experimental period, with tap water *ad libitum*.

Experimental design

Animals at 5 weeks of age were randomly divided into 2 groups of 40 animals of each sex. One group treated with low protein diet (CR-LPF, about 18% protein diet), and the other group treated with control diet (F-2, about 20% protein diet) for 26 weeks, respectively. At 4 weeks (9 weeks of age), 8 weeks (13 weeks of age), 13 weeks (18 weeks of age), and 26 weeks (31 weeks of age) of the treatment, 10 males and 10 females from each group were sacrificed and subjected to the hematology, blood biochemistry, macroscopic pathology and measurement of organ weight.

Dietary components were as follows:

Composition	Group	
	Control (F-2)	CR-LPF
Moisture (%)	7.1 – 7.3	7.9 – 8.8
Crude protein (%)	20.1 – 20.3	17.4 – 17.8
Crude fat (%)	4.4 – 4.5	4.3 – 5.4
Crude fiber (%)	3.0 – 3.5	4.7 – 5.4
Crude ashes (%)	5.0 – 5.1	6.1 – 6.5
Nitrogen-free extract (%)	59.7 – 60.0	56.8 – 59.3

Observation and examination

General signs, body weight, and food intake

General signs were observed daily throughout the experimental period. Body weight was measured on the day of initiation of the treatment, and thereafter once a week. Food intake was measured once a week for the first 4 weeks of treatment, and once every 2 weeks thereafter.

Hematology

At the end of each treatment, blood specimen was collected through inferior vena cava under pentobarbital anesthesia from 10 animals of both sexes of each group after overnight fasting of 16 hours or more, and subjected to the following hematological examinations.

Red blood cell count (RBC), hemoglobin content (Hb), hematocrit (Ht), white blood cell count (WBC) and platelet count were measured on the blood specimen prevented from coagulation by addition of EDTA-2K by using Sysmex microcell counter (F-800, Toa Medical Electronics, Kobe, Japan), and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. On the plasma specimen obtained from the blood specimen by centrifugal separation (4˚C, 3000 rpm, 10 min.) in the presence of 3.3% sodium citrate, prothrombin time (PT) , activated partial thromboplastin time (APTT) and fibrinogen were measured by using Sysmex automated blood coagulation analyzer (CA-3000, Toa Medical

Electronics, Kobe, Japan). Differential white blood cell (May-Giemsa stained) and reticulocyte (Brecher method) counts were measured visually under a light microscope.

Blood biochemistry

A part of the collected blood specimen was treated with heparin sodium to prevent coagulation and centrifuged at 3000 rpm for 10 min at 4°C. The obtained plasma specimen was subjected to the following biochemical analysis.

Transaminases (GOT and GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), glucose, total protein, albumin, blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride, phospholipid, β-lipoprotein, total bilirubin, inorganic phosphorus and calcium (Ca) were measured by using an autoanalyzer (Model 7170, Hitachi Ltd., Tokyo, Japan). Sodium (Na), potassium (K) and chloride (Cl) were measured by using an automated electrolyte analyzer (PVA αII, Analytical Instruments, Tokyo, Japan). A/G was calculated from the plasma protein fractions measured by using densitometer (HAD-501, Hiranuma Sangyo).

Macroscopic pathology and organ weight

The animals were sacrificed after the collection of the blood specimen by exsanguination from the abdominal aorta and inferior vena cava under anesthesia with pentobarbital, and subjected to the autopsy.

The following organs were removed and weighed with an electronic pan balance (Model EB-330D, Shimadzu Corp., Kyoto, Japan): brain, pituitary, thyroid (including parathyroid), submaxillary gland (including sublingual gland), thymus, heart, lung, liver, spleen, kidney, adrenal, testis, prostate, seminal vesicle, ovary and uterus. Based on the organ weight and the body weight of the day, organ weight/body weight ratio (relative organ weight) was calculated.

Statistical analysis of data

The mean and standard deviation of the value for each group were calculated including body weight, food intake, hematology, blood biochemistry, absolute and relative organ weight. The statistical significance of the difference between the control group and the CR-LPF group was assessed by Student's t-test.

RESULTS

General signs

No abnormal general signs nor deaths were detected in males and females throughout the experimental period.

Body weight

Mean body weight changes were shown in Fig. 1. Body weight of males and females in the CR-LPF group were lower than the control group throughout the experimental period. In comparison with the control group, statistical significant differences were observed at 3, 5 and 7 weeks in females.

Food intake

Mean food intake was shown in Fig. 2. Food intake of males and females in the CR-LPF group was higher than the control group throughout the experimental period. In comparison with the control group, statistical significant differences were observed from 1 week to 16 weeks, except at 14 weeks, in males, and from 1 week to 14 weeks, except at 2 weeks, in females, respectively.

Hematology

The results from hematology were shown in Table 1. In comparison with the control group, statistical significant differences were observed in the CR-LPF group as follows: in males; decrease in MCV at 4 weeks, increase in Hb, MCHC, WBC and lymphocyte ratio, and decrease in reticulocyte and segmented neutrophil ratios at 8 weeks, increase in MCHC, decrease in Hb, Ht, MCV and monocyte ratio and shortening of APTT at 13 weeks, increase in Hb, Ht and MCV, decrease in MCHC and WBC and prolonged PT at 26 weeks, and in females; increase in monocyte ratio at 4 weeks, increase in RBC and Hb at 8 weeks, shortening of PT at 13 weeks, respectively.

Blood biochemistry

The results from blood biochemistry were shown in Table 2. In comparison with the control group, statistical significant differences were observed in the CR-LPF group as follows: in males; increase in ALP, LDH, A/G ratio and BUN, and decrease in triglyceride, β-lipoprotein and Ca at 4 weeks, increase in LDH, total protein, albumin, A/G ratio, creatinine, total cholesterol, phospholipid, inorganic phosphate and Ca, and decrease in triglyceride, β-lipoprotein, total bilirubin, K and Cl at 8 weeks, increase in inorganic phosphate, and decrease in glucose, triglyceride and β-lipoprotein at 13 weeks, increase in A/G ratio, Na, K and Cl, and decrease in β-lipoprotein at 26 weeks, and in females; decrease in β-lipoprotein, total bilirubin and K at 4 weeks, increase in inorganic phosphate, and decrease in GOT, glucose, triglyceride, β-lipoprotein and total bilirubin at 8 weeks, increase in inorganic phosphate and Cl, and decrease in glucose and β-lipoprotein at 13 weeks, decrease in BUN and creatinine at 26 weeks, respectively.

Macroscopic pathology

No abnormalities were detected in any of the males and females of each group.

Organ weight

Absolute and relative organ weights were shown in Table 3 (males) and Table 4 (females). In comparison with the control group, statistical significant differences were observed in the CR-LPF group as follows: in males; decrease in absolute and relative weights of the liver, and increase in relative weights of the brain, heart, left testis and prostate at 4 weeks, increase in absolute and relative weights of the brain, and decrease in relative weight of the liver at 13 weeks, increase in relative weights of the brain, pituitary and lungs at 26 weeks, and in females; decrease in absolute and relative weights of the thymus at 4 weeks, decrease in absolute and relative weights of the liver and absolute weight of the kidney, and increase in relative weights of the brain, pituitary, submaxillary glands, heart and lungs at 8 weeks, decrease in absolute and relative weights of the uterus, absolute weights of the lungs and right adrenal, and relative weight of the liver at 26 weeks, respectively.

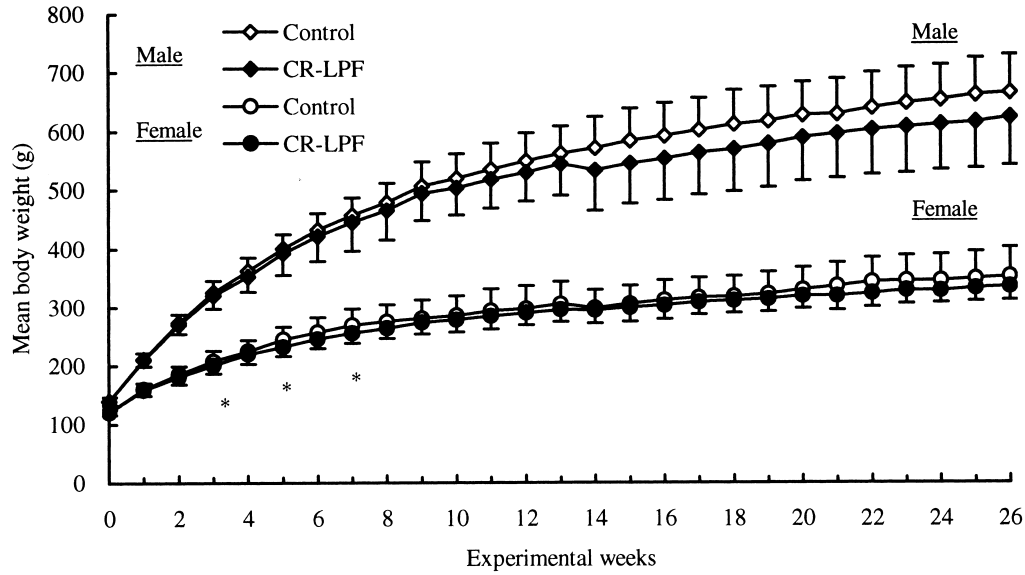

Fig. 1. Mean body weight changes in rats supplied with control or CR-LPF diets for 26 weeks
Each value represents the mean±S.D.
Significant difference from the control group, *P<0.05.

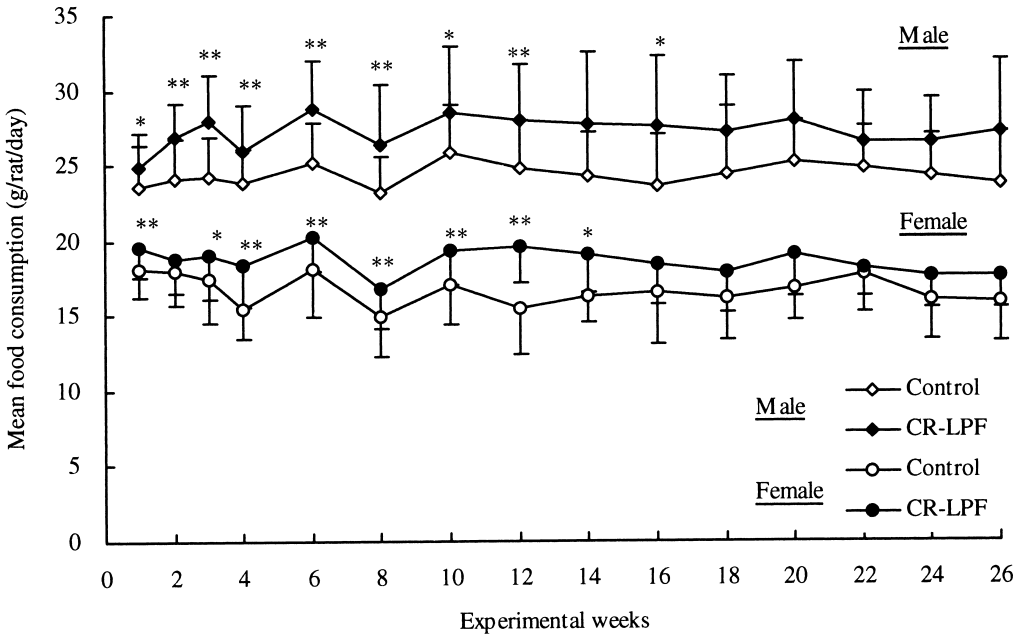

Fig. 2. Mean food intake in rats supplied with control or CR-LPF diets for 26 weeks
Each value represents the mean±S.D.
Significant difference from the control group, *P<0.05 and **P<0.01.

Table 1. Hematological findings in rats supplied with control or CR-LPF diets for 26 weeks

Items (unit)	Control				CR-LPF			
	4 weeks	8 weeks	13 weeks	26 weeks	4 weeks	8 weeks	13 weeks	26 weeks
Male								
Number of rats examined	10	10	10	10	10	10	10	10
RBC ($x10^4/\mu l$)	805 ± 27	862 ± 34	898 ± 27	921 ± 37	829 ± 30	869 ± 28	886 ± 47	946 ± 54
Hemoglobin (g/dl)	15.5 ± 0.3	14.9 ± 0.4	15.7 ± 0.5	15.5 ± 0.5	15.5 ± 0.5	15.3 ± 0.5*	15.1 ± 0.6*	16.2 ± 0.7*
Hematocrit (%)	51.9 ± 2.2	51.4 ± 1.9	57.3 ± 2.6	49.8 ± 2.5	51.1 ± 1.3	51.2 ± 1.9	51.1 ± 3.3**	54.9 ± 4.0**
MCV (fl)	64.5 ± 2.8	59.7 ± 2.2	63.8 ± 2.5	54.0 ± 2.4	61.6 ± 1.5*	59.0 ± 3.0	57.8 ± 3.1**	58.0 ± 2.9**
MCH (pg)	19.2 ± 0.7	17.3 ± 0.6	17.5 ± 0.5	16.8 ± 0.5	18.7 ± 0.3	17.7 ± 0.6	17.1 ± 0.7	17.1 ± 0.6
MCHC (g/dl)	29.8 ± 1.1	29.0 ± 0.9	27.5 ± 1.1	31.1 ± 0.9	30.3 ± 0.5	30.0 ± 1.0*	29.7 ± 1.3**	29.6 ± 1.0**
Reticulocyte (%)	0.7 ± 0.6	1.6 ± 0.7	0.4 ± 0.4	0.9 ± 0.5	0.3 ± 0.3	0.8 ± 0.6**	0.5 ± 0.4	1.2 ± 0.6
WBC ($x10^2/\mu l$)	74 ± 13	49 ± 11	75 ± 22	78 ± 18	61 ± 21	59 ± 9*	66 ± 17	54 ± 13**
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophil (%)	0.6 ± 0.8	1.0 ± 1.1	1.8 ± 1.1	0.8 ± 0.8	0.5 ± 0.5	1.5 ± 0.7	1.5 ± 1.9	1.1 ± 1.1
Stab neutrophil (%)	0.7 ± 0.7	0.4 ± 0.7	1.0 ± 1.1	0.8 ± 1.0	0.4 ± 0.5	0.7 ± 0.7	2.2 ± 1.6	1.1 ± 1.1
Segmented neutrophil(%)	9.4 ± 3.7	14.6 ± 3.9	8.1 ± 3.1	10.4 ± 6.7	9.9 ± 6.4	8.4 ± 3.3**	11.3 ± 3.7	16.0 ± 6.7
Monocyte (%)	5.3 ± 1.9	6.7 ± 2.7	8.0 ± 4.0	6.6 ± 2.8	4.5 ± 1.9	6.2 ± 2.6	4.5 ± 2.2*	5.7 ± 2.8
Lymphocyte (%)	84.0 ± 5.2	77.3 ± 5.5	81.1 ± 4.7	81.4 ± 8.4	84.7 ± 5.1	83.2 ± 4.3*	80.5 ± 5.7	76.1 ± 9.0
Platelet ($x10^4/\mu l$)	112 ± 10	114 ± 10	106 ± 11	113 ± 11	122 ± 15	113 ± 11	111 ± 12	110 ± 12
PT (second)	9.5 ± 0.2	9.6 ± 0.2	9.9 ± 0.4	9.7 ± 0.2	9.7 ± 0.3	9.5 ± 0.2	10.1 ± 0.6	10.2 ± 0.5**
APTT (second)	21.7 ± 1.2	23.8 ± 1.4	27.8 ± 2.6	20.5 ± 1.1	22.2 ± 1.5	23.3 ± 2.0	24.5 ± 4.0*	20.5 ± 2.8
Fibrinogen (mg/dl)	226 ± 17	205 ± 8	246 ± 15	230 ± 18	227 ± 15	209 ± 14	258 ± 26	224 ± 18
Female								
Number of rats examined	10	10	10	9	9	10	10	9
RBC ($x10^4/\mu l$)	806 ± 25	797 ± 45	804 ± 24	830 ± 53	813 ± 38	856 ± 30**	820 ± 31	827 ± 51
Hemoglobin (g/dl)	15.5 ± 0.5	14.4 ± 1.2	14.5 ± 0.5	15.1 ± 0.7	15.4 ± 0.4	15.4 ± 0.5*	14.7 ± 0.7	14.8 ± 0.8
Hematocrit (%)	50.4 ± 3.0	48.1 ± 3.8	49.1 ± 2.3	49.0 ± 2.9	51.1 ± 2.1	50.3 ± 1.9	50.3 ± 2.4	47.4 ± 2.5
MCV (fl)	62.5 ± 3.0	60.3 ± 2.4	61.0 ± 2.1	59.1 ± 1.5	62.8 ± 1.0	58.7 ± 2.7	61.4 ± 1.6	57.4 ± 2.2
MCH (pg)	19.2 ± 0.3	18.0 ± 0.6	18.1 ± 0.3	18.2 ± 0.5	19.0 ± 0.5	18.0 ± 0.5	18.0 ± 0.2	17.9 ± 0.4
MCHC (g/dl)	30.8 ± 1.2	29.9 ± 0.8	29.6 ± 0.8	30.8 ± 0.7	30.2 ± 0.7	30.6 ± 1.2	29.3 ± 0.6	31.2 ± 0.8
Reticulocyte (%)	0.4 ± 0.3	1.0 ± 0.4	0.6 ± 0.5	0.5 ± 0.5	0.6 ± 0.4	1.1 ± 0.5	0.8 ± 0.7	0.6 ± 0.6
WBC ($x10^2/\mu l$)	40 ± 14	47 ± 20	38 ± 10	38 ± 11	46 ± 18	34 ± 9	33 ± 12	31 ± 11
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
Eosinophil (%)	0.7 ± 0.7	0.6 ± 1.3	2.5 ± 1.9	1.9 ± 1.2	0.9 ± 0.8	0.8 ± 1.0	0.9 ± 0.7	2.0 ± 1.2
Stab neutrophil (%)	0.8 ± 0.8	0.5 ± 0.7	1.5 ± 1.1	1.3 ± 0.7	1.3 ± 1.5	0.9 ± 0.9	1.8 ± 1.8	1.3 ± 1.1
Segmented neutrophil(%)	6.4 ± 2.9	8.8 ± 4.4	9.0 ± 5.6	11.6 ± 5.0	7.2 ± 3.5	11.9 ± 5.1	10.5 ± 5.6	13.8 ± 4.5
Monocyte (%)	4.4 ± 2.0	6.2 ± 4.8	4.9 ± 2.2	8.0 ± 3.3	7.4 ± 2.9*	5.4 ± 3.1	4.9 ± 2.2	7.0 ± 3.0
Lymphocyte (%)	87.7 ± 4.6	84.7 ± 6.2	82.1 ± 7.0	77.2 ± 6.9	83.1 ± 5.1	81.0 ± 5.5	81.8 ± 7.4	75.9 ± 6.4
Platelet ($x10^4/\mu l$)	107 ± 17	104 ± 10	100 ± 6	96 ± 11	115 ± 11	105 ± 8	100 ± 10	99 ± 7
PT (second)	9.6 ± 0.4	9.5 ± 0.5	9.7 ± 0.2	9.6 ± 0.2	9.8 ± 0.7	9.4 ± 0.3	9.5 ± 0.3*	9.5 ± 0.3
APTT (second)	18.2 ± 0.8	20.8 ± 2.2	20.7 ± 2.2	15.8 ± 1.4	19.0 ± 3.3	21.3 ± 1.3	19.9 ± 2.5	15.8 ± 0.8
Fibrinogen (mg/dl)	204 ± 16	173 ± 11	176 ± 22	166 ± 16	194 ± 17	176 ± 7	190 ± 20	166 ± 11

Each value represents the mean ± S.D.

Significant difference from the control group, *$P<0.05$ and **$P<0.01$.

DISCUSSION

The general toxicity background data were collected from Crj:CD(SD)IGS rats supplied different protein contents in diets (about 18% and 20%) for 26 weeks.

In the measurement of body weight and food intake, decreased body weight gain was observed in both sexes of the CR-LPF group, whereas increased food intake was observed in both sexes of the same group. In the hematological examination performed at 4, 8, 13 and 26 weeks of the experimental period, significant differences of some parameters were observed in the CR-LPF group compared with the control group. However, these changes were inconsistent between males and females, and throughout the experimental terms. In the blood biochemical examination, decrease in glucose, triglyceride, β-lipoprotein and total bilirubin, and increase in A/G ratio and inorganic phosphate were observed in both sexes of the CR-LPF group compared with the control group. Other statistically significant changes in the blood biochemistry were inconsistent between males and females, and throughout the experimental terms. In the measurement of organ weight, decreased liver weight was observed in both sexes of the CR-LPF group compared with the control group. Other variations in organ weights were considered ascribable to decreased body weight gain in the CR-LPF group.

As described above, some significant difference were observed between low protein (CR-LPF) and control (F-2) diets. However, it is concluded that these parameters in a lower protein diet did not interference for toxicity evaluation.

ACKNOWLEDGMENT

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Table 2. Blood chemical findings in rats supplied with control or CR-LPF diets for 26 weeks

Items (unit)	Control				CR-LPF			
	4 weeks	8 weeks	13 weeks	26 weeks	4 weeks	8 weeks	13 weeks	26 weeks
Male								
Number of rats examined	10	10	10	10	10	10	10	10
GOT (IU/l)	59 ± 6	62 ± 5	67 ± 9	67 ± 10	68 ± 11	66 ± 6	67 ± 17	66 ± 25
GPT (IU/l)	24 ± 4	29 ± 1	36 ± 10	37 ± 7	27 ± 3	27 ± 4	32 ± 13	37 ± 17
ALP (IU/l)	388 ± 58	231 ± 51	173 ± 35	179 ± 52	449 ± 59*	241 ± 36	180 ± 21	194 ± 47
LDH (IU/l)	144 ± 16	133 ± 30	149 ± 42	211 ± 73	193 ± 86*	194 ± 75*	140 ± 39	161 ± 42
CPK (IU/l)	364 ± 77	335 ± 198	330 ± 150	343 ± 179	462 ± 175	461 ± 172	265 ± 127	374 ± 245
Glucose (mg/dl)	181 ± 18	183 ± 28	190 ± 18	182 ± 17	171 ± 14	174 ± 12	167 ± 13**	168 ± 15
Total protein (g/dl)	5.4 ± 0.1	5.3 ± 0.2	6.1 ± 0.3	6.1 ± 0.3	5.3 ± 0.3	5.5 ± 0.1*	5.8 ± 0.2	6.0 ± 0.3
Albumin (g/dl)	3.4 ± 0.1	3.2 ± 0.2	3.4 ± 0.2	3.3 ± 0.2	3.4 ± 0.2	3.3 ± 0.1*	3.3 ± 0.2	3.3 ± 0.2
A/G	1.33 ± 0.12	1.16 ± 0.13	1.20 ± 0.11	0.93 ± 0.08	1.49 ± 0.13**	1.33 ± 0.16*	1.16 ± 0.08	1.18 ± 0.16**
BUN (mg/dl)	13.2 ± 1.4	17.5 ± 3.4	12.4 ± 1.4	14.3 ± 1.2	15.7 ± 3.2*	16.3 ± 2.2	13.7 ± 2.9	14.3 ± 1.8
Creatinine (mg/dl)	0.21 ± 0.02	0.20 ± 0.05	0.27 ± 0.04	0.42 ± 0.10	0.23 ± 0.04	0.27 ± 0.04**	0.30 ± 0.05	0.41 ± 0.07
Total cholesterol (mg/dl)	55 ± 11	52 ± 8	70 ± 14	92 ± 25	59 ± 13	70 ± 12**	83 ± 20	93 ± 26
Triglyceride (mg/dl)	52 ± 21	46 ± 18	82 ± 37	126 ± 53	20 ± 6**	27 ± 10*	49 ± 11*	89 ± 46
Phospholipid (mg/dl)	97 ± 11	87 ± 11	111 ± 18	148 ± 29	95 ± 12	102 ± 12*	122 ± 25	142 ± 31
β-Lipoprotein (mg/dl)	47 ± 26	42 ± 19	72 ± 32	116 ± 65	8 ± 5**	17 ± 4**	27 ± 9**	47 ± 45*
Total bilirubin (mg/dl)	0.05 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.02*	0.07 ± 0.02	0.06 ± 0.02
Inorganic phosphate(mg/dl)	8.3 ± 0.6	6.8 ± 0.4	5.5 ± 0.3	5.0 ± 0.4	8.6 ± 0.7	7.4 ± 0.4**	5.9 ± 0.3**	5.1 ± 0.6
Ca (mg/dl)	9.5 ± 0.1	9.4 ± 0.2	9.6 ± 0.2	9.8 ± 0.2	9.3 ± 0.5*	9.7 ± 0.2*	9.6 ± 0.2	9.7 ± 0.2
Na (mEq/l)	144 ± 1	145 ± 2	144 ± 1	143 ± 1	141 ± 7	145 ± 1	145 ± 1	144 ± 1*
K (mEq/l)	4.67 ± 0.23	4.40 ± 0.18	4.06 ± 0.21	4.00 ± 0.15	4.58 ± 0.35	4.18 ± 0.24*	4.17 ± 0.24	4.33 ± 0.23**
Cl (mEq/l)	105 ± 1	107 ± 1	106 ± 1	104 ± 1	104 ± 5	105 ± 1**	107 ± 2	106 ± 1**
Female								
Number of rats examined	9	10	10	10	9	10	10	10
GOT (IU/l)	58 ± 9	70 ± 13	72 ± 19	76 ± 20	61 ± 5	59 ± 7*	61 ± 10	67 ± 33
GPT (IU/l)	24 ± 2	28 ± 6	34 ± 12	40 ± 10	25 ± 3	24 ± 4	28 ± 10	46 ± 27
ALP (IU/l)	262 ± 54	138 ± 39	114 ± 35	88 ± 40	229 ± 44	131 ± 20	97 ± 31	69 ± 33
LDH (IU/l)	179 ± 67	194 ± 55	168 ± 66	208 ± 99	177 ± 52	153 ± 50	159 ± 57	293 ± 127
CPK (IU/l)	449 ± 243	444 ± 315	271 ± 139	271 ± 188	301 ± 115	430 ± 195	349 ± 264	324 ± 215
Glucose (mg/dl)	160 ± 17	220 ± 72	194 ± 19	169 ± 35	146 ± 17	162 ± 15**	165 ± 16**	179 ± 25
Total protein (g/dl)	5.7 ± 0.5	6.3 ± 0.3	6.2 ± 0.4	6.5 ± 0.5	5.5 ± 0.2	6.1 ± 0.6	6.4 ± 0.5	6.4 ± 0.4
Albumin (g/dl)	3.8 ± 0.3	4.3 ± 0.3	4.1 ± 0.4	4.2 ± 0.4	3.7 ± 0.3	4.0 ± 0.5	4.3 ± 0.4	4.3 ± 0.4
A/G	1.68 ± 0.12	1.66 ± 0.26	1.83 ± 0.24	1.76 ± 0.18	1.71 ± 0.13	1.77 ± 0.14	1.84 ± 0.17	1.92 ± 0.18
BUN (mg/dl)	13.7 ± 2.4	15.6 ± 3.2	13.2 ± 1.9	15.2 ± 1.2	14.0 ± 3.1	17.9 ± 2.6	12.7 ± 1.7	13.4 ± 2.6*
Creatinine (mg/dl)	0.26 ± 0.04	0.30 ± 0.05	0.32 ± 0.04	0.43 ± 0.07	0.27 ± 0.04	0.33 ± 0.04	0.33 ± 0.03	0.36 ± 0.04*
Total cholesterol (mg/dl)	67 ± 12	73 ± 11	76 ± 7	91 ± 27	68 ± 11	81 ± 13	84 ± 18	94 ± 14
Triglyceride (mg/dl)	24 ± 18	36 ± 16	53 ± 28	119 ± 77	15 ± 8	16 ± 5**	34 ± 22	60 ± 43
Phospholipid (mg/dl)	124 ± 17	139 ± 17	144 ± 14	185 ± 44	116 ± 15	137 ± 22	153 ± 28	179 ± 24
β-Lipoprotein (mg/dl)	9 ± 7	22 ± 10	37 ± 28	87 ± 73	4 ± 2*	12 ± 1**	15 ± 13*	27 ± 24
Total bilirubin (mg/dl)	0.05 ± 0.01	0.13 ± 0.03	0.07 ± 0.02	0.07 ± 0.03	0.04 ± 0.01*	0.10 ± 0.02*	0.07 ± 0.03	0.05 ± 0.03
Inorganic phosphate(mg/dl)	7.8 ± 0.4	6.9 ± 0.7	5.3 ± 0.6	4.5 ± 0.7	8.2 ± 0.4	7.6 ± 0.6*	6.3 ± 0.9**	4.4 ± 0.9
Ca (mg/dl)	9.5 ± 0.3	10.1 ± 0.5	9.5 ± 0.4	10.0 ± 0.4	9.5 ± 0.2	9.8 ± 0.3	9.6 ± 0.3	9.8 ± 0.6
Na (mEq/l)	144 ± 1	144 ± 1	143 ± 1	143 ± 2	144 ± 2	145 ± 1	144 ± 1	144 ± 1
K (mEq/l)	4.61 ± 0.28	4.55 ± 1.43	3.98 ± 0.24	4.01 ± 0.26	4.22 ± 0.32*	4.14 ± 0.37	3.82 ± 0.35	4.13 ± 0.42
Cl (mEq/l)	105 ± 2	104 ± 2	107 ± 1	105 ± 2	106 ± 3	105 ± 2	108 ± 1*	96 ± 32

Each value represents the mean ± S.D.

Significant difference from the control group, *P<0.05 and **P<0.01.

Table 3. Absolute and relative organ weights in male rats supplied with control or CR-LPF diets for 26 weeks

Items (unit)	Control				CR-LPF			
	4 weeks	8 weeks	13 weeks	26 weeks	4 weeks	8 weeks	13 weeks	26 weeks
Absolute organ weights								
Number of rats examined	10	10	10	10	10	10	10	10
Final body weight (g)	352.6 ± 30.4	447.7 ± 15.2	542.3 ± 46.0	646.0 ± 59.9	324.4 ± 23.4*	426.6 ± 53.3	529.6 ± 32.6	602.1 ± 75.8
Brain (g)	2.02 ± 0.11	2.14 ± 0.10	2.15 ± 0.05	2.20 ± 0.04	1.99 ± 0.09	2.07 ± 0.15	2.25 ± 0.11*	2.28 ± 0.14
Pituitary (mg)	10.3 ± 1.4	12.3 ± 2.9	12.4 ± 1.7	11.1 ± 3.4	11.0 ± 2.8	12.4 ± 3.5	12.9 ± 3.8	13.4 ± 2.7
Submaxillary glands (g)	0.63 ± 0.08	0.70 ± 0.05	0.77 ± 0.08	0.74 ± 0.08	0.63 ± 0.04	0.71 ± 0.07	0.81 ± 0.09	0.76 ± 0.08
Thyroids (mg)	24.0 ± 7.6	24.0 ± 5.5	24.3 ± 7.5	24.9 ± 4.1	24.4 ± 5.2	26.6 ± 7.1	27.3 ± 6.8	27.2 ± 5.7
Thymus (g)	0.61 ± 0.11	0.48 ± 0.12	0.38 ± 0.05	0.27 ± 0.11	0.57 ± 0.07	0.44 ± 0.09	0.42 ± 0.11	0.29 ± 0.13
Heart (g)	1.21 ± 0.12	1.47 ± 0.20	1.54 ± 0.17	1.68 ± 0.16	1.31 ± 0.14	1.50 ± 0.16	1.51 ± 0.13	1.74 ± 0.28
Lungs (g)	1.32 ± 0.10	1.42 ± 0.16	1.53 ± 0.10	1.53 ± 0.14	1.28 ± 0.10	1.41 ± 0.11	1.60 ± 0.11	1.62 ± 0.13
Liver (g)	12.06 ± 1.42	12.97 ± 1.29	16.18 ± 2.41	18.27 ± 2.24	10.39 ± 0.89**	11.72 ± 1.58	14.66 ± 1.03	16.40 ± 2.70
Spleen (g)	0.69 ± 0.12	0.79 ± 0.06	0.84 ± 0.11	0.84 ± 0.10	0.67 ± 0.12	0.74 ± 0.11	0.76 ± 0.10	0.86 ± 0.12
Right kidney (g)	1.24 ± 0.08	1.36 ± 0.08	1.53 ± 0.13	1.66 ± 0.18	1.21 ± 0.09	1.27 ± 0.15	1.49 ± 0.14	1.55 ± 0.17
Left kidney (g)	1.25 ± 0.09	1.31 ± 0.10	1.54 ± 0.10	1.61 ± 0.19	1.20 ± 0.10	1.27 ± 0.14	1.53 ± 0.18	1.53 ± 0.16
Right adrenal (mg)	27.4 ± 6.6	29.7 ± 7.5	31.2 ± 5.7	26.5 ± 3.9	29.4 ± 2.8	26.9 ± 9.9	33.1 ± 9.7	25.9 ± 7.1
Left adrenal (mg)	27.1 ± 6.9	30.6 ± 8.8	29.8 ± 4.7	27.8 ± 4.3	28.0 ± 4.2	28.5 ± 8.7	34.7 ± 7.7	29.2 ± 5.9
Right testis (g)	1.52 ± 0.09	1.65 ± 0.12	1.66 ± 0.19	1.82 ± 0.11	1.55 ± 0.11	1.69 ± 0.10	1.70 ± 0.17	1.76 ± 0.16
Left testis (g)	1.52 ± 0.11	1.62 ± 0.11	1.70 ± 0.13	1.87 ± 0.18	1.56 ± 0.08	1.66 ± 0.07	1.68 ± 0.15	1.75 ± 0.14
Prostate (g)	0.85 ± 0.21	1.26 ± 0.26	1.39 ± 0.27	1.17 ± 0.44	0.96 ± 0.18	1.27 ± 0.28	1.33 ± 0.17	1.18 ± 0.27
Seminal vesicle (g)	0.85 ± 0.27	1.23 ± 0.37	1.43 ± 0.32	1.63 ± 0.32	0.89 ± 0.18	1.12 ± 0.29	1.52 ± 0.40	1.66 ± 0.14
Relative organ weights								
Number of rats examined	10	10	10	10	10	10	10	10
Brain (g%)	0.57 ± 0.04	0.48 ± 0.02	0.40 ± 0.03	0.34 ± 0.03	0.62 ± 0.04*	0.49 ± 0.05	0.43 ± 0.02*	0.38 ± 0.05*
Pituitary (mg%)	2.96 ± 0.58	2.75 ± 0.63	2.29 ± 0.27	1.73 ± 0.55	3.40 ± 0.84	2.98 ± 1.02	2.44 ± 0.70	2.25 ± 0.51*
Submaxillary glands(g%)	0.18 ± 0.02	0.16 ± 0.01	0.14 ± 0.02	0.12 ± 0.02	0.19 ± 0.02	0.17 ± 0.02	0.15 ± 0.01	0.13 ± 0.02
Thyroids (mg%)	6.86 ± 2.21	5.38 ± 1.30	4.56 ± 1.66	3.88 ± 0.72	7.57 ± 1.78	6.34 ± 1.91	5.19 ± 1.41	4.59 ± 1.17
Thymus (g%)	0.17 ± 0.03	0.11 ± 0.03	0.07 ± 0.01	0.04 ± 0.01	0.18 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.05 ± 0.02
Heart (g%)	0.34 ± 0.03	0.33 ± 0.04	0.29 ± 0.02	0.26 ± 0.03	0.40 ± 0.03**	0.36 ± 0.04	0.28 ± 0.02	0.29 ± 0.03
Lungs (g%)	0.38 ± 0.03	0.32 ± 0.04	0.29 ± 0.03	0.24 ± 0.01	0.40 ± 0.02	0.33 ± 0.03	0.30 ± 0.02	0.27 ± 0.02**
Liver (g%)	3.42 ± 0.18	2.89 ± 0.22	2.98 ± 0.25	2.83 ± 0.17	3.20 ± 0.16*	2.75 ± 0.15	2.77 ± 0.13*	2.72 ± 0.22
Spleen (g%)	0.20 ± 0.03	0.18 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	0.21 ± 0.03	0.17 ± 0.02	0.15 ± 0.02	0.14 ± 0.02
Right kidney (g%)	0.35 ± 0.03	0.31 ± 0.02	0.28 ± 0.03	0.26 ± 0.02	0.38 ± 0.02	0.30 ± 0.03	0.28 ± 0.03	0.26 ± 0.02
Left kidney (g%)	0.36 ± 0.02	0.29 ± 0.02	0.29 ± 0.03	0.25 ± 0.03	0.37 ± 0.03	0.30 ± 0.02	0.29 ± 0.04	0.26 ± 0.02
Right adrenal (mg%)	7.81 ± 1.97	6.62 ± 1.60	5.78 ± 1.03	4.12 ± 0.59	9.11 ± 1.13	6.42 ± 2.66	6.25 ± 1.71	4.35 ± 1.21
Left adrenal (mg%)	7.71 ± 1.92	6.81 ± 1.84	5.58 ± 1.26	4.30 ± 0.51	8.64 ± 1.20	6.72 ± 2.07	6.54 ± 1.24	4.84 ± 0.79
Right testis (g%)	0.43 ± 0.02	0.37 ± 0.02	0.31 ± 0.05	0.28 ± 0.03	0.48 ± 0.05	0.40 ± 0.05	0.32 ± 0.04	0.30 ± 0.04
Left testis (g%)	0.43 ± 0.03	0.36 ± 0.02	0.32 ± 0.04	0.29 ± 0.04	0.48 ± 0.05*	0.40 ± 0.05	0.32 ± 0.04	0.29 ± 0.04
Prostate (g%)	0.24 ± 0.06	0.28 ± 0.06	0.26 ± 0.05	0.18 ± 0.07	0.30 ± 0.05*	0.30 ± 0.05	0.25 ± 0.04	0.20 ± 0.04
Seminal vesicle (g%)	0.24 ± 0.09	0.28 ± 0.08	0.27 ± 0.07	0.26 ± 0.06	0.28 ± 0.08	0.27 ± 0.08	0.29 ± 0.07	0.28 ± 0.04

Each value represents the mean ± S.D.

Significant difference from the control group, *$P<0.05$ and **$P<0.01$.

Table 4. Absolute and relative organ weights in female rats supplied with control or CR-LPF diets for 26 weeks

Items (unit)	Control				CR-LPF			
	4 weeks	8 weeks	13 weeks	26 weeks	4 weeks	8 weeks	13 weeks	26 weeks
Absolute organ weights								
Number of rats examined	10	10	10	10	10	10	10	10
Final body weight (g)	211.7 ± 12.3	274.6 ± 20.4	306.5 ± 41.6	343.6 ± 46.5	205.6 ± 21.6	244.0 ± 14.2**	288.2 ± 21.1	323.5 ± 21.6
Brain (g)	1.90 ± 0.07	1.96 ± 0.10	2.01 ± 0.13	2.06 ± 0.07	1.86 ± 0.07	1.97 ± 0.08	2.04 ± 0.11	2.09 ± 0.09
Pituitary (mg)	11.6 ± 2.9	12.6 ± 2.4	15.6 ± 2.5	18.6 ± 4.1	9.6 ± 2.6	13.5 ± 2.0	15.5 ± 3.7	17.9 ± 6.5
Submaxillary glands (g)	0.45 ± 0.05	0.46 ± 0.06	0.53 ± 0.11	0.49 ± 0.04	0.44 ± 0.03	0.47 ± 0.05	0.51 ± 0.05	0.50± 0.07
Thyroids (mg)	20.5 ± 4.6	21.4 ± 5.0	23.8 ± 5.6	28.0 ± 6.1	16.7 ± 3.6	23.4 ± 6.1	22.9 ± 5.5	26.0 ± 6.2
Thymus (g)	0.52 ± 0.05	0.44 ± 0.08	0.33 ± 0.06	0.20 ± 0.07	0.45 ± 0.08*	0.40 ± 0.07	0.31 ± 0.04	0.16 ± 0.04
Heart (g)	0.88 ± 0.09	0.98 ± 0.08	1.04 ± 0.12	1.11 ± 0.11	0.87 ± 0.10	0.94 ± 0.08	0.96 ± 0.04	1.07 ± 0.12
Lungs (g)	1.05 ± 0.07	1.10 ± 0.11	1.18 ± 0.12	1.21 ± 0.05	1.02 ± 0.07	1.12 ± 0.13	1.13 ± 0.05	1.13 ± 0.09*
Liver (g)	7.17 ± 0.48	9.19 ± 1.75	8.72 ± 1.07	9.99 ± 1.62	6.74 ± 0.87	7.12 ± 0.52**	7.93 ± 0.87	8.67 ± 0.65
Spleen (g)	0.47 ± 0.03	0.49 ± 0.06	0.52 ± 0.07	0.60 ± 0.11	0.48 ± 0.09	0.49 ± 0.06	0.51 ± 0.06	0.51 ± 0.09
Right kidney (g)	0.80 ± 0.06	0.91 ± 0.10	0.94 ± 0.07	0.97 ± 0.07	0.82 ± 0.11	0.81 ± 0.06*	0.91 ± 0.08	0.93 ± 0.09
Left kidney (g)	0.80 ± 0.08	0.91 ± 0.10	0.89 ± 0.05	0.95 ± 0.09	0.79 ± 0.10	0.80 ± 0.05*	0.90 ± 0.08	0.94 ± 0.10
Right adrenal (mg)	33.0 ± 4.4	34.3 ± 5.3	36.3 ± 7.0	35.3 ± 8.0	32.1 ± 7.1	34.0 ± 9.9	33.9 ± 6.0	29.2 ± 4.3*
Left adrenal (mg)	32.2 ± 3.8	34.1 ± 5.6	38.3 ± 9.7	34.9 ± 8.0	32.5 ± 7.4	32.4 ± 9.4	37.2 ± 6.2	31.5 ± 6.3
Right ovary (mg)	53.5 ± 9.8	54.1 ± 17.1	56.4 ± 13.0	41.0 ± 12.4	46.7 ± 6.8	49.8 ± 16.9	57.5 ± 13.3	45.0 ± 8.9
Left ovary (mg)	47.4 ± 12.5	48.9 ± 15.2	56.6 ± 13.0	38.6 ± 11.5	45.9 ± 4.2	48.3 ± 20.5	56.2 ± 18.6	46.0 ± 10.5
Uterus (g)	0.48 ± 0.20	0.49 ± 0.16	0.55 ± 0.06	0.70 ± 0.09	0.50 ± 0.15	0.58 ± 0.26	0.70 ± 0.23	0.53 ± 0.07**
Relative organ weights								
Number of rats examined	10	10	10	10	10	10	10	10
Brain (g%)	0.90 ± 0.05	0.72 ± 0.05	0.67 ± 0.09	0.61 ± 0.10	0.91 ± 0.10	0.81 ± 0.02**	0.71 ± 0.04	0.65 ± 0.05
Pituitary (mg%)	5.51 ± 1.45	4.60 ± 0.91	5.20 ± 1.21	5.38 ± 0.81	4.68 ± 1.26	5.55 ± 0.84*	5.39 ± 1.32	5.56 ± 2.11
Submaxillary glands(g%)	0.21 ± 0.02	0.17 ± 0.02	0.18 ± 0.05	0.15 ± 0.01	0.21 ± 0.03	0.19 ± 0.02*	0.18 ± 0.02	0.15 ± 0.02
Thyroids (mg%)	9.71 ± 2.20	7.76 ± 1.49	8.02 ± 2.86	8.24 ± 1.93	8.18 ± 1.83	9.60 ± 2.63	7.89 ± 1.45	8.07 ± 1.91
Thymus (g%)	0.24 ± 0.02	0.16 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	0.22 ± 0.03*	0.17 ± 0.02	0.11 ± 0.02	0.05 ± 0.01
Heart (g%)	0.42 ± 0.03	0.36 ± 0.03	0.34 ± 0.03	0.33 ± 0.05	0.42 ± 0.06	0.39 ± 0.02*	0.34 ± 0.03	0.33 ± 0.03
Lungs (g%)	0.50 ± 0.03	0.40 ± 0.04	0.39 ± 0.05	0.36 ± 0.05	0.50 ± 0.03	0.46 ± 0.05**	0.40 ± 0.02	0.35 ± 0.04
Liver (g%)	3.39 ± 0.20	3.33 ± 0.43	2.85 ± 0.13	2.90 ± 0.19	3.28 ± 0.18	2.92 ± 0.11**	2.75 ± 0.18	2.69 ± 0.18*
Spleen (g%)	0.22 ± 0.01	0.18 ± 0.02	0.17 ± 0.03	0.18 ± 0.04	0.23 ± 0.03	0.20 ± 0.02	0.18 ± 0.02	0.16 ± 0.03
Right kidney (g%)	0.38 ± 0.01	0.33 ± 0.03	0.31 ± 0.04	0.29 ± 0.03	0.40 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	0.29 ± 0.03
Left kidney (g%)	0.38 ± 0.02	0.33 ± 0.03	0.30 ± 0.03	0.28 ± 0.03	0.38 ± 0.03	0.33 ± 0.01	0.31 ± 0.02	0.29 ± 0.03
Right adrenal (mg%)	15.63 ± 2.16	12.53 ± 1.99	12.00 ± 2.62	10.38 ± 2.66	15.81 ± 3.84	13.87 ± 3.71	11.77 ± 2.02	9.01 ± 1.07
Left adrenal (mg%)	15.23 ± 1.73	12.42 ± 1.79	12.68 ± 3.15	10.28 ± 2.59	15.94 ± 3.71	13.27 ± 3.80	12.93 ± 2.00	9.75 ± 1.91
Right ovary (mg%)	25.20 ± 4.01	19.78 ± 6.81	18.94 ± 5.81	12.33 ± 4.52	22.84 ± 3.61	20.46 ± 7.07	19.95 ± 4.30	13.89 ± 2.41
Left ovary (mg%)	22.42 ± 5.90	17.85 ± 5.81	18.93 ± 5.96	11.61 ± 4.30	22.48 ± 2.40	19.83 ± 8.45	19.52 ± 6.09	14.20 ± 2.98
Uterus (g%)	0.23 ± 0.10	0.18 ± 0.06	0.18 ± 0.02	0.21 ± 0.03	0.24 ± 0.07	0.24 ± 0.11	0.25 ± 0.08	0.17 ± 0.02**

Each value represents the mean ± S.D.
Significant difference from the control group, *$P<0.05$ and **$P<0.01$.

A Comparison Between the Original and International Genetic Standard Charles River (UK) Designations of Sprague-Dawley Rat, at the 13-week Time Point

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ABSTRACT. At Huntingdon Life Sciences, data obtained from the first 13 weeks of toxicity or tumorigenicity studies using the original or International Genetic Standard (IGS) Charles River (UK) designations of Sprague-Dawley rat were compared. These comparisons were made using control group data collected from 19 original and 20 IGS gang housed dietary studies given low protein maintenance diet (Special Diets Services Ltd). Bodyweight, food consumption, laboratory investigations (selected haematology, biochemistry and urinalysis parameters) and organ weights were analysed to identify any potential difference between the two strain designations. Analysis of the results obtained at the 13-week time point (when the rats were approximately 19 weeks of age) revealed that there were no statistically significant differences in bodyweight gain or food consumption between the two strain designations. However, the IGS male and female rats did show a marginally lower bodyweight growth pattern over the first 13 weeks. There were only minor differences in the laboratory investigation parameters and organ weights between the two strain designations. It can therefore be concluded that there were no remarkable differences in the parameters examined for the two strains designations of Sprague-Dawley rat at the 13-week time point. –Key words: Background data, Crl:CD® BR (VAF) IGS rat, Week 13.

CD (SD) IGS-1998: 50-56

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 studies using Charles River (UK) original or IGS designations of Sprague-Dawley rat were compared. These comparisons were made using control group data collected for the first 13 weeks of 19 original and 20 IGS gang housed dietary studies given low protein maintenance diet (Special Diets Services Ltd). Bodyweight, food consumption, laboratory investigations (selected haematology, biochemistry and urinalysis parameters) and organ weights were analysed to identify any potential difference between the two strain designations. In addition, at these laboratories, a comparison of data obtained from 104-week carcinogenicity studies using Charles River original or IGS Sprague-Dawley rats has been performed [1, 2].

MATERIALS AND METHODS

Animals: Male and female Sprague-Dawley Crl:CD® BR (VAF) rats obtained from Charles River breeding laboratories in the UK and maintained as control rats in toxicity or 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°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 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 rats via water bottles.

Study Design: The studies reviewed were the control groups of gang-housed dietary administered toxicity or tumorigenicity studies that started between 1994 and 1995 (19 studies, 645 males and 645 females) for the original strain designation of rat and between 1996 and 1998 (20 studies, 465 males and 465 females) for the IGS strain of rat.

Data presentation and statistical analysis: The bodyweight growth pattern over the first 13 weeks, comparing the original and IGS strain designations of rat, is presented graphically in Figure 1 and the weekly data are presented in Table 1. The bodyweight gain values (Weeks 0 to 13) and food consumption values (total, Weeks 1 to 13) were also compared and are presented in Table 2.

Laboratory investigations at Week 13 (haematology, biochemistry and urinalysis, selected parameters) are presented in Tables 4, 5 and 6. Standard laboratory methodologies were followed in the analysis of these parameters. The analysers used are indicated in the footnotes to the relevant tables.

Organ weights (absolute values, selected parameters) at Week 14 are presented in Table 7.

In the laboratory investigations and organ weights, the differences between the two strain designations are also presented graphically as a percentage difference from the original strain designation (Figures 2 to 5). A similar comparison is also presented for organ weights relative to bodyweight (Figure 6).

For bodyweight (absolute and gain), food consumption, laboratory parameters, and organ weights, comparisons based on a 't' distribution were made following analysis of variance to compared the potential differences between the two strain designations and the results are presented in the relevant tables.

RESULTS AND DISCUSSION

Review of the bodyweight growth pattern over the first 13 weeks (Figure 1 and Table 1) showed a marginally lower pattern for male and female IGS rats in comparison with the original strain. Statistical significance was demonstrated from

Table 1. Bodyweight (g) - Weeks 0 to 13

Week	Sex	Original/1994-95			IGS/1996-98			Significance
		Mean	SD	n	Mean	SD	n	
0	Males	212	28.2	19	200	34.1	20	ns
	Females	169	14.4	19	161	17.9	20	ns
1	Males	269	27.0	19	254	29.6	20	ns
	Females	193	14.3	19	187	14.7	20	ns
2	Males	318	25.9	19	304	25.0	20	ns
	Females	214	14.1	19	207	14.0	20	ns
3	Males	358	25.6	19	345	20.3	20	ns
	Females	232	14.7	19	225	12.8	20	ns
4	Males	393	26.2	19	377	17.0	20	P<0.05
	Females	246	14.3	19	240	11.8	20	ns
5	Males	422	27.6	19	404	17.1	20	P<0.05
	Females	259	14.9	19	251	14.0	20	ns
6	Males	449	29.8	19	429	15.0	20	P<0.05
	Females	269	16.7	19	261	12.4	20	ns
7	Males	471	29.8	19	450	16.4	20	P<0.01
	Females	278	15.7	19	270	13.0	20	ns
8	Males	492	31.2	19	468	16.2	20	P<0.01
	Females	285	15.9	19	276	10.7	20	P<0.05
9	Males	510	31.7	19	484	16.8	20	P<0.01
	Females	293	16.4	19	282	11.8	20	P<0.05
10	Males	525	33.2	19	500	17.0	20	P<0.01
	Females	299	16.6	19	288	12.8	20	P<0.05
11	Males	537	35.4	19	514	16.2	20	P<0.05
	Females	303	16.5	19	294	12.4	20	ns
12	Males	551	36.6	19	526	15.8	20	P<0.01
	Females	308	18.4	19	298	11.9	20	P<0.05
13	Males	555	37.1	19	527	21.9	20	P<0.01
	Females	307	18.2	19	296	11.3	20	P<0.05

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of studies.

Week 4 in males and from Week 8 in females.

For bodyweight gain and total food consumption over the first 13 weeks, there were no statistical differences between the two strain designations (Table 2).

Review of the haematological parameters indicated higher platelet counts in male IGS rats and higher total white blood cell counts in female IGS rats in comparison to the original strain values (Figure 2, Table 3). However, with these parameters, the number of values available for the original strain were restricted due to a change in analyser at this time.

In the biochemical parameters, male IGS rats showed higher Urea Nitrogen values, and female rats showed slightly lower glucose values and slightly higher values for glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, inorganic phosphorus and cholesterol in comparison with the original strain values (Figure 3, Table 4).

In the urinalysis parameters, the urinary volumes for male and female IGS rats, and the protein concentrations for female rats were slightly lower in comparison to the original strain values (Figure 4, Table 5).

The absolute organ weight values for IGS rats were, in general, lower than the respective values for the original strain rats. This was mainly the result of the lower terminal bodyweights recorded for the male and female IGS rats in comparison to the original strain values (Figure 5, Table 6). When analysed relative to bodyweight, the differences largely disappeared for organs that tend to follow bodyweight, such as liver weights (Figure 6).

Analysis of the results obtained at the 13-week time point (when the rats were approximately 19 weeks of age) revealed that there were no statistically significant differences in bodyweight gain or food consumption between the two strain designations. However, the IGS male and female rats did show a marginally lower bodyweight growth pattern over the first 13 weeks. There were only relatively minor differences in the laboratory investigation parameters between the two strain

Table 2. Bodyweight gain (g) and food consumption values (g/rat/week)

Parameter	Weeks	Sex	Original/1994-95			IGS/1996-1998			Significance
			Mean	SD	n	Mean	SD	n	
Bodyweight gain	0 to 13	Males	344	34.6	19	327	36.4	20	ns
(g)		Females	139	14.5	19	135	15.0	20	ns
Food consumption	1 to 13	Males	210	9.1	19	205	9.7	20	ns
(g/rat/week)		Females	152	7.3	19	153	5.7	20	ns

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of studies.

Fig. 1. Bodyweight growth pattern - Weeks 0 to 13

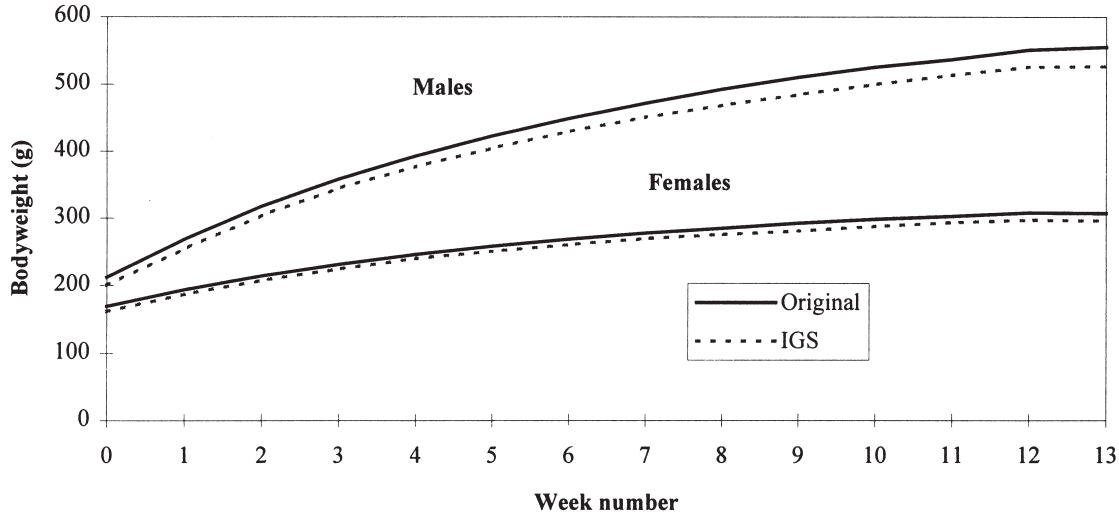


Table 3. Haematology

Parameter	Units	Sex	Original/1994-95			IGS/1996-98			Significance
(a)			Mean	SD	n	Mean	SD	n	
Packed cell volume (PCV)	%	Males	45.8	2.24	29	46.3	2.24	90	ns
		Females	44.2	1.59	29	44.7	1.99	90	ns
Haemoglobin (Hb)	g/dl	Males	15.74	0.678	29	15.91	0.627	90	ns
		Females	15.47	0.537	29	15.50	0.578	90	ns
Red blood cells (RBC)	10^{12}/l	Males	8.82	0.462	29	8.79	0.381	90	ns
		Females	8.27	0.348	29	8.22	0.405	90	ns
Mean cell haemoglobin	g/dl	Males	34.38	0.608	29	34.40	1.049	90	ns
concentration (MCHC)		Females	34.98	0.508	29	34.71	0.943	90	ns
Mean cell volume (MCV)	fl	Males	51.9	1.43	29	52.7	1.80	90	P<0.05
		Females	54.5	1.42	29	54.4	1.70	90	P<0.05
White blood cells, total (WBC)	10^9/l	Males	13.96	2.422	20	14.43	3.099	90	ns
		Females	7.67	1.633	19	9.07	2.802	90	P<0.05
Platelets	10^9/l	Males	904.7	190.86	20	1074.5	156.41	90	P<0.001
		Females	981.3	114.62	19	1065.7	130.09	90	P<0.05
Thrombotest (TT)	sec	Males	24.9	1.63	59	24.2	1.99	79	P<0.05
[4]		Females	20.7	1.04	46	20.3	1.19	80	ns

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of values.
(a) Parameters analysed using a Bayer-Technicon HIE haematology analyser, except Thrombotest [4].

Fig. 2. Haematology - Week 13

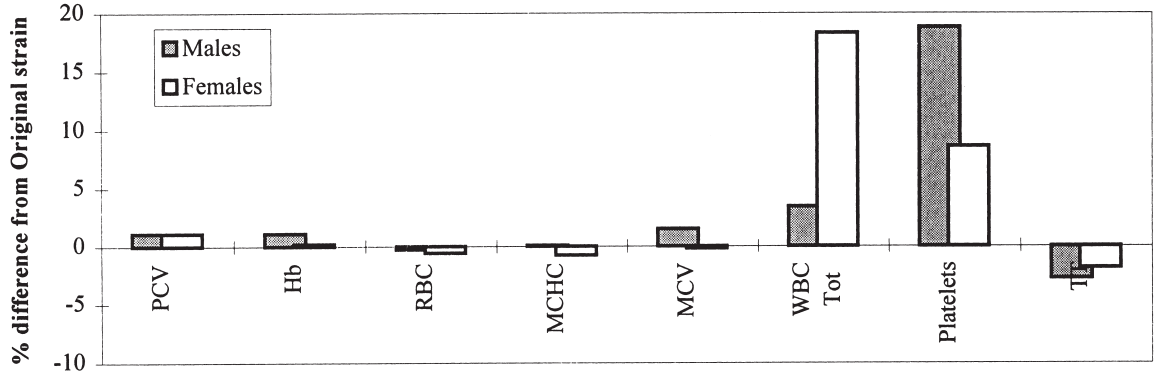


Fig. 3. Biochemistry - Week 14

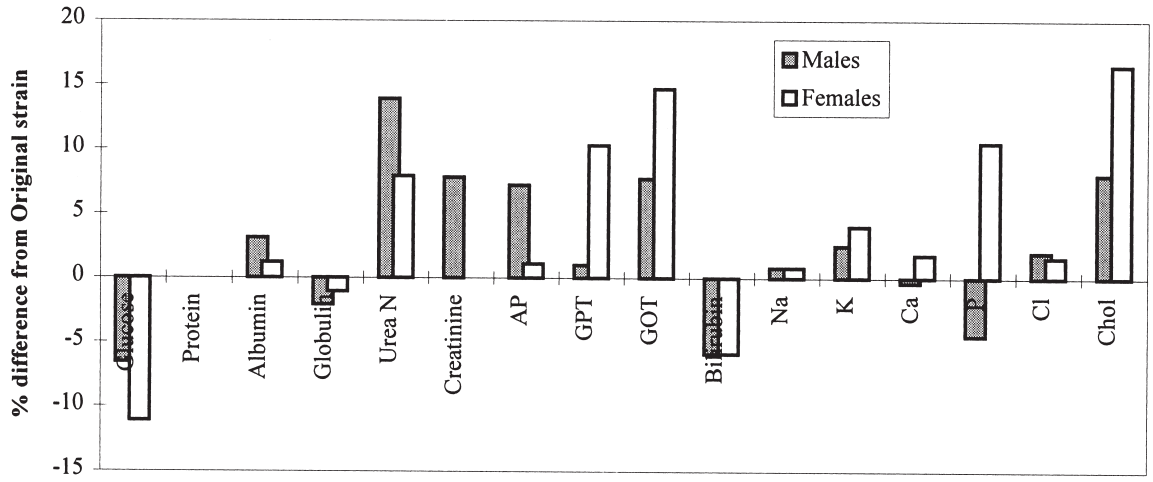


Fig. 4. Urinalysis - Week 13

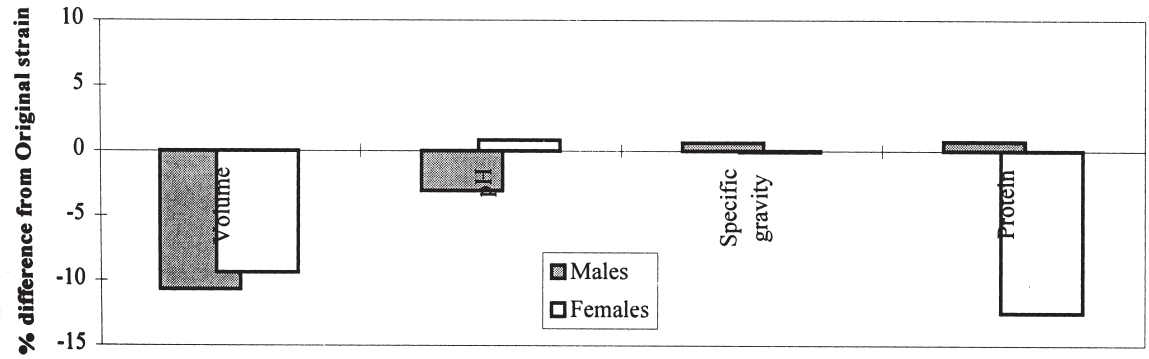


Table 4. Biochemistry

Parameter	Units	Sex	Original/1994-95			IGS/1996-98			Significance
(b)			Mean	SD	n	Mean	SD	n	
Glucose	mg/dl	Males	121.6	14.15	80	113.6	16.82	90	P<0.01
		Females	123.2	12.58	70	109.5	13.88	90	P<0.001
Protein (Total)	g/dl	Males	6.73	0.357	80	6.73	0.284	90	ns
		Females	7.01	0.428	70	7.01	0.412	90	ns
Albumin	g/dl	Males	2.86	0.126	80	2.95	0.165	90	P<0.001
		Females	3.31	0.228	70	3.35	0.265	90	ns
Globulin	g/dl	Males	3.86	0.329	80	3.78	0.248	90	ns
		Females	3.70	0.315	70	3.66	0.278	90	ns
Urea Nitrogen	mg/dl	Males	12.2	1.71	100	13.9	2.85	90	P<0.001
(Urea N)		Females	15.2	2.89	90	16.4	3.53	90	P<0.05
Creatinine	mg/dl	Males	0.51	0.061	100	0.55	0.067	90	P<0.001
		Females	0.60	0.076	90	0.60	0.063	90	ns
Alkaline Phosphatase	mU/ml	Males	164.3	41.02	80	176.1	30.74	90	P<0.0
(AP)		Females	94.5	19.63	70	95.5	20.71	90	ns
Glutamic-pyruvic	mU/ml	Males	29.1	5.71	90	29.4	5.52	100	ns
transaminase (GPT)		Females	25.3	8.97	80	27.9	11.59	100	ns
Glutamic-oxaloacetic	mU/ml	Males	57.2	8.69	90	61.6	10.80	100	P<0.01
transaminase (GOT)		Females	55.8	14.42	80	64.0	19.75	100	P<0.01
Bilirubin	mg/dl	Males	0.17	0.062	60	0.16	0.067	69	ns
		Females	0.17	0.060	59	0.16	0.065	77	ns
Sodium (Na)	mEq/l	Males	143.9	1.92	80	145.1	1.18	90	P<0.001
		Females	143.4	1.41	70	144.6	1.31	90	P<0.001
Potassium (K)	mEq/l	Males	3.55	0.258	80	3.64	0.277	90	P<0.05
		Females	3.28	0.325	70	3.41	0.316	90	P<0.05
Calcium (Ca)	mEq/l	Males	5.49	0.154	80	5.47	0.202	90	ns
		Females	5.42	0.183	70	5.52	0.218	90	P<0.01
Phosphorus (P)	mEq/l	Males	3.96	0.325	80	3.78	0.272	90	P<0.001
		Females	2.95	0.457	70	3.26	0.498	90	P<0.001
Chloride (Cl)	mEq/l	Males	100.4	2.09	80	102.4	1.73	90	P<0.001
		Females	101.8	2.00	70	103.4	1.83	90	P<0.001
Cholesterol (Chol)	mg/dl	Males	65.4	15.18	90	70.6	14.47	90	P<0.05
		Females	74.6	14.99	80	86.9	15.30	90	P<0.001

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of values.
(b) Parameters analysed using a Hitachi 737 analyser.

Table 5. Urinalysis

Parameter	Units	Sex	Original/1994-95			IGS/1996-98			Significance
			Mean	SD	n	Mean	SD	n	
Volume	ml	Males	6.94	2.589	30	6.20	2.368	80	ns
		Females	3.51	1.672	80	3.18	1.454	80	ns
pH	-	Males	7.06	0.321	30	6.84	0.430	80	P<0.05
(c)		Females	6.26	0.224	30	6.31	0.314	80	ns
Specific	-	Males	1034.3	5.32	30	1040.3	11.29	80	P<0.01
gravity(d)		Females	1045.9	16.24	30	1044.8	13.36	80	ns
Protein	mg/dl	Males	190.2	106.79	30	191.5	137.69	80	ns
(e)		Females	85.4	36.12	29	74.7	22.78	80	ns

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of values
(c) By pH meter, (d) By refractometry, compared to water with a value of 1000, (e) By Roche Cobas Centigugal Analyser using modified method [3].

Table 6. Organ weights (Absolutes)

Parameter	Units	Sex	Original/1994-95			IGS/1996-97			Significance
			Mean	SD	n	Mean	SD	n	
Terminal	g	Males	550.5	64.13	120	504.4	49.41	80	P<0.001
bodyweight		Females	307.5	27.55	99	295.0	26.71	79	P<0.01
Brain	g	Males	2.13	0.131	110	2.07	0.086	80	P<0.001
		Females	1.97	0.083	99	1.93	0.078	79	P<0.01
Pituitary	mg	Males	14.42	2.373	110	13.65	2.404	80	P<0.05
		Females	16.14	2.741	99	16.36	2.771	79	ns
Thyroids, both	mg	Males	23.311	4.4434	100	21.504	7.7402	80	ns
		Females	17.904	3.4087	99	17.587	3.4233	78	ns
Heart	g	Males	1.625	0.1960	120	1.504	0.1557	80	P<0.001
		Females	1.069	0.1027	99	1.021	0.0853	79	P<0.01
Lungs	g	Males	1.863	0.2373	70	1.761	0.1889	40	P<0.05
		Females	1.496	0.2187	60	1.352	0.1602	39	P<0.001
Liver	g	Males	22.46	3.545	120	20.16	2.489	80	P<0.001
		Females	11.84	1.549	98	11.30	1.301	79	P<0.05
Spleen	g	Males	0.956	0.1814	120	0.814	0.1185	80	P<0.001
		Females	0.621	0.0877	98	0.563	0.0892	79	P<0.001
Kidneys, both	g	Males	3.689	0.5372	120	3.375	0.4097	80	P<0.001
		Females	2.235	0.2422	99	2.184	0.2449	79	ns
Adrenals, both	mg	Males	57.42	9.503	110	58.96	12.584	80	ns
		Females	70.80	10.601	98	71.82	11.180	79	ns
Prostate	g	Males	1.110	0.1778	60	1.016	0.2209	70	P<0.01
Testis, left	g	Males	1.779	0.2059	60	1.740	0.2111	80	ns
Testis, right			1.797	0.2103	60	1.761	0.2116	80	ns
Epididymis, L	g	Males	0.633	0.0677	50	0.634	0.0860	80	ns
Epididymis, R			0.658	0.0763	50	0.652	0.0765	80	ns
Uterus	g	Females	0.600	0.1961	89	0.606	0.2318	79	ns
Ovaries, both	mg	Females	92.6	18.19	99	83.5	11.51	79	P<0.001

ns Not statistically significant (P>0.05), SD Standard deviation, n Number of values, L Left, R Right.

Fig. 5. Organ weights (absolutes) - Week 14

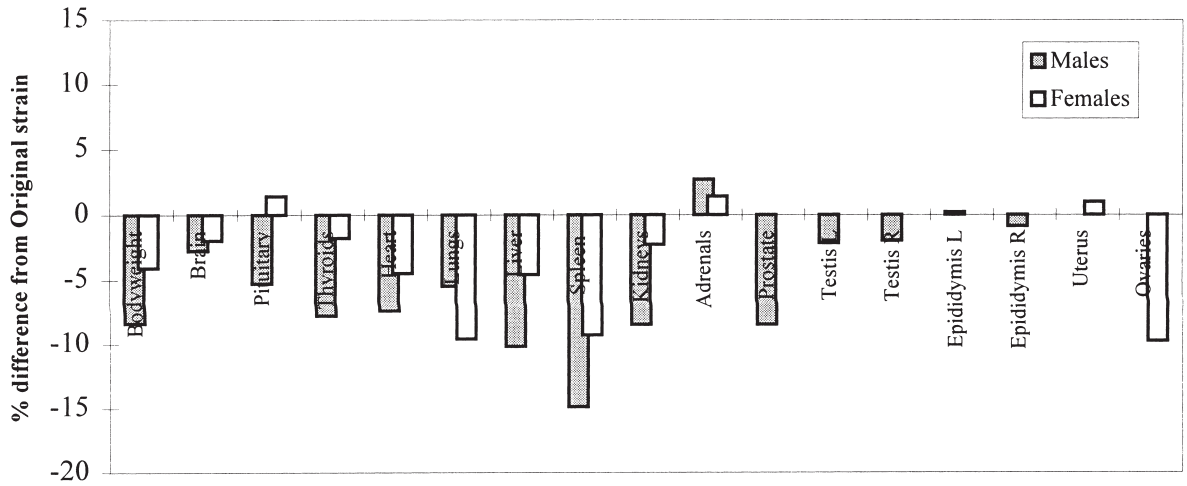
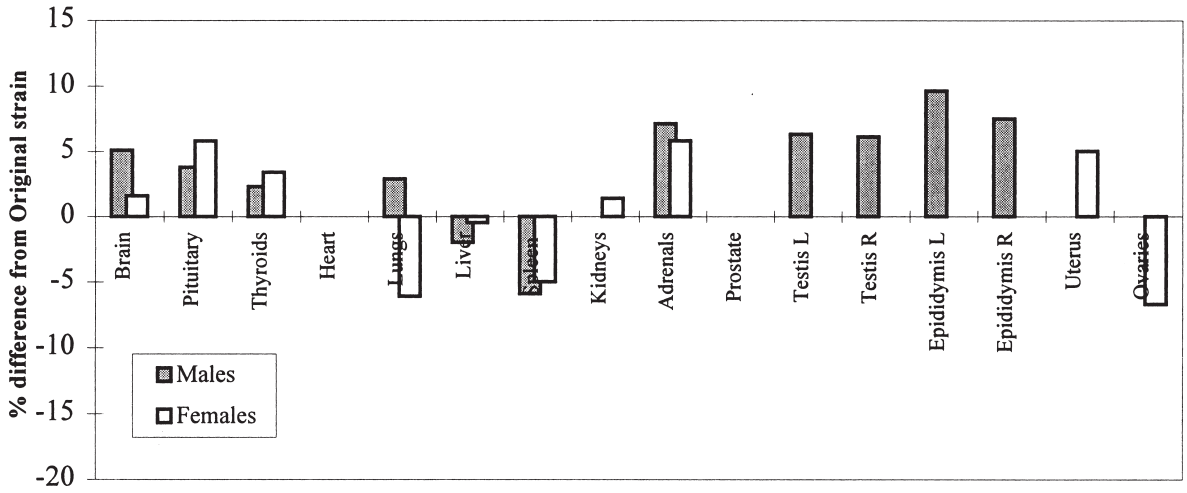


Fig. 6. Organ weights (relative to bodyweight) - Week 14



designations, especially considering that the fact that the comparisons were not contemporary in that the IGS strain superseded the original strain of rat. In general, the absolute organ weights for the IGS rats were lower than the values obtained for the original strain. This was mainly attributed to the lower terminal bodyweights recorded for the IGS male and female rats. It can therefore be concluded that there were no remarkable differences in the parameters examined for the two strains designations of Sprague-Dawley rat at the 13-week time point.

ACKNOWLEDGEMENTS. Thanks are due to John L Dyke of the Department of Statistics for assistance in generating the data and Matthew D. Saunders of the Department of Toxicological Sciences for assistance in analysis of the data. Thanks are also due Robert J. Harling and Frank W. Ross for their Sponsorship of this project, and Colin J Hardy for reviewing the manuscript.

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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

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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. 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 four 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: Crl:CD® BR (VAF) IGS rat, food consumption, mortality, tumorigenicity studies.

CD (SD) IGS-1998: 57-63

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. A comparison of data obtained from 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, at the 13-week time point [1]. However, the subject of this ongoing review 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 1998 [2]. This information has also been included in this review. Previous reviews of the longevity of the Sprague-Dawley rat at these laboratories have been published for studies completed during 1985 to 1992 [3], 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 [4].

At these laboratories, a total of 4 IGS rat studies have completed 104 weeks and there are currently 15 studies ongoing, of which, 10 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.

Study Design: The studies reviewed were dietary or oral gavage administered tumorigenicity studies that terminated between 1987 and 1998. The number of control groups reviewed were 70 completed studies for the original strain and 4 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 are compared with the original strain designation studies (1993-95 and 1996-97). The ongoing IGS rat studies comprised 10 studies at Week 52 reducing to 4

studies at Week 104.

Mean terminal (Week 104) percentage mortality values are presented over selected time periods for studies completed from 1987 to 1998, 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 1998 (Figures 4 and 5). A regression analysis of mortality against time [5] 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, 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 6 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 7 for all studies. The IGS rat studies, ongoing or completed in 1998, 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 2 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

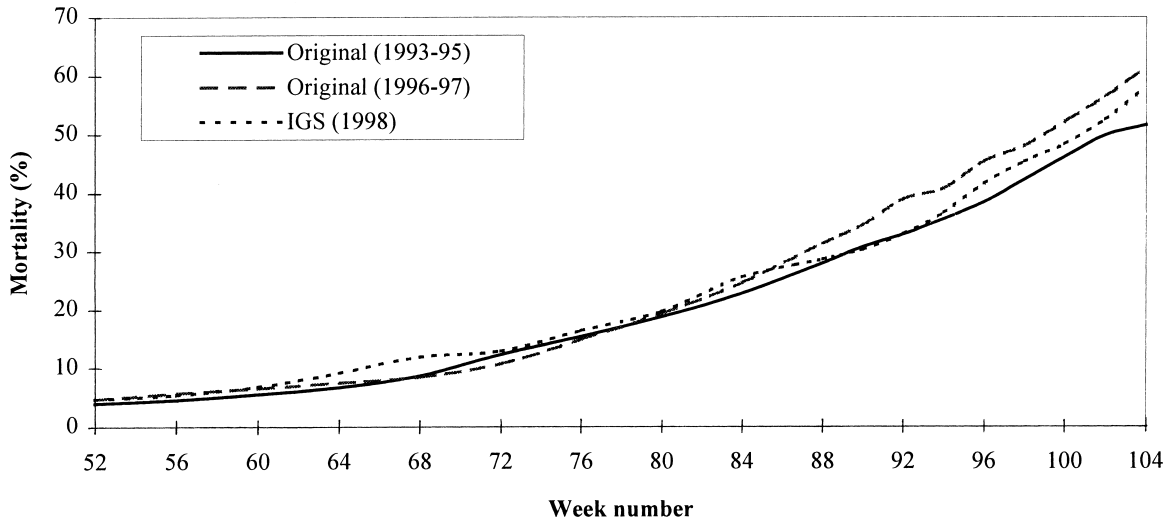
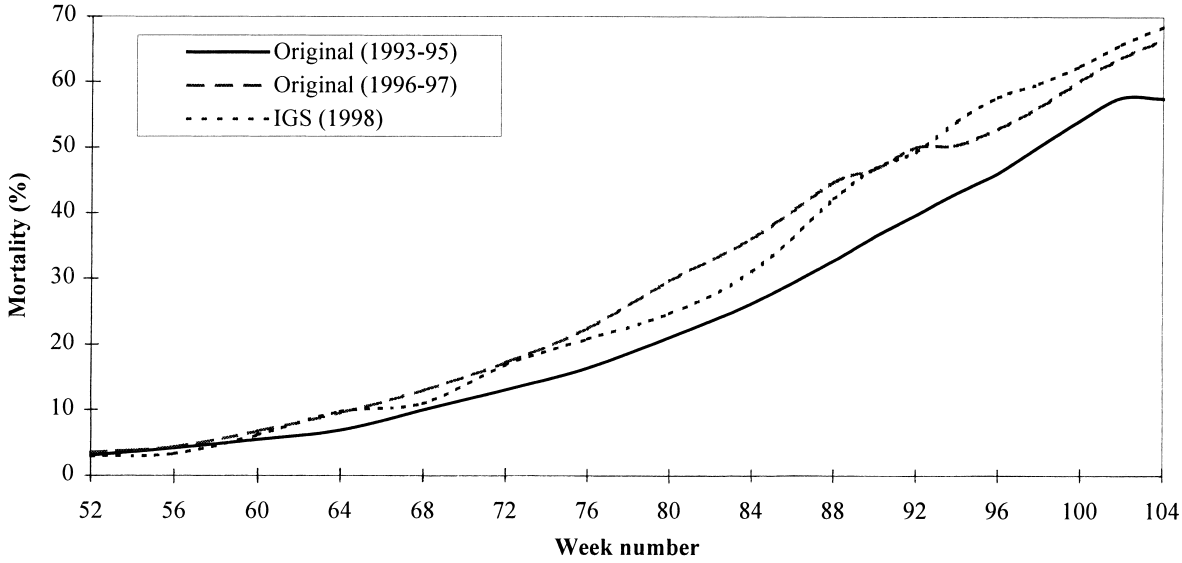
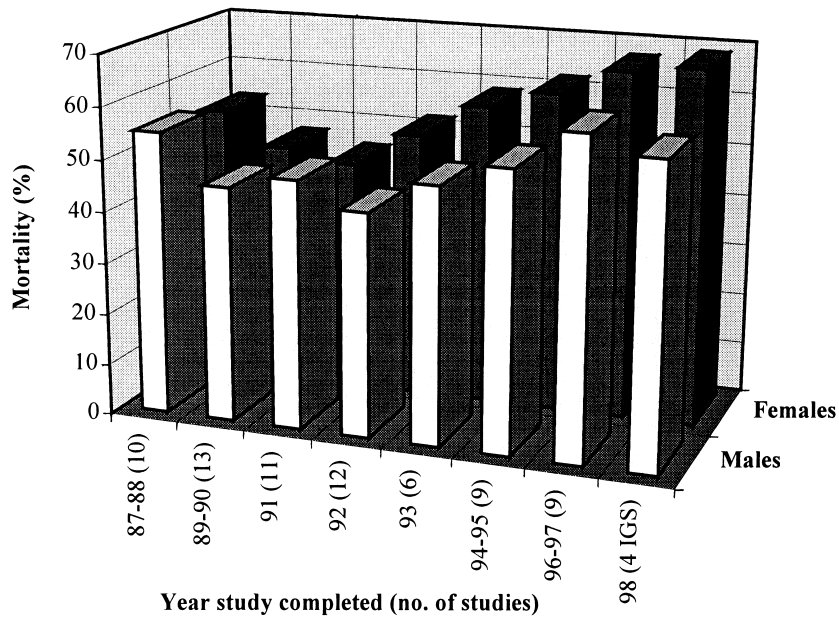


Figure 2. Mortality over Weeks 52 to 104 - females



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.

Figure 3. Mortality at Week 104 - a comparison over selected time periods



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 four IGS rat studies completed in 1998 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

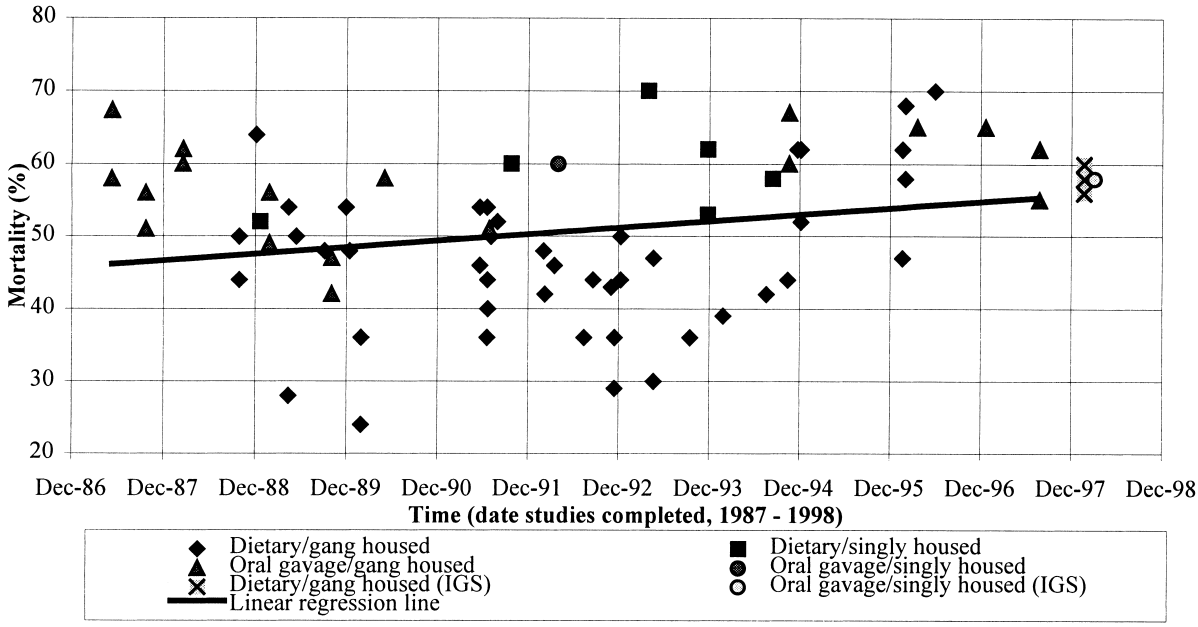
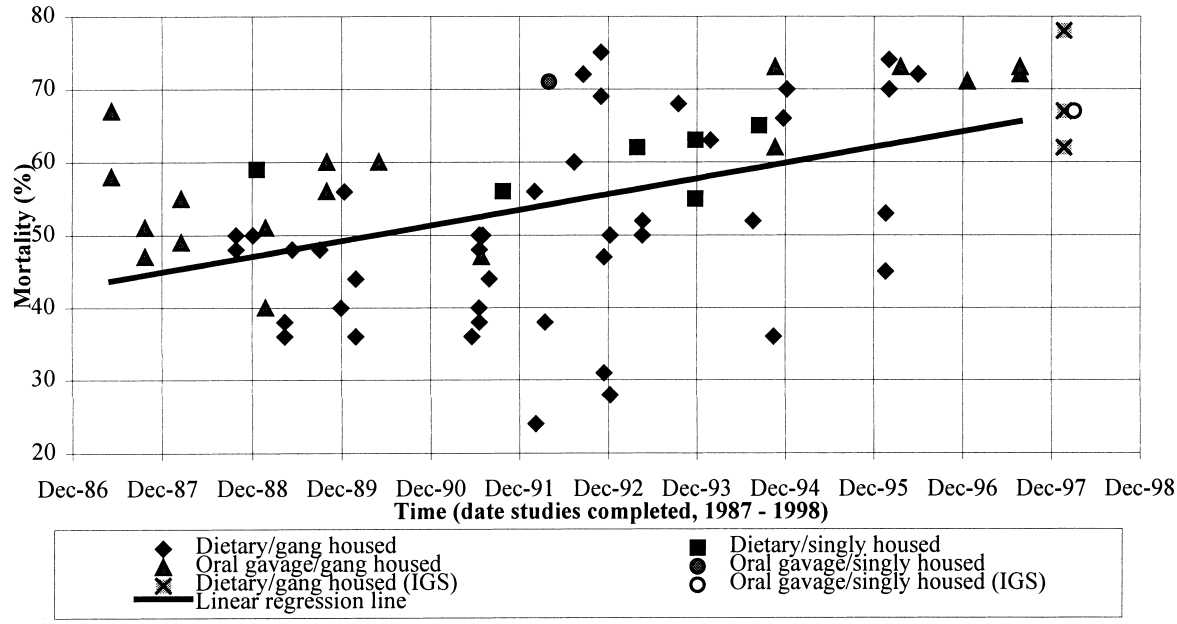


Figure 5. Mortality distribution at Week 104 - females



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.

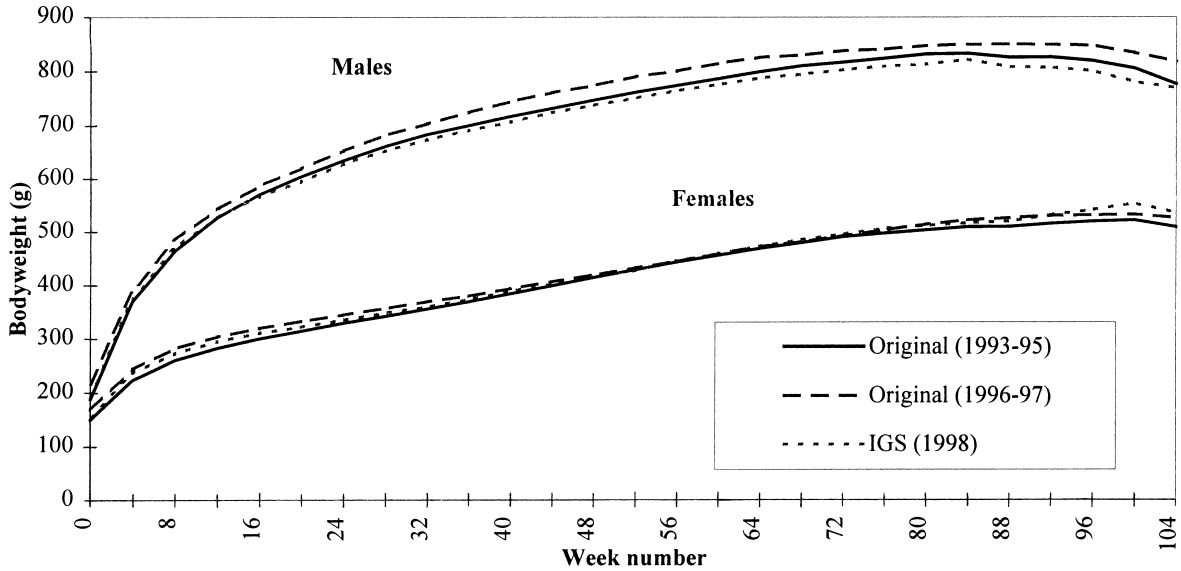
Table 1. Mortality (%) at Week 104 - dietary and oral gavage studies

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/	Mean	45	46	56	58	54	53	60	71	48	49
Original	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/	Mean	46	59	61	61	64	68			52	60
Original	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/	Mean	61	63			62	72			61	67
Original	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/	Mean	58	69					58	67	58	69
IGS	SD	2.0	8.2					-	-	1.6	6.8
	n	3	3	0	0	0	0	1	1	4	4

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.

Figure 7. Bodyweight gain - Weeks 0 to 52

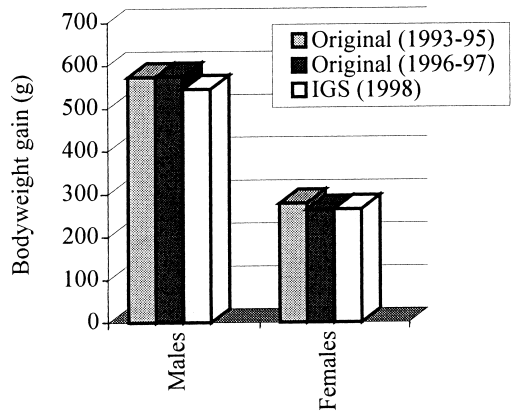


Figure 8. Food consumption - Weeks 1 to 52

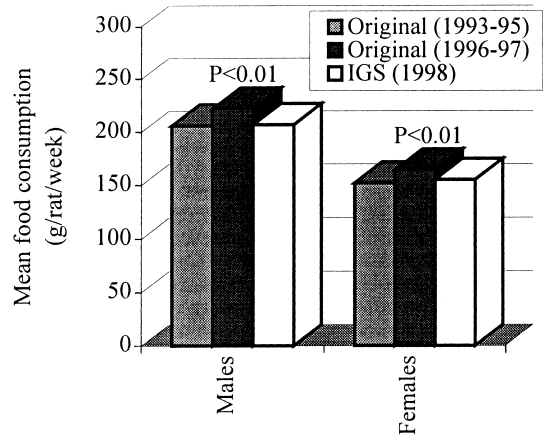


Table 2. Bodyweight gain (g) over Weeks 0 to 52 - dietary and oral gavage studies

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/	Mean	570	278	574	280	583	273			573	278
Original	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/	Mean	564	256			588	274			574	263
Original	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/	Mean	548	263			577	271	488	268	545	265
IGS	SD	24.1	14.2			-		-	-	30.5	12.8
	n	8	8	0	0	1	1	1	1	10	10

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 [4].

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/	Mean	199	150	212	152	223	158			206	152
Original	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/	Mean	214	160			235	170			222	164
Original	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/	Mean	203	152			223	160	224	170	207	155
IGS	SD	1.8	2.4			-		-	-	8.9	6.2
	n	8	8	0	0	1	1	1	1	10	10

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.

ACKNOWLEDGEMENTS

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Background Data of Crj:CD (SD) IGS Rats dosed with Distilled Water Orally for 4, 13 and 26 Weeks

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ABSTRACT. IGS rats were administered with distilled water orally for 4, 13 and 26 weeks respectively. Observations in general symptoms, body weight changes and ophthalmology showed no special points of notice. Water consumption in male showed a tendency to decrease in accordance with the rearing period. In hematological examination, tendency to decrease in leucocyte count and slight extension of PT and APTT were observed during the rearing period. In blood chemistry investigations, incidence of animals with increased levels of GOT and GPT was increased in accordance with the rearing period. In addition, TP, glucose, T-cholesterol and TG values went up slightly and on the contrary, BUN, creatinine, inorganic phosphorus and ALP values showed a slight decrease in accordance with the rearing period. Urinalysis showed a slight increase in incidence of animals showing positive occult blood and protein in accordance with the rearing period. Increase in absolute organ weights and decrease in relative organ weights in general were observed in accordance with aging. —Key words: Crj:CD (SD) IGS, Rat, Background

CD (SD) IGS-1998: 64-70

INTRODUCTION

Crj:CD (SD) IGS rats are provided by Charles River Inc.'s Gold standard system for the purpose of having a uniform quality world wide in order to keep minimum the variation factor of study results which might be arisen from the variation of animal quality. We joined Crj:CD (SD) IGSΩ study group to evaluate the characteristics of the IGS rat, and have conducted 4, 13 and 26 week-rearing study to gain various parameter values commonly used in toxicity studies performed for respective administration period. We hope the background data obtained from this study could be used for the future repeated toxicity studies using IGS rats in our facility as well as to be served for comparison with that in other facilities.

MATERIALS AND METHODS

Test system and study groups: A total of 50 male and 50 female Crj:CD (SD) IGS rats were purchased from a commercial breeder (Charles River Japan Inc., Hino, Japan) at the age of six weeks. After the quarantine and acclimation period of one week, each rat was weighed and randomly allocated to 3 groups consisted of each 15, 15 and 20 males and females per group. These groups were served for 4 weeks, 13 weeks and 26 weeks rearing respectively. Thereafter, the rats were individually marked by an ear tattoo number. All rats were 7 weeks old at the start of administration with distilled water and their mean value of body weight ± standard deviation, and body weight range (in parenthesis) were 241 ± 8 g (225-254 g) for males and 180 ± 6 g (166-192 g) for females during the respective rearing period.

The distilled water (Otsuka water for injection, Otsuka Pharma. Co., Ltd.) was administered daily to the rats by gavage at a constant volume of 1.0 ml/100 g body weight.

Accommodation: The animals were housed in individual cages under controlled environmental conditions of temperature (22 (19-24)°C), and relative humidity (55(40-75)%) with a light cycle of 12 hrs (6:00 to 18:00) in barriered animal facility. Ventilation was 15 times/hr. Rats were housed individually in standard, polycarbonate plastic cages, type CL-0105-1 (floor area per cage: 777 cm^2, Japan Clea Co., Ltd.) with Omega-dry® bedding (Oriental Yeast Co., Ltd.).

Standardized dry pellet diet CRF-1 (γ-ray irradiated diet, Charles River Japan Inc.) and municipal tap water were available *ad libitum*. Identification of the diet used and the specifications for acceptable level of contaminants were given by the producer. Periodically, the water was analyzed by Kuritaz Co., Ltd.

Observations:

General conditions: During the entire course of the experiment, clinical signs of each rat were checked and recorded daily.

Body weight: The body weight was recorded daily during 4, 13 and 26 weeks of rearing.

Food and water consumption: The food and water consumption was recorded weekly.

Ophthalmic examinations: The results of the evidence of pupillary reflex and the observations of the lens and cornea by slit lamp of all rats were recorded at the time of allocation and before the final day of rearing period on weeks 4, 13 and 26. Five male and five female rats from 26 week-group were selected for the retina examination, and performed by using fundus camera (Kowa RC-2) at the time of allocation, weeks 4, 13 and 26 for the fundus examination.

Hematology: All living rats were tested at the end of rearing period on weeks 4, 13 and 26. Food deprivation before blood sampling was not performed. For the hematological investigations, blood samples were taken under ether-anesthesia by puncturing retrobulbar venous plexus, and collected in the sampling vial containing EDTA-2K as an anticoagulating agent. For the determination of coagulation times, blood was collected in a test tube with 3.2 % sodium citrate solution. The plasma was obtained by centrifugation (3000 rpm (1710 × g), 15 min.). Hematology and coagulation studies were performed by

Microcellcounter K-4500 (Toa Med. Electronics Co., Ltd.) and Amelung KC 10A (Amelung Co., Ltd.) respectively. The methods and reagents used were shown in the following table.

Parameters, methods and reagents used in hematological examinations

Parameter	Method	Reagents (Supplier)
Erythrocytes (RBC)	Resistance detection [a)]	CELLPACK
Leucocytes	Resistance detection [a)]	STROMATOLYSER-3WP
Platelets	Resistance detection [a)]	SULFOLYSER
Hemoglobin (HGB)	SLS (sodium lauryl sulfate) [a)]	(Toa Med. Electronics)
Hematocrit (HCT)	Cumulative pulse height detection [a)]	
MCV	HCT × 10 / RBC [a)]	
MCH	HGB × 10 / RBC [a)]	
MCHC	HGB × 100 / HCT [a)]	
Reticulocytes	Brilliant cresyl blue stain	
Prothrombin time (PT)	Magnetic response [b)]	Thromboplastin · C Plus (Dade)
APTT	Magnetic response [b)]	Actin®, Activated Cephaloplastin (Dade)
Differential leukocyte counts	Wright-Giemsa stain	

a): Microcellcounter K-4500, Toa Med. Electronics Co., Ltd., b): Amelung KC 10A, Amelung Co., Ltd.,

Blood chemistry: Following to the samplings for hematology, blood for blood chemistry examination was obtained. After clotting, the serum was obtained by centrifugation 3000rpm (1710 × g), 15 min.). Blood chemistry examination was performed by Automatic Analyzer 705 (Hitachi Inc.). Methods and reagents used were listed in the following table.

Parameters, methods and reagents used in blood chemical examinations

Parameter	Method	Reagents (Supplier)
GOT	UV rate [c)]	IATROMATE GOT (DIA-IATRON)
GPT	UV rate [c)]	IATROMATE GPT (DIA-IATRON)
Alkaline phosphatase (ALP)	p-NPP rate [c)]	IATRO LQ ALP rate (DIA-IATRON)
Total protein (TP)	Biuret [c)]	AUTOSERA TP (Dai-ichi Kagaku)
Albumin	BCG [c)]	AUTOSERA ALB (Dai-ichi Kagaku)
A/G	Calculation: ALB/ (TP–ALB)	
Glucose	Glck-G6PDH [c)]	IATRO-LQ GLU (DIA-IATRON)
Blood urea nitrogen (BU/V)	Urease-GLDH [c)]	IATRO-LQ UN rate (DIA-IATRON)
Total cholesterol	CED-POD [c)]	T-choles A · 5 (Kokusai Shiyaku)
Triglyceride (TG)	GPO [c)]	Triglyceride-E (Kokusai Shiyaku)
Total bilirubin	DPD [c)]	T-BIL for Auto. Analyzer 705 (Hitachi)
Inorganic phosphorus	Enzymatic [c)]	Determiner-LIP (Kyowa Medics)
Creatinine	Enzymatic [c)]	IATROMATE CRE (DIA-IATRON)
Calcium	OCPC [c)]	IATROMATE Ca (DIA-IATRON)
Potassium, Sodium, Chloride	Electrode [d)]	Reagents for System E3A (Beckman)

c): Automatic Analyzer 705, Hitachi Inc., d): System E3A, Beckman Inc.

Urinalysis: All living rats were tested in weeks 3, 12 and 25. Urine samples were collected individually using metabolic cages 4 h and 24 h after administration. During collection time, only water was available *ad libitum* for 4 h and thereafter food was given to the rats. Urine volume and electrolytes were determined using urine samples collected for 24 h, and the other parameters were determined using samples collected for 4 h. Specific gravity was performed by Atago Uricon-S (Atago Co., Ltd.), and pH by compact pH meter b-112 (Horiba Inc.), and Protein, Glucose, Ketone, Bilirubin, Occult blood and Urobilinogen by Multi-sticks (Bayer-Sankyo Co., Ltd.). Potassium, sodium and chloride analyses were performed by electrode method of System E3A (Beckman Inc). Sediment (Squamous epithelium, Transitional epithelium, Renal epithelium, Leukocytes, Erythrocytes, Bacteria, Cylinders, Inorganic materials) was performed by microscopic examination.

Gross findings at necropsy: Rat found moribund during the experimental period was necropsied immediately. At the end of rearing period of weeks 4, 13 and 26, all survivors were anesthetized intraperitoneally with Nembutal® (Abbott Lab., USA), weighed and bled through the aorta abdominalis. At necropsy, organs or tissues were inspected macroscopically and findings were recorded. The weights of the following organs of each rat were recorded: heart, lungs, liver, kidneys, spleen, thymus, brain, adrenals, pituitary, thyroids including

parathyroids, testes, ovaries, epididymides, prostate and salivary glands (submaxillary, sublingual and parotid glands). In addition, various organs were taken out for histopathological study and fixed in phosphate buffered 10 % formalin solution or other appropriate fixing solution. Histopathological study is under progress and thus not reported in this report.

Calculations: The mean values and standard deviations were calculated in each group from the values of body weight, food and water consumption, hematology, blood chemistry, urinalysis, and absolute and relative organ weights.

Experimental period: The acclimatization of rats was started on October 14, 1997 and necropsy of 26-week rearing group was finished on April 23, 1998.

RESULTS AND DISCUSSION

Clinical observations and moribund animal: During the rearing period of weeks 4, 13 and 26, a few animals showed sporadic alopecia, crust and chromodacryorrhea. No notable clinical abnormalities were observed during the entire rearing period. The body weight of one female rat in 13 week-rearing group was retarded for about a week because of broken incisor. One male rat in 26 week-rearing group suddenly showed ventral position, tachypnea, hematuria and serous diarrhea on day 26, and moribund on day 27 because of the severe exhaustion. At necroptic observation, red urine in dilated bladder, nodulation on bladder epithelium, dilated ureter, edema of discolored kidneys (weight 5.61 g), hemorrhage in gastric mucosa and tarry stool in intestine suggested the severe lesion in urinary tract as a main cause of the moribund.

Body weight (Table 1): No notable abnormality was observed during the entire rearing period. The body weights increased well and no differences in body weights among the groups were observed.

Food consumption (Table 2): There were no differences in food consumption among the groups, and were stayed constant throughout the rearing period.

Water consumption (Table 3): There were no differences in water consumption among the groups, however, it showed a tendency to decrease in males in accordance with rearing period. The water consumption in females stayed constant throughout the rearing period.

Ophthalmic examination: Examination of the pupillary reflex as well as ocular examination by naked eye and funduscopy did not show any pathologic changes. However, in the ocular examination by slit lamp, linear fissure on the right anterior lens was observed in one animal of the 26 week-rearing group, and corneal opacity in the left eye was observed in one animal of the same group. Scobs of Omega-dry® bedding on the both cornea were observed according to rearing period, particularly, observed in all animals of the 26 week-rearing group.

Hematology (Table 4): No abnormal values were obtained during the entire rearing period. Coagulation time, PT and APTT, showed tendency to extend slightly in accordance with rearing period. Leucocyte count showed a tendency to decrease in males and females in accordance with rearing period.

Blood chemistry (Table 5): Blood chemistry investigations showed abnormal increase in GOT in three female rats (225, 626, 160 U/l) of the 13 week-rearing group. These rats also showed increased GPT value. In general, GOT, GPT, TP, glucose, T-cholesterol and TG values went up slightly and on the contrary , BUN, creatinine, inorganic phosphorus and ALP values showed a slight decrease in accordance with rearing period. These changes were rather evident between 4 and 13 weeks but fairly comparable between 13 and 26 weeks except for ALP.

Urinalysis (Table 6): Occult blood and Protein were observed in a few males in 13 and 26 week-rearing groups, and tendency to increase in the rearing period was observed. No other abnormal findings were obtained during the entire rearing period.

Organ weights (Table 7): The absolute and relative liver weights of one female rat were increased in 13 week-rearing group, which showed high serum GOT and GPT values. In all the other animals, no notable change was observed. Generally, increases in absolute weights and decreases in relative weights were observed in accordance with rearing period.

Gross findings at necropsy: At necropsy, cyst of the left kidney and petechiae of thymus was found in one male, and congestion of thymus in one female in 4 week-rearing group. Fusion between posterior lobe of right lung and thoracic wall in one male, and petechiae of mandibular lymph node or accessory spleen in two females in 13 week-rearing group were observed. In addition, tuber of the right epididymis and variated lobation of liver in two males, and swelling of pituitary in two females and infarction with uneven surface of the right kidney in one female in 26 week-rearing group were observed.

Differences between CD(SD) and IGS rats: Comparison of Crj:CD(SD) rats looking over our available background data of three 4 weeks studies in Crj:CD(SD) rats, only slightly higher values of ALP and lower values of TG were observed in Crj:CD(SD)IGS rats. In other points, no notable differences between Crj:CD(SD)IGS and Crj:CD (SD) rats were observed.

Table 1. Weekly body weight of rats during 4, 13 and 26 weeks of rearing Means ± S.D. in g

	MALE				FEMALE		
	4 Week-group	13 Week-group	26 Week-group		4 Week-group	13 Week-group	26 Week-group
Age (Weeks)	n = 15	15	20	Age (Weeks)	n = 15	15	20
7	243 ± 6	241 ± 9	240 ± 8	7	181 ± 7	180 ± 5	180 ± 6
8	292 ± 10	297 ± 12	291 ± 18	8	203 ± 8	204 ± 9	202 ± 8
9	323 ± 15	333 ± 16	324 ± 23	9	220 ± 12	222 ± 12	218 ± 10
10	349 ± 19	362 ± 20	352 ± 29	10	236 ± 13	236 ± 16	234 ± 12
11	371 ± 20	388 ± 25	374 ± 30 [a)]	11	245 ± 14	248 ± 16	243 ± 15
12		410 ± 28	395 ± 34	12		257 ± 16	253 ± 15
13		435 ± 28	420 ± 36	13		267 ± 19	262 ± 17
14		456 ± 28	440 ± 39	14		273 ± 19	268 ± 17
15		472 ± 32	455 ± 41	15		279 ± 18	273 ± 18
16		484 ± 34	468 ± 43	16		283 ± 19	277 ± 17
17		500 ± 35	481 ± 45	17		287 ± 18	281 ± 17
18		512 ± 36	497 ± 47	18		294 ± 21 [b)]	285 ± 19
19		522 ± 37	507 ± 50	19		299 ± 18 [b)]	288 ± 20
20		527 ± 39	514 ± 50	20		295 ± 21	290 ± 19
21			521 ± 50	21			294 ± 18
22			530 ± 51	22			298 ± 20
23			536 ± 52	23			298 ± 21
24			542 ± 54	24			301 ± 20
25			549 ± 52	25			303 ± 19
26			556 ± 53	26			306 ± 23
27			563 ± 54	27			309 ± 22
28			568 ± 55	28			312 ± 23
29			574 ± 55	29			315 ± 23
30			584 ± 56	30			319 ± 25
31			589 ± 57	31			323 ± 28
32			597 ± 57	32			325 ± 28
33			600 ± 58	33			325 ± 27

[a)] : n=19, One animal was moribund, accordingly number of data thereafter is basically 19.
[b)] : n=14, One female was excluded due to broken incisor.

Table 2. Weekly food consumption of rats during 4, 13 and 26 weeks of rearing Means ± S.D. in g

	MALE				FEMALE		
	4 Week-group	13 Week-group	26 Week-group		4 Week-group	13 Week-group	26 Week-group
Age (Weeks)	n = 15	15	20	Age (Weeks)	n = 15	15	20
7	177 ± 8	176 ± 9	175 ± 13	7	121 ± 7	121 ± 5	122 ± 7
8	172 ± 10	176 ± 11	174 ± 17	8	128 ± 9	130 ± 6	129 ± 7
9	166 ± 12	174 ± 14	174 ± 18	9	131 ± 7	131 ± 7	132 ± 11
10	167 ± 11	175 ± 14	176 ± 18 [a)]	10	134 ± 10	132 ± 7	133 ± 13
11		172 ± 16	174 ± 18	11		130 ± 7	132 ± 11
12		[b)]	180 ± 18	12		[b)]	133 ± 12
13		183 ± 12	185 ± 17	13		133 ± 6	134 ± 13
14		178 ± 12	179 ± 17	14		129 ± 6	127 ± 11
15		179 ± 11	179 ± 17	15		128 ± 7	129 ± 10
16		179 ± 14	177 ± 18	16		124 ± 7	127 ± 11
17		182 ± 10	183 ± 18	17		127 ± 8 [d)]	128 ± 12
18		177 ± 9	176 ± 18	18		125 ± 10 [d)]	125 ± 11
19		169 ± 10	175 ± 18	19		123 ± 10	125 ± 11
20			175 ± 16	20			126 ± 11
21			172 ± 16	21			124 ± 12
22			173 ± 17	22			124 ± 10
23			170 ± 18	23			123 ± 11
24			171 ± 15	24			123 ± 11
25			172 ± 16	25			122 ± 12
26			171 ± 17	26			122 ± 11
27			170 ± 16	27			124 ± 11
28			171 ± 17	28			122 ± 11
29			169 ± 16	29			118 ± 13
30			168 ± 16	30			120 ± 14
31			166 ± 15	31			116 ± 11
32			153 ± 14 [c)]	32			115 ± 12

[a)] : n=19, One animal was moribund, accordingly number of data thereafter is basically 19.
[b)] : Not measured by mistake.
[c)] : n=17, Data of two animals were excluded due to spilled food.
[d)] : n=14, One female was excluded due to broken incisor.

Table 3. Weekly water consumption of rats during 4, 13 and 26 weeks of rearing Means ± S.D. in g

	MALE				FEMALE		
	4 Week-group	13 Week-group	26 Week-group		4 Week-group	13 Week-group	26 Week-group
Age (Weeks) n =	15	15	20	Age (Weeks) n =	15	15	20
7	262 ± 21	263 ± 24	255 ± 39	7	200 ± 22	193 ± 22	191 ± 21
8	249 ± 24	255 ± 30	254 ± 49	8	192 ± 29	194 ± 29	190 ± 17
9	232 ± 31	243 ± 37	247 ± 54	9	193 ± 31	178 ± 29	180 ± 20
10	231 ± 28	231 ± 32	241 ± 51 [a)]	10	188 ± 35	182 ± 20	193 ± 20
11		236 ± 34	234 ± 55	11		191 ± 22	196 ± 37
12		235 ± 32	240 ± 58	12		196 ± 32	199 ± 30 [g)]
13		263 ± 34	252 ± 58	13		209 ± 25	205 ± 35 [g)]
14		252 ± 35	258 ± 59	14		217 ± 35	211 ± 44 [g)]
15		248 ± 37	235 ± 51 [b)]	15		210 ± 35	204 ± 31 [g)]
16		237 ± 34	230 ± 57	16		201 ± 38	204 ± 42
17		[c)]	[c)]	17		[c)]	[c)]
18		235 ± 34	235 ± 60	18		225 ± 47 [e)]	214 ± 47
19		208 ± 27	232 ± 55	19		205 ± 36 [f)]	217 ± 40
20			253 ± 50	20			218 ± 44
21			221 ± 51	21			209 ± 45 [g)]
22			220 ± 58 [d)]	22			205 ± 44
23			219 ± 63	23			205 ± 48
24			224 ± 52	24			212 ± 45
25			219 ± 55	25			208 ± 46
26			220 ± 49	26			211 ± 50
27			223 ± 51	27			216 ± 45
28			218 ± 57	28			224 ± 56
29			207 ± 53	29			212 ± 46
30			218 ± 61	30			211 ± 50
31			222 ± 62	31			218 ± 53
32			203 ± 46	32			210 ± 47

[a)] : n=19, One animal was moribund, accordingly number of data thereafter is basically 19.
[b)] : n=17, Data of two animals were excluded due to spilled water. [c)] : Not measured by mistake
[d)] : n=18, One animal was not measured due to spilled water. [e)] : n=14, One female was excluded due to broken incisor.
[f)] : n=14, One animal was not measured due to spilled water. [g)] : n=19, One animal was not measured due to spilled water.

Table 4. Hematology values of rats during 4, 13 and 26 weeks of rearing Means ± S.D.

MALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	FEMALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
		11	20	33			11	20	33
	n =	15	15	19		n =	15	15	20
Erythrocytes	$10^6/\mu l$	8.58 ± 0.34	9.09 ± 0.32	8.91 ± 0.44	Erythrocytes	$10^6/\mu l$	7.84 ± 0.28	8.08 ± 0.31 [c)]	7.71 ± 0.28
Hemoglobin	g/dl	16.8 ± 0.6	16.2 ± 0.4	15.8 ± 0.8	Hemoglobin	g/dl	15.8 ± 0.5	15.3 ± 0.7 [c)]	15.2 ± 0.5
Hematocrit	%	48.7 ± 2.3	48.4 ± 1.6	47.4 ± 2.7	Hematocrit	%	44.5 ± 1.7	44.8 ± 1.9 [c)]	43.6 ± 1.8
MCV	fl	57 ± 1	53 ± 1	53 ± 1	MCV	fl	57 ± 1	55 ± 1 [c)]	57 ± 1
MCH	pg	19.6 ± 0.5	17.8 ± 0.7	17.8 ± 0.6	MCH	pg	20.1 ± 0.6	18.9 ± 0.7 [c)]	19.7 ± 0.4
MCHC	g/dl	34.5 ± 0.7	33.4 ± 0.6	33.4 ± 0.5	MCHC	g/dl	35.5 ± 0.7	34.1 ± 0.7 [c)]	34.8 ± 0.8
Platelets	$10^3/\mu l$	1036 ± 108	898 ± 117	952 ± 118	Platelets	$10^3/\mu l$	1083 ± 61	969 ± 133 [c)]	924 ± 100
Leucocytes	$10^3/\mu l$	13.5 ± 2.7	11.9 ± 2.5	11.5 ± 2.5	Leucocytes	$10^3/\mu l$	10.2 ± 2.4	8.5 ± 2.4 [c)]	7.9 ± 1.7
Differential leukocyte counts					Differential leukocyte counts				
Band neutrophils	%	0.1 ± 0.3	0.1 ± 0.2	0.2 ± 0.3	Band neutrophils	%	0.0 ± 0.1	0.1 ± 0.2 [c)]	0.1 ± 0.2
Seg. neutrophils	%	10.2 ± 3.3	9.7 ± 5.1	14.2 ± 4.4	Seg. neutrophils	%	7.3 ± 3.2	9.4 ± 4.5 [c)]	10.2 ± 4.0
Lymphocytes	%	87.1 ± 3.4	87.3 ± 5.8	83.1 ± 5.1	Lymphocytes	%	90.7 ± 3.1	87.9 ± 4.6 [c)]	87.0 ± 3.9
Eosinophils	%	1.3 ± 0.7	1.0 ± 0.7	1.1 ± 0.8	Eosinophils	%	0.9 ± 1.0	1.0 ± 0.7 [c)]	1.4 ± 0.7
Basophils	%	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	Basophils	%	0.0 ± 0.1	0.1 ± 0.2 [c)]	0.1 ± 0.3
Monocytes	%	1.2 ± 0.7	1.9 ± 1.3	1.4 ± 0.9	Monocytes	%	1.1 ± 0.7	1.4 ± 1.0 [c)]	1.2 ± 0.5
Reticulocytes	‰	12 ± 2	9 ± 3	14 ± 3	Reticulocytes	‰	8 ± 3	11 ± 2 [c)]	12 ± 4
PT	second	13.5 ± 0.3 [a)]	14.4 ± 0.5 [b)]	15.1 ± 0.5	PT	second	14.4 ± 0.3	14.0 ± 1.6	15.2 ± 0.3
APTT	second	16.1 ± 1.5	16.6 ± 1.5 [b)]	15.7 ± 1.3	APTT	second	14.2 ± 0.8 [a)]	14.4 ± 1.5	15.7 ± 1.1

[a)] : n=14, One sample was excluded by inconsistency of values.
[b)] : n=13, Two samples were excluded by inconsistency of values or coagulation.
[c)] : n=14, One sample was excluded by coagulation.

Table 5. Blood chemistry values of rats during 4, 13 and 26 weeks of rearing Means ± S.D.

MALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	FEMALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
		11	20	33			11	20	33
	n =	15	15	19		n =	15	15	20
GOT	U/*l*	80 ± 17	108 ± 17	100 ± 34	GOT	U/*l*	71 ± 9	140 ± 141	120 ± 57
GPT	U/*l*	31 ± 5	41 ± 8	42 ± 23	GPT	U/*l*	33 ± 6	67 ± 60	61 ± 41
ALP	U/*l*	969 ± 210	663 ± 149	477 ± 128	ALP	U/*l*	687 ± 192	458 ± 205	314 ± 97
Total Protein	g/*l*	61 ± 2	64 ± 3	64 ± 2	Total Protein	g/*l*	60 ± 3	68 ± 5	69 ± 5
Albumin	g/*l*	25 ± 1	24 ± 1	24 ± 1	Albumin	g/*l*	26 ± 2	30 ± 3	30 ± 3
A/G		0.68 ± 0.06	0.59 ± 0.05	0.61 ± 0.04	A/G		0.77 ± 0.06	0.77 ± 0.05	0.77 ± 0.07
Glucose	mmol/*l*	7.8 ± 0.6	8.5 ± 0.8	8.2 ± 1.0	Glucose	mmol/*l*	7.5 ± 0.4	8.3 ± 0.8	7.8 ± 0.5
Urea nitrogen	mmol/*l*	6.7 ± 0.7	6.5 ± 0.8	6.1 ± 0.5	Urea nitrogen	mmol/*l*	6.8 ± 0.9	7.2 ± 1.1	6.1 ± 0.7
Total cholesterol	mmol/*l*	1.94 ± 0.36	2.24 ± 0.41	2.63 ± 0.63	Total cholesterol	mmol/*l*	2.14 ± 0.39	2.50 ± 0.59	2.57 ± 0.47
Triglycerides	mmol/*l*	1.53 ± 0.53	1.66 ± 0.51	1.84 ± 0.73	Triglycerides	mmol/*l*	0.91 ± 0.38	1.37 ± 0.44	1.76 ± 0.78
Total bilirubin	μmol/*l*	4.2 ± 0.9	4.3 ± 1.4	4.8 ± 1.1	Total bilirubin	μmol/*l*	3.8 ± 1.0	3.8 ± 0.5	4.5 ± 0.9
Creatinine	μmol/*l*	53 ± 4	26 ± 2	27 ± 4	Creatinine	μmol/*l*	51 ± 4	30 ± 3	31 ± 4
Calcium	mmol/*l*	2.57 ± 0.06	2.69 ± 0.06	2.64 ± 0.06	Calcium	mmol/*l*	2.63 ± 0.07	2.76 ± 0.12	2.74 ± 0.11
Phosphorus	mmol/*l*	2.55 ± 0.21	1.97 ± 0.21	1.82 ± 0.16	Phosphorus	mmol/*l*	1.85 ± 0.23	1.55 ± 0.20	1.49 ± 0.19
Sodium	mEq/*l*	140 ± 1	142 ± 1	139 ± 1	Sodium	mEq/*l*	139 ± 1	141 ± 1	140 ± 1
Potassium	mEq/*l*	5.03 ± 0.25	4.54 ± 0.26	4.85 ± 0.31	Potassium	mEq/*l*	4.37 ± 0.36	4.14 ± 0.31	4.33 ± 0.26
Chloride	mEq/*l*	108 ± 2	113 ± 2	107 ± 1	Chloride	mEq/*l*	108 ± 1	113 ± 2	109 ± 2

Following factors shall be multiplied to change values shown in molar concentrations to mg/dl.
Glucose: 18.15, *Urea nitrogen*: 2.80, *Total cholesterol*: 38.67, *Triglycerides*: 88.57, *Total bilirubin*: 0.058, *Creatinine*: 0.0113, *Calcium*: 4.01, *Phosphorus*: 3.097

Table 6-1. Urinarisis values of rats during 4, 13 and 26 weeks of rearing Means ± S.D.

MALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	FEMALE		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
		11	20	33			11	20	33
	n =	15	15	19		n =	15	15	20
Volume	ml/24hr	16.0 ± 4.0	18.2 ± 2.2	17.3 ± 3.3	Volume	ml/24hr	11.8 ± 2.7	17.8 ± 5.5 [a)]	15.6 ± 5.7
Potassium	mmol/24hr	3.43 ± 0.47	3.85 ± 0.45	3.40 ± 0.33	Potassium	mmol/24hr	2.62 ± 0.45	3.03 ± 0.64 [a)]	2.63 ± 0.56
Sodium	mmol/24hr	1.55 ± 0.28	1.77 ± 0.29	1.62 ± 0.28	Sodium	mmol/24hr	1.24 ± 0.26	1.61 ± 0.43 [a)]	1.31 ± 0.41
Chloride	mmol/24hr	2.48 ± 0.32	2.58 ± 0.34	2.41 ± 0.33	Chloride	mmol/24hr	1.91 ± 0.34	2.17 ± 0.54 [a)]	1.91 ± 0.51
Specific gravity		1.018 ± 0.008	1.020 ± 0.009	1.014 ± 0.007	Specific gravity		1.018 ± 0.012	1.021 ± 0.010	1.015 ± 0.007
pH		7.7 ± 0.2	7.6 ± 0.4	7.6 ± 0.4	pH		7.9 ± 0.3	7.0 ± 0.7	6.9 ± 0.7
Protein	–	0	6	0	Protein	–	6	11	5
	±	13	6	14		±	8	3	14
	+	2	3	4		+	1	1	1
	++	0	0	1		++	0	0	0
Glucose	–	15	15	19	Glucose	–	15	15	20
Bilirubin	–	14	15	19	Bilirubin	–	15	15	20
	+	1	0	0		+	0	0	0
Ketones	–	15	8	18	Ketones	–	15	15	20
	±	0	6	0		±	0	0	0
	+	0	1	1		+	0	0	0
Occult blood	–	15	11	17	Blood	–	15	15	20
	±	0	2	0		±	0	0	0
	+	0	2	0		+	0	0	0
	++	0	0	1		++	0	0	0
	+++	0	0	1		+++	0	0	0
Urobilinogen	0.1 E.U./*dl*	15	15	19	Urobilinogen	0.1 E.U./*dl*	15	15	20

[a)] : n=14, Not measured by spilled 24hr-urine

Table 6-2. Urinary sediments of rats during 4, 13 and 26 weeks of rearing

MALE			Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	FEMALE			Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
			11	20	33				11	20	33
		n =	15	15	19			n =	15	15	20
Epithelia	Squamous	0	13	14	19	Epithelia	Squamous	0	13	14	19
		1	2	1	0			1	2	1	0
		2	0	0	0			2	0	0	1
	Transitional	0	15	14	19		Transitional	0	15	15	19
		1	0	1	0			1	0	0	1
	Renal	0	15	15	19		Renal	0	15	15	20
Leucocytes		0	15	15	17	Leucocytes		0	14	14	18
		1	0	0	2			1	1	1	2
Erythrocytes		0	15	13	15	Erythrocytes		0	15	15	20
		1	0	1	2			1	0	0	0
		3	0	0	1			3	0	0	0
		4	0	1	1			4	0	0	0
Cylinders		0	15	15	19	Cylinders		0	15	15	20
Bacteria		0	2	10	12	Bacteria		0	5	13	5
		1	13	4	7			1	7	0	15
		2	0	1	0			2	3	2	0
Inorganics		0	15	15	19	Inorganics		0	15	15	20

Epithelia, Leucocytes and Erythrocytes : 0; a few in all field, 1; 1-2 in each field, 2; 3-5 in each field, 3; 6-10 in each field, 4; >11 in each field
Cylinders : 0; not detected
Bacteria and Inorganics : 0; negative, 1; slight, 2; many

Table 7-1. Absolute and relative organ weights of male rats during 4, 13 and 26 weeks of rearing Mean ± S.D.

Absolute		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	Relative		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
		11	20	33			11	20	33
	n =	15	15	19		n =	14	15	18
Body weight	g	360 ± 19 a)	515 ± 39	588 ± 59 b)					
Heart	g	1.19 ± 0.11	1.44 ± 0.18	1.57 ± 0.17	Heart	%	0.327 ± 0.025	0.278 ± 0.026	0.268 ± 0.021
Lungs	g	1.18 ± 0.11	1.34 ± 0.12	1.42 ± 0.12	Lungs	%	0.324 ± 0.024	0.261 ± 0.021	0.242 ± 0.021
Liver	g	14.43 ± 1.01	17.73 ± 2.07	19.05 ± 2.34	Liver	%	3.994 ± 0.169	3.439 ± 0.289	3.204 ± 0.181
Kidneys	g	2.59 ± 0.20	3.13 ± 0.35	3.33 ± 0.32	Kidneys	%	0.717 ± 0.035	0.606 ± 0.042	0.562 ± 0.042
Spleen	g	0.70 ± 0.12	0.74 ± 0.09	0.79 ± 0.17	Spleen	%	0.191 ± 0.031	0.144 ± 0.015	0.135 ± 0.026
Thymus	g	0.374 ± 0.092	0.297 ± 0.069	0.126 ± 0.035 b)	Thymus	%	0.103 ± 0.023	0.058 ± 0.014	0.022 ± 0.007 c)
Brain	g	2.05 ± 0.07	2.20 ± 0.07	2.21 ± 0.08	Brain	%	0.569 ± 0.024	0.429 ± 0.025	0.380 ± 0.037
Prostate	g	1.06 ± 0.19	1.29 ± 0.30	1.43 ± 0.24	Prostate	%	0.297 ± 0.057	0.252 ± 0.062	0.249 ± 0.051
Testes	g	3.14 ± 0.29	3.19 ± 0.17	3.50 ± 0.31	Testes	%	0.875 ± 0.090	0.623 ± 0.066	0.597 ± 0.076
Epididymides	g	0.93 ± 0.08	1.29 ± 0.09	1.42 ± 0.15	Epididymides	%	0.256 ± 0.017	0.251 ± 0.031	0.242 ± 0.032
Adrenals	mg	51.1 ± 6.9	47.1 ± 4.7	46.8 ± 5.9	Adrenals	‰	0.140 ± 0.018	0.092 ± 0.012	0.080 ± 0.013
Pituitary	mg	10.6 ± 1.5	11.8 ± 1.3	12.3 ± 1.3	Pituitary	‰	0.029 ± 0.003	0.023 ± 0.002	0.021 ± 0.003
Thyroids	mg	20.9 ± 4.1	23.8 ± 2.5	26.0 ± 4.0	Thyroids	‰	0.059 ± 0.011	0.046 ± 0.005	0.045 ± 0.008
Submaxillaries	g	0.52 ± 0.07	0.60 ± 0.07	0.64 ± 0.06	Submaxillaries	%	0.146 ± 0.020	0.117 ± 0.012	0.109 ± 0.011
Sublinguales	g	0.080 ± 0.010	0.094 ± 0.008	0.086 ± 0.015	Sublinguales	%	0.022 ± 0.003	0.018 ± 0.002	0.015 ± 0.003
Parotids	g	0.43 ± 0.16	0.44 ± 0.11	0.45 ± 0.17	Parotids	%	0.114 ± 0.039	0.085 ± 0.021	0.076 ± 0.031

a) : n=14, b) : n=18, c) : n=17 (Not measured)

Table 7-2. Absolute and relative organ weights of female rats during 4, 13 and 26 weeks of rearing Mean ± S.D.

Absolute		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group	Relative		Age (Weeks) 4 Week-group	13 Week-group	26 Week-group
		11	20	33			11	20	33
	n =	15	15	20		n =	15	15	20
Body weight	g	242 ± 13	291 ± 21	318 ± 26					
Heart	g	0.93 ± 0.08	0.93 ± 0.07	1.01 ± 0.08	Heart	%	0.385 ± 0.023	0.322 ± 0.025	0.320 ± 0.034
Lungs	g	1.04 ± 0.08	1.01 ± 0.05	1.07 ± 0.07	Lungs	%	0.432 ± 0.030	0.347 ± 0.022	0.337 ± 0.033
Liver	g	9.14 ± 0.74	9.94 ± 1.23	10.26 ± 1.09	Liver	%	3.787 ± 0.238	3.432 ± 0.444	3.227 ± 0.296
Kidneys	g	1.77 ± 0.14	1.95 ± 0.17	2.00 ± 0.15	Kidneys	%	0.733 ± 0.036	0.672 ± 0.061	0.630 ± 0.046
Spleen	g	0.53 ± 0.05	0.51 ± 0.07	0.49 ± 0.07	Spleen	%	0.220 ± 0.019	0.175 ± 0.028	0.155 ± 0.021
Thymus	g	0.412 ± 0.105	0.222 ± 0.062	0.139 ± 0.042	Thymus	%	0.170 ± 0.041	0.077 ± 0.022	0.044 ± 0.013
Brain	g	1.97 ± 0.06	2.02 ± 0.06	2.04 ± 0.05	Brain	%	0.820 ± 0.052	0.697 ± 0.053	0.644 ± 0.056
Ovaries	mg	82.5 ± 11.5	69.8 ± 21.5	65.8 ± 17.7	Ovaries	‰	0.342 ± 0.052	0.240 ± 0.070	0.207 ± 0.054
Adrenals	mg	59.7 ± 5.2	61.5 ± 11.8	60.9 ± 7.0	Adrenals	‰	0.247 ± 0.017	0.213 ± 0.042	0.192 ± 0.024
Pituitary	mg	14.0 ± 1.8	17.7 ± 3.7	18.9 ± 4.6	Pituitary	‰	0.058 ± 0.008	0.061 ± 0.014	0.060 ± 0.014
Thyroids	mg	17.1 ± 2.9	19.6 ± 3.3	21.0 ± 3.9	Thyroids	‰	0.071 ± 0.012	0.068 ± 0.011	0.066 ± 0.013
Submaxillaries	g	0.36 ± 0.04	0.36 ± 0.04	0.39 ± 0.03	Submaxillaries	%	0.147 ± 0.018	0.125 ± 0.018	0.124 ± 0.014
Sublinguales	g	0.075 ± 0.011	0.075 ± 0.007	0.070 ± 0.008	Sublinguales	%	0.031 ± 0.005	0.026 ± 0.004	0.022 ± 0.003
Parotids	g	0.36 ± 0.08	0.37 ± 0.04	0.36 ± 0.11	Parotids	%	0.150 ± 0.035	0.129 ± 0.013	0.115 ± 0.036

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

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ABSTRACT. To clarify the biological characteristics of Crj: CD(SD)IGS rats (IGS rats), data on the general toxicological parameters were collected. These data were compared with historical in-house data of Crj: CD(SD) rats (CD rats). The body weight was smaller in IGS rats than CD rats and low food consumption was noted in IGS rats as compared to CD rats. The levels of serum lipids in IGS rats were markedly lower than CD rats, especially in triglycerides. No apparent differences were noted between IGS and CD rats in the other parameters. In addition, the effects of diet with different protein content were also examined in IGS rats. The rats were fed standard commercial diet (CRF-1, protein content 23%) or low protein commercial diet (CR-LPF, protein content 18%). The body weight gain was less in CR-LPF group than in CRF-1 group. The output of urinary protein was less in CR-LPF group than in CRF-1 group. In addition, the severity of spontaneous renal lesions was less severe in CR-LPF group than CRF-1 group. — Key words: Biological characteristics, Crj:CD(SD)IGS rats, Low protein diet

CD (SD) IGS-1998: 71-81

INTRODUCTION

Crj:CD(SD) IGS rats newly produced by Charls River Inc. and are originally derived from Crj:CD(SD) rats. Prior to introducing this strain into toxicity studies as an experimental animal, it is necessary to know their biological characteristics. In order to understand these, the data of IGS rats relating to the general toxicological parameters were collected and were compared to those of the ordinarily used CD rats. Another purpose of this study is to examine the effects of protein content in the feed on the biological parameters. CRF-1 is one of the most widely distributed commercial diets in Japan as a rodent chow and contains approximately 23% crude protein, but it has been suggested that this is too high for rodents to maintain a healthy state and resulted in shortening their longevity by renal dysfunction due to hypernutrition. Recently, CR-LPF with approximately 18% crude protein began to be available as a new rodent chow, expecting improvement of the nutritional state. In this situation, the effects of "low" protein commercial diet were also examined in comparison to the "normal" commercial diet.

MATERIALS AND METHODS

Animals: Male and female Crj:CD(SD)IGS rats were obtained from Charles River Japan Inc.(Hino, Japan) at 4 weeks of age. The animals were acclimatized for 2 weeks and healthy animals were used at 6 weeks of age. The animals were housed in an animal room under the following conditions: temperature at 23 ± 3 °C, relative humidity at 50 ± 20%, air ventilation at 10 to 15 times per hour and 12-hour illumination (07:00 to 19:00). The animals were housed individually in hanging stainless-steel wire mesh cages. During the acclimatization period, CRF-1 diet (23% protein) and tap water were provided ad libitum. After acclimatization, either CRF-1 or CR-LPF (18% protein) was provided ad libitum. and continued up to 32 weeks of age.

Group composition and dietary components: The animals at 6 weeks of age were divided into 2 groups: one group fed CR-LPF (18% crude protein) and another group fed CRF-1 (23% crude protein). The animals of the above two groups were necropsied at weeks 4, 13 and 26 of experiment for laboratory and histopathological examinations. Main components of the two commercial diets are shown in the following.

	Diet	
	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 ashes (%)	6.5	6.3
Nitro.gen free extract (%)	53.5	58.0

In-house data of Crj:CD(SD) rats fed CRF-1: The data from the control animals in the toxicity studies carried out in our laboratory during the last 5 years were used.

Observations and examinations: *General condition*: The general condition of the animals were checked daily.

Body weight and food consumption: The body weight was recorded once weekly. One day's food consumption was calculated based on the 7 day's cumulative consumption determined weekly.

Ophthalmology: After dilating the pupil by applying a mydriatic agent (Mydrin P: Santen Pharmaceutical Co. Ltd.), external appearance of the eyes was examined macroscopically, and then the anterior portion, transparent body and fundus oculi were examined using an ophthalmoscope (BX α -type: NEITZ Instrument Co. Ltd).

Urinalysis: The parameters determined and the methods used are shown in Table 1. 4-hour urine samples were collected under deprivation of food but with free access to water and then 20-hour urine samples were collected with free access to food

and water. 24-hour urine volume was calculated by totaling the 4-hour and 20-hour urine samples. One day's output of electrolytes was calculated from the determined concentration and the 24-hour urine volume.

Hematology: The parameters determined and the methods used are shown in Table 1. At the time of necropsy, blood samples were collected from the abdominal aorta into blood collection tubes containing EDTA-2K. However, for determining coagulation parameters, blood samples treated with 3.8% sodium citrate were used.

Blood chemistry: The parameters determined and the methods used are shown in Table 1. At the time of necropsy, blood samples were collected from the abdominal aorta and the sera were used for determination; however, GOT, GPT, LDH and

Table 1. Items and method for laboratory examinations

Examination	Item	Abbreviation	Method
Urinalysis (4h-urine)	pH, Protein, Ketone body, Glucose		Test paper method (URIFLET 7A) [a]
	Occult blood, Bilirubin, Urobilinogen		Test paper method (URIFLET 7A) [a]
	Color		Macroscopic observation
	Urinary sediment		Microscopic examination
(20h-urine)	Urine volume		Measuring cylinder
	Specific gravity	S.G.	Refractometry [b]
	Osmotic pressuse		Freezing point method [c]
	Sodium	Na	Ion selective electrode method [d]
	Potassium	K	Ion selective electrode method [d]
	Chloride	Cl	Ion selective electrode method [d]
Hematology	Red blood cell	RBC	Electronic counting method [f]
	White blood cell	WBC	Electronic counting method [f]
	Platelet		Electronic counting method [f]
	Differential leukocyte count		Microscopic examination using May-Giemsa staining
	Hemoglobin	Hb	Cyanmethemoglobin method [f]
	Hematocrit	Ht	Calculated from MCV and RBC [f]
	Reticulocyte ratio		Brecher method [f]
	Mean corpuscular volume	MCV	Electronic counting method [f]
	Mean corpuscular hemoglobin	MCH	Calculated from Hb and RBC [f]
	Mean corpuscular hemoglobin concentration	MCHC	Calculated from Hb and Ht [f]
	Prothrombin time	PT	Clot method [g]
	Activated partial thromboplastin time	APTT	Clot method [g]
	Fibrinogen		Thromboplastin method [g]
Blood chemistry	Glutamic oxaloacetic transaminase	GOT	UV-rate assay method [e]
	Glutamic pyruvic transaminase	GPT	UV-rate assay method [e]
	Lactate dehydrogenase	LDH	UV-rate assay method [e]
	Creatine phosphokinase	CPK	UV-rate assay method [e]
	Alkaline phosphatase	AlP	Bessey-Lowry method [e]
	Glucose		Hexokinase-G6PD method [e]
	Blood urea nitrogen	BUN	Urease-GLDH method [e]
	Total bilirubin	T.bilirubin	Azobilirubin method [e]
	Creatinine		Jaffé method [e]
	Triglyceride	TG	UV-rate assay method [e]
	Total cholesterol	T.cho	CEH-COD-POD method [e]
	Phospholipid	PL	PLD-ChOD-POD method [e]
	Total protein	TP	Biuret method [e]
	Albumin		BCG method[e]
	Albumin-globulin ratio	A/G	Calculated from protein fractions
	Sodium	Na	Ion selective electrode method[e]
	Potassium	K	Ion selective electrode method[e]
	Chloride	Cl	Ion selective electrode method[e]
	Calcium	Ca	OCPC method[e]
	Inorganic phosphorus	P	Molybdic acid method[e]

a): mini AUTION ANALYZER MA-4210 (Kyoto Daiichi Kagaku Co., Ltd.)
b): Atago refractometer (Atago Co., Ltd.)
c): Osmotic Pressure AUTO & STAT OM-6030 (Kyoto Daiichi Kagaku Co., Ltd.)
d): Automatic Electrolyte Analyzer PVA- α Ⅱ (Analytical Instruments Inc.)
e): Automatic Analyzer Monarch (Instrumentation Laboratory)
f): Coulter Counter T890 (Coulter Electronics Inc.)
g): Coagulometer ACL 100 (Instrumentation Laboratory)

CPK were determined on the plasma obtained from blood samples treated with heparin.

Necropsy and organ weight: After collecting the blood samples, all animals were sacrificed and examined macroscopically, and then the following organs were weighed and their relative weights were also calculated: brain, thymus, heart, lungs, liver, spleen, kidneys, adrenals, ovaries, uterus, testes and prostate.

Histopathology: The following organs/tissues were fixed with phosphate buffered 10% formalin (however, the eyeballs and its accessory organs were fixed with a mixture of glutaraldehyde and formalin and the testes were fixed with Bouin's solution) and the H.E. stainined specimens were prepared by a routine method, and examined histopathologically: adrenal, aorta, brain, coagulating gland, ear, epididymis, esophagus, extraorbital lacrimal gland, eyeball, femur/marrow, Harderian gland, heart, kidney, large intestine (cecum to rectum), liver, lung, lymph node (cervical and mesenteric), mammary gland, medulla oblongata, optic nerve, pituitary, pancreas, parathyroid, prostate, spleen, salivary gland (parotid, sublingual and submandibular), sciatic nerve, seminal vesicle, skeletal muscle, skin, sternum/marrow, stomach, small intestine (duodenum to ileum), spinal cord, testis, thymus, thyroid, tongue, trachea, urinary bladder, ovary, uterus and vagina.

Statistics: Statistical analysis was not done in the present study to compare differences among 3 groups including historical in-house data.

RESULTS

In the clinical observations, no significant signs were found throughout the observation period.

The growth curves are shown in Figs. 1 and 2. The mean body weights at 32 weeks of age were as follows: CD rats, 376 ± 46 g for females and 698 ± 77 g for males; IGS rats fed CRF-339 ± 38 g for females and 610 ± 60 g for males, IGS rats fed CR-LPF, 308 ± 33 g for females and 585 ± 57 g for males. Therefore, the body weight gains were largest in CD rats, then IGS rats fed CRF-1 and smallest in IGS rats fed CR-LPF. The mean body weights of IGS rats fed CRF-1 were approximately 10% lower than those of CD rats and were approximately 10% higher than those of IGS rats fed CR-LPF. Food consumption of IGS rats fed CRF-1 was slightly lower than that of CD rats throughout the observation period; however, food consumption of IGS rats was almost the same irrespective of the kind of diet supplied.

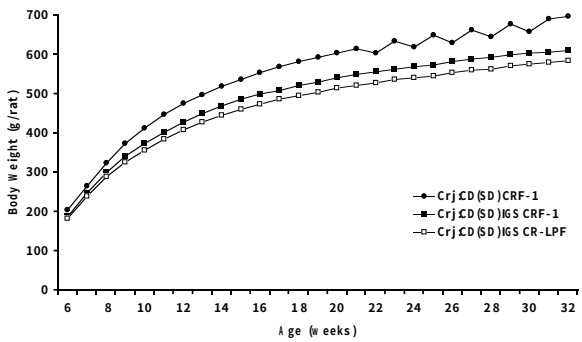

Fig. 1. Growth Curve in Crj:CD(SD) and Crj:CD(SD)IGS Male Rats Fed CRF-1 or CR-LPF

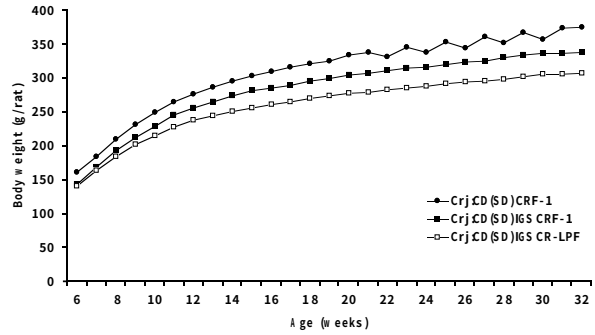

Fig. 2. Growth Curve in Crj:CD(SD) and Crj:CD(SD)IGS Female Rats Fed CRF-1 or CR-LPF

In the ophthalmological examinations, hemorrhage in the vitreous body, opacity of the cornea, abnormal running of the blood vessels in the fundus and myelinated nerve fibers were occasionally observed in IGS rats fed CRF-1 or CR-LPF; however, these lesions were observed spontaneously in CD rats and no increase in the above lesions were noted in IGS rats as compared to CD rats.

The results of urinalysis are shown in Tables 2-1 (10 weeks of age), 2-2 (16 weeks of age) and 2-3 (32 weeks of age). No apparent differences were observed between IGS rats fed CRF-1 and CD rats. When urinary parameters of IGS rats were compared between the CRF-1 and CR-LPF groups, a slight difference was noted in urinary protein. At 32 weeks of age, animals exhibiting protein more severe than 3+ were not observed in the CR-LPF group but were in the CRF-1 group.

The results of hematology are shown in Table 3. No apparent differences were observed between IGS rats fed CRF-1 and CD rats, nor between IGS rats in the CRF-1 and CR-LPF groups.

The results of blood chemistry are shown in Table 4. Remarkable differences were noted in the levels of serum lipids, especially in triglycerides (Fig. 3). The concentrations of serum triglycerides increased according to the age in both IGS and CD rats, but triglycerides in IGS rats fed CRF-1 were approximately half those in CD rats at all stages determined. The levels of total cholesterol and phospholipids in IGS rats fed CRF-1 were

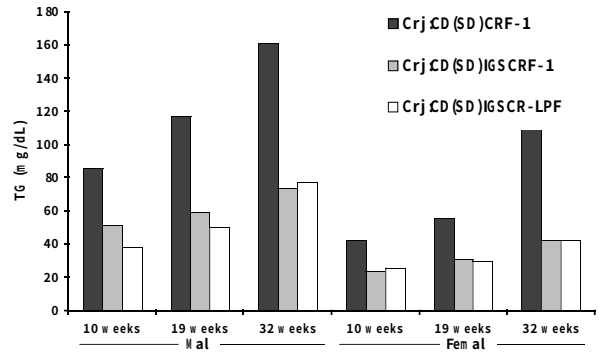

Fig. 3. Serum Triglyceride in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF

Table 2-1. Urinalysis in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF (Age : 10 weeks)

Sex	Female				Male	
Strain	Crj:CD(SD)	Crj:CD(SD)IGS	Crj:CD(SD)	Crj:CD(SD)IGS		
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR–LPF
Age : 10 weeks						
Number of animals	368	20	40	424	20	40
pH: 5.0–	0	0	0	0	0	0
6.0–	34	2	16	1	0	0
7.0–	80	6	11	30	0	6
8.0–	212	12	12	248	18	30
9.0–	42	0	1	145	2	4
Protein: 0-5mg/dL	255	9	16	93	4	0
10-20mg/dL	78	9	9	214	12	23
30-70mg/dL	33	2	15	103	4	16
100-200mg/dL	2	0	0	14	0	1
Ketone body: 0mg/dL	338	20	29	381	20	25
5mg/dL	15	0	9	31	0	12
10-20 mg/dL	15	0	2	12	0	3
Glucose: 0-10mg/dL	367	20	40	423	20	40
30-50mg/dL	1	0	0	1	0	0
Occult Blood: 0mg/dL	331	19	40	340	17	39
0.03mg/dL	11	1	0	21	1	1
0.06-0.1mg/dL	15	0	0	39	1	0
0.2-0.5mg/dL	6	0	0	19	1	0
≧1.0mg/dL	5	0	0	5	0	0
Bilirubin: 0mg/dL	363	20	40	421	20	40
0.2mg/dL	5	0	0	3	0	0
Urobilinogen: 0.2-1.0mg/dL	352	19	31	394	20	35
2.0-3.0mg/dL	16	1	9	27	0	5
4.0-6.0mg/dL	0	0	0	3	0	0
Color:Light Yellow	27	0	1	30	0	0
Yellow	341	20	39	394	20	40
Urine Sediment						
RBC:Negative(–)	367	20	40	412	20	40
Slight(+–)	1	0	0	4	0	0
Mild(+)	0	0	0	1	0	0
WBC:Negative(–)	364	20	39	413	20	40
Slight(+–)	4	0	1	4	0	0
SEC:Slight(+–)	361	20	40	400	20	40
Mild(+)	7	0	0	17	0	0
SREC:Negative(–)	313	20	40	362	19	40
Slight(+–)	5	0	0	5	1	0
Cast:Negative(–)	368	20	40	417	20	40
PS:Negative(–)	211	11	18	241	13	11
Slight(+–)	136	9	21	152	6	20
Mild(+)	14	0	1	16	1	9
Moderate(++)	7	0	0	8	0	0
CO:Negative(–)	368	20	40	417	20	40
Water Intake(mL/rat/24hrs)	34 ± 12[a)]	35 ± 9	38±14	44 ± 10	43 ± 13	36 ± 6
Urine Volume(mL/24hrs)	7.9 ± 4.3	7.8 ± 3.5	7.0 ± 3.3	12.9 ± 6.0	11.8 ± 4.5	8.9 ± 3.1
Specific Gravity	1.033 ± 0.194	1.054 ± 0.013	1.051 ± 0.013	1.063 ± 0.017	1.060 ± 0.014	1.059 ± 0.013
Osmolality(mOsm/kg H_2O)	2085 ± 502	1781 ± 428	1674 ± 430	2107 ± 522	1940 ± 428	1837 ± 418
Na(mEq/24hrs)	1.07 ± 0.49	1.00 ± 0.42	0.84 ± 0.32	1.80 ± 0.60	1.74 ± 0.44	1.34 ± 0.26
K(mEq/24hrs)	1.87 ± 0.82	1.87 ± 0.67	2.05 ± 0.69	3.19 ± 0.98	3.21 ± 0.68	3.08 ± 0.63
Cl(mEq/24hrs)	1.37 ± 0.62	1.33 ± 0.49	1.23 ± 0.40	2.38 ± 0.74	2.28 ± 0.48	1.93 ± 0.37

a): Mean ± S.D.
RBC: Red blood cell, WBC: White blood cell, SEC: Squamous epithelial cell,
SREC: Small round epithelial cell, PS: Phosphate salts, CO: Calcium oxalate

also lower than those in CD rats, but the extent of fluctuation was less than that in triglycerides. No differences were noted in the serum lipid levels in IGS rats between the CRF-1 and CR-LPF groups. No other differences were noted in any of the parameters.

The relative organ weights are shown in Table 5. At 10 weeks of age, the relative weights of the liver and kidneys in IGS rats fed CRF-1 were slightly lower than those in CD rats; however, the relative weights of the above organs became comparable in the two groups at weeks 19 and 32 of age. No other differences were noted in any of the organs.

Table 2-2. Urinalysis in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF (Age : 19 weeks)

Sex	Female			Male		
Strain	Crj:CD(SD)	Crj:CD(SD)IGS		Crj:CD(SD)	Crj:CD(SD)IGS	
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR-LPF
Age : 19 weeks						
Number of animals	306	20	40	305	20	40
pH:5.0-	0	0	0	0	0	0
6.0-	46	3	19	3	0	1
7.0-	87	4	8	45	1	8
8.0-	141	11	8	202	15	29
9.0-	27	2	5	55	4	2
Protein: 0-5mg/dL	182	9	18	56	0	3
10-20mg/dL	90	8	16	128	3	16
30-70mg/dL	30	2	5	98	14	17
100-200mg/dL	3	1	1	19	3	4
250-400mg/dL	1	0	0	3	0	0
>400mg/dL	0	0	0	1	0	0
Ketone body: 0mg/dL	275	18	31	281	13	16
5mg/dL	20	1	6	17	7	14
10-20 mg/dL	11	1	3	7	0	10
Glucose: 0-10mg/dL	303	20	40	300	17	40
30-50mg/dL	3	0	0	5	3	0
Occult Blood:0mg/dL	287	19	39	153	12	32
0.03mg/dL	9	1	0	56	5	4
0.06-0.1mg/dL	3	0	0	69	2	1
0.2-0.5mg/dL	6	0	1	24	1	2
≧1.0mg/dL	1	0	0	3	0	1
Bilirubin: 0mg/dL	301	20	40	294	20	40
0.2mg/dL	2	0	0	6	0	0
0.5-1.0mg/dL	3	0	0	5	0	0
Urobilinogen:0.2-1.0mg/dL	277	17	34	277	16	28
2.0-3.0mg/dL	24	3	6	19	4	12
4.0-6.0mg/dL	5	0	0	9	0	0
Color: Light Yellow	13	0	0	15	0	0
Yellow	293	20	40	290	20	40
Urine Sediment						
RBC: Negative(–)	306	20	40	294	20	37
Slight(+–)	0	0	0	11	0	3
WBC: Negative(–)	303	20	38	292	20	40
Slight(+–)	3	0	2	10	0	0
Mild(+)	0	0	0	3	0	0
SEC: Slight(+–)	300	20	40	293	20	40
Mild(+)	6	0	0	12	0	0
SREC:Negative(–)	249	20	39	235	20	40
Slight(+–)	2	0	1	13	0	0
Mild(+)	0	0	0	1	0	0
Cast: Negative(–)	306	20	40	305	20	40
PS: Negative(–)	179	6	14	138	6	9
Slight(+–)	112	11	24	131	13	28
Mild(+)	15	2	1	30	1	3
Moderate(++)	0	1	1	6	0	0
CO: Negative(–)	305	20	40	305	20	40
Water Intake(mL/rat/24hrs)	36 ± 12[a)]	33 ± 7	37 ± 12	43 ± 11	38 ± 7	40 ± 13
Urine Volume(mL/24hrs)	10.3 ± 5.5	10.3 ± 4.3	9.2 ± 4.6	16.2 ± 7.2	11.1 ± 3.9	11.1 ± 4.3
Specific Gravity	1.051 ± 0.014	1.048 ± 0.013	1.048 ± 0.017	1.059 ± 0.015	1.066 ± 0.013	1.058 ± 0.013
Osmolality(mOsm/kg H_2O)	1800 ± 616	1598 ± 423	1579 ± 556	2244 ± 242	2117 ± 400	1816 ± 390
Na(mEq/24hrs)	1.12 ± 0.43	1.15 ± 0.35	0.96 ± 0.27	2.05 ± 0.62	1.70 ± 0.50	1.51 ± 0.45
K(mEq/24hrs)	1.99 ± 0.67	2.14 ± 0.57	2.16 ± 0.61	3.69 ± 1.00	3.14 ± 0.83	3.46 ± 0.91
Cl(mEq/24hrs)	1.40 ± 0.53	1.46 ± 0.40	1.30 ± 0.38	2.54 ± 0.74	2.14 ± 0.59	2.06 ± 0.57

a): Mean ± S.D.
RBC: Red blood cell, WBC: White blood cell, SEC: Squamous epithelial cell,
SREC: Small round epithelial cell, PS: Phosphate salts, CO: Calcium oxalate

Table 2-3. Urinalysis in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF (Age : 32 weeks)

Sex	Female			Male		
Strain	Crj:CD(SD)	Crj:CD(SD)IGS		Crj:CD(SD)	Crj:CD(SD)IGS	
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR-LPF
Age : 32 weeks						
Number of animals	309	40	40	305	40	40
pH: 5.0-	0	2	0	0	0	1
6.0-	82	9	13	14	0	2
7.0-	90	10	8	62	5	6
8.0-	117	18	17	197	30	27
9.0-	17	1	2	31	5	4
Protein: 0-5mg/dL	117	10	11	27	2	5
10-20mg/dL	86	15	17	79	3	7
30-70mg/dL	72	10	9	75	17	23
100-200mg/dL	19	3	3	73	15	5
250-400mg/dL	10	1	0	30	2	0
>400mg/dL	5	1	0	21	1	0
Ketone body: 0mg/dL	239	28	35	247	31	26
5mg/dL	44	7	4	35	7	6
10-20 mg/dL	26	5	1	18	2	8
30-45mg/dL	0	0	0	5	0	0
Glucose: 0-10mg/dL	291	38	39	291	37	38
30-50mg/dL	18	2	1	14	3	2
Occult Blood: 0mg/dL	275	38	39	163	29	30
0.03mg/dL	15	1	0	59	7	5
0.06-0.1mg/dL	6	0	1	57	3	5
0.2-0.5mg/dL	8	1	0	21	0	0
≧1.0mg/dL	5	0	0	5	1	0
Bilirubin: 0mg/dL	300	40	40	296	40	40
0.2mg/dL	3	0	0	3	0	0
0.5-1.0mg/dL	6	0	0	4	0	0
2.0-4.0mg/dL	0	0	0	2	0	0
Urobilinogen: 0.2-1.0mg/dL	272	29	35	256	23	25
2.0-3.0mg/dL	36	11	5	42	15	14
4.0-6.0mg/dL	1	0	0	5	2	1
8.0-12.0mg/dL	0	0	0	2	0	0
Color: Light Yellow	13	0	0	5	0	0
Yellow	296	40	40	299	39	40
Dark Yellow	0	0	0	0	1	0
Urine Sediment						
RBC: Negative(-)	303	40	39	298	38	38
Slight(+-)	5	0	1	4	1	2
Mild(+)	1	0	0	0	0	0
Moderate(++)	0	0	0	3	0	0
Severe(+++)	0	0	0	0	1	0
WBC: Negative(-)	303	40	40	275	34	37
Slight(+-)	6	0	0	26	6	2
Mild(+)	0	0	0	4	0	0
Moderate(++)	0	0	0	0	0	1
SEC: Slight(+-)	300	40	40	299	40	40
Mild(+)	8	0	0	4	0	0
Moderate(++)	1	0	0	2	0	0
SREC: Negative(-)	267	40	40	251	39	39
Slight(+-)	5	0	0	17	1	1
Mild(+)	0	0	0	1	0	0
Cast: Negative(-)	309	40	40	304	40	40
Slight(+-)	0	0	0	1	0	0
PS: Negative(-)	188	23	17	187	8	10
Slight(+-)	109	17	23	110	29	27
Mild(+)	9	0	0	8	3	3
Moderate(++)	3	0	0	0	0	0
CO: Negative(-)	309	40	40	305	40	40
Water Intake(mL/rat/24hrs)	40 ± 12[a)]	37 ± 11	34 ± 7	38 ± 13	35 ± 6	36 ± 7
Urine Volume(mL/24hrs)	13.7 ± 7.2	10.4 ± 5.1	8.5 ± 4.0	18.6 ± 8.2	10.6 ± 4.5	11.8 ± 5.5
Specific Gravity	1.043 ± 0.012	1.047 ± 0.016	1.045 ± 0.014	1.055 ± 0.014	1.066 ± 0.011	1.054 ± 0.015
Osmolality(mOsm/kg H2O)	1337 ± 481	1530 ± 531	1445 ± 433	2066 ± 304	2134 ± 352	1711 ± 455
Na(mEq/24hrs)	1.16 ± 0.44	0.93 ± 0.35	0.79 ± 0.27	2.18 ± 0.62	1.46 ± 0.43	1.29 ± 0.46
K(mEq/24hrs)	2.12 ± 0.69	1.98 ± 0.70	1.90 ± 0.61	3.96 ± 0.96	3.12 ± 0.83	3.22 ± 0.98
Cl(mEq/24hrs)	1.44 ± 0.52	1.22 ± 0.44	1.08 ± 0.38	2.69 ± 0.72	1.92 ± 0.54	1.81 ± 0.59

a): Mean ± S.D.
RBC: Red blood cell, WBC: White blood cell, SEC: Squamous epithelial cell,
SREC: Small round epithelial cell, PS: Phosphate salts, CO: Calcium oxalate

Table 3. Hematology in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF

Sex	Female			Male		
Strain	Crj:CD(SD)	Crj:CD(SD)IGS		Crj:CD(SD)	Crj:CD(SD)IGS	
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR-LPF
Age : 10 weeks						
Number of animals	400	20	40	446	20	40
RBC (x104/mm^3)	738 ± 47[a)]	757 ± 30	771 ± 38	746 ± 51	780 ± 41	775 ± 33
Hemoglobin (g/dL)	15.6 ± 0.7	16.2 ± 0.6	16.3 ± 0.6	15.7 ± 0.8	16.4 ± 0.5	16.5 ± 0.6
Hematocrit (%)	45 ± 2	44 ± 2	44 ± 2	46 ± 2	45 ± 2	45 ± 2
MCV (m^3)	60.0 ± 1.9	57.6 ± 1.6	57.3 ± 1.6	60.5 ± 2.0	57.6 ± 2	57.6 ± 1.5
MCH (pg)	21.2 ± 0.9	21.4 ± 0.7	21.2 ± 0.8	21.0 ± 0.8	21.1 ± 0.9	21.2 ± 0.7
MCHC (%)	35.3 ± 1.0	37.2 ± 0.6	37.0 ± 0.7	34.7 ± 1.0	36.6 ± 0.7	36.9 ± 0.7
Reticulocyte (‰)	20 ± 7	17 ± 5	14 ± 5	23 ± 8	17 ± 3	15 ± 4
WBC (x102/mm3)	74 ± 25	74 ± 23	74 ± 19	103 ± 29	81 ± 22	85 ± 22
Lymphocyte (%)	88.1 ± 5.8	85.0 ± 6.0	84.0 ± 6.5	89.4 ± 4.6	84.6 ± 4.4	85.5 ± 6.0
Stab neutrophil (%)	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Segmented neutrophil (%)	11.0 ± 5.5	14.0 ± 6.1	15.1 ± 6.5	9.9 ± 4.4	14.3 ± 4.1	13.7 ± 6.0
Eosinophil (%)	0.6 ± 0.7	0.9 ± 0.7	0.9 ± 0.7	0.4 ± 0.5	1.0 ± 0.8	0.8 ± 0.8
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte (%)	0.1 ± 0.3	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
Platelet (x104/mm^3)	119.3 ± 14.2	118.0 ± 8.7	117.5 ± 12.2	120.5 ± 14.6	113.1 ± 13.5	112.1 ± 9.8
PT (sec)	11.4 ± 0.7	11.8 ± 0.4	11.9 ± 0.5	12.8 ± 1.3	14.1 ± 1.6	12.7 ± 0.5
APTT (sec)	13.5 ± 1.4	13.2 ± 0.9	12.3 ± 1.0	16.9 ± 2.2	16.7 ± 1.8	15.2 ± 1.2
Fibrinogen (mg/dL)	194 ± 42	212 ± 19	203 ± 20	250 ± 47	271 ± 24	246 ± 41
Age : 19 weeks						
Number of animals	103	20	40	102	20	40
RBC (x104/mm^3)	775 ± 42	804 ± 36	793 ± 36	826 ± 51	849 ± 44	853 ± 39
Hemoglobin (g/dL)	15.3 ± 0.6	16 ± 0.4	16.1 ± 0.6	15.6 ± 0.5	16.4 ± 0.6	16.4 ± 0.5
Hematocrit (%)	43 ± 2	44 ± 2	44 ± 2	45 ± 2	45 ± 2	45 ± 2
MCV (m3)	56.1 ± 1.7	54.4 ± 1.9	55.4 ± 1.6	54.3 ± 1.7	52.6 ± 1.4	52.7 ± 1.7
MCH (pg)	19.7 ± 0.8	19.9 ± 0.7	20.3 ± 0.9	18.9 ± 0.9	19.3 ± 0.7	19.2 ± 0.6
MCHC (%)	35.2 ± 1.4	36.7 ± 0.7	36.7 ± 0.9	34.8 ± 1.5	36.7 ± 0.8	36.5 ± 0.5
Reticulocyte (‰)	20 ± 6	17 ± 4	18 ± 4	20 ± 5	16 ± 4	17 ± 5
WBC (x102/mm^3)	63 ± 24	54 ± 16	56 ± 17	100 ± 33	73 ± 15	86 ± 24
Lymphocyte (%)	87.8 ± 7.0	80.7 ± 8.3	85.0 ± 5.2	87.4 ± 7.1	83.9 ± 7.3	83.6 ± 6.0
Stab neutrophil (%)	0.3 ± 0.4	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.1
Segmented neutrophil (%)	11.1 ± 6.8	18.3 ± 8.2	14.0 ± 5.2	11.5 ± 6.9	15.4 ± 7.2	15.6 ± 6.0
Eosinophil (%)	0.7 ± 0.7	0.9 ± 0.8	0.9 ± 0.6	0.6 ± 0.7	0.7 ± 0.6	0.7 ± 0.7
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte (%)	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2
Platelet (x10^4/mm^3)	111.7 ± 12.7	103.0 ± 10.9	105.0 ± 9.9	115.4 ± 10.2	102.5 ± 8.1	105.6 ± 11.8
PT (sec)	11.3 ± 0.8	11 ± 0.6	12.0 ± 0.6	12.3 ± 1.1	13.5 ± 1.0	12.0 ± 0.6
APTT (sec)	13.7 ± 1.4	13.9 ± 0.8	13.4 ± 1.1	16.4 ± 1.9	17.6 ± 1.1	14.3 ± 1.6
Fibrinogen (mg/dL)	191 ± 49	184 ± 18	168 ± 16	272 ± 53	262 ± 36	240 ± 26
Age : 32 weeks						
Number of animals	240	40	40	239	40	40
RBC (x10^4/mm^3)	770 ± 43	775 ± 39	792 ± 45	843 ± 43	866 ± 39	868 ± 60
Hemoglobin (g/dL)	15.1 ± 0.7	15.1 ± 0.7	15.2 ± 0.7	15.4 ± 0.7	15.6 ± 0.5	15.6 ± 1.0
Hematocrit (%)	43 ± 2	43 ± 2	43 ± 2	45 ± 2	45 ± 2	45 ± 3
MCV (m^3)	56.2 ± 1.8	55.2 ± 1.7	54.8 ± 2.0	53.0 ± 1.7	51.8 ± 2	52.1 ± 1.6
MCH (pg)	19.6 ± 0.8	19.5 ± 0.7	19.2 ± 0.7	18.2 ± 0.7	18 ± 0.7	18.0 ± 0.6
MCHC (%)	34.9 ± 1.0	35.3 ± 0.8	35.1 ± 0.7	34.4 ± 1.0	34.7 ± 0.8	34.5 ± 0.6
Reticulocyte (‰)	18 ± 6	19 ± 4	19 ± 4	19 ± 5	20 ± 5	21 ± 7
WBC (x10^2/mm^3)	51 ± 15	52 ± 16	50 ± 15	88 ± 25	74 ± 15	77 ± 18
Lymphocyte (%)	81.6 ± 8.3	81.4 ± 6.4	81.3 ± 6.9	80.8 ± 8.8	78.4 ± 8.2	76.6 ± 8.9
Stab neutrophil (%)	0.2 ± 0.3	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.1
Segmented neutrophil (%)	17.2 ± 8.2	17.3 ± 6.2	17.7 ± 6.9	18.1 ± 8.7	20.4 ± 8.4	22.1 ± 8.7
Eosinophil (%)	0.9 ± 0.7	1.2 ± 0.9	1.0 ± 0.8	0.7 ± 0.7	1.1 ± 0.8	1.2 ± 1.0
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte (%)	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.4	0.1 ± 0.2	0.1 ± 0.2
Platelet (x10^4/mm^3)	105.9 ± 14.0	90.7 ± 8.8	95.3 ± 11.4	115.8 ± 14.0	95.5 ± 9.6	96.5 ± 12.1
PT (sec)	11.0 ± 0.7	11.4 ± 0.6	11.7 ± 0.4	11.8 ± 0.7	12.7 ± 0.9	12.2 ± 0.6
APTT (sec)	14.1 ± 1.5	12.6 ± 1.1	12.5 ± 1.1	15.7 ± 2.3	14.8 ± 2.0	13.8 ± 1.5
Fibrinogen (mg/dL)	181 ± 42	181 ± 30	170 ± 20	265 ± 37	266 ± 30	259 ± 25

a): Mean ± S. D.

Table 4. Blood Chemistry in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF

Sex	Female			Male		
Strain	Crj:CD(SD)	Crj:CD(SD)IGS		Crj:CD(SD)	Crj:CD(SD)IGS	
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR-LPF
Age : 10 weeks						
Number of animals	400	20	40	453	20	40
GOT (IU/L)	58 ± 10[a)]	66 ± 9	60 ± 9	52 ± 13	57 ± 10	59 ± 9
GPT (IU/L)	31 ± 6	37 ± 4	37 ± 5	35 ± 7	44 ± 5	44 ± 6
LDH (IU/L)	27 ± 11	26 ± 7	23 ± 5	34 ± 11	44 ± 13	31 ± 4
CPK (IU/L)	109 ± 30	100 ± 20	94 ± 16	149 ± 34	147 ± 27	122 ± 22
ALP (IU/L)	222 ± 69	249 ± 51	241 ± 56	360 ± 99	403 ± 60	397 ± 74
T.cho (mg/dL)	75 ± 16	55 ± 13	63 ± 19	66 ± 14	54 ± 11	64 ± 14
TG (mg/dL)	42 ± 16	24 ± 5	26 ± 7	86 ± 35	51 ± 12	38 ± 14
PL (mg/dL)	142 ± 24	93 ± 15	107 ± 23	116 ± 20	87 ± 13	93 ± 14
T.bililubin (mg/dL)	0.11 ± 0.03	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
Glucose (mg/dL)	112 ± 14	108 ± 12	99 ± 9	129 ± 20	124 ± 11	112 ± 11
BUN (mg/dL)	16 ± 3	17 ± 2	16 ± 2	13 ± 2	14 ± 1	14 ± 1
Creatinine (mg/dL)	0.58 ± 0.07	0.65 ± 0.05	0.62 ± 0.04	0.58 ± 0.07	0.64 ± 0.05	0.61 ± 0.05
Na (mEq/L)	142 ± 24	142 ± 1	143 ± 1	144 ± 2	144 ± 1	145 ± 1
K (mEq/L)	4.7 ± 0.4	5.3 ± 0.4	4.6 ± 0.4	4.4 ± 0.3	4.7 ± 0.4	4.4 ± 0.3
Cl (mEq/L)	113 ± 4	113 ± 1	115 ± 2	111 ± 4	111 ± 1	114 ± 2
Ca (mEq/L)	9.4 ± 0.4	9.2 ± 0.3	9.2 ± 0.3	9.4 ± 0.3	9.3 ± 0.2	9.0 ± 0.3
P (mEq/L)	7.9 ± 0.9	7.6 ± 0.6	7.2 ± 0.5	8.7 ± 0.8	7.4 ± 0.6	6.9 ± 0.5
TP (g/dL)	6.2 ± 0.4	6.1 ± 0.4	5.9 ± 0.3	6.0 ± 0.3	5.9 ± 0.2	5.6 ± 0.2
Albumin (g/dL)	3.6 ± 0.3	3.8 ± 0.2	3.8 ± 0.2	3.3 ± 0.4	3.7 ± 0.1	3.6 ± 0.1
A/G	1.00 ± 0.10	1.02 ± 0.08	1.04 ± 0.07	0.87 ± 0.08	0.9 ± 0.08	0.93 ± 0.09
Age : 19 weeks						
Number of animals	103	20	40	102	20	40
GOT (IU/L)	69 ± 26	92 ± 56	72 ± 24	61 ± 22	60 ± 9	64 ± 11
GPT (IU/L)	40 ± 16	59 ± 45	45 ± 12	40 ± 17	44 ± 4	46 ± 6
LDH (IU/L)	31 ± 21	39 ± 28	28 ± 18	38 ± 16	38 ± 10	42 ± 11
CPK (IU/L)	56 ± 8	68 ± 10	65 ± 10	80 ± 14	77 ± 19	92 ± 15
ALP (IU/L)	90 ± 28	89 ± 19	94 ± 23	166 ± 31	200 ± 47	181 ± 33
T.cho (mg/dL)	88 ± 23	74 ± 13	76 ± 18	81 ± 22	62 ± 16	75 ± 20
TG (mg/dL)	56 ± 23	31 ± 9	30 ± 8	117 ± 60	59 ± 21	50 ± 14
PL (mg/dL)	173 ± 41	134 ± 24	133 ± 24	133 ± 33	95 ± 20	101 ± 21
T.bililubin (mg/dL)	0.13 ± 0.04	0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.03	0.10 ± 0.01	0.11 ± 0.01
Glucose (mg/dL)	129 ± 14	114 ± 10	116 ± 12	146 ± 17	127 ± 11	126 ± 14
BUN (mg/dL)	17 ± 3	15 ± 2	17 ± 3	17 ± 3	12 ± 1	15 ± 2
Creatinine (mg/dL)	0.65 ± 0.09	0.69 ± 0.06	0.65 ± 0.06	0.63 ± 0.07	0.66 ± 0.04	0.63 ± 0.05
Na (mEq/L)	143 ± 1	143 ± 2	143 ± 1	144 ± 1	146 ± 1	144 ± 2
K (mEq/L)	4.5 ± 0.4	4.6 ± 0.3	4.5 ± 0.4	4.6 ± 0.3	4.3 ± 0.2	4.6 ± 0.4
Cl (mEq/L)	117 ± 3	114 ± 10	114 ± 2	115 ± 3	113 ± 2	113 ± 2
Ca (mEq/L)	9.4 ± 0.4	9.6 ± 0.2	9.3 ± 0.4	9.4 ± 0.3	9.4 ± 0.2	9.2 ± 0.3
P (mEq/L)	5.7 ± 1.1	7.2 ± 0.7	6.0 ± 15	6.8 ± 0.8	6.2 ± 0.7	6.1 ± 0.6
TP (g/dL)	7.2 ± 0.5	7.0 ± 0.3	6.6 ± 0.5	6.7 ± 0.4	6.5 ± 0.3	6.1 ± 0.2
Albumin (g/dL)	3.9 ± 0.3	4.1 ± 0.2	4.0 ± 0.2	3.1 ± 0.2	3.8 ± 0.1	3.6 ± 0.1
A/G	1.04 ± 0.16	0.97 ± 0.06	0.96 ± 0.08	0.80 ± 0.09	0.72 ± 0.06	0.76 ± 0.05
Age : 32 weeks						
Number of animals	240	40	40	239	40	40
GOT (IU/L)	103 ± 121	129 ± 141	165 ± 414	51 ± 22	70 ± 24	77 ± 63
GPT (IU/L)	66 ± 63	73 ± 45	75 ± 100	41 ± 17	54 ± 14	56 ± 32
LDH (IU/L)	50 ± 66	60 ± 71	119 ± 478	38 ± 18	51 ± 17	52 ± 44
CPK (IU/L)	49 ± 16	62 ± 16	59 ± 16	70 ± 24	79 ± 19	78 ± 15
ALP (IU/L)	69 ± 26	58 ± 15	62 ± 20	173 ± 103	163 ± 38	171 ± 39
T.cho (mg/dL)	110 ± 31	94 ± 24	94 ± 23	105 ± 24	76 ± 21	89 ± 24
TG (mg/dL)	111 ± 80	42 ± 25	42 ± 14	161 ± 77	74 ± 33	77 ± 26
PL (mg/dL)	224 ± 52	168 ± 39	169 ± 33	168 ± 31	111 ± 24	121 ± 22
T.bililubin (mg/dL)	0.14 ± 0.06	0.11 ± 0.02	0.12 ± 0.02	0.14 ± 0.05	0.09 ± 0.01	0.11 ± 0.01
Glucose (mg/dL)	130 ± 16	125 ± 10	120 ± 13	141 ± 20	133 ± 17	137 ± 14
BUN (mg/dL)	15 ± 2	16 ± 2	16 ± 3	14 ± 2	14 ± 2	16 ± 2
Creatinine (mg/dL)	0.67 ± 0.07	0.64 ± 0.06	0.65 ± 0.05	0.66 ± 0.08	0.64 ± 0.07	0.65 ± 0.06
Na (mEq/L)	143 ± 2	143 ± 1	143 ± 1	145 ± 2	144 ± 1	144 ± 2
K (mEq/L)	4.2 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.3	4.4 ± 0.4	4.5 ± 0.3
Cl (mEq/L)	115 ± 4	113 ± 2	112 ± 1	114 ± 4	111 ± 1	111 ± 1
Ca (mEq/L)	9.8 ± 0.5	9.7 ± 0.3	9.6 ± 0.3	9.6 ± 0.3	9.3 ± 0.3	9.3 ± 0.2
P (mEq/L)	5.2 ± 1.1	5.8 ± 0.7	6.1 ± 0.7	6.4 ± 0.8	5.1 ± 0.6	5.2 ± 0.5
TP (g/dL)	7.6 ± 0.6	7.1 ± 0.6	7.1 ± 0.4	6.9 ± 0.4	6.3 ± 0.2	6.4 ± 0.2
Albumin (g/dL)	4.2 ± 0.6	4.3 ± 0.5	4.3 ± 0.3	3.4 ± 0.4	3.6 ± 0.1	3.7 ± 0.1
A/G	1.01 ± 0.14	1.0 ± 0.10	1.02 ± 0.09	0.75 ± 0.08	0.71 ± 0.07	0.74 ± 0.05

a): Mean ± S. D.

Table 5. Relative Organ Weights in Crj:CD(SD) and Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF

Sex	Female			Male		
Strain	Crj:CD(SD)	Crj:CD(SD)IGS		Crj:CD(SD)	Crj:CD(SD)IGS	
Item (unit)	CRF-1	CRF-1	CR-LPF	CRF-1	CRF-1	CR-LPF
Age : 10 weeks						
Number of animals	400	20	40	455	20	40
Final Body Weight (g)	220 ± 26[a)]	206 ± 18	197 ± 12	360 ± 51	349 ± 20	316 ± 22
Brain (g%)	0.83 ± 0.08	0.88 ± 0.08	0.92 ± 0.06	0.56 ± 0.07	0.57 ± 0.04	0.62 ± 0.05
Thymus (mg%)	204 ± 46	195 ± 39	196 ± 27	166 ± 40	135 ± 30	122 ± 27
Heart (g%)	0.38 ± 0.03	0.36 ± 0.02	0.36 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.33 ± 0.02
Lung (g%)	0.47 ± 0.04	0.46 ± 0.04	0.48 ± 0.03	0.37 ± 0.04	0.36 ± 0.03	0.37 ± 0.02
Liver (g%)	3.05 ± 0.24	2.74 ± 0.12	2.80 ± 0.14	3.21 ± 0.25	2.82 ± 0.14	2.69 ± 0.14
Spleen (g%)	0.21 ± 0.03	0.21 ± 0.03	0.21 ± 0.03	0.19 ± 0.03	0.17 ± 0.03	0.19 ± 0.03
Kidney (R+L,g%)	0.81 ± 0.08	0.76 ± 0.05	0.75 ± 0.05	0.77 ± 0.07	0.72 ± 0.04	0.74 ± 0.05
Adrenal (R+L,mg%)	31 ± 4	32 ± 5	32 ± 4	17 ± 3	18 ± 3	17 ± 2
Ovary (R+L,mg%)	39.7 ± 6.3	39.4 ± 7.1	39.7 ± 5.8	-	-	-
Uterus (g%)	191 ± 43	184 ± 36	223 ± 55	-	-	-
Testis (R+L,g%)	-	-	-	0.85 ± 0.10	0.86 ± 0.08	0.97 ± 0.09
Prostate (g%)	-	-	-	0.23 ± 0.04	0.24 ± 0.04	0.25 ± 0.05
Age : 19 weeks						
Number of animals	1332040	1322040				
Final Body Weight (g)	300 ± 36	281 ± 25	254 ± 21	560 ± 57	500 ± 44	470 ± 40
Brain (g%)	0.66 ± 0.07	0.70 ± 0.06	0.76 ± 0.06	0.39 ± 0.04	0.43 ± 0.04	0.44 ± 0.04
Thymus (mg%)	83 ± 22	87 ± 21	93 ± 21	53 ± 15	57 ± 15	54 ± 13
Heart (g%)	0.32 ± 0.03	0.32 ± 0.02	0.33 ± 0.03	0.28 ± 0.02	0.27 ± 0.02	0.28 ± 0.02
Lung (g%)	0.38 ± 0.04	0.38 ± 0.03	0.42 ± 0.03	0.28 ± 0.03	0.29 ± 0.02	0.30 ± 0.03
Liver (g%)	2.50 ± 0.18	2.45 ± 0.12	2.46 ± 0.11	2.73 ± 0.25	2.45 ± 0.19	2.44 ± 0.14
Spleen (g%)	0.17 ± 0.03	0.17 ± 0.02	0.18 ± 0.03	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.02
Kidney (R+L,g%)	0.65 ± 0.06	0.64 ± 0.05	0.68 ± 0.05	0.60 ± 0.06	0.60 ± 0.05	0.61 ± 0.05
Adrenal (R+L,mg%)	24 ± 4	24 ± 3	26 ± 3	11 ± 2	12 ± 2	11 ± 2
Ovary (R+L,mg%)	28.0 ± 6.8	28.2 ± 4.3	29.6 ± 5.3	-	-	-
Uterus (g%)	191 ± 43	199 ± 34	223 ± 45	-	-	-
Testis (R+L,g%)	-	-	-	0.61 ± 0.08	0.66 ± 0.07	0.69 ± 0.07
Prostate (g%)	-	-	-	0.23 ± 0.05	0.25 ± 0.04	0.26 ± 0.05
Age : 32 weeks						
Number of animals	266	40	40	266	40	40
Final Body Weight (g)	356 ± 47	318 ± 36	291 ± 3	674 ± 76	582 ± 60	558 ± 56
Brain (g%)	0.57 ± 0.07	0.61 ± 0.06	0.68 ± 0.07	0.33 ± 0.04	0.37 ± 0.04	0.39 ± 0.04
Thymus (mg%)	38 ± 11	47 ± 13	49 ± 11	23 ± 6	26 ± 7	27 ± 7
Heart (g%)	0.30 ± 0.03	0.30 ± 0.03	0.32 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02
Lung (g%)	0.34 ± 0.04	0.36 ± 0.04	0.37 ± 0.04	0.25 ± 0.03	0.27 ± 0.03	0.27 ± 0.02
Liver (g%)	2.43 ± 0.24	2.34 ± 0.21	2.36 ± 0.17	2.66 ± 0.22	2.36 ± 0.21	2.36 ± 0.16
Spleen (g%)	0.16 ± 0.02	0.15 ± 0.03	0.15 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.14 ± 0.02
Kidney (R+L,g%)	0.62 ± 0.07	0.61 ± 0.06	0.63 ± 0.06	0.54 ± 0.05	0.56 ± 0.05	0.54 ± 0.04
Adrenal (R+L,mg%)	21 ± 4	21 ± 3	22 ± 4	9 ± 2	9 ± 1	9 ± 1
Ovary (R+L,mg%)	29.9 ± 170.3	22.7 ± 5.7	23.9 ± 5.9	-	-	-
Uterus (g%)	200 ± 48	220 ± 42	241 ± 56	-	-	-
Testis (R+L,g%)	-	-	-	0.53 ± 0.07	0.57 ± 0.07	0.62 ± 0.05
Prostate (g%)	-	-	-	0.19 ± 0.04	0.22 ± 0.05	0.23 ± 0.04

a): Mean ± S. D.

In the necropsy, some abnormalities, such as dark red focus in the lungs or glandular stomach, white focus in the spleen, small size of the thyroids, diverticulum of the small intestine, dilatation of renal pelvis, yellow focus in the epididymides and diaphragmatic hernial nodule in the liver were observed in a few animals; however, the incidences were very low and no abnormalities suggesting specificity to IGS rats were found irrespective of the kind of diet.

The histopathological findings in the main organs from IGS rats are shown in Table 6. No apparent differences were noted between CD rats (data not shown) and IGS rats fed CRF-1 in any of the organs including the liver. When comparison was made between IGS rats in the CRF-1 and CR-LPF group, a slight difference was found in the kidney. At 32 weeks of age, mineralization in the pelvis was observed in 27/39 females (70%) in the CRF-1 group and in 10/40 females (25%) in the CR-LPF group, and the incidence in the CRF-1 group was higher than that of the CR-LPF group. Regeneration of the tubular epithelial cells was observed in 15/39 males (38%) in the CRF-1 group and in 6/40 males (15%) in the CR-LPF group, and the incidence in the CRF-1 group was higher than that of the CR-LPF group; however, no difference was found in the incidence of chronic progressive nephropathy among the two groups due to extremely low occurrence of this lesion at this age.

Table 6. Histopathology in Crj:CD(SD)IGS Rats Fed CRF-1 or CR-LPF

Sex:	Female						Male					
Diets:	CR-LPF			CRF-1			CR-LPF			CRF-1		
Organs Age (weeks):	10	19	32	10	19	32	10	19	32	10	19	32
Findings No. examined:	40	40	40	20	20	40	40	40	40	20	20	40
Adrenal												
Number examined	40	40	40	20	19	40	40	40	40	20	20	40
Not remarkable	40	39	32	20	19	37	38	31	35	18	19	35
Altered cell focus	0	0	1	0	0	0	0	2	1	0	0	1
Cell infiltration	0	0	0	0	0	0	0	0	0	1	0	0
Lipoid, increased	0	0	0	0	0	0	0	0	0	0	0	0
Peliosis adrenalis	0	1	7	0	0	3	0	0	0	0	0	0
Heart												
Number examined	40	40	40	20	20	40	40	40	40	20	20	40
Not remarkable	38	38	35	20	19	35	37	33	28	16	16	29
Arteritis	0	0	1	0	0	2	0	0	0	0	0	0
Fibrosis, myocardial	0	0	0	0	0	1	0	0	0	0	0	0
Myocarditis	2	2	4	0	1	2	3	7	12	4	4	11
Kidney												
Number examined	40	40	40	20	20	39	40	40	40	20	20	39
Not remarkable	39	35	24	18	12	8	37	33	28	16	14	16
Cell infiltration	0	1	4	0	4	0	0	1	2	0	0	4
Chromic progressive nephropathy	0	0	0	0	0	1	0	0	0	0	0	2
Cyst	0	0	0	0	0	0	0	1	1	0	0	0
Hydronephrosis	0	1	0	1	3	0	2	0	0	1	0	0
Dilatation, tubular	0	2	0	0	0	0	0	1	0	0	1	0
Fibrosis	1	0	0	0	0	0	0	0	0	1	0	0
Mineralization	0	1	0	0	1	1	0	1	0	0	0	0
Mineralization, pelvic	0	0	10	0	0	27	0	0	0	0	0	2
Pyelitis	0	0	2	0	3	5	0	1	5	0	1	8
Pyelonephritis	0	0	0	0	0	0	0	0	1	0	0	0
Regeneration, tubular cell	0	1	0	2	0	0	1	4	6	3	4	15
Urinary cast, hyaline	0	0	1	0	0	1	0	0	1	0	0	9
Liver												
Number examined	40	40	40	20	20	39	40	40	40	20	20	39
Not remarkable	28	31	32	7	17	35	29	30	30	16	18	37
Altered cell focus	0	0	0	0	0	0	0	0	2	0	0	0
Arteritis	0	0	0	0	0	0	0	0	1	0	0	0
Cell infiltration	0	0	1	0	0	0	1	0	0	0	0	0
Fibrosis	0	0	0	0	0	0	0	0	0	0	1	0
Hepatodiaphragmatic nodule	0	0	1	0	0	0	0	0	0	0	0	0
Microgranuloma	12	8	5	12	3	3	9	10	2	3	1	2
Necrosis, focal	0	0	0	0	0	0	1	0	3	1	0	0
Proliferation, bile ductular	0	0	0	0	0	0	0	0	4	0	0	0
Vacuolation, hepatocyte, periportal	0	1	1	4	0	1	0	0	0	0	0	0
Lung												
Number examined	40	40	40	20	20	39	40	40	40	20	20	39
Not remarkable	36	33	28	19	15	30	32	28	24	14	15	20
Appearence, foamy histiocytic	0	2	1	0	2	1	0	1	1	0	1	3
Arterisclerosis	1	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage, focal	0	0	0	0	0	0	1	1	0	0	0	0
Mineralization, arterial wall	2	5	11	1	4	8	7	11	16	6	5	16
Pneumonia	1	0	0	0	0	0	0	0	0	0	0	0
Ovary												
Number examined	40	40	40	20	20	40	-	-	-	-	-	-
Not remarkable	40	40	30	20	20	29	-	-	-	-	-	-
Atrophy	0	0	10	0	0	11	-	-	-	-	-	-
Spleen												
Number examined	40	40	40	20	20	39	40	40	40	20	20	39
Not remarkable	40	40	40	20	20	38	39	40	38	20	20	38
Cell infiltration	0	0	0	0	0	0	1	0	0	0	0	0
Erythropoiesis, increased	0	0	0	0	0	1	0	0	2	0	0	1
Testis												
Number examined	-	-	-	-	-	-	40	40	40	20	20	39
Not remarkable	-	-	-	-	-	-	35	40	39	20	20	36
Atrophy, tubular	-	-	-	-	-	-	0	0	1	0	0	3
Vacuolation, Sertoli cell	-	-	-	-	-	-	5	0	0	0	0	0

DISCUSSION

Some differences were observed between IGS and CD rats. Body weight gain was less in IGS rats than in CD rats, and the mean body weight was approximately 10% lower in IGS rats than CD rats. This corresponded to low food consumption in IGS rats as compared to CD rats. The most prominent difference between IGS and CD rats was noted in the serum lipid levels, especially in triglycerides. Triglycerides in IGS rats were only about half those in CD rats at each stage determined. The serum level of cholesterol and phospholipids was also lower in IGS rats than in CD rats, but the extent of deviation was less than triglycerides. Similar to the present experiment, low levels of serum lipids in IGS rats are reported by other laboratories [1] and therefore, this was thought to be one of the common characteristics of IGS rats. It is suspected that the metabolism of lipids in IGS rats may somewhat differ from ordinary CD rats and therefore, it should be mentioned that special attention will be needed when drugs with a potential to alter blood lipid levels, such as a hypolipidemic agent, is subjected to a toxicity test using IGS rats. As the liver is a major organ playing an important role in lipid metabolism, the liver was carefully examined; however, no special lesions, such as fatty change or degenerative changes, were found histopathologically. Also, no differences were found in any of the organs including the heart and testis which are suspected to have some deviations from CD rats, but we have only insufficient data at present and cannot form a hasty conclusion.

For effects of protein content in the feed, low protein commercial diet showed slight alterations in the body weight gain and renal function. Providing low protein commercial diet (CR-LPF, 18% protein) resulted in less body weight gain in IGS rats as compared to widely used CRF-1 (23% protein). In addition, low protein diet resulted in decreases in urinary protein, regeneration of the renal tubules and mineralization of the renal pelvis. These may indicate improvement of renal dysfunction advancing with age, which is a large matter in the longevity of Sprague-Dawley rats in a long term study. Therefore, it may be feasibile to use low protein commercial diet in toxicity studies, especially in the long term study. However, the possibility must be stressed that susceptibility to certain chemicals might be altered by providing low protein diet.

REFERENCES

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Characteristics of Crj:CD (SD) IGS rats compared with Crj:CD (SD) rats based on a repeated dose toxicity study

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ABSTRACT. The biological background data including body weight, food intake, urinalysis, hematology, blood biochemistry, organ weight and histopathological examination were collected from the Crj:CD (SD) IGS rats for the short-term repeated dose toxicity studies in our facility, and statistically analyzed for comparison with the data from the Crj:CD (SD) rats. Consequently, some differences between the Crj:CD (SD) IGS rats and the Crj:CD (SD) rats were found in some parameters relating to hematology, blood biochemistry and organ weight routinely examined in the repeated dose toxicity studies. Therefore, it is suggested that these parameters may be characteristics of the Crj:CD (SD) IGS rats. —Key words: Biological background data, Crj:CD (SD) IGS rats, Crj:CD (SD) rats, Repeated does toxicity study

CD (SD) IGS-1998: 82-89

INTRODUCTION

The gold standard system, a new laboratory animal breeding system, was recently established by Charles River Inc. to contribute to the globalization of research and development for the novel synthesis of chemical substances and drugs. This system can globally supply homogeneous Crj:CD (SD) IGS rats (IGS strain) at the genetic level. However, the biological characteristics of the IGS strain have not been reported nor discussed in detail previously, so that the biological background data from the IGS strain are still insufficient. The objective of the present study was to collect the biological background data at the age of 9 weeks from the IGS strain for the 1-week to 4-week repeated dose toxicity studies in our facility. Furthermore, this paper describes the statistical analysis of comparing the biological data from the IGS strain with the data from the Crj:CD (SD) rats (SD strain) as well.

MATERIALS AND METHODS

Animals: A total of 48 males and 48 females of the IGS strain from 3 repeated dose toxicity studies and 20 males and 26 females of the SD strain from 4 repeated dose toxicity studies used as controls in our facility were donated for this study (Table 1). Both the IGS strain (Tsukuba Breeding Center, Tsukuba, Japan) and the SD strain (Atsugi Breeding Center, Atsugi, Japan) were purchased from Charles River Japan, Inc., Yokohama, at the age of 4 to 7 weeks. The bacteriological grade of these animals was controlled under the specific-pathogen-free (SPF) level. Acquisition date of animals and body weight range at acquisition in each study are shown in Table 1.

Table 1. Summary of background data

Strain	Acquisition Date	Age at acquisition (week)	Body weight range at acquisition (g; sex)	No. of animals (sex)	Dosing substance	Route	Dosing volume (mL/kg)	Dosing method	Dosing period (week)
IGS strain	16 Oct. '96	6	178.4 - 195.0 (M[a)])	12 (M)	None	-	-	-	2
			161.7 - 177.8 (F [b)])	12 (F)	None	-	-	-	2
	23 Oct. '96	7	249.5 - 268.2 (M)	12 (M)	None	-	-	-	1
			175.2 - 202.3 (F)	12 (F)	None	-	-	-	1
	19 Mar. '97	4	89.0 - 102.6 (M)	12 (M)	None	-	-	-	4
			80.1 - 91.7 (F)	12 (F)	None	-	-	-	4
				12 (M)	Distilled water	Oral	10	Gavage	4
				12 (F)	Distilled water	Oral	10	Gavage	4
SD strain	15 Nov. '94	4	83.7 - 94.5 (M)	6(9[c)]) (M)	Saline	SC [e)]	5	Injection	4
			78.8 - 90.4 (F)	6(9[c)]) (F)	Saline	SC	5	Injection	4
	20 Jun. '95	6	192.2 - 213.2 (M)	6 (M)	0.5% CMC [d)]	Oral	5	Gavage	2
			139.7 - 159.6 (F)	6 (F)	0.5% CMC	Oral	5	Gavage	2
	8 Oct. '95	6	154.5 - 171.7 (F)	6 (F)	Distilled water	Oral	5	Gavage	2
	2 Apr. '96	6	207.3 - 229.2 (M)	8 (M)	Distilled water	Oral	5	Gavage	2
			147.0 - 165.3 (F)	8 (F)	Distilled water	Oral	5	Gavage	2

a): Male b): Female
c): No. of animals in body weight and food intake d): carboxymethyl cellulose sodium salt
e): subcutaneous

Animal husbandry: The animals were housed individually in wire mesh cages (260x380x180mm: Clea Japan Inc., Tokyo, Japan) and kept under standard laboratory animal conditions which were maintained at a temperature of 21 to 25°C with 40 to 70% relative humidity. The room air was ventilated 20 to 50 times per hour automatically and a 12 hr/12 hr light-dark cycle (lighting 07:00-19:00) was imposed. The animals received a commercial pellet diet (CRF-1, Oriental Yeast Industry Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*. The animals were identified by metallic ear tags.

Observation and examination: General signs, body weight and food intake: Throughout the dosing period, general signs were observed daily. Body weight and food intake for 24 hr were determined once weekly. The first day of this paper is designated as day 1 in the 4-week repeated dose toxicity study.

Urinalysis: At the age of 9 weeks, urinary samples were collected to examine specific gravity (SG), pH, protein, glucose, ketone bodies, bilirubin, occult blood, nitrates, urobilinogen and urinary sediment. Specific gravity was measured by serum-protein refractometer (SPR-N, Atago Co., Ltd., Tokyo, Japan). Urinary sediments measured by a light microscope and other parameters with dip stix (N-Multistix, Bayer-Sankyo Co., Ltd., Tokyo, Japan) were examined.

Necropsy: After the dosing period, at the age of 9 weeks, the animals were fasted before being sacrificed (18 hours or more). Blood was drawn from the abdominal aorta of each animal under ether anesthesia, using syringes as follows: 3.2% sodium citrate treatment syringes for plasma to measure prothrombin time (PT) , activated partial thromboplastin time (APTT), glucose (GLU) and aspartate aminotransferase (AST), ethylenediaminetetraacetic acid disodium salt (EDTA-2K) treatment syringes for other hematological examinations, and non-treatment syringes for serum to measure other blood biochemical parameters. The dissected animals were observed macroscopically, and the following organs were removed and weighed for post-mortem examination: brain, pituitary gland, salivary glands (submaxillary gland and sublingual gland), thymus, lung, heart, liver, spleen, kidney, adrenal gland, prostate gland, testis and ovary.

Hematology: Red blood cell counts (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular concentration (MCHC), platelet counts (PLT) and white blood cell counts (WBC) were measured by using an automated counter (K-1000, Toa Medical Electronics Co., Kobe, Japan). Differential WBC by the Wright-Giemsa method and reticulocyte counts by the Brecher method were examined with a light microscope. PT and APTT were measured by using an automated coagulation analyzer (KC-4A, Heinrich Amelung GmbH, Lemgo, Germany).

Blood biochemistry: AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC), triglyceride (TG), total bilirubin (TB), blood urea nitrogen (BUN), creatinine (CRE), GLU, total protein (TP), sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and inorganic phosphorus (IP), were measured by using an automated biochemical analyzer (JCA-RS-1100, JOEL Ltd., Tokyo, Japan). Serum protein was analyzed by an automatic electrophoresis (CTE-150-N, Joko Co., Ltd., Tokyo, Japan) and divided into albumin, α_1-, α_2-, α_3-, β- and γ-globulin fractions. The albumin/globulin ratio (A/G) was automatically calculated.

Histopathology: Following post-mortem examination, tissue samples from the cervical spinal cord, left eye, left Harderian gland, parotid gland, tongue, esophagus, trachea, thyroid gland, parathyroid gland, stomach, pancreas, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), mesenteric lymph node, urinary bladder, uterus, vagina, epididymis, seminal vesicle with coagulating gland, bulbourethral gland, skeletal muscle from the femoral region on the left side, sternum, femur on the left side, abdominal skin and weighed organs were collected and fixed in 10% buffered formalin. These samples were embedded in paraffin wax, sectioned and stained with hematoxylin-eosin (HE) for the histopathological examination.

Statistical analysis: According to the equivalence of variances between the IGS strain and the SD strain groups, student's t-test or Aspin-Welch's t-test was applied to the values for body weight, food intake, hematological parameters, blood biochemical parameters and organ weight. A cumulative chi-square test was used with respect to urinalysis, gross findings and histopathological findings. The statistical significance was set at $P<0.05$.

RESULTS

Significant general signs were not observed throughout the dosing period in each study.

Table 2. Body weight

Sex	Male		Female	
Group	IGS strain	SD strain	IGS strain	SD strain
No. of animals	48	23	48	29
Days of examination				
1	151.5 ± 5.7[a)]	147.0 ± 5.1[c)]	133.5 ± 5.5[a)]	130.6 ± 5.0[c)]
7	213.0 ± 11.6[a)]*	196.7 ± 8.8[c)]	166.2 ± 8.3[a)]*	151.8 ± 6.7[c)]
14	263.8 ± 20.3[b)]	265.2 ± 16.5	192.8 ± 11.5[b)]*	184.3 ± 10.9
21	318.8 ± 21.1	326.2 ± 19.7	213.9 ± 13.3	209.2 ± 15.1
28	354.9 ± 25.4	366.7 ± 21.9	231.1 ± 15.3	229.2 ± 16.7

Mean±S.D. Unit: g

*: Significantly different from the SD strain group, $P<0.05$ (Student's t-test)

a): No. of animals was 24. b): No. of animals was 36. c): No. of animals was 9.

The body weights are shown in Table 2. Significantly higher body weights were noted on day 7 for males and on days 7 and 14 for females of the IGS strain compared with those of the SD strain.

The food intake is shown in Table 3. For females of the IGS strain, the food intake on days 6-7 and 25-26 was higher than that of the SD strain.

The results of urinalysis are summarized in Table 4. The incidence of each finding showed no significant difference between the IGS and the SD strains.

The results of hematological examination are shown in Table 5. The hematological examination parameters in the IGS strain

Table 3. Food intake

Sex	Male		Female	
Group	IGS strain	SD strain	IGS strain	SD strain
No. of animals	48	23	48	29
Days of examination				
1 - 2	20.1 ± 1.7[a)]	18.7 ± 1.4[c)]	16.9 ± 1.6[a)]	16.1 ± 1.3[c)]
6 - 7	23.6 ± 2.5[a)]	22.5 ± 1.9[c)]	18.5 ± 1.8[a)]*	16.5 ± 1.9[c)]
13 - 14	25.9 ± 2.4[b)]	25.8 ± 2.2	18.8 ± 2.7[b)]	17.9 ± 1.7
20 - 21	26.7 ± 2.6	27.9 ± 2.6	18.3 ± 2.1	18.6 ± 2.8
25 - 26	27.8 ± 2.9	29.5 ± 4.0	20.2 ± 2.3#	18.2 ± 3.9

Mean±S.D. Unit: g

*: Significantly different from the SD strain group, P<0.05 (Student's t-test)

#: Significantly different from the SD strain group, P<0.05 (Aspin-Welch's t-test)

a): No. of animals was 24. b): No. of animals was 36. c): No. of animals was 9.

Table 4. Urinalysis

Sex		Male		Female	
Group		IGS strain	SD strain	IGS strain	SD strain
No.of animals		48	20	48	26
SG	<1.016	1	1	4	2
	<1.028	11	3	11	4
	<1.040	15	5	10	6
	<1.052	9	4	7	6
	<1.064	6	4	4	6
	<1.076	6	1	5	1
	>1.077	0	2	6	1
pH	6.0	0	0	1	0
	6.5	0	0	1	0
	7.0	0	0	0	1
	7.5	0	0	2	0
	8.0	1	0	2	1
	8.5	47	20	41	24
Protein	–	0	0	3	2
	±	9	1	19	18
	+	36	10	23	6
	++	3	9	2	0
Glucose	–	48	20	47	26
Ketones	–	30	16	46	26
	±	16	4	1	0
	++	2	0	0	0
Bilirubin	–	48	20	47	26
Occult Blood	–	48	20	47	26
Nitrites	–	48	20	47	26
Urobilinogen	0.1	48	20	47	26
RBC	–	32	4	30	9
	±	16	13	17	16
	+	0	2	0	1
	++	0	1	0	0
WBC	–	26	7	22	14
	±	22	13	25	12
Epithelial cells	–	39	10	34	19
	±	9	10	13	7
Cast	–	48	20	47	26

Table 5. Hematology

Sex	Male		Female	
Group	IGS strain	SD strain	IGS strain	SD strain
No.of animals	48	20	48	26
RBC x10e4/ μL	801 ± 40 *	749 ± 49	776 ± 35 #	730 ± 48
Hb g/dL	15.2 ± 0.7 *	14.5 ± 0.7	15.1 ± 0.7 *	14.6 ± 0.7
Ht %	47.8 ± 2.2 *	45.7 ± 2.4	45.1 ± 1.8	43.7 ± 2.5
MCV fL	59.8 ± 1.8 *	61.1 ± 1.9	58.1 ± 1.6 *	59.8 ± 2.0
MCH pg	19.0 ± 0.5 *	19.4 ± 0.6	19.4 ± 1.0	20.0 ± 0.6
MCHC %	31.8 ± 0.5	31.7 ± 0.5	33.4 ± 1.2	33.4 ± 0.7
Reticulo. %	2.6 ± 0.7	2.5 ± 0.3	1.8 ± 0.5	2.0 ± 0.6
PLT x10e4/ μL	114.6 ± 13.2	119.5 ± 9.0	115.8 ± 14.3	124.0 ± 12.1
WBC x10e2/ μL	92.6 ± 23.2 *	104.7 ± 29.4	71.4 ± 22.5	80.9 ± 29.4
Lympho. %	88.4 ± 4.0	90.6 ± 2.3	88.4 ± 4.5	88.7 ± 5.2
Mono. %	1.4 ± 1.0	1.0 ± 0.7	1.5 ± 1.0	1.3 ± 1.2
N-St. %	1.7 ± 1.3	0.6 ± 0.2	1.4 ± 1.0	0.5 ± 0.5
N-Seg. %	8.5 ± 3.2	7.8 ± 2.5	8.3 ± 3.9	8.7 ± 4.3
Eosino. %	0.8 ± 0.5	0.6 ± 0.4	1.1 ± 0.6	0.9 ± 0.7
Baso. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PT sec	15.6 ± 1.9 *	14.8 ± 1.4	14.5 ± 2.0 #	13.5 ± 0.8
APTT sec	19.1 ± 2.0 *	18.2 ± 2.4	16.1 ± 2.4	16.5 ± 2.5

Mean±S.D.

*: Significantly different from the SD strain group, P<0.05 (Student's t-test)

#: Significantly different from the SD strain group, P<0.05 (Aspin-Welch's t-test)

revealed statistical differences from those of the SD strain as follows: higher RBC, Hb and PT values in both sexes; lower MCV value in both sexes; higher Ht and APTT values in males; lower MCH and WBC values in males.

The results of blood biochemistry examination are shown in Table 6. The blood biochemistry parameters in the IGS strain revealed statistical differences from those in the SD strain as follows: lower TG and TB values in both sexes; higher ALT, ALP, BUN, α_2- and α_3-globulin values in males; lower TC, α_1-globulin, γ-globulin and Na values in males; higher Ca values in females; and lower IP value in females.

The organ weights are shown in Table 7. Organ weights of the IGS strain revealed statistical differences from those of the SD strain as follows: lower absolute and relative organ weights of the pituitary and prostate glands in males; lower absolute organ weight of the liver in males; higher relative organ weight of the testis in males; lower relative organ weight of the heart in females.

The gross findings are summarized in Table 8. The incidence of each gross finding showed no significant differences between the IGS and the SD strains.

The histopathological findings are summarized in Table 9. The incidence of each histopathological finding showed no significant differences between the IGS and the SD strains.

Table 6. Blood biochemistry

Sex	Male		Female	
Group	IGS strain	SD strain	IGS strain	SD strain
No.of animals	48	20	48	26
AST IU/L	68 ± 7	72 ± 5	62 ± 9	64 ± 9
ALT IU/L	27 ± 4 *	25 ± 4	23 ± 5	19 ± 4
ALP IU/L	443 ± 87 #	333 ± 49	244 ± 75	230 ± 58
TC mg/dL	52 ± 10 *	55 ± 7	63 ± 15	63 ± 13
TG mg/dL	35 ± 16 *	50 ± 16	14 ± 8 #	18 ± 9
TB mg/dL	0.03 ± 0.01 #	0.04 ± 0.01	0.03 ± 0.01 #	0.05 ± 0.01
BUN mg/dL	15.0 ± 2.2 #	12.7 ± 1.0	16.5 ± 1.9	16.6 ± 2.6
CRE mg/dL	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
GLU mg/dL	148.4 ± 18.1	140.0 ± 15.4	133.3 ± 14.9	130.1 ± 13.3
TP g/dL	5.0 ± 0.2	5.1 ± 0.1	5.4 ± 0.3	5.2 ± 0.3
Alb g/dL	2.7 ± 0.1	2.7 ± 0.1	3.1 ± 0.2	3.0 ± 0.4
Alb %	53.4 ± 2.2	53.3 ± 2.3	57.4 ± 2.4	57.3 ± 6.7
α_1-G %	21.7 ± 1.6 *	22.6 ± 2.0	18.7 ± 1.6	19.4 ± 1.8
α2-G %	6.0 ± 0.6 #	5.8 ± 0.8	5.1 ± 0.7	5.1 ± 3.1
α3-G %	2.7 ± 0.5 *	2.2 ± 0.4	2.3 ± 0.4	2.0 ± 0.6
β-G %	15.0 ± 1.5	14.7 ± 1.3	14.8 ± 1.7	14.6 ± 2.8
γ-G %	1.1 ± 0.5 *	1.5 ± 0.3	1.7 ± 0.6	1.6 ± 0.4
A/G	1.15 ± 0.10	1.14 ± 0.11	1.35 ± 0.13	1.38 ± 0.27
Na mmol/L	141.1 ± 1.3 *	142.6 ± 1.3	141.5 ± 1.4	142.9 ± 1.6
K mmol/L	4.5 ± 0.3	4.3 ± 0.3	4.5 ± 0.3	4.3 ± 0.4
Cl mmol/L	110 ± 2	110 ± 1	113 ± 2	112 ± 2
Ca mg/dL	9.3 ± 0.4	9.4 ± 0.3	9.6 ± 0.4 #	9.3 ± 0.3
IP mg/dL	8.8 ± 0.7	9.1 ± 0.6	7.7 ± 1.1 *	8.4 ± 0.8

Mean±S.D.

*: Significantly different from the SD strain group, P<0.05 (Student's t-test)

#: Significantly different from the SD strain group, P<0.05 (Aspin-Welch's t-test)

Table 7. Organ weight

Sex		Male		Female	
Group		IGS strain	SD strain	IGS strain	SD strain
No. of animals		48	20	48	26
Final B.W.	(g)	324.2 ± 22.5 *	339.4 ± 20.4	212.7 ± 13.1	212.0 ± 15.2
Brain	(g)	2.01 ± 0.09	2.03 ± 0.07	1.87 ± 0.09	1.90 ± 0.07
	(g%)	0.62 ± 0.05	0.60 ± 0.04	0.88 ± 0.06	0.90 ± 0.06
Pituitary gland	(mg)	10.3 ± 1.6 *	12.3 ± 1.2	12.6 ± 1.9	13.3 ± 3.0
	(mg%)	3.2 ± 0.5 *	3.6 ± 0.4	5.9 ± 0.9	6.3 ± 1.5
Salivary gland	(g)	0.61 ± 0.06	0.61 ± 0.04	0.43 ± 0.04	0.43 ± 0.05
	(g%)	0.19 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.20 ± 0.02
Thymus	(g)	0.62 ± 0.13	0.66 ± 0.12	0.50 ± 0.08	0.50 ± 0.11
	(g%)	0.19 ± 0.03	0.19 ± 0.04	0.24 ± 0.04	0.23 ± 0.04
Lung	(g)	1.40 ± 0.10	1.41 ± 0.10	1.12 ± 0.12	1.13 ± 0.12
	(g%)	0.43 ± 0.03	0.42 ± 0.02	0.53 ± 0.05	0.53 ± 0.06
Heart	(g)	1.22 ± 0.10	1.25 ± 0.11	0.79 ± 0.06	0.82 ± 0.08
	(g%)	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.02 *	0.39 ± 0.02
Liver	(g)	9.98 ± 1.22 *	10.88 ± 1.11	6.45 ± 0.52	6.60 ± 0.72
	(g%)	3.07 ± 0.21	3.21 ± 0.29	3.04 ± 0.16	3.11 ± 0.20
Spleen	(g)	0.65 ± 0.10	0.69 ± 0.09	0.47 ± 0.08	0.46 ± 0.06
	(g%)	0.20 ± 0.03	0.20 ± 0.03	0.22 ± 0.03	0.21 ± 0.03
Kidney	(g)	2.64 ± 0.26	2.71 ± 0.14	1.77 ± 0.13	1.75 ± 0.19
	(g%)	0.82 ± 0.07	0.80 ± 0.06	0.83 ± 0.06	0.83 ± 0.06
Adrenal gland	(mg)	58.4 ± 7.6	59.6 ± 8.5	67.3 ± 11.0	71.2 ± 10.1
	(mg%)	18.1 ± 2.6	17.6 ± 2.4	31.7 ± 5.3	33.6 ± 4.3
Prostate gland	(g)	0.42 ± 0.09 #	0.54 ± 0.17	–	
	(g%)	0.13 ± 0.03 #	0.16 ± 0.05	–	
Testis	(g)	2.99 ± 0.15	2.90 ± 0.23	–	
	(g%)	0.93 ± 0.06 *	0.86 ± 0.07	–	
Ovary	(mg)	–		90.5 ± 13.5	91.7 ± 16.1
	(mg%)	–		42.6 ± 6.3	43.2 ± 6.4

Mean±S.D.
*: Significantly different from the SD strain group, P<0.05 (Student's t-test)
#: Significantly different from the SD strain group, P<0.05 (Aspin-Welch's t-test)

Table 8. Gross findings

Sex		Male		Female	
Group		IGS strain	SD strain	IGS strain	SD strain
No. of animals		48	20	48	26
Organ	Findings				
Thymus					
	cervical remnant	1 (2.1)[a)]	0 (0.0)	0 (0.0)	0 (0.0)
Kidney					
	hydronephrosis	1 (2.1)	0 (0.0)	1 (2.1)	0 (0.0)
Seminal vesicle					
	hypomorphism	1 (2.1)	0 (0.0)	–	–
Uterus					
	uterine dilation	–	–	2 (4.2)	1 (3.8)

a): No. of incidences (%)

Table 9. Histopathological findings

Sex		Male		Female	
Group		IGS strain	SD strain	IGS strain	SD strain
No. of animals		48	20	48	26
Organ	Findings				
Heart					
	myocardial inflammation	1 (2.1)[a)]	0 (0.0)	0 (0.0)	0 (0.0)
Lung					
	calcification in artery	2 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
	inflammatory infiltration	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
	aggregation of lymphocytes	3 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)
	alveolar lipidosis	1 (2.1)	1 (5.0)	3 (6.2)	0 (0.0)
	osseous metaplasia	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
Stomach					
	dilated gland	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
	mineralization	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
Pancreas					
	acinar atrophy	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
	degeneration	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
	focal necrosis	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
	lobular pancreatic atrophy	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)
Small intestine					
	Ca-deposition in germinal center	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
Liver					
	fatty change	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
	focal necrosis	2 (4.2)	0 (0.0)	1 (2.1)	0 (0.0)
	vacuolar degeneration	4 (8.3)	4 (20.0)	7 (14.6)	8 (30.8)
	micro-granuloma	7 (14.6)	10 (50.0)	14 (29.2)	19 (73.1)
	hepatic hypertrophy	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
	single cell necrosis	0 (0.0)	4 (20.0)	0 (0.0)	4 (15.4)
Submaxillary gland					
	cyst formation	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
	ectopic parotid gland acini	4 (8.3)	0 (0.0)	4 (8.3)	0 (0.0)
Sublingual gland					
	ectopic parotid gland acini	5 (10.4)	0 (0.0)	10 (20.8)	1 (3.8)
	squamous metaplasia	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
Parotid gland					
	acinar atrophy	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
	basophilic cell foci	3 (6.2)	6 (30.0)	5 (10.4)	11 (42.3)

Table 9. (continued) Histopathological findings

Sex		Male		Female	
Group		IGS strain	SD strain	IGS strain	SD strain
No. of animals		48	20	48	26
Organ	Findings				
Kidney					
	hydronephrosis	1 (2.1)	0 (0.0)	1 (2.1)	0 (0.0)
	infiltration of mononuclear cell	2 (4.2)	0 (0.0)	2 (4.2)	0 (0.0)
	lymphocytic infiltration	0 (0.0)	2 (10.0)	1 (2.1)	1 (3.8)
	cortical cyst	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
	focal fibrosis	1 (2.1)	0 (0.0)	1 (2.1)	0 (0.0)
	renal mineralization	1 (2.1)	0 (0.0)	3 (6.2)	0 (0.0)
Urinary bladder					
	lymphocytic infiltration	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)
Epididymis					
	lymphocytic infiltration	0 (0.0)	1 (5.0)	–	–
Prostate gland					
	infiltration of mononuclear cell	10 (20.8)	0 (0.0)	–	–
	lymphocytic infiltration	3 (6.2)	4 (20.0)	–	–
Seminal vesicle					
	immature	1 (2.1)	0 (0.0)	–	–
Uterus					
	micro-granuloma	–	–	0 (0.0)	2 (7.7)
	uterine dilation	–	–	2 (4.2)	1 (3.8)
Thyroid gland					
	ectopic thymus	1 (2.1)	1 (5.0)	7 (14.6)	0 (0.0)
	ultimobranchial cyst	8 (16.7)	0 (0.0)	5 (10.4)	3 (11.5)
Eye					
	remnants of hyaloid vessels	0 (0.0)	3 (15.0)	1 (2.1)	1 (3.8)
Abdominal skin					
	ulceration of epidermis	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
Skeletal muscle					
	myofiber necrosis	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)
Sternum					
	chondromucinous degeneration	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)

a): No. of incidences (%)

DISCUSSION

The biological background data of the IGS strain at the age of 9 weeks for the 1-week to 4-week repeated dose toxicity studies were collected in our facility and routinely measured parameters were compared with the SD strain. Most of the parameters from the IGS strain including body weight, food intake, urinalysis, hematology, blood biochemistry, organ weight, gross findings and histopathological findings were similar to the previously reported reference data and findings in the SD strain [1-4].

Although all of the statistical differences in each examination from the SD strain could not be regarded as characteristic of the IGS strain, because of the small number of animals used to obtain the biological data on the IGS strain in our facility, differences were noted in both sexes at the age of 9 weeks, which included higher RBC, Hb and PT values, and a lower MCV value in hematology, and a higher TG value, and a lower TB value in blood biochemistry. A lower organ weight with respect to the prostate gland in the IGS strain was also confirmed.

Therefore, the hematology, blood chemistry and organ weight parameters showing statistically significant differences between the two strains may be characteristic of the IGS strain.

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Changes in body weight and organ weight with growth in Crj:CD (SD) IGS rats

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ABSTRACT. Changes in body weight and the weight of various organs, i.e., brain, pituitary gland, salivary gland, thymus, lung, heart, liver, spleen, kidney, adrenal gland, seminal vesicle, prostate, testis, epididymis, bulbourethral gland, ovary and uterus of Crj:CD (SD) IGS rats with growth were examined from 5 to 17 weeks of age. The body weight of Crj:CD (SD) IGS rats increased with growth during the period of this experiment, and gain in body weight per week was maximum at 5 to 6 weeks of age. Furthermore, the weights of all organs kept on increasing until 17 weeks of age, except for the thymus. It would appear useful to apply the results from this experiment as reference data in general toxicity studies. — Key words: Body weight, Crj:CD (SD) IGS rats, Growth, Organ weight

CD (SD) IGS-1998: 90-94

INTRODUCTION

The gold standard system, a new laboratory animal breeding system, was developed by Charles River Inc. to contribute to the globalization of research and development of new drugs and chemical substances. This system can globally supply homogeneous Crj:CD (SD) IGS rats (IGS strain) at the genetic level. However, the biological background data from the IGS strain are still insufficient. In this report, changes in body weight and the weight of various organs of IGS strain rats with growth were examined in great detail for use as reference data in general toxicity studies.

MATERIALS AND METHODS

Animals: A total 70 males and 70 females of specific-pathogen-free (SPF) IGS strain (Tsukuba Breeding Center, Tsukuba, Japan) at the age of 4 weeks were purchased from Charles River Japan, Inc., Yokohama.

Animal husbandry: The animals were housed in wire mesh cages (260x380x180 mm: Clea Japan Inc., Tokyo, Japan) and kept under standard laboratory animal conditions which were maintained at a temperature of 21 to 25°C with 40 to 70% relative humidity. The room air was ventilated 20 to 50 times per hour automatically and a 12 hr/12 hr light-dark cycle (lighting 07:00-19:00) was imposed. The animals received a commercial pellet diet (CRF-1, Oriental Yeast Industry Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*. The animals were identified by metallic ear tags.

Body weight and organ weight: At 5 weeks of age, the 70 males and 70 females were divided into 7 groups of nearly equal average body weight. The body weight of each group was measured weekly from 5 to 17 weeks of age. At 5, 7, 9 ,11, 13 ,15 and 17 weeks of age, 10 males and 10 females were euthanized by exsanguination from the abdominal aorta under ether anesthesia. The following organs were removed from these animals and weighed: brain, pituitary gland, salivary gland (submandibular and sublingual glands), thymus, lung, heart, liver, spleen, adrenal gland, kidney, seminal vesicle, prostate, testis, epididymis, bulbourethral gland, ovary and uterus. The pituitary, adrenal and bulbourethral glands and ovary were assessed in milligrams, other organs in grams. Each weight relative to final body weight was calculated.

RESULTS

Body weight changes of the IGS strain are shown in Table 1 for male and Table 2 for female. Since both the males and females were sacrificed in the course of the experiments so as to measure the organ weight, the number of animals decreased with time. Although some variations were observed among the groups, the body weight of the IGS strain increased with growth during the period of this experiment. Gains in body weight per week were maximum at 5 to 6 weeks of age for both male and female, and then decreased gradually with age.

Changes of absolute organ weight with growth are shown in Table 3 for male and Table 4 for female. As for thymus, the highest weight was recorded at 7 weeks old for both male and female, but tended to decrease after that. Except for the thymus, the weights of all organs measured in this experiment kept on increasing until 17 weeks of age. But as regards the degrees of gain in organ weight, differences were exhibited among the organs, particularly those of male and female brains were small compared with other organs. Many organs weighed in this experiment increased in almost similar degrees, except for the thymus and brain of both sexes, and the accessory sex organs of males such as seminal vesicle, prostate gland, bulbourethral gland and epididymis. The degrees of gain in weight of those organs were maximum at 5 to 7 or 7 to 9 weeks of age, and became very small after that. In the accessory sex organs of males, gains in organ weights were high until 11 weeks of age.

Changes in relative organ weight with growth are shown in Table 5 for male and Table 6 for female. As for the accessory sex organs of males, relative organ weight increased conspicuously until 9-11 weeks of age, and then became gradual or disappeared. In females, the weights of their pituitary glands, adrenal glands and ovaries tended to decrease after having increased considerably up to 9-11 weeks of age. Except for these organs, a decrease in relative organ weight was observed with growth, with a conspicuous decrease occurring at 7 weeks of age in the brains of males and at 9-11 weeks of age in the thymuses of both sexes. Although there were some differences

in terms of degree, the relative weight of other organs slowly decreased. It should also be mentioned that unilateral atrophy of kidney and anomaly in the ovary were observed in a female sacrificed at 11 weeks of age, so the weights of kidneys and ovaries at 11 weeks old were calculated based on 9 females.

DISCUSSION

The gold standard system, a new laboratory animal breeding system, was developed by Charles River Inc. to contribute to the globalization of research and development of new drugs and chemical substances. In repeated dose toxicity studies, one of the essential studies for safety evaluation, body weight and organ weight are very significant parameters. In this experiment, changes in body weight and organ weight with growth in the IGS strain from 5 to 17 weeks of age were examined and the data used as reference in repeated dose toxicity studies of less than 3 months.

Body weight of the IGS strain increased for both sexes until the end, however, the degree of gain in body weight per week decreased progressively. Because it was reported that body weight continued to increase over the life of SD strain rats [1], a similar increase naturally tended to occur in this experiment.

The weight of most of the organs measured in this experiment also increased with growth. But it was clear that weight of the thymus increased until 7 weeks of age and then decreased with respect to both sexes. This agreed with previous reports [2,4] which suggested that the thymuses of rats decrease in weight from approximately 2 months of age. The weight of brains also tended to increase with growth, but the degree of gain was small compared with that of other organs. Since it is known that half of the cell population in the cerebrum is formed in the first 3 weeks after birth [3], it has been suggested that a marked increase in brain weight occurs before 5 weeks of age. It became clear that the weights of other organs except for the accessory sex organs of males, and the pituitary glands, adrenal glands and ovaries of females showed a very similar pattern, although a tendency to decrease with growth was observed with regard to relative organ weight. It is considered that the degrees of increase in these organ weights were slightly inferior to those of body weight. In females, the relative organ weight of the pituitary glands, adrenal glands and ovaries increased until around 11 weeks of age and then decreased, while that of the accessory sex organs of males markedly increased up to 11 weeks of age. In the case of these organs, absolute organ weight exceeded body weight in terms of degree of increase until 11 weeks of age, suggesting a relationship with the puberty of rats.

Consequently, changes with growth became clear with regard to body weight and the weight of each organ. The fact that some drugs and chemical substances were found to affect the organisms of IGS strain and cause some organs to decrease in weight make the results in this experiment especially significant.

Table 1. Body weight changes of male Crj:CD (SD) IGS rats with growth

Group (weeks of age sacrificed)		5	7	9	11	13	15	17	
Number of animals examined		10	10	10	10	10	10	10	Total
Age in weeks									
5	Mean	160.4	163.4	160.3	163.9	162.3	162.7	162.5	162.2 (70)
	SD	5.1	4.8	4.2	5.5	5.5	4.7	5.8	13.5
6	Mean		233.7	226.1	230.2	225.9	230.0	230.8	229.5 (60)
	SD		6.9	10.6	6.6	11.3	8.3	7.2	21.3
7	Mean		297.2	291.8	293.8	288.8	295.9	293.8	293.6 (60)
	SD		8.8	18.4	13.4	16.7	11.4	12.0	33.9
8	Mean			349.2	346.0	345.2	352.0	346.0	347.7 (50)
	SD			26.8	17.5	20.5	16.0	15.3	44.0
9	Mean			395.3	394.2	388.3	404.2	391.9	394.8 (50)
	SD			35.1	22.3	25.3	24.1	17.6	57.1
10	Mean				431.7	422.2	446.3	433.1	433.3 (40)
	SD				26.3	28.9	30.2	15.4	51.7
11	Mean				466.9	454.5	480.9	465.9	467.1 (40)
	SD				32.2	32.5	35.0	18.1	60.4
12	Mean					476.2	503.4	488.8	489.5 (30)
	SD					35.0	41.8	19.9	58.0
13	Mean					500.8	532.7	515.2	516.2 (30)
	SD					35.3	46.8	20.5	62.1
14	Mean						553.2	534.5	543.9 (20)
	SD						55.9	20.0	59.4
15	Mean						574.9	553.0	564.0 (20)
	SD						62.0	22.9	66.1
16	Mean							562.6	562.6 (10)
	SD							24.1	24.1
17	Mean							578.0	578.0 (10)
	SD							26.9	26.9

The number of animals caluculated is shown in parenthesis. Unit: g

Table 2. Body weight changes of female Crj:CD (SD) IGS rats with growth

Group (weeks of age sacrificed)		5	7	9	11	13	15	17	
Number of animals examined		10	10	10	10	10	10	10	Total
Age in weeks									
5	Mean	138.3	134.0	135.9	135.2	135.8	133.0	135.4	135.4 (70)
	SD	4.3	5.8	3.6	5.7	5.7	4.3	5.8	13.5
6	Mean		167.3	168.1	165.6	168.1	161.4	171.7	167.0 (60)
	SD		9.8	5.2	8.5	12.4	6.2	9.7	21.9
7	Mean		195.1	192.5	188.8	193.0	181.3	190.6	190.2 (60)
	SD		15.8	5.2	14.3	18.1	8.9	12.7	32.4
8	Mean			207.4	210.2	212.8	201.0	217.8	209.8 (50)
	SD			8.8	19.3	25.7	10.0	15.8	38.2
9	Mean			226.9	229.0	231.1	216.6	239.5	228.6 (50)
	SD			12.9	21.9	28.9	11.0	15.7	43.0
10	Mean				242.5	246.4	229.2	253.8	243.0 (40)
	SD				26.0	31.7	10.3	17.7	45.8
11	Mean				257.0	258.5	239.5	268.3	255.8 (40)
	SD				24.4	35.6	13.6	21.3	50.0
12	Mean					264.5	246.4	278.6	263.2 (30)
	SD					39.1	17.4	21.9	48.1
13	Mean					276.1	254.2	292.6	274.3 (30)
	SD					39.2	17.6	22.6	48.6
14	Mean						259.1	295.9	277.5 (20)
	SD						17.6	21.6	27.9
15	Mean						266.5	303.1	284.8 (20)
	SD						22.2	24.1	32.8
16	Mean							307.4	307.4 (10)
	SD							26.8	26.8
17	Mean							317.4	317.4 (10)
	SD							29.0	29.0

The number of animals caluculated is shown in parenthesis. Unit: g

Table 3. Absolute organ weight changes of male Crj:CD (SD) IGS rats with growth

Group (weeks of age sacrificed)			5	7	9	11	13	15	17
Number of animals examined			10	10	10	10	10	10	10
Final body weight	(g)	Mean	160.4	297.2	395.3	466.9	500.8	574.9	578.0
		SD	5.1	8.8	35.1	32.2	35.3	62.0	26.9
Brain	(g)	Mean	1.72	1.91	1.97	2.04	2.10	2.14	2.14
		SD	0.07	0.09	0.05	0.07	0.06	0.09	0.09
Pituitary gland	(mg)	Mean	6.0	8.9	12.3	12.0	12.2	13.4	13.9
		SD	1.0	1.3	1.7	1.6	1.7	1.8	2.1
Salivary gland	(g)	Mean	0.33	0.52	0.67	0.69	0.75	0.83	0.82
		SD	0.02	0.04	0.08	0.07	0.06	0.11	0.05
Thymus	(g)	Mean	0.55	0.77	0.74	0.61	0.54	0.53	0.47
		SD	0.10	0.14	0.16	0.12	0.11	0.12	0.08
Lung	(g)	Mean	0.93	1.36	1.45	1.60	1.70	1.69	1.79
		SD	0.06	0.09	0.13	0.13	0.14	0.11	0.15
Heart	(g)	Mean	0.68	1.10	1.25	1.39	1.42	1.53	1.53
		SD	0.04	0.08	0.11	0.06	0.09	0.16	0.11
Liver	(g)	Mean	7.86	14.82	16.72	18.92	18.36	21.08	21.24
		SD	0.71	0.65	2.59	2.86	1.48	3.82	2.29
Spleen	(g)	Mean	0.51	0.70	0.78	0.78	0.82	0.88	0.83
		SD	0.06	0.07	0.12	0.09	0.10	0.16	0.12
Kidney	(g)	Mean	1.59	2.45	2.97	3.15	3.21	3.60	3.48
		SD	0.14	0.15	0.36	0.24	0.15	0.40	0.40
Adrenal gland	(mg)	Mean	32.5	41.7	55.8	52.2	58.4	56.0	56.0
		SD	3.9	3.3	6.3	7.5	8.1	6.4	7.6
Seminal vesicle	(g)	Mean	0.14	0.72	1.20	1.65	1.92	1.94	2.05
		SD	0.01	0.13	0.10	0.25	0.13	0.41	0.24
Prostate	(g)	Mean	0.09	0.24	0.40	0.52	0.55	0.63	0.64
		SD	0.01	0.04	0.08	0.12	0.13	0.11	0.14
Testis	(g)	Mean	1.33	2.47	3.00	3.37	3.38	3.36	3.61
		SD	0.06	0.14	0.15	0.20	0.25	0.26	0.30
Epididymis	(g)	Mean	0.19	0.42	0.76	1.09	1.28	1.37	1.47
		SD	0.02	0.06	0.07	0.06	0.15	0.12	0.11
Bulbourethral gland	(mg)	Mean	13.2	45.0	79.6	112.7	114.3	127.4	132.6
		SD	4.8	7.1	9.5	22.6	21.0	33.2	21.4

Table 4. Absolute organ weight changes of female Crj:CD (SD) IGS rats with growth

Group (weeks of age sacrificed)			5	7	9	11	13	15	17
Number of animals examined			10	10	10	10	10	10	10
Final body weight	(g)	Mean	138.3	195.1	226.9	257.0	276.1	256.9	293.5
		SD	4.3	15.8	12.9	24.4	39.2	10.6	15.4
Brain	(g)	Mean	1.67	1.78	1.83	1.90	1.90	1.92	1.99
		SD	0.09	0.07	0.06	0.07	0.07	0.09	0.08
Pituitary gland	(mg)	Mean	6.8	10.1	13.4	15.0	15.1	14.2	15.6
		SD	0.8	1.1	1.9	1.9	3.6	2.2	2.7
Salivary gland	(g)	Mean	0.29	0.39	0.46	0.46	0.49	0.49	0.44
		SD	0.03	0.04	0.04	0.03	0.09	0.05	0.04
Thymus	(g)	Mean	0.50	0.58	0.52	0.53	0.41	0.36	0.36
		SD	0.06	0.15	0.11	0.11	0.09	0.09	0.05
Lung	(g)	Mean	0.81	1.01	1.11	1.14	1.23	1.18	1.26
		SD	0.05	0.09	0.07	0.05	0.11	0.07	0.07
Heart	(g)	Mean	0.60	0.75	0.82	0.86	0.94	0.82	0.94
		SD	0.03	0.07	0.04	0.08	0.09	0.05	0.08
Liver	(g)	Mean	6.87	9.29	9.66	9.66	10.26	8.91	10.30
		SD	0.33	1.48	0.99	0.85	1.71	0.63	0.66
Spleen	(g)	Mean	0.42	0.46	0.51	0.53	0.53	0.47	0.48
		SD	0.08	0.10	0.04	0.07	0.08	0.06	0.05
Kidney	(g)	Mean	1.35	1.65	1.81	1.80 a)	1.97	1.70	1.94
		SD	0.10	0.15	0.10	0.14	0.25	0.09	0.10
Adrenal gland	(mg)	Mean	34.4	53.0	64.2	68.4	68.2	64.6	67.1
		SD	3.9	7.4	7.1	8.7	10.9	7.5	8.4
Ovary	(mg)	Mean	51.6	79.4	95.3	96.9 a)	90.0	84.9	89.2
		SD	10.9	12.6	9.4	13.3	11.8	8.8	12.0
Uterus	(g)	Mean	0.38	0.47	0.49	0.57	0.60	0.64	0.61
		SD	0.10	0.13	0.16	0.08	0.10	0.12	0.09

a): Calculation based on 9 animals owing to congenital anomaly.

Table 5. Relative organ weight changes of male Crj:CD(SD) IGS rats with growth

Group(weeks of age sacrificed)			5	7	9	11	13	15	17
Number of animals examined			10	10	10	10	10	10	10
Brain	(g%)	Mean	1.07	0.64	0.50	0.44	0.42	0.38	0.37
		SD	0.05	0.04	0.04	0.03	0.03	0.04	0.02
Pituitary gland	(mg%)	Mean	3.7	3.0	3.1	2.6	2.5	2.3	2.4
		SD	0.6	0.5	0.3	0.3	0.4	0.3	0.3
Salivary gland	(g%)	Mean	0.20	0.18	0.17	0.15	0.15	0.15	0.14
		SD	0.01	0.02	0.02	0.02	0.01	0.03	0.01
Thymus	(g%)	Mean	0.34	0.26	0.19	0.13	0.11	0.09	0.08
		SD	0.06	0.05	0.04	0.02	0.02	0.01	0.01
Lung	(g%)	Mean	0.58	0.46	0.37	0.35	0.34	0.30	0.31
		SD	0.04	0.03	0.03	0.04	0.03	0.02	0.02
Heart	(g%)	Mean	0.42	0.37	0.32	0.30	0.28	0.27	0.27
		SD	0.02	0.03	0.02	0.02	0.02	0.02	0.02
Liver	(g%)	Mean	4.90	4.98	4.21	4.04	3.67	3.65	3.67
		SD	0.33	0.17	0.33	0.35	0.17	0.31	0.32
Spleen	(g%)	Mean	0.32	0.24	0.20	0.17	0.17	0.15	0.15
		SD	0.03	0.02	0.03	0.01	0.03	0.02	0.02
Kidney	(g%)	Mean	0.99	0.83	0.75	0.67	0.64	0.63	0.60
		SD	0.08	0.05	0.09	0.04	0.03	0.04	0.07
Adrenal gland	(mg%)	Mean	20.2	14.0	14.1	11.2	11.7	9.8	9.7
		SD	2.4	1.1	1.3	1.5	1.6	0.7	1.2
Seminal vesicle	(g%)	Mean	0.09	0.24	0.31	0.36	0.39	0.34	0.36
		SD	0.01	0.04	0.03	0.06	0.04	0.09	0.05
Prostate	(g%)	Mean	0.06	0.08	0.10	0.11	0.11	0.11	0.11
		SD	0.01	0.02	0.02	0.03	0.03	0.02	0.02
Testis	(g%)	Mean	0.83	0.83	0.77	0.73	0.68	0.59	0.63
		SD	0.06	0.04	0.09	0.07	0.08	0.05	0.08
Epididymis	(g%)	Mean	0.12	0.14	0.19	0.23	0.26	0.24	0.25
		SD	0.01	0.02	0.02	0.02	0.03	0.04	0.03
Bulbourethral gland	(mg%)	Mean	8.3	15.2	20.2	24.1	22.8	22.3	23.0
		SD	3.1	2.6	2.7	4.6	3.9	6.1	3.7

Table 6. Relative organ weight changes of female Crj:CD(SD) IGS rats with growth

Group(weeks of age sacrificed)			5	7	9	11	13	15	17
Number of animals examined			10	10	10	10	10	10	10
Brain	(g%)	Mean	1.21	0.91	0.81	0.75	0.70	0.75	0.68
		SD	0.07	0.06	0.04	0.09	0.11	0.05	0.03
Pituitary gland	(mg%)	Mean	4.9	5.2	5.9	5.9	5.5	5.3	5.0
		SD	0.5	0.5	0.8	1.2	1.2	1.0	0.8
Salivary gland	(g%)	Mean	0.21	0.20	0.20	0.18	0.18	0.19	0.15
		SD	0.02	0.03	0.02	0.02	0.02	0.02	0.01
Thymus	(g%)	Mean	0.37	0.29	0.23	0.21	0.15	0.14	0.12
		SD	0.04	0.06	0.05	0.04	0.03	0.03	0.02
Lung	(g%)	Mean	0.59	0.52	0.49	0.45	0.45	0.46	0.43
		SD	0.03	0.03	0.03	0.05	0.03	0.02	0.02
Heart	(g%)	Mean	0.43	0.39	0.37	0.34	0.34	0.32	0.32
		SD	0.02	0.02	0.03	0.04	0.03	0.02	0.02
Liver	(g%)	Mean	4.97	4.68	4.26	3.77	3.71	3.47	3.51
		SD	0.15	0.40	0.30	0.21	0.23	0.22	0.16
Spleen	(g%)	Mean	0.31	0.23	0.23	0.21	0.19	0.18	0.16
		SD	0.05	0.04	0.02	0.03	0.03	0.02	0.01
Kidney	(g%)	Mean	0.97	0.83	0.80	0.71 [a)]	0.72	0.66	0.66
		SD	0.06	0.03	0.06	0.05	0.08	0.05	0.03
Adrenal gland	(mg%)	Mean	24.9	27.2	28.4	26.7	25.0	24.4	21.1
		SD	3.1	3.1	3.5	3.0	4.4	3.6	1.7
Ovary	(mg%)	Mean	37.4	41.0	42.1	38.3 [a)]	33.1	32.0	28.2
		SD	8.2	8.0	4.2	5.2	5.6	3.9	3.7
Uterus	(g%)	Mean	0.27	0.24	0.22	0.22	0.22	0.25	0.21
		SD	0.07	0.07	0.06	0.05	0.04	0.05	0.04

a): Calculation based on 9 animals owing to congenital anomaly.

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Effects of Feeding Rats (Crj:CD(SD)IGS) with CRF-1 (Protein Content: 23.1%) or CR-LPF (Protein Content: 18.4%) for Six Months

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ABSTRACT. We studied rats (Crj;CD(SD)IGS) fed with the ordinary powder feed CRF-1 (protein content: 23.1%) and rats fed with the lower-protein feed CR-LPF (protein content: 18.4%) for six months. Both feeds were treated with 30Kgy radiation.
No deaths occurred in either sex of either group. No abnormalities were observed in the general signs in either sex of either group. Males of the CR-LPF group tended to be suppressive in the body weight gain, but not female. Both sexes of the CR-LPF group showed an increase in food consumption. No difference in either sex given these feeds was found in the hematological findings, blood chemical analysis, necropsy findings, or organ weighs. As stated above, males of the CR-LPF group had lower body weight than males of the CRF-1 group, and both sexes of the CR-LPF group showed greater food consumption than the CRF-1 group, but no difference between the two feeds appeared in other findings. – Key words: Rats, Crj: CD (SD) IGS, Toxicity, Six months

CD (SD) IGS-1998: 95-99

METHODS

Test Animals and Housing Conditions: Male and female Crj:CD(SD)IGS strain rats (SPF, chares River Japan) at the age of 4 weeks were used for the present study. The animals were kept in an animal room with a 12-hour light and dark cycle (lighting: 6:00a.m. – 6:00p.m.), temperature range of 20 – 24°C, relative humidity range of 40 – 70%, and filter-sterilized fresh air changes 12 times per hour. The animals were housed individually in stainless steel cages. The animals were given free access to powder feed (CRF-1 treated with 30Kgy radiation, Oriental Yeast Co., Ltd.) from purchase to grouping.

On the day of grouping, body weights of the animals were stratified with a computer, and the animals were grouped by a random sampling method so as to distribute the mean body weights and variances among the groups. Each group consisted of 20 animals of each sex. After grouping, the CRF-1 group was given free access to CRF-1 feed containing 23.1% crude protein, 7.7% water, 5.9% crude-fat, 6.5% crude ash, and 3.3% crude fiber for six months. The CR-LPF group was likewise given free access to CR-LPF feed containing 18.4% crude protein, 7.5% water, 4.8% crude fat, 6.3% crude ash, and 5.0% crude fiber. The animals were also allowed to drink tap water freely.

Observations and Examinations: The animals were observed for general signs and mortality once daily during six months. Body weights and feed consumption were measured once a week.

After 6 months feeding, hematological examination was performed on the day of necropsy. The abdominal region of each animal was cut open under anesthesia with intraperitoneal pentobarbital sodium, and blood was collected from the abdominal aorta in a Sysmex cup coated internally with EDTA-2K. Erythrocyte count (RBC), leucocyte count (WBC), hemoglobin concentration, hematocrit, and platelet count were determined with an automatic cell counter (Systemic E-2000, Toa Medical Electronics Co., Ltd.). Mean corpuscular volume (MCV), mean corpuscular hemoglobin level (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the erythrocyte count, hemoglobin concentration, and hematocrit.

Plasma treated with trisodium citrate dihydrate (3.13%) was assayed for prothrombin time (PT), active part thromboplastin time (APTT), and fibrinogen concentration (Coagumasuter 2, Sankyo Co., LTD.).

For blood chemical analysis, serum was obtained from the blood by centrifugation at about 4°C and 3000r.p.m. for 15 minutes with an automatic high speed refrigerating centrifuge and subjected to the following tests.

GOT and GPT were determined by the modified Henry method, ALP by the p-NPP substrate method, total cholesterol by the COD.DAOS method, triglyceride by the GPO・DAOS method, total protein by the Biuret method, blood urea nitrogen (BUN) by the urease GIDH method, creatinine by Jaffe's method, total bilirubin by the azobilirubin method, glucose by the glucose dehydrogenase method, inorganic phosphorus by the molybdenum blue method, and Ca by the o-CPC method.

Na and K were measured with an ion-selective electrode and Cl by coulometric titration in an automated electrolyte analyzer (EA04, A & T LTD.).

After blood collection for hematological examination and blood chemical analysis, the animals were killed by exsanguination, and the organs and tissues were observed macroscopically. Organ weights were measured all animals. The weight of each organ relative to the body weight on the day of necropsy was calculated. The organs weighed were the brain, pituitary, salivary glands, thyroids, thymus, lungs, heart, liver, spleen, kidneys, adrenals, testes, prostate, ovaries, and uterus.

Statistical Methods: Data for the two groups were statistically analyzed as follows. After determining the equality of dispersion by F-test, data that had equal dispersion were analyzed with Student t-test, and data that did not have equal dispersion were analyzed with the Cochran-Cox t-test or the Aspin-Welch t-test.

RESULTS

General Signs: Neither death nor abnormal signs were noted in any group.

Body Weight (Table 1): Males of the CR-LPF group had body weight which was significantly lower than those of CRF-1 group on Days 85 and 155.

Food Consumption (Table 2): Both sexes of the CR-LPF group consumed more food than those of the CRF-1 group during the observation period.

Hematological Examination (Table 3): The number of WBCs in males of the CR-LPF group was significantly less than in the CRF-1 group, and the number of platelets in females of the CR-LPF group was significantly less than in the CRF-1 group, but this could not be said to be due to the difference in diet.

Blood Chemical Analysis (Table 4): Males in the CR-LPF group had significantly higher values of albumin, triglyceride, and A/G, and significantly lower values of BUN and glucose than in the CRF-1 group. Females in the CR-LPF group had significantly higher values of albumin and A/G, and significantly lower values of BUN than in the CRF-1 group.

Necropsy Findings: One male of the CR-LPF group had softness in the testis and one female of the CR-LPF group had hyperplasia of the pituitary, but we decided these findings to be incidental.

Organ Weights (Table 5): In the CR-LPF group, the absolute weight of the salivary glands of males was significantly lower, and the absolute and relative weights of the testes were significantly greater than in the CRF-1 group, and the absolute and relative weights of the salivary glands, kidneys, and adrenals of females were significantly lower, and the relative weight of the spleen of females was also significantly lower than in the CRF-1 group.

DISCUSSION

No death occurred in any group and no abnormality in general signs was seen in any group.

Though the body weight of both groups showed similar changes in females, males of the CR-LPF group had decreased growth rate throughout the observation period compared with males of the CRF-1 group. The food consumption of the CR-LPF group was greater than in the CRF-1 group in both sexes throughout the observation period. Feeding CR-LPF to rats caused a decrease in body weight of males and an increase in food consumption by both sexes.

Table 1. Body weight of rats

Sex	Male		Female	
Food	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	20	20	20	20
Days				
1	170.8 ± 6.7	170.8 ± 6.1	143.6 ± 6.3	143.6 ± 6.6
8	229.1 ± 8.8	223.9 ± 12.6	168.5 ± 10.6	168.8 ± 8.3
15	283.9 ± 12.7	278.3 ± 11.8	190.7 ± 12.2	190.7 ± 13.5
22	323.5 ± 19.5	320.4 ± 17.0	210.5 ± 13.8	212.5 ± 13.1
29	365.8 ± 24.9	358.1 ± 20.0	224.1 ± 16.5	229.2 ± 15.2
36	394.3 ± 32.9	384.6 ± 22.4	235.2 ± 17.0	239.1 ± 15.8
43	419.6 ± 31.2	406.0 ± 26.1	245.5 ± 20.1	247.8 ± 18.4
50	442.2 ± 34.8	424.8 ± 33.5	252.4 ± 19.9	257.0 ± 18.0
57	461.9 ± 37.6	443.0 ± 31.8	257.8 ± 21.5	265.5 ± 16.8
64	472.4 ± 47.0	459.6 ± 29.6	267.5 ± 23.8	272.4 ± 17.2
71	493.2 ± 44.6	474.5 ± 31.2	271.4 ± 24.4	275.7 ± 18.1
78	506.7 ± 46.5	481.3 ± 31.6	275.5 ± 25.8	281.3 ± 17.7
85	521.1 ± 48.2	494.4 ± 33.0*	278.6 ± 26.3	282.0 ± 19.8
92	530.4 ± 51.6	506.0 ± 32.1	284.8 ± 27.7	288.4 ± 19.2
99	536.0 ± 51.7	511.0 ± 30.1	289.5 ± 26.7	292.1 ± 21.8
106	549.1 ± 56.9	524.0 ± 32.0	290.5 ± 26.0	293.9 ± 21.6
113	549.0 ± 60.8	529.3 ± 32.9	293.9 ± 25.9	299.9 ± 22.2
120	562.9 ± 57.4	538.5 ± 33.7	297.4 ± 25.4	302.4 ± 23.2
127	566.7 ± 61.0	540.1 ± 40.5	301.5 ± 25.2	309.0 ± 23.3
134	569.2 ± 57.5	544.1 ± 38.3	301.2 ± 25.6	310.5 ± 24.4
141	570.7 ± 55.5	544.2 ± 38.7	300.1 ± 26.6	312.6 ± 24.7
148	582.1 ± 58.2	556.0 ± 33.7	301.8 ± 27.4	311.9 ± 25.3
155	587.9 ± 59.8	556.1 ± 34.8*	302.9 ± 27.4	310.1 ± 25.7
162	592.5 ± 59.2	563.2 ± 31.0	303.2 ± 26.8	312.7 ± 25.6
169	593.1 ± 60.1	570.5 ± 34.2	306.4 ± 27.8	314.2 ± 30.5
176	597.4 ± 63.5	573.5 ± 33.4	308.3 ± 30.0	316.4 ± 27.8
183	585.9 ± 62.6	568.8 ± 33.0	303.3 ± 29.2	311.2 ± 26.9

Each value shows mean (g) ± S.D.
Significantly different from CRF-1 control (*: $P<0.05$).

Table 2. Food consumption in rats

Sex	Male		Female	
Food	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	20	20	20	20
Days				
3	18.8 ± 1.3	21.1 ± 1.4**	14.0 ± 1.3	13.6 ± 2.0
10	21.9 ± 1.9	25.0 ± 1.6**	15.8 ± 1.4	16.4 ± 1.6
17	23.4 ± 2.4	25.6 ± 1.2**	16.9 ± 2.5	18.3 ± 1.5*
24	24.8 ± 1.7	26.5 ± 2.4*	16.5 ± 2.4	18.4 ± 2.2*
31	24.6 ± 2.2	27.3 ± 2.3**	18.0 ± 2.3	19.2 ± 2.0
38	25.0 ± 2.4	27.7 ± 2.7**	18.4 ± 1.8	19.6 ± 1.5*
45	25.3 ± 1.9	27.2 ± 3.5*	16.8 ± 2.2	19.5 ± 3.0**
52	25.0 ± 2.5	27.9 ± 2.3**	17.3 ± 2.3	19.6 ± 2.7**
59	24.7 ± 2.2	27.1 ± 2.6**	17.3 ± 1.6	19.0 ± 1.5**
66	26.2 ± 3.1	26.9 ± 3.3	17.7 ± 2.5	18.8 ± 1.8
73	27.3 ± 2.7	27.6 ± 3.2	18.2 ± 2.3	19.3 ± 1.6
80	25.8 ± 2.8	28.0 ± 3.2*	19.0 ± 3.4	19.8 ± 2.3
87	25.0 ± 2.4	26.4 ± 2.3	16.7 ± 2.2	18.3 ± 2.4*
94	24.7 ± 2.5	26.0 ± 2.2	17.5 ± 2.0	19.1 ± 2.3*
101	25.5 ± 2.5	26.3 ± 3.3	15.7 ± 2.0	17.1 ± 1.9*
108	26.0 ± 2.5	26.8 ± 3.3	17.0 ± 2.0	18.3 ± 2.0*
115	24.9 ± 3.0	25.9 ± 2.6	16.5 ± 1.6	18.5 ± 2.0**
122	24.0 ± 2.0	25.6 ± 1.8*	15.5 ± 1.0	18.6 ± 1.7**
129	23.5 ± 2.6	24.8 ± 2.9	15.9 ± 2.9	18.1 ± 1.5**
136	24.1 ± 2.5	24.9 ± 2.5	15.0 ± 2.5	17.5 ± 3.2**
143	24.9 ± 3.1	27.9 ± 3.0**	17.4 ± 2.2	19.9 ± 2.5**
150	23.5 ± 2.5	26.3 ± 3.0**	16.4 ± 2.3	18.4 ± 2.2**
157	23.2 ± 2.3	26.2 ± 2.7**	15.9 ± 3.1	18.5 ± 2.5**
164	24.2 ± 2.7	26.6 ± 3.1*	16.9 ± 3.8	19.4 ± 3.8*
171	25.5 ± 2.8	27.7 ± 2.8*	16.8 ± 2.5	18.2 ± 2.7
178	23.5 ± 3.2	27.1 ± 3.5**	17.1 ± 3.7	19.4 ± 3.5

Each value shows mean (g/day) ± S.D.
Significantly different from CRF-1 control (*: P<0.05, **: P<0.01).

Table 3. Hematological finding in rats

Sex	Male		Female	
Food	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	20	20	20	20
RBC (10^4/mm^3)	843.7 ± 63.5	822.5 ± 46.6	746.4 ± 34.6	745.1 ± 37.6
Hemoglobin (g/dl)	15.03 ± 0.95	14.97 ± 0.89	14.33 ± 0.68	14.18 ± 0.49
Hematocrit (%)	45.13 ± 2.70	44.57 ± 2.70	41.50 ± 2.08	41.27 ± 1.77
MCV (μm^3)	53.60 ± 2.17	54.19 ± 1.59	55.62 ± 1.46	55.43 ± 1.42
MCH (Pg)	17.85 ± 0.63	18.22 ± 0.59	19.21 ± 0.62	19.05 ± 0.60
MCHC (g/dl)	33.31 ± 0.70	33.60 ± 0.41	34.55 ± 0.60	34.38 ± 0.72
Platelet (10^4/mm^3)	105.18 ± 17.90	102.92 ± 8.53	106.38 ± 11.79	98.69 ± 11.21*
Reticulocyte (‰)	20.8 ± 6.2	23.5 ± 9.1	21.3 ± 4.2	20.0 ± 4.6
PT (sec.)	14.95 ± 0.45	15.17 ± 0.37	14.09 ± 0.21	14.10 ± 0.38
APTT (sec.)	26.90 ± 1.86	25.95 ± 2.24	23.80 ± 1.47	23.68 ± 1.39
Fibrinogen(mg/dl)	276.2 ± 41.7	261.8 ± 15.0	200.3 ± 43.3	182.7 ± 17.3
WBC (10^2/mm^3)	56.6 ± 20.7	45.4 ± 12.8*	46.4 ± 29.7	34.1 ± 14.1
Differential leukocyte (%)				
Lymphocyte	82.8 ± 11.9	85.1 ± 6.9	80.9 ± 10.7	85.8 ± 7.3
Neutrophil	16.1 ± 11.7	13.7 ± 6.5	17.9 ± 10.5	13.5 ± 7.1
Eosinophil	0.8 ± 0.9	0.7 ± 0.7	0.7 ± 0.9	0.4 ± 0.7
Basophil	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	0.5 ± 0.6	0.6 ± 0.8	0.6 ± 0.7	0.5 ± 0.7

Each value shows mean ± S.D.
Significantly different from CRF-1 control (*: P<0.05).

Table 4. Blood chemical analysis in rats

Sex		Male		Female	
Food		CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals		20	20	20	20
GOT	(IU/l)	71.01 ± 18.80	63.12 ± 10.57	81.77 ± 58.65	70.44 ± 20.09
GPT	(IU/l)	34.05 ± 12.76	29.59 ± 5.58	40.36 ± 39.88	36.20 ± 13.71
ALP	(IU/l)	92.07 ± 20.53	89.38 ± 21.03	60.58 ± 18.81	66.34 ± 26.82
T-protein	(g/dl)	5.79 ± 0.25	5.80 ± 0.23	6.40 ± 0.72	6.61 ± 0.46
Albumin	(g/dl)	3.024 ± 0.196	3.177 ± 0.153**	3.894 ± 0.616	4.277 ± 0.364*
A/G		1.101 ± 0.109	1.219 ± 0.100**	1.568 ± 0.260	1.842 ± 0.152**
T-bilirubin	(mg/dl)	0.061 ± 0.015	0.066 ± 0.011	0.079 ± 0.019	0.074 ± 0.015
BUN	(mg/dl)	14.06 ± 2.83	12.46 ± 1.83*	14.72 ± 1.92	13.40 ± 1.53*
Creatinine	(mg/dl)	0.416 ± 0.053	0.408 ± 0.046	0.427 ± 0.063	0.437 ± 0.040
Glucose	(mg/dl)	153.89 ± 17.74	142.96 ± 9.81*	149.33 ± 7.40	149.78 ± 8.12
T-cholesterol	(mg/dl)	67.55 ± 13.90	75.27 ± 11.41	79.05 ± 17.54	78.64 ± 17.85
Triglyceride	(mg/dl)	63.66 ± 18.12	87.50 ± 44.25*	62.33 ± 27.60	67.68 ± 33.84
Na	(mEq/l)	154.13 ± 3.05	154.52 ± 3.02	148.76 ± 2.94	149.55 ± 1.99
K	(mEq/l)	4.671 ± 0.243	4.743 ± 0.245	4.379 ± 0.369	4.380 ± 0.252
Cl	(mEq/l)	105.57 ± 1.50	106.29 ± 0.85	107.08 ± 1.76	107.65 ± 1.70
Ca	(mg/dl)	9.61 ± 0.37	9.61 ± 0.31	10.41 ± 0.50	10.44 ± 0.50
I-phosphorus	(mg/dl)	5.22 ± 1.13	5.05 ± 0.89	4.40 ± 1.29	4.39 ± 1.23

Each value shows mean ± S.D.
Significantly different from CRF-1 control (*: P<0.05, **: P<0.01).

Table 5. Organ weights in rats

Sex		Male		Female	
Food		CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals		20	20	20	20
Body weight	(g)	586.0 ± 62.6	568.8 ± 33.0	303.3 ± 29.2	311.2 ± 26.9
Brain	(g)	2.151 ± 0.101	2.110 ± 0.094	1.924 ± 0.057	1.944 ± 0.067
	(g%)	0.370 ± 0.032	0.372 ± 0.028	0.640 ± 0.062	0.629 ± 0.050
Pituitary	(mg)	16.65 ± 3.01	16.33 ± 3.73	20.39 ± 6.25	20.53 ± 3.94
	(mg%)	2.85 ± 0.42	2.90 ± 0.77	6.67 ± 1.64	6.64 ± 1.33
Salivary glands	(g)	0.913 ± 0.084	0.823 ± 0.090**	0.584 ± 0.056	0.537 ± 0.046**
	(g%)	0.157 ± 0.018	0.146 ± 0.016	0.195 ± 0.018	0.174 ± 0.017**
Thyroids	(mg)	22.92 ± 3.78	22.41 ± 4.29	17.06 ± 1.85	16.07 ± 3.80
	(mg%)	3.93 ± 0.58	3.94 ± 0.73	5.66 ± 0.75	5.17 ± 1.11
Thymus	(mg)	145.67 ± 33.49	149.39 ± 39.39	159.51 ± 42.37	143.61 ± 34.28
	(mg%)	25.04 ± 6.19	26.38 ± 7.03	53.00 ± 14.44	46.19 ± 10.40
Lungs	(g)	1.522 ± 0.147	1.487 ± 0.087	1.113 ± 0.103	1.111 ± 0.112
	(g%)	0.262 ± 0.021	0.262 ± 0.018	0.369 ± 0.037	0.358 ± 0.039
Heart	(g)	1.538 ± 0.181	1.515 ± 0.189	0.977 ± 0.089	0.954 ± 0.088
	(g%)	0.263 ± 0.014	0.266 ± 0.027	0.324 ± 0.032	0.308 ± 0.027
Liver	(g)	17.105 ± 2.336	16.800 ± 1.948	9.946 ± 1.263	9.753 ± 1.023
	(g%)	2.918 ± 0.267	2.948 ± 0.236	3.277 ± 0.252	3.139 ± 0.252
Spleen	(g)	0.858 ± 0.170	0.769 ± 0.107	0.546 ± 0.075	0.507 ± 0.055
	(g%)	0.146 ± 0.021	0.137 ± 0.020	0.181 ± 0.023	0.164 ± 0.021*
Kidneys	(g)	3.455 ± 0.402	3.309 ± 0.266	2.199 ± 0.225	2.019 ± 0.188**
	(g%)	0.591 ± 0.050	0.582 ± 0.041	0.728 ± 0.061	0.651 ± 0.069**
Adrenals	(mg)	54.03 ± 6.37	50.25 ± 9.17	72.70 ± 8.01	65.72 ± 6.77**
	(mg%)	9.31 ± 1.40	8.84 ± 1.57	24.14 ± 3.30	21.29 ± 2.99**
Testes	(g)	3.478 ± 0.227	3.764 ± 0.393**	—	—
	(g%)	0.600 ± 0.072	0.663 ± 0.066**	—	—
Prostate	(g)	0.659 ± 0.224	0.630 ± 0.152	—	—
	(g%)	0.115 ± 0.043	0.112 ± 0.029	—	—
Ovaries	(mg)	—	—	76.75 ± 19.50	74.61 ± 14.57
	(mg%)	—	—	25.56 ± 6.83	24.14 ± 5.05
Uterus	(g)	—	—	0.803 ± 0.253	0.765 ± 0.196
	(g%)	—	—	0.267 ± 0.087	0.247 ± 0.063

Each value shows mean ± S.D.
Significantly different from CRF-1 control (*: P<0.05, **: P<0.01).

In blood chemical analysis of the CR-LPF group, albumin and A/G were increased in both sexes, BUN was decreased in both sexes, and triglyceride and glucose were decreased in males. However, these difference between the two diets were slight, so we decided them not to be due to the difference in diet.

At necropsy, there was no difference between the two diets.

In organ weights, there was an increase in absolute and relative weights of the testes in males, a decrease in absolute weight of the salivary glands in both sexes, and a decrease in relative weight of the salivary glands in females. But these findings were not remarkably different from those of the CRF-1 group. The kidneys and adrenals of females in the CR-LPF group were decreased in absolute and relative weights. As these are not marked differences from the CRF-1 group, we conclude that the difference between CRF-1 and CR-LPF has little effect.

As stated above, there was a decrease in body weight of males and an increase in food consumption by both sexes in the CR-LPF group compared with the CRF-1 group, but no difference between the two diets appeared in other findings.

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

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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 clinical observation, body weight, food consumption, water intake, urine volume, ophthalomoscopy, urinalysis, hematology, blood chemistry, blood coagulation test results and activities of hepatic drug-metabolizing enzymes. Sex-related changes were observed in the pH, protein, ketone bodies and leukocytes upon urinalysis; the leukocyte count and reticulocyte count upon hematological examination; the A/G ratio, albumin, triglyceride, total bilirubin, ALP, CK, sodium and inorganic phosphorus upon blood chemical examination; the APTT and fibrinogen upon blood coagulation testing; and aminopyrine-*N*-demethylase and aniline hydroxylase activities upon determination of hepatic drug-metabolizing enzymes. Age-related changes were observed in the erythrocyte count, hematocrit value, hemoglobin concentration, MCV, reticulocyte count upon hematological examination; creatinine, ALP, CK and inorganic phosphorus in both sexes, triglyceride in males and total bilirubin in females upon blood chemical examination; and aniline hydroxylase activity upon determination of hepatic drug-metabolizing enzymes. These changes have also been observed in Crj:CD(SD) rats and other strains, F344 and Wistar rats, used commonly in repeated-dose toxicity studies. —Key words: Background data, CD(SD)IGS rat, Low protein diet

CD (SD) IGS-1998: 100-107

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 [4, 5]. 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 [6]. 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 [2].

The standard protein levels in laboratory animal diets, 20-25%, are appropriate for growth and reproduction but are thought to be too high for long-term maintenance [1]. It has been reported that using an 18% protein diet poses no nutritional problems in rats [11, 12] and can effectively prolong their life span by reducing the incidence and retarding the development of spontaneous lesions [7, 8, 16]. This study was designed to collect and evaluate 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

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).

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°C, a relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (light on from 07:00 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 60Co 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 60Co source).

Grouping and diet components:

The experimental groups were as follows.

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

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 of 10 and 20, respectively, for the 2-, 4- and 13-week

study. Seven, 9 and 18 weeks of age are abbreviated as 7w, 9w and 18w, respectively.

The diet components were as follows.

Composition	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

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 (PM4800, 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 (PM4800, 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 and 18w. 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 (PM4800, Mettler GmbH).

5) Ophthalmoscopy

Ophthalmoscopic examination was performed for all animals in each group once before the experiment commenced (5w) and at 9w and 18w. 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 each group at 7w, 9w and 18w. Animals were denied access to food and water, and a 4-hr morning urine sample was collected and centrifuged at 400 xg 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) Hematology, blood chemistry and blood coagulation

Hematological, blood chemical and blood coagulation analyses were conducted for each of the 10 males and 10 females or 20 males and 20 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), heparin sodium (blood chemistry) or 3.13% sodium citrate (blood coagulation test) under ether anesthesia. The heparinized blood was centrifuged at 7500xg for 10 min to obtain plasma for blood chemistry. The blood containing sodium citrate was centrifuged at 1500xg for 10 min to obtain plasma for blood coagulation testing.

a) Hematology

The values of the following were determined or calculated with an automated hematology analyzer (E5000, Toa Medical Electronics Co., Ltd.) and an automated reticulocyte analyzer (R-1000, Toa Medical Electronics Co., Ltd.). 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.

- total protein (biuret method)
- albumin (BCG method)
- A/G ratio
- glucose (glucokinase-G-6-PDH method)
- total cholesterol (COD-DAOS method)
- triglyceride (GPO-DAOS method)
- urea nitrogen (urease-GlDH method)
- creatinine (method of Jaffé)
- total 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)

c) Blood coagulation

The values of the following were determined with an automated blood coagulation analyzer (CA-1000, Toa Medical Electronics Co., Ltd.).

- prothrombin time (PT, light scattering method)
- activated partial thromboplastin time (APTT, light scattering method)
- fibrinogen (light scattering method)

8) 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 9000xg for 20 min, and the supernatant was assayed for aminopyrine-*N*-demethylase (Cochin & Axelrod method) and aniline hydroxylase (modified method of Wills) activity.

Statistical analysis: The data on body weight, food consumption, water intake, urine output, hematology, blood chemistry, blood coagulation and activity of hepatic drug-metabolizing enzymes were analyzed statistically as follows. An analysis of variance was performed to see whether there were effects of age, sex and/or lot [15].

RESULTS

Mortality: No animals died during the experimental period.

Clinical signs (Table 1): In males, incrustation was observed during the early stage of the experimental period and hair loss, stained fur around the eyes and malinterdigitation tended to increase during the late stage of the experimental period. Lacrimation was observed in some males. In females, the incidence of findings was low as compared with that in males. Stained fur around the eyes, malinterdigitation and lacrimation were observed in only 1 female each.

Body weight (Table 2): In both sexes, body weight increased rapidly up to 9w. Thereafter, it increased gradually, leveling off and almost reaching a plateau at 18w.

Food consumption (Table 3): No change was observed during the experimental period. The average weekly food consumption value was 172 g in males and 118 g in females.

Water intake and urine output (Table 4): In both sexes, urine output was lower at 7w than at 9w and 18w. No change in the water intake was observed at any age.

Ophthalmoscopy (Table 5): In both sexes at 5w, persistent hyaloid vessels were observed in more than 60% of the animals, but thereafter, the incidence of this finding decreased gradually with age. At 5w and 9w, corneal opacity and adhesion were observed in some animals; however, these changes were no longer observed at 18w.

Urinalysis (Table 6): The pH level was higher in males than in females. The level of leukocytes was lower in males than in females. Age-related increases in the levels of protein and ketone bodies were observed in both sexes but especially in males.

Hematology (Table 7): The reticulocyte count and leukocyte count were higher in males than in females at each examination time point. Age-related increases in the erythrocyte count in males were observed. Slight age-related decreases in the MCV and reticulocyte count were observed in both sexes. The hematocrit value and hemoglobin concentration were increased slightly at 10w as compared with the values at 8w; however, a tendency towards a decrease was seen at 19w.

Blood chemistry (Table 8): The triglyceride, ALP, CK, sodium and inorganic phosphorus and were higher in males than in females at each examination time point. The albumin, A/G ratio and total bilirubin were lower in males than in females. The creatinine in both sexes, the triglyceride in males and the total bilirubin in females increased slightly with age. The ALP, CK and inorganic phosphorus decreased slightly with age. Marked increases in the AST, ALT and LDH (200-500% of the average value) were observed in some females at 19w.

Blood coagulation (Table 9): The APTT and fibrinogen were higher in males than in females. No age-related changes were observed.

Activity of the hepatic drug-metabolizing enzymes (Table 10): The aminopyrine-*N*-demethylase and aniline hydroxylase activities were higher in males than in females at each examination time point. The aniline hydroxylase activity at 8w was the highest among ages examined, and the activity at 10w was almost the same as that at 19w.

DISCUSSION

Sex-related changes were observed in the levels of pH, protein, ketone bodies and leukocytes upon urinalysis; the leukocyte count and reticulocyte count upon hematological examination; the albumin, A/G ratio, triglyceride, total bilirubin, ALP, CK, sodium and inorganic phosphorus upon blood chemical examination; the APTT and fibrinogen upon blood coagulation testing; and aminopyrine-*N*-demethylase and aniline hydroxylase activities upon the determination of hepatic drug-metabolizing enzymes. It is well known that there are sex differences in the hepatic drug-metabolizing enzymes activities in rodents, and these activities are controlled by androgen [3]. Increased urinary protein in male rats is commonly observed and is reported to be due to an increase in α_{u2}-globulin [14].

Age-related changes were observed in the erythrocyte count, hematocrit value, hemoglobin concentration, MCV and reticulocyte count upon hematological examination; creatinine, ALP, CK and inorganic phosphorus in both sexes, triglyceride in males and total bilirubin in females upon blood chemical examination; and aniline hydroxylase activity upon the determination of hepatic drug-metabolizing enzymes. Age-related changes in hematological parameters and plasma ALP have also been seen in various strains of rats used commonly in

repeated-dose toxicity studies [9, 11]. However, the leukocyte count and platelet count have been found to be higher in Sprague-Dawley rats, including Crj:CD(SD)IGS rats, than in F344 and Wistar rats [11]. An increase in urinary protein with age is commonly observed and is considered to be caused by renal injury [13].

Plasma ALT, AST and LDH were increased in Crj:CD(SD)IGS rat at 19w; however, no lesions were seen in the corresponding animals upon histopathological examination of the liver. Although the reason for the increases in the hepatic leakage enzymes was obscure, the same findings were observed in a similar study using the same lot of Crj:CD(SD)IGS rats at the Hikari Branch in our laboratories [10] and in Jcl:SD rats aged 3 to 15 months [9]. No changes in AST or ALT have been seen in F344 rats in our laboratories [11]. Therefore, an increase in plasma transaminases and LDH without hepatic lesions is considered to be a common finding in aged Sprague-Dawley rats.

From these results, it is concluded that there are no great differences among Crj:CD(SD)IGS rats and 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.

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Table 1. Clinical signs - Incidence of individual signs

Male	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	150	150	100	100	50	50	50	50	50	50	50	50	50
Incrustation	4	5	2	1	0	0	0	0	0	0	0	0	0
Hair loss	0	0	1	0	0	0	0	0	0	1	2	2	1
Fur around eyes, stained	1	1	0	0	0	1	1	0	1	1	2	3	2
Malinterdigitation	0	0	0	0	0	1	1	1	1	1	2	2	2
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	1
Wound	0	1	0	0	0	0	0	0	0	0	0	0	0
Erosion	0	1	0	0	0	0	0	0	0	0	0	0	0

Female	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	150	150	100	100	50	50	50	50	50	50	50	50	50
Fur around eyes, stained	0	0	0	0	0	0	0	0	0	0	0	1	0
Malinterdigitation	0	0	0	0	0	0	0	0	0	0	1	1	1
Lacrimation	1	0	0	0	0	0	0	0	0	0	0	0	0

Table 2 Body weight

Age (weeks)	No. of animals	Male	Female
6	150	199 ± 10	152 ± 6
7	150	254 ± 12	173 ± 11
8	150	302 ± 17	194 ± 13
9	100	345 ± 23	213 ± 15
10	100	378 ± 26	226 ± 18
11	50	400 ± 28	238 ± 17
12	50	423 ± 31	249 ± 16
13	50	444 ± 35	259 ± 17
14	50	458 ± 37	264 ± 18
15	50	477 ± 40	271 ± 20
16	50	492 ± 43 (49)	278 ± 18
17	50	505 ± 45 (49)	283 ± 20 (49)
18	50	514 ± 48	286 ± 20
19	50	518 ± 47 (49)	285 ± 20

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

Table 3. Food consumption

Age (weeks)	No. of animals	Male	Female
6	150	163 ± 11	114 ± 10
7	150	170 ± 14	116 ± 12 (149)
8	100	180 ± 17	121 ± 11
9	100	178 ± 17	121 ± 12
10	100	173 ± 14	121 ± 12
11	50	175 ± 17	124 ± 10
12	50	178 ± 20	126 ± 11
13	50	171 ± 18	117 ± 11
14	50	170 ± 18	119 ± 12
15	50	172 ± 20	119 ± 11
16	50	170 ± 17 (49)	116 ± 11 (49)
17	50	167 ± 18 (49)	112 ± 10 (49)
18	50	165 ± 16	112 ± 9

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

Age(weeks)	No. of animals	Water intake (g)		Urine output (g)	
		Male	Female	Male	Female
7	50	32 ± 5 (49)	28 ± 5 (49)	13 ± 3 (49)	12 ± 4 (49)
9	50	35 ± 6	31 ± 6	16 ± 4	14 ± 4
18	50	29 ± 7 (49)	27 ± 6	17 ± 5 (49)	15 ± 4

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

Table 5. Ophthalmoscopy

Sex	Age (weeks)	No. of animals	Persistent hyaloid vessel		Corneal opacity	Abhesion of iris
			+	++		
Male	5	150	112	6	6	2
	9	100	46	1	2	1
	18	50	23	0	0	0
Female	5	150	118	1	1	0
	9	100	46	0	1	0
	18	50	13	0	1	0

Table 6. Urinalysis

Sex	Age (weeks)	Number of animals	pH				Protein				Glucose	Ketones				Occult blood			Urobili–nogen
			6	7	8	9	–	±	+	++	–	–	±	+	++	–	±	+	±
Male	7	50	0	1	7	42	1	10	35	4	50	21	20	9	0	50	0	0	50
	9	50	1	3	13	33	2	7	35	6	50	4	20	26	0	49	0	1	50
	18	49	0	2	9	38	2	3	22	22	49	6	11	30	2	48	0	1	49
Female	7	50	1	4	16	29	24	11	15	0	50	34	15	1	0	49	1	0	50
	9	50	1	10	19	20	22	14	14	0	50	28	21	1	0	50	0	0	50
	18	47	5	9	12	21	18	8	18	3	47	21	22	4	0	47	0	0	47

Sex	Age (weeks)	Number of animals	Casts	Epithelial cells		Leukocytes			Erythrocytes		
			–	–	+	–	+	++	–	+	+++
Male	7	50	50	49	1	50	0	0	50	0	0
	9	50	50	50	0	50	0	0	50	0	0
	18	49	49	46	3	49	0	0	48	0	1
Female	7	50	50	50	0	37	12	1	50	0	0
	9	50	50	49	1	36	13	1	50	0	0
	18	49	49	49	0	42	6	1	48	1	0

Table 7. Hematology

Sex	Age (weeks)	Number of animals	Erythrocyte count ($x10^4/\mu L$)	Hematocrit value (%)	Hemoglobin concentration (g%)	MCH (pg)	MCHC (%)	MCV (c μ)	Platelets ($x10^4/\mu L$)	Leukocytes ($x10^2/\mu L$)	Retculocytes (%)
Male	8	50	778 ± 26	47.7 ± 1.7	15.7 ± 0.6	20.1 ± 0.6	32.8 ± 0.6	61 ± 2	136.5 ± 11.3	124 ± 35	4.2 ± 0.6
	10	50	841 ± 43	48.8 ± 1.8	16.1 ± 0.6	19.2 ± 0.6	33.1 ± 0.5	58 ± 2	123.3 ± 12.3	122 ± 27	3.0 ± 1.3
	19	50	888 ± 44	47.3 ± 1.8	15.6 ± 0.6	17.6 ± 0.6	33.0 ± 0.5	53 ± 2	114.4 ± 11.3	111 ± 27	2.3 ± 0.4
Female	8	48	798 ± 34	46.6 ± 1.7	15.7 ± 0.6	19.7 ± 0.7	33.7 ± 0.5	59 ± 2	128.3 ± 11.6	101 ± 30	2.6 ± 0.6
	10	50	829 ± 38	46.7 ± 1.9	15.8 ± 0.6	19.1 ± 0.6	33.9 ± 0.5	56 ± 1	122.1 ± 10.9	92 ± 22	2.2 ± 0.4
	19	50	815 ± 34	44.8 ± 1.7	15.1 ± 0.6	18.6 ± 0.5	33.8 ± 0.7	55 ± 1	111.8 ± 11.2	79 ± 18	2.1 ± 0.4

Sex	Age (weeks)	Number of animals	Leukocyte, differential count (%)				
			Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Male	8	50	7.5 ± 6.6	91.7 ± 6.8	0.4 ± 0.6	0.4 ± 0.7	0.0 ± 0.0
	10	50	7.4 ± 3.9	90.9 ± 4.5	0.7 ± 0.9	1.1 ± 1.1	0.0 ± 0.0
	19	50	13.1 ± 5.5	85.0 ± 5.8	0.7 ± 0.8	1.1 ± 1.2	0.0 ± 0.0
Female	8	48	6.4 ± 3.1	92.2 ± 3.3	0.6 ± 0.9	0.8 ± 0.8	0.0 ± 0.0
	10	50	6.9 ± 3.2	91.4 ± 3.4	0.7 ± 0.7	1.0 ± 0.9	0.0 ± 0.0
	19	50	9.9 ± 4.1	88.0 ± 4.1	0.4 ± 0.6	1.7 ± 1.4	0.0 ± 0.0

Data are expressed as mean ± S.D.

Table 8. Blood chemistry

Sex	Age (weeks)	Number of animals	Total protein (g/dL)	Albumin (g/dL)	A/G ratio	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Urea nitrogen (mg/dL)	Creatinine (mg/dL)	Total bilirubin (mg/dL)
Male	8	49	6.01 ± 0.17	3.41 ± 0.15	1.32 ± 0.14	142 ± 18	73 ± 22	48 ± 15	14.5 ± 1.9	0.4 ± 0.1	0.06 ± 0.01
	10	50	6.16 ± 0.17	3.33 ± 0.14	1.18 ± 0.11	151 ± 15	74 ± 14	55 ± 21	14.0 ± 1.9	0.5 ± 0.1	0.07 ± 0.01
	19	49	6.44 ± 0.28	3.27 ± 0.21	1.04 ± 0.13	165 ± 19	79 ± 15	70 ± 26	14.3 ± 1.6	0.6 ± 0.1	0.08 ± 0.02
Female	8	47	6.14 ± 0.25	3.62 ± 0.20	1.44 ± 0.10	131 ± 13	79 ± 14	35 ± 11	14.8 ± 2.2	0.5 ± 0.1	0.06 ± 0.01
	10	50	6.25 ± 0.34	3.67 ± 0.22	1.43 ± 0.13	129 ± 14	81 ± 14	32 ± 17	14.7 ± 1.9	0.5 ± 0.1	0.10 ± 0.02
	19	50	6.96 ± 0.44	4.03 ± 0.35	1.39 ± 0.16	140 ± 15	88 ± 22	41 ± 15	15.7 ± 1.9	0.6 ± 0.1	0.11 ± 0.03

Sex	Age (weeks)	Number of animals	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)	CK (U/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mg/dL)
Male	8	49	64 ± 6	23 ± 5	77 ± 23	520 ± 117	179 ± 33	144 ± 1	3.8 ± 0.3	111 ± 2	10. 16 ± 0.34
	10	50	59 ± 7	24 ± 3	69 ± 21	361 ± 60	135 ± 21	143 ± 1	3.7 ± 0.3	111 ± 2	10.11 ± 0.29
	19	49	59 ± 15	28 ± 10	74 ± 47	169 ± 27	80 ± 23	144 ± 1	3.8 ± 0.2	114 ± 2	9.67 ± 0.29
Female	8	47	59 ± 6	18 ± 2	67 ± 18	302 ± 66	122 ± 16	142 ± 1	3.8 ± 0.3	113 ± 3	10.20 ± 0.33
	10	50	57 ± 7	20 ± 3	59 ± 14	205 ± 48	97 ± 12	142 ± 1	3.7 ± 0.3	113 ± 2	10.04 ± 0.40
	19	50	84 ± 81	34 ± 35	81 ± 57	85 ± 25	60 ± 11	142 ± 1	3.7 ± 0.3	114 ± 2	9.95 ± 0.41

Sex	Age (weeks)	Number of animals	Inorganic phosphorus (mg /dL)
Male	8	49	8.5 ± 0.5
	10	50	7.9 ± 0.4
	19	49	6.1 ± 0.4
Female	8	47	7.8 ± 0.5
	10	50	7.1 ± 0.6
	19	50	5.6 ± 0.5

Data are expressed as mean ± S.D

Table 9. Blood coagulation

Sex	Age (weeks)	Number of animals	Prothrombin time (sec)	Activated partial thromboplastin time (sec)	Fibrinogen (mg/dL)
Male	8	49	12.1 ± 0.6	18.6 ± 1.1	276 ± 33
	10	48	12.3 ± 0.5	18.2 ± 1.5	274 ± 19
	19	48	12.4 ± 0.6	18.7 ± 2.7	270 ± 21
Female	8	45	12.0 ± 0.5	16.6 ± 1.4	231 ± 16
	10	50	12.3 ± 0.3	16.5 ± 0.9	218 ± 15
	19	50	12.0 ± 0.3	16.5 ± 1.1	207 ± 20

Data are expressed as mean ± S.D.

Table 10. Activity of hepatic drug-metabolizing enzymes

Sex	Age (weeks)	Number of animals	Aminopyrine-*N*-demethylase		Aniline hydroxylase	
			(mU/g liver)	(mU/mg protein)	(mU/g liver)	(mU/mg protein)
Male	8	50	227.6 ± 42.0	1.258 ± 0.181	22.0 ± 7.4	0.121 ± 0.036
	10	50	216.6 ± 49.3	1.111 ± 0.218	15.8 ± 3.6	0.082 ± 0.018
	19	50	238.9 ± 35.0	1.337 ± 0.233	15.9 ± 6.4	0.088 ± 0.037
Female	8	50	140.5 ± 23.6	0.827 ± 0.156	16.1 ± 4.5	0.096 ± 0.030
	10	50	129.7 ± 27.3	0.721 ± 0.189	12.0 ± 2.9	0.066 ± 0.018
	19	50	150.7 ± 21.7	0.918 ± 0.128	11.9 ± 5.1	0.072 ± 0.031

Data are expressed as mean ± S.D.

Crj:CD(SD)IGS Rats and the Effects of a Commercial Low Protein Diet on the Biological Parameters Used in Repeated-Dose Toxicity Studies

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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 or 13 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, hematology, blood chemistry, blood coagulation test results, hepatic drug-metabolizing enzyme activities or gross pathology. Body weight in males in the LPF group was lower than that in the F-1 group beginning at 12w, although food consumption was higher in males in the LPF group until 10w. The pituitary weight was slightly higher in females in the LPF group than in the F-1 group without any histopathological lesions at 19w. Upon histopathological examination of the heart, lungs, liver, spleen, testes, ovaries, prostate, pituitary and adrenals, no differences were observed in incidence or severity of the findings between the 2 groups. In the kidneys, the incidences of the basophilic tubules and mononuclear cell infiltration were higher in the LPF group than in the F-1 group at 10w. However, no differences between the 2 groups were observed in the kidneys at 8w or 19w. 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 13 weeks. – Key words: Biological parameter, CD(SD)IGS rat, Low protein diet

CD (SD) IGS-1998: 108-120

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 [4, 6]. These 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 [7].

Diets for experimental rodents are manufactured and sold by many food suppliers in accordance with the nutritional requirements for laboratory animals published by the National Research Council in the United States [16]. Nutritional requirements for mature rodents used in long-term investigations however are virtually unknown [10]. It has recently been suggested that the current nutritional standard results in hypernutrition, which in turn adversely affects health conditions. The standard protein levels in laboratory animal diets, 20-25%, are appropriate for growth and reproduction but too high for long-term maintenance [1]. It has also been reported that using an 18% protein diet poses no nutritional problems in rats [10, 15] and can effectively prolong their life span by reducing the incidence and retarding the development of spontaneous lesions [8, 9, 18].

In the past, we have used a 23-24% protein diet for general toxicity and carcinogenicity studies in rodents. In the present study, an 18% protein diet which is expected to reduce the development of lesions caused by hypernutrition was assessed in rats. This study was also designed to collect background data on Crj:CD(SD)IGS rats at 6-19 weeks of age for repeated-dose toxicity studies. CD(SD)IGS rats were 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 ramification and to promote internationalization of the research and development of new drugs [2].

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 the Takatsuki Laboratories for 1 week. One hundred and twenty animals, 60 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 30 males and 30 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 weight from 196 to 226 g (males) and from 138 to 169 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˚C, a relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (light 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		
			2weeks	4weeks	13weeks	2weeks	4weeks	13weeks
1	CRF-1	24	10	10	10	10	10	10
2	CR-LPF	18	10	10	10	10	10	10

The 30 males and 30 females in each group were randomly divided into 3 groups of 10 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.

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 (PM4800, 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 (PM4800, 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 and 18w. 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 (PM4800, Mettler GmbH).

5) Ophthalmoscopy

Ophthalmoscopic examination was performed for all animals in each group once before the experiment commenced (5w) and at 9w and 18w. 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 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 and 18w. 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 and 18w. 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 (ONE-TEN, Fiske Associate). Calcium and inorganic phosphorus were determined at 9w and 18w.

- 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, blood chemistry and blood coagulation

Hematological, blood chemical and blood coagulation 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), heparin sodium (blood chemistry) or 3.13% sodium citrate (blood coagulation test) under ether anesthesia. The heparinized blood was centrifuged at 7500 ×g for 10 min to obtain plasma for blood chemistry. The blood containing sodium citrate was centrifuged at 1500 ×g for 10 min to obtain plasma for blood coagulation testing.

a) Hematology

The values of the following were determined or calculated with an automated hematology analyzer (E5000, Toa Medical Electronics Co., Ltd.) and an automated reticulocyte analyzer (R-1000, Toa Medical Electronics Co., Ltd.). 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. 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)

c) Blood coagulation

The values of the following were determined with an automated blood coagulation analyzer (CA-1000, Toa Medical Electronics Co., Ltd.).

- prothrombin time (PT; light scattering method)
- activated partial thromboplastin time (APTT; light scattering method)
- fibrinogen (light scattering method)

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) activity.

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 (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.

11) Histopathology

The liver, kidneys, heart, lungs, spleen, testes, prostate, ovaries, pituitary and adrenals from all animals in each group 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.

5. Statistical analysis

The data on body weight, food consumption, water intake, urine output, hematology, blood chemistry, blood coagulation, 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 [3]. 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 1 male in each group. In the F-1 group, incrustation, hematuria and malinterdigitation were observed in 1-3 animals; 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. The mean value in males in the LPF group was lower than that in the F-1 group beginning at 12w. At 19w, the value for males in the LPF group was 9% lower than that in the F-1 group. In females, there were no significant differences between the 2 groups.

4. Food consumption (Table 3)

In males, food consumption in the LPF group was increased when compared with the value in the F-1 group from 6w to 10w. In females, there were no significant differences between the 2 groups.

5. Water intake and urine output (Table 4)

In males, there were no significant differences between the 2

groups. In females, there were no significant differences between the 2 groups at 7w or 9w; however, decreases in water intake and urine output as compared with the values in the F-1 group were observed in the LPF group at 18w.

6. Ophthalmoscopy (Table 5)

There were no significant differences between the 2 groups. In both sexes in each group, persistent hyaloid vessels were observed, and incidence of this finding decreased with age. Corneal opacity was observed in each group, especially at 5w.

7. Urinalysis (Table 6)

In females in the LPF group, an increased level of ketone bodies at 7w and a decreased level of protein at 18w were observed when compared with the corresponding levels in the F-1 group. The levels of protein and ketone bodies increased with age in males in both groups.

8. Urine chemistry (Table 7)

In females in the LPF group, an increase in potassium at 9w and calcium at 18w were observed when compared with the values in the F-1 group.

9. Hematology (Table 8)

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

10. Blood chemistry (Table 9)

There were no significant differences between the 2 groups. Direct bilirubin increased with age and ALP and inorganic phosphorus decreased with age in both sexes in both groups.

11. Blood coagulation (Table 10)

There were no significant differences between the 2 groups.

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

There were no significant differences between the 2 groups.

13. Gross pathology

At 8w, dilatation of the pelvis in the right kidney was observed in 1 male in each group. At 10w, the same finding in both kidneys was observed in 1 male in the LPF group. At 19w, focal white discoloration in the prostate was observed in 1 male in the LPF group. However, no significant differences were observed between the 2 groups because of the low incidence of these findings.

14. Organ weights (Tables 12, 13)

In males, there were no significant differences between the 2 groups. In females at 19w, the pituitary weight was slightly higher in the LPF group than in the F-1 group. The thymus weight decreased with age in both sexes in both groups.

15. Histopathology (Table 14)

There were no significant differences between the 2 groups.

Liver: Vacuolization of the centrilobular hepatocytes was observed in females in both groups at 8w and 10w and in both sexes in both groups at 19w. Mononuclear cell infiltration was observed occasionally in both sexes in both groups at 8w, in almost all animals in each group at 10w and in almost all animals in the F-1 group and in almost all males and half the females in the LPF group at 19w. Proliferation of the hematopoietic cells was observed in some males in each group at 8w. Other changes as shown in Table 14 were observed in 1 animal each at 19w.

Kidney: Basophilic tubules and mononuclear cell infiltration were occasionally observed in both sexes in both groups at 8w and 10w and in some animals at 19w. Hyperplasia of the pelvic epithelium was observed occasionally in males in both groups at 8w and in some animals at 10w and 19w. Hyaline droplets in the tubules were observed in 1 animal at 8w, in 3 males in the LPE group at 10w and in males in both groups at 19w. Cyst formation at 8w and hydronephrosis at 8w, 10w and 19w were observed in some animals. Calcification in the medulla at 10w and 19w, focal glomerulosclerosis at 10w and hyaline casts at 19w were observed in 1 animal each.

Heart: Cardiomyopathy was occasionally observed in males in both groups at 8w and 10w and in both sexes in both groups at 19w.

Spleen: Proliferation of the hematopoietic cells was occasionally observed in males in both groups at 8w and in some animals at 10w and 19w. Brown pigmentation was observed in almost all animals in each group at 19w.

Prostate: Mononuclear cell infiltration was observed in some males in each group at 8w and occasionally at 10w and 19w. Abscess formation was observed in 1 male in the F-1 group at 19w.

Lung: Mononuclear cell infiltration was observed in 1 male in the F-1 group at 8w.

Others: The other changes shown in Table 14 were observed in 1 animal each.

DISCUSSION

Caloric restriction is a major factor for increasing the longevity of rats, and the effects of protein restriction, without caloric restriction, on longevity are small [4]. However, protein restriction has been shown to inhibit the development of severe chronic nephropathy [5, 6]. In the present study, a low protein diet which was expected to reduce the development of lesions caused by hypernutrition was assessed in rats.

No significant differences were observed between the low and normal protein diet groups in clinical signs, hematology, blood chemistry, blood coagulation, hepatic drug-metabolizing enzyme activities or gross pathology.

Body weight in the LPF group was lower than in the F-1 group during the late stage of the experimental period, although increased food consumption was observed in males in the LPF group during the early stage of the period. The values in the LPF group were only 9% lower than the values in the F-1 group at 19w. Although suppression of body weight gain in the LPF group was small in the present study, low protein diets have been shown to cause a significant reduction in body weight gain in longer-term studies [14, 15].

Decreases in water intake and urine output were observed in females in the LPF group when compared with the values in the F-1 group at 18w. The reason for this difference between the 2 groups is obscure; however, the difference is not considered to be significant because no significant differences in these values were observed between the 2 groups at 18w or 32w in our studies using the same lot of Crj:CD(SD)IGS rats at the Hikari

Branch in our laboratories [13].

Upon urinalysis, a higher level of urinary ketone bodies at 7w and a lower level of protein at 18w were observed in females in the LPF group when compared to the levels in 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 the level of ketone bodies at 7w or protein at 18w were observed between the 2 groups in our studies using the same lot of Crj:CD(SD)IGS rats at the Hikari Branch [13]. 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 in the new nutritional environment [17].

The pituitary weight in females in the LPF group at 19w was higher than that in the F-1 group. The difference between the 2 groups was small, and no abnormalities were observed upon histopathological examination of the pituitary in the LPF 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 the pituitary weight at 19w or 32w were observed between the 2 groups in our studies using the same lot of Crj:CD(SD)IGS rats at the Hikari Branch [13].

Upon histopathological examination of the heart, lungs, liver, spleen, testes, ovaries, prostate, pituitary and adrenals, no differences were observed in incidence or severity of the findings between the 2 groups. In the kidneys at 10w, the incidences of basophilic tubules and mononuclear cell infiltration were higher in the LPF group than in the F-1 group. However, no differences between the 2 groups in the present study were observed in the kidney at 19w. In our other studies using the same lot or other lots of Crj:CD(SD)IGS rats and CR-LPF (lower protein diet) at the Takatsuki Laboratories [11] and Hikari Branch [12, 13] in our laboratories, the incidences of basophilic tubules and mononuclear cell infiltration in the kidneys were lower than those in the present study. Therefore, it is judged that there were no noteworthy differences in the renal lesions between the 2 groups.

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 13 weeks. However, further investigation including a long-term study is considered necessary to fully evaluate the effects of a low protein diet on the biological parameters and to determine an optimal dietary protein content.

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

CRF-1/Male	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	30	30	20	20	10	10	10	10	10	10	10	10	10
Incrustation	1	3	1	1	0	0	0	0	0	0	0	0	0
Hair loss	0	0	0	0	1	1	1	1	1	1	1	1	1
Hematuria	0	0	0	1	0	0	0	0	0	0	0	0	0
Malinterdigitation	0	0	0	0	1	1	1	1	1	0	0	0	0

CR-LPF/Male	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	30	30	20	20	10	10	10	10	10	10	10	10	10
Incrustation	0	0	0	0	0	0	0	0	0	0	0	0	0
Hair loss	0	0	0	0	0	0	0	0	0	1	1	1	1
Hematuria	0	0	0	0	0	0	0	0	0	0	0	0	0
Malinterdigitation	0	0	0	0	0	0	0	0	0	0	0	0	0

CRF-1/Female	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	30	30	20	20	10	10	10	10	10	10	10	10	10
Incrustation	0	1	0	0	0	0	0	0	0	0	0	0	0

CR-LPF/Female	Age (weeks)												
	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of animals	30	30	20	20	10	10	10	10	10	10	10	10	10
Incrustation	0	0	0	0	0	0	0	0	0	0	0	0	0

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

Sex	Group	No. of animals	Initial body weight	Age (weeks)			
				7	8	9	10
Male	CRF-1	30	210 ± 8	264 ± 13	317 ± 19	356 ± 25 (20)	389 ± 29 (20)
	CR-LPF	30	210 ± 7	262 ± 11	310 ± 15	346 ± 21 (20)	374 ± 25 (20)
Female	CRF-1	30	153 ± 8	172 ± 11	194 ± 16	214 ± 18 (20)	229 ± 19 (20)
	CR-LPF	30	153 ± 7	168 ± 11	188 ± 15	208 ± 18 (20)	219 ± 20 (20)

Sex	Group	No. of animals	Age (weeks)				
			11	12	13	14	15
Male	CRF-1	30	415 ± 33 (10)	442 ± 37 (10)	464 ± 40 (10)	479 ± 43 (10)	489 ± 40 (10)
	CR-LPF	30	389 ± 20 (10)	412 ± 24 (10)*	430 ± 26 (10)*	441 ± 29 (10)*	455 ± 29 (10)*
Female	CRF-1	30	242 ± 24 (10)	251 ± 22 (10)	261 ± 24 (10)	266 ± 22 (10)	275 ± 23 (10)
	CR-LPF	30	239 ± 23 (10)	248 ± 22 (10)	257 ± 22 (10)	262 ± 22 (10)	270 ± 24 (10)

Sex	Group	No. of animals	Age (weeks)			
			16	17	18	19
Male	CRF-1	30	509 ± 43 (10)	522 ± 44 (10)	522 ± 46 (10)	535 ± 47 (10)
	CR-LPF	30	468 ± 31 (10)*	479 ± 33 (10)*	479 ± 33 (10)*	489 ± 34 (10)*
Female	CRF-1	30	280 ± 25 (10)	285 ± 25 (10)	288 ± 27 (10)	290 ± 27 (10)
	CR-LPF	30	277 ± 24 (10)	279 ± 23 (10)	279 ± 23 (10)	283 ± 23 (10)

Data are expressed as mean ± S.D. (g).
Significantly different from the corresponding value in the CRF-1 group (*:$p \leqq 0.05$)
Number in the parentheses indicates the number of animals examined.

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

Sex	Group	No. of animals	Age (weeks)				
			6	7	8	9	10
Male	CRF-1	30	151 ± 10	161 ± 13	162 ± 15 (20)	164 ± 16 (20)	159 ± 13 (10)
	CR-LPF	30	165 ± 9**	173 ± 12**	176 ± 16 (20)**	175 ± 16 (20)*	171 ± 10 (10)*
Female	CRF-1	30	103 ± 9	105 ± 11	108 ± 11 (20)	113 ± 12 (20)	115 ± 14 (10)
	CR-LPF	30	106 ± 9	108 ± 11	112 ± 11 (20)	115 ± 11 (20)	124 ± 13 (10)

Sex	Group	No. of animals	Age (weeks)				
			11	13	14	15	16
Male	CRF-1	30	165 ± 15 (10)	156 ± 15 (10)	143 ± 35 (10)	159 ± 20 (10)	157 ± 17 (10)
	CR-LPF	30	172 ± 12 (10)	161 ± 11 (10)	158 ± 15 (10)	159 ± 14 (10)	158 ± 12 (10)
Female	CRF-1	30	113 ± 10 (10)	106 ± 11 (10)	104 ± 11 (10)	107 ± 11 (10)	107 ± 11 (10)
	CR-LPF	30	118 ± 11 (10)	110 ± 11 (10)	110 ± 10 (10)	109 ± 8 (10)	108 ± 10 (10)

Sex	Group	No. of animals	Age (weeks)	
			17	18
Male	CRF-1	30	147 ± 15 (10)	150 ± 14 (10)
	CR-LPF	30	150 ± 9 (10)	151 ± 11 (10)
Female	CRF-1	30	105 ± 14 (10)	103 ± 10 (10)
	CR-LPF	30	110 ± 8 (10)	107 ± 8 (10)

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)
Number in the parentheses indicates the number of animals examined.

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

Sex	Age (weeks)	No. of animals	Water intake		Urine output	
			CRF-1	CR-LPF	CRF-1	CR-LPF
Male	7	10	31 ± 4	33 ± 5 (9)	13.7 ± 4.8	13.0 ± 2.8 (9)
	9	10	34 ± 8	35 ± 7	17.4 ± 8.4	16.1 ± 4.5
	18	10	28 ± 6 (9)	25 ± 6	17.9 ± 5.8 (9)	17.4 ± 5.2
Female	7	10	27 ± 4 (9)	26 ± 4	11.7 ± 3.6 (9)	9.9 ± 3.5
	9	10	28 ± 5	28 ± 6	13.5 ± 4.1	12.7 ± 4.3
	18	10	33 ± 8	25 ± 6*	20.8 ± 6.2	13.7 ± 4.3**

Data are expressed as mean ± S.D. (g).
Significantly different from the corresponding value in the CRF-1 group (*:p≦0.05 or **:p≦0.01)
Number in parentheses indicates the number of animals examined.

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

Sex	Age (weeks)	No. of animals	CRF-1				CR-LPF			
			Persistent hyaloid vessel		Corneal opacity	Abhesion of iris	Persistent hyaloid vessel		Corneal opacity	Abhesion of iris
			+	++			+	++		
Male	5	30	26	1	1	0	17	0	4	0
	9	20	14	3	0	0	7	0	1	0
	18	10	7	0	0	0	4	0	0	0
Female	5	30	18	0	3	0	16	0	1	0
	9	20	5	0	0	0	6	0	1	0
	18	10	3	0	0	0	2	0	1	0

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

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

Sex	Group	Age (weeks)	Number of animals	Casts	Epithelial cells	Leukocytes		Erythrocytes		
				-	-	-	+	-	+	++
Male	CRF-1	7	10	10	10	10	0	10	0	0
		9	10	10	10	10	0	9	0	1
		18	10	10	10	10	0	10	0	0
	CR-LPF	7	10	10	10	10	0	10	0	0
		9	10	10	10	10	0	10	0	0
		18	9	9	9	9	0	9	0	0
Female	CRF-1	7	10	10	10	6	4	10	0	0
		9	10	10	10	8	2	10	0	0
		18	10	10	10	8	2	10	0	0
	CR-LPF	7	10	10	10	6	4	10	0	0
		9	10	10	10	5	5	10	0	0
		18	9	9	9	9	0	9	0	0

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

Sex	Group	Age (weeks)	Number of animals	Creatinine (mg/dL)	Total protein (mg/mg)	*N*-acetyl-β-D-glucosaminidase (mU/mg)	Sodium (mmol/mg)	Potassium (mmol/mg)	Chloride (mmol/mg)	Calcium (mg/mg)	Inorganic phosphorus (mg/mg)	Osmolality (mOsmol/kg)
Male	CRF-1	7	10	53.7 ± 13.1	0.93 ± 0.38	23.9 ± 8.8	0.193 ± 0.088	0.253 ± 0.098	0.237 ± 0.105	ND	ND	1357 ± 320
		9	10	71.2 ± 19.9	0.98 ± 0.31	14.8 ± 8.2	0.153 ± 0.057	0.231 ± 0.033	0.200 ± 0.055	0.14 ± 0.13	0.03 ± 0.02	1274 ± 311
		18	10	122.4 ± 47.7	0.63 ± 0.45	9.1 ± 6.6	0.076 ± 0.024	0.127 ± 0.014	0.072 ± 0.017	0.14 ± 0.10	0.02 ± 0.02	1453 ± 546
	CR-LPF	7	10	55.8 ± 16.1	1.02 ± 0.48	24.3 ± 8.2	0.230 ± 0.041	0.295 ± 0.143	0.249 ± 0.127	ND	ND	1521 ± 577
		9	10	68.4 ± 18.3	1.09 ± 0.36	18.4 ± 6.5	0.130 ± 0.070	0.300 ± 0.056**	0.223 ± 0.056	0.09 ± 0.04	0.22 ± 0.026*	1136 ± 323
		18	9	129.7 ± 55.4	0.50 ± 0.19	11.4 ± 5.6	0.071 ± 0.031	0.141 ± 0.035	0.074 ± 0.029	0.08 ± 0.07	0.08 ± 0.07*	1340 ± 560
Female	CRF-1	7	10	47.5 ± 26.1	0.14 ± 0.29	24.9 ± 5.3	0.213 ± 0.068	0.286 ± 0.088	0.283 ± 0.097	ND	ND	1376 ± 555
		9	10	43.2 ± 14.7	0.01 ± 0.05	26.1 ± 4.3	0.194 ± 0.069	0.213 ± 0.047	0.233 ± 0.060	0.31 ± 0.14	0.21 ± 0.24	927 ± 263
		18	9	136.4 ± 42.4	0.07 ± 0.08	17.9 ± 7.3	0.059 ± 0.049	0.104 ± 0.054	0.057 ± 0.037	0.46 ± 0.18	0.17 ± 0.17	1574 ± 300
	CR-LPF	7	10	45.4 ± 15.5	0.03 ± 0.11	22.6 ± 5.9	0.258 ± 0.073	0.337 ± 0.130	0.275 ± 0.119	ND	ND	1357 ± 541
		9	10	53.7 ± 13.5	0.00 ± 0.00	18.0 ± 7.3**	0.186 ± 0.073	0.340 ± 0.094**	0.254 ± 0.085	0.20 ± 0.13	0.42 ± 0.50	1151 ± 235
		18	9	96.2 ± 76.7	0.00 ± 0.00*	14.3 ± 4.8	0.089 ± 0.047	0.164 ± 0.064*	0.092 ± 0.065	0.33 ± 0.17	0.16 ± 0.11	1428 ± 977

Data are expressed as excretion per mg of creatinine (mean ± S.D.).

Significantly different from the corresponding value in the CRF-1group (*: $p \leqq 0.05$ or **: $p \leqq 0.01$)

ND: Not determined

Table 8. Hematology in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Group	Age (weeks)	Number of animals	Erythrocyte count ($x10^4/\mu L$)	Hematocrit value (%)	Hemoglobin concentration (g%)	MCH (pg)	MCHC (%)	MCV (c μ)	Platelets ($x10^4/\mu L$)	Leukocytes ($x10^2/\mu L$)	Reticulocytes (%)
Male	CRF-1	8	10	782 ± 28	47.5 ± 1.4	15.6 ± 0.4	20.0 ± 0.7	33.0 ± 0.5	61 ± 3	131.8 ± 10.2	127 ± 22	3.8 ±0.6
		10	10	827 ± 19	46.7 ± 1.1	15.5 ± 0.6	18.8 ± 0.5	33.3 ± 0.6	56 ± 1	128.8 ± 10.7	101 ± 15	2.5 ±0.3
		19	10	919 ± 38	47.3 ± 1.8	15.8 ± 0.6	17.2 ± 0.9	33.4 ± 0.7	52 ± 2	114.5 ± 15.2	112 ± 18	2.0 ± 0.2
	CR-LPF	8	10	785 ± 18	48.1 ± 1.5	15.8 ± 0.5	20.1 ± 0.5	32.8 ± 0.7	61 ± 2	134.0 ± 11.2	128 ± 30	4.1 ± 0.4
		10	10	850 ± 36	49.3 ± 1.7	16.3 ± 0.5	19.2 ± 0.6	33.2 ± 0.4	58 ± 2	119.9 ± 11.0	104 ± 17	2.7 ± 0.5
		19	10	906 ± 37	47.6 ± 2.1	15.7 ± 0.7	17.3 ± 0.7	33.0 ± 0.5	53 ± 2	112.4 ± 14.6	92 ± 34	2.3 ± 0.6
Female	CRF-1	8	10	799 ± 34	46.5 ± 1.4	15.9 ± 0.4	19.9 ± 0.6	34.1 ± 0.6	58 ± 1	125.1 ± 13.3	81 ± 23	2.1 ± 0.6
		10	10	835 ± 24	46.3 ± 1.6	15.8 ± 0.5	18.9 ± 0.9	34.1 ± 0.8	55 ± 2	126.9 ± 14.4	87 ± 16	2.1 ± 0.6
		19	10	816 ± 23	44.2 ± 1.7	15.1 ± 0.6	18.5 ± 0.6	34.1 ± 0.4	54 ± 2	116.1 ± 13.8	95 ± 55	2.1 ± 0.3
	CR-LPF	8	10	808 ± 41	46.9 ± 2.4	15.9 ± 0.8	19.7 ± 0.5	33.8 ± 0.4	58 ± 1	128.1 ± 6.5	89 ± 23	2.3 ± 0.6
		10	10	826 ± 43	46.0 ± 1.6	15.6 ± 0.5	18.9 ± 0.7	33.9 ± 0.5	56 ± 1	127.5 ± 10.7	90 ± 19	2.4 ± 0.4
		19	10	824 ± 29	45.3 ± 1.0	15.4 ± 0.4	18.7 ± 0.4	34.0 ± 0.7	55 ± 1	115.1 ± 9.8	82 ± 17	2.3 ± 0.4

Group	Sex	Age (weeks)	Number of animals	Leukocytes, differential count (%)				
				Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Male	CRF-1	8	10	7.2 ± 2.3	91.9 ± 2.9	0.4 ± 0. 5	0.5 ± 0.7	0.0 ± 0.0
		10	10	7.7 ± 2.7	90.4 ± 2.5	0.6 ± 1.0	1.3 ± 1.3	0.0 ± 0.0
		19	10	12.7 ± 5.6	85.1 ± 6.0	0.4 ± 0.5	1.8 ± 1.5	0.0 ± 0.0
	CR-LPF	8	10	5.3 ± 3.4	94.0 ± 3.8	0.2 ± 0.4	0.5 ± 0.8	0.0 ± 0.0
		10	10	6.8 ± 2.7	91.6 ± 3.5	0.7 ± 0.9	0.9 ± 1.1	0.0 ± 0.0
		19	10	15.0 ± 6.0	83.2 ± 5.5	0.7 ± 0.8	1.1 ± 1.5	0.0 ± 0.0
Female	CRF-1	8	10	6.2 ± 3.7	92.4 ± 4.0	0.1 ± 0.3	1.3 ± 0.8	0.0 ± 0.0
		10	10	6.8 ± 2.3	90.6 ± 2.8	0.9 ± 1.2	1.7 ± 1.6	0.0 ± 0.0
		19	10	15.1 ± 12.4	82.6 ± 12.5	0.6 ± 0.7	1.7 ± 1.7	0.0 ± 0.0
	CR-LPF	8	10	7.1 ± 3.2	91.7 ± 2.6	0.2 ± 0.4	1.0 ± 1.1	0.0 ± 0.0
		10	10	6.7 ± 2.7	91.6 ± 2.7	0.6 ± 0.5	1.1 ± 0.9	0.0 ± 0.0
		19	10	9.8 ± 4.9	88.8 ± 4.8	0.5 ± 0.7	0.9 ± 1.2	0.0 ± 0.0

Data are expressed as mean ± S.D.

No significant differences from the corresponding value in the CRF-1 group

Table 9. Blood chemistry in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Group	Age (weeks)	Number of animals	Total protein (g/dL)	Albumin (g/dL)	A/G ratio	Glucose (mg/dL)	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Triglyceride (mg/dL)	Phospholipids (mg/dL)	Urea nitrogen (mg/dL)
Male	CRF-1	8	10	5.94 ± 0.10	3.45 ± 0.12	1.39 ± 0.12	155 ± 17	56 ± 9	38 ± 6	49 ± 14	92 ± 11	13.8 ± 1.4
		10	10	6.10 ± 0.17	3.31 ± 0.09	1.19 ± 0.09	148 ± 9	55 ± 9	35 ± 6	51 ± 18	90 ± 12	14.3 ± 1.2
		19	10	6.41 ± 0.19	3.31 ± 0.14	1.07 ± 0.09	158 ± 13	68 ± 10	42 ± 7	76 ± 33	106 ± 14	12.5 ± 1.9
	CR-LPF	8	9	6.01 ± 0.14	3.38 ± 0.11	1.29 ± 0.10	146 ± 15	76 ± 21*	47 ± 12*	47 ± 19	109 ± 24	14.2 ± 0.6
		10	10	6.07 ± 0.12	3.34 ± 0.09	1.22 ± 0.10	154 ± 10	77 ± 25	44 ± 9	63 ± 24	114 ± 29	15.3 ± 2.4
		19	9	6.49 ± 0.19	3.42 ± 0.10	1.10 ± 0.05	146 ± 12	78 ± 18	50 ± 11	58 ± 23	113 ± 21	12.5 ± 1.2
Female	CRF-1	8	10	5.98 ± 0.24	3.58 ± 0.21	1.50 ± 0.14	140 ± 14	76 ± 20	47 ± 11	28 ± 4	128 ± 27	15.0 ± 2.6
		10	10	6.14 ± 0.30	3.57 ± 0.26	1.40 ± 0.15	129 ± 12	70 ± 9	45 ± 5	27 ± 4	122 ± 12	13.8 ± 1.1
		19	10	6.65 ± 0.47	3.87 ± 0.44	1.41 ± 0.22	135 ± 15	79 ± 20	56 ± 13	38 ± 14	141 ± 26	14.9 ± 1.3
	CR-LPF	8	9	5.97 ± 0.16	3.51 ± 0.18	1.42 ± 0.10	129 ± 9	74 ± 9	44 ± 5	28 ± 5	126 ± 12	16.6 ± 1.4
		10	10	6.00 ± 0.25	3.59 ± 0.23	1.49 ± 0.13	125 ± 10	77 ± 12	46 ± 7	33 ± 19	127 ± 18	15.1 ± 2.2
		19	10	6.80 ± 0.41	4.01 ± 0.38	1.45 ± 0.16	138 ± 13	71 ± 14	50 ± 8	33 ± 11	131 ± 26	14.1 ± 2.0

Sex	Group	Age (weeks)	Number of animals	Urea nitrogen (mg/dL)	Creatinine (mg/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)	CK (U/L)	Sodium (mmol/L)
Male	CRF-1	8	10	13.8 ± 1.4	0.4 ± 0.1	0.05 ± 0.01	0.04 ± 0.01	66 ± 6	25 ± 2	84 ± 16	549 ± 113	204 ± 53	144 ± 1
		10	10	14.3 ± 1.2	0.5 ± 0.1	0.05 ± 0.01	0.04 ± 0.01	60 ± 5	26 ± 2	66 ± 19	394 ± 84	127 ± 19	143 ± 1
		19	10	12.5 ± 1.9	0.5 ± 0.1	0.05 ± 0.01	0.06 ± 0.01	62 ± 6	28 ± 2	57 ± 14	191 ± 22	78 ± 10	144 ± 2
	CR-LPF	8	9	14.2 ± 0.6	0.4 ± 0.0	0.06 ± 0.02*	0.04 ± 0.01	62 ± 6	24 ± 2	76 ± 21	432 ± 85*	169 ± 24	145 ± 1*
		10	10	15.3 ± 2.4	0.4 ± 0.1	0.06 ± 0.01	0.05 ± 0.00	59 ± 4	25 ± 2	75 ± 20	343 ± 56	122 ± 15	142 ± 1
		19	9	12.5 ± 1.2	0.5 ± 0.1	0.07 ± 0.02*	0.07 ± 0.02	56 ± 8	27 ± 4	57 ± 17	173 ± 19	68 ± 6*	144 ± 1
Female	CRF-1	8	10	15.0 ± 2.6	0.5 ± 0.0	0.06 ± 0.01	0.04 ± 0.01	61 ± 5	17 ± 2	64 ± 14	289 ± 44	128 ± 33	144 ± 1
		10	10	13.8 ± 1.1	0.4 ± 0.0	0.07 ± 0.01	0.05 ± 0.01	59 ± 5	21 ± 3	63 ± 13	201 ± 57	101 ± 15	142 ± 1
		19	10	14.9 ± 1.3	0.5 ± 0.0	0.09 ± 0.02	0.08 ± 0.01	66 ± 23	24 ± 10	61 ± 29	84 ± 30	55 ± 7	141 ± 1
	CR-LPF	8	9	16.6 ± 1.4	0.5 ± 0.0	0.08 ± 0.01*	0.05 ± 0.01**	62 ± 6	19 ± 2	80 ± 27	280 ± 68	116 ± 11	143 ± 1
		10	10	15.1 ± 2.2	0.4 ± 0.0	0.08 ± 0.02	0.05 ± 0.01	55 ± 6	19 ± 2	61 ± 18	214 ± 50	98 ± 11	141 ± 1
		19	10	14.1 ± 2.0	0.5 ± 0.1	0.10 ± 0.03	0.09 ± 0.03	108 ± 152	46 ± 71	79 ± 67	87 ± 20	52 ± 9	142 ± 1

Data are expressed as mean ± S.D.

Significantly different from the corresponding value in the CRF-1 group (*: $p \leqq 0.05$)

Table 9. Blood chemistry in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Group	Age (weeks)	Number of animals	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mg /dL)	Inorganic phosphorus (mg/dL)	T4 (μg/dL)	T3 (ng/mL)
Male	CRF-1	8	10	3.8 ± 0.2	114 ± 1	10.39 ± 0.26	8.7 ± 0.5	4.70 ± 0.83	1.26 ± 0.14
		10	10	3.6 ± 0.2	109 ± 1	10.19 ± 0.11	8.0 ± 0.5	4.02 ± 0.44	1.16 ± 0.09
		19	10	3.8 ± 0.2	112 ± 1	9.70 ± 0.31	6.2 ± 0.4	4.21 ± 0.33	1.67 ± 0.15
	CR-LPF	8	9	3.7 ± 0.3	115 ± 1	10.47 ± 0.18	8.3 ± 0.2*	4.59 ± 0.33	1.27 ± 0.13
		10	10	3.6 ± 0.3	110 ± 1	10.18 ± 0.29	7.5 ± 0.4	3.90 ± 0.37	1.05 ± 0.11
		19	9	3.8 ± 0.2	113 ± 1*	9.72 ± 0.18	6.0 ± 0.3	4.53 ± 0.70	1.62 ± 0.25
Female	CRF-1	8	10	3.9 ± 0.4	116 ± 1	10.42 ± 0.30	8.3 ± 0.4	3.92 ± 0.49	1.41 ± 0.16
		10	10	3.9 ± 0.4	112 ± 2	10.08 ± 0.23	7.3 ± 0.4	3.21 ± 0.50	1.35 ± 0.16
		19	10	3.7 ± 0.3	112 ± 1	10.15 ± 0.29	5.9 ± 0.4	4.04 ± 0.28	1.51 ± 0.21
	CR-LPF	8	9	3.7 ± 0.3	116 ± 2	10.44 ± 0.45	7.9 ± 0.7	4.46 ± 0.52*	1.47 ± 0.19
		10	10	3.7 ± 0.2	112 ± 2	10.08 ± 0.53	7.3 ± 0.4	2.75 ± 0.45	1.31 ± 0.14
		19	10	3.6 ± 0.2	114 ± 2	10.01 ± 0.32	5.6 ± 0.5	4.26 ± 0.52	1.29 ± 0.19*

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

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

Sex	Group	Age (weeks)	Number of animals	Prothrombin time (sec)	Activated partial thromboplastin time (sec)	Fibrinogen (mg/dL)
Male	CRF-1	8	10	14.4 ± 1.6	21.1 ± 2.3	262 ± 9
		10	10	13.2 ± 1.1	20.3 ± 1.7	264 ± 17
		19	10	13.9 ± 1.2	20.7 ± 1.8	282 ± 20
	CR-LPF	8	9	12.7 ± 0.7**	18.4 ± 1.0**	274 ± 14*
		10	10	12.5 ± 0.7	18.0 ± 2.5	269 ± 30
		19	9	12.4 ± 0.6**	18.4 ± 1.5**	276 ± 24
Female	CRF-1	8	10	12.0 ± 0.3	16.1 ± 0.3	229 ± 15
		10	10	12.2 ± 0.4	16.4 ± 0.8	213 ± 12
		19	10	11.8 ± 0.3	16.0 ± 0.9	222 ± 49
	CR-LPF	8	7	12.6 ± 0.5*	16.9 ± 1.4	220 ± 22
		10	10	12.4 ± 0.4	16.4 ± 1.0	211 ± 15
		19	10	12.1 ± 0.2**	16.5 ± 1.0	210 ± 17

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

Table 11. Activity of hepatic drug-metabolizing enzymes in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Group	Age	Number of	Liver weight	Relative liver weight	Aminopyrine-*N*-demethylase		Aniline hydroxylase	
		(weeks)	animals	(g)	(%)	(mU/ g liver)	(mU/mg protein)	(mU/ g liver)	(mU/mg protein)
Male	CRF-1	8	10	9.08 ± 0.80	3.15 ± 0.17	201.0 ± 19.9	1.305 ± 0.139	16.8 ± 2.3	0.109 ± 0.015
		10	10	10.70 ± 1.53	2.92 ± 0.20	186.8 ± 32.7	1.221 ± 0.233	14.5 ± 2.5	0.094 ± 0.014
		19	10	12.74 ± 1.14	2.48 ± 0.09	272.0 ± 28.7	1.545 ± 0.186	27.2 ± 2.9	0.155 ± 0.019
	CR-LPF	8	10	8.93 ± 0.43	3.21 ± 0.13	170.2 ± 19.1**	1.158 ± 0.117*	13.9 ± 2.2*	0.095 ± 0.016
		10	10	10.48 ± 0.81	2.94 ± 0.08	208.9 ± 38.9	1.182 ± 0.187	17.1 ± 3.1	0.097 ± 0.016
		19	10	11.25 ± 0.96**	2.42 ± 0.12	245.8 ± 30.2	1.450 ± 0.148	24.2 ± 4.8	0.142 ± 0.023
Female	CRF-1	8	10	5.41 ± 0.60	3.08 ± 0.23	133.6 ± 23.6	0.855 ± 0.120	15.6 ± 3.7	0.100 ± 0.022
		10	10	6.00 ± 0.67	2.78 ± 0.14	139.6 ± 21.0	0.890 ± 0.122	13.2 ± 2.4	0.084 ± 0.015
		19	10	6.60 ± 0.67	2.38 ± 0.16	152.4 ± 26.3	0.969 ± 0.140	16.1 ± 3.7	0.102 ± 0.020
	CR-LPF	8	10	5.23 ± 0.54	3.17 ± 0.16	136.5 ± 34.9	0.925 ± 0.232	15.6 ± 4.4	0.106 ± 0.028
		10	10	5.77 ± 0.54	2.87 ± 0.13	145.2 ± 20.0	0.982 ± 0.143	12.6 ± 3.6	0.085 ± 0.022
		19	10	6.45 ± 0.68	2.41 ± 0.20	149.0 ± 21.1	0.974 ± 0.129	16.3 ± 3.5	0.106 ± 0.022

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

Table 12. Organ weights in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Group	Age (weeks)	No. of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)
Male	CRF-1	8	10	288 ± 15	1.93 ± 0.07	1.09 ± 0.09	1.08 ± 0.08	9.08 ± 0.80	2.31 ± 0.08
		10	10	365 ± 30	2.02 ± 0.05	1.23 ± 0.15	1.27 ± 0.10	10.70 ± 1.53	2.74 ± 0.30
		19	10	514 ± 45	2.11 ± 0.08	1.50 ± 0.17	1.42 ± 0.14	12.74 ± 1.14	3.09 ± 0.19
	CR-LPF	8	10	278 ± 10	1.90 ± 0.04	1.03 ± 0.06	1.08 ± 0.08	8.93 ± 0.43	2.38 ± 0.16
		10	10	356 ± 24	2.01 ± 0.08	1.18 ± 0.11	1.21 ± 0.10	10.48 ± 0.81	2.64 ± 0.29
		19	10	465 ± 32*	2.11 ± 0.08	1.36 ± 0.15	1.31 ± 0.09	11.25 ± 0.96**	2.79 ± 0.19**

Sex	Group	Age (weeks)	No. of animals	Spleen (g)	Pituitary gland (mg)	Adrenal gland (mg)	Thymus (mg)	Testes (g)	Ventral prostate (mg)
Male	CRF-1	8	10	0.60 ± 0.07	9.6 ± 1.6	49.6 ± 4.3	616.2 ± 128.3	2.82 ± 0.17	351.1 ± 52.8
		10	10	0.65 ± 0.09	10.5 ± 2.2	54.5 ± 5.9	615.1 ± 119.0	3.33 ± 0.22	492.4 ± 154.0
		19	10	0.72 ± 0.08	11.1 ± 2.1	51.2 ± 4.3	258.7 ± 37.4	3.39 ± 0.14	565.0 ± 191.6
	CR-LPF	8	10	0.57 ± 0.08	10.1 ± 1.2	49.9 ± 5.3	589.4 ± 84.3	2.86 ± 0.20	338.2 ± 50.4
		10	10	0.63 ± 0.06	10.6 ± 1.5	56.9 ± 11.3	549.2 ± 89.3	3.21 ± 0.17	469.2 ± 65.5
		19	10	0.67 ± 0.08	11.1 ± 2.6	52.4 ± 11.4	246.2 ± 62.9	3.44 ± 0.19	526.3 ± 80.2

Sex	Group	Age (weeks)	No. of animals	Body weight (g)	Brain (g)	Heart (g)	Lungs (g)	Liver (g)	Kidneys (g)
Female	CRF-1	8	10	175 ± 13	1.75 ± 0.07	0.67 ± 0.08	0.83 ± 0.08	5.41 ± 0.60	1.48 ± 0.13
		10	10	215 ± 19	1.87 ± 0.06	0.75 ± 0.07	0.92 ± 0.06	6.00 ± 0.67	1.62 ± 0.19
		19	10	278 ± 25	1.94 ± 0.07	0.84 ± 0.06	0.99 ± 0.07	6.60 ± 0.67	1.72 ± 0.15
	CR-LPF	8	10	164 ± 11	1.78 ± 0.06	0.65 ± 0.07	0.79 ± 0.06	5.23 ± 0.54	1.46 ± 0.13
		10	10	202 ± 18	1.83 ± 0.09	0.75 ± 0.08	0.92 ± 0.06	5.77 ± 0.54	1.55 ± 0.11
		19	10	268 ± 23	1.97 ± 0.07	0.87 ± 0.07	1.01 ± 0.10	6.45 ± 0.68	1.64 ± 0.13

Sex	Group	Age (weeks)	No. of animals	Spleen (g)	Pituitary gland (mg)	Adrenal gland (mg)	Thymus (mg)	Ovaries (mg)
Female	CRF-1	8	10	0.36 ± 0.07	9.5 ± 1.8	52.3 ± 7.0	470.3 ± 90.9	66.4 ± 19.7
		10	10	0.44 ± 0.06	11.9 ± 2.4	57.9 ± 10.6	461.1 ± 57.8	86.1 ± 16.6
		19	10	0.46 ± 0.08	12.9 ± 3.2	60.1 ± 7.3	246.1 ± 43.7	74.5 ± 11.1
	CR-LPF	8	10	0.38 ± 0.09	8.6 ± 2.3	57.3 ± 5.6	449.9 ± 75.1	69.1 ± 9.4
		10	10	0.43 ± 0.07	11.7 ± 1.5	63.3 ± 13.1	411.1 ± 92.9	83.1 ± 16.0
		19	10	0.45 ± 0.10	15.8 ± 2.4*	63.7 ± 6.4	297.0 ± 61.4*	68.8 ± 10.6

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

Table 13. Organ weights (body weight ratio) in CrjCD(SD)IGS rats fed a low protein or normal protein diet

Sex	Group	Age (weeks)	No. of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys
Male	CRF-1	8	10	288 ± 15	0.67 ± 0.03	0.38 ± 0.03	0.38 ± 0.02	3.15 ± 0.17	0.80 ± 0.04
		10	10	365 ± 30	0.56 ± 0.04	0.34 ± 0.03	0.35 ± 0.02	2.92 ± 0.20	0.75 ± 0.03
		19	10	514 ± 45	0.41 ± 0.04	0.29 ± 0.03	0.28 ± 0.03	2.48 ± 0.09	0.60 ± 0.02
	CR-LPF	8	10	278 ± 10	0.68 ± 0.02	0.37 ± 0.03	0.39 ± 0.03	3.21 ± 0.13	0.86 ± 0.06*
		10	10	356 ± 24	0.57 ± 0.03	0.33 ± 0.03	0.34 ± 0.02	2.94 ± 0.08	0.74 ± 0.07
		19	10	465 ± 32*	0.46 ± 0.04*	0.29 ± 0.02	0.28 ± 0.02	2.42 ± 0.12	0.60 ± 0.06

Sex	Group	Age (weeks)	No. of animals	Spleen	Pituitary gland (x 10^{-3})	Adrenal gland (x 10^{-3})	Thymus (x 10^{-3})	Testes	Ventral prostate (x 10^{-3})
Male	CRF-1	8	10	0.21 ± 0.02	3.3 ± 0.5	17.2 ± 1.5	213 ± 38	0.98 ± 0.09	122 ± 18
		10	10	0.18 ± 0.02	2.9 ± 0.4	15.0 ± 1.4	169 ± 32	0.92 ± 0.09	134 ± 38
		19	10	0.14 ± 0.02	2.2 ± 0.3	10.0 ± 1.1	51 ± 9	0.67 ± 0.08	111 ± 40
	CR-LPF	8	10	0.21 ± 0.03	3.6 ± 0.4	17.9 ± 1.8	212 ± 29	1.03 ± 0.08	122 ± 17
		10	10	0.18 ± 0.02	3.0 ± 0.4	16.0 ± 3.1	154 ± 21	0.90 ± 0.05	132 ± 16
		19	10	0.14 ± 0.01	2.4 ± 0.5	11.3 ± 2.7	53 ± 12	0.74 ± 0.06*	114 ± 20

Sex	Group	Age (weeks)	No. of animals	Body weight (g)	Brain	Heart	Lungs	Liver	Kidneys
Female	CRF-1	8	10	175 ± 13	1.00 ± 0.07	0.38 ± 0.03	0.48 ± 0.03	3.08 ± 0.23	0.84 ± 0.06
		10	10	215 ± 19	0.87 ± 0.08	0.35 ± 0.02	0.43 ± 0.03	2.78 ± 0.14	0.75 ± 0.06
		19	10	278 ± 25	0.70 ± 0.05	0.30 ± 0.02	0.36 ± 0.02	2.38 ± 0.16	0.62 ± 0.04
	CR-LPF	8	10	164 ± 11	1.08 ± 0.07*	0.40 ± 0.03	0.48 ± 0.03	3.17 ± 0.16	0.89 ± 0.06
		10	10	202 ± 18	0.91 ± 0.05	0.37 ± 0.03	0.46 ± 0.03	2.87 ± 0.13	0.77 ± 0.07
		19	10	268 ± 23	0.74 ± 0.05	0.32 ± 0.03	0.38 ± 0.01*	2.41 ± 0.20	0.61 ± 0.04

Sex	Group	Age (weeks)	No. of animals	Spleen	Pituitary gland (x 10^{-3})	Adrenal gland (x 10^{-3})	Thymus (x 10^{-3})	Ovaries (x 10^{-3})
Female	CRF-1	8	10	0.21 ± 0.04	5.4 ± 0.8	29.8 ± 3.3	267 ± 38	37.9 ± 10.7
		10	10	0.21 ± 0.03	5.5 ± 1.1	27.0 ± 5.0	215 ± 30	40.0 ± 6.9
		19	10	0.17 ± 0.02	4.6 ± 1.0	21.9 ± 3.9	89 ± 18	27.0 ± 4.3
	CR-LPF	8	10	0.23 ± 0.05	5.2 ± 1.2	35.0 ± 4.3**	273 ± 39	42.2 ± 6.1
		10	10	0.21 ± 0.02	5.8 ± 0.8	31.5 ± 6.9	203 ± 37	41.3 ± 7.9
		19	10	0.17 ± 0.03	5.9 ± 0.9**	23.9 ± 2.9	111 ± 24*	25.6 ± 3.1

The values are expressed as a percentage of body weight (mean ± S.D.).
Significantly different from the corresponding value in the CRF-1 group (*:$p \leqq 0.05$ or **:$p \leqq 0.01$)

Table 14-1. Histopathology in male Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age	8 weeks								10 weeks								19 weeks							
Group	CRF-1 (N=10)				CR-LPF (N=10)				CRF-1 (N=10)				CR-LPF (N=10)				CRF-1 (N=10)				CR-LPF (N=10)			
Grade of the finding	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Liver																								
Hematopoietic cell proliferation	8	2	0	0	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0
Infiltrative cell, Mononuclear cell	3	7	0	0	1	9	0	0	3	7	0	0	0	10	0	0	1	9	0	0	1	9	0	0
Vacuolization intracytoplasmic, Hepatocyte	9	1	0	0	10	0	0	0	9	1	0	0	10	0	0	0	6	4	0	0	8	2	0	0
Heart																								
Cardiomyopathy	9	1	0	0	8	2	0	0	8	2	0	0	8	2	0	0	4	5	1	0	6	4	0	0
Adrenal gland																								
Dilatation, Sinus, Cortex	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0
Pituitary gland																								
Cyst, Pars distalis	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Prostate																								
Abscess	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Infiltrative cell, Mononuclear cell	9	1	0	0	9	1	0	0	6	4	0	0	8	2	0	0	6	4	0	0	7	3	0	0
Testis																								
Atrophy, Seminiferous tubule, Focal	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Giant cell, Seminiferous tubule	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0	10	0	0	0
Spleen																								
Hematopoietic cell proliferation	2	8	0	0	2	8	0	0	10	0	0	0	9	1	0	0	8	2	0	0	8	2	0	0
Pigmentation, Brown	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	1	9	0	0	1	9	0	0
Lung																								
Infiltrative cell, Mononuclear cell	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Kidney																								
Basophilic, Renal tubule	3	7	0	0	3	7	0	0	8	2	0	0	1	9	0	0	7	3	0	0	9	1	0	0
Calcification, Medulla	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0	10	0	0	0
Cast	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Hyaline droplet, Renal tubule	9	1	0	0	10	0	0	0	10	0	0	0	7	3	0	0	7	3	0	0	8	2	0	0
Hydronephrosis, Bilateral	10	0	0	0	9	1	0	0	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Hydronephrosis, Right	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	8	2	0	0	10	0	0	0
Hyperplasia, Epithelium, Pelvis	8	2	0	0	8	2	0	0	10	0	0	0	9	1	0	0	8	2	0	0	10	0	0	0
Infiltrative cell, Mononuclear cell	6	4	0	0	8	2	0	0	8	2	0	0	5	5	0	0	9	1	0	0	10	0	0	0

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

Table 14-2. Histopathology in female Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age	8 weeks								10 weeks								19 weeks							
Group	CRF-1 (N=10)				CR-LPF (N=10)				CRF-1 (N=10)				CR-LPF (N=10)				CRF-1 (N=10)				CR-LPF (N=10)			
Grade of the finding	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	+++	+++	-	+	++	+++
Liver																								
Hematopoietic cell proliferation	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Infiltrative cell, Mononuclear cell	6	4	0	0	6	4	0	0	3	7	0	0	2	8	0	0	1	9	0	0	4	6	0	0
Necrosis, Hepatocyte, Focal	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Thrombus, Sinusoid	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0
Vacuolization intracytoplasmic, Hepatocyte	6	4	0	0	4	6	0	0	5	5	0	0	8	2	0	0	7	3	0	0	9	1	0	0
Heart																								
Cardiomyopathy	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	7	3	0	0	9	1	0	0
Pituitary gland																								
Cyst, Pars distalis	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Cyst, Pars intermedia	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0
Ovary																								
Hemorrhage, Corpus luteum	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Spleen																								
Hematopoietic cell proliferation.	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Pigmentation, Brown	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	0	10	0	0	0	10	0	0
Kidney																								
Basophilic, Renal tubule	4	6	0	0	6	4	0	0	7	3	0	0	7	3	0	0	9	1	0	0	10	0	0	0
Calcification, Medulla	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Cyst	10	0	0	0	9	1	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Glomerulosclerosis, Focal	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0	10	0	0	0
Hyperplasia, Epithelium, Pelvis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	8	2	0	0	9	1	0	0
Infiltrative cell, Mononuclear cell	9	1	0	0	9	1	0	0	9	1	0	0	10	0	0	0	9	1	0	0	10	0	0	0
Inflammation, Pelvis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	1	0	0	10	0	0	0

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

Background Data for Clinical and Pathological Parameters in Crj:CD(SD)IGS Rats Bred for Long Time

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ABSTRACT. This study was performed in order to compare general toxicological parameters between Crj:CD(SD) rats (SD) and Crj:CD(SD)IGS rats (IGS), fed normal diet (CRF-1), and to investigate the effects of a low protein (CR-LPF) diet in IGS rats. Differences between IGS and SD rats were found in some of the examination items, particularly body weights and food consumption. Moreover, IGS rats fed CR-LPF demonstrated differences in some of the examination items too, particularly body weights and food consumption, as compared with IGS rats fed CRF-1. — Key words: Crj:CD(SD)IGS Rat, Crj:CD(SD) Rats, Low protein diet

CD (SD) IGS-1998: 121-130

INTRODUCTION

The gold standard system, providing as far as possible uniform animals worldwide, has been developed by Charles River Laboratories Inc. (Wilmington, USA) for the internationalization of research and development of new drugs. We have performed numerous toxicity tests with Crj:CD(SD)IGS rats (IGS) bred using this system and have collected background data for clinical and pathological parameters. In the present study, the data were compared with those for Crj:CD(SD) rats (SD) obtained in past toxicity tests performed in our laboratory.

Recently, the question has been raised whether a high calorie/protein diet results in a decrease in survival rate. In fact, it has been reported that a low protein diet causes no nutritional problems in rats, with reduction and retardation of spontaneous lesions and prolongation of the life span. We have used a low protein diet (CR-LPF, Oriental Yeast Co., Tokyo, Japan) recommended by the National Institutes of Health (NIH), for a long-term examination. We have compared the data with those for IGS rats fed a regular high protein diet (CRF-1, Oriental Yeast Co., Tokyo, Japan).

MATERIALS AND METHODS

Crj:CD(SD)IGS rats (IGS) were purchased from Charles River Japan Inc. (Tsukuba, Japan) at 4 weeks of age and acclimated for 1 week. They were housed in animal rooms conditioned to 23 ± 1°C and a relative humidity of 55 ± 5% and ventilated 10-18 times/hour, and provided with light for 12 hours/day. The animals were allowed free access to autoclaved CRF-1 pellet food and tap water. Normal animals, without any abnormalities in general condition and weight were selected and divided as following at 5 weeks of age and given CRF-1 or CR-LPF for various periods.

Test housing period		10 days		1 month		3 months		6 months	
Age at autopsy		7 weeks		9 weeks		18 weeks		31 weeks	
Food		CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF	CRF-1	CR-LPF
No. of Animals	Male	–	–	16	16	16	16	16	16
	Female	16	16	16	16	16	16	16	16

The components of the diets were as follows:

	CRF-1	CR-LPF
Moisture (%)	8.2	7.9
Crude protein (%)	22.3	17.0
Crude fat (%)	5.4	4.2
Crude ashes (%)	6.6	6.2
Crude fiber (%)	3.0	4.8
Nitrogen-free extract (%)	54.5	59.9

The animals were observed for general condition twice or more weekly. Body weights and food consumption were measured once or twice weekly. Water intake was measured at 9, 18 and 31 weeks. Urinalysis was performed in the final week of the observation period, urine being collected for 5 hours under fasting but water supply conditions, and then for 24 hours with access to both feed and water. For the 5-hour urine samples, urinary pH, protein, bilirubin, urobilinogen, glucose, occult blood and ketone bodies were examined using test paper (Multistix, Bayer·Sankyo Co. Ltd., Tokyo, Japan), and sediment was examined by the microscopic method. Urine volume, specific gravity and electrolytes were measured for 24-

hour urine samples. Specific gravity was measured using a clinical refractometer (Erma Co., Tokyo, Japan) and the electrolytes; sodium (Na), potassium (K) and chlorine (Cl), using an automatic electrolytic analyzer (710, Hitachi Ltd., Tokyo, Japan). Ophthalmological observations were performed macroscopically at final week. The animals were fasted for approximately 24 hours, then exsanguinated from the abdominal vena cava under ether anesthesia. Thereafter autopsies were performed, and the following organs were weighed : brain, heart, lung, liver, kidney, spleen, submandibular gland and testis, especially adrenal, thymus, thyroid gland, hypophysis, prostate, seminal vesicle, ovary and uterus after being fixed in formalin for one day. Hematological examinations were performed using EDTA-treated blood samples with a hematology system (H-1E, Technicon, NY, USA). The red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count and white blood cell differential count were examined. Reticulocyte counts were made with an automated reticulocyte analyzer (R-2000, Toa Medical Electronics, Kobe, Japan). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using blood samples treated with tri-sodium citrate solution with the aid of an automated blood coagulation analyzer (CA-1000, Toa Medical Electronics, Kobe, Japan). Blood biochemical examinations were performed with an automatic analyzer (7250, Hitachi Ltd., Tokyo, Japan). Serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), creatinine, calcium (Ca), inorganic phosphorus (IP), total cholesterol, phospholipid (PL), triglyceride (TG), glucose, uric acid and total protein were assessed. Sodium (Na), potassium (K) and chlorine (Cl) were measured using an automatic electrolytic analyzer (710, Hitachi Ltd., Tokyo, Japan) and protein fractions (albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin and A/G ratio) were measured with a densitometer (D-606N, Cosmo Co. LTD., Tokyo, Japan).

Statistical analyses of data for body weight, food consumption, water intake, urinalysis (urine volume, specific gravity and electrolytes), hematological examination, blood biochemical examination and organ weights were performed using the Student's t-test. The significance of differences in test data between Crj:CD(SD) rats fed CRF-1 (SD(CRF-1)) and IGS(CRF-1), and between IGS(CRF-1) and IGS(CR-LPF) were assessed using data for SD rats from past toxicity studies performed in our laboratory.

RESULTS

1. Comparison between IGS (CRF-1) and SD(CRF-1)

Data for body weight changes with age are shown in Figure 1. IGS(CRF-1) values were obviously lower than these for SD(CRF-1) throughout the observation period in males. However, no obvious differences were noted for females. Data for food consumption are shown in Figure 2. There was continuously lower food consumption in both sexes of IGS(CRF-1) throughout the observation period.

The results of urinalysis are summarized in Table 1. Significant increases in some parameters, namely K and Cl at 9 weeks in males and specific gravity at 9 and 18 weeks in females, were found in IGS(CRF-1) as compared to SD(CRF-1). Volume at 9 weeks and Na at 18 and 31 weeks were significantly lower in IGS(CRF-1) than in SD(CRF-1) females.

The results of hematological examination are summarized in Table 2. Significant differences in some parameters were found between IGS(CRF-1) and SD(CRF-1) males. Reticulocytes, PT, HCT and MCV were higher at 9 weeks, from 9 to 31 weeks, and at 31 weeks, respectively, in IGS(CRF-1), HGB and APTT, MCH and MCHC, and platelets were decreased at 9 and 18 weeks, from 9 to 31 weeks, and at 18 and 31 weeks, respectively. In females, significantly higher values were also found for reticulocytes at 7 weeks, platelets at 7 and 9 weeks, RBC at 9 weeks, HCT at 9 and 31 weeks, MCV at 7, 18 and 31 weeks, and PT at 9-31 weeks, and lower values were noted for RBC at 7 weeks, WBC at 18 weeks, HGB at 7 and 18 weeks, MCH at 9 and 18 weeks, MCHC at 7-31 weeks, and APTT at 9-31 weeks.

The results of blood biochemical examination are summarized in Table 3. Significantly greater values were found in IGS(CRF-1) as compared to SD(CRF-1) males for total bilirubin, PL, TG and creatinine at 9 weeks, Na at 9 and 18 weeks, GPT, Cl and the α_2-globulin fraction at 18 weeks, Ca and the α_1-globulin fraction at 18 and 31 weeks. GPT and Cl at 9 weeks, total cholesterol, PL, TG, IP, total protein, the albumin fraction and A/G at 18 and 31 weeks, and total bilirubin, creatinine, K, the α_2-globulin fraction and the γ-globulin fraction at 31 weeks were lower in IGS(CRF-1) males. In females, the γ-globulin fraction at 7 weeks, total bilirubin at 7 and 9 weeks, Cl at 9 weeks, Na at 9 and 18 weeks, ALP, glucose and the β-globulin fraction at 18 weeks, the α_2-globulin fraction at 7-18 weeks, Ca and the α_1-globulin fraction at 9-31 weeks, and creatinine at 7-31 weeks were higher, and glucose at 7 weeks, the albumin fraction and A/G at 7 and18 weeks, GPT at 9 weeks, GOT and total bilirubin at 18 weeks, PL, IP and total protein at 18 and 31 weeks, K at 9-31 weeks, the γ-globulin fraction at 31 weeks, and total cholesterol at 7-31 weeks were lower in IGS(CRF-1).

Organ weights are summarized in Table 4. In males, no apparent differences were observed between IGS(CRF-1) and SD(CRF-1), although significant variation in absolute or relative values was noted. On the other hand, brain and submandibular gland weights in IGS(CRF-1) were lower than in SD(CRF-1) females at 18 and 31 weeks and 31 weeks, respectively. In addition, ovary and thyroid gland weights in IGS(CRF-1) were lower and higher respectively, than in SD(CRF-1) females at 18 weeks. Hypophysis weights were higher in IGS(CRF-1) females at 9 and 18 weeks, but this was not the case at other observation points. No other consistent significant differences in absolute or relative values were evident.

2. Comparison between IGS(CRF-1) and IGS(CR-LPF)

No abnormal clinical signs were observed throughout the

experimental period. Body weight curves are shown in Figure 1. Significantly lower body weights were noted for IGS(CR-LPF) than IGS(CRF-1) throughout the observation period in males. However, no significant differences were noted in females. Food consumption data are summarized in Figure 2. Food consumption in IGS(CR-LPF) was continuously higher than with IGS(CRF-1) in both sexes, this being especially marked in females. Statistically significant differences were apparent from 6 to 7 weeks and at 24 weeks in males and from 6 to 31 weeks in females. Water intake did not significantly vary in either sex except at 9 and 18 weeks when values were higher for IGS(CRF-1) in males (data not shown).

The urinalysis results are shown in Table 1. Significant decreases in volume and Cl at 9 and 18 weeks, and Na from 9 to 31 weeks, were found in IGS(CR-LPF) as compared to IGS(CRF-1) males. In females, Na was increased significantly at 18 weeks in IGS(CR-LPF).

Ophthalmological abnormalities were not observed in any of the animals.

The results of hematological examination are summarized in Table 2. In males, MCHC at 31 weeks and PT and APTT from 9 to 31 weeks were lower, and reticulocytes were higher at 9 and 18 weeks in IGS(CR-LPF). In IGS(CR-LPF) females, RBC at 7 weeks, WBC, lymphocyte and LUC at 9 weeks, PT at 9 and 18 weeks were significantly higher, and MCV, MCH and reticulocytes at 7 weeks and LUC was significantly lower at 18 weeks.

The results of blood biochemical examination are summarized in Table 3. In males, GOT, glucose and BUN at 9 weeks, GPT at 18 weeks, Na and the α_1-globulin fraction at 9 and 18 weeks were significantly lower in IGS(CR-LPF) as compared to IGS(CRF-1). On the other hand, total cholesterol, PL and Cl at 9 weeks, total bilirubin at 18 weeks, the α_2-globulin fraction at 31 weeks, and the albumin fraction and A/G from 9 to 31 weeks were significantly higher in IGS(CR-LPF) than in IGS(CRF-1) males. In females, glucose from 7 to 18 weeks, the α_1-globulin fraction at 7 and 18 weeks, uric acid at 18 weeks, and the albumin fraction and A/G at 31 weeks were significantly lower in IGS(CR-LPF) as compared to IGS(CRF-1). On the other hand, GPT, TG and Ca at 7 weeks, Na, Cl and uric acid at 9 weeks, total bilirubin and A/G at 18 weeks, total cholesterol and PL at 7, 9 and 31 weeks, and the albumin fraction at 7 and 18 weeks were significantly higher in IGS(CR-LPF) females.

Organ weight data are shown in Table 4. In IGS(CR-LPF) males, both absolute and relative liver weights were lower than in IGS(CRF-1) from 9 to 31 weeks. Brain weights and kidney weights were also lower at 9 weeks and at 31 weeks. On the other hand, testis weights were higher at 31 weeks. The weights of seminal vesicles and the prostate were lower than in IGS(CRF-1) at 9 weeks. The hypophysis exhibited higher values at 18 weeks. Other organs only demonstrated variation in absolute or relative weights. In IGS(CR-LPF) females, kidney weights were lower than in IGS(CRF-1) at 31 weeks with significance for both absolute and relative values, similarly to the male case. Absolute and relative ovary weights were also significantly lower at 7 weeks.

DISCUSSION

As described above, significant differences were found in some parameters examined in toxicity studies between IGS(CRF-1) and SD(CRF-1). Body weights in IGS(CRF-1) males were obviously lower in association with a low values in food consumption. However, no obvious differences for body weights were noted in females, although there was continuously lower food consumption in IGS(CRF-1) as compared to SD(CRF-1). It is supposed that differences in body weights would be easily happened in males than in females. Blood biochemical examination revealed that lipids such as total cholesterol, triglyceride and phospholipid, tend to be lower in both sexes of IGS(CRF-1) than SD(CRF-1). This effect can be considered to be caused by the decreased intake of lipid based on a difference of food consumption.

A comparison between IGS(CRF-1) and IGS(CR-LPF) showed differences in same parameters, namely body weights, food consumption and lipid. Significantly lower body weights were noted in IGS(CR-LPF) than in IGS(CRF-1) males throughout the observation period, although there were little differences between both food consumption. On the other hand, no significant differences for body weights were noted in females, because the significantly increased food consumption were noted in IGS(CR-LPF) as compared to IGS(CRF-1). In blood biochemical examination, lipids tend to be higher in IGS(CR-LPF) than in IGS(CRF-1) females. These differences were probably caused by the increased intake of lipid because of an increased food consumption in IGS(CR-LPF).

Based on these findings, we conclude that IGS rats are more useful than SD rats as experimental animals in long-term toxicity test, because a low values for body weights and serum lipid levels due to lower food consumption probably lead to decrease in factors interfering with toxicity evaluation such as mortality rate and spontaneous lesions, especially in males. Furthermore, it may be better to supply a low protein diet to IGS rats in order to improve more their usefully, especially in females. However, further investigations are needed to qualify this conclusions.

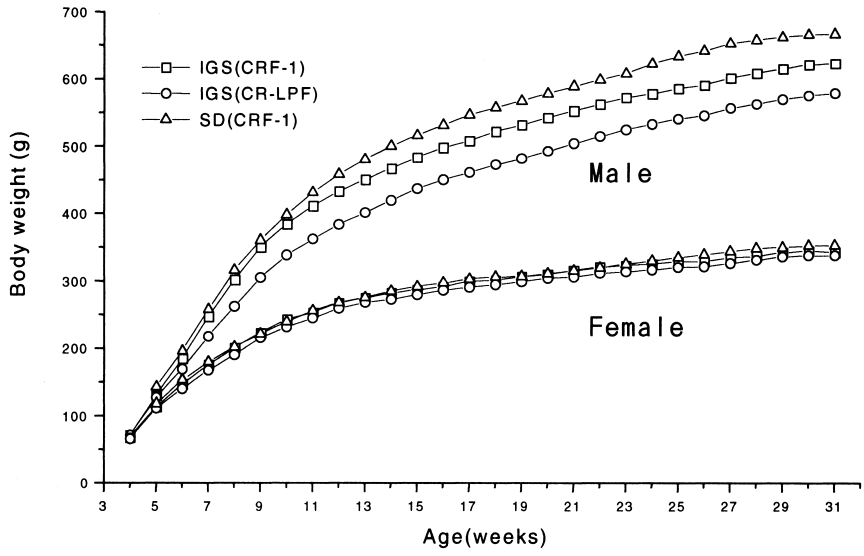

Fig. 1. Body weight changes with age

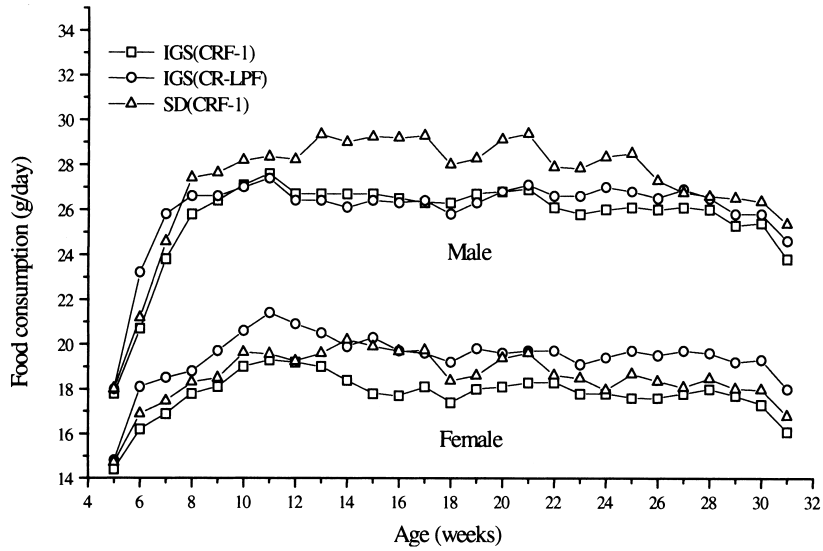

Fig. 2. Food consumption

Table 1. Urinalysis data

	7 weeks			9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Male												
Animal No.				16	106	16	16	68	16	16	53	16
Volume				11.5	9.2	6.6**	13.5	15.7	8.4*	16.0	14.0	13.9
(ml)				5.6	4.3	1.8	8.1	6.3	4.3	10.9	7.3	5.8
Specific				1.061	1.069	1.063	1.064	1.056	1.074	1.054	1.062	1.049
gravity				0.022	0.023	0.012	0.022	0.018	0.020	0.014	0.018	0.015
Na				1.8	1.7	1.1**	1.7	2.2	1.2**	1.6	1.6	1.3*
(mEq/24 hr)				0.4	0.4	0.3	0.3	1.1	0.3	0.3	0.4	0.3
K				3.4	2.9*	3.0	3.5	3.6	3.4	3.6	3.5	3.6
(mEq/24 hr)				0.7	0.7	0.7	0.5	0.7	0.8	0.5	0.8	0.7
Cl				2.5	2.2*	1.8**	2.4	2.3	2.0*	2.2	2.1	2.0
(mEq/24 hr)				0.5	0.5	0.4	0.4	0.6	0.4	0.3	0.5	0.4
Female												
Animal No.	16		16	16	104	16	16	68	16	16	53	16
Volume	0.9		1.1	6.7	10.5*	7.2	10.0	13.3	7.2	9.0	11.8	10.8
(ml)	0.5		1.0	3.4	6.3	5.0	6.3	7.3	5.0	4.7	7.7	6.1
Specific	1.036		1.034	1.078	1.052**	1.067	1.063	1.050*	1.060	1.056	1.056	1.048
gravity	0.014		0.015	0.033	0.020	0.028	0.025	0.019	0.025	0.021	0.022	0.012
Na				1.1	1.2	0.9	1.1	1.6*	0.8*	0.9	1.1*	0.9
(mEq/24 hr)				0.3	0.3	0.2	0.4	0.8	0.3	0.3	0.4	0.3
K				2.2	2.2	2.6	2.4	2.6	2.2	2.2	2.4	2.7
(mEq/24 hr)				0.6	0.5	0.6	0.7	0.7	0.9	0.8	0.8	0.8
Cl				1.5	1.6	1.5	1.6	1.6	1.2	1.3	1.5	1.5
(mEq/24 hr)				0.4	0.4	0.3	0.5	0.4	0.6	0.5	0.5	0.5

* : P<0.05, ** : P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

Table 2-1. Hematological data for male rats

	9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
animal No.	16	86	16	16	60	16	16	51	16
RBC	7.71	7.62	7.66	8.70	8.72	8.53	8.88	8.74	8.67
($10^6/\mu l$)	0.30	0.39	0.32	0.33	0.40	0.42	0.39	0.43	0.33
HGB	15.4	16.1**	15.2	15.4	16.1**	15.1	15.8	16.0	15.4
(g/dl)	0.40	0.6	0.4	0.6	0.7	0.4	0.8	0.5	0.4
HCT	46.3	45.3	46.0	46.2	45.2	45.2	46.3	44.0**	46.1
(%)	1.1	2.0	1.4	1.7	2.6	1.6	2.6	1.5	1.5
MCV	60.1	59.6	60	53.1	51.9	53.1	52.1	50.4**	53.2
(fl)	2.1	1.9	2	1.3	2.6	1.6	1.3	2.0	2.0
MCH	20.0	21.1**	19.9	17.7	18.5**	17.8	17.8	18.3*	17.8
(pg)	0.6	0.7	0.7	0.5	0.8	0.6	0.5	0.7	0.7
MCHC	33.2	35.4**	33.1	33.4	35.6**	33.5	34.2	36.3**	33.4**
(g/dl)	0.4	0.8	0.6	0.8	1.4	0.5	0.6	0.6	0.5
Platelet	1136	1173	1126	1030	1153*	1019	1075	1278**	1041
($10^3/\mu l$)	142	140	97	117	189	86	141	162	84
Reticulocyte	30	24**	35**	20	21	24*	22	22	21
(‰)	4	7	5	3	5	5	9	4	3
WBC	9.32	10.41	9.80	9.28	11.29**	9.37	9.98	10.07	9.39
($10^3/\mu l$)	2.15	2.70	1.78	1.83	2.83	1.72	2.04	1.97	1.52
Eosinophil	0.09		0.09	0.12		0.15	0.16		0.18
($10^3/\mu l$)	0.03		0.02	0.04		0.06	0.06		0.07
Neutrophil	0.85		0.87	1.29		1.38	1.61		1.66
($10^3/\mu l$)	0.22		0.39	0.59		0.56	0.79		0.60
Lymphocyte	8.12		8.57	7.39		7.37	7.62		7.01
($10^3/\mu l$)	2.06		1.64	1.86		1.36	1.85		1.24
Basophil	0.03		0.03	0.02		0.02	0.02		0.02
($10^3/\mu l$)	0.01		0.01	0.01		0.01	0.01		0.01
Monocyte	0.20		0.22	0.37		0.35	0.45		0.41
($10^3/\mu l$)	0.07		0.09	0.17		0.15	0.17		0.11
LUC	0.03		0.03	0.09		0.10	0.12		0.12
($10^3/\mu l$)	0.01		0.01	0.03		0.04	0.03		0.04
PT	15.8	11.9**	13.7**	15.5	11.3**	14.2**	16.3	10.7**	15*
(sec)	1.7	1.8	0.5	1.8	1.4	0.4	1.9	1.0	1.0
APTT	22.2	25.1**	19.0**	18.4	25.9**	16.1**	23.1	25.1	21.6**
(sec)	1.5	4.3	1.4	1.4	4.1	1.3	1.3	4.2	1.5

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

Table 2-2. Hematological data for female rats

	7 weeks			9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Animal No.	16	36	16	16	86	16	16	60	16	16	54	16
RBC	6.85	7.21**	7.15**	7.77	7.29**	7.79	8.01	8.11	7.98	7.89	7.78	7.76
($10^6/\mu l$)	0.19	0.37	0.30	0.22	0.34	0.34	0.29	0.49	0.22	0.37	0.41	0.41
HGB	14.5	15.1**	14.8	15.4	15.6	15.4	15.1	15.8**	15.2	15.5	15.3	15.1
(g/dl)	0.5	0.7	0.5	0.4	0.6	0.4	0.4	0.7	0.6	0.6	0.6	0.8
HCT	44.9	45.2	45.6	45.0	42.3**	44.8	44.7	44.0	44.5	43.6	42.1*	43.0
(%)	1.4	1.8	1.4	1.2	1.8	1.5	1.4	3.7	1.7	1.7	2.1	2.1
MCV	65.6	62.7**	63.9**	57.9	58.0	57.6	55.9	54.2*	55.7	55.2	54.1*	55.5
(fl)	1.8	2.1	1.6	1.2	1.9	1.4	1.9	2.6	1.4	2.0	1.4	1.5
MCH	21.2	20.9	20.7*	19.8	21.4**	19.7	18.9	19.6**	19.0	19.6	19.7	19.5
(pg)	0.6	0.7	0.6	0.4	0.7	0.5	0.5	0.9	0.5	0.8	0.6	0.4
MCHC	32.4	33.3**	32.4	34.2	36.9**	34.3	33.9	36.1**	34.1	35.5	36.4**	35.2
(g/dl)	0.3	1.0	0.5	0.5	0.9	0.7	0.6	2.1	0.6	0.4	0.8	0.6
Platelet	1386	1274*	1405	1219	1306*	1257	1036	1046	1089	1064	1078	1072
($10^3/\mu l$)	138	142	132	156	161	106	126	170	144	111	119	92
Reticulocyte	52	46**	47*	24	22	25	22	19	23	20	22	23
(‰)	7	8	6	5	6	6	4	6	4	5	4	17
WBC	6.71	7.78	7.49	8.08	8.52	10.74**	6.47	7.57*	5.77	6.54	6.04	7.04
($10^3/\mu l$)	1.90	2.00	1.89	2.11	2.43	3.14	1.27	2.01	1.41	1.38	1.96	2.22
Eosinophil	0.06		0.07	0.11		0.11	0.10		0.09	0.12		0.13
($10^3/\mu l$)	0.02		0.03	0.04		0.04	0.04		0.05	0.04		0.05
Neutrophil	0.43		0.38	0.69		0.66	0.90		0.65	0.87		1.02
($10^3/\mu l$)	0.19		0.10	0.18		0.28	0.65		0.24	0.19		0.61
Lymphocyte	5.99		6.80	7.05		9.68**	5.20		4.81	5.17		5.42
($10^3/\mu l$)	1.69		1.77	2.06		2.91	1.14		1.18	1.32		1.63
Basophil	0.02		0.02	0.02		0.03	0.01		0.01	0.01		0.01
($10^3/\mu l$)	0.01		0.01	0.01		0.02	0.01		0.01	0.01		0.01
Monocyte	0.18		0.20	0.20		0.23	0.20		0.17	0.29		0.34
($10^3/\mu l$)	0.07		0.07	0.09		0.10	0.07		0.07	0.12		0.19
LUC	0.02		0.03	0.02		0.04**	0.05		0.03*	0.08		0.12
($10^3/\mu l$)	0.01		0.01	0.01		0.02	0.02		0.02	0.03		0.07
PT				13.9	8.7**	14.3**	13.9	9.2**	14.4**	14.0	9.4**	14.3
(sec)				0.4	0.4	0.4	0.4	0.3	0.4	0.5	0.3	0.5
APTT				17.3	19.2**	17.9	17.4	23.5**	17.7	18.3	21.1**	18.4
(sec)				1.5	2.8	1.0	1.1	4.6	0.9	1.5	2.7	1.5

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

Table 3-1. Blood biochemical data for male rats

	9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Animal No.	16	86	16	16	58	16	16	51	16
GOT	103	99	85**	96	91	80	85	87	81
(IU/l)	23	20	12	35	21	15	20	25	23
GPT	30	33*	30	39	30*	28*	31	32	29
(IU/l)	5	5	6	25	6	5	6	10	8
ALP	575	557	577	227	195	207	149	143	153
(IU/l)	108	105	102	38	73	30	21	28	20
T-Bilirubin	0.04	0.03**	0.05	0.05	0.05	0.06*	0.06	0.07*	0.06
(mg/dl)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
T-Cholesterol	51	52	70**	59	79**	62	69	91**	69
(mg/dl)	10	9	14	8	21	12	11	17	18
PL	94	83**	116**	101	140**	105	108	142**	107
(mg/dl)	14	12	16	14	22	14	14	26	23
TG	39	31*	38	50	95**	52	73	111*	68
(mg/dl)	19	13	14	20	51	25	41	56	31
Glucose	107	112	91**	135	140	143	142	136	147
(mg/dl)	15	15	17	12	24	17	18	15	17
BUN	15.2	15.0	12.9**	14.7	13.8	14.5	14.8	14.1	14.2
(mg/dl)	2.2	2.0	1.9	1.5	2.0	1.3	1.9	1.9	1.5
Creatinine	0.5	0.4**	0.5	0.6	0.5	0.6	0.6	0.5*	0.6
(mg/dl)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ca	10.0	9.4**	10.2	10.6	10.0**	10.8	10.4	10.1**	10.4
(mg/dl)	0.4	0.4	0.3	0.2	0.3	0.3	0.4	0.3	0.2
IP	8.5	8.6	8.5	6.3	7.1**	6.5	5.4	6.1**	5.8
(mg/dl)	1.0	0.7	1.0	0.5	0.9	0.5	0.6	0.8	0.6
Na	147	145**	145**	147	144**	145**	145	145	145
(mEq/l)	1	2	1	1	1	1	1	1	1
K	5.1	5.0	5.0	4.7	4.8	4.7	4.4	4.9**	4.6
(mEq/l)	0.5	0.5	0.2	0.3	0.5	0.3	0.4	0.4	0.4
Cl	105	106*	107**	107	106*	106	106	106	106
(mEq/l)	1	2	1	1	1	1	2	1	1
T-Protein	5.6	5.7	5.5	6.1	6.6**	6.0	6.1	6.6**	6.1
(g/dl)	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.3	0.1
Albumin	62.3	62.0	64.0**	55.8	61.1**	57.2*	54.0	57.2**	55.3*
(%)	1.5	4.2	1.8	1.6	3.4	1.6	1.5	2.5	2.1
α_1-globulin	14.8	14.5	13.4*	17.2	13.6**	16.0*	19.6	14.8**	18.8
(%)	2.0	2.4	1.4	1.5	2.4	1.2	1.2	1.7	2.2
α_2-globulin	7.4	7.1	7.1	7.5	6.7**	7.3	5.9	6.4**	6.3*
(%)	0.4	1.1	0.4	0.7	1.2	0.5	0.4	0.6	0.5
β-globulin	12.5	13.2	12.5	14.3	13.9	14.2	15.6	15.7	15.3
(%)	0.8	1.9	0.9	0.9	1.7	0.6	0.7	1.1	1.0
γ-globulin	3.1	3.3	3.0	5.2	4.7	5.3	4.9	5.9**	4.3
(%)	0.6	0.9	1.0	1.1	1.2	1.2	1.3	1.2	0.9
A/G	1.65	1.66	1.78**	1.26	1.59**	1.34*	1.18	1.35**	1.24*
	0.11	0.25	0.15	0.09	0.22	0.09	0.07	0.14	0.11
Uric acid	1.6		1.6	1.4	1.5	1.4	1.4		1.5
(mg/dl)	0.4		0.4	0.4	0.6	0.3	0.2		0.3

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

Table 3-2. Blood biochemical data for female rats

	7 weeks			9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Animal No.	16	108	16	16	86	16	16	59	16	16	53	16
GOT	96	106	93	96	103	105	76	107**	88	170	128	171
(IU/l)	10	25	24	31	26	30	16	36	40	120	96	160
GPT	24	25	26*	20	25**	22	24	30	28	64	49	65
(IU/l)	3	6	4	3	6	7	6	15	17	53	44	93
ALP	460	490	525	302	300	322	112	97*	101	61	66	67
(IU/l)	108	85	114	87	55	84	34	20	23	19	19	19
T-Bilirubin	0.05	0.04**	0.06	0.05	0.04**	0.05	0.07	0.08*	0.08*	0.10	0.09	0.10
(mg/dl)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.03	0.03	0.05
T-Cholesterol	64	74*	85**	51	63**	66**	67	85**	75	72	96**	95**
(mg/dl)	14	17	11	8	9	13	11	18	15	19	23	26
PL	116	119	148**	106	102	120*	139	170**	147	152	200**	183*
(mg/dl)	21	28	19	16	14	22	18	30	27	32	43	50
TG	27	34	38*	14	15	14	28	34	20	54	80	50
(mg/dl)	10	23	15	10	7	5	19	20	13	36	55	30
Glucose	99	109*	85**	106	112	92**	127	116*	109**	125	119	127
(mg/dl)	12	17	8	9	15	10	14	19	19	14	15	14
BUN	14.7	16	13.3	17	17.8	17.6	16.4	16.4	17.7	16.5	16.4	17.3
(mg/dl)	1.8	2.9	2.3	2.9	3.1	2.5	1.7	3.0	3.1	2.4	3.4	2.3
Creatinine	0.5	0.3**	0.5	0.6	0.5**	0.6	0.7	0.6**	0.7	0.7	0.6**	0.7
(mg/dl)	0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0	0.1	0.1	0.1
Ca	10.1	9.9	10.5**	10.2	9.4**	10.2	11.1	10.1**	10.9	10.8	10.4**	10.8
(mg/dl)	0.3	0.5	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.6
IP	8.8	9.1	9.2	7.5	7.5	7.8	5.0	6.0**	5.1	4.2	4.9**	4.3
(mg/dl)	0.7	0.7	0.7	0.7	0.9	1.0	0.5	1.2	0.6	0.6	0.9	0.9
Na	145	145	145	146	144**	149**	145	144**	145	144	144	143
(mEq/l)	1	2	1	1	3	2	1	1	1	1	2	1
K	5.3	5.3	5.2	4.8	5.1*	5.0	4.2	4.6**	4.2	4.0	4.5**	4.1
(mEq/l)	0.4	0.4	0.3	0.4	0.5	0.5	0.3	0.4	0.3	0.3	0.4	0.5
Cl	105	105	104	109	108*	110*	108	108	109	108	108	108
(mEq/l)	1	2	2	1	2	1	1	2	1	1	2	3
T-Protein	5.6	5.5	5.6	5.9	5.9	5.8	6.6	6.9**	6.5	6.7	7.2**	6.8
(g/dl)	0.1	0.2	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.5	0.7
Albumin	65.5	67.0*	66.9*	62.8	64.2	64.0	62.5	67.1**	64.5*	66.6	66.0	64.8*
(%)	2.2	2.4	1.6	2.7	2.8	2.3	2.1	3.5	3.1	1.9	4.9	2.8
α_1-globulin	11.8	12.5	10.8*	14.0	13.0*	13.0	13.2	9.6**	11.1**	11.0	9.3**	12.0
%)	1.3	2.2	1.4	1.3	1.7	1.8	1.2	1.7	1.3	1.3	1.8	2.0
α_2-globulin	7.6	6.3**	7.4	6.9	6.2*	6.4	6.2	5.4**	6.0	5.1	5.3	5.3
(%)	0.6	0.9	0.9	0.8	1.0	1.0	0.5	0.7	0.7	0.3	0.7	0.5
β-globulin	12.0	11.8	12.0	12.4	12.7	12.9	13.1	12.3*	13.1	12.4	13.1	12.7
(%)	1.1	0.7	0.7	1.4	1.7	0.7	0.8	1.5	1.2	1.0	1.8	1.2
γ-globulin	3.2	2.4**	2.9	3.9	3.9	3.8	5.1	5.6	5.4	4.9	6.3**	5.2
(%)	0.8	0.8	1.2	1.3	1.2	1.1	1.1	1.4	1.1	0.9	1.6	0.9
A/G	1.91	2.05*	2.03	1.70	1.81	1.78	1.67	2.07**	1.84*	2.00	2.00	1.86*
	0.19	0.23	0.15	0.19	0.22	0.18	0.14	0.33	0.23	0.17	0.41	0.23
Uric acid				1.4	1.4	1.7*	1.7	1.6	1.5*	1.3		1.4
(mg/dl)				0.3	0.3	0.3	0.2	0.4	0.3	0.3		0.3

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are means and S.D. values.

Table 4-1. Organ weights for male rats

	9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Animal No.	16	86	16	16	60	16	16	52	16
Final body weight	300.7	291.8	273.5**	473.2	496.3	457.6	594.1	593	549.1*
(g)	21.9	29.0	20.9	39.3	50.3	28.9	65.3	78.7	43.1
Liver	8.84	8.46	7.69**	11.65	12.81*	10.91*	13.99	14.61	12.33**
(g)	1.04	1.04	0.76	0.98	2.12	1.06	1.83	2.65	1.05
Liver	2.93	2.90	2.81*	2.46	2.57	2.38	2.35	2.46	2.25*
(g/100 g)	0.17	0.15	0.12	0.12	0.22	0.15	0.14	0.24	0.11
Kidney	2.42	2.28*	2.18*	2.99	2.98	2.81*	3.37	3.27	2.93**
(g)	0.28	0.21	0.23	0.25	0.33	0.18	0.34	0.45	0.31
Kidney	0.81	0.78	0.80	0.63	0.60*	0.62	0.57	0.55	0.53*
(g/100 g)	0.07	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.04
Lung	1.2	1.21	1.12*	1.43	1.46	1.40	1.64	1.62	1.51**
(g)	0.11	0.12	0.09	0.08	0.14	0.12	0.11	0.18	0.11
Lung	0.40	0.41	0.41	0.30	0.30	0.31	0.28	0.27	0.28
(g/100 g)	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02	0.02
Heart	1.08	1.06	0.95**	1.37	1.37	1.28*	1.48	1.48	1.42
(g)	0.08	0.11	0.08	0.14	0.12	0.08	0.15	0.16	0.11
Heart	0.36	0.36	0.35	0.29	0.28	0.28	0.25	0.25	0.26
(g/100 g)	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02
Spleen	0.56	0.57	0.56	0.69	0.74	0.69	0.79	0.78	0.71
(g)	0.04	0.10	0.09	0.12	0.11	0.07	0.15	0.10	0.12
Spleen	0.19	0.20	0.20*	0.15	0.15	0.15	0.13	0.13	0.13
(g/100 g)	0.02	0.03	0.02	0.02	0.02	0.01	0.03	0.02	0.02
Brain	1.90	1.89	1.82**	2.01	2.08**	2.02	2.11	2.20	2.08
(g)	0.05	0.08	0.07	0.07	0.08	0.07	0.07	0.20	0.08
Brain	0.63	0.65	0.67*	0.43	0.42	0.44	0.36	0.38	0.38
(g/100 g)	0.05	0.06	0.05	0.04	0.04	0.03	0.04	0.06	0.03
Submandibular G.	0.56	0.57	0.53	0.67	0.70	0.66	0.73	0.75	0.68*
(g)	0.05	0.07	0.05	0.07	0.08	0.08	0.07	0.08	0.06
Submandibular G.	0.19	0.19	0.20	0.14	0.14	0.14	0.13	0.13	0.12
(g/100 g)	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.01
Thymus	0.52	0.50	0.45	0.29	0.32	0.28	0.18	0.18	0.17
(g)	0.11	0.09	0.07	0.06	0.07	0.06	0.04	0.05	0.04
Thymus	0.17	0.17	0.17	0.06	0.06	0.06	0.03	0.03	0.03
(g/100 g)	0.03	0.03	0.03	0.01	0.02	0.01	0.01	0.01	0.01
Testis	3.00	2.90	2.98	3.30	3.30	3.37	3.20	3.46*	3.54*
(g)	0.26	0.33	0.18	0.38	0.21	0.24	0.57	0.35	0.22
Testis	1.00	1.00	1.09**	0.70	0.67	0.74	0.55	0.59	0.65**
(g/100 g)	0.09	0.12	0.09	0.09	0.07	0.05	0.12	0.07	0.05
Seminal vesicle	0.73	0.67	0.56**	1.19	1.26	1.17	1.39	1.38	1.38
(g)	0.06	0.11	0.13	0.14	0.19	0.18	0.10	0.22	0.23
Seminal vesicle	0.24	0.23	0.20**	0.25	0.26	0.26	0.24	0.24	0.25
(g/100 g)	0.02	0.04	0.04	0.04	0.04	0.04	0.03	0.05	0.05
Prostate	0.85	0.84	0.66**	1.41	1.50	1.34	1.58	1.58	1.51
(g)	0.12	0.17	0.12	0.22	0.23	0.19	032	0.30	0.35
Prostate	0.28	0.29	0.24*	0.30	0.30	0.30	0.27	0.27	0.27
(g/100 g)	0.04	0.05	0.04	0.05	0.05	0.05	0.06	0.06	0.06
Adrenal	48	49	44	54	53	51	51	52	48
(mg)	10	7	8	6	8	9	7	7	6
Adrenal	16	17	16	12	11*	11	9	9	9
(mg/100 g)	3	2	3	1	1	2	1	2	1
Thyroid gland	18	17	16*	22	21	24	26	25	26
(mg)	3	4	3	4	4	4	5	5	5
Thyroid gland	6.2	5.8	6.0	4.6	4.3	5.4*	4.3	4.2	4.8
(mg/100 g)	1.0	1.3	1.1	0.8	0.9	0.9	0.6	1.0	0.9
Hypophysis	12	10**	11	12	13	14*	14	14	12**
(mg)	2	2	2	2	2	2	2	2	2
Hypophysis	3.9	3.6	3.9	2.6	2.7	3.1**	2.4	2.3	2.2
(mg/100 g)	0.7	0.6	0.6	0.3	0.6	0.4	0.2	0.5	0.3

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

Table 4-2. Organ weights for female rats

	7 weeks			9 weeks			18 weeks			31 weeks		
	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF	CRF-1		CR-LPF
	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS	IGS	SD	IGS
Animal No.	16	108	16	16	86	16	16	60	16	16	54	16
Final body weight	140.4	137.8	133.3*	198.6	193.6	194.5	283.7	274.8	267.4	323	309.2	316.5
(g)	7.2	7.8	8.8	17.1	16.7	14.3	29.5	33.7	38.1	38.4	44.3	30.7
Liver	4.93	4.90	4.59*	5.95	5.76	5.67	6.80	6.80	6.31	7.39	7.32	7.28
(g)	0.35	0.41	0.47	0.58	0.63	0.63	0.68	1.13	0.91	0.93	1.12	1.03
Liver	3.51	3.56	3.44	3.00	2.97	2.91	2.40	2.47	2.36	2.29	2.38	2.30
(g/100 g)	0.16	0.19	0.22	0.24	0.18	0.17	0.13	0.17	0.14	0.14	0.25	0.19
Kidney	1.38	1.33	1.32	1.64	1.56	1.54	1.74	1.82	1.75	2.05	2.01	1.89*
(g)	0.11	0.11	0.11	0.20	0.17	0.14	0.17	0.26	0.20	0.24	0.26	0.19
Kidney	0.98	0.97	0.99	0.83	0.81	0.79	0.62	0.66**	0.66*	0.64	0.66	0.60**
(g/100 g)	0.06	0.06	0.06	0.07	0.06	0.07	0.04	0.05	0.06	0.04	0.06	0.03
Lung	0.83	0.81	0.80	0.96	1.00	0.96	1.12	1.12	1.10	1.18	1.15	1.19
(g)	0.06	0.08	0.07	0.09	0.11	0.08	0.09	0.12	0.09	0.12	0.12	0.11
Lung	0.59	0.59	0.60	0.48	0.52*	0.49	0.40	0.41	0.42	0.37	0.37	0.38
(g/100 g)	0.03	0.05	0.03	0.03	0.05	0.03	0.03	0.04	0.05	0.02	0.04	0.04
Heart	0.61	0.60	0.58	0.73	0.73	0.71	0.88	0.89	0.84	0.95	0.95	0.94
(g)	0.04	0.05	0.04	0.05	0.07	0.06	0.06	0.11	0.09	0.11	0.10	0.08
Heart	0.43	0.44	0.44	0.37	0.38	0.37	0.31	0.32	0.32	0.30	0.31*	0.30
(g/100 g)	0.02	0.03	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.03	0.02
Spleen	0.37	0.35	0.35	0.42	0.43	0.42	0.48	0.49	0.46	0.48	0.50	0.50
(g)	0.08	0.05	0.06	0.05	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.06
Spleen	0.26	0.25	0.26	0.21	0.22	0.22	0.17	0.18	0.17	0.15	0.16*	0.16
(g/100 g)	0.05	0.03	0.04	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.02
Brain				1.80	1.78	1.79	1.89	1.96*	1.89	1.95	2.02*	1.95
(g)				0.08	0.06	0.05	0.08	0.11	0.09	0.09	0.09	0.07
Brain				0.91	0.93	0.92	0.67	0.72*	0.72	0.61	0.66*	0.62
(g/100 g)				0.08	0.07	0.06	0.06	0.07	0.09	0.07	0.08	0.06
Submandibular G.	0.33	0.33	0.32	0.39	0.39	0.38	0.44	0.46	0.44	0.45	0.48*	0.46
(g)	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.06	0.03	0.05	0.04	0.05
Submandibular G.	0.24	0.24	0.24	0.20	0.20	0.20	0.15	0.17**	0.17**	0.14	0.16**	0.15
(g/100 g)	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.01
Thymus	0.45	0.48	0.45	0.47	0.48	0.5	0.29	0.29	0.25	0.14	0.15	0.16
(g)	0.05	0.09	0.09	0.07	0.09	0.09	0.07	0.07	0.06	0.04	0.04	0.03
Thymus	0.32	0.35*	0.34	0.24	0.25	0.26	0.10	0.11	0.09	0.05	0.05	0.05
(g/100 g)	0.03	0.06	0.05	0.04	0.04	0.04	0.02	0.02	0.02	0.01	0.01	0.01
Ovary	57	57	49*	72	76	71	74	86*	76	73	74	74
(mg)	10	9	8	16	13	16	15	19	10	14	18	21
Ovary	41	42	37	36	39	37	26	32**	29	23	24	23
(mg/100 g)	7	7	6	7	6	8	5	6	4	5	6	6
Uterus	0.36	0.30*	0.31	0.40	0.41	0.37	0.56	0.54	0.58	0.65	0.72	0.62
(g)	0.10	0.11	0.12	0.15	0.13	0.17	0.20	0.18	0.22	0.14	0.41	0.22
Uterus	0.25	0.21	0.23	0.20	0.21	0.19	0.20	0.20	0.22	0.21	0.24	0.20
(g/100 g)	0.08	0.08	0.09	0.09	0.07	0.09	0.07	0.07	0.08	0.06	0.16	0.07
Adrenal	42	42	38*	62	59	57*	71	65*	63*	67	66	64
(mg)	4	5	4	6	9	7	11	9	7	7	10	8
Adrenal	30	31	29	31	30	29	25	24	24	21	22	20
(mg/100 g)	3	3	4	3	4	3	3	3	4	3	4	2
Thyroid gland	12	13	12	16	16	16	20	17**	19	20	19	22
(mg)	2	2	3	4	3	2	4	3	3	5	4	5
Thyroid gland	8.6	9.3	8.7	8.2	8.1	8.1	7.0	6.2*	7.1	6.4	6.1	7.1
(mg/100 g)	1.4	1.7	1.9	1.9	1.9	1.3	1.2	1.0	1.1	1.5	1.1	1.5
Hypophysis				15	13**	14	18	15*	17	20	18	20
(mg)				2	2	3	3	3	3	6	6	3
Hypophysis				7.8	6.7**	7.1	6.2	5.6*	6.6	6.3	5.9	6.2
(mg/100 g)				1.1	1.1	1.5	1.0	1.1	1.1	1.7	1.6	1.1

* : P<0.05, ** P<0.01 (significant difference from IGS rats fed CRF-1) Data are mean and S.D. values.

The Effect of Controlled Feeding on the Growth of Crl:CD(SD)IGS Rats

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ABSTRACT. The growth of ad-litum fed CD[R] rats and IGS CD[R] rats was compared to that of IGS CD[R] rats given daily controlled quantities of food over the first 52 weeks of 2 year carcinogenicity studies. The growth of males fed 21g/day showed body weight gains lower than ad-libitum fed males for 0 to 24 weeks, whereas males fed 24 or 25g/day showed lower weight gains for the first 12 weeks. Females fed 17g/day showed weight gains that were lower than ad-libtum fed animals at all intervals. – Key words: Crl:CD(SD)IGS, Controlled feeding

CD (SD) IGS-1998: 131-134

INTRODUCTION

The rat is a required species in toxicology testing. Throughout the 1980's there was a recognized reduction in the longevity of rats strains commonly used in carcinogenicity studies. It has been demonstrated that feeding rats a daily ration produces smaller, healthier animals with lower incidence of obesity, renal pathologies and greater longevity and delays the onset of spontaneous tumors. [1,2,3].

One of the most commonly used strains of rat has been the "CD" rat from Charles River Laboratories (CRL). Debate as to the cause of poor survival consisted of environmental, nutrition and animal breeding issues. The International Genetic Standard (IGS) CD rats have been produced to improve survival and to provide consistent animals from the various breeding facilities.

The IGS rats have been used in this laboratory since November 1995 for acute, chronic and carcinogenicity studies. Projects have been conducted using either ad-libitum or controlled daily feeding.

MATERIALS AND METHODS

Animals were obtained from CRL facilities at Raleigh NC in the USA, and St Constant, Quebec in Canada. For two of the projects described (Projects A and B), the animals obtained prior to the IGS[R] designation were identified as Crl:CD(SD)BR, rats (CD) and were obtained from Canada; the IGS rats (Crl:CD(SD)IGS), for 3 projects (C, D and E) were also obtained from Canada and one set of animals (Project F) was obtained from Raleigh NC. All six projects represent the control groups from 2 year carcinogenicity studies. Projects A, B, D and E consist of 2 groups of 60 males and 60 females, Project F consists of 2 groups of 50 males and 50 females and Project C consists of 1 group of 60 males and 60 females.

All animals were housed individually in stainless steel wire mesh-bottomed cages, equipped with an automatic watering valve. Each cage was clearly labelled with a colour-coded cage card indicating project, group, animal numbers, sex and dose level. Each animal was uniquely identified using a tail tattooing system.

The conditions for animal room environment and photoperiod were temperature 22 ± 3°C; humidity 50 ± 20% and light cycle 12 hours light and 12 hours dark. The animals were housed in either rooms in which the air was changed with fresh air 10-15 times per hour or in laminar air flow rooms in which the air was changed approximately 100 times per hour.

In Projects A, B and C, animals had free access to a standard certified pelleted commercial laboratory diet (PMI Certified Rodent Chow 5002: PMI Feeds Inc. St Louis MO U.S.A.), Project F was fed daily powdered PMI Certified Chow 5002 - 24g for males and 17g for females, Projects D and E were fed Certified Rodent Diet Extruded 5L35 4.2g pellets : PMI Feeds Inc. St Louis MO U.S.A. - Project D animals were fed daily, 21g for males and 17g for females, Project E animals were fed daily 25g for males and 17g for females.

Municipal tap water which had been softened, purified by reverse osmosis and sterilized by ultraviolet light was freely available.

Animals were examined twice daily for mortality and signs of ill health or reaction to treatment on all studies. A complete detailed examination was performed weekly and palpation for masses was first performed in week 26 and weekly thereafter. The food intake for ad-libitum fed animals was determined weekly. Body weights were recorded weekly.

RESULTS

The average daily food intake for ad-libitum fed animals was 29 to 32g for males and 19 to 25g for females. Thus males of Project D fed 21g/day received 65 to 80% of ad-libitum fed whereas males fed 24g or 25g/day received 70 to 80% and 75% to 85%, respectively. The females of Projects D, E and F received 68% to 89% of ad libitum fed animals.

The body weight gains for the periods 0 to 4, 4 to 12, 12 to 24 and 24 to 52 weeks were compared for all projects. For all periods, the weight gains of the CD males and females (Projects A and B) given food ad-libitum were similar the those of the IGS ad-libitum fed animals (Project C). The body weight gain of males fed daily were lower than ad-libitum fed animals mainly during the first 12 weeks, thereafter the body weight gain for ad-litum and daily fed animals were essentially similar.

For females, difference in the body weight gain for the daily fed animals compared to ad-litum animals was greatest during the first 4 weeks and the magnitude of the difference decreased between weeks 4 and 24 but the magnitude increased for the period of weeks 24 to 52.

Table 1. Food Consumption of Ad Libitum Fed Rats (g/day)

	Males			Females		
	Project A n=120	Project B n=120	Project C n=60	Project A n=120	Project B n=120	Project C n=60
Week						
0-4	29	29	26	20	20	19
4-8	31	31	30	22	21	21
8-12	32	29	31	23	20	20
12-16	31	29	30	22	20	20
16-20	30	29	29	22	20	20
20-24	30	29	30	22	21	21
24-28	31	29	31	23	21	21
28-32	30	29	30	23	21	21
32-36	31	29	29	23	22	22
36-40	31	29	30	24	22	22
40-44	31	29	31	24	23	22
44-48	32	30	30	24	23	22
48-52	32	29	30	25	22	23

Table 2. Body Weight (g)
Males

Week	Project A	Project A	ProjectB	ProjectB	Project C	Project D	Project D	Project E	Project E	Project F	Project F
	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=50	n=50
0	197.3	196.6	172.6	171.3	194.6	170.5	165.8	196.6	196	197.2	200.8
4	385.7	390.5	382.7	375.6	383.7	302.4	300.2	331.9	324.8	301.4	301.5
8	480.1	491.9	495.5	485.1	482.3	344.4	335.7	406.3	404.1	394.1	383.6
12	536.8	552.7	550.7	537.0	547.4	376.0	371.5	456.8	458.3	452.8	440.6
16	571.5	595.5	588.6	572.4	590.1	403.2	410.9	487.6	497.5	493.3	479.2
20	603.9	616.3	613.1	597.4	619.9	437.9	438.2	518.5	529.4	527.2	505.0
24	631.7	640.7	636.1	624.0	645	454.7	462.6	557.8	557.5	559.3	524.42
8	651.8	666.5	656.6	645.1	667.7	481.4	479.1	572.2	564.1	578.9	543.6
32	665.2	685.7	677.4	662.5	692.4	501.3	499.5	602.0	583.8	582.9	546.7
36	677.7	697.7	693.3	681.5	709.5	524.5	521.3	629.4	602.9	585.5	548.8
40	696.1	708.2	706.3	697.8	720.3	536.3	528.3	653.1	617.5	596.6	565.5
44	714.2	720.8	716.7	703.7	739.1	555.2	546.2	665.6	629.0	635.6	607.9
48	731.4	741.1	736.6	728.1	753.8	565.0	554.7	682.9	651.2	647.5	623.1
52	740.2	756.3	751.5	739.2	766.4	574.0	567.9	684.6	659.6	653.8	644.3

Females

Week	Project A	Project A	Project B	Project B	Project C	Project D	Project D	Project E	Project E	Project F	Project F
	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=50	n=50
0	147.9	147.9	134.6	135.0	152.5	152.2	153.9	138.8	139	136.1	134.3
4	227.7	230.2	223.2	225.9	229.7	192.8	202.9	188.7	189.9	201.4	198.7
8	272.1	278.0	263.4	263.4	265.0	239.0	238.0	221.1	222.5	213.5	216.7
12	300.1	307.8	286.4	286.4	288.0	274.0	263.6	242.5	239.7	232.0	234.4
16	316.0	322.4	304.7	311.7	302.1	293.5	279.5	256.9	254.6	252.6	253.3
20	326.0	334.1	315.2	325.1	316.5	306.9	288.8	271.9	268.9	263.5	265.6
24	339.7	350.7	329.8	338.1	330.0	315.3	301.9	283.6	282.9	272.2	272.4
28	356.8	369.3	341.1	348.5	346.1	323.7	307.8	285.2	286.7	283.1	285.6
32	372.8	386.8	359.0	365.9	359.3	325.3	307.9	295.7	299.6	290.6	293.5
36	386.6	395.1	373.9	385.5	368.3	323.9	300.8	304.3	309.9	304.7	305.4
40	397.3	407.9	389.0	396.9	380.9	327.2	307.3	312.3	319.8	310.1	311.0
44	407.2	421.4	410.1	418.5	398.0	342.8	331.0	316.6	321.3	318.7	320.7
48	420.3	439.8	427.9	434.2	404.6	347.1	339.8	324.6	331.2	325.6	327.8
52	443.6	455.3	432.3	438.5	419.8	358.2	345.0	325.9	334.9	333.2	339.0

Table 3. Group Mean Body Weight Gain (g) - males

Males	Weeks			
	0-4	4-12	12-24	24-52
Ad-libitum				
Project A	188.4	151.1	94.9	108.5
Project A	193.9	162.2	88.0	115.6
Project B	210.1	168.0	85.4	115.4
Project B	204.3	161.4	87.0	115.2
Project C	189.1	163.7	97.6	121.4
Mean	197.2	161.3	90.6	115.2
21g/day				
Project D	131.9	73.6	78.7	119.3
Project D	134.4	71.3	91.1	105.3
Mean	133.2	72.5	84.9	112.3
25g/day				
Project E	135.3	124.9	101.0	126.8
Project E	128.8	133.5	99.2	102.1
Mean	132.1	129.2	100.1	114.5
24g/day				
Project F	104.2	151.4	106.5	94.5
Project F	100.7	139.1	83.8	119.9
Mean	102.5	145.3	95.1	107.2
	Body weight gain as percent of Ad-libitum fed animals			
21g/day	68%	45%	94%	97%
24g/day	52%	90%	105%	93%
25g/day	67%	80%	111%	99%

Table 3. Group Mean Body Weight Gain (g) - females

Females	Weeks			
	0-4	4-12	12-24	24-52
Ad-libtum				
Project A	79.8	72.4	39.6	103.9
Project A	82.3	77.6	42.9	104.6
Project B	88.6	63.2	43.4	102.5
Project B	90.9	60.5	51.7	100.4
Project C	77.2	58.3	42.0	89.8
Mean	83.8	66.4	43.9	100.2
17g/day				
Project D	65.3	30.6	40.2	61.0
Project D	64.4	35.7	38.0	66.6
Project E	49.9	53.8	41.1	42.3
Project E	50.9	49.8	43.2	51.3
Project F	40.6	81.2	41.3	42.9
Project F	49.0	60.7	38.3	43.1
Mean	53.4	52.0	40.4	51.2
	Body weight gain as percent of Ad-libitum fed animals			
17g/day	64%	78%	92%	51%

Fig. 1.

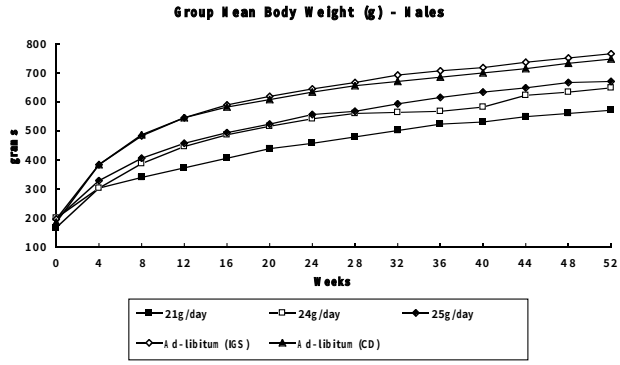
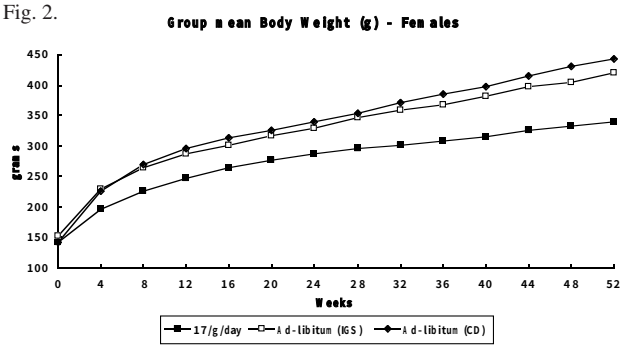


Fig. 2.



DISCUSSION

The growth and food consumption of ad-libitum fed CD strain rats was comparable to that of ad-litum fed IGS strain rats and it is speculated that the survival will be similar. The differences in the weight gain of males fed 21g/day was noticeably lower than those fed 24 or 25g/day, which in turn were lower than those of the ad-litum regime. It remains to be seen as to whether 21g/day is too low to maintain health for the full duration of a 2 year study. Indeed, the benefits of controlled feeding will only be determined upon the completion of the projects with respect to survival and ultimately to the reduction of spontaneous tumours.

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Study on Clinical Chemistry Values in Crj:CD(SD)IGS Rats

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ABSTRACT. A comparative study was conducted of clinical chemistry values in Crj:CD(SD)IGS rats and conventionally used Crj:CD(SD) rats. In a comparison of median values in the reference intervals, AST, ALP, TP, Alb and K were higher in Crj:CD(SD)IGS rats than Crj:CD(SD) rats, while TC, PL, TG and A/G were lower. In a comparison of the RANGE (difference between lower and upper limits of the reference intervals), the RANGE was wider for Crj:CD(SD)IGS rats than Crj:CD(SD) rats for ALP only. Other parameters were the same or narrower. In a comparison of the degree of variation, ALP, Glu and Cl exhibited larger variation in Crj:CD(SD)IGS rats than Crj:CD(SD) rats, while TC, PL, TG, Ca, IP and K exhibited smaller variation. —Key words: Clinical Chemistry, Rats, IGS, SD

CD (SD) IGS-1998: 135-138

INTRODUCTION

For the evaluation process of the clinical chemistry values on the rodent toxicology study, an understanding of reference intervals from historical background data is very important regardless of which test data is analyzed. When changes occur to the background data, it is necessary to determine the cause of those changes as well as be familiar with precautions that must be taken as a result of those changes. Charles River Inc. recently introduced a new animal breeding system known as the International Genetic Standard System for the production of Sprague-Dawley rats. This new system was developed by Charles River Inc. for the purpose of supplying laboratory animals that are as genetically uniform as possible. Although the use of genetically uniform animals supplied by this system is expected to lead to improve reliability of animal studies, there is also the possibility of changes occurring in the background data as a result of supplying animals using this new system. Since we have been using Crj:CD(SD)IGS rats (abbreviated as IGS rats) supplied by this new system for more than a year, we compared clinical chemistry values between IGS rats and conventionally used Crj:CD(SD) rats (abbreviated as SD rats) to assess any precautions that must be taken relative to clinical chemistry in toxicology studies using IGS rats.

MATERIALS AND METHODS

Data used: Data from control groups used in previous toxicology studies conducted at our testing facility were used for the clinical chemistry data. Data determined for the three year period from 1993 to 1995 was used for SD rats, while that determined for the one year period during 1996 was used for IGS rats.

Test parameters and animal ages: The test parameters used for comparison consisted of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen (UN), creatinine (CRE), glucose (Glu), total cholesterol (TC), phospholipids (PL), triglycerides (TG), total protein (TP), albumin (Alb), albumin-globulin ratio (A/G), calcium (Ca), inorganic phosphorous (IP), sodium (Na), potassium (K) and chloride (Cl). All parameters except for Alb and A/G were measured with an automated biochemical analyzer (Hitachi 736-10, Hitachi, Ltd.). A/G was determined using an automated electrophoresis system (AES-600, Olympus Optical Co., Ltd.), and Alb was calculated from TP and A/G. Data from 7 to 9 week old rats were used for ALP, Glu, Ca and IP, while that from 7 to 23 week old rats were used for other parameters.

Reference intervals and their indices: Reference intervals were determined in the manner described below. Outlying values were first excluded with a box-and-whisker plot. The 95% range was determined according to the normal probability paper method. The median, reference limits (lower limit and upper limit) of the reference intervals were determined for use as indices, after which the RANGE was determined from the difference between the lower and upper limits. Different reference intervals were used according to sex.

Index of variation: Studies in which the control group consisted of 6 animals were extracted, and the standard deviation of each study was totaled (calculation of mean value). There were no distinctions made based on sex.

Difference index:

(1) The following equation was used to compare median values of the reference intervals.
(Median of IGS rat - Median of SD rat)/Median of SD rat x 100 (%)

(2) The following equation was used to compare the RANGE of the reference intervals.
(Range of IGS rat - Range of SD rat)/Median of SD rat x 100 (%)

(3) The following equation was used to compare the degree of variation of one study.
(S.D. of IGS rat - S.D. of SD rat)/S.D. of SD rat x 100 (%)

RESULTS

Table 1 shows the reference intervals and their RANGEs determined in IGS and SD rats. Only in the K data of male IGS rats, the data which were excluded from the box-and-whisker plot, were observed. These excluded K data consisted of 4 animals with low values in the same study.

Table 1. Comparison of reference intervals and RANGE of IGS and SD rats.

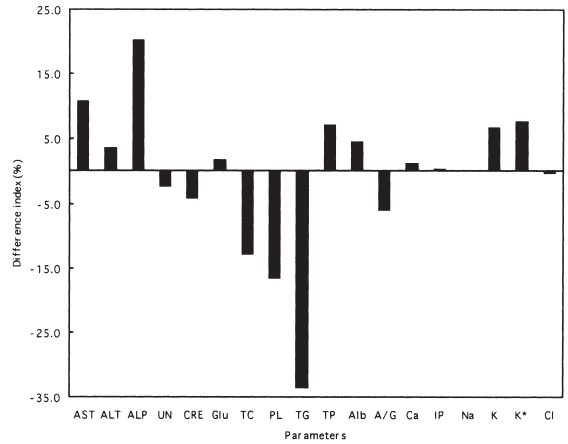
Item	Strain	Male			Female		
		Reference intervals [a)]	RANGE	n	Reference intervals [a)]	RANGE	n
AST	IGS	64 (115.4) 163	99	89	64 (112.4) 155	91	84
u/l	SD	64 (108.4) 175	111	291	55 (97.6) 156	101	280
ALT	IGS	21 (27.7) 40	19	89	16 (21.7) 32	16	84
u/l	SD	19 (26.9) 42	23	291	15 (20.8) 32	17	280
ALP	IGS	320 (525.1) 917	597	89	194 (349.1) 643	449	84
u/l	SD	238 (441.6) 786	548	270	164 (287.4) 561	397	259
UN	IGS	7.3 (10.29) 13.8	6.5	89	8.9 (12.00) 17.3	8.4	84
mg/dl	SD	7.3 (10.54) 15.1	7.8	291	8.4 (12.27) 18.4	10.0	280
CRE	IGS	0.3 (0.32) 0.4	0.1	89	0.3 (0.37) 0.5	0.2	84
mg/dl	SD	0.3 (0.34) 0.4	0.1	246	0.3 (0.38) 0.5	0.2	241
Glu	IGS	62 (86.3) 122	60	89	67 (87.7) 111	44	84
mg/dl	SD	65 (85.7) 118	53	270	69 (85.3) 113	44	259
TC	IGS	31 (46.1) 70	39	89	35 (55.6) 81	46	84
mg/dl	SD	34 (55.1) 83	49	291	37 (61.4) 97	60	280
PL	IGS	57 (75.6) 105	48	89	70 (95.8) 140	70	84
mg/dl	SD	66 (91.6) 132	66	219	73 (113.4) 177	104	208
TG	IGS	17 (28.4) 68	51	89	17 (26.8) 58	41	84
mg/dl	SD	17 (46.7) 97	80	291	16 (37.2) 90	74	280
TP	IGS	5.3 (5.77) 6.4	1.1	89	5.4 (5.90) 6.6	1.2	84
g/dl	SD	4.86 (5.448) 6.18	1.32	291	4.92 (5.445) 6.27	1.35	280
Alb	IGS	2.9 (3.17) 3.4	0.5	89	3.0 (3.40) 3.8	0.8	84
g/dl	SD	2.73 (3.062) 3.41	0.68	291	2.88 (3.218) 3.69	0.81	280
A/G	IGS	1.06 (1.213) 1.45	0.39	89	1.20 (1.369) 1.59	0.39	84
	SD	0.98 (1.295) 1.61	0.63	291	1.18 (1.451) 1.72	0.54	280
Ca	IGS	9.2 (9.68) 10.5	1.3	89	9.0 (9.79) 10.3	1.3	84
mg/dl	SD	8.9 (9.60) 10.3	1.4	270	9.0 (9.63) 10.2	1.2	259
IP	IGS	8.1 (9.06) 10.3	2.2	89	6.8 (8.33) 9.4	2.6	84
mg/dl	SD	7.1 (9.12) 10.7	3.6	270	6.5 (8.24) 9.8	3.3	259
Na	IGS	141 (143.3) 146	5	89	140 (142.0) 145	5	84
mmol/l	SD	140 (143.2) 146	6	291	140 (142.0) 144	4	280
K	IGS	4.2 (4.53) 5.1[b)]	0.9	85	3.4 (4.32) 4.9	1.5	84
mmol/l		3.9 (4.61) 5.1	1.2	89			
	SD	3.3 (4.16) 5.3	2.0	291	3.2 (4.13) 4.9	1.7	280
Cl	IGS	96 (10.4) 104	8	89	96 (101.8) 105	9	84
mmol/l	SD	97 (10.6) 104	7	291	98 (102.1) 106	8	280

SD : Crj:CD(SD) Rat, IGS : Crj:CD(SD)IGS Rat
a) : Figures represent the lower and upper limit of reference intervals, median values were in parentheses.
b) : Excluded the data from 4 animals by box-and-whisker plot.

Comparisons were made of the median values of the reference intervals between IGS rats and SD rats using the "difference index" (Fig. 1). The median values of IGS rats showed higher values for parameters including AST, ALP, TP, Alb and K compared with those of SD rats. While in the case of TC, PL, TG as well as A/G, the median values of IGS rats were lower than those of SD rats. The median values of the other parameters were comparable between IGS rats and SD rats.

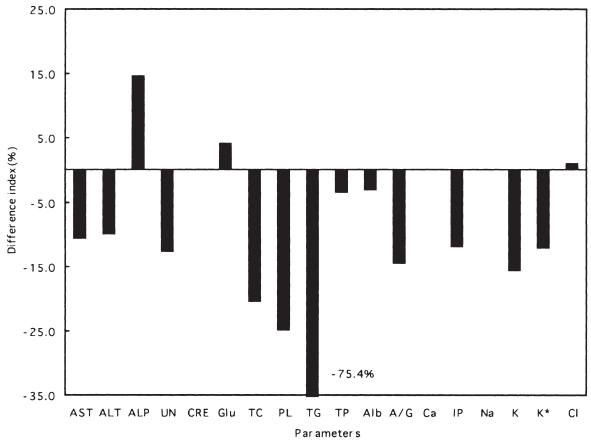
The RANGEs of IGS rats were compared with those of SD rats using the "difference index" (Fig. 2). In the case of ALP, the RANGE of IGS rats became wider than that of SD rats. However, that of the other parameters of IGS rats remained the almost same values or narrowed in comparison with SD rats.

Table 2 shows the mean values of the standard deviation of the data from each study. Comparisons were made of those mean values between IGS rats and SD rats using the "difference index" (Fig. 3). Standard deviations of IGS rats became larger than those of SD rats for parameters including ALP, Glu and Cl, and smaller for parameters including TC, PL, TG, Ca, IP as well as K. The other parameters of the IGS rats were comparable to that of SD rats.



Difference index = (Median of IGS rat-Median of SD rat) / Median of SD rat X 100(%)
K* : Excluded the data from 4 animals by box-and-whisker plot.

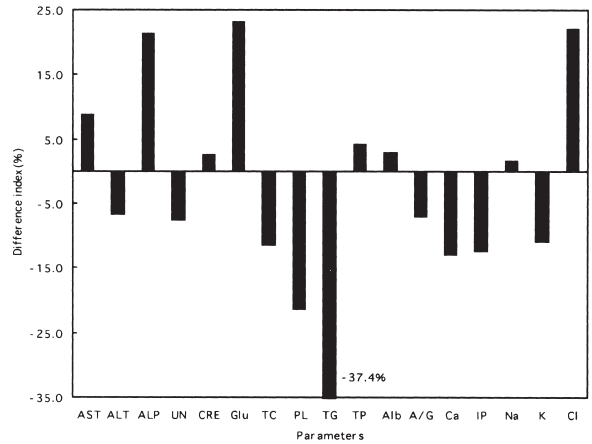
Fig. 1. Difference Index of the Median Values. Parameters having a positive difference index indicate that the median value of the reference intervals of IGS rats are higher in comparison with those of SD rats, while those having a negative one indicate the opposite of the former.



Difference index = (RANGE of IGS rat-RANGE of SD rat) / Median of SD rat x 100(%)

K* : Excluded the data from 4 animals by box-and-whisker plot.

Fig. 2. Difference Index of the RANGEs. Parameters having a positive difference index indicate that the RANGE of IGS rats widened in comparison with that of SD rats, while those having a negative difference index indicate that the RANGE of IGS rats was narrowed compared with that of SD rats.



Difference index = (S.D. of IGS rat-S.D. of SD rat) / S.D. of SD rat x 100(%)

Fig. 3. Difference Index of the Standard Deviations. Parameters having a positive difference index indicate that the variation in a single study of IGS rats was larger than that of SD rats. Those having a negative difference index indicate that the variation of IGS rats was smaller than that of SD rats.

Table 2. Comparison of standard deviation (S.D.) of the data from each study.

Item	IGS		SD	
AST	18.89 ± 8.19	(28)	17.37 ± 7.91	(74)
ALT	4.30 ± 2.72	(28)	4.61 ± 2.40	(74)
ALP	96.87 ± 45.04	(28)	79.82 ± 38.68	(74)
UN	1.634 ± 0.638	(28)	1.769 ± 0.721	(74)
CRE	0.039 ± 0.022	(28)	0.038 ± 0.026	(62)
Glu	9.39 ± 3.89	(28)	7.63 ± 3.02	(72)
TC	10.04 ± 3.38	(28)	11.33 ± 4.07	(74)
PL	13.38 ± 5.56	(28)	17.02 ± 6.97	(50)
TG	9.69 ± 5.95	(28)	15.49 ± 8.33	(74)
TP	0.225 ± 0.075	(28)	0.216 ± 0.079	(74)
Alb	0.140 ± 0.055	(28)	0.136 ± 0.051	(74)
A/G	0.0809 ± 0.0268	(28)	0.0870 ± 0.0316	(74)
Ca	0.216 ± 0.065	(28)	0.248 ± 0.081	(72)
IP	0.451 ± 0.167	(28)	0.514 ± 0.178	(72)
Na	1.28 ± 0.48	(28)	1.26 ± 0.490	(74)
K	0.244 ± 0.099	(28)	0.274 ± 0.12	(74)
Cl	2.00 ± 0.66	(28)	1.64 ± 0.52	(74)

Values represent mean ± S.D. (n)

DISCUSSION

Changes in median values of the reference intervals are naturally worth noting from the viewpoint of the background data of study facilities. These changes do not actually present a problem in toxicology studies. This is because control groups are usually assigned for each study. There is the possibility, however, of changes in median values indicating a change in the reactivity of an animal to a drug. For example, the increased background values of Alb may have an effect by increasing the amount of Alb bound to the drug [6]. In this study, decreased back ground values of serum TC, PL and TG levels in IGS rats may cause a change in the reactivity and sensitivity of the conventional SD rats in the case of drugs such as hypolipidemic drugs that affect lipid metabolism.

In the cases of RANGE and variation, changes are considered to have a significant effect on the data evaluation process of toxicology studies. Because the assessment of the effects of a drug in a toxicology study is usually made based on the magnitude of spontaneously occurring error. In this study, the unit study variations of ALP, Glu and Cl of IGS rats were increased, and the RANGE became wider for ALP of IGS rats in particular in comparison with the conventional SD rats. It is believed that the error for these parameters increased, thereby

making it easier to overlook effects of drug administration. In the case of parameters of IGS rats including TC, PL, TG, Ca, IP as well as K, for which the unit study variation became smaller compared with SD rats, it became easier to find even slight changes due to the smaller degree of error. There are still many cases, however, in which even slight changes do not necessarily mean an effect caused by drug administration. Careful toxicological assessment based on the reference intervals therefore becomes even more important in these cases.

In this report, data collected over a period of three years was used for SD rats, while that collected over a one year period was used for IGS rats. Thus, there is a considerable difference in the size of the data. More detailed studies will be able to be conducted in the future as more data on IGS rats is gathered. There are also differences in clinical chemistry values between the facilities performing the tests. Values from other facilities or reference values frequently cannot be applied directly to one's own facility. Although it is possible to have some idea of the tendency of a change, the degree of that tendency is likely to vary between each facility. Particular caution is required with respect to enzyme activity, since the types of reagents used (substrate, buffer composition, etc.) can have a considerable effect on the outcome [1-5].

Careful consideration should therefore be given to the factors described above when assessing the results of clinical chemistry using IGS rats to ensure that the effects of drug administration are accurately determined.

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

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ABSTRACT. Hematological 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 indicated that both IGS and CD rats had similar biological values for the almost all parameters examined. However, white blood cell count (WBC) in males and percentage of monocytes and APTT in both sexes of IGS rats were greater than those of CD rats. The percentages of segmented neutrophils in both sexes of IGS rats were smaller than those of CD rats. The hematological parameters in IGS rats obtained in the present study may be useful for toxicity studies. —Key words: Crj:CD(SD)IGS, Hematology, Rat

CD (SD) IGS-1998: 139-142

INTRODUCTION

The gold 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 gold 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 hematological parameters of IGS rats have not yet been fully accumulated. Therefore, we investigated hematological parameters of IGS rats and compared the results thus obtained 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, Chiba, Japan). The animals were divided into three groups of 30 males and 30 females each and acclimated to laboratory animal facilities at our institute for 12 days. The general conditions of all rats were a good state of health during an acclimatization period. The animals were housed individually in a stainless wire cage (21W x 35D x 20H cm) during the acclimatization and study periods. The animal room was maintained at a temperature of 21-25 ºC and relative humidity of 30-70% 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). Animals were allowed free access to diet sterilized by radiation of 30 kGy (CR-LPF, Oriental Yeast Co., Tokyo, Japan) and tap water.

Hematological 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. Ethylenediaminetetraacetic acid disodium salt was used as anticoagulant for the determination of red blood cell counts (RBC), WBC, hemoglobin concentration (HGB), hematocrit value (HT), platelet counts (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) using a microcell counter (CC-180A, Toa Medical Electronics, Hyogo, Japan). Citric acid disodium salt was used as anticoagulant for the determination of prothrombin time (PT) and activated partial thromboplastin time (APTT) using an automated coagulation analyzer (ST4, Boehringer Mannheim, Tokyo, Japan). Differential leukocyte counts were determined microscopically with May-Gruenwald Giemsa stain. Reticulocyte counts were determined microscopically by the method of Brecher.

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's t-test. Differences were considered significant at $p<0.05$ and $p<0.01$.

RESULTS

The hematological parameters in the male and female IGS rats determined in the present study are shown in Tables 1 and 2. The hematological parameters in male and female CD rats that have been determined on the other occasions at our institute are shown in Tables 3 and 4. The results of hematological 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 9 weeks of age, the values of RBC, HGB, HT, WBC, band neutrophils, lymphocytes, monocytes and APTT were significantly larger than those of their CD counterparts; conversely, the values of MCH, MCHC, segmented neutrophils, eosinophils, PLT and PT were significantly smaller than those of CD rats. In male IGS rats at 18 weeks of age, the values of MCHC, WBC, lymphocytes and

Table 1. Hematological parameters of male Crj:CD (SD) IGS rats

Parameters	Weeks of age (No. of rats)		
	9 (30)	18 (30)	31 (30)
RBC (x10^{4}count/mm^3)	778 ± 26 **	851 ± 61	869 ± 55
HGB (g/dL)	14.9 ± 0.5 **	14.8 ± 0.8	14.7 ± 0.8
HT (%)	47.4 ± 1.9 **	45.4 ± 2.6	45.3 ± 2.4
MCV (μm^3)	61 ± 2	54 ± 3	52 ± 3
MCH (pg)	19.1 ± 0.5 *	17.4 ± 0.8	16.9 ± 0.7
MCHC (%)	31.4 ± 0.9 **	32.6 ± 0.8 **	32.5 ± 0.7 **
WBC (x10^{2}count/mm^3)	135 ± 29 **	132 ± 27 **	131 ± 31 **
Differential leucocyte counts (%)			
Band neutrophil	0.6 ± 0.4 **	0.4 ± 0.4	0.8 ± 1.0 *
Segmented neutrophil	6.8 ± 2.7 **	7.9 ± 2.5 **	12.3 ± 8.5
Lymphocyte	90.2 ± 2.9 *	88.5 ± 3.2 **	82.7 ± 9.6
Monocyte	1.9 ± 0.7 **	2.3 ± 1.1 **	3.2 ± 1.7 **
Eosinophil	0.5 ± 0.4 **	0.7 ± 0.6 **	0.9 ± 0.8 **
Basophil	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.3
Others	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PLT (x10^{4}count/mm^3)	100.5 ± 9.4 **	98.3 ± 8.9 *	103.4 ± 14.0
PT (sec)	16.0 ± 1.1 **	14.5 ± 1.1 **	15.1 ± 1.2 **
APTT (sec)	17.4 ± 1.4 **	15.0 ± 2.8	15.0 ± 2.0
Reticulocyte counts (‰)	24 ± 6	21 ± 4	23 ± 5 **

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 2. Hematological parameters of female Crj:CD (SD) IGS rats

Parameters	Weeks of age (No. of rats)		
	9 (30)	18 (30)	31 (30)
RBC (x10^{4}count/mm^3)	748 ± 47 **	757 ± 41 **	755 ± 36 **
HGB (g/dL)	14.4 ± 0.7	14.1 ± 0.7 *	14.0 ± 0.7 **
HT (%)	43.6 ± 2.0	42.1 ± 2 **	42.0 ± 2.2 *
MCV (μm^3)	58 ± 3 **	56 ± 2	56 ± 2
MCH (pg)	19.3 ± 0.8 **	18.6 ± 0.6 **	18.6 ± 0.6 *
MCHC (%)	33.0 ± 0.9	33.4 ± 0.9 **	33.3 ± 0.6
WBC (x10^{2}count/mm^3)	91 ± 27 *	64 ± 14	59 ± 13
Differential leucocyte counts (%)			
Band neutrophil	0.4 ± 0.4	0.6 ± 0.8	0.9 ± 0.6 **
Segmented neutrophil	7.7 ± 3.5 *	9.7 ± 2.8 **	14.4 ± 5.4
Lymphocyte	89.4 ± 3.9	86.3 ± 4.2 *	81.3 ± 5.7
Monocyte	1.6 ± 0.9 **	1.8 ± 1 **	1.8 ± 0.8 **
Eosinophil	0.9 ± 0.8 **	1.5 ± 1.2	1.5 ± 0.9
Basophil	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.4
Others	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
PLT (x10^{4}count/mm^3)	105.4 ± 9.2 **	90.6 ± 10.3 **	92.6 ± 9.9
PT (sec)	15.3 ± 0.6 **	14.2 ± 0.8 **	14.9 ± 1.1 **
APTT (sec)	15.8 ± 1.2 **	13.2 ± 1.7	14.3 ± 1.8
Reticulocyte counts (‰)	20 ± 3 **	19 ± 4	20 ± 4

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

monocytes were significantly larger than the corresponding values of CD rats; on the other hand, the values of segmented neutrophils, eosinophils, PLT and PT were significantly smaller than those of CD rats. In male IGS rats at 31 weeks of age, the values of WBC, band neutrophils, monocytes and reticulocyte counts were significantly larger than the corresponding values of CD rats; in contrast, the values of MCHC, eosinophils and PT were significantly smaller than those of CD rats (Tables 1 and 3). The values of WBC and the percentages of monocytes in IGS rats at 9, 18 and 31 weeks of age were 1.2- to 1.4-fold and 2.6- to 3.6-fold larger than those of CD rats, respectively. The percentages of segmented neutrophils in IGS rats at 9 and 18 weeks of age accounted for 70% and 60% of the corresponding values of CD rats. APTT in IGS rats at 9 weeks of age was 1.2-fold longer than that of CD rats.

In female IGS rats at nine weeks of age, the values of RBC, WBC, monocytes and APTT were significantly larger than the corresponding values of CD rats; conversely, the values of MCV, MCH, segmented neutrophils, eosinophils, PLT, PT and reticulocyte counts were significantly smaller in IGS rats. At 18

Table 3. Hematological parameters of male Crj:CD (SD) rats

Parameters	Weeks of age					
	8 - 12		14 - 18		20 - 24	
RBC (x10⁴count/mm³)	754 ± 52	(192) [a]	855 ± 60	(115)	856 ± 61	(72)
HGB (g/dL)	14.5 ± 0.5	(192)	14.7 ± 0.4	(115)	14.7 ± 0.6	(72)
HT (%)	45.3 ± 1.6	(192)	45.8 ± 1.3	(115)	44.4 ± 2.1	(72)
MCV (μm³)	61 ± 4	(186)	54 ± 3	(115)	52 ± 3	(72)
MCH (pg)	19.4 ± 1.1	(186)	17.3 ± 1.0	(115)	17.2 ± 1.1	(72)
MCHC (%)	32.1 ± 0.7	(186)	32.1 ± 0.5	(115)	33.0 ± 0.9	(72)
WBC (x10²count/mm³)	113 ± 29	(192)	102 ± 25	(115)	97 ± 27	(78)
Differential leucocyte counts (%)		(192)		(115)		(72)
Band neutrophil	0.3 ± 0.3		0.3 ± 0.4		0.3 ± 0.4	
Segmented neutrophil	9.4 ± 3.5		13.0 ± 4.7		14.9 ± 4.5	
Lymphocyte	88.7 ± 3.8		84.7 ± 5.0		82.3 ± 5.3	
Monocyte	0.7 ± 0.6		0.9 ± 0.8		0.9 ± 0.7	
Eosinophil	0.9 ± 0.7		1.2 ± 0.7		1.5 ± 0.9	
Basophil	0.1 ± 0.2		0.1 ± 0.1		0.1 ± 0.1	
Others	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
PLT (x10⁴count/mm³)	112.9 ± 14.2	(192)	103.0 ± 11.9	(115)	101.9 ± 12.5	(72)
PT (sec)	16.6 ± 0.9	(172)	15.9 ± 0.8	(115)	16.3 ± 1.1	(72)
APTT (sec)	14.0 ± 0.8	(12)	N.E.		13.6 ± 2.4	(10)
Reticulocyte counts (‰)	26 ± 6	(180)	21 ± 4	(115)	19 ± 6	(72)

Each value represents mean ± S.D..
a: No. of rats.
N.E.: Not examined.

Table 4. Hematological parameters of female Crj:CD (SD) rats

Parameters	Weeks of age					
	8 - 12		14 - 18		20 - 24	
RBC (x10⁴count/mm³)	719 ± 55	(190) [a]	799 ± 58	(115)	795 ± 49	(73)
HGB (g/dL)	14.4 ± 0.6	(190)	14.4 ± 0.4	(115)	14.4 ± 0.5	(73)
HT (%)	44.1 ± 1.7	(190)	43.7 ± 1.1	(115)	43.0 ± 1.5	(73)
MCV (μm³)	62 ± 4	(184)	55 ± 4	(115)	55 ± 3	(73)
MCH (pg)	20.2 ± 1.3	(184)	18.0 ± 1.3	(115)	18.2 ± 0.9	(73)
MCHC (%)	32.7 ± 0.6	(184)	32.8 ± 0.5	(115)	33.5 ± 0.7	(73)
WBC (x102count/mm³)	80 ± 22	(190)	68 ± 19	(115)	63 ± 19	(79)
Differential leucocyte counts (%)		(190)		(115)		(73)
Band neutrophil	0.3 ± 0.4		0.4 ± 0.4		0.4 ± 0.5	
Segmented neutrophil	9.7 ± 4.1		13.4 ± 4.9		15.7 ± 6.7	
Lymphocyte	87.9 ± 4.4		84.2 ± 5.5		81.5 ± 7.0	
Monocyte	0.7 ± 0.6		0.6 ± 0.7		0.8 ± 0.6	
Eosinophil	1.4 ± 0.9		1.5 ± 1.1		1.6 ± 0.9	
Basophil	0.1 ± 0.2		0.1 ± 0.1		0.2 ± 0.2	
Others	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0	
PLT (x10⁴count/mm³)	113.1 ± 14.1	(190)	102.9 ± 9.6	(115)	95.9 ± 10.4	(73)
PT (sec)	17.4 ± 1.3	(171)	16.0 ± 0.8	(115)	15.9 ± 0.9	(73)
APTT (sec)	13.6 ± 1.4	(12)	N.E.		13.6 ± 1.4	(10)
Reticulocyte counts (‰)	25 ± 7	(177)	20 ± 5	(115)	20 ± 5	(73)

Each value represents mean ± S.D..
a: No. of rats.
N.E.: Not examined.

weeks of age, the values of MCH, MCHC, lymphocytes and monocytes were significantly larger in IGS rats than in CD rats; in contrast, the values of RBC, HGB, HT, segmented neutrophils, PLT and PT were significantly smaller in IGS rats. At 31 weeks of age, the values of MCH, band neutrophils and monocytes were significantly larger in IGS rats; on the other hand, the values of RBC, HGB, HT and PT were significantly smaller in IGS rats (Tables 2 and 4). The percentages of monocytes in IGS rats at 9, 18 and 31 weeks of age were 2.3- to 3-fold greater than those of CD rats. The percentages of segmented neutrophils in IGS rats at 9 and 18 weeks of age accounted for 80% and 72% of those in CD rats, respectively. APTT in the IGS rats at 9 weeks of age were 1.2-fold longer than that of CD rats. RBC and percentages of segmented neutrophils in males and females of both strains and percentages of monocytes in males of both strains tended to increase with age. On the other hand, percentages of lymphocytes in both sexes of both strains tended to decrease with age.

DISCUSSION

The results obtained in the present study revealed that many of hematological parameters of IGS rats differed significantly from those of CD rats. The values of hematological parameters in IGS rats differing more than 20% from those in CD rats were as follows: WBC in males and, percentages of monocytes and APTT in both sexes of IGS rats were greater than those of CD rats, whereas percentages of segmented neutrophils in both sexes of IGS rats were smaller than those of CD rats. The remaining parameters in IGS rats were observed to be in the biological range of CD rats. Wolford et al. [3] have reported the reference range data for hematological values in Crl:COBSCD(SD) rats in the age groups of <6 months, 6-18 months and >18 months. The hematological values in IGS rats obtained from the present study were in their reference range except for HT, MCV, MCHC and WBC. The hematological reference data for rats have also been described by the other authors [1, 2]. When compared with their data, the hematological parameters of IGS rats were comparable to those described by these authors except for percentages of segmented neutrophils and APTT. In comparison with the aforementioned three reference range data, there were no constant trends in appearance of variation in the hematological values of IGS rats. It thus appeared that the values of the hematological parameters observed in IGS rats may be in the biological range of the Sprague-Dawley strain.

Age-related changes in hematological parameters in Sprague-Dawley rats have been well documented [1, 4]. According to these authors, RBC increased between the ages of 2 and 3 months, and subsequently decreased with age; neutrophil counts (absolute and percentages) began to increase after approximately 6 months; lymphocyte number and its percentages decreased with age. In the hematological data of IGS rats and our background data of CD rats, RBC, percentages of segmented neutrophils and lymphocytes roughly agreed with those data.

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

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General Toxicological Parameters in Crj:CD (SD)IGS Rats and Crj:CD (SD) Rats

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ABSTRACT. Charles River Inc. established a new breeding system of laboratory animals called Gold Standard System. In this experiment, we measured for general toxicological parameters in Crj:CD (SD) IGS rats bred under this system (IGS rat) to make a part of our background data. Also, how the values of parameters differ from those of conventionally used Crj:CD rats (SD rat) were compared.

Body weights and food consumption in male and female IGS rats were significantly lower than those of SD rats. Normal estrus cycles were appeared oftener in female IGS rats than SD rats at 29-31 weeks of age. In ophthalmological examination, some abnormal findings known to occur spontaneously were observed in IGS rats. In urinalysis, electrolyte and protein excretions in IGS rats of both sexes were lower than those of SD rats. In hematological findings, remarkable difference was not observed between IGS rats and SD rats. In biochemical findings, concentration of triglyceride, total cholesterol, and phospholipid in IGS rats of both sexes were lower than those of SD rats. In organ weights, weights of pituitary, thyroid, adrenal gland, liver and spleen in IGS rats of both sexes were lower than those of SD rats.

As described above, some of general toxicological parameters in IGS rats were significantly different from those of SD rats. We concluded that further investigation is needed to make a more reliable background data. –Key words: Crj:CD (SD) IGS, Rats, Historical data, Toxicology

CD (SD)IGS-1998: 143-154

INTRODUCTION

Charles River Inc. established a new breeding system of laboratory animals called Gold Standard System. Since these animals are bred by parent species supplied periodically from reference colony of Charles River U.S. Inc. in this system, it is said that Charles River Japan Inc. can supply internationally homogeneous laboratory animals. Accordingly, it is expected that the Sprague-Dawley rats (Crj:CD (SD) IGS rats) bred under this system is going to be used generally in several toxicity studies. In this experiment, we measured for general toxicological parameters in Crj:CD (SD) IGS rats (IGS rats) to make a part of our background data. Also, how the values of parameters differ from those of conventionally used Crj:CD rats (SD rat) were compared.

MATERIALS AND METHODS

Animals : Specific-pathogen-free (SPF) IGS rats and SD rats of both sexes were individually obtained at 5 weeks of age from Charles River Japan Inc.. These animals were housed under the barrier-sustained condition in a well-ventilated animal room maintained at a temperature of 22 ± 2°C, a relative humidity of 60 ± 10%, and lightening cycles of 12 hours (8:00-20:00) light and 12 hours dark. They were housed in polycarbonate cages (265 mm ×425 mm ×200 mm, CLEA Japan Inc.) in which White Flake (Charles River Japan Inc.) was spread for bedding. Commercial pellet diet (CE-2, CLEA Japan Inc.) and tap water sterilized by ultra-violet rays were given ad libitum, except for about 18 hours fasting before necropsy.

Observation and examination : Clinical signs were observed every day for all animals. Body weights and food consumption were determined once a week until the age of each animal reaches 32 weeks and every 4 weeks thereafter. Water consumption and estrus cycles were estimated for 10 animals each of both IGS and SD rats at about 10 and 30 weeks of age, and also 10 animals of IGS rats at about 60 and 90 weeks of age. Ophthalmological examinations were carried out for 10 animals of SD rats at 6 and 32 weeks of age, and also 50 animals of IGS rats at 6, 32, 67 and 90 weeks of age. Urinalysis were performed for 10 animals of each rats at 10 and 31 weeks of age, and also all surviving animals of IGS rats at 58 and 88 weeks of age. 10 animals of each rats at 10, 32 and 60 weeks of age, and also all surviving animals at 91 weeks of age were anesthetized with penthobarbital sodium (50mg/kg, ip), and were drawn blood for hematology through the internal jugular vein and for blood chemistry through the abdominal aorta, then necropsied.

Estrus cycle : Vaginal smears were made of vaginal lavages collected from female rats every morning for more than 10 days, and were examined after the Giemsa staining. Types of estrus cycle were classified as follows, normal type that estrus reappeared regularly at 4 or 5 days interval, continuous diestrus type (CD) and continuous estrus type (CE) that mainly diestrus and estrus respectively reappeared, prolongation type (P) that estrus reappeared at more than 6 days interval, and irregular type that the other abnormal cycles than CD, CE and P were included in this type.

Ophthalmological examination : Ocular reflexes (pupillary, palpebral and corneal) were tested before the instillation of a mydriatic. After the application of a mydriatic, eyes were examined by means of a direct ophthalmoscope and a fundus camera. The examination was further performed using a slit lamp biomicroscopy in the case some changes were detected in the anterior segment or the vitreous body.

Urinalysis : Fresh urine samples were collected for about 4 hours under fasting, and analyzed for color, pH, sediment, and protein, glucose, ketone bodies, bilirubin, occult blood, urobilinogen using colorimetric strips (Multistix, Bayer-Sankyo Co., Ltd.). Also, urine samples collected for 24 hours were analyzed for volume, specific gravity, concentration of Na, K and Cl by an automatic analyzer with ion-selective electrodes

(SERA-520, Horiba, Ltd.), and concentration of Ca (OCPC), iP (Fiske-Subbarow), total protein (pyrogarol red), NAG (MCP-NAG substrate) by an automatic analyzer (7050, Hitachi, Ltd.).

Hematology : Hemoglobin concentration (Hb), hematocrit (Hct), erythrocyte count (RBC), leukocyte count (WBC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and also mean corpuscular hemoglobin concentration (MCHC) were determined by a hematological autoanalyzer (Sysmex CC-180A, Toa Medical Electronics Co., Ltd.). Prothrombin time (PT) and activated partial thromboplastin time (APTT) of plasma were determined by a blood coagulation analyzer (KC 10A, Amelung GMBH). Differential leukocyte count (Wright-Giemsa stain) and reticulocyte count (Brecher's method) were determined on the blood smears.

Blood chemistry : Plasma levels of GOT, GPT (IFCC), LDH (Wröbreuski ladue), CPK (creatine phosphate substrate/UV), ALP (Bessy-Lowry), γ-glutamyl transpeptidase (γ-glutamyl CPA substrate), total bilirubin (azobilirubin), total protein (Biuret), albumin (BCG), A/G ratio (calculated), urea nitrogen (urease indophenol), creatinine (Jaffë), glucose (hexokinase), TG (free glycerol elimination method), total cholesterol (enzymatic), phospholipid (enzymatic), Ca (OCPC), iP (Fiske-Subbarow) were determined by an automatic analyzer (7050, Hitachi, Ltd.). Plasma concentration of Na, K and Cl were determined by an automatic analyzer with ion-selective electrodes (SERA-520, Horiba, Ltd.). Also plasma concentration of luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH), insulin, progesterone, estradiol and testosterone were determined by using enzyme immunoassay kits (Amersham International plc., Morinaga Science Research Institute and Cayman Chemical Company).

Organ weights : Organ weights of cerebral, pituitary, thyroid, adrenal gland, heart, lung, submandibular gland, liver, kidney, thymus, spleen, testis, seminal vesicle, prostate, ovary and uterus were determined.

Statistical analysis : The significant difference in test data

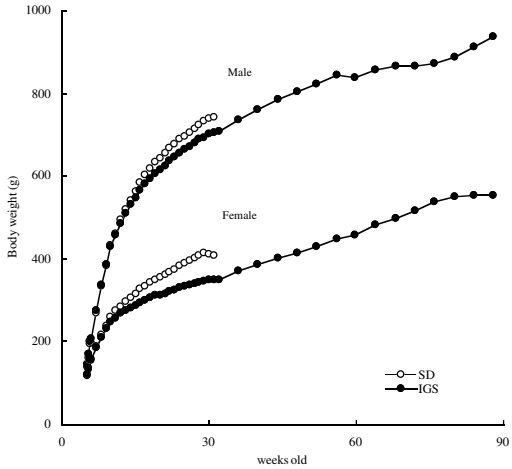

Fig. 1. Body weight changes in SD rats and IGS rats.

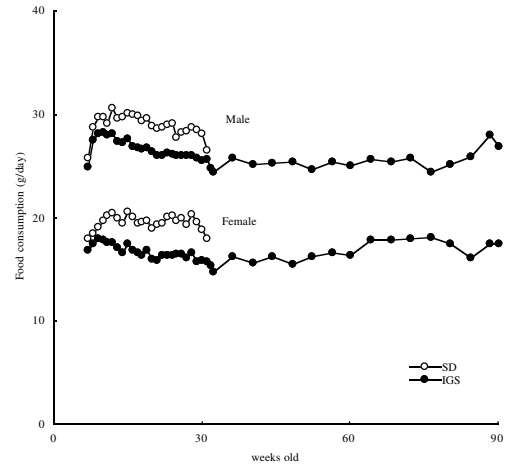

Fig. 2. Food consumption in SD rats and IGS rats.

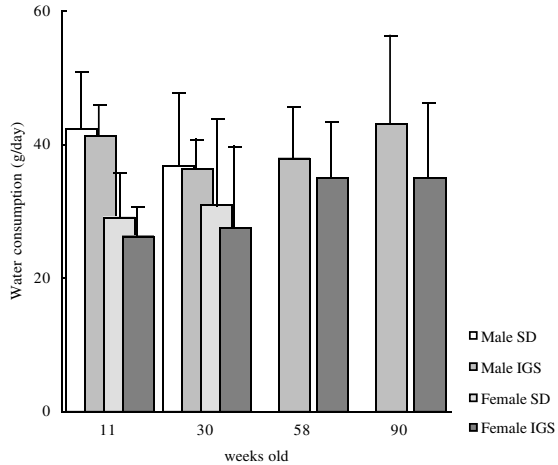

Fig. 3. Water consumption in SD rats and IGS rats. Each values represents mean+S.D

between IGS rats and SD rats were examined by Student's t-test. The frequency of type of estrus cycle between IGS rats and SD rats were analyzed using the chi-square test.

RESULTS AND DISCUSSION

No abnormal clinical signs were observed throughout the experimental period. Body weight changes, food and water consumption are shown in Fig. 1-3. Body weights in male and female IGS rats were significantly lower than those of SD rats from about 23 and 10 weeks of age, respectively. Food consumption in IGS rats of both sexes were significantly lower than those of SD rats from about 6 weeks of age. Because of no difference in their food converting efficiency, this lower body weights observed in IGS rats were considered to be caused by lower food consumption mainly. Water consumption in IGS rats was slightly lower than that of SD rats, but significant difference was not observed.

The results of estrus cycle are shown in Table 1. No abnormal types of estrus cycle were observed in IGS rats and SD rats at 11-12 weeks of age. At 29-31 weeks of age, frequency of normal type in IGS rats was still slightly higher than that of SD rats, though statistical difference was not observed. This result suggested that IGS rats might maintain regular estrus cycle longer than SD rats. All of IGS rats showed abnormal types at 59-61 weeks and 83-86 weeks of age. Especially frequencies of continuous diestrus type and prolongation type increased with an age related manner.

In the ophthalmological examination, no abnormality was observed in SD rats of both sexes at 6 and 32 weeks of age. Abnormal iris (hemorrhage and absence of pupillary light reflex) was found in two male and one female IGS rats at 6 weeks of age. One focal retial atrophy from 6 to 90 weeks of age, and one abnormality of the iris (insufficient mydriasis) from 32 to 90 weeks of age were found in male IGS rats. Tortuosity of the retinal arteriole was found in two male IGS

Table 1. Estrus cycles in SD rats and IGS rats

weeks of age		female SD	female IGS
11-12	n	10	20
	Normal type	10	20
	Continuous diestrus type	0	0
	Continuous estrus type	0	0
	Prolongation type	0	0
	Irregular type	0	0
29-31	n	10	10
	Normal type	3	6
	Continuous diestrus type	1	0
	Continuous estrus type	4	1
	Prolongation type	1	1
	Irregular type	1	2
59-61	n		20
	Normal type		0
	Continuous diestrus type		2
	Continuous estrus type		4
	Prolongation type		7
	Irregular type		7
83-86	n		20
	Normal type		0
	Continuous diestrus type		7
	Continuous estrus type		2
	Prolongation type		10
	Irregular type		1

rats at 67 weeks, in three male IGS rats at 90 weeks and in one female IGS rat at 67 and 90 weeks of age. Lenticular opacity was found in one male IGS rat at 67 weeks, in two male IGS rats at 90 weeks, in one female IGS rat at 32 and 67 weeks and in two female IGS rats at 90 weeks of age. These findings obserbed in IGS rats were not uncommon and known to occur spontaneously[3].

The results of urinalysis are shown in Table 2. Electrolyte and protein excretions in male and female IGS rats were lower than those of SD rats. Significance of lower excretions were

Table 2. Urinalysis in SD rats and IGS rats

	weeks of age	male SD	male IGS	female SD	female IGS
n	10	10	10	10	10
	31	10	10	10	10
	58		48		50
	88		36		40
Volume (ml/24hrs)	10	20.9 ± 4.3	22.1 ± 2.9	16.7 ± 5.2	13.8 ± 4.2
	31	22.6 ± 7.1	19.7 ± 2.0	17.1 ± 9.2	10.9 ± 2.6
	58		20.8 ± 7.0		15.9 ± 5.2
	88		25.9 ± 7.8		18.9 ± 6.4
Volume/BW (ml/100gBW)	10	4.80 ± 0.87	5.14 ± 0.61	6.35 ± 1.89	5.53 ± 1.60
	31	3.03 ± 0.88	2.81 ± 0.25	4.24 ± 2.27	3.02 ± 0.65
	58		2.47 ± 0.79		3.57 ± 1.38
	88		2.74 ± 0.80		3.40 ± 1.17
Specific gravity	10	1.049 ± 0.007	1.044 ± 0.006	1.044 ± 0.011	1.047 ± 0.012
	31	1.045 ± 0.012	1.039 ± 0.006	1.048 ± 0.016	1.058 ± 0.010
	58		1.041 ± 0.012		1.043 ± 0.010
	88		1.030 ± 0.007		1.036 ± 0.009
pH	10	7.4 ± 0.4	7.5 ± 0.4	7.4 ± 0.5	7.6 ± 0.4
	31	7.8 ± 0.4	8.0 ± 0.3	7.8 ± 0.4	8.1 ± 0.3*
	58		7.7 ± 0.4[a)]		7.8 ± 0.5
	88		7.2 ± 0.5[b)]		7.1 ± 0.5[c)]
Total Na (mmol/24hrs)	10	1.81 ± 0.33	1.83 ± 0.38	1.30 ± 0.25	1.19 ± 0.27
	31	1.31 ± 0.50	1.25 ± 0.43	1.17 ± 0.40	0.66 ± 0.18**
	58		3.22 ± 0.96		1.80 ± 0.44
	88		0.84 ± 0.37		1.14 ± 0.36
Total K (mmol/24hrs)	10	3.94 ± 0.45	3.84 ± 0.34	2.70 ± 0.42	2.35 ± 0.26*
	31	4.77 ± 0.96	3.40 ± 0.71**	3.18 ± 0.74	2.72 ± 0.40
	58		3.00 ± 0.67		2.07 ± 0.52
	88		2.23 ± 0.43		2.19 ± 0.56
Total Cl (mmol/24hrs)	10	2.11 ± 0.31	2.11 ± 0.35	1.41 ± 0.20	1.29 ± 0.21
	31	2.28 ± 0.61	1.85 ± 0.37	1.81 ± 0.57	1.36 ± 0.32*
	58		1.10 ± 0.53		1.27 ± 0.28
	88		0.38 ± 0.31		1.00 ± 0.32
Total Ca (mg/24hrs)	10	0.94 ± 0.36	0.77 ± 0.27	1.55 ± 0.62	0.80 ± 0.18**
	31	1.12 ± 0.58	0.50 ± 0.15*	3.35 ± 1.81	1.99 ± 1.35
	58		0.81 ± 0.35		2.94 ± 1.42
	88		2.18 ± 1.27		4.35 ± 1.65
Total iP (mg/24hrs)	10	40.3 ± 6.6	34.2 ± 3.0*	27.6 ± 4.9	23.9 ± 2.8
	31	37.0 ± 7.6	23.4 ± 4.0**	27.5 ± 5.9	22.5 ± 5.2
	58		27.2 ± 5.8		25.0 ± 5.3
	88		29.6 ± 6.0		23.0 ± 5.0
Total NAG (U/24hrs)	10	0.43 ± 0.06	0.43 ± 0.08	0.28 ± 0.06	0.22 ± 0.04*
	31	0.49 ± 0.09	0.45 ± 0.07	0.33 ± 0.12	0.22 ± 0.04*
	58		0.51 ± 0.11		0.28 ± 0.06
	88		0.59 ± 0.18		0.35 ± 0.08
Total protein (mg/24hrs)	10	16.8 ± 9.6	13.3 ± 4.3	1.2 ± 0.8	1.2 ± 1.1
	31	48.9 ± 56.5	22.5 ± 15.0	8.5 ± 9.7	3.3 ± 2.2
	58		77.0 ± 92.4		11.8 ± 22.2
	88		171.0 ± 220.7		18.8 ± 33.6

Each value represents mean±S.D.

a) n=47. b) n=34. c) n=36.

*,** Significantly different from SD rats ($p<0.05$, 0.01).

Table 2. continued

	weeks of age	male SD	male IGS	female SD	female IGS
Multistix® grade		- ± + 2+ 3+	- ± + 2+ 3+ 4+	- ± + 2+ 3+	- ± + 2+ 3+ 4+
Protein	10	0 8 2	0 10	8 2	7 3
	31	0 3 3 3 1	0 3 5 2	1 6 2 0 1	2 5 3
	58		0 4 9 16 15 3		4 19 19 4 4
	88		0 0 2 6 6 20		0 7 20 3 5 1
Glucose	10	10	10	10	10
	31	10	10	10	10
	58		47		50
	88		34		36
Ketone bodies	10	10	10	10	10
	31	10	10	10	10
	58		39 8		50
	88		32 2		35 1
Bilirubin	10	10	10	10	10
	31	10	10	10	10
	58		47		50
	88		34		36
Occult blood	10	10	10	10	10
	31	7 2 1	6 3 0 1	10	10
	58		45 1 0 1		42 4 0 0 4
	88		32 2		34 1 0 0 1
Urobilinogen	10	10	10	10	10
	31	10	10	10	10
	58		47		50
	88		34		36
Urinary sediment grade		- ± + 2+ 3+	- ± + 2+ 3+	- ± + 2+ 3+	- ± + 2+ 3+
Erythrocyte	10	10	10	10	10
	31	10	9 1	10	10
	58		46 0 0 1		44 2 1 1 2
	88		32 2		34 0 1 0 1
Leukocyte	10	10	8 2	9 0 1	6 3 1
	31	7 2 1	8 1 1	8 1 1	6 2 1 1
	58		31 14 2		28 11 5 6
	88		15 11 7 1		17 10 5 3 1
Epitherial cell	10	6 4	7 3	8 2	6 4
	31	4 4 2	2 4 4	5 3 1 1	7 1 2
	58		20 23 4		20 24 6
	88		9 13 11 1		16 14 6
Cast	10	10	10	10	10
	31	10	10	10	10
	58		47		50
	88		22 4 8		35 1
Crystal	10	9 0 1	10	10	8 0 2
	31	10	10	10	8 0 2
	58		36 1 4 6		41 0 6 3
	88		32 0 1 1		33 0 3
Color		yt pt pc	yt pt pc	yt pt pc	yt pt pc
	10	0 10	0 10	0 10	0 10
	31	0 10	0 10	0 10	0 10
	58		7 39 1		2 43 5
	88		3 31		0 36

Each value represents number of animal.
Color : pt, pale yellow transparent ; pc, pale yellow cloudy ; bt, pale yellow brown transparent.

observed in K, Ca, iP (male) and Na, K, Cl, Ca, NAG (female). Also, age related increase of Ca, NAG, protein excretions were observed in IGS rats of both sexes. This proteinurea observed in old animals was considered to be caused by the chronic progressive nephropathy of aging rat[1].

The results of hematological findings are shown in Table 3. Remarkable difference was not observed between IGS rats and SD rats.

The results of blood chemistry are shown in Table 4. Lower concentration of triglyceride, total cholesterol, and phospholipid was observed in male and female IGS rats at 10 and 32 weeks of ages. At 10 weeks of age, significantly lower concentration of total bilirubin (male and female), LH, insulin (male) and PRL (female), and also significantly higher value of A/G ratio (female), and concentration of FSH and TSH (male) were observed in IGS rats. At 32 weeks of age, significantly lower concentration of TG, Ca (male and female), and total protein (female) and also significantly higher concentration of urea nitrogen (female) were observed in IGS rats. Furthermore, the tendencies to increase in GPT activities and concentration of creatinine, TG, total cholesterol, phospholipid and LH, and the tendencies to decrease in ALP activities and concentration of insulin, iP, FSH, GH and testosterone were observed in IGS rats as an age related changes. An age related remarkable increase of LH was observed in male rats . As LH release is suppressed by testosterone[2], an increase of plasma LH concentration observed in these old animals was thought due to a decrease in plasma testosterone concentration. Although remarkable decrease in ALP activities was observed in younger IGS rats from 10 weeks to 32 weeks of age, decrease in ALP activities was not observed in animals after 32 weeks of age.

The results of organ weights are shown in Table 5. Weights of pituitary, thyroid, adrenal gland, liver and spleen in IGS rats of both sexes were lower than those of SD rats. Relative lower values of these organ weights were also reported in mildly diet-restricted animals[4, 5], suggesting the relation to the decrease

Table 3. Hematological findings in SD rats and IGS rats

	weeks of age	male SD	male IGS	female SD	female IGS
n	10	10	10	10	9
	32	10	10	10	10
	58		10		10
	91		36		35
Hct (%)	10	47.7 ± 1.6	48.5 ± 3.1	46.4 ± 2.8	46.0 ± 2.2
	32	47.3 ± 4.8	50.8 ± 2.6	44.8 ± 2.7	45.3 ± 2.7
	58		48.1 ± 1.7		47.0 ± 3.4
	91		45.3 ± 6.5		45.0 ± 4.2
Hb (g/dl)	10	15.7 ± 0.5	15.7 ± 0.7	15.1 ± 0.5	15.5 ± 0.7
	32	14.9 ± 1.3	16.3 ± 0.4**	14.5 ± 0.7	14.9 ± 0.5
	58		15.4 ± 0.5		15.1 ± 0.7
	91		14.5 ± 1.6		14.3 ± 1.3
RBC (10^4/mm^3)	10	798 ± 34	825 ± 54	793 ± 41	817 ± 48
	32	903 ± 104	985 ± 80	791 ± 47	830 ± 46
	58		898 ± 44		841 ± 54
	91		847 ± 93		777 ± 80
MCV (m^3)	10	59.7 ± 1.9	58.7 ± 1.3	58.5 ± 1.4	56.4 ± 0.9**
	32	52.5 ± 1.3	51.8 ± 2.4	56.5 ± 1.1	54.7 ± 1.2**
	58		53.7 ± 2.2		55.9 ± 2.7
	91		53.9 ± 2.2		57.9 ± 2.5
MCH (pg)	10	19.6 ± 0.6	19.0 ± 0.7	19.0 ± 0.7	19.0 ± 0.5
	32	16.5 ± 0.8	16.6 ± 1.0	18.4 ± 0.7	18.1 ± 0.8
	58		17.2 ± 0.8		18.0 ± 1.1
	91		17.2 ± 0.8		18.5 ± 0.7
MCHC (%)	10	32.8 ± 0.5	32.3 ± 1.1	32.5 ± 1.2	33.8 ± 0.5*
	32	31.4 ± 1.3	32.1 ± 0.9	32.4 ± 0.8	33.1 ± 1.4
	58		32.1 ± 0.9		32.2 ± 1.4
	91		31.9 ± 0.9		32.0 ± 0.8
Reticulocyte (‰)	10	25.8 ± 4.9	25.3 ± 3.8	26.2 ± 5.5	24.7 ± 4.6
	32	34.1 ± 15.2	28.7 ± 4.8	27.4 ± 4.1	26.2 ± 3.4
	58		31.4 ± 3.5		28.5 ± 4.2
	91		26.6 ± 10.3		25.5 ± 6.6
WBC (10^2/mm^3)	10	102.5 ± 26.8	76.1 ± 14.2*	61.2 ± 21.6	47.4 ± 12.2
	32	63.9 ± 16.0	53.8 ± 11.7	49.9 ± 15.1	41.3 ± 10.6
	58		68.3 ± 23.7		29.7 ± 5.8
	91		57.0 ± 13.9		42.7 ± 18.1

Each value represents mean±S.D.

*,** Significantly different from SD rats ($p<0.05$, 0.01).

Table 3. continued

	weeks of age	male SD	male IGS	female SD	female IGS
Differential leukocyte count (%)					
Basophile	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	32	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	58		0.0 ± 0.0		0.0 ± 0.0
	91		0.0 ± 0.0		0.0 ± 0.0
Eosinophile	10	0.6 ± 0.8	0.5 ± 0.8	0.4 ± 0.7	0.7 ± 0.9
	32	1.0 ± 1.2	1.4 ± 1.4	1.0 ± 0.8	1.2 ± 0.8
	58		2.7 ± 2.2		3.2 ± 1.7
	91		1.5 ± 1.3		1.5 ± 1.6
Neutrophile	10	7.8 ± 4.0	7.8 ± 4.8	5.8 ± 2.6	8.2 ± 6.1
	32	16.6 ± 8.8	18.0 ± 7.2	12.8 ± 5.6	13.2 ± 3.4
	58		22.9 ± 7.4		26.8 ± 9.2
	91		24.7 ± 10.1		26.9 ± 8.4
Lymphocyte	10	91.3 ± 4.0	91.4 ± 4.7	93.5 ± 2.5	90.7 ± 6.6
	32	81.9 ± 9.5	80.0 ± 8.1	85.7 ± 5.9	85.2 ± 3.7
	58		73.8 ± 8.8		69.4 ± 9.7
	91		73.4 ± 10.6		71.1 ± 8.8
Monocyte	10	0.3 ± 0.5	0.3 ± 0.7	0.3 ± 0.5	0.4 ± 0.5
	32	0.5 ± 0.5	0.6 ± 0.5	0.5 ± 0.5	0.4 ± 0.5
	58		0.6 ± 0.7		0.6 ± 0.7
	91		0.4 ± 0.6		0.5 ± 0.6
Platelet (10^4/mm^3)	10	126.1 ± 13.3	127.3 ± 11.7	120.9 ± 14.0	111.3 ± 10.9
	32	137.9 ± 20.7	135.3 ± 20.6	108.1 ± 11.5	103.9 ± 10.6
	58		129.3 ± 14.1		107.9 ± 11.1
	91		120.1 ± 27.3		101.8 ± 14.0
PT (sec.)	10	20.6 ± 2.2	19.8 ± 1.6	19.8 ± 2.4	18.8 ± 1.7
	32	18.8 ± 1.3	19.4 ± 1.7	16.5 ± 1.1	17.2 ± 1.6
	58		18.2 ± 2.0		16.4 ± 0.9
	91		21.2 ± 4.9		25.7 ± 6.7
APTT (sec.)	10	36.0 ± 4.1	36.0 ± 3.0	30.5 ± 2.9	30.0 ± 3.6
	32	34.2 ± 4.9	32.5 ± 5.8	28.4 ± 2.3	25.7 ± 6.0
	58		32.1 ± 4.4		26.6 ± 2.3
	91		27.0 ± 3.8		31.0 ± 5.0

Each value represents mean±S.D.

Table 4. Biochemical findings in SD rats and IGS rats

	weeks of age	male SD	male IGS	female SD	female IGS
n	10	10	10	10	9
	32	10	10	10	10
	58		10		10
	91		10		10
AST (U/l)	10	127.2 ± 80.9	175.1 ± 100.9	70.4 ± 9.7	69.9 ± 13.8
	32	144.7 ± 83.5	118.1 ± 57.8	103.4 ± 59.3	185.5 ± 186.7
	58		162.7 ± 139.7		141.8 ± 113.0
	91		133.6 ± 84.6		144.2 ± 80.5
ALT (U/l)	10	31.1 ± 4.9	31.9 ± 5.6	26.0 ± 7.3	34.8 ± 27.0
	32	30.5 ± 4.2	41.2 ± 15.8	39.7 ± 16.7	89.4 ± 104.9
	58		43.5 ± 10.6		57.9 ± 51.6
	91		47.3 ± 15.3		47.1 ± 20.2
LDH (U/l)	10	95.7 ± 33.1	107.1 ± 41.6	171.8 ± 70.3	344.0 ± 297.0
	32	132.1 ± 52.2	142.6 ± 38.0	228.2 ± 255.2	207.8 ± 94.5
	58		190.2 ± 168.2		146.5 ± 70.1
	91		108.6 ± 41.6		193.6 ± 152.1
CPK (U/l)	10	166.9 ± 73.1	149.3 ± 47.4	204.0 ± 95.0	451.3 ± 507.2
	32	113.1 ± 51.6	106.0 ± 25.2	92.5 ± 54.9	88.6 ± 23.3
	58		119.8 ± 21.6		76.2 ± 19.6
	91		264.7 ± 378.4		78.2 ± 19.5
ALP (U/l)	10	369.2 ± 96.5	373.9 ± 86.7	229.3 ± 53.5	227.4 ± 38.6
	32	155.7 ± 34.0	168.0 ± 21.8	62.9 ± 20.8	50.7 ± 13.5
	58		157.6 ± 39.7		53.5 ± 20.0
	91		160.1 ± 35.6		61.9 ± 15.6
γ-GTP (U/l)	10	1.95 ± 0.23	2.19 ± 0.41	1.92 ± 0.26	2.23 ± 0.63
	32	2.14 ± 0.34	2.02 ± 0.20	1.93 ± 0.39	1.82 ± 0.24
	58		2.99 ± 0.94		2.48 ± 0.34
	91		2.14 ± 0.96		1.79 ± 0.42
Total bilirubin (mg/dl)	10	0.07 ± 0.01	0.06 ± 0.01**	0.10 ± 0.01	0.09 ± 0.01**
	32	0.07 ± 0.02	0.06 ± 0.01	0.13 ± 0.02	0.11 ± 0.03
	58		0.07 ± 0.02		0.10 ± 0.02
	91		0.09 ± 0.03		0.12 ± 0.03
Total protein (g/dl)	10	5.55 ± 0.16	5.68 ± 0.26	5.58 ± 0.22	5.68 ± 0.43
	32	6.04 ± 0.27	6.03 ± 0.18	6.98 ± 0.30	6.59 ± 0.43*
	58		6.05 ± 0.35		6.52 ± 0.21
	91		6.00 ± 0.17		6.43 ± 0.21
Albumin (g/dl)	10	2.08 ± 0.10	2.18 ± 0.13	2.20 ± 0.13	2.32 ± 0.18
	32	2.08 ± 0.09	2.16 ± 0.10	2.90 ± 0.12	2.77 ± 0.25
	58		1.98 ± 0.17		2.57 ± 0.14
	91		1.99 ± 0.13		2.47 ± 0.16
A/G ratio	10	0.60 ± 0.04	0.62 ± 0.04	0.65 ± 0.03	0.69 ± 0.04*
	32	0.53 ± 0.04	0.56 ± 0.04	0.71 ± 0.05	0.72 ± 0.05
	58		0.49 ± 0.04		0.65 ± 0.06
	91		0.50 ± 0.04		0.63 ± 0.06
Urea N. (mg/dl)	10	16.3 ± 2.4	17.9 ± 1.8	20.3 ± 1.8	21.7 ± 3.9
	32	15.2 ± 2.0	16.7 ± 1.6	14.6 ± 2.2	17.5 ± 2.9*
	58		15.0 ± 1.3		15.8 ± 2.3
	91		20.0 ± 7.5		16.7 ± 1.2

Each value represents mean±S.D.

*,** Significantly different from SD rats ($p<0.05$, 0.01).

Table 4. continued

	weeks of age	male SD	male IGS	female SD	female IGS
Creatinine (mg/dl)	10	0.40 ± 0.04	0.42 ± 0.05	0.48 ± 0.07	0.51 ± 0.05
	32	0.52 ± 0.07	0.53 ± 0.08	0.52 ± 0.04	0.57 ± 0.07
	58		0.60 ± 0.07		0.56 ± 0.05
	91		0.62 ± 0.20		0.61 ± 0.06
Uric acid (mg/dl)	10	0.37 ± 0.07	0.36 ± 0.16	0.45 ± 0.15	0.84 ± 0.94
	32	0.49 ± 0.11	0.43 ± 0.08	0.47 ± 0.18	0.37 ± 0.08
	58		0.58 ± 0.33		0.37 ± 0.11
	91		0.67 ± 0.59		0.57 ± 0.19
Glucose (mg/dl)	10	135.8 ± 9.8	144.6 ± 13.3	125.1 ± 11.6	122.3 ± 8.7
	32	142.9 ± 12.5	140.9 ± 17.8	134.2 ± 15.1	138.7 ± 6.9
	58		160.5 ± 19.7		132.3 ± 15.0
	91		157.2 ± 13.2		130.6 ± 18.3
TG (mg/dl)	10	49.2 ± 23.8	32.2 ± 17.2	17.2 ± 9.6	9.9 ± 9.5
	32	92.9 ± 34.2	59.4 ± 25.9*	99.3 ± 70.1	35.9 ± 23.4*
	58		164.6 ± 94.8		86.5 ± 42.1
	91		199.0 ± 238.5		100.6 ± 78.6
Total cholesterol (mg/dl)	10	56.8 ± 8.3	51.8 ± 10.9	65.3 ± 11.0	56.9 ± 7.5
	32	74.0 ± 21.6	67.6 ± 18.1	82.9 ± 17.8	76.3 ± 16.9
	58		97.5 ± 25.6		78.0 ± 10.4
	91		116.0 ± 62.8		88.6 ± 12.3
Phospholipid (mg/dl)	10	103.9 ± 11.0	95.8 ± 14.1	130.1 ± 20.4	110.1 ± 15.0*
	32	124.2 ± 24.9	106.4 ± 23.3	187.9 ± 38.8	159.3 ± 37.2
	58		161.8 ± 38.8		165.5 ± 19.2
	91		186.4 ± 92.7		173.9 ± 22.0
Ca (mg/dl)	10	9.64 ± 0.26	9.73 ± 0.36	9.48 ± 0.37	9.65 ± 0.31
	32	9.53 ± 0.17	9.30 ± 0.18**	10.17 ± 0.30	9.82 ± 0.29*
	58		9.58 ± 0.23		9.68 ± 0.15
	91		9.58 ± 0.39		9.63 ± 0.25
iP (mg/dl)	10	8.74 ± 0.40	8.45 ± 0.57	10.76 ± 1.31	9.56 ± 1.72
	32	4.87 ± 0.29	4.98 ± 0.33	5.15 ± 0.61	5.09 ± 0.50
	58		4.12 ± 0.35		4.16 ± 0.48
	91		4.24 ± 0.82		3.52 ± 0.42
Na (mmol/dl)	10	142.0 ± 2.4	141.5 ± 1.7	142.6 ± 1.7	141.9 ± 2.0
	32	142.7 ± 1.4	142.5 ± 1.1	145.3 ± 1.3	144.7 ± 1.9
	58		143.3 ± 1.9		147.5 ± 2.0
	91		142.3 ± 1.0		141.0 ± 1.0
K (mmol/dl)	10	2.99 ± 0.13	3.03 ± 0.25	3.17 ± 0.36	3.90 ± 1.89
	32	3.11 ± 0.15	3.13 ± 0.21	3.13 ± 0.40	3.04 ± 0.24
	58		3.21 ± 0.21		3.06 ± 0.40
	91		3.26 ± 0.19		3.01 ± 0.37
Cl (mmol/dl)	10	104.2 ± 0.6	104.7 ± 1.2	104.7 ± 1.3	104.4 ± 1.5
	32	108.6 ± 2.2	108.7 ± 2.5	106.8 ± 1.2	106.4 ± 1.5
	58		104.9 ± 1.2		104.0 ± 2.1
	91		103.4 ± 1.0		102.5 ± 1.6

Each value represents mean±S.D.

*,** Significantly different from SD rats ($p<0.05$, 0.01)

Table 4. continued

	weeks of age	male SD	male IGS	female SD	female IGS
LH (ng/ml)	10	11.6 ± 3.2	8.9 ± 2.0*	4.5 ± 1.3	4.5 ± 1.6
	32	7.6 ± 4.1	7.7 ± 3.6	8.0 ± 5.0	6.1 ± 1.7
	58		12.2 ± 1.6		9.7 ± 3.0
	91		24.3 ± 42.2		6.6 ± 4.4
FSH (ng/ml)	10	306.2 ± 70.0	368.8 ± 62.5*	288.3 ± 59.0	275.3 ± 90.1
	32	205.8 ± 98.4	233.1 ± 81.1	190.9 ± 79.9	141.2 ± 35.5
	58		414.2 ± 104.4		230.3 ± 155.0
	91		157.6 ± 142.5		165.4 ± 177.8
GH (ng/ml)	10	269.7 ± 164.9	205.2 ± 73.7	236.6 ± 36.0	289.2 ± 110.6
	32	322.6 ± 57.6	385.1 ± 147.7	331.5 ± 119.8	167.7 ± 167.7
	58		234.5 ± 100.9		157.0 ± 34.3
	91		132.6 ± 54.8		110.3 ± 145.9
PRL (ng/ml)	10	62.6 ± 2.1	58.5 ± 15.9	523.9 ± 288.9	215.6 ± 120.1*
	32	62.6 ± 30.3	56.6 ± 28.6	689.0 ± 958.7	430.9 ± 392.5
	58		80.3 ± 34.2		554.1 ± 492.6
	91		28.8 ± 21.2		305.8 ± 281.2
TSH (ng/ml)	10	9.60 ± 2.15	13.50 ± 2.40**	6.86 ± 1.13	7.47 ± 1.42
	32	7.62 ± 3.96	9.27 ± 3.24	5.78 ± 2.25	5.82 ± 1.70
	58		23.57 ± 7.91		8.08 ± 2.77
	91		12.02 ± 6.17		6.43 ± 7.31
Insulin (pg/ml)	10	0.936 ± 0.162	0.695 ± 0.319*	0.460 ± 0.226	0.448 ± 0.243
	32	1.187 ± 0.180	1.131 ± 0.339	1.080 ± 0.608	0.855 ± 0.331
	58		1.165 ± 0.189		1.156 ± 0.287
	91		1.332 ± 0.254		1.107 ± 0.268
Testosterone (ng/ml)	10	1.847 ± 1.466	3.441 ± 3.511		
	32	0.917 ± 0.335	0.828 ± 0.455		
	58		0.595 ± 0.461		
	91		0.443 ± 0.142		
Progesterone (ng/ml)	10			172.9 ± 44.4	175.7 ± 58.6
	32			91.5 ± 34.0	137.8 ± 66.9
	58				96.5 ± 65.5
	91				121.4 ± 67.5
Estradiol (pg/ml)	10			20.2 ± 5.9	20.4 ± 2.6
	32			22.4 ± 5.1	21.3 ± 6.2
	58				19.9 ± 4.2
	91				18.3 ± 5.5

Each value represents mean±S.D.
*,** Significantly different from SD rat ($p<0.05$, 0.01).

Table 5. Absolute organ weights in SD rats and IGS rats

	weeks of age	male SD	male IGS	female SD	female IGS
n	10	10	10	10	10
	32	10	10	10	10
	58		10		10
	91		36		37
Body weight (g)	10	412.0 ± 21.6	410.9 ± 20.4	241.1 ± 18.1	231.8 ± 18.1
	32	708.7 ± 53.1	675.7 ± 57.8	393.7 ± 67.9	341.6 ± 29.2
	58		849.6 ± 116.7		410.0 ± 72.5
	91		915.6 ± 111.4		558.5 ± 125.9
Crerebrum (g)	10	1.49 ± 0.08	1.49 ± 0.07	1.41 ± 0.06	1.36 ± 0.04*
	32	1.57 ± 0.10	1.63 ± 0.09	1.44 ± 0.05	1.43 ± 0.09
	58		1.60 ± 0.08		1.42 ± 0.07
	91		1.66 ± 0.08		1.48 ± 0.07
Pituitary gland (mg)	10	14.0 ± 1.8	12.8 ± 1.2	14.0 ± 1.6	14.2 ± 2.1
	32	15.9 ± 1.9	13.4 ± 1.1**	28.7 ± 12.5	21.3 ± 4.3
	58		18.3 ± 7.9		26.7 ± 5.4
	91		25.4 ± 37.1		68.1 ± 69.1
Thyroid (mg)	10	22.4 ± 4.0	24.1 ± 2.8	16.9 ± 2.3	15.6 ± 3.9
	32	33.4 ± 7.5	27.0 ± 4.6*	23.7 ± 6.1	18.4 ± 4.4*
	58		38.5 ± 11.1		23.8 ± 2.7
	91		46.6 ± 11.7		31.2 ± 7.2
Adrenal gland (mg)	10	60.9 ± 9.2	61.9 ± 7.5	63.8 ± 7.2	57.9 ± 7.1
	32	62.9 ± 10.1	55.8 ± 5.7	75.0 ± 12.9	61.7 ± 7.3*
	58		56.2 ± 7.2		73.6 ± 9.0
	91		70.3 ± 16.0		91.1 ± 34.9
Heart (g)	10	1.35 ± 0.10	1.38 ± 0.05	0.87 ± 0.05	0.84 ± 0.07
	32	1.79 ± 0.09	1.75 ± 0.15	1.20 ± 0.14	1.04 ± 0.10**
	58		2.02 ± 0.16		1.14 ± 0.13
	91		2.12 ± 0.23		1.35 ± 0.22
Lung (g)	10	1.46 ± 0.14	1.40 ± 0.06	1.04 ± 0.07	0.98 ± 0.04*
	32	1.75 ± 0.21	1.68 ± 0.17	1.22 ± 0.05	1.18 ± 0.06
	58		1.84 ± 0.16		1.26 ± 0.07
	91		2.07 ± 0.20		1.42 ± 0.16
Submandibular gland (g)	10	0.65 ± 0.07	0.63 ± 0.05	0.44 ± 0.04	0.42 ± 0.04
	32	0.76 ± 0.09	0.75 ± 0.07	0.49 ± 0.08	0.45 ± 0.09
	58		0.79 ± 0.03		0.49 ± 0.04
	91		0.80 ± 0.10		0.52 ± 0.09
Liver (g)	10	13.38 ± 1.34	13.72 ± 1.96	6.91 ± 0.80	6.64 ± 0.80
	32	19.44 ± 1.74	16.57 ± 1.87**	9.66 ± 1.79	7.65 ± 1.11**
	58		20.35 ± 3.67		8.48 ± 1.37
	91		21.50 ± 3.07		11.77 ± 2.37
Kidney (g)	10	3.22 ± 0.22	3.28 ± 0.28	1.80 ± 0.14	1.74 ± 0.16
	32	3.91 ± 0.33	3.78 ± 0.34	2.21 ± 0.24	2.03 ± 0.20
	58		4.46 ± 0.46		2.14 ± 0.24
	91		5.11 ± 1.11		2.63 ± 0.32
Thymus (g)	10	0.70 ± 0.14	0.61 ± 0.11	0.47 ± 0.12	0.45 ± 0.04
	32	0.19 ± 0.10	0.26 ± 0.08	0.15 ± 0.05	0.19 ± 0.04
	58		0.14 ± 0.06		0.10 ± 0.03
	91		0.18 ± 0.10		0.12 ± 0.05

Each value represents mean±S.D.
*,** Significantly different from SD rat ($p<0.05$, 0.01).

Table 5. continued

	weeks of age	male SD	male IGS	female SD	female IGS
Spleen (g)	10	0.85 ± 0.10	0.79 ± 0.11	0.57 ± 0.08	0.47 ± 0.08*
	32	0.99 ± 0.11	0.88 ± 0.12	0.63 ± 0.06	0.54 ± 0.04**
	58		1.10 ± 0.31		0.58 ± 0.09
	91		1.34 ± 0.35		0.76 ± 0.24
Testis (g)	10	3.12 ± 0.24	3.19 ± 0.21		
	32	3.59 ± 0.17	3.45 ± 0.66		
	58		3.74 ± 0.38		
	91		3.89 ± 0.46		
Prostate (g)	10	0.53 ± 0.11	0.50 ± 0.08		
	32	0.75 ± 0.28	0.88 ± 0.33		
	58		0.74 ± 0.25		
	91		0.69 ± 0.23		
Ovary (g)	10			91.7 ± 15.0	85.6 ± 14.9
	32			62.7 ± 21.3	69.9 ± 19.8
	58				56.7 ± 14.6
	91				92.4 ± 123.1
Uterus (g)	10			0.45 ± 0.07	0.45 ± 0.18
	32			0.73 ± 0.12	0.68 ± 0.15
	58				0.96 ± 0.24
	91				0.90 ± 0.25

Each value represents mean±S.D.
*,** Significantly different from SD rat ($p<0.05$, 0.01).

of food consumption. In other organs, marked weight difference was not recognized.

As described above, some of general toxicological parameters such as body weight, plasma lipids and organ weights in IGS rats were significantly different from those of SD rats. We concluded that further investigation is needed to make a more reliable background data.

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CHAPTER 3

Reproduction Toxicology

Comparison between Crj:CD(SD)IGS and Crj:CD(SD) rats in Reproductive and Developmental Parameters

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ABSTRACT. The gold standard system is an animal breeding system with the purpose of providing experimental animals having a uniform quality worldwide. It was developed by Charles River Inc. for internationalization of scientific research and development of new drugs. We have studied the parameters commonly evaluated in general and reproductive/developmental toxicity tests using Crj:CD(SD)IGS rats bred by the gold standard system. The data were compared with those of Crj:CD(SD) rats.

Differences between SD and (SD)IGS rats were found in some of the examination items commonly performed in toxicity tests. Consequently, it is considered to be important that the results obtained in (SD)IGS rats should be carefully evaluated when (SD)IGS rats are used in reproductive and developmental toxicity studies. –Key words: CD(SD)IGS, Rat, Reproduction, Development

CD (SD) IGS-1998: 155-163

INTRODUCTION

The gold standard system, a new animal breeding system, has been developed by Charles River Inc. to cope with the internationalization of research and development of new drugs by supplying, as much as possible, uniform experimental animals by minimizing the genetic ramifications. We performed examinations usually employed in general toxicity as well as reproductive and developmental toxicity studies to obtain reproductive and developmental background data on Crj:CD(SD)IGS rats produced by this system, with results compared to those from Crj:CD(SD) rats.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats (Tsukuba Breeding Center, hereafter referred to as "IGS strain") and Crj:CD(SD) rats (Atsugi Breeding Center, hereafter referred to as "SD strain"), which were 7 weeks of age, were twice obtained from Charles River Japan, Inc. The first lot (Jan. 24, 1996) consisting of 30 animals/sex/strain was assigned to the caesarean section groups to collect data mainly on fetal development. The second lot (Feb. 21, 1996) consisting of 25 animals/sex/strain was assigned to natural delivery groups to collect data mainly on delivery, nursing of dams and postnatal development of the subsequent generations.

The animals were housed individually in metal cages in an animal room which was maintained at a temperature of 24 ± 1°C and a relative humidity of 50-65%, ventilated approximately 15 times/hour and provided with light for 12 hours/day. They were allowed free access to pellet food (CRF-1, Oriental Yeast, Co., Ltd.) and tap water. The females assigned for delivery were housed in rat breeding cages from day 14 of gestation (day 0 of gestation is the day on which presence of sperm is confirmed) until day 10 of lactation (day 0 of lactation is the day on which delivery is confirmed) with adequate paper pulp tip bedding.

The animals were observed for clinical signs once daily. All the males were weighed on a weekly basis. The females were weighed on day 0 of gestation, daily on days 6-20 of gestation and on days 0, 4, 7, 14 and 21 of lactation (animals in the natural delivery groups). Food intake was measured once weekly for males and daily on days 1-20 of gestation for females in the caesarean section groups. All the females were examined for estrous cycles using the vaginal smear method daily from the age of 9 weeks. At the age of 11 weeks, they were paired on a one-to-one basis with males of the same strain for a maximum of 2 weeks. The day on which the presence of a vaginal plug or sperm in the vaginal smear was confirmed was designated as day 0 of gestation. The copulation index, fertility index, and number of days required for mating were determined. Males in the caesarean section and natural delivery groups, at the ages of 16 and 20 weeks, respectively, were fasted for 18 to 24 hours, then exsanguinated to death from the posterior vena cava under pentobarbital sodium anesthesia with blood collected, and examined gross pathologically. Organ weight measurement and hematological, blood biochemistry and sperm examinations were performed on all the males. Hematological parameters examined using EDTA-treated whole blood with Coulter Counter Model S-PLUS IV (Coulter Electronics) included red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), mean corpuscular volume (MCV), hematocrit (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count. Venous blood smears were used to determine the white blood cell differential count. Heparin-treated plasma samples were used to determine the following blood biochemistry parameters: levels of total protein (TP), albumin (ALB), total cholesterol (T-Cho), triglyceride (TG), glucose (GLU), urea nitrogen (BUN), creatinine (CREA), total bilirubin (T-Bill), calcium (Ca) and inorganic phosphorus (IP) and activities of alkaline phosphatase (ALP), lactic dehydrogenase (LDH), GOT and GPT using a centrifugal biochemical autoanalyzer (COBAS-FARA, Roche); sodium (Na), potassium (K) and chloride (Cl) levels using an automated analyzer (EA05, A&T) with ion-selective electrodes; and A/G ratio by calculation. Epididymis fluid collected from a duct of the right caudal epididymis with a needle at necropsy

was used for determination of the percentages of motile sperm and progressive motile sperm using a computer-assisted sperm analysis (CASA) (HTM-IVOS, Hamilton-Thorne). The right caudal epididymis was kept frozen until thawing and smashing for sperm examination. After staining the head of sperm (Modified IDENT STAIN KIT, Hamilton-Thorne), the number of sperm (number of sperm in the right caudal epididymis / weight of the right caudal epididymis) was determined using CASA.

Females in the caesarean section groups were necropsied on day 20 of gestation. Embryos/fetuses were removed and the ovaries and uterus were examined for the number of corpora lutea and implantations. Organs were weighed in all the females. The number of implantations in the uterus was confirmed using a modified Salewski's method[4]. The number of live fetuses and dead embryos was counted and the embryo/fetal mortality was determined based on the number of implantations. After measurement of body and placental weights, live fetuses were sexed and observed for external anomalies. Approximately half of the live fetuses in each litter was assigned for preparation of skeletal specimens[2] for observation of skeletal anomalies and the number of ossified sacrococcygeal vertebrae. The remaining live fetuses were observed for visceral anomalies[3,5].

All the females in the natural delivery groups were allowed to deliver. They were observed for abnormalities in delivery and the duration of gestation was determined. They were also observed for nursing behavior daily after delivery and necropsied on day 22 of lactation. Offspring were observed for external anomalies on the day of birth (day 0 of lactation). The birth index and viability index on day 0 of lactation were determined based on the litter size (total number of live and dead newborn). The litter size was determined daily after birth to calculate the viability index on day 4 of lactation and weaning index. On day 4 of lactation, the litter size was adjusted to 4 animals of each sex. Dead offspring were observed for external, skeletal and visceral anomalies. Male and female offspring were weighed separately on a litter basis on days 0, 4, 7 and 14 of lactation. They were weighed individually on day 21 of lactation and once weekly from weaning until the age of 9 weeks. During the lactation period, 1 male and 1 female 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. Two male and 2 female offspring from each litter were necropsied on day 22 of lactation, while the remaining offspring were weaned. After weaning, an open field test and a multiple T-shaped water maze test were performed on 1 animal/sex/litter at the ages of 4-5 weeks and 5-6 weeks, respectively. The open field test was performed in a round field apparatus (150 cm diam., 35 cm height) to examine emotionality using an image analyzer 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. One female was selected from each litter at the age of 8 weeks and the vaginal smears were examined for estrous cycles for 2 weeks. F_1 females were assigned for mating with non-sibling males of the same strain on a one-to-one basis at the age of 10 weeks. All the females with confirmed copulation were allowed to deliver. They were weighed on days 0, 7, 14, 17 and 20 of gestation and on days 0 and 4 of lactation. F_2 offspring were observed for viability and anomalies in the same manner as in the F_1 generation. They were weighed on days 0 and 4 of lactation. F_1 dams and F_2 offspring were necropsied during days 4-6 of lactation.

Statistical analyses of data were performed using the following methods. Chi-square test with Yate's correction was used to analyze contingency-type data such as copulation and fertility indices. Numerical data such as body weight and food intake were analyzed using Student's t-test. Statistical analyses were made between SD and IGS strains in the caesarean section and natural delivery groups at two-tailed 5% and 1% levels of significance.

RESULTS AND DISCUSSION

Clinical observation of males revealed hair loss including sparse fur in 5 animals in the SD strain and 8 animals in the IGS strain, and kinky tail tip and chromodacryorrhea in 1 animal each in the IGS strain.

Body weight and food consumption in males are shown in Table 1. Significantly lower body weight than SD males was noted in IGS males in the caesarean section group from 7 weeks to 15 weeks of age. Body weight of IGS males in the natural delivery group was lower than SD males from 7 weeks to 9 weeks of age; however, no significant differences were noted from 10 weeks to 19 weeks of age. Food intake in IGS males in the caesarean group was lower than in SD males from 9 weeks to 15 weeks of age. No significant differences in food intake were observed between IGS and SD strains in the natural delivery groups.

Organ weights in males are presented in Table 2. Weights of the heart, lung, liver, kidneys and spleen in IGS males in the caesarean section group and weight of the liver in IGS males in the natural delivery group were significantly lower than those in the SD males in the corresponding groups. Testicular weight of IGS males in the natural delivery group was significantly higher than in SD males.

At necropsy, granuloma were observed in the caudal epididymis in 2 IGS males. No gross lesions were observed in any of the other animals.

The hematological and blood biochemical findings, and the result of sperm examinations in males are presented in Table 3. Hematological examination revealed the following significant differences from SD animals in IGS animals: increases in the RBC and Hb in the caesarean section group and in the RBC, Hb, Ht and percentage of monocytes in the natural delivery group; and a decrease in the percentage of lymphocytes in the natural delivery group. In the blood biochemistry examination, significant decreases in the levels of glucose, total cholesterol and triglycerides and significant increases in the ALP and GPT

Table 1. Body Weights (g) and Food Consumption (g/day) of Male Rats

Group	Caesarean section		Natural delivery	
Strain	SD	IGS	SD	IGS
Body weights				
Week 7	246.2 ± 6.4	217.5 ± 18.9 **	249.3 ± 4.6	240.0 ± 8.2 **
8	322.4 ± 10.5	292.7 ± 10.6 **	324.2 ± 7.9	316.7 ± 14.9 *
9	376.2 ± 15.4	338.5 ± 14.5 **	377.4 ± 14.4	364.0 ± 20.7 *
10	424.9 ± 22.6	380.9 ± 22.3 **	421.4 ± 18.7	408.0 ± 27.9
11	462.8 ± 28.4	411.7 ± 28.5 **	460.0 ± 22.5	445.7 ± 32.8
12	489.8 ± 32.2	438.4 ± 31.8 **	488.3 ± 25.7	474.8 ± 36.8
13	521.8 ± 37.5	466.0 ± 35.8 **	520.2 ± 28.9	506.5 ± 42.1
14	552.4 ± 42.5	491.4 ± 40.2 **	550.7 ± 31.5	533.9 ± 47.5
15	572.6 ± 45.9	510.4 ± 44.8 **	568.9 ± 34.7	553.9 ± 51.9
16	NE	NE	589.5 ± 37.8	575.8 ± 55.2
17	NE	NE	610.2 ± 42.8	597.0 ± 56.5
18	NE	NE	625.6 ± 43.3	608.8 ± 59.0
19	NE	NE	638.1 ± 45.7	622.5 ± 59.5
Food consumption				
Week 9 33.7 ± 2.7	29.6 ± 2.7 **	32.9 ± 2.5	30.2 ± 7.0	
10	32.7 ± 3.5	28.4 ± 2.6 **	31.7 ± 3.1	30.6 ± 3.5
13	34.0 ± 4.1	29.7 ± 2.8 **	32.1 ± 2.8	31.2 ± 3.5
14	33.8 ± 3.7	29.3 ± 2.6 **	32.0 ± 3.0	31.1 ± 3.8
15	31.7 ± 3.8	29.1 ± 3.0 **	30.9 ± 5.5	31.0 ± 3.9
16	NE	NE	31.8 ± 2.8	31.3 ± 3.7
17	NE	NE	30.8 ± 2.7	29.7 ± 3.6
18	NE	NE	30.6 ± 3.0	29.6 ± 3.3
19	NE	NE	29.9 ± 2.8	28.4 ± 3.3

Values represent mean ± S.D. *: $p<0.05$, **: $p<0.01$ NE: Not examined

Table 2. Organ Weights in Male Rats at 16 and 20 Weeks of Age

Group	Caesarean section (16W)		Natural delivery (20W)	
Strain	SD	IGS	SD	IGS
Body weight (g)	561.9 ± 47.9	499.1 ± 46.3 **	621.4 ± 47.7	603.7 ± 58.6
Heart (g)	1.45 ± 0.10	1.33 ± 0.10 **	1.42 ± 0.10	1.38 ± 0.10
Lung (g)	1.44 ± 0.09	1.38 ± 0.12 *	1.46 ± 0.11	1.46 ± 0.09
Liver (g)	16.89 ± 2.13	14.12 ± 2.04 **	18.24 ± 2.52	16.22 ± 2.99 *
Kidneys (g)	3.48 ± 0.35	3.22 ± 0.30 **	3.47 ± 0.32	3.42 ± 0.30
Spleen (g)	0.88 ± 0.14	0.76 ± 0.12 **	0.89 ± 0.15	0.84 ± 0.13
Adrenals (mg)	60.7 ± 8.7	58.1 ± 8.7	51.0 ± 8.0	51.5 ± 8.2
Thymus (mg)	317.0 ± 70.2	322.9 ± 74.7	41.2 ± 48.2	267.1 ± 81.4
Testes (g)	3.31 ± 0.30	3.34 ± 0.22	3.33 ± 0.28	3.49 ± 0.24 *
Epididymides (g)	1.18 ± 0.11	1.21 ± 0.11	1.29 ± 0.11	1.34 ± 0.12

Values represent mean ± S.D. *: $p<0.05$, **: $p<0.01$

activities were noted in IGS males in the caesarean section group compared to those in SD males. The total cholesterol, triglyceride and potassium levels in IGS males in the natural delivery group were significantly decreased, while calcium, sodium and chloride levels and ALP activity in these males were significantly increased compared to SD males. No significant differences were noted in the sperm motility rate, progressive motile sperm rate or number of sperm in the caudal epididymis between IGS and SD males.

In the clinical observation of females, the following signs were noted: hair loss including sparse fur in 5 animals, swelling of the hindlimb in 1 animal, vaginal bleeding with piloerection in 1 animal in the SD strain; hair loss including sparse fur in 10 animals and a tumor in the axillary region in 1 animal in the IGS strain.

Body weight in females are shown in Table 4. Body weight of IGS females in both the caesarean and natural delivery groups was significantly lower than that of SD females through the gestation and lactation periods.

Food intake in females is presented in Table 5. Food intake in IGS females was significantly lower than in SD females throughout the gestation period. No abnormalities in delivery or nursing behavior were observed in any of the dams. No significant differences in the duration of gestation were noted between SD females (22.0 ± 0.4 days) and IGS females (21.8 ± 0.4 days).

Organ weights in females are presented in Table 6. The weights of the heart, lung, liver, kidneys and spleen in IGS females were significantly lower than in SD females. Gross pathology revealed thickening of the right hindlimb and dark red discoloration of the thymus in 2 animals in the SD strain and a tumor in the right axillary region in 1 animal in the IGS strain.

Estrous cycles and the result of mating in F_0 animals are presented in Table 7. Estrous cycles other than a 4-day cycle were observed in 13 of 55 females in the SD strain, while in 1 of 55 females in the IGS strain. There were no significant

Table 3. Hematological and Blood Biohemical Findings, and Sperm Motion in Male Rats

Group	Caesarean section (16W)		Natural delivery (20W)	
Strain	SD	IGS	SD	IGS
Hematology				
RBC (x10⁴/mm³)	840 ± 51	866 ± 36 **	868 ± 46	903 ± 50 *
Hb	15.3 ± 0.7	15.6 ± 0.5 *	15.3 ± 0.6	15.8 ± 0.7 *
Ht	44.6 ± 2.1	45.4 ± 1.8	44.0 ± 1.9	45.6 ± 2.1 **
MCV	53.1 ± 1.7	52.5 ± 1.5	50.7 ± 1.8	50.5 ± 1.5
MCH	18.3 ± 0.7	18.1 ± 0.5	17.7 ± 0.7	17.5 ± 0.6
MCHC	34.4 ± 0.6	34.5 ± 0.5	34.9 ± 0.6	34.6 ± 0.4
WBC (x100/mm³)	76 ± 34	73 ± 20	86 ± 22	60 ± 15
Band (%)	0	0	0	0
Segmented (%)	17 ± 10	15 ± 6	12 ± 6	15 ± 7
Eosinophil (%)	1 ± 1	1 ± 1	1 ± 1	1 ± 1
Basophil (%)	0	0	0	0
Monocyte (%)	2 ± 2	3 ± 2	2 ± 2	4 ± 3 **
Lymphocyte (%)	79 ± 10	80 ± 7	85 ± 7	80 ± 8 *
Platelets (x10⁴/mm³)	12.7 ± 8.9	109.7 ± 10.7	103.1 ± 11.8	103.8 ± 17.3
Blood biochemistry				
TP (g/dl)	5.5 ± 0.3	5.5 ± 0.4	5.7 ± 0.2	5.8 ± 0.3
ALB (g/dl)	2.8 ± 0.2	2.8 ± 0.2	2.9 ± 0.2	3.0 ± 0.2
A/G	1.04 ± 0.11	1.07 ± 0.10	1.05 ± 0.11	1.10 ± 0.14
BUN (mg/dl)	18 ± 2	17 ± 2	16 ± 2	19 ± 3
CREA (mg/dl)	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.2
GLU (mg/dl)	155 ± 20	144 ± 17 *	176 ± 23	168 ± 25
T-Cho (mg/dl)	49 ± 11	42 ± 10 *	61 ± 15	50 ± 13 **
TG (mg/dl)	71 ± 24	47 ± 21 **	112 ± 73	69 ± 44 *
ALP (U/l)	166 ± 35	189 ± 35 *	146 ± 28	165 ± 30 *
LDH (U/l)	302 ± 456	288 ± 212	234 ± 79	234 ± 101
GPT (U/l)	21 ± 6	24 ± 6 *	30 ± 9	30 ± 7
GOT (U/l)	65 ± 11	67 ± 10	64 ± 11	69 ± 14
T-Bill (mg/dl)	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
IP (mg/dl)	5.8 ± 0.8	5.9 ± 0.8	5.7 ± 0.7	5.9 ± 0.6
Ca (mg/dl)	8.7 ± 0.4	8.6 ± 0.4	8.7 ± 0.3	8.9 ± 0.3 *
Na (mEq/l)	143.0 ± 1.6	143.3 ± 1.1	141.7 ± 0.8	143.4 ± 1.1 **
K (mEq/l)	4.21 ± 0.31	4.13 ± 0.41	4.59 ± 0.38	4.35 ± 0.37*
Cl (mEq/l)	105.5 ± 1.8	105.5 ± 1.5	104.3 ± 1.2	105.8 ± 1.4 **
Sperm examination				
Motile sperm (%)				
0 h	95.9 ± 4.6	96.3 ± 2.8	97.9 ± 1.8	98.5 ± 1.7
3 h	91.2 ± 5.2	90.8 ± 5.3	NE	NE
Progressive motile sperm (%)				
0 h	54.5 ± 8.5	54.3 ± 8.7	65.9 ± 9.5	70.5 ± 8.1
3 h	20.6 ± 4.1	20.8 ± 6.1	NE	NE
Sperm count (x10⁶/g)	1760.8 ± 516.4	1913.5 ± 632.9	1710.8 ± 357.0	1815.0 ± 481.1

Values represent mean ± S.D. *: $p<0.05$, **: $p<0.01$ NE: Not examined

differences between SD and IGS strains in the copulation index, fertility index or the number of days required for mating.

Findings at the caesarean section are presented in Table 8. The mean number of corpora lutea, implantations and live fetuses on a litter basis was significantly decreased in IGS females compared to SD females. The embryo/fetal mortality and sex ratio were comparable between both strains. Fetal weight was significantly higher, but placental weight was significantly lower in the IGS strain than in the SD strain.

External, skeletal and visceral findings in live fetuses are presented in Table 9. No external anomalies were observed in either SD or IGS strain. Skeletal anomalies included multiple anomalies including fusion of external occipital bone and atlas, fused thoracic vertebral arches, defect of thoracic vertebral bodies and defect of lumbar vertebral arches in 1 fetus and those including fusion/hypoplasia of thoracic vertebral arches and fused ribs in another fetus of the SD strain. No skeletal anomalies were observed in any fetus of the IGS strain. Skeletal variations such as dumbbell-shaped thoracic vertebral bodies, splitting of thoracic vertebral bodies and lumbar ribs were observed in both SD and IGS strains. The incidence of lumbar ribs was significantly greater in the IGS strain. In addition, cervical ribs, unilateral ossification of thoracic vertebral bodies, shortening of 13th rib, variation in the number of lumbar vertebrae, saclarization of lumbar vertebrae and unilateral ossification of lumbar vertebral bodies were observed in the SD strain. However, no significant difference in the total incidence of skeletal variations was noted in either strain. The number of ossified sacrococcygeal vertebrae was significantly greater in the IGS strain than in the SD strain. The only visceral

Table 4. Body Weights (g) of Female Rats

Group	Caesarean section		Natural delivery	
Strain	SD	IGS	SD	IGS
Body weights				
GD 0	305.4 ± 17.8	249.5 ± 13.3 **	292.3 ± 18.6	259.2 ± 16.5 **
6	337.6 ± 18.7	281.0 ± 17.4 **	324.7 ± 22.5	288.3 ± 18.3 **
7	341.5 ± 19.7	283.8 ± 19.0 **	328.5 ± 22.2	292.1 ± 18.8 **
8	345.8 ± 19.6	288.6 ± 18.8 **	333.4 ± 24.0	294.9 ± 18.4 **
9	349.7 ± 20.0	292.6 ± 18.6 **	337.8 ± 23.9	299.4 ± 19.7 **
10	356.6 ± 20.6	297.5 ± 19.2 **	344.2 ± 23.4	303.8 ± 19.6 **
11	363.0 ± 21.6	303.8 ± 18.9 **	351.3 ± 24.4	312.0 ± 19.8 **
12	370.1 ± 21.4	310.8 ± 19.2 **	355.2 ± 24.6	315.4 ± 21.7 **
13	373.9 ± 22.1	314.1 ± 19.9 **	359.9 ± 25.2	318.4 ± 21.6 **
14	380.0 ± 21.7	319.3 ± 20.4 **	365.9 ± 25.5	323.5 ± 22.2 **
15	387.9 ± 23.6	326.2 ± 22.0 **	372.5 ± 25.0	329.6 ± 22.5 **
16	400.3 ± 23.5	334.1 ± 23.2 **	382.9 ± 24.8	338.7 ± 22.9 **
17	415.1 ± 25.0	347.1 ± 24.8 **	396.1 ± 25.2	350.1 ± 23.8 **
18	432.4 ± 26.5	362.5 ± 25.8 **	411.1 ± 26.4	364.5 ± 25.1 **
19	451.4 ± 28.5	376.0 ± 28.3 **	427.9 ± 27.1	378.8 ± 27.4 **
20	470.8 ± 29.5	393.0 ± 30.5 **	444.9 ± 28.5	393.6 ± 28.2 **
LD 0	NE	NE	339.8 ± 33.1	297.4 ± 28.8 **
4	NE	NE	341.9 ± 30.1	308.9 ± 21.4 **
7	NE	NE	349.3 ± 23.5	312.9 ± 23.0 **
14	NE	NE	360.4 ± 25.4	330.6 ± 21.2 **
21	NE	NE	333.3 ± 27.0	310.2 ± 21.0 **

Values represent mean±S.D. *: $p<0.05$, **: $p<0.01$ NE: Not examined
GD: Gestational day LD: Lactational day

Table 5. Food Consumption (g/day) of Female Rats during Gestation

Strain	SD	IGS
Food consumption		
GD 1～2	26.6 ± 3.3	23.0 ± 2.2 **
2～3	27.1 ± 3.5	23.6 ± 2.3 **
3～4	27.3 ± 3.5	23.3 ± 2.6 **
4～5	28.6 ± 4.4	25.0 ± 2.9 **
5～6	29.6 ± 3.8	25.2 ± 3.1 **
6～7	28.8 ± 4.3	24.4 ± 3.2 **
7～8	29.4 ± 4.0	25.0 ± 2.8 **
8～9	28.5 ± 3.9	25.1 ± 3.0 **
9～10	28.7 ± 3.8	24.3 ± 2.7 **
10～11	28.9 ± 4.5	24.6 ± 2.2 **
11～12	29.7 ± 3.6	25.6 ± 2.7 **
12～13	29.1 ± 4.0	26.2 ± 3.6 **
13～14	27.9 ± 3.3	24.6 ± 2.6 **
14～15	27.4 ± 4.0	25.0 ± 3.7 *
15～16	28.7 ± 3.2	24.6 ± 3.0 **
16～17	29.0 ± 3.5	26.2 ± 3.4 **
17～18	30.3 ± 3.6	26.7 ± 2.7 **
18～19	30.2 ± 3.4	27.4 ± 3.0 **
19～20	28.0 ± 3.1	24.9 ± 2.8 **

Values represent mean±S.D. *: $p<0.05$, **: $p<0.01$
GD: Gestational day

Table 6. Organ Weights in Female Rats on Day 20 of Gestation

Strain	SD	IGS
Body weight (g)	470.8 ± 29.5	393.0 ± 30.5 **
Heart (g)	0.94 ± 0.06	0.83 ± 0.08 **
Lung (g)	1.31 ± 0.14	1.19 ± 0.15 **
Liver (g)	20.35 ± 1.85	16.99 ± 1.68 **
Kidneys (g)	2.39 ± 0.22	2.01 ± 0.16 **
Spleen (g)	0.74 ± 0.08	0.59 ± 0.10 **
Adrenals (mg)	82.7 ± 11.7	77.5 ± 12.9
Thymus (mg)	311.7 ± 70.8	292.0 ± 68.6

Values represent mean±S.D. *: $p<0.05$, **: $p<0.01$

Table 7. Estrous Cycles and Fertility in F_0 Rats

Group		Caesarean section		Natural delivery	
Strain		SD	IGS	SD	IGS
No. of animals examined		30	30	25	25
Estrous cyclres	4 day cycle	22	29	20	25
	5 day cycle	3	0	2	0
	irregular cycle	3	1	3	0
	no estrus	2	0	0	0
Copulation index (%)		100.0 (30/30)	100.0 (30/30)	100.0 (25/25)	100.0 (25/25)
Fertility index (%)		96.7 (29/30)	96.7 (29/30)	96.0 (24/25)	100.0 (25/25)
Pre-coital period (Mean ± SD)		2.7 ± 1.4	1.6 ± 0.9	1.7 ± 0.8	1.8 ± 1.1

Copulation index = (No. of animals copulated successfully / no. of mated animals)x100
Fertility index = (No. of pregnant animals / no. of animals copulated successfully)x100

Table 8. Reproductive and Fetal Parameters Obtained with Caesarean Section

Strain		SD	IGS
No. of pregnant animals		29	29
No. of corpora lutea		19.4 ± 2.3	16.6 ± 2.8 **
No. of implantations		17.6 ± 2.2	14.4 ± 2.9 **
Implantation loss (%)		8.3 ± 6.9	6.0 ± 8.1
No. of live fetuses		16.1 ± 2.1	13.6 ± 2.9 **
Body weights (g)			
	♂	3.48 ± 0.24	3.81 ± 0.27 **
	♀	3.33 ± 0.23	3.61 ± 0.23 **
Placental weights (g)			
	♂	0.53 ± 0.06	0.47 ± 0.05 **
	♀	0.50 ± 0.05	0.45 ± 0.04 **
Sex ratio (%)		46.6 ± 12.9	46.3 ± 12.3

Values represent mean± S.D. **: $p<0.01$
Implantation loss = (No. of intrauterine deaths / no. of implantations)x100
Sex ratio = (No. of live male fetuses / no. of live fetuses)x100

anomaly was a ventricular septal defect in 1 fetus in the SD strain. No visceral anomalies were noted in the IGS strain. Visceral variations such as thymic remnant in the neck, supernumerary coronary ostium, dilatation of the renal pelvis, and dilatation of the ureter were observed in both SD and IGS strains. The incidence of thymic remnant in the neck was significantly decreased in the IGS strain. Persistent left umbilical artery was observed only in the SD strain. The total incidence of visceral variations in the IGS strain was significantly decreased compared to the SD strain.

Viability indices from birth until weaning and morphological findings in offspring are presented in Table 10. There were no significant differences between SD and IGS strains in the litter means of the number of implantations, total number of newborn, number of live newborn, birth index or viability index on day 0, 4 or 21 of lactation. No external anomalies were observed in either strain. Visceral anomalies included renal agenesis in 1 dead SD offspring and hydronephrosis in 1 IGS weaned offspring. Visceral variations included dilatation of the renal pelvis in 1 SD dead offspring and in 6 IGS weaned offspring. One SD offspring with a visceral anomaly had multiple skeletal anomalies including fusions of cervical vertebral arches, thoracic vertebral arches, thoracic vertebral bodies and ribs, and rib shortening. Skeletal variations such as dumbbell-shaped thoracic vertebral bodies, unilateral ossification of the thoracic vertebral bodies, variation of the lumbar vertebrae, lumbar ribs and cervical ribs were observed in dead SD offspring.

Body weight during the lactation period and the results of early behavioral and physical development observation are presented in Table 11. Body weight of IGS offspring was significantly lower than that of SD offspring on days 0, 14 and 21 of lactation. The completion days of surface righting reflex in males and females and negative geotaxis in females were significantly shorter in the IGS strain than in the SD strain. The completion day of cliff drop aversion response was significantly prolonged in IGS male offspring. No significant differences in physical development were noted between IGS and SD strains in either males or females.

Body weight of offspring after weaning is presented in Table 12. Body weight of IGS offspring was significantly lower than that of SD offspring from 3 weeks to 9 weeks of age.

The results of the open field test and T-shaped water maze test are presented in Table 12. In the open field test, an increasing tendency was noted in the ambulation and frequency of rearing and defecation in IGS males compared to SD males. The frequency of defecation attained statistical significance. A similar tendency was noted in females with no statistical

Table 9. Morphological Observations in Fetuses

Strain	SD		IGS	
External abnormality (%)	0	(0/468)	0	(0/393)
Skeletal malformations (%)	0.8 ± 3.0	(2/242)	0	(0/206)
Fusion of exoccipital bone and atlas	0.4 ± 2.1	(1/242)		
Fused thoracic vertebral arch	0.8 ± 3.0	(2/242)		
Hypoplasia of thoracic vertebral arch	0.4 ± 2.3	(1/242)		
Thoracic vertebral body defect	0.4 ± 2.1	(1/242)		
Lumbar vertebral arch defect	0.4 ± 2.1	(1/242)		
Fused rib	0.4 ± 2.3	(1/242)		
Skeletal variations (%)	21.4 ± 20.7	(48/242)	21.9 ± 21.4	(47/206)
Cervical rib	0.8 ± 3.0	(2/242)		
Dumbbell-shaped thoracic vertebral body	14.1 ± 19.6	(30/242)	9.2 ± 17.1	(21/206)
Splitting of thoracic vertebral body	1.5 ± 6.4	(4/242)	0.4 ± 2.3	(1/206)
One side ossification of thoracic vertebral body	0.4 ± 2.1	(1/242)		
13th rib shortening	1.3 ± 5.1	(3/242)		
Lumbar rib	2.8 ± 8.0	(7/242)	14.5 ± 14.5	(30/206) **
5 lumbar vertebrae	0.9 ± 3.2	(2/242)		
Sacralization (one side)	0.8 ± 2.9	(2/242)		
One side ossification of lumbar vertebral body	0.4 ± 2.1	(1/242)		
No. of ossified sacral-caudal vertebrae	7.5 ± 0.4	(242)	7.9 ± 0.4	(206) **
Visceral malformations (%)	0.4 ± 2.3	(1/226)	0	(0/187)
Ventricular septum defect	0.4 ± 2.3	(1/226)		
Visceral variations (%)	18.6 ± 16.7	(44/226)	9.0 ± 15.9	(18/187) **
Thymic remnant in neck	12.2 ± 14.9	(29/226)	5.0 ± 12.8	(10/187) **
Supernumerary of coronary ostium	0.4 ± 2.1	(1/226)	0.4 ± 2.3	(1/187)
Dilatation of renal pelvis	6.5 ± 9.2	(15/226)	3.6 ± 8.8	(7/187)
Dilatation of ureter	0.4 ± 2.3	(1/226)	0.6 ± 3.1	(1/187)
Remnant of left umbilical artery	0.4 ± 2.1	(1/226)		

Values represent mean ± S.D. **: $p<0.01$

Table 10. Viability and Morphological Observations in F_1 Pups

Strain		SD	IGS
No. of dams with live pups		24	25
No. of implantations		16.6 ± 2.9	15.6 ± 1.9
No. of pups delivered		15.3 ± 2.7	14.3 ± 2.7
No. of live pups		14.7 ± 2.8	14.2 ± 2.7
Birth index (%)		88.6 ± 9.0	90.7 ± 12.5
Viability index (%)			
	at birth	96.1 ± 5.7	99.2 ± 2.9
	day 4	98.4 ± 3.9	99.0 ± 2.9
	day 21	99.5 ± 2.6	100.0 ± 0.0
Abnormalities of dead pups (%)			
Aplasia of kidney		7.7 (1/13)	
Dilatation of renal pelvis		7.7 (1/13)	
Multiple skeletal malformations		7.7 (1/13)	
Skeletal variations		15.4 (2/13)	
Abnormalities of wealings (%)			
Hydronephrosis		1.0 (1/98)	
Dilatation of renal pelvis		6.1 (6/98)	

Values represent mean ± S.D.
Birth index = (No. of live pups / no. of implantations)x100
Viability index at birth = (No. of live pups / no. of pups delivered)x100
Viability index on day 4 = (No. of live pups on day 4 before culling / no. of live pups)x100
Viability index on day 21 = (No. of live pups on day 21 / no. of live pups after culling)x100

Table 11. Body Weights, Behavioral and Physical Development of F_1 Pups

Strain	SD		IGS	
Sex	♂	♀	♂	♀
Pup body weights (g)				
at birth	6.8 ± 0.5	6.5± 0.5	6.4 ± 0.6 **	6.1 ± 0.6 *
day 4	10.6 ± 1.7	10.2± 1.6	10.5 ± 1.5	10.1 ± 1.4
day 7	18.1 ± 2.4	17.5± 2.2	17.6 ± 2.1	17.0 ± 2.0
day 14	38.8 ± 3.1	37.8± 2.5	35.9 ± 3.1 **	34.8 ± 3.3 **
day 21	65.5 ± 4.9	62.9± 4.0	58.1 ± 5.7 **	55.8 ± 5.4 **
Behavioral development (Day of achievement)				
Surface righting	3.1 ± 1.1	3.2 ± 1.0	2.2 ± 0.7 **	2.3 ± 1.1 **
Cliff aversion	6.8 ± 1.1	7.0 ± 1.2	7.6 ± 1.0 *	7.5 ± 0.8
Negative geotaxis	10.5 ± 0.8	10.5 ± 0.8	10.3 ± 1.0	10.0 ± 0.7 *
Physical development (Day of achievement)				
Incisor eruption	9.7 ± 0.8	9.5 ± 1.0	10.0 ± 0.9	10.0 ± 0.9
Ear opening	12.2 ± 0.4	12.1 ± 0.4	12.5 ± 0.7	12.3 ± 0.5
Eye opening	14.3 ± 0.5	14.3 ± 0.5	14.5 ± 0.5	14.6 ± 0.6

Values represent mean ± S.D. *: $p<0.05$, **: $p<0.01$

Table 12. Body Weights after Weaning, Open Field Test and Water-Filled Multiple T-Maze Test of F_1 Rats

Strain			SD		IGS	
Sex			♂	♀	♂	♀
Body weights after weaning (g)						
	Week	3	65.5 ± 5.6	62.7 ± 4.7	58.1 ± 6.0 **	55.7 ± 5.8 **
		4	112.8 ± 9.0	101.8 ± 7.0	99.4 ± 9.6 **	89.8 ± 11.1 **
		5	177.9 ± 13.1	148.2 ± 10.7	157.6 ± 14.6 **	134.2 ± 9.8 **
		6	246.8 ± 17.6	183.7 ± 14.5	220.7 ± 20.0 **	168.9 ± 12.8 **
		7	318.8 ± 22.1	213.9 ± 16.6	282.8 ± 24.6 **	196.6 ± 16.9 **
		8	387.2 ± 26.8	241.5 ± 20.4	340.8 ± 29.7 **	221.0 ± 20.3 **
		9	443.8 ± 30.4	265.4 ± 24.0	387.4 ± 33.6 **	242.2 ± 24.5 **
Open field test						
Latency		D1	45.7 ± 58.0	50.9 ± 54.0	42.3 ± 50.7	26.8 ± 22.6
(sec)		D2	44.4 ± 56.8	33.3 ± 56.4	22.8 ± 18.9	18.1 ± 17.6
		D3	34.3 ± 60.8	19.0 ± 41.3	21.6 ± 37.7	8.7 ± 7.1
Ambulation		D1	488.7 ± 388.5	676.2 ± 458.4	646.4 ± 356.2	858.7 ± 431.9
(cm)		D2	518.1 ± 351.9	697.3 ± 575.9	803.8 ± 433.6	914.7 ± 585.1
		D3	472.7 ± 516.2	651.0 ± 577.7	660.2 ± 520.7	1011.0 ± 756.3
Rearing		D1	1.8 ± 2.7	2.3 ± 2.9	2.6 ± 3.2	2.4 ± 2.4
		D2	1.8 ± 2.6	1.7 ± 3.1	2.7 ± 2.8	3.6 ± 3.8
		D3	1.2 ± 1.7	2.1 ± 2.9	2.3 ± 3.2	3.0 ± 2.5
Grooming		D1	0.1 ± 0.5	0.3 ± 0.5	0.1 ± 0.3	0.5 ± 0.7
		D2	0.7 ± 1.0	0.8 ± 0.8	0.8 ± 0.9	1.1 ± 1.0
		D3	0.8 ± 1.2	1.1 ± 1.1	1.0 ± 1.3	1.5 ± 1.3
Defecation		D1	2.5 ± 1.5	3.3 ± 1.9	4.3 ± 2.0 **	2.9 ± 1.5
		D2	1.7 ± 1.5	2.7 ± 1.6	2.9 ± 2.3 *	3.0 ± 2.2
		D3	2.2 ± 2.2	3.2 ± 2.2	3.8 ± 1.8 **	3.0 ± 2.0
Urination		D1	0.4 ± 0.7	0.2 ± 0.4	0.5 ± 0.7	0.1 ± 0.4
		D2	0.0 ± 0.2	0.04 ±0.2	0.4 ± 0.7	0.2 ± 0.4
		D3	0.3 ± 0.6	0.2 ± 0.4	0.1 ± 0.3	0.04 ± 0.2
Water-filled multiple T-maze test						
Error	D1	1st	12.8 ± 8.6	11.7 ± 5.2	16.5 ± 6.1	10.9 ± 6.2
		2nd	12.5 ± 8.7	14.3 ± 10.4	13.8 ± 10.1	12.8 ± 9.5
		3rd	8.3 ± 8.5	6.8 ± 5.0	8.2 ± 6.2	10.5 ± 4.9 *
	D2	1st	13.9 ± 14.9	6.3 ± 5.2	10.8 ± 9.6	11.3 ± 9.0 *
		2nd	5.4 ± 5.2	6.1 ± 5.4	4.6 ± 3.6	7.8 ± 5.7
		3rd	3.8 ± 3.7	4.0 ± 2.9	3.2 ± 3.9	3.7 ± 2.9
	D3	1st	8.1 ± 11.8	4.9 ± 6.0	4.1 ± 5.8	3.7 ± 3.7
		2nd	3.4 ± 5.7	4.2 ± 4.3	1.1 ± 1.4	3.9 ± 4.5
		3rd	1.9 ± 3.1	1.8 ± 2.2	0.7 ± 1.2	2.7 ± 3.8
Time	D1	1st	66.1 ± 35.8	52.1 ± 15.2	70.8 ± 24.9	47.9 ± 26.3
(sec)		2nd	60.5 ± 41.1	66.0 ± 43.0	66.2 ± 43.5	63.3 ± 41.1
		3rd	43.6 ± 34.5	41.1 ± 20.3	41.8 ± 24.9	54.0 ± 23.8
	D2	1st	57.6 ± 46.9	36.1 ± 16.9	55.8 ± 40.4	55.1 ± 36.1 *
		2nd	31.9 ± 20.2	31.5 ± 18.4	28.0 ± 12.9	42.5 ± 22.6
		3rd	28.4 ± 22.8	24.7 ± 9.5	23.2 ± 12.8	27.3 ± 15.2
	D3	1st	46.6 ± 42.8	28.6 ± 14.7	32.6 ± 28.1	26.7 ± 15.5
		2nd	25.7 ± 18.5	26.8 ± 10.7	16.8 ± 9.0 *	22.6 ± 15.5
		3rd	21.4 ± 19.7	22.3 ± 7.5	15.4 ± 7.1	25.2 ± 30.0

Values represent mean± S.D. *: $p<0.05$, **: $p<0.01$

significance in both strains. In the T-shaped water maze test, the required time to the goal was significantly shorter in IGS males during the second trial on the third day. In females, the number of errors during the third trial on the first day and the number of errors and required time to the goal during the first trial on the second day were significantly increased in the IGS strain.

The results of reproductive function are presented in Table 13. Nine of 24 SD females and 6 of 25 IGS females had estrous cycles other than a 4-day cycle. There were no significant differences between the IGS and SD strains in the copulation index, fertility index or the number of days required for mating.

Body weight and weight gain of F_1 dams during the gestation and lactation periods are presented in Table 14. Body weight of IGS dams was significantly lower than SD dams from day 0 of gestation until day 4 of lactation. The duration of gestation tended to be shortened in IGS dams (21.5 ± 0.5 days) compared to SD dams (21.9 ± 0.5 days). No abnormalities were observed in delivery, nursing behavior or gross pathology on days 4-6 of lactation in either IGS or SD dams.

Table 13. Estrous Cycles and Fertility in F_1 Rats

Strain		SD	IGS
No. of animals examined		24	25
Estrous cycles	4 day cycle	15	19
	5 day cycle	4	0
	irregular cycle	4	5
	no estrus	1	1
Copulation index (%)		91.7 (22/24)	92.0 (23/25)
Fertility index (%)		77.3 (17/22)	91.3 (21/23)
Pre-coital period (Mean ± SD)		3.8 ± 2.8	3.1 ± 1.9

Copulation index = (No. of animals copulated successfully / no. of mated animals) x100
Fertility index = (No. of pregnant animals / no. of animals copulated successfully)x100

Table 14. Body Weights (g) of F1 Dams

Strain		SD	IGS
Body weights			
GD	0	309.4 ± 28.5	276.7 ± 32.9 **
	7	349.8 ± 33.0	314.8 ± 33.7 **
	14	389.2 ± 38.8	350.7 ± 36.1 **
	17	421.7 ± 39.1	375.0 ± 34.4 **
	20	469.2 ± 40.2	417.2 ± 35.7 **
LD	0	373.6 ± 41.3	334.9 ± 37.8 **
	4	372.5 ± 40.9	338.6 ± 35.5 **

Values represent mean ± S.D. **: $p<0.01$
GD: Gestational day LD: Lactational day

The viability index and body weight of F_2 offspring are presented in Table 15. There were no significant differences between IGS and SD strains in the litter means of the number of implantations, litter size, number of live newborn, birth index, body weight or viability index on day 0 or 4 of lactation. Morphological observation of F_2 offspring which died during the lactation period revealed dilatation of the renal pelvis in 1 SD offspring, but no abnormalities in the other offspring. Nor were any abnormalities observed in any F_2 offspring necropsied on days 4-6 of lactation. No gross lesions in the genital organs were observed in F_1 males after mating.

Table 15. Viability and Body Weights of F_2 Pups

Strain		SD	IGS
No. of dams with live pups		17	21
No. of implantations		15.6 ± 4.0	14.0 ± 2.5
No. of pups delivered		14.5 ± 3.9	13.0 ± 3.5
No. of live pups		14.1 ± 4.1	12.8 ± 3.5
Birth index (%)		89.9 ± 9.9	91.9 ± 18.8
Viability index (%)			
at birth		96.8 ± 6.2	99.0 ± 3.4
day 4		99.2 ± 2.1	99.6 ± 1.8
Pup body weights (g)			
at birth	♂	6.7 ± 0.7	6.5 ± 0.7
	♀	6.4 ± 0.6	6.1 ± 0.6
day 4	♂	10.8 ± 1.9	10.3 ± 1.2
	♀	10.8 ± 2.6	10.1 ± 1.3

Values represent mean±S.D.
Birth index = (No. of live pups / no. of pups delivered)x100
Viability index at birth = (No. of live pups / no. of pups delivered)x100
Viability index on day 4 = (No. of live pups on day 4 / no. of live pups)x100

As described above, some significant differences were noted in some parameters examined in toxicity studies between IGS and SD strains. Therefore, parameters with significant differences from the SD strain should be evaluated carefully in toxicity studies using IGS strain rats.

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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 rat 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 caesarean sectioning and fetal examinations. And these data were compared with that of Crj:CD(SD) rats which we have used.

There were no significant differences in the results of caesarean sectioning and fetus examinations between Crj: CD(SD)IGS rats and Crj:CD(SD) rats, though the gestation index was slightly lower in the former than in the latter. –Key words: Crj:CD(SD)IGS rat, Crj:CD(SD) rat, Background data, Reproduction, Development

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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 drugs. 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 caesarean sectioning and fetal examinations. And these data were compared with that of Crj:CD(SD) rats which we have used.

MATERIALS AND METHODS

Ten male and 35 female Crj: CD(SD)IGS rats at 9 weeks of age were purchased from Charles River Japan, Inc., Hino Breeding Center, on January 16, 1997. The body weight range at arrival was from 282 g to 297 g for males and from 198 g to 217 g for females. The healthy animals, which showed the normal clinical condition and body weight changes during 7-day quarantine and acclimatization periods, were employed for the present study. The body weight range of females on day 0 of gestation was from 246 g to 307 g.

The animals were individually housed in an animal room environmentally maintained under the following conditions throughout the study period: temperature; 20-26°C, humidity; 30-70%, lighting; 12-hour lighting and frequency of air changes; 10 times or more per hour.

The animals were given CA-1 (Clea Japan Inc.) and tap water via an automatic water supplier *ad libitum*.

Each animal was identified with an ear tag and each cage with a label described animal number, study No., etc.

In females at 11 weeks of age, impedance of vaginal mucous epithelium was examined by an impedance checker for determining appropriate mating time. One or two females, which showed 3kΩor more in this test, and one male were housed together in each cage overnight. In the following morning, the females were examined for the presence of sperm in the vaginal smear, and the day on which sperm was observed, was designated as day 0 of gestation. The mating was continued until the number of animals mated was 26.

In females mated, the clinical signs were observed daily, and body weight and food intake were measured once a week.

On day 21 of gestation, the dams were exsanguinated to death from the posterior aorta and vena cava under ether anesthesia, and observed for successful pregnancy and visceral anomalies. In the successfully pregnant rats, the number of corpora lutea and the number of implantation were counted, and the number of live fetuses, absorbed embryos and dead fetuses and their positions in the uterus were examined. As for live fetuses, external anomalies, sex and body weights were examined, and about 1/2 fetuses in each litter were fixed in Bouin solution and the rest of fetuses in 95% ethanol. In fetuses fixed in Bouin solution, the head and abdomen were examined by Wilson's method, and the thorax by a microdissection method to detect visceral anomalies. In fetuses fixed in 95% ethanol, the double stained specimens were prepared by Inouye's method, and the skeletal anomalies and variations, and the degree of ossification were examined.

RESULTS AND DISCUSSION

Since 5 of 26 females mated were nonpregnant, the number of pregnant rats was 21. Therefore the pregnant index was 80.8% (21/26), which was lower compared with that (89.1~99.0%) in Crj: CD(SD) rats (hereinafter abbreviated as CD rats).

No abnormalities were observed in the clinical signs, body weight changes and food intake.

Caesarean sectioning data was shown in Table 1.

The number of corpora lutea and the number of implantation were 17.5 ± 2.1 and 15.9 ± 1.7, respectively. Therefore the implantation index was 91.4 ± 7.3%. On the other hand, the number of corpora lutea and the number of implantation in CD rats tested in this laboratory were 17.5 and 15.0, respectively, indicating that there were no differences in these numbers between Crj:CD(SD)IGS rats and CD rats.

The incidence of absorption was 3.8 ± 6.7%, and all

Table 1. Caesarean sectioning data of Crj: CD(SD) IGS rats

Animal Number	No. of corpora lutea			No. of implantation			Implant. ratio(%)	No. of live fetuses			No. of resorption					Total(%)	No. of dead fetuses(%)	Body weight(g) of fetuses	
	(L)	(R)	Total	(L)	(R)	Total		Male	Female	Total	G	P	E	L	M			Male	Female
3951	6	11	17	6	11	17	100	5	12	17	0	0	0	0	0	0(0)	0(0)	4.96	4.85
3952	9	10	19	9	9	18	94.7	10	7	17	0	1	0	0	0	1(5.6)	0(0)	4.84	4.60
3953		Infertile																	
3954	11	8	19	11	5	16	84.2	6	10	16	0	0	0	0	0	0(0)	0(0)	5.69	5.35
3955	8	14	22	7	11	18	81.8	13	5	18	0	0	0	0	0	0(0)	0(0)	5.19	4.70
3956		Infertile																	
3957	7	9	16	7	9	16	100	9	7	16	0	0	0	0	0	0(0)	0(0)	5.24	4.83
3958	9	10	19	9	8	17	89.5	10	7	17	0	0	0	0	0	0(0)	0(0)	4.90	4.67
3959		Infertile																	
3960	8	10	18	7	8	15	83.3	8	7	15	0	0	0	0	0	0(0)	0(0)	5.55	5.12
3961	11	8	19	8	7	15	78.9	4	11	15	0	0	0	0	0	0(0)	0(0)	5.29	5.29
3962	6	9	15	6	9	15	100	9	6	15	0	0	0	0	0	0(0)	0(0)	5.34	5.24
3963	9	6	15	9	4	13	86.7	7	6	13	0	0	0	0	0	0(0)	0(0)	5.42	5.23
3964		Infertile																	
3965	5	10	15	5	9	14	93.3	5	8	13	0	1	0	0	0	1(7.1)	0(0)	5.71	5.47
3966	7	8	15	5	8	13	86.7	4	6	10	0	3	0	0	0	3(23.1)	0(0)	5.55	4.68
3967	12	9	21	12	8	20	95.2	9	11	20	0	0	0	0	0	0(0)	0(0)	5.13	4.81
3968	10	7	17	10	6	16	94.1	8	8	16	0	0	0	0	0	0(0)	0(0)	5.07	4.70
3969	8	8	16	8	8	16	100	10	3	13	0	3	0	0	0	3(18.8)	0(0)	5.62	5.49
3970	9	9	18	9	8	17	94.4	10	7	17	0	0	0	0	0	0(0)	0(0)	5.49	5.00
3971	10	9	19	9	8	17	89.5	7	10	17	0	0	0	0	0	0(0)	0(0)	5.57	5.12
3972	11	8	19	8	7	15	78.9	10	4	14	0	1	0	0	0	1(6.7)	0(0)	5.79	5.57
3973		Infertile																	
3974	7	9	16	7	9	16	100	8	7	15	0	1	0	0	0	1(6.3)	0(0)	5.32	5.35
3975	6	8	14	6	8	14	100	2	12	14	0	0	0	0	0	0(0)	0(0)	5.65	5.39
3976	10	8	18	9	7	16	88.9	8	6	14	1	1	0	0	0	2(12.5)	0(0)	5.27	4.88
Min.	5	6	14	5	5	13	78.9	2	3	10	0	0	0	0	0	0(0.0)		4.84	4.60
Max.	12	14	22	12	12	20	100	13	12	20	1	3	0	0	0	3(23.1)		5.79	5.57
Mean			17.5			15.9	91.4			15.3						(3.8)		5.36	5.06
±S.D.			±2.1			±1.7	±7.3			±2.2						(±6.7)		±0.28	±0.31

(L) : Left side of uterus horn, (R) : Right side of uterus horn, Mini. : Minimal value per litter, Max. : Maximal value per litter

Implant. : Implantations, G : Maternal gland, P : Placental remnant, E : Early resorption, L : Late resorption, M : Macerate fetus

absorption occurred at early stage of the gestation period. The number of live fetuses was 15.3 ± 2.2. These values were not different from those of CD rats. The body weight of live fetuses was 5.36 ± 0.28 g for males and 5.06 ± 0.31 g for females.

Results of external, visceral and skeletal examinations of fetuses were shown in Table 2.

In the external, visceral and skeletal examinations of live fetuses, the external and skeletal anomalies were not observed in any fetus. As the visceral anomalies, the combined anomaly, hydronephrosis and megaroureter, was observed in one fetus. As a visceral variation, thymic remnant in the neck was in 2 fetuses (1.3%). As the skeletal variations, separation of thoracic vertebral body, separation of lumbar vertebral body, rudimentary lumbar rib, separation of costicartilage and accessory sternebra were observed in total 18 fetuses (12.9%), which was lower compared with the incidence (28.13%) in CD rats tested in this laboratory.

As described above, there were no significant differences in the results of caesarean sectioning and fetus examinations between Crj: CD(SD)IGS rats and CD rats, though the gestation index was slightly lower in the former than in the latter .

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Table 2. External, visceral and skeletal examinations of fetuses from Crj: CD (SD) IGS rats

External examination		
No. of fetuses examined (Litter)		322 (21)
No. of fetuses with anomalies		0
Visceral examination		
No. of fetuses examined (Litter)		159 (21)
No. of fetuses with anomalies (Litter)		3 (3)
Type		
Hydronephrosis & Megaroureter		1 (1)
No. of fetuses with visceral variations (Litter)		
Type		
Thymic remnant in the neck		2 (2)
Skeletal examination		
No. of fetuses examined (Litter)		163 (21)
No. of fetuses with anomalies		0
No. of fetuses with skeletal variation (Litter)		18 (9)
Type		
Separation of thoracic vertebral body		2 (1)
Separation of lumbar vertebral body		1 (1)
Rudimentary lumbar rib		1 (1)
Separation of costicartilage		16 (8)
Accessory sternebra (7th)		1 (1)
No. of ossification sites (Mean ± S. D.)		
Sacro-cocygeal		9.7 ± 0.8
Sternebrae		5.9 ± 0.2
Metacarpi	Right	4.3 ± 0.5
	Left	4.2 ± 0.4
Metatarasi	Right	4.6 ± 0.4
	Left	4.6 ± 0.4
Carpal proximal pharanges	Right	2.4 ± 1.0
	Left	2.2 ± 1.0
Tarsal proximal pharanges	Right	0.8 ± 0.9
	Left	0.9 ± 1.0

Reproductive and Developmental Data in Crj:CD(SD) IGS Rats

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ABSTRACT. In order to collect background data on reproduction and development, pregnant Crj:CD(SD)IGS rats were given tap water by oral gavage from day 6 to day 17 (cesarean section group) or to day 21 of lactation (natural delivery group), and fetal and neonatal examinations were carried out. The results showed a high incidence of renal pelvic dilatation in fetuses and neonates. ––Key words: CD(SD)IGS rats, Reproduction, Development

CD (SD) IGS-1998: 167-169

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 some examinations usually employed in reproductive and developmental toxicity studies to obtain background data.

MATERIALS AND METHODS

Crj:CD(SD) IGS rats were purchased from Charles River Japan Inc. (Tsukuba Breeding Center, Japan) and acclimatized for 2 weeks. Healthy animals that had no abnormalities in appearance and showed normal weight gain were selected. The animals were housed in a room maintained at 23±2 and 50-60% relative humidity. Room air was ventilated 15 times per hr and a 12 hr/12hr light-dark cycle (lighting 7:00-19:00) was imposed. The animals were given a commercially available pellet diet and sterilized tap water ad libitum.

At 10 weeks of age females were paired with 12-week old males of the same strain for a maximum of 2 weeks. The day on which the presence of a vaginal plug or sperm in the vaginal smear was confirmed, was designated as day 0 of gestation.

Throughout the experimental period, females were observed for clinical signs once daily. All females were weighed on day 0, 6, 8, 10, 13, 17and 20 of gestation and those of the natural delivery group were weighed on days 1, 4, 7, 10, 14, 17and 21 of lactation. Food intake per day was determined on the same days on which animals were weighed except day 0 of gestation.

Females in the cesarean section groups 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. Placenta and amniotic fluid were observed grossly. Also, the visceral organs of dams were observed grossly. After measurement of fetal body weights, 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 bone-cartilage double staining method [1] with Alizarin-red and Alcian-blue, and observed for abnormalities, variations (cervical rib, 14th rib, asymmetrical sternebra, shortening of 13th rib, extra lumbar vertebra, dummbell shaped vertebral body) and ossification pattern (5^{th} or 6^{th} sternebra, supraoccipital bone, odontoid process, 7^{th} cervical body, 2^{nd} sternebra, hyoid body, number of ossified sacral and caudal vertebrae, number of ossified metacarpal, number of ossified metatarsal). The remaining fetuses were fixed in Bouin's solution for visceral examination by Wilson's method [2] and modified Nishimura's method [3].

All females 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 animals of each sex. Total male and total female weights per litter were determined on day 0, 4 of lactation, and after culling all fetuses were individually weighed on day 4, 7, 14 and 21 of lactation.

As a postnatal developmental test, completion of ear unfolding, palmar grasp reflex, incisors eruption, auditory startle reflex and eyelid opening were examined once or twice until weaning.

On day 22 of lactation, dams were necropsied and implantation sites were counted. Neonates were also necropsied and their visceral organs were observed grossly.

Part of the present results were compared to our Slc:SD rat's background data.

RESULTS AND DISCUSSION

No abnormal clinical signs were observed during pregnancy and lactation periods. Also no abnormalities on body weight and food intake were noted (Table 1 and 2). At necropsy, no abnormal findings were observed.

The number of corpora lutea, implantations, live fetuses, ratio of pre-implantation loss and fetal weight were slightly higher than our Slc:SD background data. Frequency of fetal resorptions and sex ratio were similar to those of the background data (Table 3).

One incidence of anal atresia and one of rudimentary tail were seen as external anomalies (Table 4). However, the frequency was low. In visceral examination of fetuses, a high incidence of renal pelvic dilatation was seen. Most of them were accompanied by dilatation of the ureter (Table 5). The

frequency of skeletal anomalies was considered to be normal (Table 6). There were no characteristic changes in skeletal variation (Table 7). The number of ossified sacral and caudal vertebrae were slightly low, but probably within normal range (Table 8).

There were no abnormalities in delivery and nursing of dams (Table 9). Neonatal body weight changes seemed to be normal (Table 10). The results of post-natal developmental tests were considered to be normal (Table 11). Necropsy of pups at weaning showed a high incidence of dilatation of renal pelvis as observed in fetuses (Table 12).

In conclusion, we consider that it is necessary to accumulate a larger amount of background data for IGS rats in order to evaluate the reproduction toxicity data adequately.

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Table 1. Body weight during gestation and lactation in dams

During of Gestation		
Number of animals		45
Day of gestation	0	250.0 ± 13.5
	6	282.8 ± 12.8
	8	291.8 ± 12.5
	10	302.0 ± 14.1
	13	320.3 ± 15.8
	17	355.3 ± 18.6
	20	401.6 ± 22.6
	0-20 (gain)	151.5 ± 19.0
During of Lactation		
Number of animals		22
Day of lactation	1	293.0 ± 17.2
	4	315.0 ± 15.6
	7	324.5 ± 14.8
	10	335.0 ± 14.6
	14	344.2 ± 17.2
	17	341.8 ± 18.3
	21	324.5 ± 14.9
	1-21 (gain)	31.5 ± 15.9

Values : Mean (g)±S.D.

Table 2. Food intake during gestation and lactation in dams

During of Gestation		
Number of animals		45
Day of gestation	6	24.3 ± 2.7
	8	25.0 ± 3.0
	10	25.2 ± 3.1
	13	26.2 ± 2.9
	17	27.6 ± 2.7
	20	24.6 ± 2.8
During of Lactation		
Number of animals		22
Day of lactation	1	17.3 ± 7.6
	4	42.8 ± 8.0
	7	46.8 ± 5.6
	10	56.4 ± 6.5
	14	61.2 ± 6.5
	17	64.6 ± 8.0
	21	74.5 ± 8.7

Values : Mean (g)±S.D.

Table 3. Cesarean section findings in dams

Strain		Crj:IGS	Slc:SD Background data
No. of dams examined		23	780
No. of corpora lutea	Total	403	
	Mean±S.D.	17.5 ± 2.9	15.0 ± 0.5
No. of implantations	Total	354	
	Mean± S.D.	15.4 ± 3.2	14.0 ± 0.6
Percent of preimplantation losses		12.2	6.8
No. of dams with all resorptions(%)		0 (0.0)	-
Postimplantation losses	(%)	16 (5.0)	2.0-9.2%
Early resorptions	(%)	15 (4.7)	
Late resorptions	(%)	0 (0.0)	
Dead fetuses	(%)	1 (0.3)	
No. of live fetuses	Total	338	
	Mean± S.D.	14.7 ± 3.3	13.1 ± 0.6
Sex ratio (Male / Male+Female)		0.45	0.50
Fetal weight(g)	Mean± S.D.	3.61 ± 0.49	3.42 ± 0.09

Table 4. External anomalies of fetuses

No. of dams	23
No. of fetuses	338
No. of fetuses with anomalies (%)	1 (0.3)
Anal atresia	1 (0.3)
Rudimentary tail	1 (0.3)

Table 5. Visceral anomalies of fetuses

Strain	Crj:IGS	Slc:SD Background data
No. of dams	23	
No. of fetuses	165	
No. of fetuses with anomalies (%)	38 (26.3)	
Dilatation of renal pelvis	38 (26.3)	0-1.0%
Dilatation of ureter	30 (20.6)	0-1.0%

Table 6. Skeletal anomalies of fetuses

No. of dams	23
No. of fetuses	172
No. of fetuses with anomalies (%)	9 (5.2)
Separation of cervical vertebral body	2 (1.2)
Separation of thoracic vertebral body	3 (1.7)
Fusion of 2nd carpal bone and 2nd metacarpals	5 (2.9)

Table 7. Skeletal variations of fetuses

No. of dams	23
No. of fetuses	172
No. of fetuses with variations(%)	22(13.0)
Cervical rib	2 (1.2)
Asymmetrical sternebra	1 (0.6)
Extra lumbar vertebra	0 (0.0)
Dumbbell shaped vertebral body	5 (3.0)
Shortening of the 13th rib	0 (0.0)
14th rib	
Total	15 (8.9)
Rudimentary	15 (8.9)
Extra	0 (0.0)

Rudimentary: Less than half the length of the 14th rib
Extra: Half or greater than half the length of the 14th rib

Table 8. Ossification patterns of fetuses

Strain	Crj:IGS	Slc:SD Background data
No. of dams	23	
No.of fetuses	172	
No. of fetuses with unossified(%)		
5th sternebra	73 (41.2)	
6th sternebra	60 (33.9)	
Supraoccipital bone	0 (0.0)	
Odontoid process	150 (83.1)	
7th cervical body	50 (27.6)	
2nd sternebra	9 (5.4)	
Hyoid body	0 (0.0)	
No. of ossified bones (Mean ± S.D.)		
Sacral & caudal vertebrae	7.8 ± 1.1	8.3 ±0.3 (7.7-8.9)
Metacarpal	7.3 ± 0.7	
Metatarsal	8.0 ± 0.6	

Table 9. Nursing and lactation behavior of dams

No. of pregnant females		22
No. of dams with live newborns		22
Delivery index		100.0
Gestation period (days)	Mean ± S.D.	22.1 ± 0.4
No. of implantation sites	Mean ± S.D.	15.2 ± 2.6
No. of delivered pups	Mean ± S.D.	14.3 ± 2.7
No. of live newborns	Mean ± S.D.	14.0 ± 2.9
No. of stillborns (%)		2.2
Birth index		91.7
Sex ratio at birth	(Male/Male+Female)	0.50
No. of live pups		
At birth	Mean ± S.D.	14.0 ± 2.9
Day 4 before culling	Mean ± S.D.	13.9 ± 2.9
Viability index (day 4)		98.8
Weaning index		100.0

Delivery index: (No. of dams with live newborns / No. of pregnant females) ×100
Birth index: (No. of live newborns / No. of implantation sites) ×100
Viability index: (No. of live pups on days 4 after birth / No. of live newborns) ×100
Weaning index: (No. of live pups on days 21 after birth / No. of live pups after culling on days 4) ×100

Table 10. Postnatal development of offspring during lactation period

Male		
No. of pups positive/examined(%)		
Ear unfold	(day 2)	52 / 155 (35.5)
	(day 4)	155 / 155 (100.0)
Palmar grasp	(day 5)	85 / 85 (100.0)
Incisors eruption	(day 11)	85 / 85 (100.0)
Eyelid opening	(day 15)	80 / 85 (94.3)
	(day 17)	85 / 85 (100.0)
Female		
No. of pups positive/examined(%)		
Ear unfold	(day 2)	57 / 150 (40.8)
	(day 4)	149 / 150 (99.4)
Palmar grasp	(day 5)	87 / 87 (100.0)
Incisors eruption	(day 11)	87 / 87 (100.0)
Eyelid opening	(day 15)	84 / 87 (96.6)
	(day 17)	87 / 87 (100.0)

Table 11. Body weight of offspring during lactation period

Male	Day	1	7.3 ± 0.5
		4	10.5 ± 1.1
		4C	10.6 ± 1.1
		7	17.6 ± 1.4
		14	35.3 ± 1.9
		21	56.0 ± 3.0
Female	Day	1	6.9 ± 0.6
		4	10.0 ± 1.1
		4C	10.0 ± 1.0
		7	16.6 ± 1.5
		14	34.0 ± 1.9
		21	53.6 ± 3.4

C : After culling Values : Mean (g)±S.D.

Table 12. Macroscopic findings of offspring on day 22 of lactation

No. of offspring examined	172
Dilatation of renal pelvis (%)	31 (18.0)

Background Control Data of Reproductive and Developmental Toxicity Studies in Crj:CD(SD)IGS Rats

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ABSTRACT. The objective of this study was to accumulate data of the reproductive and developmental parameters in four studies using Crj:CD(SD)IGS rats. The data included sperm analysis, testis weight, mating, fertility, estrous cycle, fetal morphological alterations (external, visceral and skeletal findings) and other observations performed at the terminal cesarean section on day 20 of gestation in this strain of rats. –Key words: CD(SD)IGS Rat, Development, Reproduction

CD (SD) IGS-1998: 170-173

INTRODUCTION

We accumulated the reproductive and developmental background data (sperm analysis, testis weight, estrous cycle, mating, fertility, reproductive and fetal parameters, fetal morphological observations) in Crj:CD(SD)IGS rats. The data of each item in the four studies were combined in this report. The studies were performed from November 1996 to December 1997.

MATERIALS AND METHODS

1. Animals and rearing conditions

Crj:CD(SD)IGS strain male and female rats were purchased from Charles River Japan, Inc. (Hino Farm). The animals were acclimated and quarantined for one week or more in the same conditions as the study, and healthy males aged 12 to 14 weeks and females aged 9 to 11 weeks were used for the study. The date of receipt, number of animals, rearing and environmental conditions were as follows:

Date of receipt	:	Nov. 1996 ～ Sep. 1997
Age and body weight of females at start	:	10 ～ 12 weeks 213 ～ 306 g
No. of animals per study	:	20 ～ 24 / sex
Rearing room	:	Animal facility with barrier system
Cage	:	Made of aluminum with stainless steel mesh floor
No. of animals per cage	:	2 to 3 / sex (pre-mating period) 1 male and 1 female (1 pair, mating period) 1 (gestation period)
Room temperature & relative humidity	:	22.4 ～ 23.9°C and 47 ～ 67%
Ventilation & lighting	:	14 ～ 20 air changes / hour and 12 hours from 7:00 a.m. to 7:00 p.m.
Food	:	Free access to solid food (CRF-1,autoclaved, Oriental Yeast, Co., Ltd.)
Drinking water	:	Free access to filtered tap water containing 2±1 ppm of chlorine adjusted with sodium hypochlorite from an automatic dispenser
Cage exchange	:	Once or more in 2 weeks

2. Observation items and methods

1) General signs

The animals were observed for general signs of ill health and abnormal behavior once daily during the study period.

2) Estrous cycles

Vaginal smears were collected for estrous cycles daily from the age of 10 weeks for 2 weeks and examined microscopically after Wright's staining.

3. Mating

The males (15-week-old) and females (12-week-old) were mated on a one-to-one basis for a maximum of two weeks. During the mating period the animals were observed daily for general signs, and vaginal smears were taken daily from the females. Copulation was regarded as established when a vaginal plug was found in the vagina or sperm was present in the smear, and the day was considered day 0 of gestation. At this time, the females were separated from the males. Copulation index was calculated from the following formula.

Copulation index (%) =[No. of animals with confirmed copulation / No. of animals mated] × 100.

4. Observation of males after confirmed copulation

All the males with confirmed copulation were bled to death under ether anesthesia after weighing the body on the day of necropsy, and were examined gross pathologically. The testes and epididymides were removed, and the testes were weighed (combined right and left weights) and the relative weight was calculated. Sperm count and sperm viability in the epididymis cauda were examined in about two thirds of the males in accordance with the modified manual method of Suzuki [4].

5. Observation of pregnant animals

All the dams were bled to death under ether anesthesia on day 20 of gestation and dissected for gross pathological observation, and cesarean section was performed to confirm pregnancy and to determine the number of corpora lutea, implantations, resorptions (early or late) and live fetuses. The fertility index, implantation rate, rate of resorptions and dead fetuses were calculated from these values by the following formulas.

Fertility index (%) = [No. of pregnant animals / No. of animals with confirmed copulation] × 100.

Implantation rate (%) = [No. of implantations / No. of corpora lutea] × 100.

Rate of resorption and dead fetuses (%)
= [No. of resorptions and dead fetuses / No. of implantations] ×100.

6. Observation of live fetuses
The live fetuses were individually weighed, sexed and examined for external abnormalities. Each placenta was weighed. Mean male and female fetal weights in each litter were calculated independently and collectively, and mean placental weight was calculated without distinction of sex. Sex ratio and incidence of fetuses with abnormalities were calculated as follows.
Sex ratio (%) = [No. of live male fetuses / No. of live fetuses] ×100.
Incidence of fetuses with abnormalities (%) = [No. of fetuses with abnormalities / No. of examined fetuses] × 100.
About half of the live fetuses from each dam were fixed in Bouin's solution and gross slice specimens were prepared by the modified method of Wilson [5] or Nishimura [3]. The specimens were examined for visceral abnormalities and variations with a stereomicroscope.
The other fetuses were fixed in 99% ethanol, stained with alizarin red S [1] after removing the external skin, and examined for skeletal abnormalities, variations and ossifications with a stereomicroscope.

RESULTS AND DISCUSSION

1. General signs
Piloerection and decrease in spontaneous motility were observed in one 12-weeks-old female(1.2%). Thereafter, the animal showed debility and was euthanized due to moribundity. Necropsy of this animal revealed dilatation of the ureter, white foci in the kidney and urethral calculus in the urethra. From these findings, the cause of death was considered to be aggravation of general signs due to urethral calculus. There were no abnormal signs in any other animals.
2. Estrous cycles, mating and fertility (Table 1)
Estrous cycles other than a 4-day cycle were observed in 2 of 84 females. One of the 2 females had a 5-day cycle, and the other, the aforementioned 1 female with urethral calculus had an irregular cycle. Copulation was confirmed in all the pairs expect 1 pair and mean days until copulation were 2.7. Among the females with confirmed copulation, only 1 animal did not become pregnant, and copulation and fertility indices were 98.8 and 97.6%, respectively.
3. Sperm analysis and testes weight (Table 2)
Sperm viability scarcely varied among individuals. On the other hand, sperm count varied relatively widely, and the difference between min. and max. values was 2.5 times

Table 1. Estrous Cycle and Fertility in Crj : CD(SD) IGS Rats

Item	Mean[a)]	(Min.- Max./Study)
Estrous cycles (84 females)		
4 - day cycle (%)	97.5	(95.0 - 100)
5 - day cycle (%)	1.25	(0.0 - 5.0)
Irregular cycle (%)	1.25	(0.0 - 5.0)
Fertility (84 pairs)		
Copulation index (%)	98.8	(95.0 - 100)
Fertility index (%)	97.6	(94.7 - 100)
Days until copulation	2.7	(2.5 - 3.0)

a) :Values represent mean percentage of 4 tests

Table 2. Testes Weight and Sperm Analysis in Crj : CD(SD) IGS Rats

Item	Mean[b)]	(Min.- Max./Study)	(Min.- Max./Male)
Testes weight (84 males)[a)]			
Actual (mg)	3431	(3364 - 3521)	(2836 - 4231)
Relative (%)	0.68	(0.65 - 0.72)	(0.58 - 0.82)
Body weight (g)	505.5	(473 - 523)	(467 - 560)
Sperm analysis (54 males)			
Sperm count[c)]	4.08	(3.61 - 4.58)	(2.60 - 6.53)
Sperm viability (%)	93.7	(92.6 - 95.4)	(88.4 - 96.5)

a) : Sacrificed at 15 - 16 weeks
b) : Values represent mean of 4 tests
c) : Sperm count :x 10^8/g epididymis cauda

Table 3. Reproductive and Fetal Parameters Obtained by Cesarean Section in Crj : CD(SD) IGS Rats

Item	Mean[a)]	(Min.-Max./Study)	(Min.-Max./Litter)
No. of dams examined	21.3	(18 - 24)	
No. of corpora lutea	16.0	(15.7 - 16.4)	(13 - 21)
No. of implantations	15.2	(14.9 - 15.4)	(8 - 20)
Implantation rate (%)	94.8	(93.9 - 96.2)	(57.1 - 100)
No. of resorptions and dead fetuses			
Early	0.7	(0.5 - 1.0)	(0 - 3)
Late	0.01	(0 - 0.04)	(0 - 1)
Total	0.8	(0.5 - 1.0)	(0 - 4)
Rate of resorptions and dead fetuses (%)	4.8	(3.2 - 6.0)	(0 - 26.7)
No. of live fetuses			
Male	7.1	(6.7 - 7.7)	(2 - 12)
Female	7.3	(7.0 - 7.6)	(1 - 13)
Total	14.5	(14.2 - 14.8)	(8 - 19)
Sex ratio (%)	49.4	(47.2 - 52.6)	(14.3 - 91.7)
Fetal weight (g)			
Male	4.06	(3.96 - 4.13)	(3.25 - 4.68)
Female	3.84	(3.76 - 3.92)	(2.94 - 4.29)
Total	3.95	(3.85 - 4.02)	(3.10 - 4.31)
Placental weight (g)	0.46	(0.45 - 0.47)	(0.35 - 0.58)

a) : Values represent mean of 4 tests

among individuals, and that between mean min. and max. values was 1.3 times among studies.

4. Observation at cesarean section (Table 3)
The mean number of corpora lutea, implantations, live fetuses and mean implantation rate were 16.0, 15.2, 14.5 and 94.8%, respectively. Mean fetal weight (combined male and female) was 3.95g: (male: 4.06g, female: 3.84g). Mean placental weight was 0.46g and no gross pathological abnormalities appeared in the placenta.
5. Fetal morphological observation (external, visceral and skeletal findings) (Table 4)
No external abnormalities were observed in any fetus. The only visceral abnormality was dilatation of the cerebral ventricle in one fetus. As visceral variations, thymic remnant in the neck was observed in one fetus (incidence: 0.16%) and dilatation of renal pelvis in three fetuses (incidence: 0.47%). No skeletal abnormalities were observed in any fetus. Skeletal variations such as splitting of the thoracic vertebral body, 5 lumbar vertebrae, 7 lumbar vertebrae, cervical rib, 14th rib, wavy ribs and shortening of the rib were observed, and the incidence of fetuses with skeletal variations was 7.9%. The most frequent skeletal variation was 14th rib (rudimentary), and the incidence was 5.7%.
6. Skeletal ossifications (Table 5)
No delayed ossifications were observed in any of 594 fetuses. The number of sternebrae, caudal vertebrae, metacarpal and metatarsal bones as indices of ossification was 5.9, 4.3, 3.9 and 4.0, respectively.

As above-mentioned, we investigated the reproductive and developmental parameters of Crj:CD(SD)IGS rats. When the parameters were compared with our background data of Jcl:SD rats, the greatest difference was shown in the skeletal variations. Mean incidence of 14th rib, the most frequently observed skeletal variation in Crj:CD(SD)IGS rats (about 8%) was reduced to about one-forth of that in Jcl:SD rats (about 30%) (Table 6). It is known that there is a wide differences among strains or bleeders in the incidence of 14th rib (lumbar rib) in rats [2]. There were no remarkable differences between Crj:CD(SD)IGS and Jcl:SD rats in any other parameters.

Table 4. Morphological Observation in Crj : CD(SD) IGS Rat Fetuses

Item	Number	(Min.-Max./Study)	Incidence (%)	(Min.- Max./Study)
No. of fetuses examined	1231			
External abnormality	0		0	
No. of fetuses examined	637			
Visceral abnormality	1	(0 - 1)	0.16	(0 - 0.8)
・Dilatation of cerebral ventricle	1	(0 - 1)	0.16	(0 - 0.8)
Visceral variations	4	(0 - 3)	0.63	(0 - 1.6)
・Thymic remnant in neck	1	(0 - 1)	0.16	(0 - 0.5)
・Dilatation of renal pelvis	3	(0 - 2)	0.47	(0 - 1.1)
No. of fetuses examined	594			
Skeletal abnormality	0		0	
Skeletal variations	47	(8 - 16)	7.91	(4.9 - 11.7)
・Splitting of thoracic vertebral body	9	(1 - 4)	1.52	(0.6 - 3.3)
・5 lumbar vertebrae	1	(0 - 1)	0.17	(0 - 0.8)
・7 lumbar vertebrae	2	(0 - 2)	0.34	(0 - 1.5)
・Cervical rib	1	(0 - 1)	0.17	(0 - 0.7)
・14 th rib (rudimentary)	34	(6 - 13)	5.72	(4.3 - 9.5)
・Wavy ribs	2	(0 - 1)	0.34	(0 - 0.8)
・Shortening of rib	1	(0 - 1)	0.17	(0 - 0.7)

Table 5. Skeletal Ossification in Crj : CD(SD) IGS Rat Fetuses

Item	Number	Incidence (%)
No. of fetuses examined	594	
No. of fetuses with delayed ossification	0	0
Item	Mean[a)]	(Min.-Max./Study)
No. of sternebrae	5.9	(5.8 - 5.9)
No. of caudal vertebrae	4.3	(4.2 - 4.5)
No. of metacarpal bones (L)	3.9	(3.8 - 3.9)
(R)	3.9	(3.8 - 3.9)
No. of metatarsal bones (L)	4.0	(4.0 - 4.0)
(R)	4.0	(4.0 - 4.0)

a) : Values represent mean of 4 tests

Table 6. Skeletal Variations in Jcl : SD Rat Fetuses

Item	Number	(Min.-Max./Study)	Incidence (%)	(Min.-Max./Study)
No. of fetuses examined	406			
Skeletal variations	125	(24 - 53)	30	(21.4 - 35.8)
・Splitting of thoracic vertebral body	1	(0 - 1)	0.3	(0.0 - 0.9)
・7 lumbar vertebrae	2	(0 - 2)	0.5	(0.0 - 1.4)
・14 th rib (extra)	3	(0 - 2)	0.7	(0.0 - 1.4)
(rudimentary)	121	(24 - 51)	29.6	(21.4 - 35.8)
・Splitting of sternebra	1	(0 - 1)	0.2	(0.0 - 0.7)

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Comparison between Crj:CD(SD)IGS and Crj:CD(SD) Rats in Behavioral Observations

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ABSTRACT. The gold standard system is an animal breeding system of which purpose is to provide experimental animals having uniform quality in the world. It was developed by Charles River Inc. for internationalization of research and development of new drugs. The authors have examined on the growth as well as the learning ability and memory evaluated in reproductive and developmental toxicity study using Crj:CD(SD)IGS rat bred by the gold standard system. The data were compared with those of Crj:CD(SD) rats.

Although significantly lower body weight in male F_1 offspring than Crj:CD(SD) rats was noted in Crj:CD(SD)IGS rats during the postweaning period, there were no differences between these two strains regarding body weight gains in female F_1 offspring during the lactation and postweaning periods. The sensitivity for the learning ability and memory of F_1 offspring were significantly higher in Crj:CD(SD)IGS rats than in Crj:CD(SD) rats. —Key words: CD(SD)IGS Rat, F_1 Offspring, Leaning Ability, Memory

CD (SD) IGS-1998: 174-177

INTRODUCTION

The gold standard system, a new animal breeding system, has been developed by Charles River Inc. to cope with the internationalization of research and development of new drugs by supplying, as much as possible, uniform experimental animals by minimizing the genetic ramifications.

In the reproductive and developmental toxicity studies of new drugs, the effects of new drugs on behavioral observations have been usually evaluated in F_1 generation. In our laboratory, the learning ability and memory, spontaneous motor activity and emotionality have been examined in male and female F_1 rats in order to assess the effects of new drugs on behavioral observations. The authors performed the shuttle box avoidance test to estimate the leaning ability and memory in F_1 generation using CD(SD)IGS rats produced by the above described new system, and the data were compared with those of CD(SD) rats.

MATERIALS AND METHODS

Animals: Crj:CD(SD)IGS rats (Tsukuba Breeding Center, hereafter referred to as "IGS strain") and Crj:CD(SD) rats (Hino Breeding Center, hereafter referred to as "SD strain"), which were 10 and 11 weeks of age, respectively, were purchased from Charles River Japan Inc. They 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°C and a relative humidity of 40-60%, ventilated 10-20 times/hour and provided with light for 12 hours a day. These animals were allowed free access to pellet food (CRF-1, Oriental Yeast Co., Ltd.) and sterilized tap water *ad libitum*. Male and female rats of both strains were mated after one-week acclimation. One female rat paired with one male rat overnight in the same cage and females with spermatozoa observed in the vaginal smears were considered on day 0 of gestation. Pregnant female rats were housed in rat breeding cages (W220 × D380 × H200 mm) on day 20 of gestation.

Measurements and observations: *Parturition*: All pregnant dams were allowed to litter normally and nurtured the offspring. The day of parturition was considered on postpartum day 0. On postpartum day 0, all litters were examined for gross external malformations, sexed, and 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. Litter size was reduced to 8 pups/litter on postpartum day 4 with equal numbers per sex when possible. On postpartum day 21, the pups were weaned and the dams, which were euthanatized by exsanguination under the anesthesia with halothane or carbon dioxide, were examined for the number of implants.

Body weight changes in F_1 rats: Male and female F_1 rats were weighed once a week until 7 week-old.

Shuttle box avoidance test: The shuttle box avoidance test was performed at 5 and 7 weeks of age in order to examine the learning ability and memory in 10 male and 10 female F_1 rats of each strain. The shuttle box apparatus (MSB-001, Toyo Sangyo Co., Ltd.) consisted of two separate compartments and each compartment was equipped with grids for foot shock. Each animal was tested for 50 trials per day over 3 days at 5 weeks of age and again once at 7 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 the unconditioned stimulus (1400 V, 0.5 mA of electric current). There was a 20-second interval between each trial (intertrial interval). The numbers of avoidance, escape and intertrial interval responses for each animal were recorded. The latency time of avoidance and escape for each animal was also recorded. 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.

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. Statistical analyses of data were performed using the following methods.

Fisher's exact test [1] was used to analyze incidence data. Numerical data were analyzed using Student's t-test [1] or Aspin-Welch test [1]. Statistical analyses were made between IGS and SD rats at two-tailed 5% and 1% levels of significance.

RESULTS

Delivery status in F_0 dams and postnatal viability in F_1 offspring: The delivery status in F_0 dams of IGS and SD strains and the postnatal viability in their F_1 offspring are shown in Table 1.

There were no differences between IGS and SD rats with regard to the gestation period as well as the number of implants, stillborn pups, live pups and their sex ratio. Further, the gestation and birth indices in IGS rats were comparable to those in SD rats. On the other hand, the viability indices on postnatal day 4 and weaning indices in F_1 offspring of IGS rats were not different from those of SD rats. Besides, no F_1 pups with external malformations were observed for both IGS and SD rats.

Body weight changes in F_1 offspring: Body weight changes in F_1 offspring of IGS and SD rats are presented in Table 2.

No significant differences of body weight gains were observed for both IGS and SD males during the lactation period. However, significantly lower body weight than SD males was noted in IGS males from 4 weeks to 7 weeks of age.

There were no differences between IGS and SD females with respect to body weight gains during the lactation and postweaning periods.

Shuttle box avoidance test in F_1 offspring: The results of shuttle box avoidance test in F_1 offspring of IGS and SD rats are shown in Table 3.

Significantly higher avoidance rates than SD males were noted in IGS males on Days 1, 2, 3 and 15. In addition, the escape rates were significantly lower in IGS males than in SD males on Days 1, 2, 3 and 15. On the other hand, the avoidance times were significantly shorter in IGS males than in SD males on Days 1 and 2. Further, the escape times were shortened in IGS males on Days 1, 2, 3 and 15 as compared with SD males. Besides, the numbers of intertrial interval responses were significantly increased in IGS males in comparison with SD males on Days 1, 2, 3 and 15.

The avoidance rates were higher in IGS females than in SD females on Days 1 and 2. The escape rates were lower in IGS females than in SD females on Days 1 and 15. Moreover, significantly shorter avoidance times than SD females were observed for IGS females on Days 1, 2 and 15. In addition, the escape times were shortened in IGS females on Days 1 and 3 compared with SD females. However, the numbers of intertrial interval responses in IGS females were comparable to those in SD females.

DISCUSSION

Though the authors have previously performed the reproductive and developmental toxicity studies of new drugs using SD rats, we are going to conduct these studies employing IGS rats produced by the gold standard system, a new animal breeding system developed by Charles River Inc., in future. Thus, we carried out the shuttle box avoidance test in order to evaluate the leaning ability and memory in F_1 generation using IGS rats, and the results of IGS rats were compared with those of SD rats.

There were no differences between IGS and SD rats with regard to the gestation indices, birth indices and gestation period as well as the numbers of implants, stillbirths and live pups and their sex ratio. Further, the viability indices on postnatal day 4 and weaning indices in IGS rats were comparable to those in SD rats. Therefore, it was considered that there were no differences between IGS and SD rats regarding the delivery status in F_0 dams and postnatal viability in their F_1 offspring.

Although no significant differences of body weight gains in F_1 generation were observed for both IGS and SD male rats during the lactation period, significantly lower body weight than SD males was noted in IGS males from 4 weeks to 7 weeks of age. However, there were no differences between IGS and SD females with respect to body weight gains in F_1 generation during the lactation and postweaning periods. Matsumoto et al. [3] reported that body weight gains in both male and female F_1 rats were lowered in IGS strain as compared with SD strain. Our findings in male F_1 rats were consistent with their results, but those in female F_1 rats differed from their results. These findings indicated that there were differences between IGS and SD strains regarding the growth of male F_1 offspring.

Regarding the results of the shuttle box avoidance test in male F_1 rats, it was observed 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 strain compared with SD strain. Further, the results in female F_1 rats were similar to those in male F_1 rats excluding the numbers of intertrial interval responses. These findings also suggested that there were differences between IGS and SD strains with respect to the learning ability and memory in F_1 progeny.

In conclusion, it was considered that there were differences between IGS and SD rats, especially in male offspring, with regard to their growth as well as the leaning ability and memory.

The results obtained from the study using IGS rats have been already reported in the Japanese Pharmacology and Therapeutics [2].

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Table 1. Delivery status in F_0 rats, and postnatal viability in F_1 offspring.

Groups		SD strain	IGS strain
No. of pregnant dams		12	11
No. of dams with live offspring		11	11
Gestation index (%)		91.67	100.00
Gestation period	Mean ± S.D., day	22.18 ± 0.40	22.45 ± 0.52
No. of implants	Total	184	175
	Mean ± S.D.	15.33 ± 4.68	15.91 ± 2.55
No. of stillbirths (%)		5 (2.72)	0 (0.00)
No. of offspring born alive	Total	175	167
	Mean ± S.D.	15.91 ± 0.70	15.18 ± 2.32
Birth index (%)		95.11	95.43
Sex ratio (males/females)		0.79 (77/98)	1.06 (86/81)
No. of offspring alive on postnatal day 4		173	166
Viability index on postnatal day 4 (%)		98.86	99.40
No. of offspring alive immediately after culling		88	83
No. of live weanlings		88	83
Weaning index (%)		100.00	100.00
External malformations:			
No. of offspring with external malformations (%)		0 (0.00)	0 (0.00)

Gestation index (%) = (No. of dams with live offspring/No. of pregnant dams) $\times$100.
Birth index (%) = (No. of offspring born alive/No. of implants) $\times$ 100.
Viability index on postnatal day 4 (%) = (No. of offspring alive on postnatal day 4/No. of offspring born alive) $\times$ 100.
Weaning index (%) = (No. of live weanlings/No. of offspring alive immediately after culling) $\times$100.

Table 2. Body weight changes in male and female F_1 rats.

	Groups		SD strain	IGS strain
Male	No. of litters examined		11	11
	Postnatal day	0	6.19 ± 0.55	6.66 ± 0.52
		7	18.35 ± 1.61	18.45 ± 2.24
		14	37.47 ± 2.63	39.34 ± 3.40
		21	60.09 ± 3.95	62.42 ± 4.23
	No. of F_1 rats examined		22	21
	Postnatal week	4	94.77 ± 7.71	89.19 ± 9.03*
		5	156.32 ± 11.24	139.29 ± 13.18**
		6	225.45 ± 14.64	205.33 ± 16.92**
		7	293.00 ± 18.73	268.24 ± 20.07**
Female	No. of litters examined		11	11
	Postnatal day	0	5.93 ± 0.57	6.46 ± 0.53
		7	17.64 ± 1.69	17.72 ± 2.14
		14	36.40 ± 2.60	37.90 ± 3.37
		21	57.72 ± 3.48	59.82 ± 4.33
	No. of F_1 rats examined		22	22
	Postnatal week	4	85.86 ± 6.09	88.82 ± 8.54
		5	133.86 ± 9.01	128.14 ± 10.34
		6	168.91 ± 12.35	165.18 ± 11.67
		7	194.73 ± 17.09	194.50 ± 14.28

Each value shows mean ± S.D.
* $p<0.05$, ** $p<0.01$: Significant difference from SD strain rats (Student's t-test).

Table 3. Shuttle box avoidance test in male and female F_1 rats.

Groups			SD strain	IGS strain
	No. of F_1 rats examined		10	10
	Avoidance rate (%)	Day 1	15.40 ± 11.89	64.20 ± 19.99**
		2	25.40 ± 27.37	85.20 ± 14.76**
		3	36.00 ± 29.21	92.40 ± 7.88**
		15	15.00 ± 19.98	90.00 ± 14.05**
	Avoidance time (sec.)	Day 1	2.97 ± 0.93	1.98 ± 0.44**
		2	2.95 ± 0.67	1.64 ± 0.24**
		3	2.38 ± 0.90	1.78 ± 0.41
		15	2.17 ± 1.29	1.89 ± 0.39
	Escape rate (%)	Day 1	50.00 ± 26.72	25.80 ± 11.56*
Male		2	57.80 ± 31.00	14.60 ± 14.61**
		3	46.00 ± 24.15	7.60 ± 7.88**
		15	65.20 ± 27.68	10.00 ± 14.05**
	Escape time (sec.)	Day 1	1.98 ± 0.85	1.02 ± 0.50**
		2	0.96 ± 0.43	0.42 ± 0.17**
		3	0.97 ± 0.58	0.24 ± 0.12**
		15	1.31 ± 0.87	0.31 ± 0.18**
	No. of intertrial interval response	Day 1	0.20 ± 0.11	0.65 ± 0.39**
		2	0.18 ± 0.12	0.59 ± 0.32**
		3	0.22 ± 0.15	0.58 ± 0.39*
		15	0.12 ± 0.10	0.45 ± 0.32*
	No. of F_1 rats examined		10	10
	Avoidance rate (%)	Day 1	43.40 ± 23.67	70.20 ± 18.29*
		2	62.80 ± 30.01	92.00 ± 12.22*
		3	67.40 ± 34.49	92.80 ± 14.52
		15	72.80 ± 30.29	90.00 ± 14.05
	Avoidance time (sec.)	Day 1	2.47 ± 0.45	1.86 ± 0.51**
		2	2.28 ± 0.64	1.46 ± 0.46**
		3	1.94 ± 0.86	1.57 ± 0.36
		15	2.22 ± 0.65	1.38 ± 0.37**
	Escape rate (%)	Day 1	49.40 ± 19.57	28.60 ± 18.09*
Female		2	35.20 ± 26.82	14.60 ± 14.61
		3	31.80 ± 33.92	7.20 ± 14.52
		15	26.80 ± 29.65	3.40 ± 3.13*
	Escape time (sec.)	Day 1	1.25 ± 0.28	0.82 ± 0.52*
		2	0.54 ± 0.21	0.39 ± 0.17
		3	0.71 ± 0.40	0.28 ± 0.19*
		15	0.49 ± 0.27	0.31 ± 0.18
	No. of intertrial interval response	Day 1	0.36 ± 0.31	0.35 ± 0.27
		2	0.41 ± 0.36	0.53 ± 0.30
		3	0.58 ± 0.48	0.55 ± 0.31
		15	0.68 ± 0.63	0.69 ± 0.36

Each value shows mean ± S.D.
* $p<0.05$, ** $p<0.01$: Significant difference from SD strain rats (Student's t-test or Aspin-Welch test).

Effects on Reproductive Function in Rats (Crj:CD(SD)IGS) Fed with CRF-1 (Protein Content: 23.1%) or CR-LPF (Protein Content: 18.4%)

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ABSTRACT. We studied the effects of feeding two kinds of solid diets, CRF-1 (protein content: 23.1%) and CR-LPF (protein content: 18.4%) on reproductive function of rat dams and development of offspring.

In dams, the CR-LPF group showed an increase in food consumption and a decrease in body weight gain, but no abnormalities in general signs, or necropsy findings.

Regarding the reproductive function of dams, there were no abnormalities in the maintenance of pregnancy, delivery, or nursing. The length of gestation , number of implantation scars, and gestation index did not show any difference between the CRF-1 group and the CR-LPF group.

The body weight gain of offspring showed a tendency to be inhibited in the CR-LPF group. In the number of live offspring, sex ratio, number of dead offspring, viability index, weaning index, general signs, external anomalies, postnatal development, necropsy findings and organ weights, there was no difference between the CRF-1 group and the CR-LPF group.

As described above, the rats fed with CR-LPF showed an increase in food consumption and a decrease in the body weight gain of dams compared with those fed CRF-1. However, we concluded that there were no effects on the reproductive function of dams. CR-LPF inhibited the body weight gain of offspring, as well as of dams. But, we concluded that there were no effects on the mortality, postnatal development, necropsy, or organ weights of offspring. —Key words: Rats, Crj:CD(SD)JGS, Reproduction, Low protein.

CD (SD) IGS-1998: 178-182

METHODS

Test Animals and Housing Conditions: Male and female Crj:CD(SD)IGS strain rats (SPF, Charles River Japan) at the age of 10 weeks 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.), temperature range of 20 – 24°C , relative humidity range of 40-70%, and filter-sterilized fresh air changes 12 times per hour. Pregnant dams were transferred to individual plastic cages with a substrate of autoclaved wood chips on the day 20 of pregnancy. They were allowed to deliver spontaneously and nurse their litters. After weaning the offspring were housed in stainless steel cages, two cages per litter, one for the males and the other for the females.

The animals were given free access to a commercial diet (CRF-1, Oriental Yeast) from the day of purchase to the day of grouping, and given tap water for drinking.

Males and females aged 12 weeks were paired at the ratio of 1 to 1 from the evening to the following morning. Confirmation of copulation was done at the same time every morning. Females having vaginal plugs or sperm in the vaginal smear were regarded as having copulated. The day when either of these signs was found was counted as Day 0 of pregnancy. Females were assigned to each group on day 0 of pregnancy.

Care was taken to equalize the number of dams with the same day of pregnancy and the mean body weights among the groups. After grouping, the CRF-1 group was given free access to CRF-1 diet containing 23.1% crude protein, 7.7% water, 5.9% crude fat, 6.5% crude ash, and 3.3% crude fiber, and the CR-LPF group was given CR-LPF diet containing 18.4% crude protein, 7.5% water, 4.8% crude fat, 6.3% crude ash, and 5.0% crude fiber.

Observations and Examinations:

(1) Dams

The animals were observed for general signs once a day during the pregnancy and lactation periods. The body weight was measured on days 0 and 4, daily from days 7 to 20 of pregnancy, and on days 0, 4, 7, 14 and 21 after delivery. Daily food consumption was measured every day from days 1 to 20 of pregnancy, and on days 4, 7, 14 and 21 after delivery.

Dams were allowed to deliver spontaneously. They were observed for delivery 3 times a day (9:00 a.m., 1:30 p.m. and 4:00 p.m.) on day 21 of pregnancy and thereafter. When a litter was found, the dam was examined for abnormality at delivery. Nursing behavior was observed daily until day 21 after delivery. Dams were sacrificed by exsanguination from the abdominal aorta under anesthesia with ether on day 21 after delivery, and necropsied, and implantation scars were counted.

(2) Offspring

At birth, the number of live and dead offspring, sex, and external anomalies were ascertained. General signs and mortality of offspring were observed once a day. Four offspring of each sex were randomly selected from each litter. Remaining offspring were killed on day 4 after birth. Live offspring were weaned on day 21 after birth.

Offspring were weighed on day 0 after birth (day of birth) and on days 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84 after birth.

To assess postnatal development, all offspring were examined for separation of the auricles and hair growth 4 days after birth and for separation of the eyelids and eruption of lower incisors 14 days after birth.

Offspring on day 56 after birth were killed by exsanguination from the abdominal aorta under anesthesia with ether, necropsied, and each organ was weighed. The weight of each organ relative to the body weight on the day of necropsy was

calculated.

Statistical Methods: The body weight, food consumption, length of pregnancy, number of implantation scars, number of live offspring, number of dead offspring, and organ weights were analyzed with a statistical t-test. The body weight of offspring was analyzed with a statistical t-test to calculate the means of each dam and each sex. The birth index, viability index, weaning index, appearance rate of external anomalies, sex ratio, and rate of postnatal development were calculated for each dam with Wilcoxon's order test.

RESULTS

Effects on Dams: Neither dead nor moribund animals were found in any group throughout the pregnancy and lactation periods. No abnormalities in general signs were found in any group.

Body weights are shown in Tables 1 and 2. Body weights in the CR-LPF group were lower than those in the CRF-1 group from the late stage of pregnancy to the early stage after delivery, and there was significant difference on day 20 of pregnancy and on the day of delivery.

Table 1. Body weight of dams during pregnancy period

Food	CRF-1	CR-LPF
Number of dams	24	21
Days of pregnancy		
0	267.4 ± 13.3	267.9 ± 15.0
4	290.6 ± 14.6	290.3 ± 15.4
7	303.6 ± 15.2	303.4 ± 16.6
8	304.5 ± 16.5	307.4 ± 18.4
9	309.1 ± 14.6	310.0 ± 18.2
10	314.0 ± 15.8	313.8 ± 18.9
11	320.9 ± 17.5	320.0 ± 18.7
12	325.6 ± 17.5	325.0 ± 19.9
13	330.7 ± 16.3	328.2 ± 21.0
14	335.3 ± 17.3	333.0 ± 20.7
15	341.6 ± 16.2	338.0 ± 21.4
16	351.6 ± 17.2	347.2 ± 21.1
17	366.0 ± 18.0	360.0 ± 23.3
18	382.0 ± 19.1	375.3 ± 23.3
19	397.7 ± 19.9	388.1 ± 25.8
20	412.5 ± 21.2	397.7 ± 27.8*

Each value shows mean (g) ± S.D.
Significantly different from CRF-1 control (*: P<0.05).

Table 2. Body weight of dams during lactation period

Food		CRF-1	CR-LPF
Number of dams		24	21
Days after delivery	0	309.8 ± 23.7	291.2 ± 29.9
	4	327.1 ± 17.7 (23)	314.4 ± 24.0
	7	331.2 ± 15.0 (23)	321.1 ± 21.5
	14	355.6 ± 16.0 (23)	353.1 ± 22.1
	21	353.9 ± 17.4 (23)	354.2 ± 19.8

Each value shows mean (g) ± S.D.
Figures in parentheses indicate number of dams.
Significantly different from CRF-1 control (*: P<0.05).

Food consumption is shown in Tables 3 and 4. Food consumption in the CR-LPF group was greater than in the CRF-1 group through pregnancy, and there was significant difference from days 9 to 18 of pregnancy. In the lactation period, a significantly higher value of food consumption was obtained on day 21 after delivery in the CR-LPF group as compared with the CRF-1 group.

At necropsy on day 21 after delivery, no abnormalities were found in either group.

Table 3. Food consumption of dams during pregnancy period

Food	CRF-1	CR-LPF
Number of dams	24	21
Days of pregnancy		
1	23.1 ± 2.5	23.4 ± 2.5
2	26.6 ± 2.3	26.5 ± 3.2
3	26.8 ± 2.5	27.0 ± 2.3
4	26.6 ± 2.0	27.3 ± 2.4
5	26.6 ± 2.1	28.0 ± 2.5
6	27.3 ± 2.5	28.8 ± 3.3
7	27.0 ± 2.8	28.6 ± 2.6
8	26.7 ± 2.1	28.6 ± 3.4
9	26.3 ± 2.7	28.2 ± 2.7 *
10	26.2 ± 2.9	28.4 ± 2.9 *
11	26.1 ± 3.2	28.6 ± 3.0 *
12	26.8 ± 2.4	29.0 ± 3.5 *
13	27.1 ± 1.7	29.4 ± 3.0 **
14	26.3 ± 2.1	28.2 ± 2.9 *
15	26.1 ± 1.5	28.5 ± 2.9 **
16	27.6 ± 2.4	30.1 ± 2.9 **
17	28.3 ± 2.8	30.8 ± 3.2 **
18	27.9 ± 3.2	30.8 ± 3.0 **
19	28.1 ± 2.4	29.6 ± 3.5
20	25.7 ± 2.4	26.6 ± 4.9

Each value shows mean (g/day) ± S.D.
Significantly different from CRF-1 control (*: P<0.05, **: P<0.01).

Table 4. Food consumption of dams during lactation period

Food		CRF-1	CR-LPF
Number of dams		23	21
Days after delivery	4	32.0 ± 5.6	31.1 ± 8.4
	7	41.6 ± 4.1	42.0 ± 8.0
	14	62.6 ± 7.9	58.3 ±13.5
	21	72.8 ± 6.2	79.5 ±10.9 **

Each value shows mean (g/day) ± S.D.
Significantly different from CRF-1 control (**: P<0.01).

Effects on Reproductive Functions of Dams: Neither abortion nor pre-mature delivery occurred in any group. In the observations on nursing behavior, no abnormalities were found in any group. The results of observations at delivery and on day 21 after delivery are shown in Table 5. There was no difference between the two groups in length of gestation and number of implantation scars. The gestation index of each group was 100%.

Effects on Offspring: The results of observations of offspring are shown in Table 5. The number of live offspring in the CR-LPF group was significantly smaller than that of the CRF-1 group. There was no significant difference in the sex ratio, number of dead offspring, birth index, viability index, or weaning index. Offspring of both groups showed no external anomalies. In observations on general signs, no abnormalities were found in the offspring of either group.

The body weights of offspring are shown in Table 6. There was no significant difference on any days till the day of weaning between the CRF-1 and CR-LPF groups. After the day of weaning, the body weights of both sexes in the CR-LPF group were lower than those in the CRF-1 group. In males from days 28 to 49 after birth, and females from days 28 to 56 after birth, there were significant differences.

Table 5. Observation of offspring

Food	CRF-1	CR-LPF
Number of dams	24	21
Length of gestation (days)		
Mean±S.D. per dam	21.98 ± 0.38	22.10 ± 0.52
Implantation scars		
Mean±S.D. per dam	16.0 ± 3.3	15.0 ± 2.5
Gestation index (%) a)	100.0	100.0
Live offspring at birth		
Mean±S.D. per dam	14.7 ± 3.5	13.3 ± 2.2 *
Sex ratio b)		
Mean±S.D. per dam	0.45 ± 0.14	0.49 ± 0.16
Dead offspring at birth		
Mean±S.D. per dam	0.4 ± 0.9	0.5 ± 0.8
Birth index c)		
Mean%±S.D. per dam	89.9 ± 11.2	89.2 ± 8.0
Viability index d)		
Mean%±S.D. per dam	92.3 ± 21.0	94.9 ± 16.0
Weaning index e)		
Mean%±S.D. per dam	100.0 ± 0.0 (23)	100.0 ± 0.0
External anomalies		
Mean%±S.D. per dam	0.0 ± 0.0	0.0 ± 0.0

Significantly different from CRF-1 control (*: P<0.05).
Figures in parentheses indicate number of dams.
a): (Number of dams with live offspring / number of pregnant dams) ×100.
b): Number of male offspring / number of live offspring.
c): (Number of live offspring at birth / number of implantation scars) ×100.
d): (Number of live offspring on day 4 / number of live offspring at birth) ×100.
e): (Number of live offspring on day 21 / number of live offspring after culling) ×100.

Table 6. Body weights of offspring

Sex	Male		Female	
Food	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of dams	23	21	24	21
Days after birth				
0	6.1 ± 0.5	6.2 ± 0.5	5.9 ± 0.6	5.8 ± 0.5
4	9.7 ± 1.2	9.9 ± 1.4	9.2 ± 1.2 (23)	9.1 ± 1.4
7	15.8 ± 1.8	15.7 ± 1.6	14.8 ± 1.7 (23)	14.6 ± 1.7
14	32.6 ± 3.0	31.9 ± 2.5	31.1 ± 2.9 (23)	30.3 ± 2.7
21	51.8 ± 4.4	51.7 ± 3.9	49.7 ± 4.1 (23)	49.1 ± 3.9
28	85.0 ± 7.0	79.9 ± 6.1 *	77.8 ± 5.7 (23)	73.6 ± 6.0 *
35	140.5 ± 10.4	130.9 ± 9.5 **	120.7 ± 7.4 (23)	114.0 ± 9.4 *
42	203.1 ± 12.9	192.7 ± 13.4 *	159.2 ± 8.0 (23)	152.3 ± 11.0 *
49	266.9 ± 16.9	255.3 ± 17.1 *	187.4 ± 8.2 (23)	181.3 ± 13.4 *
56	329.7 ± 20.6	318.1 ± 22.4	214.7 ± 9.6 (23)	207.5 ± 17.6 *
63	381.8 ± 27.1	365.7 ± 30.6	239.6 ± 14.7 (23)	229.0 ± 24.7
70	424.0 ± 30.9	404.3 ± 35.3	252.0 ± 17.9 (23)	242.7 ± 24.9
77	459.9 ± 34.7	438.8 ± 38.0	263.7 ± 17.9 (23)	253.6 ± 26.0
84	488.3 ± 39.0	463.9 ± 41.8	272.6 ± 18.3 (23)	263.3 ± 27.0

Each value shows mean(g) ± S.D. per dam.
Figures in parentheses indicate Number of dams.
Significantly different from CRF-1 control (*: P<0.05, **: P<0.01).

Results of observations on postnatal development are shown in Table 7. No significant difference in the ratio of offspring showing a positive response in separation of the aunicles, hair growth, eruption of the lower incisors, separation of the eyelids, descent of the testes, or opening of the vagina were seen between the CRF-1 and CR-LPF groups on any day of observation.

Organ weights measured on day 56 after birth are shown in Table 8. In males of the CR-LPF group, the relative weight of the brain and spleen was significantly greater than in the CRF-1 group, and the absolute weight of the kidney was significantly lower. We decided that these values were due to the difference in the body weight, since they were not different in both the absolute and relative weights. In females of the CR-LPF group, the absolute and relative weights of the kidneys were significantly lower than those in the CRF-1 group. The relative weight of the brain of females in the CR-LPF group was significantly greater than in the CRF-1 group, and the absolute weight of the adrenals was significantly lower. We decided that these values also were due to the difference in the body weights, since they were not different in both the absolute and relative weights.

Findings at necropsy did not show any difference between the CRF-1 and CR-LPF groups.

Table 7. Postnatal development of offspring

Food		CRF-1	CR-LPF
Number of dams		23	21
Separation of auricles (%)	day 4	100.0 ± 0.0	98.8 ± 5.5
	5	100.0 ± 0.0	100.0 ± 0.0
Hair growth (%)	day 4	100.0 ± 0.0	98.8 ± 5.5
	5	100.0 ± 0.0	100.0 ± 0.0
Eruption of lower incisors (%)	day 14	100.0 ± 0.0	100.0 ± 0.0
Separation of eyelids (%)	day 12	0.0 ± 0.0	0.0 ± 0.0
	13	3.9 ± 11.1	7.8 ± 23.9
	14	56.1 ± 34.1	42.3 ± 41.0
	15	90.8 ± 18.9	84.6 ± 24.3
	16	99.5 ± 2.5	98.9 ± 3.6
	17	100.0 ± 0.0	99.4 ± 2.6
	18	100.0 ± 0.0	100.0 ± 0.0
Descent of testes (%)	day 21	0.0 ± 0.0	0.0 ± 0.0
	22	6.5 ± 18.8	7.1 ± 16.1
	23	67.4 ± 36.5	61.9 ± 40.8
	24	88.8 ± 18.5	83.7 ± 21.8
	25	95.3 ± 10.6	93.7 ± 14.1
	26	100.0 ± 0.0	98.4 ± 7.2
	27	100.0 ± 0.0	100.0 ± 0.0
Opening of vagina (%)	day 35	89.1 ± 18.2	76.0 ± 36.1
	36	97.8 ± 7.2	89.3 ± 25.7
	37	98.9 ± 5.2	94.0 ± 15.6
	38	100.0 ± 0.0	100.0 ± 0.0

Each value shows mean ± S.D. per dam.

Table 8. Organ weights of offspring on day 56 after birth

Sex		Male		Female	
Food		CRF-1	CR-LPF	CRF-1	CR-LPF
Number of offspring		44	38	48	41
Body weight	(g)	329.2 ± 23.9	318.9 ± 25.4	213.2 ± 18.7	206.0 ± 20.7
Brain	(g)	1.862 ± 0.087	1.863 ± 0.085	1.742 ± 0.093	1.742 ± 0.096
	(g%)	0.568 ± 0.035	0.586 ± 0.045 *	0.820 ± 0.053	0.850 ± 0.069 *
Lungs	(g)	1.211 ± 0.114	1.193 ± 0.093	0.948 ± 0.084	0.933 ± 0.092
	(g%)	0.368 ± 0.031	0.375 ± 0.028	0.445 ± 0.034	0.453 ± 0.026
Heart	(g)	1.185 ± 0.107	1.138 ± 0.113	0.832 ± 0.079	0.804 ± 0.082
	(g%)	0.360 ± 0.026	0.357 ± 0.027	0.391 ± 0.027	0.391 ± 0.028
Liver	(g)	14.791 ± 1.775	14.533 ± 1.834	9.072 ± 1.064	8.970 ± 1.265
	(g%)	4.488 ± 0.361	4.560 ± 0.477	4.253 ± 0.260	4.340 ± 0.341
Spleen	(g)	0.730 ± 0.124	0.762 ± 0.115	0.519 ± 0.094	0.501 ± 0.086
	(g%)	0.222 ± 0.034	0.239 ± 0.027 *	0.243 ± 0.038	0.243 ± 0.030
Kidneys	(g)	2.526 ± 0.211	2.422 ± 0.250 *	1.729 ± 0.171	1.619 ± 0.214 **
	(g%)	0.769 ± 0.054	0.761 ± 0.073	0.812 ± 0.053	0.784 ± 0.060 *
Adrenals	(mg)	47.03 ± 6.80	44.83 ± 8.04	57.61 ± 8.84	53.13 ± 9.49 *
	(mg%)	14.32 ± 2.06	14.05 ± 2.03	27.10 ± 3.97	25.76 ± 4.00
Testes	(g)	2.783 ± 0.391	2.777 ± 0.447	—	—
	(g%)	0.849 ± 0.124	0.873 ± 0.142	—	—
Ovaries	(mg)	—	—	84.31 ± 13.54	81.04 ± 13.97
	(mg%)	—	—	39.84 ± 7.18	39.32 ± 6.14

Each value shows mean ± S.D.
Significantly different from CRF-1 control (*: $P<0.05$, **: $P<0.01$).

DISCUSSION

We tested the common solid feed CRF-1, which contains 23.1% protein and CR-LPF feed which contains 18.4% protein. We studied the influence on the development of offspring and on the reproductive function of dams when these diets were given to rats.

The body weight of dams from the later stage of pregnancy to the early stage of delivery was lower, and the food consumption during pregnancy and the later stage of delivery was greater in the CR-LPF group than in the CRF-1 group. No abnormality was found in either group in general signs or at necropsy.

Reproductive function, delivery, and nursing were normal in both groups. Pregnancy, the number of implantation scars and the gestation index did not show any difference between the two groups. Therefore, we decided that the animals kept on a low-protein diet had a lower body weight gain in dams, however there was no influence on pregnancy or reproductive function.

In the offspring, there was a lower number of live offspring in the CR-LPF group, but there was no difference in the number of dead offspring, and the total number of pups (the number of live offspring plus the number of dead offspring) in both groups was in the range of the average number of fetuses1), 2). We decided that the number of live offspring resulted accidentally, and not from the difference in diet. The other parameters, i.e., the sex ratio, number of dead offspring, birth index, viability index, weaning index, postnatal development, external anomalies, general signs, and necropsy showed no difference between the two groups. The body weight did not differ in the two groups till weaning. However, after weaning, the body weights of the CR-LPF group were lower than those of the CRF-1 group in both sexes. This difference in offspring as well as dams, was due to difference in the quantity of protein in the diet. On day 56 after birth, the absolute and relative weights of the kidneys of females in the CR-LPF group were lower, but not in the males.

Thus, feeding a low-protein diet inhibits the body weight gain of offspring, but has no effect on the mortality of offspring.

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Background Control Data of Reproductive and Developmental Toxicity Study in Crj:CD(SD)IGS Rats.

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ABSTRACT. The background control data in reproductive and developmental toxicity study of rats bred in the inhalation chamber were investigated using Crj:CD(SD)IGS rats through last one year. These include body weights and food consumption of pregnant females, fertility, implantation rate and loss obtained with cesarean section, sex ratio of live fetuses, fetal body weights and placental weights, fetal morphological observations, natural delivery parameter and pups viability, and pups body weights. Although the variation among the studies was recognized in some results, these background data will contribute to evaluate reproductive and developmental inhalation toxicity of chemical compounds in Crj:CD(SD)IGS rats. —Key words: CD(SD)IGS Rat, Development, Reproduction

CD (SD) IGS-1998: 183-187

INTRODUCTION

SD or Wistar rats, being various differences among the breeders or strain, have been used for toxicity studies including reproductive and developmental study. For the purpose minimizing these differences Crj:CD(SD)IGS rats are being recently used[2]. Adequate historical control data are, therefore, very important. Thus, we investigated the background control data of reproductive and developmental toxicity study of rats bred in the inhalation chamber for definite periods, because the inhalation toxicity studies have been mainly conducted in our laboratory.

MATERIALS AND METHODS

Nine-week-old females and 10-week-old males of Crj:CD(SD)IGS rat were purchased from Charles River Japan, Atsugi. A total of 190 females and 190 males in 10 studies performed during 10-month (May 1997 - February 1998) were presented in this study. Following quarantine for 1 week, they were kept in the inhalation chamber (1.06m3). They were individually housed in the suspended stainless-steel wire-mesh cage except for mating and lactation period. Animals allowed to deliver were out of the inhalation chamber on day 20 of gestation, animal cage was covered with an aluminium plate, and added paper pulp tip as nesting material (ALPHA-dri., Shepherd Specialty Paper, Inc., USA). Room temperature and humidity were maintained at 22 ± 2°C, 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°C, 55 ± 10%, and 12 ± 1th/h ventilation, respectively. Tap water and commercial pellet (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 and 4 of lactation. Food consumption was measured once a week during the gestation period, and once during the lactation period (4 days). All females were daily examined for estrus 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. At the presence of a vaginal plug or sperm 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 in 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 [4]. 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 anomalies. Two-third of live fetuses in each litter was assigned for preparation of skeletal specimens by Dowson's methods [1], skeletal anomalies and the number of ossified sacral-caudal vertebrae were examined. The remaining live fetuses were observed for visceral anomalies by Wilson's and Nishimura's methods [3,5].

Six pregnant females in each study were allowed to deliver for examination of the abnormalities in delivery and the duration of gestation was determined. Pups were examined for external anomalies 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 litter size was counted daily after birth to determined the viability index on day 4 of lactation. Male and female pups were weighed separately on a litter basis on days 0 and 4 of lactation.

RESULTS AND CONCLUSION

No abnormal clinical signs were observed in all male and female rats. Changes of body weights and food consumption of pregnant females are shown in Table 1 and 2, respectively. Body weight on day 20 of gestation (the day conducted cesarean sectioning) was highest through gestational period. Food consumption mildly increased through gestational period, and after delivery the value increased remarkably.

One or two estrus were observed in all female rats through the examination of estrus cycles (at least 8 days). Fertility in female rats is shown in Table 3. The average copulation (for 4 days) index of ten studies was 94.7%, and the copulation index of each study ranged from 89.5% to 100%. The average fertility index of ten studies was 94.9%, and the fertility of each study ranged from 88.9% to 100%. The average pre-coital period was 2.3 days.

Table 1. Body Weights(g) of Pregnant Females

	Gestational day					Lactational day		
Study No.	No.of rats	0	7	14	20	No.of rats	0	4
97-5	19	235 ± 13 [a]	278 ± 13	315 ± 15	374 ± 20	6	281 ± 10	315 ± 15
97-6	19	253 ± 9	290 ± 14	331 ± 15	411 ± 30	6	321 ± 17	330 ± 18
97-7	16	230 ± 12	271 ± 15	306 ± 19	371 ± 24	6	291 ± 25	295 ± 14
97-8	18	240 ± 10	281 ± 13	319 ± 18	390 ± 23	6	290 ± 45	314 ± 20
97-9	17	228 ± 13	272 ± 18	309 ± 25	385 ± 29	6	279 ± 32	301 ± 22
97-10	16	236 ± 8	276 ± 13	316 ± 16	393 ± 18	6	276 ± 17	302 ± 20
97-11	16	250 ± 15	291 ± 18	325 ± 22	403 ± 31	6	294 ± 27	316 ± 27
97-12	18	233 ± 12	270 ± 16	303 ± 17	379 ± 31	5	307 ± 28	315 ± 25
98-1	16	249 ± 9	286 ± 13	325 ± 18	402 ± 24	6	297 ± 21	320 ± 6
98-2	16	249 ± 15	291 ± 16	333 ± 16	406 ± 21	6	314 ± 26	327 ± 11
Total	171 [b]	240 ± 9	281 ± 9	318 ± 10	391 ± 14	59	295 ± 10	314 ± 11

[a] Mean ± S.D
[b] Total number of animals of ten studies

Table 2. Food Consumption(g) of Pregnant Females

	Gestational day				Lactational day	
Study No.	No.of rats	0 - 7	7 - 14	14 - 20	No.of Rat	0 - 4
97-5	19	22 ± 1 [a]	24 ± 2	25 ± 2	6	34 ± 4
97-6	19	24 ± 2	25 ± 2	27 ± 2	6	32 ± 5
97-7	16	23 ± 2	24 ± 2	24 ± 2	6	25 ± 2
97-8	18	24 ± 2	26 ± 3	27 ± 3	5	35 ± 3
97-9	17	23 ± 3	24 ± 4	26 ± 3	6	31 ± 5
97-10	16	23 ± 2	25 ± 2	26 ± 2	6	29 ± 6
97-11	16	23 ± 2	24 ± 3	25 ± 2	6	28 ± 9
97-12	18	23 ± 2	24 ± 2	26 ± 3	5	29 ± 6
98-1	16	24 ± 2	26 ± 2	27 ± 2	6	32 ± 4
98-2	16	25 ± 2	26 ± 2	27 ± 2	6	30 ± 5
Total	171 [b]	23 ± 1	25 ± 1	26 ± 1	58	30 ± 3

[a] Mean ± S.D
[b] Total number of animals of ten studies

Table 3. Fertility in Female Rats

Study No.	No.of mated rats	Copulation index(%)	Fertility index(%)	Pre-coital period(day)
97-5	19	100	100	2.4± 1.0 [a]
97-6	19	100	100	2.3± 0.7
97-7	19	89.5	94.1	2.1± 1.1
97-8	19	100	94.7	2.1± 1.0
97-9	19	94.7	94.4	2.7± 1.0
97-10	19	94.7	88.9	2.4± 1.2
97-11	19	89.5	94.1	2.2± 1.1
97-12	19	100	94.7	2.3± 1.2
98-1	19	89.5	94.1	2.4± 1.2
98-2	19	89.5	94.1	2.4± 1.0
Total	180 [b]	94.7± 5.0	94.9± 3.2	2.3± 0.2

Copulation index(%) = (No.of animals copulated successfully/no. of mated animals) x 100
Fertility index(%) = (No.of pregnant animals/no.of animals copulated successfully) x 100
[a] Mean ± S.D [b] Total number of animals of ten studies

Implantation rate and loss determined by the cesarean sectioning are shown in Table 4. The average implantation rate of ten studies was 91.1%, and the implantation rate of each study ranged from 80.3% to 96.1%. The average frequency of implantation loss of ten studies was 6.3%, and the frequency of implantation loss of each study ranged from 3.2% to 9.6%. All intrauterine death of all cases were placental remnants.

The number and sex ratio of live fetuses are shown in Table 5. The average number of live fetuses per itter of ten studies was 13.7 animals, and sex ratio was 1.1.

Body weights and placental weights of live fetuses are shown in Table 6. The average body weights in male and female fetuses of ten studies were 3.90g and 3.69g, those of individual placental weight were 0.49g and 0.47g, respectively. Both weight in males was slightly higher than that in females.

Morphological findings in fetuses are shown in Table 7. External anomalies included dwarf, craniorachishisis, open eyelid, microphthalmia, and filamentous tail. Total incidence of

Table 4. Implantation Rate and Loss Obtained with Cesarean Section

Study No.	No. of dams	No. of corpora lutea	No. of implantations	Implantation rate(%)	Intrauterine death	Implantation loss(%)
97-5	19	16.3 ± 1.7[a]	15.2 ± 2.0	93.9 ± 11.9	1.4 ± 1.8	9.6 ± 13.9
97-6	19	17.2 ± 2.1	14.6 ± 5.1	83.9 ± 27.7	0.8 ± 0.8	7.3 ± 9.2
97-7	16	15.2 ± 1.0	12.3 ± 5.0	80.3 ± 31.7	1.2 ± 2.1	7.7 ± 13.5
97-8	18	15.5 ± 2.5	14.4 ± 3.5	91.9 ± 13.0	1.2 ± 1.2	9.3 ± 12.4
97-9	17	15.6 ± 0.9	15.0 ± 0.9	96.1 ± 5.0	0.9 ± 1.3	6.2 ± 8.8
97-10	16	16.2 ± 2.0	14.9 ± 1.6	92.6 ± 10.0	0.6 ± 0.7	4.2 ± 4.7
97-11	16	16.0 ± 2.1	15.0 ± 1.6	94.2 ± 6.6	0.5 ± 0.5	3.2 ± 3.3
97-12	18	15.8 ± 2.5	14.8 ± 1.6	94.9 ± 6.9	0.5 ± 0.5	3.5 ± 3.7
98-1	16	15.7 ± 2.7	14.6 ± 2.6	93.2 ± 10.3	0.8 ± 0.8	5.6 ± 6.0
98-2	16	16.6 ± 2.3	14.9 ± 1.8	90.3 ± 8.5	1.0 ± 0.8	6.7 ± 5.5
Total	171[b]	16.0 ± 0.6	14.6 ± 0.8	91.1 ± 5.1	0.9 ± 0.3	6.3 ± 2.3

Implantation rate(%) = (No.of corpora lutea/no.of implantations) x 100
Implantation loss(%) = (No.of intrauterine death/no.of implantations) x 100
[a] Mean ± S.D [b] Total number of animals of ten studies

Table 5. Sex Ratio of Live Fetuses

Study No.	No. of dams	Males	Females	Total	Sex ratio
97-5	19	6.5 ± 2.6[a]	7.4 ± 2.0	13.8 ± 2.9	1.0 ± 0.6
97-6	19	6.5 ± 3.1	7.2 ± 2.9	13.8 ± 5.0	1.0 ± 0.5
97-7	16	5.6 ± 3.3	5.5 ± 2.6	11.1 ± 4.5	1.1 ± 0.8
97-8	18	6.8 ± 2.6	6.4 ± 2.4	13.3 ± 3.8	1.1 ± 0.5
97-9	17	7.2 ± 2.1	6.9 ± 1.4	14.1 ± 1.7	1.1 ± 0.6
97-10	16	7.2 ± 1.5	7.2 ± 1.4	14.4 ± 1.7	1.1 ± 0.4
97-11	16	6.8 ± 2.0	7.8 ± 1.4	14.6 ± 1.5	0.9 ± 0.4
97-12	18	6.8 ± 2.4	7.5 ± 2.0	14.3 ± 1.7	1.1 ± 0.7
98-1	16	7.4 ± 2.0	6.4 ± 2.2	13.8 ± 2.7	1.3 ± 0.4
98-2	16	8.3 ± 2.5	5.7 ± 1.6	14.0 ± 1.9	1.7 ± 1.0
Total	171[b]	6.9 ± 0.7	6.8 ± 0.8	13.7 ± 1.0	1.1 ± 0.2

Sex ratio = No.of females/no.of males
[a] Mean ± S.D [b] Total number of animals of ten studies

Table 6. Body Weights and Placental Weights in Fetuses

	Body weight(g)		Placental weight(g)	
Study No.	Males	Females	Males	Females
97-5	3.96 ± 0.28[a]	3.80 ± 0.27	0.50 ± 0.17	0.46 ± 0.07
97-6	4.06 ± 0.55	3.85 ± 0.57	0.51 ± 0.08	0.47 ± 0.04
97-7	3.77 ± 0.30	3.51 ± 0.31	0.48 ± 0.06	0.47 ± 0.07
97-8	3.75 ± 0.19	3.51 ± 0.23	0.47 ± 0.05	0.45 ± 0.05
97-9	3.75 ± 0.36	3.61 ± 0.37	0.50 ± 0.06	0.48 ± 0.05
97-10	3.90 ± 0.30	3.65 ± 0.32	0.48 ± 0.03	0.48 ± 0.03
97-11	3.90 ± 0.30	3.66 ± 0.29	0.47 ± 0.04	0.45 ± 0.05
97-12	4.03 ± 0.21	3.85 ± 0.17	0.49 ± 0.03	0.46 ± 0.03
98-1	3.95 ± 0.33	3.73 ± 0.28	0.52 ± 0.05	0.49 ± 0.07
98-2	3.89 ± 0.20	3.70 ± 0.20	0.48 ± 0.04	0.46 ± 0.03
Total	3.90 ± 0.11	3.69 ± 0.11	0.49 ± 0.02	0.47 ± 0.01

[a] Mean ± S.D

Table 7. Morphological Observations in Fetuses

	Case/no.examined	%(Min. - Max.)[a]
External anomalies	4/1525	0.2(0-0.7)
Dwarf	1/1525	0.1(0-0.5)
Craniorachishisis	1/1525	0.1(0-0.4)
Open eyelid	1/1525	0.1(0-0.4)
Microphthalmia	1/1525	0.1(0-0.7)
Filamentous tail	1/1525	0.1(0-0.7)
Skeletal malformation	1/1054	0.1(0-0.6)
Wavy rib	1/1054	0.1(0-0.6)
Skeletal variations	151/1054	14.2(6.7-22.9)
Dumbbell-shaped thoracic vertebral body	50/1054	4.8(0-9.2)
Lumbar rib	96/1054	9.0(1.7-17.8)
Cervical rib	5/1054	0.4(0-2.8)
13th rib shortening	1/1054	0.1(0-0.8)
Splittingofthoraticvertebralbody	6/1054	0.5(0-1.8)
Asymmetrysternebrae	1/1054	0.1(0-0.7)
No.ofossifiedsacral-caudalvertebrae		7.9(7.4-8.1)[b]
Visceralmalformations	13/471	3.0(1.7-6.7)
Patentforamenovale	3/471	0.6(0-1.3)
Ventricularseptaldefect	9/471	2.2(0-6.7)
Microphthalmia	1/471	0.2(0-2.5)
Visceralvariations	28/471	7.3(2.8-13.3)
Persistentleftumbilicalartery	4/471	2.2(0-10.0)
Diaphragmatichernia	1/471	0.2(0-2.0)
Thymicremnantinneck	19/471	4.2(0-11.7)
Dilatationofrenalpelvis	2/471	0.4(0-2.5)
Supernumerarycoronaryostium	2/471	0.3(0-1.7)

[a] Averageincidenceoftenstudies(minimumandmaximumincidenceoftenstudies)
[b] Averagenumberoftenstudies(minimumandmaximumnumberoftenstudies)

external anomalies was 0.2%. A fetus with craniorachisisis also included open eyelid. Skeletal malformation observed was only wavy rib of 1 fetus. Skeletal variations included dumbbell-shaped thoracic vertebrae body, lumbar rib, cervical rib, 13th rib shortening, splitting of thoratic vertebral, and asymmetry sternebrae. Total incidence of skeletal variations was 14.2%, that of each study ranged 6.7% to 22.9%. Most frequent variation was lumbar rib (including rudimentary rib). Dumbbell-shaped thoracic vertebral body was often observed, as well. The number of ossified sacral-caudal vertebrae was almost 7 or 8 (average value was 7.9). Visceral malformations included patent foramen ovale, ventricular septal defect, and microphthalmia. Total incidence of visceral malformations was 3.0%, that of each study ranged from 1.7% to 6.7%. Most frequent malformations was ventricular septal defect. Visceral variations included persistent left umbilical artery, diaphragmatic hernia, thymic remnant in neck, dilatation of renal pelvis, and supernumerary coronary ostium. Total incidence of visceral variations was 7.3%, that of each study ranged from 2.8% to 13.3%. Most frequent visceral variations was thymic remnant in neck, followed by persistent left umbilical artery.

The natural delivery parameter and pups viability are shown in Table 8, and body weight of pups in Table 9. The gestation length of almost animals were 22 days. The average delivery index of ten studies was 94.8%, that of each study ranged from 88.9% to 100%. The average birth index was 92.7%, that of each study ranged from 82.2% to 97.7%. The average live birth index was 97.9%, that of each study ranged from 82.2% to 100%. The average viability index on day 4 of lactation was 99.2%, that of each study ranged from 97.2% to 100%. The average of individual body weight of male and female pups at birth were 6.6g and 6.2g, respectively, and those on day 4 of lactation were 10.7g and 10.1g. These values in males were higher than those in females. No abnormalities were observed in delivery and lactational condition of dams.

These data would contribute to evaluate the results in reproductive and developmental toxicity study in Crj:CD(SD)IGS rats.

Table 8. Natural Delivery Parameter and Pups Viability

Study No.	No.of dams	Gestation length(day)	Implantations	Pups delivered	Delivery index(%)
97-5	6	22.0± 0[a)]	16.0 ± 2.4	15.8 ± 2.6	98.8± 2.9
97-6	6	22.0± 0	16.0 ± 1.7	15.2 ± 1.5	95.0± 4.7
97-7	6	22.0± 0	14.3 ± 2.0	13.5 ± 1.2	94.8± 6.9
97-8	6	22.3± 0.8	13.0 ± 5.5	13.0 ± 5.5	100 ± 0
97-9	6	22.0± 0	14.5 ± 0.8	14.0 ± 0	96.8± 5.3
97-10	6	22.2± 0	14.3 ± 1.0	13.8 ± 0.8	96.7± 5.3
97-11	6	22.3± 0.5	16.8 ± 1.2	15.5 ± 1.5	92.1± 5.9
97-12	5	22.0± 0	14.4 ± 1.1	13.6 ± 1.1	94.6± 5.6
98-1	6	21.8± 0	15.5 ± 1.6	14.0 ± 2.0	90.3± 8.1
98-2	6	22.3± 0.5	14.7 ± 2.0	13.0 ± 2.0	88.9± 10.3
Total	59[b)]	22.1± 0	15.0 ± 1.1	14.1 ± 1.0	94.8± 3.5

live pups	Birth index(%)	Live birth index(%)	Sex ratio	Day 4 of lactation viability index(%)
15.7± 2.7	97.7 ± 3.6	98.9 ± 2.7	3.2 ± 4.5	97.2 ± 4.4
15.2± 1.5	95.0 ± 4.7	100 ± 0	1.0 ± 0.4	99.0 ± 2.4
13.3± 1.2	93.7 ± 6.5	98.8 ± 2.9	1.9 ± 2.0	100 ± 0
12.5± 6.3	82.2 ± 40.4	82.2 ± 40.4	1.4 ± 1.1	100 ± 0
14.0± 0	96.8 ± 5.3	100 ± 0	1.1 ± 0.4	100 ± 0
13.8± 0.8	96.7 ± 5.3	100 ± 0	1.3 ± 0.7	98.9 ± 2.7
15.3± 1.6	91.0 ± 6.1	98.9 ± 2.7	1.2 ± 0.7	99.1 ± 2.3
13.6± 1.1	94.6 ± 5.6	100 ± 0	0.7 ± 0.5	98.7 ± 3.0
14.0± 2.0	90.3 ± 8.1	100 ± 0	1.0 ± 0.5	98.9 ± 2.7
13.0± 2.0	88.9 ± 10.3	100 ± 0	1.3 ± 1.5	100 ± 0
14.0± 1.0	92.7 ± 4.7	97.9 ± 5.5	1.4 ± 0.7	99.2 ± 0.9

Delivery index = (No.of pups delivered / no.of implantation) x 100
Birth index = (No. of live pups / no.of implantations) x 100
Live birth index = (No. of live pups / no.of pups delivered) x 100
Sex ratio = No. of live male pups / no.of live female pups
Viability index on day 4 = (No. of live pups on day 4 / no. of live pups) x 100
a) Mean ± S.D b) Total animal number of ten studies

Table 9. Body Weights(g) of Pups

Study No.	Day 0		Day 4	
	Males	Females	Males	Females
97-5	6.2 ± 0.6[a)]	5.9 ± 0.7	10.3 ± 1.3	9.7 ± 1.3
97-6	6.6 ± 0.2	6.3 ± 0.2	10.6 ± 0.4	10.1 ± 0.3
97-7	6.7 ± 0.2	6.4 ± 0.3	10.5 ± 0.7	10.1 ± 0.7
97-8	6.4 ± 0.4	5.9 ± 0.4	10.7 ± 1.4	10.0 ± 1.4
97-9	6.6 ± 0.6	6.0 ± 0.4	10.6 ± 0.7	10.0 ± 0.7
97-10	6.6 ± 0.4	6.3 ± 0.3	10.4 ± 0.8	10.1 ± 0.7
97-11	6.3 ± 0.6	5.9 ± 0.6	10.1 ± 1.6	9.2 ± 1.3
97-12	6.8 ± 0.4	6.4 ± 0.2	11.0 ± 1.1	10.7 ± 1.0
98-1	6.3 ± 0.4	6.1 ± 0.4	11.5 ± 1.6	9.7 ± 1.4
98-2	7.5 ± 0.2	6.8 ± 0.4	11.6 ± 1.3	11.0 ± 1.7
Total	6.6 ± 0.4	6.2 ± 0.3	10.7 ± 0.4	10.1 ± 0.5

[a)] Mean ± S.D

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Reference Data of Reproductive and Developmental Toxicity Studies in CD(SD)IGS Rats

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ABSTRACT. International Genetic Standard (IGS) system is a new system of animal breeding proposed by Charles River, Inc. We have conducted this study to assess reproductive and developmental parameters in CD(SD)IGS rats bred by the international genetic standard system. And these results were compared to the data in CD(SD) rats in our laboratories.

No significant differences were observed in most parameters between CD(SD)IGS rats and CD(SD)IGS rats. Skeletal variations in CD(SD)IGS rat fetuses, however, were higher than those in CD(SD) rats. Therefore, this changes should be taken into consideration when CD(SD)IGS rats are used instead of CD(SD) rats in reproductive and developmental toxicity studies and more background data in CD(SD)IGS rats should be collected. —Key words: CD(SD)IGS Rat, Reproductive and developmental toxicity.

CD (SD) IGS-1998: 188-190

INTRODUCTION

Charles River, Inc. has proposed a new animal breeding system called International Genetic Standard (IGS) system for the standardization of the research and development of new medical drugs. The purpose of the new system is that experimental results from individual laboratories can be shared in the world by using uniform genetic experimental animals. We therefor conducted a study to assess reproductive and developmental parameters in CD(SD)IGS rats produced by the new system. And these results were compared to the data in CD(SD) rats in our laboratories.

MATERIALS AND METHODS

Male and female CD(SD)IGS rats were obtained from Charles River Japan, Inc.(Kanagawa, Japan) twice. In the first lot (CD(SD)IGS-1), males were approximately 27 weeks old, 614-757g of body weight and females were approximately 11 weeks old, 227-310g of body weight, while in the second lot (CD(SD)IGS-2), males were approximately 11 weeks old, 395-439g of body weight and females were approximately 11weeks old, 216-282g of body weight at the start of mating. The animals were housed individually in stainless-steel cages in the animal room which was controlled at a temperature of 23±2°C and relative humidity of 55±10%, and with a 13-hr light/11-dark cycle(8:00-21:00). Pellet food (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and municipal tap water were available ad libitum. The females were cohabited overnight with males. The day on which sperm was found in a vaginal smear was designated as day 0 of gestation. The general condition and behavior of all dams were checked daily during gestation. Maternal body weight and food consumption were also recorded on day 0, 7, 8, 10, 12, 15, 18 and 20 of gestation.

Dams were sacrificed by exsanguination from abdominal aorta under anesthesia with ether on day 20 of gestation, and then examined macroscopically. Uteri and ovaries were excised, and the number of implantations, live and dead fetuses, resorptions and corpora lutea were determined. The live fetuses were sexed, weighted and examined for external macroscopic abnormalities including examination of the palate. Each placenta of live fetuses were also weighed. Approximately one-half of live fetuses in each litter were fixed in Bouin's solution and were examined for visceral abnormalities using a microdissection methods [2]. The remaining live fetuses were fixed in acetone and stained with alizarin red S [1] for skeletal examination.

The results of CD(SD) rats were obtained from 4 different lots examined using the methods described above in our laboratories.

RESULTS AND DISCUSSION

No clinical signs were observed. Body weights, body weight gains and food consumptions of dams are showed in Table 1. No gross pathological abnormalities were observed in any dams.

Gestational parameters for CD(SD)IGS and CD(SD) rats are presented in Table 2. There was no significant difference between the CD(SD)IGS and CD(SD) rats in the number of corpora lutea, implantations, live fetuses and the percentage of pre- and post-implantation loss. Mean fetal body weights and placental weights in CD(SD)IGS rats were also not significantly different from the values in CD(SD) rats.

The Incidences and types of fetal malformations and variations are presented in Table 3. The incidences of external, visceral and skeletal malformations were comparable between CD(SD)IGS and CD(SD) rats. The abnormalities found in both CD(SD)IGS and CD(SD) rat fetuses were of type reported as a spontaneous malformation in Sprague Dawley rats and the incidence of malformations were well within the historical control data [4]. In addition, no significant differences in the incidences of visceral variations, thymic remnant in neck, dilatation of renal pelvis and dilatation of ureter, were observed in CD(SD)IGS compared to CD(SD) rat fetuses. However, a tendency of increased incidence of skeletal variations were observed in CD(SD)IGS rat fetuses compare to CD(SD). It is resulted from higher percentages at 14th ribs and splitting of thoracic vertebral body. The mean number of ossified sternebrae and sacral-caudal vertebrae was not significantly defferent between CD(SD)IGS and CD(SD) rat fetuses.

From the result of our findings, differences between CD(SD)IGS rats and CD(SD) rats in the reproductive parameters were hardly observed. However, the incidence of skeletal variations in CD(SD)IGS rat fetuses were higher than those in CD(SD) rat fetuses. Therefore, this changes should be taken into consideration when CD(SD)IGS rats are used instead of CD(SD) rats in reproductive and developmental toxicit y studies and more background data in CD(SD)IGS rats should be collected.

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Table 1. Body Weights and Food Consumptions in Pregnant IGS Rats

	CD(SD)IGS-1	CD(SD)IGS-2
Body weights(g)		
Day 0	278.6 ± 12.5	251.8 ± 14.4
7	314.4 ± 16.3	296.0 ± 18.0
8	316.8 ± 16.2	299.8 ± 18.2
10	328.4 ± 15.8	309.9 ± 19.2
12	340.1 ± 18.8	322.2 ± 19.5
15	356.8 ± 18.5	339.5 ± 20.0
18	392.5 ± 23.6	375.5 ± 21.4
20	429.2 ± 26.4	407.1 ± 24.7
Body weight gains(g)		
Days 0- 7	35.3 ± 7.28	44.2 ± 8.76
7- 8	2.2 ± 2.54	3.8 ± 3.43
8-10	1.6 ± 2.20	10.1 ± 4.54
10-12	11.4 ± 4.53	12.3 ± 4.21
12-15	16.6 ± 3.30	17.3 ± 3.13
15-18	35.9 ± 6.92	36.0 ± 6.16
18-20	36.4 ± 6.67	31.7 ± 7.09
Food consumption(g/rat/day)		
Days 0- 7	23.2 ± 2.32	24.5 ± 2.18
7- 8	24.7 ± 3.01	25.7 ± 3.08
8-10	24.9 ± 2.33	26.0 ± 2.74
10-12	25.0 ± 3.07	28.3 ± 2.82
12-15	25.0 ± 2.23	26.4 ± 3.01
15-18	26.0 ± 2.79	28.0 ± 2.70
18-20	26.6 ± 2.65	27.8 ± 2.20

Values represent means±S.D. IGS-1:n=18, IGS-2:n=28

Table 2. Gestational Parameters Obtained at Cesarean Section.

	CD(SD)IGS-1	CD(SD)IGS-2	CD(SD)
Observations of dams			
No. of pregnant animal	18	28	35
No. of corpora lutea	16.6 ± 1.79	17.1 ± 2.85	17.9 ± 0.35
No. of implantations	15.7 ± 1.93	14.1 ± 3.95	16.8 ± 0.42
Observations of fetuses			
Pre-implantation loss(%)	5.0	17.6	6.1
Post-implantation loss(%)	6.7	4.5	4.9
No. of live fetuses	14.7 ± 2.42	13.4 ± 3.71	15.9 ± 0.47
Sex ratio(male/female)	1.4	1.2	0.9
Body weights(g)			
male	3.67 ± 0.21	3.61 ± 0.36	3.38 ± 0.04
female	3.43 ± 0.21	3.42 ± 0.30	3.29 ± 0.04
Placental weights(g)			
male	0.47 ± 0.04	0.47 ± 0.06	0.50 ± 0.01
female	0.45 ± 0.03	0.47 ± 0.06	0.48 ± 0.01

Values represent means±S.D.

Table 3. Morphological Observations in Fetuses.

	CD(SD)IGS-1	CD(SD)IGS-2	CD(SD)
External examination			
Number of fetuses examined	265	375	539
Malformations(%)	0(0.00)	1(0.27)	2(0.56)
Vestigial tail		1(0.27)	1(0.12)
Myeloschisis			1(0.12)
Visceral examination			
Number of fetuses examined	128	183	266
Malformations	3(2.34)	2(1.09)	7(2.63)
Hypoplasia of thyroid		1(0.55)	
Agenesis of lung			1(0.38)
Abnormal lobation of lung			1(0.38)
Atrial septum defect	2(1.56)		
Defect of atrioventricular septum			1(0.38)
Abnormal origin of right subclavian artery	1(0.78)		1(0.38)
Abnormal origin of right common carotid artery			1(0.38)
Agenesis of testis			1(0.38)
Hermaphroditism	1(0.55)		
Situs inversus			1(0.38)
Variations	20(15.6)	30(16.4)	23(8.65)
Thymic remnant in neck	7(5.47)	22(12.0)	23(8.65)
Dilatation of renal pelvis	6(3.28)	5(1.88)	
Dilatation of ureter	6(3.28)	3(1.13)	
Skeletal examination			
Number of fetuses examined	137	192	273
Skeletal malformations	0(0.00)	1(0.52)	0(0.00)
Supernumerary rib		1(0.52)	
Skeletal variations	26(18.9)	27(14.1)	15(5.4)
Cervical rib	3(2.19)		2(0.73)
13th rib shortening	2(1.46)	2(1.04)	
14th ribs	10(7.30)	15(7.81)	1(0.37)
Splitting of thoracic vertebral body	7(5.11)	6(3.13)	4(1.47)
Dumbbell shaped thoracic vertebral body	4(2.92)	4(2.08)	6(2.20)
Scralization of lumbar vertebrae			1(0.37)
Lumbarization of sacral vertebra			1(0.52)
No. of sternebrae[a)]	5.02 ± 0.45	5.22 ± 0.70	5.28 ± 0.09
No. of sacral-caudal vertebrae[a)]	7.45 ± 0.43	7.67 ± 0.46	7.39 ± 0.06

Mean(%), a)Mean±S.D.

Characteristics of Crj:CD (SD) IGS rats compared with Crj:CD (SD) rats based on a study for effects on embryo-fetal development

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ABSTRACT. We performed examinations usually employed in the study for effects on embryo-fetal development using Crj:CD(SD)IGS rats. The data were compared with the reproductive and developmental background data on Crj:CD(SD) rats in our facility. Differences between Crj:CD(SD)IGS rats and Crj:CD(SD) rats were found in some parameters such as body weight of dams and fetuses, placental weight, skeletal variation and ossification. Therefore, it is suggested that these parameters may be characteristics of Crj:CD(SD)IGS rats. —Key words: Biological background data, Crj:CD(SD) rats, Crj:CD(SD)IGS rats, Development, Reproduction

CD (SD) IGS-1998: 191-193

INTRODUCTION

The gold standard system, a new laboratory animal breeding system, was developed by Charles River Inc. to contribute to the globalization of research and development for novel synthesis of chemical substances and drugs. This system can globally supply homogeneous Crj:CD(SD)IGS rats (IGS strain) at the genetic level. However, the biological characteristics of the IGS strain have not been reported nor discussed in detail previously, so that the biological background data from the IGS strain are still insufficient. We performed experiments using IGS strain to collect background data mainly on fetal development, and compared the results with to reproductive and developmental background data on Crj:CD(SD) rats (SD strain) in our facility.

MATERIALS AND METHODS

Animals: A total 38 females of the IGS strain from 2 studies for effects on embryo-fetal development and 29 females of the SD strain used as controls in our facility were donated for this study. Ten females of the IGS strain were subcutaneously injected with saline to the dorsum and all the SD strain were orally administrated 0.5% carboxymethyl cellulose sodium salt or water at a constant volume of 0.5 ml/100 g of body weight from day 7 to17 of pregnancy. The doses were based on the body weight of each rat on day 7 of pregnancy. The other IGS strain were non-treated during pregnancy. Both the IGS strain (Tsukuba Breeding Center, Tsukuba, Japan) and the SD strain (Atsugi Breeding Center, Atsugi, Japan) were purchased from Charles River Japan, Inc., Yokohama, which were 11 weeks old for male and 10 weeks old for female. The bacteriological grade of these animals was controlled under specific-pathogen-free conditions.

Animal husbandry: The animals were housed individually (prior to mating and during pregnancy) or in pairs (during mating) in wire mesh cages (260x380x180 mm: Clea Japan Inc., Tokyo, Japan). The room was maintained at a temperature of 21 to 25°C with 40 to 70% relative humidity. The room air was ventilated 20 to 50 times per hour automatically and a 12 hr/12 hr light-dark cycle (lighting 07:00 h-19:00 h) was imposed. The animals received a commercial pellet diet (CRF-1, Oriental Yeast Industry Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*. The animals were identified by metallic ear tags.

Mating: Mating was done on a one-to-one basis. A female animal was placed in the cage of a male animal in the evening. On the following morning, cohabitating females were examined for a vaginal plug and removed from the male's cage. If a vaginal plug was found, the animals were considered to have been fertilized. The day on which a vaginal plug was found was designated as day 0 of pregnancy.

Observation and examination: The general signs were observed once daily for non-treated dams and twice daily for treated dams (before and after treatment) during pregnancy. Body weights were measured on days 0 and 7-20 of pregnancy and food intake on days 0, 5, 7, 11, 16 and 19 of pregnancy. Cesarean sections were performed on day 20 of pregnancy. The dams were anesthetized with ether and sacrificed by exsanguination from the abdominal aorta. After removing the ovaries and uterus, the internal organs were then carefully examined for gross pathologically. The number of corpora lutea in each ovary was counted. The uterus was examined for the number of implantations, live fetuses and dead embryos/fetuses. The dead embryos/fetuses were classified into three categories such as early, middle and late. The live fetuses were removed from the uterus, sexed, individually weighed, and examined for external anomalies, including those in the oral cavity. The placentas were also individually weighted after fetal membrane residues had been removed. Approximately half of the fetuses in each litter were fixed in 95% ethanol, and stained with Alizarin red by Dawson's technique [1]. The skeletal specimens were examined for skeletal anomalies, variations such as cervical rib, wavy rib, shortened 13th rib, 14th rib, splitting of vertebral body, 5 or 7 lumbar vertebrae, asymmetry or splitting of sternebra as well as the number of ossified metacarpals and sacrococcygeal vertebral bodies. The remaining fetuses were fixed in 10% buffered formalin, examined for internal organ anomalies by Wilson's and Nishimura's methods [2-3] and variations such as thymic remnant in the neck and left umbilical artery.

Statistical analysis: According to the equivalence of

variances between the IGS strain and SD strain groups, Student's t-test or Aspin-Welch's t-test was applied to the appropriate values. Statistical significance was set at $p<0.05$. Data on fetal parameters were calculated by litter as a unit.

RESULTS AND DISCUSSION

Significant general signs of dams were not observed during pregnancy. Body weight in dams is shown in Table 1. Body weight of the IGS strain was lower than for the SD strain during pregnancy and significantly lower on days 0, 7-9, 12, 17 and 19 of pregnancy. Food intake in dams is shown in Table 2. Food intake was comparable between both strains, except on day 16 of pregnancy, when the IGS strain showed a significantly higher level.

Table 1. Body weights of dams in Crj:CD(SD) and IGS rat

Group	Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams	38	29
Days of pregnancy		
0	256.9 ± 15.4 *	268.7 ± 16.7
7	299.8 ± 17.0 *	310.0 ± 21.0
8	304.2 ± 17.5 *	318.2 ± 21.6 a)
9	308.0 ± 17.7 *	323.1 ± 21.4 a)
10	314.5 ± 18.0	324.8 ± 24.9
11	322.0 ± 17.7	330.1 ± 24.0
12	326.8 ± 17.9 *	339.3 ± 23.8 a)
13	333.2 ± 19.6	343.0 ± 24.0 a)
14	340.1 ± 19.7	348.5 ± 23.9 a)
15	346.0 ± 19.0	356.6 ± 24.2 a)
16	355.1 ± 21.6	366.8 ± 23.9 a)
17	368.8 ± 22.1 *	382.1 ± 25.9 a)
18	386.0 ± 23.3	396.4 ± 27.9 a)
19	402.3 ± 24.1 *	416.8 ± 27.9 a)
20	417.9 ± 25.5	428.0 ± 32.9

Mean±SD Unit:g

*: Significantly different from the SD strain group ,P<0.05 (Student's t-test)

a): Examined on 21 dams.

Table 2. Food intake of dams in Crj:CD(SD) and IGS rats

Group	Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams	38	13
Days of pregnancy		
0-1	21.7 ± 1.7	21.2 ± 3.0
5-6	25.3 ± 2.6 a)	26.4 ± 3.7
7-8	25.1 ± 2.4	25.6 ± 3.1
11-12	26.9 ± 2.5	25.4 ± 2.5
16-17	27.7 ± 2.3 *	26.1 ± 2.2
19-20	26.8 ± 2.6	25.5 ± 2.5

Mean ± SD Unit:g

*: Significantly different from the SD strain group ,P<0.05 (Student's t-test)

a): Examined on 27 dams.

Reproductive findings of dams are shown in Table 3. The mean number of corpora lutea, implantations, total embryolethality and live fetuses on a litter basis revealed no significant differences between the SD and IGS strains. Among other litter parameters, implantation rate, embryolethality rate and sex ratio were comparable between both strains, but fetal body weight was significantly higher and placental weight was significantly lower in the IGS strain than in the SD strain. External examination of fetuses is shown in Table 4. In the external examinations, polydactry with short tail and cleft palate were observed in each fetus of the SD strain. There was no significant differences between the SD and IGS strains in the incidence of fetuses with anomalies.

Table 3. Reproductive findings of dams in Crj:CD(SD) and IGS rats

Group	Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams	38	29
Number of corpora lutea	17.0 ± 2.4	17.8 ± 1.9
Number of implantations	15.6 ± 2.3	16.5 ± 2.1
Implantation rate [A]	91.9 ± 10.0	93.0 ± 7.8
Number of embryolethality		
Early	0.7 ± 1.1	1.1 ± 1.2
Middle	0.1 ± 0.3 #	0.0 ± 0.0
Late	0.1 ± 0.3	0.0 ± 0.2
Total	0.9 ± 0.3	1.1 ± 1.2
Embryolethality rate [B]	5.7 ± 9.0	6.5 ± 6.6
Number of live fetuses	14.7 ± 2.5	15.4 ± 2.2
Sex ratio [C]	48.4 ± 12.7	52.7 ± 11.0
Mean fetal body weight(g)		
Male	3.94 ± 0.25 *	3.60 ± 0.31
Female	3.75 ± 0.23 *	3.37 ± 0.26
Mean placental weight(g)		
Male	0.46 ± 0.04 *	0.49 ± 0.05
Female	0.44 ± 0.04 *	0.48 ± 0.05

Mean ± SD

[A]: (Number of implantations /Number of corpora lutea) x 100 (%)

[B]: (Total number of embryolethality / Number of implantations) x 100 (%)

[C]: (Number of live males / Number of live fetuses) x 100 (%)

*: Significantly different from the SD strain group ,P<0.05 (Student's t-test)

#: Significantly different from the SD strain group ,P<0.05 (Aspin-Welch's t-test)

Table 4. External examination of fetuses in Crj:CD(SD) and IGS rats

Group	Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams	38	29
Number of fetuses examined	557	447
Number of fetuses with anomalies	0	2
Incidence of fetuses with anomalies (%)	0.0 ± 0.0	0.4 ± 1.5
Type and number of external anomalie		
polydactyly	0	1 a)
short tail	0	1 a)
cleft patate	0	1

Mean±SD

a): Observed in the same fetus.

Internal organ examination of fetuses is shown in Table 5. In the internal organ examination of fetuses, one anomalous fetus

was observed in both strains, cleft palate for SD and microphthalmia for IGS. There were no significant differences between the SD and IGS strains in the incidence of fetuses with anomalies as well as internal organ variations such as thymic remnant in the neck and left umbilical artery. Skeletal examination of fetuses is shown in Table 6. In the skeletal examination of fetuses, no skeletal anomalies were observed in any fetus of either strain. Among the incidence of fetuses with skeletal variations, cervical rib, wavy rib, shortened 13th rib, splitting of vertebral body, 7 lumbar vertebrae, asymmetry or splitting of sternebra were comparable between both strains, but that of 14th rib and 5 lumber vertebrae were significantly higher in the IGS strain than in the SD strain. The number of ossified bones in metacarpals was comparable between both strains, but sacrococcygeal vertebral bodies was significantly higher in the IGS strain than in the SD strain.

As described above, significant differences were noted in the body weights of dams, fetal body weight, placental weight, incidence of fetuses with skeletal variations such as 14th rib and 5 lumber rib and number of fetal ossified sacrococcygeal vertebral bodies between both strains. Therefore, it is suggested that these parameters may be characteristic of the IGS strain.

Table 5. Internal organ examination of fetuses in Crj:CD(SD) and IGS rats

Group	Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams	38	29
Number of fetuses examined	279	224
Number of fetuses with anomalies	1	1
Incidence of fetuses with anomalies (%)	0.3 ± 2.0	0.6 ± 3.1
Type and number of internal organ anomalies		
microphthalmia	1	0
cleft palate	0	1
Incidence of fetuses with variations (%)		
Thymic remnant in the neck	7.3 ± 11.5	12.5 ± 14.8
Left umbilical artery	0.3 ± 1.8	1.3 ± 3.9

Mean±SD

Table 6. Skeletal examination of fetuses in Crj:CD(SD) and IGS rat

Group	Crj:CD(SD) IGS	Crj:CD(SD)
No. of dams	38	29
Number of fetuses examined	278	223
Number of fetuses with anomalies	0	0
Incidence of fetuses with anomalies (%)	0.0±0.0	0.0±0.0
Incidence of fetuses with variations (%)		
Cervical rib	0.3±0.2	0.9±3.5
Wavy rib	0.0±0.0	0.0±0.0
Shortened 13th rib	0.0±0.0	4.2±12.2
14th rib	9.4±16.0 #	1.4±4.1
Splitting of vertebral body	1.8±5.8	1.6±5.2
5 lumbar vertebrae	9.4±16.0 #	3.2±7.8
7 lumbar vertebrae	0.0±0.0	0.0±0.0
Asymmetry of sternebra	0.3±2.0	0.5±2.7
Splitting of sternebra	0.7±2.8	0.0±0.0
Number of ossified bones		
Metacarpals	7.4±0.7	7.1±0.7
Sacrococcygeal vertebral bodies	8.1±0.3 #	7.7±0.6

Mean ± SD

#: Significantly different from the SD strain group ,P<0.05(Aspin-Welch's t-test)

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Variability in Offspring Survival and Reproductive Parameters in Recent Pre- and Postnatal and Multigeneration Studies in the Crl:CD (SD) IGS Rat

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ABSTRACT. An examination of the performance of the Crl:CD (SD) IGS Rat purchased from Charles River UK during 1997 has shown marked differences in offspring bodyweight at birth and subsequent survival to Day 4 of age between the animals paired at 11 weeks of age (pre-and post natal studies) and those paired at 16 weeks of age (multigeneration studies). Typically the older females have slightly longer gestation lengths but give birth to lighter offspring. Litter size is similar in rats paired at 11 or 16 weeks and there is little change in pup weight relative to live litter size at Day 1. However, females paired at 16 weeks show an increased proportion of litters where male pup weight (used as a benchmark criterion) is below 5.5 g and these litters show a much increased level of litter death in the first four days after birth. While it is normal for offspring born after a longer gestation length to show higher bodyweights at Day 1 of age, it has been found that the litters in which total litter loss occurs before Day 4 commonly show no weight gain and may be considered vulnerable as "small for date" pups. — Key words: Crl:CD (SD) IGS Rat; Litter survival; Maternal age

CD (SD) IGS-1998: 194-199

INTRODUCTION

Two basic breeding study types are performed on a regular basis at our laboratories: the pre- and post-natal study, typically conducted to ICH guidelines in which virgin female rats are paired at approximately 11 weeks of age to proven stock males to provide time mated females, and multigeneration studies where virgin males and females are paired at approximately 16 weeks of age. In the past, litter mortality within our facility has been approximately 2% of litters born for all types of breeding studies, but recently we have experienced an increase in the incidence of litter deaths with the older females used in multigeneration studies. A review of performance of animals in similar studies in other laboratories within the United Kingdom (IRDG meeting March 1998) indicated that this was not an isolated observation. The animal supplier (Charles River UK, Margate, Kent, England) had not experienced any decline in breeding output. In their animal production colony, the supplier first breeds their females at an age of about 9 weeks and expects to obtain 5 litters before seeing a significant decline in performance. Under this regime of continuous production the female's effective breeding life would be at least 37 weeks, much older than the animals used in multigeneration studies. A decline in fertility of virgin females with increasing age is well known (Bottomley and Leeming 1980) and fertility problems have been seen with the original (pre IGS) CD rat in multigeneration studies where females were first paired at 20 weeks of age.

In this report we first present background data on litter survival in the original Charles River CD rat in multigeneration studies and then compare maternal bodyweight during gestation, offspring bodyweight and litter survival of the more recent supplies of the Crl:CD (SD) IGS rat used in pre- and post-natal studies with those used in the first generation of multigeneration studies.

MATERIALS AND METHODS

Animals: All animals were purchased from Charles River UK, Margate, Kent, England and were of the Crl:CD strain. Prior to 1996 the original CD strain was used but from 1996 animals were of the re-derived International Genetic Standard (Crl:CD®(SD) IGS BR).

Females for the pre- and post-natal studies were purchased at approximately 10 weeks of age (200 – 220g) and were allowed about one week of acclimatisation before pairing with stock male animals from the same supplier.

Animals for multigeneration studies were purchased as identified siblings at the age of 4 weeks and at a bodyweight of approximately 80 – 110g (males) or 70 – 90 g (females). The animals were allowed about 2 weeks to acclimatise to the laboratory conditions before allocation to study.

Husbandry conditions: Animals were housed in a fully barriered rodent facility, maintained at a temperature of 19 - 23°C and 40 - 70 % relative humidity with at least 15 air changes per hour. The lighting was controlled to provide 12 hours light:12 hours dark. For mating, polypropylene cages with floors and lids of stainless steel grid were used. The same cage type was used through to Day 17 or 20 of gestation before mated females were transferred to solid bottomed polypropylene cages to allow littering to take place. During the littering phase females were supplied with wood flakes (Lignocell 3-4 grade) for bedding. When the offspring were approximately 2 weeks of age, the dams and litters were transferred to larger stainless steel cages with grid floors and lids. Multigeneration animals were group housed (4 per cage) in the large stainless steel cages during the maturation phase which preceded pairing. Cages with grid floors were suspended above absorbent paper. The absorbent paper and wood flake bedding were changed at least twice weekly.

All animals were fed on a high protein breeding diet (LAD SQC) from Special Diet Services Ltd., UK. For the pre-and post-natal studies the diet was provided as solid pellets in food hoppers (LAD 1) while the multigeneration study animals received the same diet in powdered form (LAD 2) which was provided in either metal tins or in glass jars. Water from the public drinking supply was provided via water bottles with sipper tubes. Both food and water were available to the animals without restriction.

Females in the pre- and post-natal studies received test materials daily, either by oral gavage or by intravenous injection into the tail vein from Day 6 after mating until Day 20 of lactation. Control animals received the vehicle. Animals in the multigeneration studies received test materials as admixtures in the powdered diet throughout the period of investigation. Except where specifically mentioned, there were no apparent effects of treatment upon the parameters presented in this report and data presented includes data from control and treated animals.

Mating procedures: For pre- and post-natal studies each female was paired with a proven stock male of the same strain when the females were approximately 11 weeks of age. For the multigeneration studies one male was paired with one female after 10 weeks of treatment when the females were approximately 16 weeks of age. Pairing was controlled so that sibling mating was avoided but each group had matched mating pairs derived from the original litter supply. The numbers of ejected copulation plugs and an estimation of the numbers of sperm in the vaginal smear were recorded as evidence of mating and the day these were observed was designated Day 0 of gestation.

Observations: All animals in both pre- and post-natal and multigeneration studies were monitored at least twice daily for evidence of reaction to treatment or ill-health. Bodyweights were recorded at least weekly and food consumption was monitored continuously over weekly or shorter intervals. From Day 20 of gestation females were checked three times per day (09.00, 13.00 and 17.00 hrs) for evidence of the start and completion of parturition. Gestation length is reported to the nearest half day as the interval between the day preceding confirmation of mating and the start of the parturition process. Detailed records of litter size and offspring bodyweight were started between 8 and 24 hours after parturition was completed (Day 1 of lactation). Litter size was monitored daily and offspring were sexed and weighed on Days 1, 4, 7, 14 and 21 or at more frequent intervals. On Day 4 of age litter size was reduced to 8 (where possible 4 males and 4 females per litter) by using a non-selective random culling procedure. Offspring which died prior to weaning were subjected to a gross necropsy. The only consistent finding at these examinations was the high incidence of absence of milk/food in the stomach and these data are not reported.

Data reported here is limited to the performance of the animals during gestation and parturition and through to Day 4 of age of the offspring. By this time, the majority of effects upon offspring survival have been established, although there may be a small number of individual offspring deaths and cases of total litter loss after Day 4, particularly where early development was prejudiced. Some of the studies reported in this paper are still in progress and no data is presented on the performance of animals from the second generation.

RESULTS

Survival data for multigeneration studies conducted in the original CD rat: 1988 – 1992 (Table 1)

During the period between 1988 and 1992 thirteen multigeneration studies were performed and, for the first generation paired at 16 weeks of age, the maximum incidence of total litter loss in Control groups and in low and intermediate dosage groups was two litters per group (approximately 9% incidence per study group) with an overall mean incidence of between 0.7% and 2.0%. Three studies showed 3 or more litter deaths in the high dose groups (12 - 17% per group) and it was considered likely that these were an expression of a treatment related effect upon litter viability. The overall incidence of litter deaths in the high dose groups amounted to 5.8%, and even with the inclusion of data from the high dosage treated groups, which may have been affected by treatment, the overall incidence of litter death was less than 3% of nearly 1200 litters examined.

Current levels of litter survival data in the IGS rat : studies initiated in 1997 (Table 2)

Pre- and post-natal studies conducted on females paired at approximately 11 weeks of age show a similar frequency of litter deaths in the new strain as previously recorded in the original strain. The maximum incidence of litter death is 2 per group (about 8%) and, in the absence of any apparent treatment related effects, the overall incidence of litter losses up to Day 4 of age was 1.7% of nearly 500 litters. Multigeneration studies conducted at the same time, however, show increased levels of litter deaths. Of the five studies in the current series, the first showed an overall incidence of 21.6% of litter deaths and the four subsequent studies showed incidences of between 5.7% and 7.3% of litter deaths within the first four days after birth. None of these studies showed any indications that the test article might be a cause of the litter deaths.

Comparison between IGS rats mated at 11 weeks and 16 weeks of age (Table 3)

At the start of gestation, females used for the pre- and post-natal studies (1l weeks of age) were approximately 60 g lighter than the females used for the multigeneration studies (16 weeks of age). There were no apparent differences in maternal weight at conception for the classes of females which failed to give birth to live young, whose litters died before Day 4 of age or those where the litters survived beyond Day 4 of age.

Bodyweight gain between Days 0 and 20 of gestation for females which had surviving litters at Day 4 was approximately 7% greater in the younger females than recorded for the older animals but there were no obvious relationships between maternal weight gain to Day 20 and litter survival.

The females mated at 11 weeks of age had marginally shorter gestation lengths than females mated at 16 weeks of age for all survival categories. In both age groups the average gestation lengths were marginally shorter for litters which survived beyond Day 4 of age than for those which died.

Implantation counts (where included), total young at Day 1, live offspring at Day 1 and Day 4 for litters which survived were similar in both the females paired at 11 weeks and those paired at 16 weeks of age. Among the litters which died before Day 4, there was increased between litter variation in litter size

Table 1. Incidence of total litter losses in multigeneration studies in the original CD strain 1988-92

Date of purchase	Incidence (%) of litters dying before weaning				Overall litter data		
of animals	Control	Low dose	Mid dose	High dose	Born	Died	% Died
Jan 88	0/22 (0)	0/19 (0)	0/18 (0)	1/20 (5)	79	1	1.3
Mar 88	0/23 (0)	0/23 (0)	2/22 (9)	0/23 (0)	91	2	2.2
Aug 88	2/23 (9)	2/22 (9)	2/22 (9)	2/21 (10)	88	8	9.1
Mar 89	2/25 (8)	0/26 (0)	0/26 (0)	4/26 (15)	103	6	5.8
Jul 89	0/19 (0)	0/22 (0)	1/20 (5)	0/23 (0)	84	1	1.2
Sep 89	1/19 (5)	0/20 (0)	0/19 (0)	0/20 (0)	78	1	1.3
Jan 90	0/24 (0)	0/24 (0)	0/23 (0)	0/23 (0)	94	0	0.0
Jul 90	0/19 (0)	0/20 (0)	0/21 (0)	1/26 (4)	86	1	1.2
Oct 90	0/26 (0)	0/30 (0)	1/29 (3)	5/29 (17)	114	6	5.3
Jan 91	1/23 (4)	0/24 (0)	1/23 (4)	3/25 (12)	95	5	5.3
Feb 91	0/26 (0)	0/21 (0)	1//25 (4)	0/26 (0)	98	1	1.0
Mar 91	0/23 (0)	0/21 (0)	0/23 (0)	0/23 (0)	90	0	0.0
Feb 92	0/21 (0)	0/22 (0)		2/28 (7)	71	2	2.8
Total	6/293	2/294	8/271	18/313	1181	34	
Mean	2.0	0.7	0.7	5.8			2.9
Low	0.0	0.0	0.0	0.0			0.0
High	9	9	9	17			9.1

Table 2. Incidence of total litter losses before Day 4 of age in the IGS rat

	Females paired at 11 weeks of age (Pre- and Post natal studies)						
Date of purchase	Incidence (%) of litters dying before Day 4 of age				Overall litter data		
of animals	Control	Low dose	Mid dose	High dose	Born	Died	% Died
Jul 97	2/25 (8)	0/24 (0)	1/25 (4)	1/24 (4)	98	4	4.1
Sep 97	0/22 (0)	0/20 (0)	0/22 (0)	0/22 (0)	86	0	0.0
Oct 97	0/22 (0)	0/20 (0)	0/21 (0)	0/22 (0)	85	0	0.0
Oct 97	1/30 (3)	0/30 (0)	0/27 (0)	1/30 (3)	116	2	1.7
Dec 97	0/22 (0)	0/22 (0)	0/22 (0)	2/24 (8)	89	2	2.2
Total	3/121	0/116	1/117	4/122	476	8	
Mean	2.5	0.0	0.9	0.8			1.7
Low	0	0	0	0			0.0
High	8	0	4	8			4.1
	Females paired at 16 weeks of age (Multigeneration studies)						
May 97	8/26 (31)	3/24 (13)	6/24 (25)	5/28 (18)	102	22	21.6
Aug 97	1/28 (4)	4/26 (15).	1/25 (4)	0/27 (0)	106	6	5.7
Sep 97	2/26 (8)	2/26 (8)	1/26 (4)	2/26 (8)	104	7	6.7
Oct 97	2/29 (7)	2/32 (6)	2/31 (6)	0/32 (0)	124	6	4.8
Dec 97	3/26 (12)	1/24 (4)	1/26 (4)	2/26 (8)	102	7	6.9
Total	16/135	12/132	11/132	9/139	538	48	
Mean	11.9	9.1	8.3	6.5			8.9
Low	4	4	4	0			4.8
High	31	115	25	18			21.6

and no firm conclusions could be reached with regard to an effect of maternal age on litter size where the litters did not survive.

Offspring bodyweight at Day 1 of age was lower for the older females, despire their slightly longer gestation lengths: the offfspring weight difference between females mated at 11 weeks and those mated at 16 weeks amounted to about 5% for litters which survived and about 12% for litters which died. For litters dying by Day 4 of age, as compared with those that survived, bodyweight at Day 1 of age was lower by about 8/13% for male/female offspring from the younger dams and by about 11/18% for male/female offspring for the older dams. This bodyweight deficit, coupled with changes in gestation length, was considered to be critical to the survival of the offspring in the first four days after birth.

The relationship between offspring bodyweight at Day 1 and subsequent survival (Table 4)

At Day 1 of age male offspring are normally about 0.3 grams heavier than their female siblings, and for simplicity the data have been presented and analysed based on male offspring bodyweight only.

For females paired at 11 weeks of age a mean bodyweight of less than 5.5g in male offspring occurs in fewer than 5% of litters, whilst among females paired at 16 weeks of age approximately 23% of litters fall into this weight category. It is within this group of low bodyweight litters that the majority of the litter deaths occur. In the older females nearly 80% of the

litter deaths were from these low weight litters. Where male offspring bodyweight was above 5.5g the incidence of litter deaths was less than 5% and was similar in both the younger and older dams, falling to less than 2% of litters when male offspring weight at Day 1 was greater than 6.0g.

Table 3. Maternal and litter parameters for females paired at either 11 or 16 weeks of age

		Females paired at 11 weeks of age (Pre- and Post natal studies)			Females paired at 16 weeks of age (Multigeneration studies)		
Parameter		Litter dead at Day 1	Litter dead by Day 4	Litter survived Day 4	Litter dead at Day 1	Litter dead by Day 4	Litter survived Day 4
Number in category		3	7	468	10	38	494
Percentage of total litters		0.6	1.5	97.9	1.8	7.0	91.1
Gestation weight (g) Day 0	Mean	241.3	248.4	245.1	301.4	309.8	306.7
	SD	12.3	12.9	11.3	46.2	33.0	30.1
	n	3	7	468	10	38	494
Gestation weight (g) Day 20	Mean	370.3	411.1	390.6	418.8	440.7	442.7
	SD	27.1	51.3	27.9	62.8	39.2	38.6
	n	3	7	468	10	38	494
Gestation weight gain (g) 0 - 20	Mean	129.0	162.7	145.6	117.4	130.9	136.0
	SD	18.3	39.3	22.4	52.2	18.8	18.3
	n	3	7	468	10	38	494
Gestation length (Days)	Mean	22.8	22.7	22.4	23.2	23.0	22.6
	SD	0.4	0.5	0.4	1.0	0.3	0.4
	n	2	7	468	8	38	494
Implantation site count	Mean	15.0	16.5	15.3	10.0	16.7	15.6
	SD	4.6	1.0	2.2	8.2	2.7	2.5
	n	3	6	467	4	15	316
Total litter size at Day 1	Mean	4.0	12.6	14.0	5.5	14.7	14.3
	SD	6.9	4.0	2.3	7.0	3.2	2.6
	n	3	7	468	10	38	494
Live litter size at Day 1	Mean	0.0	11.6	13.9	0.0	11.9	14.0
	SD		4.0	2.3		4.1	2.6
	n		7	468		38	494
Live litter size at Day 4	Mean		0.0	13.6		0.0	13.2
	SD			2.4			3.0
	n			468			494
Male pup weight (g) at Day 1	Mean		6.0	6.5		5.2	6.2
	SD		0.3	0.7		0.5	0.8
	n		6	467		38	494
Female pup weight (g) at Day 1	Mean		5.4	6.2		4.9	6.0
	SD		0.4	0.6		0.5	0.7
	n		6	468		37	493

SD Standard deviation, n Number of animals evaluated

Table 4. Distribution of male offspring weight# at Day 1 and incidence of litter death by Day 4 of age in females paired at 11 or 16 weeks of age

Male pup weight classification	Females paired at 11 weeks of age (Pre- and Post natal studies)				Females paired at 16 weeks of age (Multigeneration studies)			
	Litters born		Litters dying before Day 4		Litters born		Litters dying before Day 4	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%
< 5.0 g	3	0.6	0	0.0	39	7.3	15	40.5
> 5.0 < 5.5 g	18	3.9	0	0.0	83	15.6	15	18.1
> 5.5 < 6.0 g	88	18.0	4	4.5	138	25.9	6	4.3
> 6.0 < 6.5 g	138	29.3	1	0.7	128	24.1	2	1.6
> 6.5 < 7.0 g	126	26.8	1	0.8	85	16.0	0	0.0
> 7.0 < 7.5 g	62	13.3	0	0.0	34	6.4	0	0.0
> 7.5 < 8.0 g	27	5.8	0	0.0	14	2.6	0	0.0
> 8.0 g	11	2.4	0	0.0	11	2.1	0	0.0

Litter mean of male offspring weight used as a bench mark.

The relationship between gestation length, offspring bodyweight at Day 1 and survival (Table 5)

The range of gestation lengths, mostly 22 to 23.5 days, was similar in females mated at 11 and 16 weeks, but within this range the distribution favoured earlier parturition in the younger rats. For litters that survived beyond Day 4 of age, offspring from younger females were consistently slightly heavier than those of the older dams and male offspring weight increased with increasing gestation length. For the same gestational age, litters which died were lighter than those which lived and this difference in weight increased with increasing gestational age. The highest incidence of litter loss was seen in animals where gestation length was 23.5 days, and particularly in the older females where the mean weight deficit was over a gram per male pup.

Table 5. The relationship between gestation length, offspring weight and survival

Gestation length (days)	Females paired at 11 weeks of age (Pre- and Post natal studies)					Females paired at 16 weeks of age (Multigeneration studies)				
	% incidence gestation length	Litter dies before Day 4		Litter survives Day 4		% incidence gestation length	Litter dies before Day 4		Litter survives Day 4	
		n	Mean male pup wt (g)	n	Mean male pup wt (g)		n	Mean male pup wt (g)	n	Mean male pup wt (g)
21.5	0.2	0 0.0% loss	-	1	6.2	-	0	-	0	-
22.0	35.2	1 0.6% loss	5.9	167	6.2	21.7	0 0.0% loss	-	117	6.0
22.5	41.3	4 2.0% loss	6.2	193	6.5	37.4	11 5.5% loss	5.0	190	6.0
23.0	21.4	3 2.9% loss	5.8	99	7.1	37.4	27 13.4% loss	5.2	175	6.5
23.5	1.7	1 12.5% loss	5.9	7	6.9	3.5	7 36.8% loss	5.2	12	6.4
24.5	0.2	0 0.0% loss	-	1	5.4	-	0	-	0	-
25.5	-	0		0		0.2	1 100% loss		0	-

DISCUSSION

The observation that low offspring weight is associated with reduced litter viability in the rat is not a startling finding. However, the increased incidence of low bodyweight litters (from about 5% to 22%) between naive females which are approximately 5 weeks different in age at mating and the consequent reduction in litter survival have potentially serious consequences for the conduct of long term breeding studies. As the background frequency of litter deaths rises, it becomes necessary to increase starting group sizes so that the integrity of the study is maintained into the second generation. It also becomes more difficult to distinguish between possible treatment related effects and coincidental litter losses.

At the UK IRDG meeting of March 1998 a number of inherent differences between pre- and post-natal and multigeneration studies were identified as possible contributory factors to the difference in performance, these included:

Of these suggestions, the age of the rats was considered the most likely significant variable.

Pre and post natal studies
Raised by the supplier on supplier's diet to 10 weeks of age.
Mated to proven (older) males at 11 weeks of age.
Animals maintained on pelleted diet.
Animals handled and dosed daily.

Multigeneration studies
Raised by the user laboratory on high protein diet from about 4 weeks of age.
Mated to unproven males from the same supply as the females at 16 weeks of age.
Animals maintained on powdered diet.
Handling less frequent.

The effect on offspring bodyweight appears to be mediated through the mother during the last few days of pregnancy with low offspring weight gain in this period being associated with a minor extension of gestation length. It has been suggested that this may be the result of a genetic predisposition and although this is speculation at present it will be interesting to see the outcome of the second generation breeding of the studies in progress. The performance of the more recently obtained batches of animals in their first generation may also help identify whether the phenomenon reported in this paper is a short term variation in the performance of the Charles River CD rat or a more significant long term problem.

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CONCLUSION

It is concluded that the latest version of the Charles River CD rat (International Genetic Standard) has shown a reduction in litter viability when first pairing of the females occurs at 16 weeks of age, compared to the performance of females paired at 11 weeks of age and with females from the original UK supply of CD rat paired at 16 weeks of age. It remains to be seen if this is a short term variation in the performance of the Charles River CD rat or a more significant long term problem.

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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

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ABSTRACT. Data from embryo-fetal toxicity studies have been used to compare observations between in-house and supplier mated Crl:CD(SD) and Crl:CD(SD)IGS rats. The change from the CD(SD) to the CD(SD)IGS rat appears to have been accompanied by lower maternal weight gain during pregnancy. At Day 20 of gestation, consistent differences are still apparent in litter parameters when in-house and supplier mated rats are compared, but in both situations corpora lutea and implantation counts are lower, litter size and mean fetal weight is smaller than previously. A low incidence of major fetal abnormalities is seen in control group animals, with two syndromes - the squat fetus and mottled fetus - being markedly decreased on introducing the CD(SD)IGS rat while incidence of vertebral column termination with anury has been seen to increase. Incidence of 14th rib has also been compared as this is a sensitive area of change during fetal development and more variation study to study is seen with the CD(SD)IGS compared to CD(SD) and with in-house mated compared to supplier mated.
—Key words: Crl:CD(SD)IGS rat, embryo-fetal, developmental parameters

CD (SD) IGS-1998: 200-205

INTRODUCTION

Rats of the CD strain, supplied by Charles River UK Limited have been extensively used at these laboratories for reproductive toxicity studies. The change from the original CD(SD) designation to the CD(SD)IGS designation, following introduction of a re-derived strain, was monitored carefully to establish whether there were any changes in fetal parameters that might affect interpretation of data generated in developmental toxicity studies. This paper speculates on the effect that re-derivation has had on litter parameters routinely recorded in our laboratories at Day 20 of gestation and presents the incidence of spontaneous structural changes recorded in the fetuses following visceral and skeletal examination. The comparison is based both upon embryo-fetal studies performed at the Huntingdon Life Sciences laboratory at Eye, Suffolk and a similar number of studies performed at the Huntingdon laboratory. Use of information from both laboratories also provided an opportunity to compare data from in-house and supplier mated animals. Control groups from studies where treatment was administered during organogenesis (i.e. between Day 6 and Day 17 of gestation) were primarily used in these comparisons.

MATERIALS AND METHODS

The data reported in this paper are from studies conducted in support of regulatory submissions of new materials or data up-grading of old 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, between 22 and 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 UK Limited, Margate, Kent, England. At Huntingdon Life Science they were housed in a controlled room temperature of 21 ± 3°C and relative humidity 55 ± 15%. Artificial light gave 12 hours light and 12 hours darkness. All animals were given free access to tap water and to Special Diet Services (SDS) Laboratory Animal Diet No. 1 which were analysed on a regular basis for nutrients, contaminants and micro-organisms. The day of mating is 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, food and water consumption were recorded according to protocol requirements.

Supplier mated rats - Sexually mature 8-10 week old Specific Pathogen Free female rats with weight range 150-260g were mated to identified males of the same strain. Individual studies varied within these ranges. On arrival at the laboratory the animals were examined and given group allocation by computerised stratified randomisation to give comparable mean group body weight and acceptable distribution of males to which females were mated. They were ear marked for individual identification before being housed 5 to a cage during gestation.

In-house mated rats - Adult virgin female rats were allowed 1-4 weeks acclimatisation before being mated with stock males of the same strain; at study commencement dams were in the weight range 200-310g and 10-12 weeks of age, again these parameters varied within this range for each individual study. On group allocation they were assigned a number identified by tail tattoo. During acclimatisation dams were housed 5 to a cage, during mating and gestation they were individually housed.

Autopsy - On Day 20 of gestation dams were killed by CO_2 asphyxiation and examined for macroscopic pathological changes in maternal organs and congenital abnormalities. The uterus and ovaries were removed and examined in detail; embryo-fetal deaths were classified as 'early' where only placenta is apparent and 'late' where both placenta and embryonic remnants were visible. Placental weight was routinely recorded for in-house mated studies, not supplier mated. Uteri of apparently non-pregnant dams were examined

for evidence of implantation sites using a modified Salewski technique [6]. Live young were examined externally, weighed and individually identified. Half the fetuses in each litter were preserved in Bouins solution for examination by Wilson technique [10], half were preserved in 74 OP industrial methylated spirit for skeletal examination after staining by a modified Dawson technique [5]. Fetuses allocated for skeletal examination were macroscopically viscerally examined and sexed, either prior to fixation or after 4 days fixation.

Data shown here are taken from control group animals (Table 1), with the exception of Table 4 where test groups not considered to show treatment-related bias are included for these same studies in order to enlarge the pool of data. Statistical analysis was performed as a pairwise comparison between Crl:CD(SD) vs Crl:CD(SD)IGS for supplier and in house mated studies. Table 2 was analysed by a Two-sided Student's t-tests performed in SAS 6.11 [7] with Two-sided Wilcoxon-Mann-Whitney test performed in StatXact 3.0.1 for in utero deaths; Tables 3 and 4 were analysed by the Two-sided Fisher's Exact tests performed in StatXact for Windows [2].

RESULTS AND DISCUSSION

Autopsy parameters - Supplier mated rats had lower corpora lutea and implantation counts, smaller litter size and higher mean fetal weight than in-house mated rats, shown in Table 2. This may be due to the difference in age and weight range between the two groups [1]. However, early, late and total in utero deaths amongst in-house mated rats were also lower in comparison with supplier mated rats.

On comparing CD(SD) and CD(SD)IGS rats, there was a lower maternal weight at Day 20 and lower weight gain during pregnancy in the new rat with a decrease in litter size and mean fetal weight common to both supplier mated and in house mated animals. A lower incidence of in utero deaths was seen in CD(SD)IGS rats with a significant decrease in early and total in utero deaths in supplier mated studies (Table 5).

Fetal abnormalities - There is a very low incidence of major abnormalities, categorised by being rare and/or potentially lethal, that are observed during detailed visceral and skeletal examinations; these are compared in Table 3, with statistical analysis in Table 5. There are specifically three abnormalities whose incidences we notice at bench level have altered on changing to CD(SD)IGS rats which are considered separately in Table 4. As they occur at a low incidence all groups from each category of 10 studies have been included to demonstrate this change. Two syndromes which were previously seen as clusters within litters have markedly decreased on the introduction of CD(SD)IGS rat. The Squat fetus shown in Figure 1, previously observed in the CD(SD) rat [8],[9], is characterised by a hunched posture with downward flexed forelimbs. Skeletal examination revealed thickened ribs with the sternum ventrally distorted. The Mottled fetus (Figure 2) reported in 1994, [4], also occurred in the CD(SD) rat with multiple abnormalities including white cutis with brown mottling, subcutaneous oedema, brachygnathia, cleft palate, syndactyly, irregularly ossified and kinked long bones and ribs. On the other hand, the incidence of anury as identified at autopsy shows a statistically significant increase in the CD(SD)IGS rat. At autopsy the tail may be fleshy, short, constricted at the base or absent, and no vertebrae are apparent in all cases. On skeletal examination termination of the vertebral column is seen in either the thoracic, lumbar or sacral regions.

14^{th} rib - The incidence of 14th rib is a sensitive area during fetal development [3]. Control group % litter incidence has shown a greater variation since introducing the CD(SD)IGS rat demonstrated by comparing correlation coefficients (r). In Figure 3, CD(SD) in-house mated r = - 0.5, while CD(SD)IGS r = 0.0. Supplier mated CD(SD) r = -0.8 also show an increase in variability on introducing the CD(SD)IGS rat r = 0.6. With either rat, in-house mated incidence of 14th rib is more variable than supplier mated incidence within this period.

It is felt that there are sufficient differences between the CD(SD), CD(SD)IGS, in-house and supplier mated rat that individual study data should only be interpreted using background control data for rats of a similar origin.

Table 1. Details of data used in Crl:CD(SD) and Crl:CD(SD)IGS comparison.

	Crl:CD(SD)		Crl:CD(SD)IGS	
	In-house mated	Supplier mated	In-house mated	Supplier mated
Period covered by autopsies	3/95 to 3/96	9/94 to 10/95	11/96 to 7/97	11/95 to 11/96
Number of studies	10	10	10	10
Number of control litters examined	222	234	218	234
Number of control fetuses examined	3231	3086	3121	2933

Fig. 1. Squat fetus and normal litter mate:
Hunched posture with ventrally distorted rib cage

Fig. 2. Mottled fetus and normal litter mate:
Mottled and thin cutis, brachygnathia, cleft palate subcutaneous oedema, syndactyly and oligodactyly

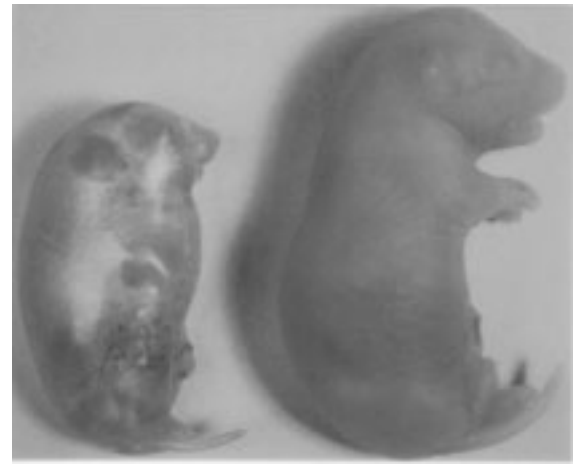

Table 2. Comparison of control group data for Crl:CD(SD) and Crl:CD(SD)IGS caesarian autopsy parameters

		Crl:CD(SD)		Crl:CD(SD)IGS	
		In-house mated	Supplier mated	In-house mated	Supplier mated
Group mean ± SD values:					
Day 3 maternal body weight (g)		271.3 ± 13.43	236.3a ± 8.50	264.3 ± 6.45	228.5a ± 11.87
Day 20 maternal body weight (g)		429.5 ± 13.18	402.1 ± 8.85	405.3 ± 14.32	378.0 ± 12.28
Average weight gain (g)		158.2	165.8	141.0	149.5
Corpora lutea		16.5 ± 0.70	15.1 ± 0.59	16.0 ± 0.43	13.9 ± 0.87
Implantations		15.57 ± 0.66	13.97 ± 0.55	15.21 ± 0.48	13.11 ± 0.87
Number live young		14.6 ± 0.66	13.2 ± 0.61	14.3 ± 0.55	12.5 ± 0.84
Fetal weight (g)		3.77 ± 0.10	3.85 ± 0.06	3.66 ± 0.07	3.79 ± 0.07
Placental weight (g)		0.53 ± 0.02	b	0.54 ± 0.01	b
Litter incidence:					
In utero deaths early: n =	0	91	128	108	155
	1	76	70	68	54
	2	40	31	27	19
	3	7	5	5	2
	4	4		2	1
	5	2		4	2
	6			1	1
	7			2	
	9	1			
	10	1		1	
In utero deaths late: n =	0	219	209	218	218
	1	3	22		16
	2		2		
	6		1		
In utero deaths total: n =	0	90	114	108	143
	1	76	76	68	63
	2	40	35	27	21
	3	8	5	5	3
	4	4	3	2	1
	5	2		4	2
	6		1	1	1
	7			2	
	9	1			
	10	1		1	

a) Excludes 3 studies weighed at Days 2,4.
b) Placental weights not routinely recorded in supplier mated studies
n = Number of litters with 0,1,2…embryo and/or fetal deaths

Table 3. Comparison of control group data for Crl:CD(SD) and Crl:CD(SD)IGS major fetal abnormalities at detailed examination (Individual fetuses may occur in more than one category)

	Crl:CD(SD)		Crl:CD(SD)IGS	
	In-house mated	Supplier mated	In-house mated	Supplier mated
Total number fetuses(litters) affected	18(14)	18(12)	14(11)	19(16)
Multiple facial abnormalities	-	-	-	1(1)
Hydrocephaly	2(2)	-	-	2(2)
Meningocoele	-	-	1(1)	1(1)
Misshapen pituitary	-	1(1)	-	-
Microphthalmia/small orbital socket	4(4)	-	-	2(2)
Retinal/lenticular irregularities	-	-	5(3)	-
Cleft palate	1(1)	1(1)	-	1(1)
Brachygnathia	-	-	1(1)	-
Fused mandibles/lower incisors	-	-	1(1)	-
Agenesis of trachea	-	-	1(1)	-
Systemic/pulmonary artery	1(1)	1(1)	2(2)	2(2)
Cervico/thoracic artery /vein	-	1(1)	1(1)	-
Interventricular/atrial septal defect	1(1)	2(2)	2(2)	3(3)
Double outlet ventricle	-	-	1(1)	-
Cardinal vein	1(1)	-	1(1)	-
Diaphragmatic hernia	-	-	1(1)	-
Umbilical hernia	-	-	-	1(1)
Situs inversus complete	-	-	1(1)	-
Split sternum	-	-	-	1(1)
Thickened/kinked ribs	3(2)	-	3(2)	4(2)
Multiple vertebral/rib irregularities	-	1(1)	-	-
Termination of vertebral column	-	-	-	4(4)
Interrupted vertebral column	-	1(1)	-	-
Vertebral irregularities	1(1)	1(1)	-	-
Right sided duodenum and pancreas	1(1)	-	-	-
Small stomach	1(1)	-	-	-
Misshapen/displaced adrenal	-	1(1)	-	-
Anury/brachury	-	-	1(1)	2(2)
Ano-rectal atresia	1(1)	-	1(1)	1(1)
Misshapen clavicles	1(1)	-	-	-
Forelimb flexure	1(1)	-	-	-
Hindlimb talipes/malrotation	2(2)	-	1(1)	1(1)
Short/thickened long bones	1(1)	-	-	-
Polydactyly	1(1)	-	-	-
Conjoined twins	1(1)	-	-	-
Mottled fetus	1(1)	-	-	-
Squat fetus syndrome	3(1)	10(4)	-	-

Table 4. Comparison of control and test group data for Crl:CD(SD) and Crl:CD(SD)IGS fetal abnormalities at autopsy

	Crl:CD(SD)		Crl:CD(SD)IGS	
	In-house mated	Supplier mated	In-house mated	Supplier mated
Total fetuses(litters) examined [a]	12348(845)	12950(980)	12016(837)	12536(1007)
Squat fetus syndrome	21(6)	33(10)	-	1(1)
Mottled fetus syndrome	6(2)	11(4)	-	-
Anury [b]	2(2)	2(2)	5(5)	19(17)

a) Test group excluded where these findings may be due to treatment

b) Identified as termination of vertebral column above 1st caudal at skeletal examination

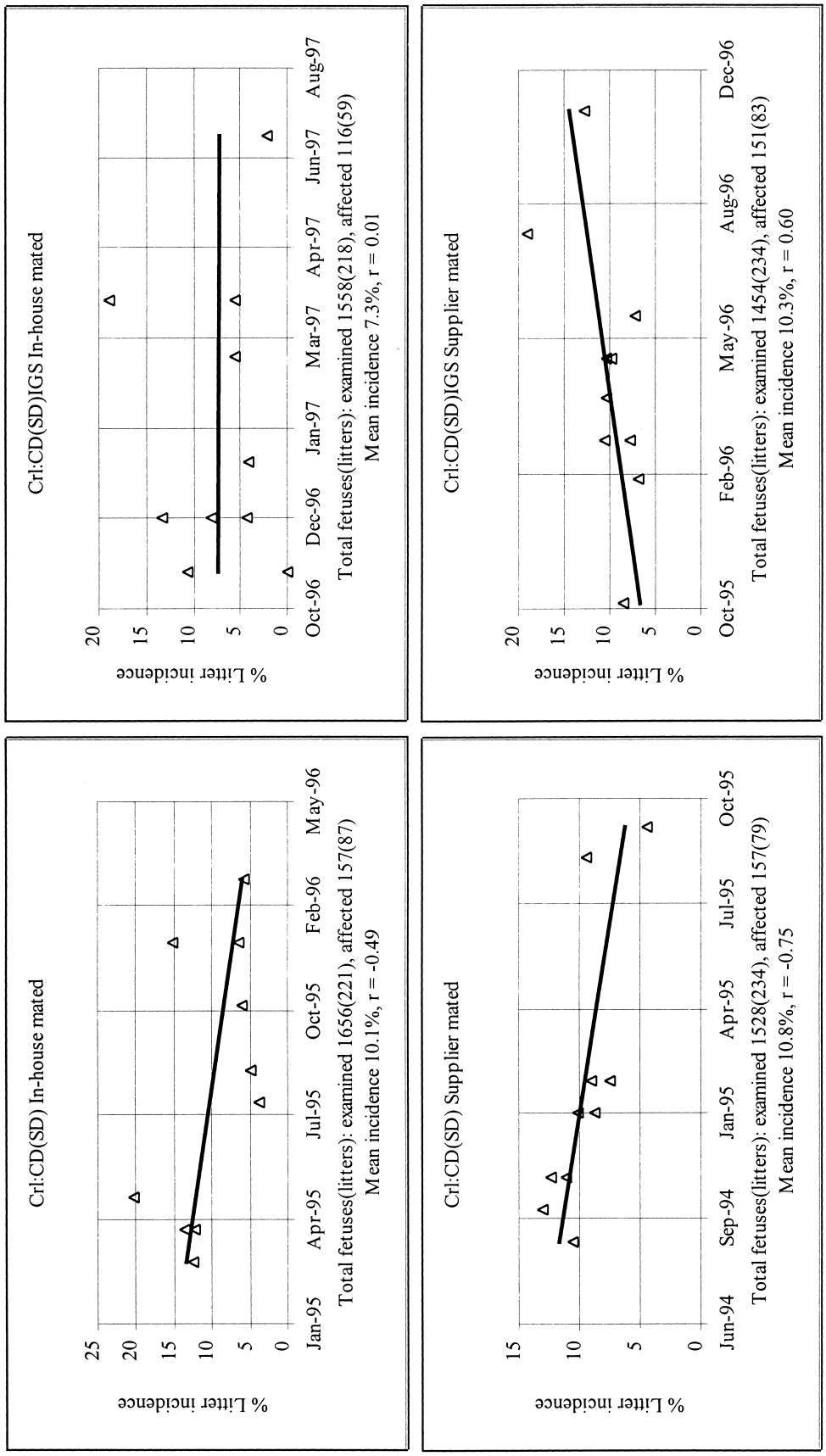

Fig. 3. Comparison of control group data for Crl:CD(SD) and Crl:CD(SD)IGS rat 14th rib observations

Table 5. Results of statistical analysis

	Crl:CD(SD) v Crl:CD(SD)IGS	
	In-house mated	Supplier mated
Table 2:		
Day 20 maternal body weight	p=0.001	p<0.001
Corpora lutea	NS	p=0.002
Implantations	NS	p=0.016
Fetal weight	p=0.012	NS
In utero death early	NS	p=0.012
In utero death total	NS	p=0.005
Table 3: Fetal incidence:		
Retinal/lenticular irregularities	p=0.025	-
Thickened/kinked ribs	NS	p=0.048
Termination vertebral column	-	p=0.048
Squat fetus syndrome	NS	p=0.001
Table 4: Fetal incidence :		
Squat fetus syndrome	p<0.001	p<0.001
Mottled fetus syndrome	p=0.015	p<0.001
Anury	NS	p<0.001
Litter incidence:		
Squat fetus syndrome	p=0.014	p=0.005
Anury	NS	p<0.001

NS Not significant

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Comparison of Reproductive and Developmental Parameters between Crj:CD (SD) rat and Crj:CD (SD) IGS rats

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ABSTRACT. In order to obtain background data for reproduction studies using Crj:CD (SD) IGS rats which were developed by Charles River Inc., we conducted a study under the same schedule as a single study design in Guidelines for Toxicity Studies of Drugs to obtain various reproductive / developmental parameters. The data were compared with those of Crj:CD (SD) rats.

Various parameters on reproduction and developmental toxicity obtained from Crj:CD (SD) IGS rats were almost the same as those from Crj:CD (SD) rats and there were no big differences between Crj:CD (SD) IGS rats and Crj:CD (SD) rats. —Key words: Crj:CD (SD) IGS rats, Reproductive and developmental parameters

CD (SD) IGS-1998: 206-213

INTRODUCTION

The gold standard system has been developed by Charles River Inc. to cope with the internationalization of research and development of new pharmaceutical compounds. We conducted a study in order to obtain reproductive and developmental background data on Crj:CD (SD) IGS rats and the data obtained from them were compared to those from Crj:CD (SD) rats.

MATERIALS AND METHODS

Twenty male and 40 female Crj:CD (SD) IGS rats were obtained at 5 weeks of age from Charles River Inc. The animals were housed in an animal room which was maintained at a temperature of 23±3°C 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 except for the mating period. The females assigned for delivery were housed in plastic Econ cages with bedding from day 17 of gestation until weaning and weanlings were housed 2 animals of the same sex per litter in the same wire mesh cages. Pellet diet (NMF: Oriental Yeast, Co., Ltd.) and tap water were provided ad libitum.

Males were weighed once a week. Females were weighed once a week prior to mating and during the mating period, on days 0, 4, 7, 11, 14, 17 and 20 of gestation, and on days 0, 4, 7, 11, 14, 17 and 21 of lactation. The estrous cycles of all females were examined daily by the vaginal smear method for 2 weeks prior to mating. Females were paired with males on a one-to-one basis at 11 weeks of age for a maximum of 2 weeks. The day on which the presence of vaginal plugs or sperm in the vaginal smear was observed was designated as day 0 of gestation. The indices of copulation, insemination and fertility were determined. Males were sacrificed by exsanguination under ether anesthesia after completion of delivery. The testes and epididymides were weighed and the testes and the left epididymis were fixed and preserved in Bouin's solution. The right epididymis was used for the sperm examination in which total numbers of sperm and numbers of immotile sperm, live and dead sperm, and sperm with abnormalities and the motility, survival index and the incidence of sperm with abnormalities were determined.

Females in the cesarean section group were sacrificed by exsanguination under ether anesthesia on day 20 of gestation. For pregnant animals, their ovaries and uterus were removed, and the numbers of the corpora lutea, implantations, the numbers of live fetuses and resorbed or dead fetuses were counted and the indices of implantation and resorbed or dead fetuses were determined. The live fetuses were examined for external abnormalities including the oral cavity. Their body weights were measured after sex determination. Approximately one half of the fetuses in each litter were fixed in Bouin's solution and examined for visceral abnormalities [1, 2]. Of the remaining half of the fetuses in each litter, clear skeletal specimens stained with Alizarin-red S were prepared and examined for skeletal abnormalities, variations and progress of ossification [3].

Females in the natural delivery group were allowed to deliver. They were observed for the presence or absence of abnormalities in delivery, and the duration of the gestation and the delivery index were determined. They were allowed to nurse their pups for 21 days after delivery and their nursing behavior was observed daily. They were necropsied on day 22 of lactation and the number of implantation sites was counted.

On the day of birth (day 0 of lactation), the numbers of live and stillborn pups were counted and live born pups were examined for sex and the presence or absence of external abnormalities. Body weight of nurslings was measured on days 0, 4, 7, 14 and 21 of lactation. On day 4 of lactation, the nurslings were culled to 8 pups (4 males and 4 females, as a rule). They were examined for physical development: pinna detachment on day 4 of lactation, abdominal hair growth and eruption of the lower incisors on days 11 and 14 of lactation and opening of the eyelids on days 14 and 17 of lactation. For the ontogeny of the sensory and reflex functions during the nursing period, 2 males and 2 females in each litter were examined for surface righting reflex on day 10 of lactation, air righting reflex on day 15 of lactation and pupillary, Preyer's and pain reflexes on day 21 of lactation. The stillborn index, birth index, survival index on days 4 and 21 of lactation were determined.

On day 21 of lactation, 2 males and 2 females from each litter

were selected for various examinations after weaning and the remainder were necropsied. After weaning, an open field test and a multiple water T-maze test were conducted on 1 male and 1 female from each litter at 5 weeks of age and at 7 – 8 weeks of age, respectively. For the open field test, a Behavioral Tracing Analyzer (BTA-2A: Muromachi Kikai Co., Ltd.) was used and latency, the amount of ambulation and the numbers of rearing, grooming and defecation were recorded for 3 minutes once a day. For the water T-maze test, the animals were examined using Biel's water maze in 3 trials a day and the time taken to the goal and the number of errors were recorded. The reproductive performance of the weanlings was examined in 1 male and 1 female from each litter at 10 – 12 weeks of age. Males and females were housed together overnight on a one-to-one basis avoiding sibling mating. All females with confirmed copulation were weighed on days 0, 3, 7, 11 and 15 of gestation and necropsied by exsanguination under ether anesthesia on day 15 of gestation. For pregnant animals, the numbers of the corpora lutea, implantations, the numbers of live or dead embryos were counted. From the results of the reproductive performance, the indices of copulation, insemination, fertility, implantation and dead embryos were determined.

For statistical analyses, parametric data such as body weight were analyzed by Student's t-test or Aspin-Welch's t-test. Non-parametric data such as the indices regarding dams, fetuses and F1 generation were analyzed by the Wilcoxon rank sum method except that data such as copulation, fertility and sex ratio were analyzed by the Chi-square test. Statistical analyses were made between SD and IGS strain rats at two-tailed 5 and 1% levels of significance.

RESULTS AND DISCUSSION

Body weights of males are shown in Table 1. Body weights of IGS rats were significantly high in comparison with those of SD rats on days 0, 7 and 14 of the pre-mating period (6, 7 and 8 weeks of age) and body weight gain between day 0 and day 70 in IGS rats showed significantly high value. No significant differences between IGS rats and SD rats were noted in body weights on or after day 21 of the pre-mating period.

Table 1. Body weight (g) of males during the pre-mating and post-mating periods

Strain	SD	IGS
No. of animals	88	20
Pre-mating period		
Day 0 (6 weeks of age)	289.8 ± 12.7	316.3 ± 15.8**
7 (7 weeks of age)	342.9 ± 15.7	363.8 ± 23.5**
14 (8 weeks of age)	382.4 ± 21.0	401.6 ± 30.4*
21 (9 weeks of age)	416.7 ± 26.1	431.2 ± 35.2
28 (10 weeks of age)	446.8 ± 30.3	456.9 ± 41.3
Mating and post-mating periods		
Day 35 (11 weeks of age)	469.5 ± 33.2	468.8 ± 42.1
42 (12 weeks of age)	495.1 ± 35.2	492.6 ± 47.3
49 (13 weeks of age)	515.0 ± 38.7	507.5 ± 47.9
56 (14 weeks of age)	533.4 ± 38.7	528.4 ± 51.2
63 (15 weeks of age)	550.1 ± 42.4	546.5 ± 54.3
70 (16 weeks of age)	564.8 ± 44.4	558.6 ± 56.9
Body weight gain (day 0 – 70)	275.0 ± 37.8	242.3 ± 44.8**

Values represent mean ± S.D.
* : Significant difference from SD rats at $P<0.05$
** : Significant difference from SD rats at $P<0.01$

Testis and epididymis weights and the results of sperm examination are shown in Table 2. No significant differences between IGS rats and SD rats were noted in weights of testes or epidydimides. In the results of the sperm examination, there were no significant differences between IGS rats and SD rats on the number of sperm, survival index or incidence of sperm with abnormalities. Mortality of the sperm was significantly low in IGS rats. However, mortality of sperm in each male IGS rats was within the range of SD rats and it was considered that there was no big difference between IGS rats and SD rats on mortality of sperm.

Table 2. Testis and epididymis weights and results of sperm examination

Strain		SD	IGS
No. of animals		88	20
Absolute organ weights			
Testis (g)	Right	1.78 ± 0.15	1.72 ± 0.37
	Left	1.77 ± 0.14	1.72 ± 0.38
Epididymis(mg)	Right	700 ± 65	676 ± 127
	Left	686 ± 68	651 ± 122
Sperm examinations			
No. of sperm		456.7 ± 90.4	497.2 ± 65.9
Motility (%)		82.5 ± 3.7	80.1 ± 2.9*
Survival index (%)		80.6 ± 2.2	80.8 ± 2.9
Abnormalities (%)		0.3 ± 0.3	0.2 ± 0.4

Values represent mean ± S.D.
* : Significant difference from SD rats at P<0.05

Body weights of females in the pre-mating, gestation and lactation periods are shown in Table 3. No significant differences between IGS rats and SD rats were noted in body weights in the pre-mating, gestation or lactation periods. Body weight gains in the pre-mating and gestation periods of IGS rats were almost the same as those in SD rats and no significant differences from the SD rats were noted. Body weight gain in the lactation period in IGS rats showed a significantly high value.

Table 3. Body weight (g) of females during the pre-mating, gestation and lactation periods

Strain	SD	IGS	SD	IGS
	Cesarean section group		Delivery group	
No. of animals	88	20	88	20
Pre-mating period				
Day 0 (6 weeks of age)	255.0 ± 16.2	250.2 ± 18.6	274.3 ± 16.1	271.2 ± 24.7
7 (7 weeks of age)	265.5 ± 17.8	262.7 ± 20.9	282.9 ± 18.3	275.3 ± 26.6
14 (8 weeks of age)	277.4 ± 18.9	272.2 ± 23.2	290.5 ± 19.2	283.6 ± 28.5
Body weight gain (day 0 – 14)	22.4 ± 9.8	22.1 ± 8.5	16.2 ± 10.8	12.4 ± 8.2
Gestation period				
Day 0	284.0 ± 17.7	280.2 ± 24.6	296.6 ± 18.8	286.3 ± 30.6
4	306.9 ± 20.5	302.8 ± 25.0	319.9 ± 20.2	310.3 ± 31.3
7	318.4 ± 22.7	313.2 ± 25.1	331.6 ± 21.1	321.6 ± 32.2
11	338.7 ± 24.4	332.8 ± 26.4	352.0 ± 22.3	342.6 ± 34.7
14	353.2 ± 24.7	346.9 ± 26.8	365.7 ± 23.2	356.8 ± 32.9
17	383.6 ± 26.6	381.9 ± 29.8	396.5 ± 26.1	390.1 ± 38.4
20	435.0 ± 29.9	435.2 ± 33.6	443.9 ± 31.4	432.4 ± 41.3
Body weight gain (day 0 – 20)	150.9 ± 20.8	154.9 ± 16.2	147.4 ± 23.5	146.1 ± 21.7
Lactation period				
Day 0			346.2 ± 24.9	336.2 ± 31.7
4			354.2 ± 23.5	345.0 ± 31.5
7			359.8 ± 24.5	353.3 ± 33.4
11			365.1 ± 22.8	367.3 ± 31.0
14			367.7 ± 22.6	369.5 ± 33.9
17			363.6 ± 22.0	368.8 ± 31.1
21			346.6 ± 21.0	357.1 ± 30.7
Body weight gain (day 0 – 21)			0.4 ± 20.4	20.1 ± 15.6 **

Values represent mean ± S.D.
** : Significant difference from SD rats at P<0.01

Estrous cycle, the results of mating and fertility are shown in Table 4. Estrous cycle and indices of mating and fertility of IGS rats showed similar values in comparison with those of SD rats and there were no significant differences between IGS rats and SD rats in estrous count, duration of cycle, days until successful mating or indices of copulation or fertility. Findings at cesarean section are shown in Table 5. The numbers of corpora lutea and implantations and implantation index of IGS rats were similar to those of SD rats and no significant differences from the SD rat were noted. In parameters obtained from fetuses, there were no significant differences from SD rats in the number of live fetuses, mortality or sex ratio. Body weights of the fetuses in both sexes showed significantly high values in IGS rats in comparison with those in SD rats. Body

weights of the fetuses of both sexes in each dam of IGS rats were within the range of SD rats except the values of fetuses from 2 dams in IGS rats exceeded the upper limit of SD rats.

Table 4. Estrous cycle and results of mating and fertility in females

Strain	SD	IGS	SD	IGS
	Cesarean section group		Delivery group	
No. of animals	88	20	88	20
Estrous cycle				
Estrous count [a)]	3.3 ± 0.5	3.5 ± 0.5	3.3 ± 0.5	3.4 ± 0.6
Duration of cycle [a)]	4.2 ± 0.4	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.2
Mating and fertility				
Days until copulation [a)]	2.7 ± 1.9	3.1 ± 1.6	2.5 ± 1.3	2.4 ± 1.0
Copulation index (%)	97.7 (86/88)	95.0 (19/20)	97.7 (86/88)	90.0 (18/20)
Fertility index (%)	95.3 (82/86)	89.5 (17/19)	96.5 (83/86)	88.9 (16/18)

a) : Values represent mean ± S.D.

Table 5. Reproductive and fetal parameters obtained from cesarean section

Strain		SD	IGS
No. of animals		88	20
Data from dams			
No. of pregnant animals		82	17
No. of corpora lutea [a)]		17.1 ± 2.4	16.7 ± 1.9
No. of implantations [a)]		15.9 ± 2.2	15.9 ± 2.2
Implantation index (%)		93.4 ± 9.0	95.4 ± 9.2
Data from fetuses			
No. of live fetuses [a)]		14.9 ± 3.1	15.7 ± 2.2
Mortality (%)		7.0 ± 10.2	1.4 ± 2.6
Body weights (g)	males	3.61 ± 0.34	4.03 ± 0.23**
	females	3.41 ± 0.33	3.80 ± 0.22**
Sex ratio (males/females)		1.02	1.21

a) : Values represent mean ± S.D.
** : Significant difference from SD rats at $P<0.01$

Findings obtained from external, visceral and skeletal examinations of live fetuses are shown in Table 6. No fetuses with external abnormalities were observed in IGS rats. Fetuses with visceral abnormalities were observed in 10 out of 129 fetuses in IGS rats and the incidence (8.0%) of fetuses with visceral abnormalities in IGS rats was significantly higher than that in SD rats (1.5%). Although abnormalities observed in SD rats were abnormal origin of the artery from the aortic arch, ventricular septal defect and diaphragmatocele, the abnormality observed in IGS rats was ventricular septal defect. In visceral variations, no significant differences were noted between IGS rats and SD rats. No fetuses with skeletal abnormalities were noted in IGS rats. Incidence of fetuses with skeletal variations in IGS rats was significantly higher than that in SD rats and the skeletal variation observed in IGS rats was only 14th rib. In the progress of ossification, no significant differences from SD rats were noted in the metacarpi. However, the ossification in the 5th sternebra, metatarsi and sacral and caudal vertebrae showed significantly high values in IGS rats.

Table 6. External, visceral and skeletal examinations in live fetuses

Strain		SD	IGS
External abnormalities (%)		0.1 ± 0.7	0.0 ± 0.0
Visceral abnormalities (%)		1.5 ± 4.5	8.0 ± 9.8**
Abnormal origin of left carotid artery		0.2 ± 1.4	0.0 ± 0.0
Anomaly of right subclavian artery		0.3 ± 1.8	0.0 ± 0.0
Ventricular septal defect		0.9 ± 3.2	8.0 ± 9.8**
Diaphragmatocele		0.3 ± 1.9	0.0 ± 0.0
Visceral variations (%)		16.0 ± 18.2	10.7 ± 14.2
Thymic remnant in neck		14.0 ± 17.1	10.7 ± 14.2
Double azygos vein		0.3 ± 1.9	0.0 ± 0.0
Dilatation of renal pelvis		1.0 ± 4.9	0.7 ± 2.7
Left umbilical artery		1.4 ± 4.3	0.0 ± 0.0
Skeletal abnormalities (%)		0.3 ± 1.9	0.0 ± 0.0
Bifurcation of rib		0.2 ± 1.4	0.0 ± 0.0
Decreased number of ribs and thoracic vertebrae		0.2 ± 1.4	0.0 ± 0.0
Skeletal variations (%)		5.2 ± 10.2	9.8 ± 11.8*
Cervical rib		1.5 ± 5.0	0.0 ± 0.0
Wavy ribs		0.1 ± 1.1	0.0 ± 0.0
Shortened 13th rib		0.2 ± 2.2	0.0 ± 0.0
14th rib		2.5 ± 7.7	9.8 ± 11.8**
Splitting of thoracic vertebral body		0.5 ± 2.8	0.7 ± 3.0
Sacralization of lumbar vertebra		0.3 ± 1.8	0.0 ± 0.0
Ossifications			
Sternebrae	1st	99.7 ± 1.9	100.0 ± 0.0
	2nd	98.7 ± 6.2	100.0 ± 0.0
	3rd	99.7 ± 1.9	100.0 ± 0.0
	4th	99.3 ± 3.0	100.0 ± 0.0
	5th	87.1 ± 17.1	95.8 ± 9.6*
	6th	97.9 ± 10.0	100.0 ± 0.0
Metacarpi	right	4.0 ± 0.0	4.0 ± 0.0
	left	4.0 ± 0.1	4.0 ± 0.0
Metatarsi	right	4.4 ± 0.3	4.9 ± 0.2**
	left	4.4 ± 0.3	4.9 ± 0.1**
Sacral and caudal vertebrae		7.9 ± 0.7	8.2 ± 0.5*

a) : Values represent mean ± S.D.
* : Significant difference from SD rats at P<0.05
** : Significant difference from SD rats at P<0.01

Delivery data on F0 dams and examination of F1 pups are shown in Table 7. No significant differences were noted in delivery index, the numbers of implantation and liveborns, incidence of stillborn or live birth index in IGS rats and SD rats except that the gestation period was significantly shorter in IGS rats than in SD rats. However, the differences in the gestation period between IGS rats and SD rats were slight and the gestation period of each dam in IGS rats was within the normal range of SD rats. Regarding the data from pups, no newborns with external abnormalities were observed in IGS rats or no significant differences from SD rats were noted in the indices of viability on day 4 or weaning on day 21 in IGS rats.

Table 7. Delivery data on F0 dams and examinations of F1 pups

Strain	SD	IGS
No. of pregnant animals	88	16
Data from dams		
No. of dams with live pups	78	16
Delivery index (%) [a)]	96.3	100.0
Gestation period	22.3 ± 0.3	22.0 ± 0.4**
No. of implantations	16.7 ± 2.1	16.7 ± 2.2
Incidence of stillborn [b)]	3.0 ± 5.1	4.7 ± 6.7
No. of liveborns	15.0 ± 2.1	14.6 ± 2.5
Live birth index (%) [c)]	90.2 ± 8.5	87.4 ± 10.2
Data from pups		
Sex ratio (males/females)	0.99	0.97
External abnormalities(%) [d)]	0.0 ± 0.0	0.0 ± 0.0
Viability index on day 4 [e)]	97.5 ± 5.6	92.1 ± 19.7
Weaning index on day 21 [f)]	98.1 ± 7.0	90.6 ± 27.2

Values represent mean ± S.D.
a) : (No. of dams with live pups / No. of pregnant animals) x 100
b) : (No. of stillborn pups / No. of stillborn pups and live born pups) x 100
c) : (No. of live born pups / No. of implantations) x 100
d) : (No. of live born pups with external abnormalities / No. of live born pups) x 100
e) : (No. of live pups on day 4 / No. of live born pups) x 100
f) : (No. of live pups on day 21 / No. of live pups on day 4 after culling) x 100
** : Significant difference from SD rats at P<0.01

Body weight of F1 rats during the lactation period and the period after weaning is shown in Table 8. Body weight of IGS rats tended to be lower than that of SD rats, with significant differences in males on day 0 and in both sexes on day 4. Physical developments and reflex responses are shown in Table 9. In IGS rats, significantly low values were observed for physical development in pinna detachment on day 4, appearance of the abdominal hair and eruption of the lower incisor on day 11 and opening of the vagina on day 35. However, physical development for appearance of the abdominal hair and on eruption of the lower incisor on day 14 and opening of the vagina on day 42 were completed in all F1 rats. No significant differences between IGS rats and SD rats were noted in righting reflex, air righting reflex, pupillary reflex, preyer's reflex or pain reflex.

Table 8. Body weight (g) of F1 during lactation period and period after weaning

Strain	SD		IGS	
Sex	Males	Females	Males	Females
Day 0 (at birth)	6.7 ± 0.4	6.3 ± 0.4	6.2 ± 0.4**	6.0 ± 0.6
4	10.1 ± 1.0	9.5 ± 1.0	8.7 ± 1.8**	8.4 ± 1.8*
7	16.6 ± 1.8	15.7 ± 1.7	15.2 ± 1.7	14.6 ± 2.0*
14	31.4 ± 4.8	29.8 ± 4.8	31.9 ± 3.3	30.9 ± 2.9
21	51.4 ± 7.6	49.0 ± 7.3	50.0 ± 4.5	47.7 ± 4.5
28	94.2 ± 12.1	86.4 ± 11.6	90.0 ± 11.0	82.1 ± 6.7
35	156.5 ± 16.7	134.7 ± 14.5	147.9 ± 20.5	129.9 ± 8.2
42	223.5 ± 20.7	174.1 ± 16.8	213.0 ± 23.6	168.9 ± 11.5
49	290.7 ± 24.8	203.6 ± 18.1	278.9 ± 31.5	198.2 ± 14.3
56	352.3 ± 27.5	230.4 ± 20.5	336.3 ± 39.7	225.9 ± 18.3
63	399.7 ± 30.3	254.2 ± 21.2	377.0 ± 38.7	248.6 ± 23.0
70	442.2 ± 33.7	274.2 ± 23.6	413.5 ± 39.2	267.4 ± 22.0

Values represent mean ± S.D.
* : Significant difference from SD rats at P<0.05
** : Significant difference from SD rats at P<0.01

Table 9. External differentiation and reflex responses of F1 animals

Strain		SD		IGS	
External differentiation					
No. of litters		78		16	
Pinna detachment (%)	Day 4	100.0 (1140/1140)		98.6 (212/215) **	
Appearance of	11	89.7 (551/614)		1.7 (2/118) **	
abdominal hair (%)	14	100.0 (613/613)		100.0 (117/117)	
Eruption of lower incisor (%)	11	62.5 (384/614)		11.0 (13/118) **	
	14	99.8 (612/613)		100.0 (117/117)	
Opening of eyelids (%)	14	42.3 (259/613)		49.8 (58/117)	
	17	100.0 (610/610)		100.0 (116/116)	
Opening of vagina (%)	35	100.0 (156/156)		90.0 (27/30) **	
	42	100.0 (156/156)		100.0 (30/30)	
Cleavage of	49	100.0 (66/66)		96.7 (29/30)	
the balanopreputial gland (%)	56	100.0 (66/66)		96.7 (29/30)	
Reflex responses / Sex		Males	Females	Males	Females
No. of animals		66	66	30	30
Righting reflex (sec.)	Day 10	2 ± 2	2 ± 2	1 ± 1	1 ± 0
Air righting reflex (%)	15	65.2 (43/66)	60.6 (40/66)	90.0 (27/30)	93.3 (28/30)
Pupillary reflex (%)	21	100.0 (66/66)	100.0 (66/66)	100.0 (30/30)	100.0 (30/30)
Preyer's reflex (%)	21	100.0 (66/66)	100.0 (66/66)	100.0 (30/30)	100.0 (30/30)
Pain reflex (%)	21	100.0 (66/66)	100.0 (66/66)	100.0 (30/30)	100.0 (30/30)

Values represent mean ± S.D.
* : Significant difference from SD rats at P<0.05
** : Significant difference from SD rats at P<0.01

The results of the open field test and water-filled multiple T-maze test in F1 animals are shown in Table 10. In the open field test, ambulation and rearing showed significantly low values in males of IGS rats in comparison with those of SD rats. However, these values in females of IGS rats were almost the same as those of SD rats. In the water-filled multiple T-maze test, no significant differences between IGS rats and SD rats were noted in the time required to reach the goal or the number of errors in each of 3 trials for 3 days.

Table 10. Open field test and water-filled multiple T-maze test in F1 animals

Strain			SD		IGS	
Sex			Males	Females	Males	Females
Open field test						
No. of animals			76	78	15	15
Latency (sec.)			6.3 ± 6.4	6.5 ± 9.7	9.9 ± 10.5	6.8 ± 5.8
Ambulation (cm)			1548 ± 395	1796 ± 417	1280 ± 429*	1620 ± 283
Rearing			6.6 ± 3.3	7.0 ± 3.3	4.7 ± 2.5*	7.7 ± 3.7
Grooming			0.3 ± 0.6	0.3 ± 0.5	0.1 ± 0.3*	0.1 ± 0.4
Defecation			1.9 ± 1.4	1.2 ± 1.3	1.6 ± 1.5	0.9 ± 0.7
Water-filled multiple T-maze test						
No. of animals			76	78	15	15
Time (sec.)	Day 1	Trial 1	56.3 ± 29.4	63.7 ± 34.4	58.0 ± 23.4	46.1 ± 17.3
		2	54.2 ± 29.5	51.0 ± 28.2	43.2 ± 22.4	41.9 ± 31.1
		3	42.3 ± 24.4	38.3 ± 22.2	44.3 ± 36.1	41.1 ± 21.4
	Day 2	Trial 1	30.4 ± 16.5	28.1 ± 13.3	25.1 ± 13.5	52.0 ± 45.4
		2	23.5 ± 10.0	26.6 ± 15.2	21.3 ± 6.9	30.1 ± 14.7
		3	23.2 ± 11.7	23.4 ± 15.0	20.2 ± 7.3	33.0 ± 28.0
	Day 3	Trial 1	23.2 ± 12.0	23.2 ± 9.8	20.3 ± 5.5	21.1 ± 7.3
		2	20.1 ± 8.2	26.0 ± 12.2	15.6 ± 2.7	21.3 ± 11.8
		3	20.0 ± 8.1	25.6 ± 16.6	19.6 ± 9.3	22.8 ± 7.8
Error	Day 1	Trial 1	3.6 ± 1.8	3.5 ± 2.1	4.3 ± 1.3	2.7 ± 1.2
		2	3.1 ± 1.8	3.2 ± 2.0	2.8 ± 1.4	2.5 ± 1.6
		3	2.4 ± 2.0	2.3 ± 1.9	2.5 ± 1.6	2.0 ± 1.6
	Day 2	Trial 1	1.9 ± 1.9	1.7 ± 1.5	1.5 ± 1.3	3.5 ± 3.7
		2	1.1 ± 1.2	1.4 ± 1.6	1.1 ± 0.5	1.7 ± 1.3
		3	0.9 ± 1.4	0.9 ± 1.4	0.7 ± 0.7	2.0 ± 2.8
	Day 3	Trial 1	0.9 ± 1.2	0.8 ± 1.2	0.5 ± 0.7	0.8 ± 0.9
		2	0.5 ± 0.8	0.8 ± 1.0	0.3 ± 0.6	0.9 ± 1.8
		3	0.4 ± 0.7	0.7 ± 1.1	0.5 ± 0.8	0.7 ± 1.2

Values represent mean ± SD

The results of mating and fertility in F1 animals are shown in Table 11. Days until successful mating and indices of copulation and fertility of IGS rats were almost the same as those of SD rats and no significant differences were noted in any parameters.

Reproductive and embryo parameters in F1 animals are shown in Table 12. There were no significant differences between IGS rats and SD rats in the numbers of corpora lutea, implantations, dead embryos or live embryos or implantation index.

Table 11. Results of mating and fertility in F1 animals

Strain	SD	IGS
No. of pairs	78	15
Days until copulation[a)]	3.4 ± 2.5	3.1 ± 1.5
Copulation index (%)	96.2 (75/78)	100.0 (15/15)
Fertility index (%)	93.3 (70/75)	93.3 (14/15)

a) : Values represent mean ± S.D.

Table 12. Reproductive and embryo parameters in F1 animals

Strain	SD	IGS
No. of pregnant animals	70	14
No. of corpora lutea	18.0 ± 3.0	17.1 ± 2.1
No. of implantations	16.8 ± 3.2	16.6 ± 2.0
Implantation index (%)	93.2 ± 10.1	97.2 ± 3.8
No. of dead embryos	6.7 ± 6.8	2.8 ± 3.4
No. of live embryos	15.7 ± 3.3	16.1 ± 2.2

Values represent mean ± S.D.

From above results, although significant differences were noted in some parameters between Crj:CD (SD) IGS rats and Crj:CD (SD) rats, various parameters on reproduction and developmental toxicity obtained from Crj:CD (SD) IGS rats were almost the same as those from Crj:CD (SD) rats and there were no big differences between Crj:CD (SD) IGS rats and Crj:CD (SD) rats. However, we need to obtain more data on Crj:CD (SD) IGS rats in order to conduct reproduction studies using the rats because the sample size of the rats was too small in this experiment.

ACKOWLEDGMENTS: We thank the members of the reproduction section in the Gotemba Laboratory of Bozo Research Center Inc.

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Effects of Food Restriction on Developmental Toxicity Study in Crj:CD(SD)IGS Rats.

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ABSTRACT. The effects of restricted food intake on both dams and fetal development during organogenesis (days 7 to 17 of gestation) were studied using pregnant Crj:CD(SD)IGS rats. Two groups of animals comprised restricted feeding groups : one restricted to 60% of the control-group food amount (100%), the other 30%. Actual food consumption values for these groups were 56.3% and 29.0%, respectively. The control group was fed ad libitum. Seven to eight pregnant animals were used in each group. The effect of food restriction on dams was marked decrease in body weight in both 60% and 30% feeding groups. However, there were no deaths in either group, nor were there effects on maintenance of gestation such as increases in spontaneous abortion or premature delivery. Gross examination revealed no effects of food restriction on the number of corpora lutea, implantations, live fetuses and dead embryos, implantation loss, or sex ratio. External, visceral and skeletal examinations of fetuses showed no increase of anomalies or variations that were attributable to food restriction. From these results, it was concluded that effects of food restriction down to 30% of control amounts produced marked decreases in maternal body weight but did not influence abortion or premature delivery rates, or induce fetal malformation or death. —Key words: Crj:CD(SD)IGS Rats, Food restriction, Development

CD (SD) IGS-1998: 214-218

INTRODUCTION

In reproductive and developmental toxicity studies, some test substances may cause reduced food intake accompanied by suppressed body weight gain. These changes, in turn, may induce reproductive, physiologic or embryologic changes especially when pregnant animals are malnourished during the period of organogenesis. Such undernourishment may result in significantly retarded fetal development or growth. To properly evaluate the toxicity of a test substance, it is important to determine whether the changes observed are produced directly by the test substance or secondarily by malnourishment induced by the test substance. In the present study, Crj:CD(SD)IGS rats bred at Charles River Japan Inc. using a newly established system were used. The effect of restricted food intake on dams and fetal development during the period of organogenesis were investigated.

MATERIALS AND METHODS

Animals and maintenance: Fifteen male and thirty female SD [Crj:CD(SD)IGS] rats were obtained from Charles River Japan Inc. (Tsukuba Breeding Center). After quarantine and acclimatization for more than one week, healthy rats were paired day and night at a ratio of 1 male to 2 females. The females were examined for evidence of copulation every morning. Dams, which had a vaginal plug or sperm in their vaginal smears were considered at day 0 of gestation and used in the experiment. Seven to eight animals were assigned to each group using a stratified randomization method based on body weight measured on day 0 of gestation. The age at the start of mating was 12 weeks old for both sexes, and body weight of dams on day 0 of gestation ranged from 238 g to 294 g.

Animals were kept in an animal room set at a temperature of 22 ± 2°C, a relative humidity of 55 ± 15 % with 12 air changes/hour, and lit for 12 hours per day (7:00 - 19:00). The animals were individually housed in polycarbonate cages (265W x 426D x 200Hmm, Tokiwa Kagaku Kikai Co., Ltd.) lined with bedding (Beta Chip, Charles River Japan Inc.) . The animals were fed with autoclave-sterilized pelleted food (CRF-1, Oriental Yeast Co., Ltd.) and given tap water that had been passed through a 5 μm filter and UV-irradiated.

A daily food consumption average was calculated from the historical control data from this laboratory. The amount of food given to the restricted feeding groups was set at 60% and 30% of the historical control food consumption. The control group was fed ad libitum. The period of food restriction corresponded to the period of organogenesis, i.e. from day 7 to 17 of gestation. Beginning the morning of day 18 of gestation, all groups were fed ad libitum.

Group formation:

Group	Amount of normal food intake(%)	Dams
Control	100[1)]	8
60% feeding	60	7
30% feeding	30	8

1) The control value was regarded as 100 percent.

Examination method: *Observation of dams*: The animals were observed for clinical signs once daily. Body weight was recorded on day 0 and daily from day 7 to 20 of gestation. Body weight gains were calculated using the body weight on day 7 of gestation as the initial value and body weights for each day measured. Food consumption was determined on days 0, 7, 10, 14, 17 and 20 of gestation for the control group, and the consumption per day was calculated. In addition, the actual ratio of food consumed in the 60% and 30% feeding groups to the food consumed by the control group was calculated and expressed as a percentage.

The dams were sacrificed by exsanguination from the abdominal aorta under sodium thiopental anesthesia on day 20 of gestation. Postmortem examination of dams was performed macroscopically on thoracic and abdominal organs and tissues.

Observation of embryos and fetuses: After sacrifice on day 20

of gestation, ovaries and uterus were removed and examined for the numbers of corpora lutea, implantations, live fetuses, early dead embryos and late dead embryos. In addition, pre-implantation loss [{(number of corpora lutea - number of implantations)/number of corpora lutea}x100], post-implantation loss [{(number of implantations - number of live fetuses)/ number of implantations}x100], and total implantation loss [{(number of corpora lutea - number of live fetuses)/number of corpora lutea}x100] were calculated.

Live fetuses were examined for sex and external anomalies including in the oral cavity, and individually weighed. About half the male and female live fetuses in each litter were fixed with acetone, and used for skeletal examination. The remaining fetuses were fixed in a mixture of formalin and acetic acid solution and used for visceral examination.

Fetuses used in visceral examination were examined for cranial anomaly by Wilson's method [1] and for thoracic and abdominal anomalies by the microdissection method [2]. Fetuses used in skeletal examination were stained with alizarin red S [3] then examined for skeletal anomalies and variations. They were also examined for degree of ossification of the cervical and sacro-caudal vertebral bodies, sternebrae, and bones of the fore and hind limbs.

Statistical analysis: The fetal data were analyzed based on mean litter values. Data were tested for homogeneity of variance by Bartlett's test. When the variance was homogenous, a one-way analysis of variance was performed; when not homogenous, Kruskal-Wallis test was used. When significant inter-group differences were found, data were analysed by Dunnett's test or Dunnett's type multiple comparison. For embryonic and fetal mortality and incidences of external, visceral and skeletal anomalies and variations, however, Kruskal-Wallis analysis was first applied. Enumerated data were analyzed by Fischer's exact probability test.

RESULTS

*Effects on dam*s: *Clinical signs*: No death occurred in any of the restricted feeding groups. No changes in clinical signs were noted either.

Body weights: Figs. 1 and 2 and Table 1 show changes in body weight. Significant decreases in body weight and body weight gain were noted soon after the start of food restriction in both the 60% and 30% feeding groups. The decreases persisted to the day of cesarean section. Body weight gain in these groups decreased on each day measured during the period of food restriction, but after the end of this period body weight gains increased markedly.

The percent decrease in body weight compared to the control group in each restricted feeding group was highest on day 18 of gestation when food restriction ended, being 18.7% in the 60% feeding group and 31.6% in the 30% feeding group. The average percent decrease in body weight during the food restriction period was 14.2% in the 60% feeding group and 22.3% in the 30% feeding group.

Food consumption: The actual food restriction percentage to the control group was 56.3% in the 60% feeding group and 29.0% in the 30% feeding group, almost achieving the targeted amount of food consumption.

Necropsy: No abnormalities were noted in the control, 60% feeding or 30% feeding group.

Effects on embryos and fetuses: *Observation at cesarean section*: Table 2 shows the findings at cesarean section. A trend towards decreased fetal body weight was observed in male and female live fetuses in the 30% feeding group. However, the number of corpora lutea, implantation sites, live fetuses and dead embryos, and implantation loss did not differ significantly between the control and restricted feeding groups. The sex ratio in the 30% feeding group differed significantly from that in the control group. External examination of fetuses showed one fetus with gastroschisis without eventration in the 30% feeding group. The other fetuses showed no anomalies.

Visceral and skeletal examination of fetuses: Tables 3 and 4 show the results of the visceral and skeletal examination of fetuses. Visceral examination showed only one fetus with a ventricular septal defect in the control group. Visceral variation was observed in thymic remnant in the neck, left umbilical artery and dilatation of renal pelvis. The incidences of these variations in the restricted feeding groups were not statistically significant when compared with those in the control group.

Skeletal examination showed the following anomalies: absence of lumbar vertebral arches in one fetus of the control group and malformation complex (absence, fusion or bifurcation of the ribs and hemivertebra) in one fetus of the 30% feeding group. Skeletal variations included occlusion of the closure of transverse foramen of one or more cervical vertebrae arches, splitting of ossification centers of the thoracic vertebral bodies and 14 ribs, but their incidences were not significantly higher than those in the control group. Examination for degree of ossification in the 30% feeding group showed a trend towards delayed ossification in all of bones used as indexes.

DISCUSSION

Significant effects due to restricted food intake during pregnancy on dams, fetuses or offspring have been reported elsewhere[4)-9)]. When pregnant animals were undernourished during the period of organogenesis, effects secondary to undernourishment may be expected in fetuses. In the present study, 12-week-old Crj:CD(SD)IGS rats were undernourished from day 7 to 17 of gestation, and effects of restricted food intake on dams and embryonic and fetal development were investigated. Two groups of animals were established in which food was restricted to 60% and 30% of the control food consumption (100%). The actual food consumption values for these groups were 56.3% and 29.0%, respectively, of the food consumed by the control group.

The effect on dams was marked decrease in body weight in the 60% and 30% feeding groups. The greatest percentage decrease in body weight from the control group was 18.7% in the 60% feeding group and 31.6% in the 30% feeding group. Neither death nor any other abnomalities related to the maintenance of gestation such as increases in spontanous abortion or premature delivery occurred in any of the restricted

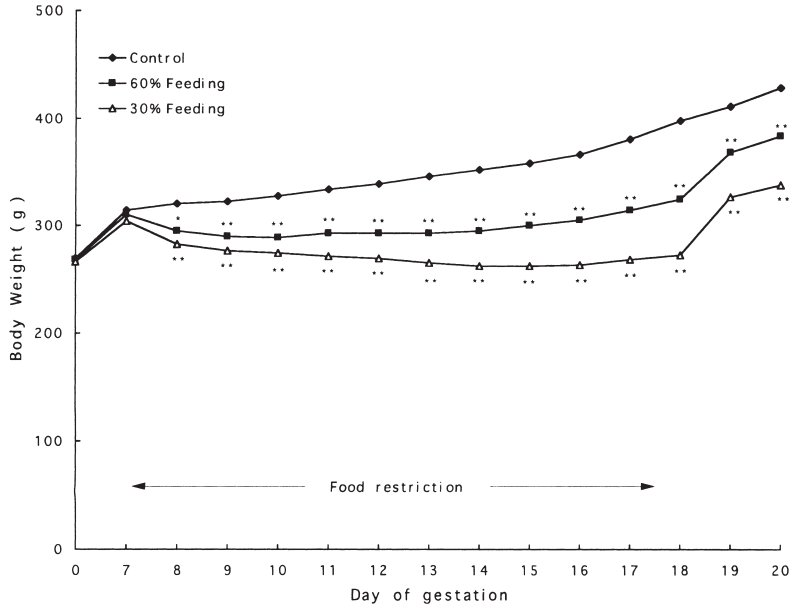

Fig. 1. Body weight changes following restricted feeding during gestation

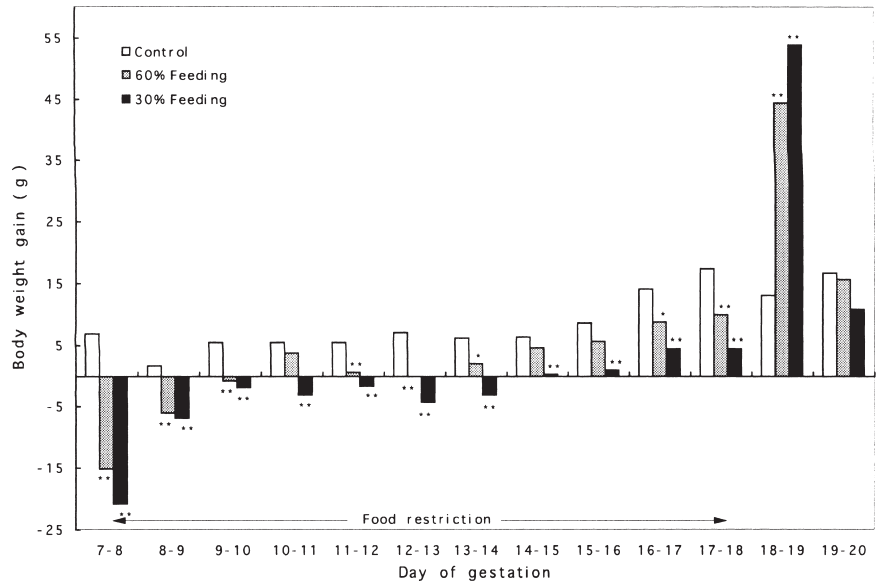

Fig. 2. Body weight gain at intervals of each measured days

feeding groups.

Examination at cesarean section revealed no influence of food restriction on the number of corpora lutea, implantation sites, live fetuses and dead embryos, implantation loss or sex ratio. External, visceral and skeletal examinations of fetuses revealed no food restriction-related increase in anomalies or variations. Although body weight of live fetuses and the degree of ossification showed a decrease, this trend was not statistically significant.

Ikemi et al.[6] previously reported that fetal body weight decreased, and suppression of fetal growth was suggested when pregnant rats were fed at 40% normal food intake, which was close to the 30% food amount used in the present study. In contrast, the present study showed in spite of slightly more severe experimental conditions fetal body weight was only slightly affected, suggesting only a slight suppression of fetal

Table 1. Effects of food restriction on body weight changes and food consumtion following restricted feeding during gestation in Crj:CD(SD)IGS rats

		Control	60% feeding	30% feeding
No. of dams		8	7	8
Body weight (g)	Day 7	313.9 ± 11.0	310.4 ± 21.4	303.6 ± 16.0
	Day 14	351.8 ± 15.4	295.0 ± 23.0 **	262.4 ± 15.2 **
	Day 18	398.3 ± 23.0	324.0 ± 20.7 **	272.5 ± 17.3 **
	Day 20	428.1 ± 30.5	384.1 ± 21.1 **	337.4 ± 17.4 **
Body weight gain (g)	Day 7 - 14	37.9 ± 7.9	-15.4 ± 9.8 **	-41.3 ± 6.7 **
	Day 14 - 18	46.5 ± 10.2	29.0 ± 3.7	10.1 ± 5.1 **
	Day 18 - 20	29.9 ± 9.2	60.1 ± 3.8 **	64.9 ± 6.0 **
Loss of	Day 14	16.1	25.4	
body weight (%)	Day 18	–	18.7	31.6
	Day 20	–	10.3	21.2
	Mean (Day 8 - 18)	14.2 ± 3.34	22.3 ± 6.53	
Percentage of food consumption (%)	Mean (Day 7 - 17)	–	56.3 ± 3.84	29.0 ± 1.22

Significantly different from control : *, P < 0.05 ; **,P < 0.01

Table 2. Observations at cesarean section of food restriction in Crj:CD(SD)IGS rats

	Control	60%-feeding	30%-feeding
No. of dams examined	8	7	8
No. of corpora lutea	16.9 ± 1.1[a)]	16.1 ± 2.2	16.5 ± 1.4
No. of implantations	14.9 ± 4.3	15.9 ± 2.0	15.1 ± 3.2
No. of deaths	0.4 ± 0.7	0.1 ± 0.4	0.6 ± 1.4
Early deaths	0.4 ± 0.7	0.1 ± 0.4	0.6 ± 1.4
Late deaths	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Pre-implantation loss (%)	11.84 ± 24.6	1.61 ± 2.78	8.91 ± 14.51
Post-implantation loss (%)	2.66 ± 5.5	0.76 ± 2.00	4.74 ± 10.82
Total implantation loss (%)	14.14 ± 25.1	2.33 ± 4.12	12.83 ± 19.11
No. of live fetuses	14.5 ± 4.4	15.7 ± 1.7	14.5 ± 3.9
Sex ratio (male/female)	0.61(44/72)	0.77(48/62)	1.00(58/58)*
Live fetal weight (g)			
Male	3.54 ± 0.28	3.48 ± 0.21	3.29 ± 0.35
Female	3.36 ± 0.2	3.30 ± 0.18	3.15 ± 0.33
No. of fetuses with external anomalies	0	0	1 (0.7)[b)]
Gastroschisis	0	0	1 (0.7)

Significantly different from control : *, P < 0.05 ; **,P < 0.01
a) Mean ± S.D.
b) Mean litter incidence (%)

Table 3. Visceral examination of fetuses with food restriction in Crj:CD(SD)IGS rats

	Control	60% feeding	30% feeding
Visceral examination			
No. of dams	8	7	8
No. of fetuses examined	61	58	60
No. of fetuses with any anomalies	5 (7.6)[a)]	7 (11.1)	8 (13.3)
Malformations			
Ventricular septal defect	1 (1.4)	0 (0.0)	0 (0.0)
Variations			
Thymic remnant in neck	1 (1.4)	0 (0.0)	6 (10.2)
Left umbilical artery	1 (2.1)	1 (1.6)	0 (0.0)
Dilatation of renal pelvis	2 (2.8)	6 (9.5)	3 (4.7)

a) Mean litter incidence (%)

Table 4. Skeletal examination of fetuses with food restriction in Crj:CD(SD)IGS rats

	Control	60% feeding	30% feeding
Skeletal examination			
No. of dams	8	7	8
No. of fetuses examined	55	52	56
No. of fetuses with any anomalies	1 (1.4)	0 (0.0)	1 (1.4)
Malformations			
Absence of lumbar vertebral arches	1 (1.4)	0 (0.0)	0 (0.0)
Absence of ribs	0 (0.0)	0 (0.0)	1 (1.4)
Fusion of ribs	0 (0.0)	0 (0.0)	1 (1.4)
Bifurcation of ribs	0 (0.0)	0 (0.0)	1 (1.4)
Hemivertebra	0 (0.0)	0 (0.0)	1 (1.4)
Variations			
Closure of transverse foramen of one or more cervical vertebral arches	4 (6.1)	2 (3.6)	1 (1.8)
Splitting of ossification centers of the thoracic vertebral bodies	0 (0.0)	0 (0.0)	2 (3.2)
14th ribs	3 (4.7)	0 (0.0)	3 (5.2)
Degree of ossification			
Cervical vertebrae	0.54 ± 0.43	0.51 ± 0.59	0.44 ± 0.59
Sternebrae	5.63 ± 0.40	5.50 ± 0.39	5.16 ± 0.76
Metacarpi	7.35 ± 0.68	7.20 ± 0.40	6.84 ± 0.64
Metatarsi	8.00 ± 0.00	8.00 ± 0.00	7.98 ± 0.07
Sacral and caudal vertebrae	7.81 ± 0.34	7.81 ± 0.27	7.64 ± 0.59

a) Mean litter incidence (%)
b) Mean ± S.D.

growth. However, under more severe conditions, as used by Ikemi et al. [6] such as fasting or feeding at 10% normal level, increased embryonic resorption, decreased body weight of live fetuses and degree of ossification and increase of visceral abnomalies were observed. In an experiment reported by Chapin et al. [8] in which animals were maintained so as to have body weight corresponding to 70% of that of control animals before mating through the gestation period, the number of embryonic resorptions did not increase. The influence of food restriction on embryos and fetuses, therefore, was thought to depend on maternal nutritional conditions related to the items controlled (i.e. body weight or food consumption) and the duration or stage of food restriction.

In conclusion, food restriction down to 30% of normal levels during the period of fetal organogenesis in pregnant rats did not increase embryonic resorption, fetal visceral and skeletal abnormalities or fetal deaths or anomalies.

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A Comparison study for embryo-fetal development of the Crj:CD (SD) IGS and Crj:CD (SD) rats

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ABSTRACT. We performed a study to collect fetal historical control data of the Crj:CD (SD) IGS rats, and compared with those of the Crj:CD (SD) rats in our laboratory. Pregnant Crj:CD (SD) IGS rats were euthanized on Day 20 of pregnancy and external, visceral and skeletal anomalies of fetuses were observed. No significant differences were observed in the number of corpora lutea, implantation, dead embryo/fetus and living fetus between the Crj:CD (SD) IGS rat group and the Crj:CD (SD) rat group. An average body weight of living fetuses in the Crj:CD (SD) IGS rat group significantly increased in comparison with that in the Crj:CD (SD) rats group. In the morphological observation of fetuses, slight increases in the incidence of the ventricular septal defect as visceral anomalies, lumbar rib as anatomical variation and the average number of ossified caudal vertebrae were observed in the Crj:CD (SD) IGS rat group in comparison with the Crj:CD (SD) rat group.

We found no clear differences between the Crj:CD (SD) rats and the Crj:CD (SD) IGS rats in the reproductive parameters. It is considered that Crj:CD (SD) IGS rats can be used in our teratogenicity studies. —Key words: Crj:CD (SD) IGS Rat, Teratogenicity, Fetal anomaly

CD (SD) IGS-1998: 219-222

INTRODUCTION

The gold standard system, a new animal breeding system, has been developed by Charles River, Inc. for supplying uniform experimental animals with minimizing the genetic variations. We performed a study to collect fetal historical control data of the Crj:CD (SD) IGS rats, and compared with those of the Crj:CD (SD) rats in our laboratory.

MATERIALS AND METHODS

Experimental Animals: Crj:CD (SD) IGS rats produced by Charles River Japan, Inc. were purchased at 8 weeks of age, male and female were cohabited at 1:1 at 10 weeks of age, and the day when the vaginal plug was confirmed was designated as day 0 of pregnancy. The number of pregnant animals actually used in the study was 27. Animals were housed in an animal room with barrier system controlled at 23 ± 2˚C, RH55 ± 10%, 13-hour illumination/day (6:00 to 19:00), about 200 luxes of intensity of illumination and frequency of ventilation 10 - 13 times/hr.

During the acclimatization period, females in groups of five were housed in an automatic washing bracket cage produced by Nippon Cage Co., Ltd., and males were housed individually in R-1 type bracket cages produced by Shin Toyo Seisakusho. During the copulation period, together one male and female each were put in a R-1 type bracket cage, and the female during pregnancy were individually housed in tapered bracket cages produced by Shin Toyo Seisakusho. Animals were fed with NMF solid diet radiosterilized with 30 kGy ^{60}Co- γ ray, produced by Oriental Yeast Co., Ltd. and tap water *ad libitum*.

Test Article and Method of Dosing: The route of administration was determined to be oral, and by using a metallic stomach tube for rats, 0.5 ml/100 g of body weight of 0.5 % CMC (carboxymethyl cellulose sodium) salt solution was successively given once a day for 11 days from Day 7 to 17 of pregnancy. The dosing volume was calculated from the body weight that scaled on the nearest day of administration.

Observation and Examination: The general conditions of dams were observed dairy. In all dams, on Day 0, 3, 7, 9, 11, 13, 15, 17 and 20 of pregnancy, body weights were measured with electron balances EB-3300DW, produced by Shimadzu Corporation. The solid diet was given on days of body weight measurement except on day 0 of pregnancy, the remaining amount was measured on the following day with electron balances of EB-3300DW. Food intake was obtained as the difference. All dams were euthanized on Day 20 of pregnancy by injection of pentobarbital sodium (Dainippon pharmaceutical Co., Ltd.) into the peritoneal viscera, and an autopsy was done on the thoracic/peritoneal viscera macroscopically. Thereafter, the number of corpus lutea, implantations, living fetuses, and dead embryos/fetuses were counted. The placenta was examined macroscopically. For apparently no implantation sites observed in the uterus, an implantation test originally described by Salewski[1] was performed to identify peri-implantation death embryo. After the living fetuses were examined in the presence or absence of abnormality of external appearance including the oral cavity and sex type with a stereoscopic microscope produced by Nippon Kogaku K. K., their body weights were measured with an EB-340DW electron even balance produced by Shimadzu Corporation. At least half of the fetuses in each litter were examined in the presence or absence of visceral abnormality by concomitant use of Nishimura's microscopic anatomical method[2] and the Wilson method[3]. Using the remaining fetuses, skeletal specimens were prepared according to the Dawson method[4] for examination in the presence or absence of skeletal abnormality.

Statistical Analysis: Results obtained from each examination were expressed as mean and standard error values or as percentages. For calculation of the mean values of body weights of living fetuses and the number of ossification centers of the fetal caudal vertebrae, a litter was treated as 1 calculation unit. Results were compared with the background data (0.5% CMC treated Crj:CD rats) of the teratogenicity studies in our laboratory. Differences in values between the Crj:CD (SD) rat group and Crj:CD (SD) IGS rat group were tested, according to

the prescribed program (BMDP-P3D and Sord), and when there was no difference in variance of the mean value, the student t-test was used, and when there was a difference in variance, Aspin- Welch's test was used. The incidence examined for the presence or absence of significant difference by the χ^2 -test at 1% level of significance. The values showing significant differences in the above tests were subjected further Mann-Whitney's rank sum test (BMDP-P3S), and only those that showed a significant difference in the last test at a 1% level of significance were judged as being significantly different. Regarding the sex ratio of living fetuses, the conformity was examined by the χ^2 -test, and the presence or absence of significant difference was judged at a 1% level of significance.

RESULT AND DISCUSSION

1. General Conditions, Body Weight and Food Intake in Dams

In general conditions, there were no abnormalities observed in any group.

Body weights of dams of the Crj:CD (SD) IGS and Crj:CD (SD) rat groups during the test period are shown in Table 1. In comparison with the Crj:CD (SD) rat group, Crj:CD (SD) IGS rat group showed a tendency of decrease in body weight gain, and significant differences were observed on Day 0, 3, 7, 15, 17 and 20 of pregnancy. Food intake of dams is shown in Table 2. In comparison with the Crj:CD (SD) rat group, significant decreases in food intake were observed on Day 3, 7, 9, 11 and 15 of pregnancy in the Crj:CD (SD) IGS rat group.

Table 1. Body weight changes in rat dams

Strain		Crj:CD (SD) IGS	Crj:CD (SD)
Number of pregnant females		27	125
Gestational day	0	247.0 ± 2.74 *	256.6 ± 1.25
	3	268.4 ± 2.58 *	278.6 ± 1.36
	7	289.4 ± 2.72 *	299.2 ± 1.55
	9	299.3 ± 2.86	308.7 ± 1.62
	11	312.1 ± 3.18	320.9 ± 1.68
	13	321.5 ± 3.04	330.9 ± 1.80
	15	333.0 ± 3.26 *	345.3 ± 1.90
	17	357.2 ± 3.64 *	370.5 ± 2.08
	20	407.2 ± 4.38 *	423.4 ± 2.58

Data are expressed as mean±S.E.
* Significant at 1% level compared with the Crj:CD (SD) rat group

Table 2. Food intake in rat dams

Strain		Crj:CD (SD) IGS	Crj:CD (SD)
Number of pregnant females		27	125
Gestational day	3	25.2 ± 0.35 *	26.9 ± 0.27
	7	26.0 ± 0.39 *	28.4 ± 0.28
	9	27.0 ± 0.37 *	28.9 ± 0.29
	11	26.9 ± 0.42 *	28.6 ± 0.26
	13	27.4 ± 0.36	28.9 ± 0.29
	15	26.4 ± 0.43 *	28.7 ± 0.30
	17	29.4 ± 0.59	30.9 ± 0.30
	20	29.1 ± 0.60	29.5 ± 0.27

Data are expressed as mean±S.E.
* Significant at 1% level compared with the Crj:CD (SD) rat group

2. Observation at Autopsy

The results of observation at autopsy of dams are shown in Table 3. Compared with the Crj:CD (SD) rat group, no significant differences in the number of corpora lutea, implantation, living fetus and dead embryo/fetus were observed in the Crj:CD (SD) IGS rat group. The body weight of living fetuses in the Crj:CD (SD) IGS rat group significantly increased in comparison with that of the Crj:CD (SD) rat group. In the sex ratios of living fetus, no significant difference was observed. No abnormality in the external appearance of fetuses was observed. In the macroscopic examination at autopsy of dams, no abnormality of thoracic/peritoneal organs was observed.

Table 3. Pregnancy status in rat dams

Strain		Crj:CD (SD) IGS	Crj:CD (SD)
Number of pregnant females		27	125
Number of corpora lutea	(Mean ± S.E.)	458 (17.0 ± 0.40)	2128 (17.0 ± 0.20)
Number of implantations	(Mean ± S.E.)	434 (16.1 ± 0.35)	1978 (15.8 ± 0.25)
Number of living fetuses	(Mean ± S.E.)	405 (15.0 ± 0.38)	1885 (15.1 ± 0.25)
Pre-implantation loss [1)]	(%)	24 (5.2)	150 (7.1)
Post-implantation loss [2)]	(%)	29 (6.7)	93 (4.7)
Body wight of fetuses	(Mean ± S.E.)	3.90 ± 0.029 *	3.65 ± 0.022
Sex ratio	(Male/Female)	1.23 (223 / 182)	1.01 (949 / 936)
Type and number of external malformations	(%)	0	6 (0.3) a)

1) $\frac{\text{(Number of corpora lutea - Number of implantations)}}{\text{Number of corpora lutea}} \times 100$

2) $\frac{\text{(Number of implantations - Number of living fetuses)}}{\text{Number of implantations}} \times 100$

a) Micro- or anophthalmia, edema, oligodactyly, anal atresia and brachyury : 1
Edema : 1
Kinky tail : 1
Anal atresia and brachyury : 1
Omphalocele : 2

* Significant at 1% level compared with the Crj:CD (SD) rat group

3. Observation of Fetuses

The results of visceral observation in the Crj:CD (SD) and Crj:CD (SD) IGS rat groups are shown in Table 4. As abnormal visceral appearances, 1 case of dilatation of latebral ventricle, 7 cases of ventricular septal defect, 8 cases of thymic remnant in neck and 1 case of supernumerary coronary orifice in the Crj:CD (SD) IGS rat group. In comparison with the Crj:CD (SD) rat group increase in the incidence of ventricular septal defect was observed in the Crj:CD (SD) IGS rat group. However, the incidence of visceral anomalies was not significantly difference between the Crj:CD (SD) rat group and Crj:CD (SD) IGS rat group. Dilatation of renal pelvis was observed in the Crj:CD (SD) rat group, but not observed in the Crj:CD (SD) IGS rat group.

The results of the skeletal observation in the Crj:CD (SD) and Crj:CD (SD) IGS rat groups are shown in Table 5. Skeletal anomalies were not observed in the Crj:CD (SD) IGS rat group. As anatomical variations, 3 cases of cervical rib, 2 cases of wavy rib, 13 cases of lumbar rib were observed in the Crj:CD (SD) IGS rat group. An increase in the incidence of lumbar rib was observed in the Crj:CD (SD) IGS rat group in comparison with the Crj:CD (SD) rat group. However, the incidence of anatomical anomalies was not significantly difference between the Crj:CD (SD) rat group and Crj:CD (SD) IGS rat group. Further, compared with the Crj:CD (SD) rat group, the average number of ossified caudal vertebrae in the Crj:CD (SD) IGS rat group was increased, but it was not significant.

Table 4. Visceral observation in fetuses derived from rat dams

Strain	Crj:CD (SD) IGS	Crj:CD (SD)
Number of fetuses examined	208	666
Type and number of malformations (%)		
Dilatation of lateral ventricle	1 (0.5)	1 (0.2)
Thymic remnant in neck	8 (3.8)	34 (5.1)
Vetricular septal defect	7 (3.4)	5 (0.8)
Supernumerary coronary orifice	1 (0.5)	1 (0.2)
Right subclavian artery arising from aortic arch	0	1 (0.2)
Abnormal origin of right subclavian artery	0	3 (0.5)
Vascular ring	0	1 (0.2)
Right aortic arch	0	1 (0.2)
Persistent left umbilical artery	0	8 (1.2)
Dilatation of renal pelvis	0	31 (4.7)
Situs inversus viscerum totalis	0	1 (0.2)

Table 5. Skeletal observation in fetuses derived from rat dams

Strain	Crj:CD (SD) IGS	Crj:CD (SD)
Number of fetuses examined	197	869
Malformations (%)		
Fusion of exoccipital bone and atlas	0	1 (0.1)
Hypoplasia of lumbar vertebral body and arch	0	1 (0.1)
Fusion and deformity of sternebrae	0	1 (0.1)
Variations (%)		
Cervical rib	3 (1.5)	19 (2.2)
Splitting of ossification centers of thoracic vertebral bodies	0	9 (1.0)
Wavy rib	2 (1.0)	0
Shotening of 13th rib	0	5 (0.6)
Lumbar rib	13 (6.6)	19 (2.2)
25 or 27 presacral vertebrae	0	9 (1.0)
Number of ossified caudal vertebrae (Mean ± S.E.)	4.32 ± 0.059	4.13 ± 0.042

We found that no clear difference between the Crj:CD (SD) rat and the Crj:CD (SD) IGS rat in the reproductive parameters. It is considered that the Crj:CD (SD) IGS rats can be used in our studies. However, we should continue to collect historical control data when Crj:CD (SD) IGS rats used in teratogenicity studies.

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Fertility and General Reproductive Performance of Crj:CD(SD)IGS Rats Fed a Low Protein Diet

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ABSTRACT. A low protein diet (containing 18% protein) was given to three lots of Crj:CD(SD)IGS rats, and the effects of this diet on fertility and general reproductive performance of parental animals and development of fetuses were studied. In parental animals, body weight gain in males fed the low protein diet was less than that in males fed commonly used diet (containing 24% protein). Food consumption values in males and females fed the low protein diet were higher than those in animals fed the commonly used diet. There were no significant differences between the control animals and those fed the low protein diet in copulation index, fertility index, estrous cycles, sperm characteristics, necropsy findings or organ weights. In fetal and placental observations, there were no significant differences in embryo/fetal mortality, number of live fetuses, sex ratio, fetal or placental weight, external, visceral or skeletal findings in live fetuses or morphologic features of placentae. Consequently, the use of this low protein diet poses no problems with regard to the evaluation of reproductive performance including the development of fetuses. However, if animals used in a reproductive toxicity study are fed this diet, the above-mentioned effects on the parental animals should be taken into consideration when interpreting the study results even though use of the diet does not seem to affect the results of fetal examinations. – Key words: Crj:CD(SD)IGS, Low protein diet

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INTRODUCTION

Laboratory animal diets are produced by food suppliers in accordance with the nutrient requirements for animal food recommended by the National Research Council (NRC). Recently, it has been shown that using a low protein (18%) diet does not result in nutritional problems and in fact lengthens the life span of animals by suppressing excessive body weight gains. Therefore, it is thought that this diet will be useful for long-term studies in adult rats. However, we have no data on the effects of a low protein diet on reproductive function in rats. In this study, we examined fertility and general reproductive performance of animals to obtain background data on the use of a low protein diet, and the results were compared to those in animals fed a commonly used diet.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats (9-week-old males and 8-week-old females) were obtained three times (Lot A: Oct. 3, 1995, Lot B: Nov. 14, 1996 and Lot C: Dec. 6, 1996) from Charles River Japan Inc. Each lot of animals was divided into two groups comprised of 15 animals per sex and acclimatized to the environmental conditions for about 2 weeks.

The animals were housed individually in metal cages in a clean booth. The booth was placed in an animal room with a room temperature of 20-26˚C, relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (light on from 7:00 to 19:00). The animals were allowed free access to tap water and a laboratory diet. One group (group name: C) was given CRF-1, a commonly used diet containing 24% protein (Oriental Yeast, Co. Ltd.), and the other group (group name: LP) was given CR-LPF, a new low protein diet containing 18% protein (Oriental Yeast, Co. Ltd.).

All animals were observed for survival and clinical signs once daily. Males were weighed twice a week throughout the study period. Females were weighed twice a week before mating and on days 0, 3, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. A 24-hour food consumption value was determined for all males and females once a week before mating and for pregnant females on days 0, 3, 6, 8, 10, 12, 14, 16 and 18 of gestation. Vaginal smears were made for all females daily in the morning for 4 weeks before mating and continuing to the day before copulation. These smears were stained with Giemsa's solution after ethanol fixation and examined for signs of estrous cycle anomalies. Estrous cycles were judged as follows: Regular, animals showing 4- or 5-day cycles; Irregular, animals showing shorter-than-4-day or longer-than-5-day cycles; and No cycle, animals showing no estrous cycles. Males were mated with females for 3 weeks on a one-to-one basis. Females were examined daily for the presence of a copulation plug, and the day on which it was found was designed day 0 of gestation. The copulatory index (number of animals that copulated x 100/number of mated animals) and fertility index (number of pregnant females x 100/number of females that copulated) were calculated for each group.

On day 20 of gestation, all females in each group were exsanguinated under carbon dioxide anesthesia, and then the uterus, ovaries and other main internal organs of the dams were observed macroscopically. The carcass, liver, kidneys, spleen and adrenal glands were weighed, and the relative organ weights (% of body weight) were calculated. The numbers of corpora lutea, implants, resorptions, placental remnants, dead embryos/fetuses and live fetuses were recorded. Placentae, amniotic fluid and amnions were observed macroscopically, and placentae were weighed. The sex ratio (number of males x 100/number of males and females) was determined. The live fetuses were examined for external abnormalities and were weighed. Approximately half of the live fetuses were fixed in

10% formalin for visceral examination. Visceral examination was carried out according to the methods of Wilson [3] and Barrow and Taylor [1]. The remaining half of the live fetuses were eviscerated and then fixed in ethyl alcohol and stained with alizarin red S by the modified Dawson technique [2]. These skeletal specimens were examined for skeletal abnormalities and variations, and the degree of ossification was determined by counting the number of ossified sacro-caudal vertebrae.

All males in each group were necropsied after reproductive performance was evaluated. They were exanguinated under carbon dioxide anesthesia. Their reproductive and main organs were examined macroscopically. The carcass, liver, kidneys, spleen, adrenal glands, testes, epididymides, seminal vesicles and ventral prostate were weighed, and the relative organ weights (% of body weight) were calculated. The right cauda epididymis was weighed and speared with a surgical knife. An appropriate amount of the semen that leaked out was scooped up with a glass rod and diffused in medium for examination of sperm motility. The remainder of the caudal part of the epididymis was minced in medium for examination of sperm concentration. Both media were kept at 37°C. Sperm concentration and motility were measured using CASA (Cell Soft™ 4000, CRYO Resource Ltd.). Both the testes and the left epididymis were fixed in Bouin's solution for 2 or 3 days and then postfixed in 10% neutral buffered formalin.

Statistical analysis of data was performed for each lot using the following methods. The data on estrous cycles, copulatory and fertility indices were evaluated by Fisher's exact test. The data on external, visceral, skeletal and placental abnormalities/variations were transformed into ranked data as one experimental unit. The Wilcoxon test was performed to compare the mean rank in the C group with that in the LP group. The other data were analyzed statistically as one experimental unit as follows. First, the F test was performed for homogeneity of variance between the groups. When the variances were homogeneous, the Student's t test was applied, and when the variances were heterogeneous, the Aspin & Welch t test was performed to compare the mean in the C group with that in LP group. The F test was conducted at the significant level of 0.20, and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01.

RESULTS

No deaths occurred in any group. No abnormalities in clinical signs were observed in any animals except for one male in 1 LP group that showed hematuria.

Body weight and body weight gain are shown in Tables 1 to 4. Lower male body weight was noted in the LP group from Lots B and C on day 0, indicating that body weight decreased in these two groups over the first two weeks following the switch to the low protein diet, i.e. during the acclimation period. In males, significant decreases or a tendency towards decreases in body weight gain during the study period were noted in two LP groups when compared to the respective C groups. In females, there were no effects on body weight gain in any lot during the study period.

Table 1. Body weight of males (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Number of animals		15	5	15	15	5	15
Days	0	375 ± 22	370 ± 23	397 ± 21	375 ± 13 **	400 ± 18	386 ± 21
	3	391 ± 24	384 ± 24	406 ± 23	385 ± 13 **	412 ± 20	397 ± 22
	7	412 ± 25	402 ± 27	424 ± 26	401 ± 15 **	430 ± 19	413 ± 24 *
	10	422 ± 25	414 ± 29	37 ± 27	410 ± 16 **	442 ± 19	23 ± 25 *
	14	435 ± 28	423 ± 29	453 ± 30	421 ± 15 **	457 ± 22	435 ± 26 *
	17	449 ± 28	435 ± 30	459 ± 32	427 ± 16 **	467 ± 22	444 ± 27 *
	21	460 ± 30	445 ± 30	474 ± 32	438 ± 17 **	478 ± 23	454 ± 29 *
	24	469 ± 33	452 ± 32	482 ± 36	444 ± 17 **	483 ± 27	462 ± 31
	28	480 ± 35	463 ± 33	497 ± 37	456 ± 19 **	494 ± 27	471 ± 33 *
	31	481 ± 36	466 ± 37	500 ± 37	454 ± 19 **	497 ± 29	474 ± 33 *
	35	492 ± 38	475 ± 39	514 ± 38	567 ± 19 **	509 ± 28	485 ± 33 *
	38	502 ± 40	483 ± 40	523 ± 40	473 ± 24 **	518 ± 28	493 ± 34 *
	42	512 ± 43	491 ± 42	534 ± 43	483 ± 21 **	525 ± 30	500 ± 34 *
	45	519 ± 44	497 ± 41	542 ± 43	490 ± 22 **	536 ± 31	508 ± 34 *
	49	532 ± 47	507 ± 44	550 ± 45	496 ± 23 **	546 ± 34	513 ± 33 *
	52	538 ± 49	511 ± 44	555 ± 46	496 ± 21 **	551 ± 35	519 ± 31 *
	56	548 ± 51	519 ± 44	572 ± 51 (7)	504 ± 17 * (7)	561 ± 35	525 ± 34 **
	59	551 ± 53	524 ± 44				
	63	540 ± 35 (8)	544 ± 52 (8)				

*,**: Significantly different from C group (P≤0.05, P≤0.01)
Number in parenthesis indicates the number of animals examined.

Table 2. Body weight gain in males (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Number of animals		15	15	15	15	15	15
Days	0	375 ± 22	370 ± 23	397 ± 21	375 ± 13 **	400 ± 18	386 ± 21
	3	16 ± 4	14 ± 4	9 ± 4	10 ± 5	12 ± 6	11 ± 4
	7	21 ± 3	18 ± 6	18 ± 8	16 ± 5	18 ± 4	16 ± 5
	10	10 ± 3	11 ± 5	13 ± 3	8 ± 5 **	12 ± 3	10 ± 4
	14	13 ± 6	10 ± 6	16 ± 6	12 ± 5 *	15 ± 4	12 ± 4 *
	17	14 ± 5	12 ± 4	6 ± 4	5 ± 5	10 ± 3	9 ± 3
	21	11 ± 4	9 ± 4	14 ± 3	11 ± 5	10 ± 5	10 ± 5
	24	9 ± 4	7 ± 5	9 ± 5	6 ± 4	5 ± 7	8 ± 4
	28	11 ± 6	11 ± 4	14 ± 4	11 ± 3 *	11 ± 6	9 ± 5
	31	1 ± 8	3 ± 5	3 ± 5	-2 ± 5 **	3 ± 6	3 ± 6
	35	11 ± 9	9 ± 4	14 ± 5	13 ± 6	12 ± 5	11 ± 7
	38	10 ± 5	7 ± 3	9 ± 4	6 ± 8	9 ± 5	9 ± 4
	42	9 ± 5	8 ± 4	11 ± 6	10 ± 8	8 ± 5	6 ± 3
	45	8 ± 4	7 ± 4	9 ± 4	7 ± 5	11 ± 4	9 ± 4
	49	13 ± 5	10 ± 6	8 ± 4	6 ± 4	10 ± 5	4 ± 5 **
	52	5 ± 4	4 ± 4	5 ± 4	-1 ± 7 *	5 ± 5	6 ± 7
	56	10 ± 4	8 ± 3	6 ± 9 (7)	10 ± 4 (7)	9 ± 5	6 ± 6
	59	4 ± 3	5 ± 5				
	63	12 ± 4 (8)	9 ± 5 (8)				

*,**: Significantly different from C group ($p \leq 0.05$, $P \leq 0.01$)
Number in parenthesis indicates the number of animals examined.

Table 3. Body weight of dams (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Pre-mating period							
Number of animals		15	15	15	15	15	15
Days	0	220 ± 11	224 ± 11	252 ± 12	251 ± 8	245 ± 15	246 ± 13
	3	230 ± 11	233 ± 13	260 ± 12	259 ± 7	253 ± 16	254 ± 13
	7	240 ± 12	245 ± 15	270 ± 13	270 ± 7	265 ± 18	264 ± 13
	10	244 ± 15	248 ± 15	277 ± 14	276 ± 8	271 ± 19	267 ± 13
	14	250 ± 14	253 ± 17	284 ± 16	282 ± 10	278 ± 20	273 ± 12
	17	254 ± 15	258 ± 17	285 ± 17	286 ± 7	281 ± 22	275 ± 16
	21	257 ± 16	261 ± 18	293 ± 20	291 ± 9	285 ± 22	279 ± 16
	24	261 ± 16	265 ± 19	299 ± 21	296 ± 9	292 ± 21	285 ± 15
	28	265 ± 16	270 ± 19	304 ± 21	303 ± 8	298 ± 22	290 ± 15
Gestation period							
Number of animals		13	15	15	14	13	14
Days	0	267 ± 20	267 ± 17	305 ± 20	304 ± 9	299 ± 22	289 ± 16
	3	285 ± 19	288 ± 18	325 ± 23	324 ± 9	317 ± 24	307 ± 16
	6	293 ± 20	296 ± 19	338 ± 24	334 ± 9	329 ± 26	320 ± 18
	8	301 ± 21	304 ± 20	344 ± 26	341 ± 9	337 ± 26	328 ± 18
	10	309 ± 21	312 ± 20	352 ± 25	349 ± 10	347 ± 27	337 ± 21
	12	318 ± 22	321 ± 22	364 ± 27	361 ± 11	357 ± 28	350 ± 21
	14	325 ± 22	330 ± 21	372 ± 27	368 ± 11	363 ± 26	357 ± 23
	16	340 ± 20	344 ± 22	388 ± 30	387 ± 15	377 ± 25	373 ± 24
	18	366 ± 21	378 ± 25	419 ± 32	416 ± 19	404 ± 30	404 ± 26 (13)
	20	397 ± 23	408 ± 28	454 ± 35	450 ± 21	433 ± 37	435 ± 29

No statistical significance
Number in parenthesis indicates the number of animals examined.

Table 4. Body weight gain in dams (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Pre-mating period							
Number of animals		15	15	15	15	15	15
Days	0-3	10 ± 4	9 ± 4	8 ± 3	8 ± 4	8 ± 4	8 ± 4
	3-7	10 ± 4	12 ± 3	10 ± 4	11 ± 3	12 ± 3	10 ± 3
	7-10	4 ± 5	3 ± 4	7 ± 5	6 ± 4	6 ± 5	4 ± 3
	10-14	6 ± 3	6 ± 5	7 ± 4	7 ± 4	8 ± 3	6 ± 5
	14-17	5 ± 4	5 ± 6	1 ± 5	3 ± 5	3 ± 4	1 ± 7
	17-21	3 ± 3	3 ± 3	9 ± 4	6 ± 3	4 ± 3	4 ± 4
	21-24	4 ± 4	4 ± 6	5 ± 3	4 ± 4	7 ± 4	6 ± 3
	24-28	4 ± 2	4 ± 3	5 ± 3	7 ± 3	7 ± 3	6 ± 3
Gestation period							
Number of animals		13	15	15	14	13	14
Days	0-3	18 ± 6	20 ± 5	21 ± 9	19 ± 8	19 ± 6	18 ± 5
	3-6	8 ± 5	9 ± 4	12 ± 6	10 ± 3	12 ± 5	13 ± 5
	6-8	7 ± 4	8 ± 3	7 ± 5	8 ± 4	8 ± 3	8 ± 3
	8-10	9 ± 3	8 ± 3	8 ± 4	8 ± 4	10 ± 4	9 ± 5
	10-12	8 ± 2	9 ± 4	12 ± 4	12 ± 4	10 ± 4	13 ± 4
	12-14	7 ± 4	9 ± 3	8 ± 4	7 ± 4	6 ± 9	7 ± 4
	14-16	15 ± 5	14 ± 7	17 ± 6	19 ± 5	14 ± 7	16 ± 5
	16-18	26 ± 4	34 ± 8 **	31 ± 6	30 ± 6	27 ± 12	30 ± 5 (13)
	18-20	31 ± 7	30 ± 5	35 ± 7	34 ± 5	29 ± 13	33 ± 5 (13)

**: Significantly different from C group (P≤0.01)
Number in parenthesis indicates the number of animals examined.

Food consumption values are shown in Tables 5 and 6. A significant increase or a tendency towards an increase in food consumption was noted in both males and females in all LP groups during the study period when compared to the values in the respective C groups.

Estrous cycles are shown in Table 7. One animal in each of one C and two LP groups showed an irregular cycle. However, there were no diet-related effects on estrous cycles.

Mating performance is shown in Table 8. There were no diet-related effects on the copulatory or fertility index or mean copulatory interval in any lot of animals.

Table 5. Food consumption in males (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Number of animals		15	15	15	15	15	15
Days	0	26 ± 3	28 ± 3	24 ± 5	28 ± 3 *	27 ± 43	30 ± 3
	7	25 ± 2	27 ± 3 * (14)	27 ± 3	27 ± 3	27 ± 2	31 ± 3 **
	14	27 ± 3	28 ± 3	27 ± 4	29 ± 2	29 ± 2	32 ± 2 **
	21	24 ± 3	26 ± 3	28 ± 3	28 ± 3	28 ± 3	33 ± 2 **(14)

*,**: Significant different from C group (p≤0.05, p≤0.01).
Number in parenthesis indicates the number of animals examined.

Table 6. Food consumption in dams (g, Mean ± S.D.)

Lot of animals		A		B		C	
Group		C	LP	C	LP	C	LP
Pre-mating period							
Number of animals		15	15	15	15	15	15
Days	0	17 ± 4	17 ± 4 (14)	18 ± 3 (14)	20 ± 3	19 ± 4 (12)	21 ± 4 (13)
	7	20 ± 2	22 ± 2 ** (14)	21 ± 3 (14)	24 ± 2 *	21 ± 2	24 ± 3 **(13)
	14	20 ± 2	24 ± 3 ** (14)	24 ± 4 (14)	24 ± 2	23 ± 3	26 ± 2 **(13)
	21	16 ± 3	17 ± 3 (13)	22 ± 3 (14)	21 ± 2 (14)	23 ± 3	24 ± 3 (13)
Gestation period							
Number of animals		13	15	15	14	13	14
Days	0	20 ± 3	20 ± 4 (14)	21 ± 4 (14)	23 ± 4	22 ± 2	23 ± 5
	3	23 ± 3	25 ± 2 (14)	27 ± 3 (14)	28 ± 4	26 ± 3	26 ± 3
	6	23 ± 3	25 ± 3 *	28 ± 4 (14)	29 ± 4	27 ± 4	28 ± 3
	8	23 ± 3	26 ± 2 *	27 ± 4 (14)	29 ± 4	26 ± 3	29 ± 3
	10	24 ± 3	26 ± 3 *	27 ± 3 (13)	29 ± 3	26 ± 4	29 ± 4 *
	12	23 ± 3	27 ± 2 **	28 ± 4 (14)	30 ± 3	26 ± 3	28 ± 4
	14	23 ± 4	27 ± 4 *	27 ± 3	29 ± 3	23 ± 3	27 ± 3 **
	16	26 ± 3	30 ± 4 **	29 ± 3	32 ± 4 *	26 ± 5	29 ± 4 *
	18	26 ± 3	31 ± 3 **	29 ± 3	32 ± 4 *	25 ± 3	32 ± 4 **

*,**: Significant different from C group ($p \leq 0.05$, $p \leq 0.01$).
Number in parenthesis indicates the number of animals examined.

Table 7. Estrous cycles

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of animals	15	15	15	15	15	15
Number of regular cycles	15	14	15	14	14	15
% of regular cycles	100.0	93.3	100.0	93.3	93.3	100.0
Number of irregular cycles	0	1	0	1	1	0
% of irregular cycles	0.0	6.7	0.0	6.7	6.7	0.0
Number of no cycles	0	0	0	0	0	0
% of no cycles	0.0	0.0	0.0	0.0	0.0	0.0

No statistical significance

Table 8. Mating performance

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of pairs (A)	15	15	15	15	15	15
Number of copulate pairs (B)	15	15	15	15	15	14
Copulatory index (%) (B/A)	100.0	100.0	100.0	100.0	100.0	93.3
Mean copulatory interval (days)	2.4 ± 1.6	1.5 ± 0.6	1.9 ± 1.7	2.9 ± 2.9	2.9 ± 2.9	1.6 ± 0.8
Number of fertile pairs (C)	13	15	15	14	13	14
Fertility index (%) (C/B)	86.7	100.0	100.0	93.3	86.7	100.0

No statistical significance

Necropsy findings and organ weights in males are shown in Tables 9 to 11. Foci in the epididymides, small testes, renal cysts and deformity of the kidney were each observed in one or two animals in the C groups. Small epididymides, small testes, hydronephrosis and deformity of the kidney were each observed in one to three animals in the LP groups. However, there was no tendency towards an increase or decrease in the appearance of these abnormal findings in the LP groups in any lot of animals. There were no diet-related effects on organ weights in any lot of animals.

Table 9. Necropsy findings in males

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of animals	15	15	15	15	15	15
Normal	12	14	14	13	13	13
Small epididymis	0	0	0	2	0	1
Focus in the epididymis	1	0	0	0	0	0
Small testis	0	0	1	0	0	1
Hydronephrosis	0	1	0	2	0	0
Renal cyct	2	0	0	0	0	0
Deformity of the kidney	0	0	0	0	2	1

Table 10. Organ weights in males (Mean ± S.D.)

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of animals	15	15	15	15	15	15
Body weight (g)	555 ± 53	525 ± 44	552 ± 45	494 ± 20 ** (14)	552 ± 35	516 ± 33
Carcass (g)	436 ± 38	414 ± 35	436 ± 32	394 ± 15 **	436 ± 25	410 ± 27
Testis (Right) (g)	1.75 ± 0.13	1.72 ± 0.20	1.80 ± 0.18	1.68 ± 0.49	1.79 ± 0.19	1.65 ± 0.26
Testis (Left) (g)	1.76 ± 0.12	1.72 ± 0.18	1.82 ± 0.17	1.76 ± 0.37	1.76 ± 0.23	1.69 ± 0.17
Epididymis (Right) (mg)	646 ± 72	648 ± 54	653 ± 38	623 ± 144	630 ± 66	622 ± 101
Epididymis (Left) (mg)	629 ± 72	630 ± 45	634 ± 46	624 ± 107	612 ± 65	611 ± 59
Ventral prostate (mg)	593.2 ± 121.7	537.6 ± 103.4	581.8 ± 121.7	505.1 ± 102	583.1 ± 161.2	507.4 ± 78.8
Seminal vesicles (g)	1.47 ± 0.25	1.59 ± 0.25	1.68 ± 0.28	1.67 ± 0.24	1.70 ± 0.23	1.64 ± 0.191
Liver (g)	19.32 ± 3.37	17.56 ± 2.12	18.46 ± 3.05	15.77 ± 1.36 **	18.57 ± 1.57	16.46 ± 1.19
Kidney (Right) (g)	1.68 ± 0.20	1.58 ± 0.15	1.67 ± 0.20	1.57 ± 0.18	1.73 ± 0.17	1.56 ± 0.12
Kidney (Left) (g)	1.68 ± 0.18	1.60 ± 0.18	1.68 ± 0.20	1.56 ± 0.16	1.72 ± 0.17	1.56 ± 0.15
Spleen (g)	0.78 ± 0.20	0.71 ± 0.16	0.73 ± 0.14	0.73 ± 0.11	0.70 ± 0.05	0.70 ± 0.09
Adrenal glands (mg)	46.4 ± 977	53.1 ± 9.2	48.3 ± 12.6	47.5 ± 5.5	53.5 ± 9.0	49.7 ± 9.6

**: Significantly different from C group ($p \leq 0.01$)
Number in parenthesis indicates the number of animals examined.

Table 11. Relative organ weights in males

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of animals	15	15	15	15	15	15
Body weight (g)	555 ± 53	525 ± 44	552 ± 45	494 ± 20 ** (14)	552 ± 35	516 ± 33
Carcass	79 ± 2	79 ± 1	79 ± 2	79 ± 1	79 ± 1	79 ± 1
Testis (Right)	0.32 ± 0.03	0.33 ± 0.04	0.33 ± 0.05	0.34 ± 0.10	0.33 ± 0.04	0.32 ± 0.05
Testis (Left)	0.32 ± 0.03	0.33 ± 0.04	0.33 ± 0.04	0.36 ± 0.08	0.32 ± 0.05	0.33 ± 0.03
Epididymis (Right) (x 10^{-3})	117 ± 18	124 ± 14	119 ± 12	127 ± 31	115 ± 13	121 ± 21
Epididymis (Left) (x 10^{-3})	114 ± 18	121 ± 12	116 ± 12	127 ± 22	111 ± 13	119 ± 14
Ventral prostate (x 10^{-3})	107 ± 23	103 ± 22	106 ± 23	100 ± 19	106 ± 29	99 ± 16
Seminal vesicles	0.27 ± 0.05	0.30 ± 0.05 *	0.31 ± 0.05	0.34 ± 0.05	0.31 ± 0.04	0.32 ± 0.03
Liver	3.46 ± 0.32	3.34 ± 0.22	3.36 ± 0.36	3.16 ± 0.21	3.36 ± 0.15	3.19 ± 0.22
Kidney (Right)	0.30 ± 0.03	0.30 ± 0.02	0.30 ± 0.02	0.31 ± 0.02	0.31 ± 0.03	0.30 ± 0.02
Kidney (Left)	0.30 ± 0.02	0.30 ± 0.03	0.30 ± 0.02	0.31 ± 0.02	0.31 ± 0.03	0.30 ± 0.02
Spleen	0.14 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.02
Adrenal glands (x 10^{-3})	8.5 ± 2.0	10.1 ± 1.5 *	8.7 ± 2.2	9.6 ± 1.1	9.7 ± 1.7	9.6 ± 1.7

*,**: Significantly different from C group ($p \leq 0.05$, $p \leq 0.01$)
Number in parenthesis indicates the number of animals examined.

Sperm examination results are shown in Table 12. There were no diet-related effects on sperm motility or count in any lot of animals.

The results of necropsy on day 20 of gestation are shown in Tables 13 to 15. Enlargement of the spleen, adhesion of the spleen, accessory spleen and deformity of the kidney were each observed in one or two animals in the C groups. Enlargement of the spleen, adhesion of the spleen and pancreas, deformity of the kidney and duplication of renal vein were each observed in one animal in the LP groups. There was no tendency towards an increase or decrease in the appearance of the abnormal findings in the LP groups. There were no diet-related effects on organ weights in any lot of animals.

Table 12. Epididymal sperm motility and count (Mean ± S.D.)

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of animals	15	15	15	15	15	15
Sperm motility (%)	73.2 ± 27.4	83.2 ± 7.8	79.9 ± 12.2	69.0 ± 24.5	69.9 ± 23.8	74.1 ± 23.0
Sperm count						
x 10^6 cauda epididymis	297 ± 108	298 ± 52	296 ± 66	284 ± 160	265 ± 102	2633 ± 96
x 10^6 /g cauda epididymis	910 ± 265	913 ± 138	923 ± 213	853 ± 437	870 ± 269	828 ± 294

No statistically significance

Table 13. Necropsy findings in dams - On day 20 of gestation

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Normal	11	15	13	14	13	13
Adhesion of the spleen	1	0	0	0	0	0
Adhesion of the spleen and pancreas	0	0	0	1	0	0
Enlargement of the spleen	1	0	0	1	0	0
Accessory spleen	1	0	0	0	0	0
Deformity of the kidney	0	0	2	0	0	1
Duplication of renal vein	0	0	0	0	0	1

Table 14. Organ weights in dams (Mean ± S.D.)

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Body weight (g)	376 ± 45	403 ± 27	449 ± 36	444 ± 38	430 ± 37	431 ± 28
Carcass (g)	237 ± 19	238 ± 14	269 ± 19	264 ± 9	258 ± 28	248 ± 28
Liver (g)	14.67 ± 2.16	15.47 ± 1.13	17.00 ± 1.77	6.69 ± 1.92	15.74 ± 1.84	16.49 ± 1.98
Kidney (Right) (g)	0.94 ± 0.09	0.95 ± 0.07	1.10 ± 0.09	1.03 ± 0.08 *	1.01 ± 0.06	0.97 ± 0.12
Kidney (Left) (g)	0.94 ± 0.09	0.94 ± 0.07	1.10 ± 0.08	1.03 ± 0.08 *	0.97 ± 0.06	0.95 ± 0.11
Spleen (g)	0.62 ± 0.18	0.64 ± 0.07	0.65 ± 0.07	0.68 ± 0.23	0.62 ± 0.07	0.65 ± 0.13
Adrenal glands (mg)	66.8 ± 12.3	64.9 ± 8.0	68.5 ± 14.6	69.1 ± 9.1	69.3 ± 10.4	67.1 ± 11.9

*: Significantly different from C group ($p \leq 0.05$)

Table 15. Relative organ weights in dams (Mean ± S.D.)

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Body weight (g)	376 ± 45	403 ± 27	449 ± 36	444 ± 38	430 ± 37	431 ± 28
Carcass	64 ± 6	59 ± 2 *	60 ± 2	60 ± 6	60 ± 4	58 ± 5
Liver	3.89 ± 0.34	3.84 ± 0.13	3.78 ± 0.23	3.76 ± 0.31	3.65 ± 0.20	3.82 ± 0.29
Kidney (Right)	0.25 ± 0.03	0.23 ± 0.01	0.25 ± 0.02	0.23 ± 0.03	0.24 ± 0.02	0.22 ± 0.02
Kidney (Left)	0.25 ± 0.03	0.23 ± 0.02	0.25 ± 0.02	0.23 ± 0.03	0.23 ± 0.02	0.22 ± 0.02
Spleen	0.17 ± 0.04	0.16 ± 0.02	0.14 ± 0.02	0.15 ± 0.05	0.14 ± 0.02	0.15 ± 0.03
Adrenal glands (x 10^{-3})	17.9 ± 3.5	16.2 ± 2.4	15.3 ± 3.3	15.6 ± 1.9	16.2 ± 2.1	15.5 ± 2.3

*: Significantly different from C group ($p \leq 0.05$)

The results of intrauterine observation are shown in Table 16. There were no diet-related effects on numbers of corpora lutea or implants, embryo/fetal mortality, sex ratio, numbers of live fetuses or fetal or placental weights in any lot of animals.

Gross placental findings are shown in Table 17. Fused placentae were observed in one C and one LP groups. In addition, enlargement of the placentae was observed in two LP groups. However, there were no significant difference in the incidence of the placental anomalies between the two diet groups in any lot of animals.

External findings in fetuses are shown in Table 18. Club foot and kinky tail were observed in one fetus in the C and LP groups from lot A, respectively. However, there were no significant differences in the incidence of the external anomalies between the two diet groups in any lot.

Table 16. Intrauterine observation in dams and fetuses

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Mean number of corpora lutea (A)	15.2 ± 1.5	16.5 ± 2.0	17.9 ± 1.4	17.2 ± 1.6	17.6 ± 1.8	16.8 ± 1.9
Mean number of implants (B)	13.8 ± 2.7	15.1 ± 2.0	15.6 ± 2.6	16.2 ± 1.4	16.4 ± 1.9	15.8 ± 1.6
Mean pre-implantation loss						
(A-B)/(A) (%)	9.7 ± 15.3	8.1 ± 8.6	12.7 ± 14.5	5.6 ± 5.4	7.0 ± 4.5	5.7 ± 4.9
Mean number of dead implants						
Resorbed	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Placental remnants	0.7 ± 0.9	0.9 ± 1.8	0.7 ± 0.7	0.4 ± 0.5	0.5 ± 0.7	0.4 ± 0.6
Dead fetus	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.0
Total (C)	0.8 ± 0.9	0.9 ± 1.8	0.8 ± 0.7	0.4 ± 0.5	0.5 ± 0.7	0.4 ± 0.6
Mean post-implantation loss						
(C)/(B) (%)	6.5 ± 9.1	6.4 ± 12.7	5.6 ± 4.9	2.7 ± 3.2	3.2 ± 3.9	2.3 ± 4.1
Mean number of live fetuses	13.0 ± 3.0	14.2 ± 2.8	14.8 ± 2.8	15.8 ± 1.5	15.8 ± 1.9	15.4 ± 1.7
Sex ratio						
[Male / (Male + Female) (%)]	52.4 ± 11.0	48.3 ± 13.8	52.5 ± 19.3	53.9 ± 16.6	49.4 ± 7.4	48.5 ± 11.2
Mean weight of fetuses						
(g, mean ± S.D.) Male	3.36 ± 0.21	3.46 ± 0.35	3.45 ± 0.25	3.35 ± 0.40	3.43 ± 0.28	3.46 ± 0.20
Female	3.23 ± 0.17	3.28 ± 0.28	3.37 ± 0.19	3.19 ± 0.37	3.31 ± 0.24	3.27 ± 0.16
Mean weight of placentae						
(mg, mean ± S.D.) Male	436 ± 47	465 ± 84	460 ± 57	460 ± 60	422 ± 35	429 ± 41
Female	426 ± 50	455 ± 124	440 ± 45	444 ± 43	415 ± 40	416 ± 38

No statistical significance

Table 17. Gross findings in placentae

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Number of placentae examined	169	213	222	221	206	216
Abnormalities						
Mean frequencies (%)	0.0 ± 0.0	4.7 ± 14.7	0.4 ± 1.6	2.2 ± 8.4	0.0 ± 0.0	0.0 ± 0.0
Types and frequencies (%)						
Fused placentae	0.0 ± 0.0	0.9 ± 2.5	0.4 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Enlargement of the placenta	0.0 ± 0.0	3.8 ± 14.8	0.0 ± 0.0	2.2 ± 8.4	0.0 ± 0.0	0.0 ± 0.0

No statistical significance

Table 18. External findings in fetuses

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Number of fetuses examined	169	213	222	221	206	216
Abnormalities						
Mean frequencies (%)	0.6 ± 2.1	0.5 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Types and frequencies (%)						
Club foot	0.0 ± 0.0	0.5 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Kinky tail	0.6 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

No statistical significance

Visceral findings in fetuses are shown in Table 19. Visceral abnormalities such as abnormal origin of pulmonary artery were observed in all C and LP groups. Right aortic arch, persistent atrioventricular canal, single ventricle, membranous and muscular ventricular septal defect, agenesis of pulmonary intermedial lobe and abnormal lobulation of the lung were observed in the LP group in 1 lot. Space in adrenal medulla was observed in one C group. The incidence of visceral anomalies observed was low except for the incidence of abnormal origin of pulmonary artery observed in all groups (2.1% to 10.8%). However, there were no significant differences in the incidence of the visceral anomalies between the two diet groups in any lot of animals. Visceral variations such as left umbilical artery were observed in all C and LP groups. Dilatation of renal pelvis and dilatation of the ureter were observed in two C and two LP groups. Discoloration of the adrenal gland was observed in one LP group. However, there were no significant differences in the incidence of the visceral variations between the two diet groups in any lot of animals.

Skeletal findings in fetuses are shown in Table 20. No skeletal anomalies were observed in any fetus. Skeletal variations such as dumb-bell-shaped thoracic vertebral bodies and lumber ribs were observed in all C and LP groups. Cervical ribs were observed in one LP group. Dumb-bell-shaped lumbar vertebral bodies were observed in one C group. However, there were no significant differences in the incidence of the skeletal variations between the two diet groups in any lot of animals. There were no significant differences in the number of ossified sacro-caudal vertebrae between the two diet groups in any lot of animals.

Table 19. Visceral findings in fetuses

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Number of fetuses examined	90	113	120	118	110	113
Abnormalities						
Mean frequencies (%)	2.1 ± 5.0	10.7 ± 18.2	3.3 ± 7.2	5.5 ± 8.2	11.8 ± 16.5	3.2 ± 5.3
Types and frequencies (%)						
Right aortic arch	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Persistent atrioventricular canal	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Abnormal origin of pulmonary artery	2.1 ± 5.0	6.8 ± 15.6	3.3 ± 7.2	5.5 ± 8.2	10.8 ± 16.0	3.2 ± 5.3
Single ventricle	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Membranous ventricular septal defect	0.0 ± 0.0	3.0 ± 6.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Muscular ventricular septal defect	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Agenesis of pulmonary intermedial lobe	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Abnormal lobulation of the lung	0.0 ± 0.0	1.0 ± 3.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Space in adrenal medulla	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 3.5	0.0 ± 0.0
Variations						
Mean frequencies (%)	13.0 ± 21.0	9.1 ± 11.8	7.4 ± 10.4	3.2 ± 6.8	13.4 ± 20.8	5.3 ± 10.6
Types and frequencies (%)						
Left umbilical artery	1.0 ± 3.5	1.5 ± 4.0	0.8 ± 3.2	3.2 ± 6.8	0.9 ± 3.1	0.9 ± 3.3
Dilatation of renal pelvis	3.0 ± 7.7	1.9 ± 5.2	6.5 ± 10.4	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 2.7
Dilatation of the ureter	11.0 ± 17.6	4.8 ± 8.6	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 20.6	4.4 ± 10.5
Discoloration of the adrenal gland	0.0 ± 0.0	1.7 ± 6.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

No statistical significance

Table 20. Skeletal findings in fetuses

Lot of animals	A		B		C	
Group	C	LP	C	LP	C	LP
Number of dams	13	15	15	14	13	14
Number of fetuses examined	79	100	102	103	96	103
Abnormalities						
Mean frequencies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Variations						
Mean frequencies (%)	10.6 ± 20.0	17.0 ± 22.8	20.0 ± 23.7	27.0 ± 25.8	8.2 ± 12.2	16.4 ± 17.5
Types and frequencies (%)						
Dumb-bell-shaped thoracic vertebral body	7.1 ± 17.5	11.6 ± 20.5	3.7 ± 8.3	7.0 ± 9.9	1.1 ± 4.0	1.9 ± 4.9
Cervical rib	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 3.8	0.0 ± 0.0	0.0 ± 0.0
Lumbar rib	2.4 ± 5.8	6.3 ± 11.4	18.1 ± 20.9	20.9 ± 29.0	7.1 ± 12.2	14.5 ± 18.5
Dumb-bell-shaped lumbar vertebral body	3.0 ± 7.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean number of ossified s.c.v.	7.7 ± 0.3	7.6 ± 0.7	7.8 ± 0.3	7.4 ± 1.3	7.9 ± 0.2	8.0 ± 0.4

No statistical significance
s.c.v.: Sacro-caudal vertebrae

DISCUSSION

The effects of a low protein diet on fertility and reproductive performance of parental animals and development of fetuses was studied.

In parental animals, lower body weight gain in males and higher food consumption value in males and females were noted in the low protein diet groups. With regard to lower body weight gain in males, the same change was reported in the data from a similar study in Crj:CD(SD) rats (Oriental Yeast, Co. Ltd., unpublished data). In general, it is considered that nutritional requirements are high during the gestation and lactation periods in female animals. However, there were no effects of the lower protein content on female body weight or body weight gain throughout the study period. The low protein diet had no effects on reproductive performance in parental animals and development of fetuses.

As described above, it is concluded that the low protein diet does not effect reproductive performance in parental animals including development of their fetuses. However, if animals used in a reproductive toxicity study are fed this diet, the above-mentioned effects on the parental animals should to be taken into consideration when interpreting the study results, even though use of the diet does not seem to affect the results of fetal examinations.

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Historical Control Data for Crj:CD(SD)IGS Rats Used in Teratology Studies

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ABSTRACT. Fetuses from pregnant Crj:CD(SD)IGS rats were examined to collect historical control data for future teratology studies using this strain. Data were obtained from the control group of six teratology studies (118 dams and 1697 fetuses) performed during 1996-1998 in our laboratory. The mean numbers of corpora lutea and implants were 16.1 and 15.0, respectively. The pre- and post-implantation loss rates were 6.5% and 4.3%, respectively. The mean fetal body weight was 3.45 g for males and 3.27 g for females. The frequencies of external, visceral and skeletal abnormalities were 0.4%, 0.3% and 0.4%, respectively. The frequencies of visceral and skeletal variations were 1.9% and 16.2%, respectively. No lot difference was observed except that the incidence of lumbar rib varied from 5.7% to 21.2%. — Key words: Crj:CD(SD)IGS, Historical control data, Teratology study

CD (SD) IGS-1998: 233-235

INTRODUCTION

In order to more accurately evaluate the toxicity of new compounds in a specific animal species, it is important to accumulate historical control data for that species. Crj:CD(SD)IGS rats are produced by the gold standard system, a new breeding system developed by Charles River Inc. in order to minimize genetic ramifications as well as to promote internationalization of research and development of new drugs. We have used this particular animal for teratology studies for two years in our laboratory. In this report, we present the results of an analysis of the cumulative teratological control data for Crj:CD(SD)IGS rats.

MATERIALS AND METHODS

The data from the control groups of six teratology studies conducted in our laboratory (1996-1998) were used in this study. Thirteen- to 14-week-old male and 10- to 11-week old female Crj:CD(SD)IGS rats were obtained from Hino Farms of Charles River Japan Inc. They were acclimatized to the environmental conditions for more than a week. Rats which showed no abnormalities in appearance or general conditions were used. Virgin females were mated with males overnight on a one-to-one basis. The day on which a copulation plug was found was considered day 0 of gestation. Animals were housed individually in metal cages in a clean booth. The booth was placed in an animal room with a room temperature of 20-26˚C, relative humidity of 40-70%, air exchange 8-25 times/hour and a 12-hr light/dark cycle (light on from 7:00 to 19:00). The animals were allowed free access to tap water and a laboratory animal diet (CR-LPF, Oriental Yeast, Co. Ltd., γ-ray irradiated).

On day 20 of gestation, all dams were killed by cervical dislocation. The numbers of corpora lutea, implants, resorptions, placental remnants, dead embryos/fetuses and live fetuses were recorded. The live fetuses were examined for external abnormalities and were weighed. Approximately half of the live fetuses were fixed in 10% formalin for visceral examination. Visceral organs were examined according to the methods of Wilson[5] and Barrow and Taylor[1]. The remaining half of the live fetuses were eviscerated and then fixed in ethyl alcohol for skeletal examination. The fetuses fixed in ethyl alcohol were stained with alizarin red S by the modified Dawson technique[2]. These skeletal specimens were examined for skeletal abnormalities and variations, and the degree of ossification was examined by counting the number of ossified sacro-caudal vertebrae.

RESULTS

Intrauterine observations are shown in Table 1. The mean numbers of corpora lutea and implants were 16.1 and 15.0, respectively. The mean pre- and post- implantation loss rates were 6.5% and 4.3%, respectively. In one study, the mean pre-implantation loss rate was 14.3% which is higher than that in the other studies (ranging from 3.3% to 6.5%). This high value was due to the fact that 2 out of the 20 dams in this study showed a pre-implantation loss of more than 50% (data not shown in this paper). The mean number of dead implants was 0.7. The mean number of live fetuses was 14.4. The sex ratio was 50.6%. The mean weights of male and female fetuses were 3.45 g and 3.27 g, respectively.

External findings in fetuses are shown in Table 2. Six out of the 1697 fetuses examined (0.4%) showed at least one abnormality. Microphtalmia, ankyloglossia, cleft jaw, kinky tail, abnormality of external genitalia and edema were each observed in one or two fetuses. The distribution of incidence in each teratology study were relatively homogeneous.

Visceral findings in fetuses are shown in Table 3. Three out of the 905 fetuses examined (0.3%) showed abnormalities. Membranous ventricular septal defect was seen in 2 of these fetuses, and hypoplasia of the kidney was observed in the other fetus. Seventeen out of the 905 fetuses (1.4%) showed visceral variations. Left umbilical artery, dilatation of renal pelvis, dilatation of the ureters, and discoloration of the adrenal gland were sporadically observed. The distribution of the incidence for the six teratology studies was relatively homogeneous.

Skeletal findings in fetuses are shown in Table 4. Three out of the 792 fetuses examined (0.4%) showed abnormalities. One

of these 3 fetuses showed various combinations of the following abnormalities; segmentation defect of thoracic vertebrae, a decrease in the number of thoracic vertebrae, fused ribs and a decrease in the number of ribs. The other 2 fetuses showed an increase in the number of lumbar vertebrae or a decrease in the number of lumbar vertebrae. Skeletal variations were observed in 128 out of the 792 fetuses (16.2%). Dumb-bell-shaped thoracic vertebral bodies and lumbar ribs were frequently observed in all studies. On the other hand, shortening of the 13th rib, cervical ribs, wavy ribs, dumb-bell-shaped lumbar vertebral bodies and peduncular fusion of sternebrae were observed at low frequencies. The number of ossified sacrocaudal vertebrae was ranged from 7.6 to 7.9.

DISCUSSION

Control data from teratology studies that utilized Crj:CD(SD)IGS rats and were conducted during 1996-1998 were examined and analyzed to collect historical control data for future studies using this strain.

Upon fetal observation, the frequencies of external, visceral and skeletal abnormalities were 0.4%, 0.3% and 0.4%, respectively. Fourteen abnormalities were observed in this study. Each finding was observed in only 1 or 2 fetuses, and no novel or rare abnormality was observed. Nakatsu et al.[4] and Kurishita et al.[3] reported historical control data in our laboratory for spontaneous abnormalities in Wistar rats in which the incidences of external, visceral and skeletal abnormalities were 0.09%-0.41%, 0.14%-0.30% and 0.00%-0.12%, respectively. These values are similar to those in the current study. The frequencies of visceral and skeletal variations were 1.9% and 16.2 %, respectively. Lumbar ribs were observed most frequency in this strain, and the frequency (10.7%) was higher than that in the Wistar rats previously reported in our laboratory (2.31%-4.70%)[3, 4]. Additionally, the incidence varied from 5.7 to 21.2%. Currently, it is not clear whether these high values and variations are specific to Crj:CD(SD)IGS rats. It is though necessary to continuously accumulate background data.

In conclusion, no remarkable teratological data was observed in the Crj:CD(SD)IGS rats six studies examined, but since this is a newly developed strain, it is necessary to continuously accumulate background data and it should be evaluated carefully in teratology studies.

Table 1. Intrauterine observation

Study No.	1	2	3	4	5	6	Total & Mean
No. of animals	20	20	19	20	20	19	118
Mean no. of corpora lutea (A)	16.4	16.4	16.3	16.2	16.2	15.3	16.1
Mean no. of implants (B)	15.8	15.5	15.2	13.8	15.4	14.5	15.0
Mean pre-implantation loss (A-B)/(A) (%)	3.3	5.2	6.1	14.3	5.1	4.9	6.5
Mean no. of dead implants							
Resorbed	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Placental remnants	1.0	0.8	0.6	0.7	0.3	0.5	0.7
Dead fetuses	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Total (C)	1.0	0.8	0.6	0.7	0.3	0.5	0.7
Mean post-implantation loss (C)/(B) (%)	6.4	4.7	3.7	5.3	1.9	3.5	4.3
Mean no. of live fetuses	14.8	14.8	14.6	13.1	15.1	14.0	14.4
Sex ratio M/(M+F) (%)	52.5	52.1	49.2	47.1	52.1	50.3	50.6
Mean weight of fetuses (g) Male (M)	3.42	3.51	3.41	3.41	3.54	3.43	3.45
Mean weight of fetuses (g) Female (F)	3.28	3.26	3.21	3.26	3.38	3.21	3.27

Table 2. External findings in fetuses

Study No.	1	2	3	4	5	6	Total & Mean
No. of animals (F0)	20	20	19	20	20	19	118
No. of fetuses examined	295	295	278	262	301	266	1697
Abnormalities							
Mean frequencies (%)	0.7	0.0	0.4	0.3	0.7	0.0	0.4
Types and frequencies (%)							
Microphthalmia	0.0	0.0	0.0	0.0	0.3	0.0	0.1
Ankyloglossia	0.3	0.0	0.0	0.0	0.0	0.0	0.1
Cleft jaw	0.3	0.0	0.0	0.0	0.0	0.0	0.1
Kinky tail	0.0	0.0	0.4	0.0	0.0	0.0	0.1
Abnormality of the external genitalia	0.0	0.0	0.0	0.3	0.4	0.0	0.1
Edema	0.0	0.0	0.0	0.0	0.3	0.0	0.1

Table 3. Visceral findings in fetuses

Study No.	1	2	3	4	5	6	Total & Mean
No. of animals (F0)	20	20	19	20	20	19	118
No. of fetuses examined	155	160	149	141	158	142	905
Abnormalities							
Mean frequencies (%)	1.3	0.0	0.0	0.0	0.0	0.6	0.3
Types and frequencies (%)							
Membranous ventricular septal defect	0.6	0.0	0.0	0.0	0.0	0.6	0.2
Hypoplasia of the kidney	0.6	0.0	0.0	0.0	0.0	0.0	0.1
Variations							
Mean frequencies (%)	1.9	2.5	0.7	6.2	0.6	1.5	1.9
Types and frequencies (%)							
Left umbilical artery	1.9	2.5	0.0	2.1	0.0	0.6	1.2
Dilatation of renal pelvis	0.0	0.0	0.0	4.0	0.0	0.0	0.3
Dilatation of the ureter	0.0	0.0	0.7	1.7	0.0	0.0	0.2
Discoloration of the adrenal gland	0.0	0.0	0.0	0.0	0.6	0.9	0.2

Table 4. Skeletal findings in fetuses

Study No.	1	2	3	4	5	6	Total & Mean
No. of animals (F0)	20	20	19	20	20	19	118
No. of fetuses examined	140	135	129	121	143	124	792
Abnormalities							
Mean frequencies (%)	0.0	1.5	0.8	0.0	0.0	0.0	0.4
Types and frequencies (%)							
Segmentation defect of thoracic vt.	0.0	0.8	0.0	0.0	0.0	0.0	0.1
Decrease in the number of thoracic vt.	0.0	0.8	0.0	0.0	0.0	0.0	0.1
Fused ribs	0.0	0.8	0.0	0.0	0.0	0.0	0.1
Decrease in the number of ribs	0.0	0.8	0.0	0.0	0.0	0.0	0.1
Increase in the number of lumbar vt.	0.0	0.0	0.8	0.0	0.0	0.0	0.1
Decrease in the number of lumbar vt.	0.0	0.6	0.0	0.0	0.0	0.0	0.1
Variations							
Mean frequencies (%)	16.0	12.5	25.6	11.1	16.2	11.1	16.2
Types and frequencies (%)							
Dumb-bell-shaped thoracic vt. body	5.7	6.8	4.3	4.0	6.7	2.4	5.3
Cervical rib	0.7	0.0	0.0	0.0	0.0	0.9	0.3
Shortening of the 13th rib	0.0	0.6	0.0	0.8	0.0	0.0	0.3
Lumber rib	9.8	5.7	21.2	7.1	9.6	8.7	10.7
Wavy ribs	0.0	0.0	0.8	0.0	0.0	0.0	0.1
Dumb-bell-shaped lumbar vt. body	0.6	0.0	0.0	0.0	0.7	0.0	0.3
Peduncular fusion of sternebrae	0.0	0.0	0.8	0.0	0.0	0.0	0.1
Mean no. of s.c.v. ossified	7.8	7.9	7.7	7.6	7.9	7.8	7.8

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Historical Control Data for Reproductive and Developmental Toxicity Studies in Crj:CD(SD)IGS Rats - Fertility Study -

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ABSTRACT. In 1996-1997, Ina Research Inc. conducted several fertility and early embryonic developmental studies using Crj:CD(SD)IGS rats. In this paper, control data were compiled from these studies and compared to those of Crj:CD(SD) rats from studies performed at our laboratories during the last 5 years. Comparisons revealed an increase in the number of rats with 4-day estrous cycles and a decrease in the number of corpora lutea in IGS rats. —Key words: Crj:CD(SD)IGS, Rats, Fertility

CD (SD) IGS-1998: 236-237

INTRODUCTION

Charles River Japan, Inc. began supplying Crj:CD(SD)IGS rats (IGS rats) bred by the gold standard system in 1995. In reproductive and developmental studies using this strain of rats, historical control data play an important role in the accurate toxic evaluation of test substances. Control data concerning on estrous cycles, mating ability, fertility, sperm examinations, male genital organ weights and intrauterine findings obtained from several studies using IGS rats performed at Ina Research Inc. during 1996-1997 are presented. These studies were conducted in accordance with the standard operating procedures of INA Research Inc.with the exception of some differences in vehicle, feed and age of animals.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats purchased from the Tsukuba Breeding Center, Charles River Japan, Inc. were used for the studies. Animals were housed individually in stainless steel wire mesh cages in an animal room which was maintained at a temperature of 21-25°C with a relative humidity of 40-70% and provided with air changes 16 times per hour with a 12-hour light-dark cycle (7 AM to 7 PM). Animals were given free access to autoclaved pelleted feed (NMF, CRF-1 or CR-LPF, Oriental Yeast Co., Ltd.) and tap water.

The estrous phase of each female was determined every morning for 2 weeks by microscopic examination of vaginal smears 10 weeks of age. The number of cycles and the length of the cycle in days were calculated. Mating ability was observed in males and females aged 13 and 12 weeks or more, respectively. Animals were co-housed on a one-to-one basis from the evening until the following morning for a maximum of 14-21 days. Mating was determined by the presence of a vaginal plug in the vagina or sperm in the vaginal smear. The day on which evidence of mating was confirmed was designated as Day 0 of gestation. Males were euthanized at 16-19 weeks of ages and their testes, epididymides, seminal vesicles and prostates were weighed.

Sperm examinations were performed using semen collected from the cauda epididymis. A loop of semen was collected and diluted. A few drops of diluted semen were placed on a sperm motility slide which had been warmed to approximately 37°C and sperm motility was examined under a microscope, both at 40 and 240 minutes after dilution. The sperm motility rate was calculated from the total sperm and non-motile sperm counts. Malformations were recorded following inactivation.

Mated females were euthanized on Day 14 or 15 of gestation and intrauterine examinations were performed. The uteri in which implantations were not visible were immersed in a 10% solution of ammonium sulfide to confirm implantation sites. The numbers of corpora lutea, implantations, live fetuses and dead embryos were recorded in each pregnant animal. Dead embryos were classified into the following two categories: early resorptions (implantation sites only or unformed embryos) and late resorptions (formed embryos with placentas or macerated embryos).

RESULTS AND DISCUSSION

The number of animals observed was 66-116 per parameter and the minimum to maximum mean values from the studies are presented for each parameter. The estrous cycle, mating and fertility indices, sperm examinations, male genital organ weights and embryonic data are shown in Tables 1, 2, 3, 4 and 5, respectively. Background data on IGS rats were compared to those on Crj:CD(SD) rats (CD rats), bred at the Atsugi Breeding Center, from 15 studies conducted at Ina Research Inc. during the last 5 years (1993-1997).

The mean length of the estrous cycle and the number of estrous cycles were comparable between the two strains. The incidence of a 4-day estrous cycle was 80% in IGS rats and approximately 67% in CD rats, suggesting a slight difference between strains. There were no remarkable differences between both strains in the mating index, fertility index, sperm motility, sperm motility rate, sperm count and the incidence of sperm malformations. Comparison of male genital organ weights could not be performed due to absence of available data on CD rats. Reproductive findings at Caesarean sectioning during the middle stages of gestation revealed slight decreases in the number of corpora lutea in IGS rats (16.7-17.9 vs 17.5-20.9 in CD rats). There were no differences evident in the number of implantations, implantation index, number of live embryos and post-implantation loss between two strains.

The intrastrain differences in the estrous cycle and number of corpora lutea have attracted considerable interest. However, it cannot be concluded whether these differences were incidental or specific to IGS rats since the number of IGS rats examined was minimal. Therefore, more historical, background data in IGS rats are necessary for more accurate comparisons.

Table 1. Estrous cycle in female rats

Number of studies	4
Number of animals	80
Mean length of estrous cycle (days)	4.13～4.33
Mean number of cycles	3.08～3.20
Estrous cycle pattern	
4 days	64
4～5	5
5	5
3～4	1
4～6	3
4～8	1
4～9	1

Table 2. Mating and fertility in rats

Number of studies	6
Number of males	116
females	116
Males	
Successfully mated (%)	95.0～100.0
Impregnated (%)	100.0
Females	95.0～100.0
Successfully mated (%)	100.0
Pregnant (%)	
Precoital time (days)	1.6～3.1

Table 3. Sperm examination of male rats

Number of studies	4
Number of animals	66
Sperm motility after dilution:	
40 min	3.2 ～3.8
240 min	3.3 ～3.9
Number of sperms ($\times 10^6$/mL)	2149 ～2415
Motility ratio (%)	75.5 ～80.2
Number of sperms observed	500 sperms / animal
Number of sperms with malformation (%)	3～177 (0 ～3.6)
Head	1～146 (0 ～2.9)
Neck	0～45 (0 ～0.5)
Middle piece	0～37 (0 ～0.4)
Tail	0 (0.0)

Table 4. Organ weights in male rats of 16～19 weeks of age

Number of studies	4	
Number of animals	79	
	Absolute (g)	Relative (g%)
Testes	3.33～3.51	0.618～0.710
Epididymides	1.28～1.35	0.248～0.268
Seminal vesicles	2.15～2.34	0.415～0.473
Prostate	0.539～0.621	0.0993～0.1260

Table 5. Reproductive findings at cesarean section in dams on Day 14 or 15 of gestation

Number of studies	4
Number of animals	76
Number of corpora lutea	16.7 ～17.9
Number of implantations	15.1 ～16.8
Implantation index (%)	90.8 ～94.1
Number of live embryos (%)	14.4 ～15.3
Post implantation loss (%)	3.0 ～8.6
Early resorptions (%)	2.7 ～8.2
Late resorptions (%)	0.0 ～0.7

Historical Control Data for Reproductive and Developmental Toxicity Studies in Crj:CD(SD)IGS Rats - Teratology Study-

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ABSTRACT. Control data obtained from several embryo/fetal developmental studies performed at Ina Research Inc. using Crj:CD(SD)IGS rats during 1996-1997 are presented. This data was also compared to background control data on Crj:CD(SD) rats from studies conducted at our laboratories during the last 5 years (1993-1997). Decreases in the number of corpora lutea, implantations and live fetuses, and increases in the fetal weight, incidence of lumbar ribs and number of sacral and caudal vertebrae and digit bones were observed in Crj:CD(SD)IGS rats. — Key words: Crj:CD(SD)IGS, Rats, Teratology

CD (SD) IGS-1998: 238-239

INTRODUCTION

Charles River Japan, Inc. began supplying Crj:CD(SD)IGS rats (IGS rats) bred by the gold standard system in 1995. In reproductive and developmental toxicity studies using this strain of rats, historical control data play an important role for accurate study evaluation. Control data on intrauterine findings and morphological observations of fetuses from several studies using IGS rats performed during 1996-1997 are presented. The studies were conducted in accordance with the standard operating procedures of INA Research Inc. with the exception of some differences in vehicles, feed and age of the animals.

MATERIALS AND METHODS

IGS rats purchased from the Tsukuba Breeding Center, Charles River Japan, Inc. were housed individually in stainless steel wire mesh cages. Animal rooms were maintained at a temperature of 21-25°C with a relative humidity of 40-70% and provided with air changes 16 times per hour with a 12-hour light-dark cycle (7 AM to 7 PM). Animals were given free access to autoclaved pelleted feed (NMF, CRF-1 or CR-LPF, Oriental Yeast Co., Ltd.) and tap water.

Males and females, aged 12 and 11 weeks or more respectively, were co-housed overnight on a one-to-one basis to achieve pregnancy. The day on which evidence of copulation (sperm in vaginal smears) was confirmed was designated as day 0 of gestation. Females were euthanized on Day 20 of gestation and intrauterine examinations were performed. The numbers of corpora lutea, implantations and live and dead fetuses were counted. Dead fetuses were classified into the following 2 categories: early deaths (implantation sites only or unformed fetuses) and late deaths (formed fetuses with placentae or macerated fetuses). Live fetuses were observed for external anomalies including those in the oral cavity, and were sexed and weighed. Placental weight was also recorded. Approximately one-half of the live fetuses were fixed in Bouin's solution and their internal organs/tissues were examined under a stereoscopic microscope according to the modified methods of Wilson[1] and Nishimura[2]. The remaining live fetuses were cleared and stained with alizarin red S by the modified Dawson's method[3], and examined for skeletal anomalies and variations under a stereoscopic microscope. The number of ossified bones of the sacral and caudal vertebrae, sternebrae and digits was recorded as an index of the degree of ossification.

RESULTS AND DISCUSSION

The number of animals observed was 91-93 dams and 667-700 fetuses for each parameter. The minimum to maximum mean values from studies are presented for each parameter as follows: the numbers of corpora lutea, implantations and live fetuses, implantation index, male proportion, fetal and placental weights and fetal mortality in Table 1; visceral anomalies in Table 2; skeletal anomalies in Table 3; skeletal variations in Table 4 and skeletal ossification in Table 5.

Historical control data on IGS rats were compared to those on CD rats, supplied from the Atsugi Breeding Center, compiled from approximately 30 studies conducted at INA Research during the last 5 years.

The numbers of corpora lutea, implantations and live fetuses were 15.8-17.3, 14.8-16.5 and 13.7-15.7 in IGS rats and 16.5-21.2, 14.0-18.1 and 13.0-16.8 in CD rats, respectively. These parameters were slightly lower in IGS rats than in CD rats. In conjunction with these values, slightly increased fetal weights (3.92-4.22 g in male and 3.67-4.00 g in female IGS rats vs 3.50-4.12 g in male and 3.41-3.85 g in female CD rats) were noted; whereas placental weights (488-526 mg in IGS rats vs 497-576 mg in CD rats) were somewhat decreased in IGS rats. The implantation index, male proportion and fetal mortality rate were comparable between both strains. The incidence in skeletal variations was higher in IGS rats than in CD rats, especially in the incidence of lumbar ribs which was 8.0-16.2% in IGS rats and 0.5-8.9% in CD rats. Increased numbers of sacral and caudal vertebrae (8.3-8.5 in IGS rats vs 7.9-8.4 in CD rats) and proximal phalanges in the forelimbs (1.3-2.0 in IGS rats vs 0.2-0.9 in CD rats), probably related to increased fetal weight, were noted in IGS rats. There were negligible differences between IGS and CD rats in the incidences of visceral and skeletal anomalies and morphological findings.

Though comparison of background data between IGS and CD rats is rather difficult due to minimum historical data in IGS

rats, it is suggested that IGS rats may have a specific characteristic of an increased number of corpora lutea since similar results were obtained in fertility studies [4]. An increased incidence of lumbar ribs is likely to be another characteristic of IGS rats. However, more historical data is necessary to accurately characterize the IGS rat.

Table 1. Reproductive findings at cesarean section in dams on Day 20 of gestation

Number of studies	5
Number of dams	95
Number of corpora lutea	15.8 ～ 17.3
Number of implantations	14.8 ～ 16.5
Implantation index (%)	89.2 ～ 95.9
Number of live fetuses	13.7 ～ 15.7
Male proportion (%)	44.4 ～ 52.1
Body weight (g) males	3.92 ～ 4.22
females	3.67 ～ 4.00
Placental weight (mg)	488 ～ 526
Dead fetuses (%)	3.2 ～ 10.6
Early deaths	3.2 ～ 8.7
Late deaths	0.0 ～ 1.8
Number of fetuses with external anomalies	0

Table 2. Visceral anomalies in fetuses (F_1)

Number of studies	5
Number of dams	93
Number of fetuses	667
Number of fetuses with anomalies (%)	10～21(8.2～19.2)
Hypoplasia of aorta	0～2 (0.0～1.9)
Persistent left umbilical artery	0～5 (0.0～3.7)
Persistent right azygos vein	0～3 (0.0～2.0)
Hypoplasia of left atrioventricular canal	0～2 (0.0～1.9)
Hypoplasia of left ventricular	0～2 (0.0～1.9)
Ventricular septal defect	0～2 (0.0～1.9)
Thymic remnant in neck	6～15 (4.7～14.8)
Accessory spleen	0～1 (0.0～0.8)
Situs inversus totalis	0～1 (0.0～0.8)

Table 3. Skeletal anomalies in fetuses (F_1)

Number of studies	5
Number of dams	92
Number of fetuses	700
Number of fetuses with anomalies (%)	0～1 (0.0～0.7)
Hypoplasia of thoracic vertebral body	0～1 (0.0～0.7)

Table 4. Skeletal variations in fetuses (F_1)

Number of studies	5
Number of dams	92
Number of fetuses	700
Number of fetuses with variations (%)	15～30 (11.2～19.0)
Vertebrae	
Cervical rib	0～4 (0～3.1)
Splitting of thoracic vertebral body	0～2 (0.0～1.3)
Lumbar rib	11～24 (8.0～16.2)
7 lumbar vertebrae	0～1 (0～0.8)
Ribs	
Wavy rib	0～3 (0～1.9)
Sternebrae	
Splitting of sternebra	0～2 (0～1.5)
Asymmetry of sternebra	0～1 (0～0.6)

Table 5. Skeletal ossification in fetuses (F_1)

Number of studies	3
Number of dams	54
Number of fetuses	401
Number of sacral and caudal vertebrae	8.3～8.5
Number of sternebrae	5.9
Forelimbs	
Number of metacarpi	7.7～7.9
Number of proximal phalanges	1.3～2.0
Number of distal phalanges	10.0
Hindlimbs	
Number of metatarsi	8.0
Number of distal phalanges	10.0

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Background Data for Reproductive and Developmental Toxicity Studies in Crj:CD(SD)IGS Rats

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ABSTRACT. To collect background data for reproductive and developmental toxicity studies, female Crj:CD(SD)IGS (IGS) rats were clinically observed during the gestation period and external, internal and skeletal malformations in their fetuses were examined. The data obtained were compared with those of the Crj:CD(SD) (CD) strain. Estrous cycles before mating and body weight gains and food consumption during the gestation period in the IGS strain were similar to those of the CD strain. The numbers of ovulation and implantation, and the embryo/fetal mortality in the IGS strain were not significantly different from those in the CD strain. The number of live fetuses, fetal body weights, placental weights, and sex ratio in the IGS strain did not differ significantly from those in the CD strain. The observations of external, visceral and skeletal malformations in fetuses revealed no noticeable differences in the incidences of these parameters between the IGS and CD strains. Consequently, IGS rats seemed to be comparable to CD rats in reproductive and developmental toxicity studies. −Key words: Background, CD(SD)IGS Rat, Development, Reproduction

CD (SD) IGS-1998: 240-243

INTRODUCTION

The gold standard system, which has been developed by Charles River, Inc., is a new breeding procedure of laboratory rats. By this system, it is possible to produce uniform laboratory rats because of the genetic ramification control. Crj:CD(SD)IGS (IGS) rats have been produced by the gold standard system and have been widely used in various toxicity studies. The animals are expected to fit internationalization of research and development of new drugs. However, background data on reproductive and developmental parameters of IGS rats have not yet been fully accumulated. Therefore, we investigated reproductive conditions of adult animals of the IGS strain and spontaneous malformations in their offspring. The results thus obtained were compared with those of the Crj:CD(SD) strain.

MATERIALS AND METHODS

Twenty 10-week-old male and 20 9-week-old female Crj:CD(SD)IGS (IGS) rats were purchased from Tsukuba Breeding Center (Charles River Japan, Inc., Chiba, Japan). The rats were housed individually in a wire-mesh cage (21 ×35 ×20 cm) and were maintained in a barrier-sustained room controlled at 21 - 23°C 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 standard laboratory diet for rats (CR-LRF with γ-ray irradiation, Oriental Yeast Co., Tokyo, Japan) and tap water.

All IGS rats were observed for clinical signs and mortality twice daily. All females were examined for estrous cycles using the vaginal smear method during 2 weeks before mating completed. At 13 and 12 weeks of age in males and females, respectively, they were paired on a one-to-one basis for a maximum of 8 days. Males which copulated were sacrificed by exsanguination under ether anesthesia. The day on which the presence of a vaginal plug or sperm in vaginal smears was confirmed was designated as day 0 of gestation. Pregnant females were weighed on gestational days 0, 6, 8, 10, 12, 14, 16, 18, and 20. Food consumption (per day) of the females was measured on gestational days 1, 7, 9, 11, 13, 15, 17, 19, and 20. On gestational day 20, the females were sacrificed by exsanguination under ether anesthesia and necropsied, and they were laparotomized for counting of corpora lutea as an index of the number of ova shed. The numbers of implantations and, of live and dead fetuses were counted. Dead embryos and fetuses were classified into following three groups: Implantation sites represent embryo-death in an early stage of pregnancy showing embryo-site less than 3 mm in diameter; placental remnants represent embryo-death in the middle stage of pregnancy showing embryo-site equal to or larger than 3 mm in diameter but less than the placental diameter; and dead fetuses represent fetal death in the late stage of pregnancy showing fetal size larger than the placental diameter. After measurements of placental and body weights, live fetuses were sexed for calculation of the sex ratio and observed for grossly visible external anomalies including oral cavity. Approximately half the live fetuses from each litter were fixed in Bouin's solution and observed for head and abdominal abnormalities according to the method of Barrow and Taylor [1] and for the thoracic visceral abnormalities by the method of Nishimura [2]. The remaining live fetuses were fixed in ethanol, then cleared and stained by the Dawson's method [3], and observed for skeletal anomalies. Forty-seven male and female Crj:CD(SD) (CD) rats served as the control animals were maintained and examined by the same method as that for IGS rats. The statistical significance was assessed by Student's t-test for numerical data.

RESULTS AND DISCUSSION

None of the rats in both IGS and CD strains developed any clinical signs throughout the observation period. Body weight gain of the pregnant rats of the IGS strain during the gestation period was similar to that of the CD strain and there were no

significant differences between body weights of both strains (Table 1). Food consumption of the pregnant rats of the IGS strain during the gestation period did not differ significantly from that of the CD strain (Table 2).

The estrous cycle of the IGS strain was 4.1 ± 0.3 days and that of the CD strain was 4.2 ± 0.4 days and no significant differences were observed between the estrous cycles of both strains (Table 3). The copulation and fertility indices of the IGS strain were 100%. Grossly, a gray nodule, 10 mm in diameter, was observed in the subcutis of the neck of an IGS rat and was diagnosed histopathologically as adenocarcinoma of the mammary gland. In observations of reproductive parameters, the mean numbers of corpora lutea and implants, and a percentage of the implantation loss in the IGS strain were smaller than those in the CD strain (Table 4). The number of corpora lutea in the IGS strain was significantly ($p < 0.05$) smaller than in the CD strain. On the other hand, no significant differences were observed in the number of live fetuses between the IGS and CD strains. This was in agreement with the absence of significant differences between the numbers of implantation and dead embryos or fetuses of both strains. The number of dead embryos and fetuses in the IGS strain was 1.0 ± 1.2 (6.8%) and that in the CD strain was 1.0 ± 1.1 (6.1%) (Table 4). In observations of fetal development, the number of live fetuses, the placental weight, and the sex ratio (% of male) in the IGS strain were smaller than those in the CD strain, whereas the fetal body weights of the IGS strain were greater than those of the CD strain (Table 5). However, no significant differences were present between these values of both strains. All parameters collected from the IGS strain in the present study were in the range of the minimal and maximal values from different experiments using the CD strain in a previous paper [4].

No grossly visible abnormalities were detected by external and oral cavity examinations on fetuses of the IGS strain (Table 6). Visceral observations revealed abnormalities in 19 of 195 fetuses (9.8%) of the IGS strain (Table 6). In this strain, there were persistent left umbilical artery in two fetuses (1.0%), accessory liver in one fetus (0.5%), dilatation of renal pelvis in 13 fetuses (6.7%), and thymic remnant in the neck in 4 fetuses (2.3%). When compared with the CD strain, dilatation of renal pelvis was observed in higher frequency, whereas accessory liver and thymic remnant in the neck occurred in lower frequency. However, these values in the IGS strain were in the range from the minimal to maximal values from different

Table 1. Body Weights (g) of Female Rats of the IGS and CD Strains during Gestation

Strain	No. of rat examined	Day of gestation 0	6	8	10	12	14	16	18	20
IGS	30	236 ± 13	268 ± 14	276 ± 15	284 ± 16	295 ± 16	305 ± 17	320 ± 18	346 ± 21	374 ± 23
CD	47	233 ± 12	265 ± 14	273 ± 14	283 ± 15	296 ± 15	308 ± 16	325 ± 18	354 ± 21	383 ± 27

Values represent mean ± S.D.

Table 2. Food Consumption (g/day) of Female Rats of the IGS and CD Strains during Gestation

Strain	No. of rat examined	Day of gestation 1	7	9	11	13	15	17	19	20
IGS	30	22 ± 3	27 ± 3	26 ± 3	26 ± 3	27 ± 2	27 ± 3	28 ± 3	29 ± 3	27 ± 2
CD	47	21 ± 2	26 ± 2	27 ± 3	27 ± 3	28 ± 3	27 ± 4	29 ± 3	29 ± 3	29 ± 3

Values represent mean ± S.D.

Table 3. Estrous Cycles and Fertility in Female Rats of the IGS and CD Strains

Strain	No. of rat examined	Estrous cycle (days)	Fertility Copulation index (%)	Fertility index (%)
IGS	30	4.1 ± 0.3	100.0 (30/30)	100.0 (30/30)
CD	47	4.2 ± 0.4	100.0 (47/47)	97.9 (46/47)

Values represent mean ± S.D.

Table 4. Ovulation and Implantation in Rats of the IGS and CD Strains

Strain	No. of pregnant rats	No. of corpora lutea	No. of implantations	Implantation loss (%)	No. of dead embryos and fetuses IS	PR	DF	Total	(%)
IGS	30	15.7 ± 2.0[a]	14.6 ± 1.7	6.5 ± 9.3	1.0 ± 1.1	0.1 ± 0.3	0.0 ± 0.0	1.0 ± 1.2	(6.8)
CD	46	19.2 ± 2.8	16.3 ± 2.0	13.9 ± 12.5	1.0 ± 1.0	0.1 ± 0.3	0.0 ± 0.0	1.0 ± 1.1	(6.1)

Values represent mean ± S.D.
IS: Implantation sites.
PR: Placental remnants.
DF: Dead fetuses.
[a]: $p<0.05$.

Table 5. Fetal Developments in Rats of the IGS and CD Strains

Strain	No. of pregnant rats	No. of live fetuses		Body weights (g)		Placental weights (mg)		Sex ratio
		Male	Female	Male	Female	Male	Female	(% male)
IGS	30	5.9 ± 1.8	7.6 ± 2.3	3.82 ± 0.25	3.61 ± 0.20	456 ± 47	448 ± 48	44.4 ± 14.1
CD	46	7.6 ± 2.3	7.7 ± 2.2	3.64 ± 0.26	3.47 ± 0.24	508 ± 50	493 ± 44	49.5 ± 14.1

Values represent mean ± S.D.

Table 6. Morphological Observations on Fetuses of Rats of the IGS and CD Strains

Strain	IGS	CD
No. of litters	30	46
External observation		
No. of fetuses examined	406	619
No. of fetuses with abnormalities (%)	0 (0.0)	2 (0.3)
Visceral observation		
No. of fetuses examined	195	299
No. of fetuses with abnormalities (%)		
Persistent left umbilical artery	2 (1.0)	3 (1.6)
Accessory liver	1 (0.5)	9 (3.5)
Dilatation of renal pelvis	13 (6.7)	2 (0.3)
Thymic remnant in neck	4 (2.3)	13 (4.2)
Total	19 (9.8)	27 (9.9)
Skeletal observation		
No. of fetuses examined	211	320
No. of fetuses with abnormalities (%)		
Wavy rib	1 (0.5)	2 (0.6)
Total	1 (0.5)	2 (0.6)
No. of fetuses with variations		
Shortening of cervical vertebral arch	2 (1.0)	19 (5.2)
Splitting thoracic vertebral body	1 (0.5)	3 (0.8)
Dumbbell shape of thoracic vertebral body	11 (5.4)	36 (11.0)
20 thoracic and lumbar vertebrae	1 (0.5)	0 (0.0)
Asymmetry of sternebrae	1 (0.5)	1 (0.3)
Cervical rib	1 (0.5)	2 (0.6)
Rudimentary 14th rib	27 (12.8)	3 (1.6)
Total	43 (20.6)	87 (27.6)
Degree of ossification		
Cervical's corpus vertebrae	1.0 ± 0.7 [a]	0.2 ± 0.2
Sacral and coccygeal bone	8.0 ± 0.3	7.4 ± 0.5

[a]: Values represent mean ± S.D.

experiments using the CD strain in a previous survey [4]. In skeletal observations, 1 of 211 fetuses (0.5%) in the IGS strain had skeletal abnormalities and 43 fetuses (20.6%) showed skeletal variations. These findings, however, did not differ significantly from those of the CD strains (Table 6). In the IGS strain, skeletal variations, such as rudimentary 14th rib, dumbbell shape of thoracic vertebral body, shortening of cervical vertebral arch, splitting thoracic vertebral body, 20 thoracic and lumber vertebrae, asymmetry of sternebrae, and cervical rib were observed in 27 (12.8%), 11 (5.4%), 2 (1.0%), 1 (0.5%), 1 (0.5%), 1 (0.5%), and 1 (0.5%) fetuses, respectively. The incidence of rudimentary 14th rib in the IGS strain was markedly higher than in the CD strain. The incidences of shortening of cervical vertebral arch and dumbbell shape of thoracic vertebral body in the IGS strain were markedly lower than in the CD strain. However, these values in the IGS strain were in the range of the minimal and maximal values from different experiments reported in a previous survey [4]. In observations of ossification, the numbers of cervical's corpus vertebrae and sacral/coccygeal bone in the IGS strain were 1.0 ± 0.7 and 8.0 ± 0.3, respectively, but these figures did not differ significantly from those of the CD strains.

The results from the present study revealed that there were no noticeable differences in the reproductive and developmental parameters between the IGS and CD strains. Furthermore, all parameters described here were in the range of the minimal and maximal values obtained from different experiments using the CD strain [4]. It is, therefore, likely that the IGS strain is comparable to the CD strain in reproductive and developmental toxicity studies.

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Reproductive Toxicology Control Data for Crl:CD(SD)IGS Rats

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ABSTRACT. Comparisons of reproductive toxicology historical control data for parameters required in the ICH guidelines from CD[R] and the IGS CD[R] rats were made. For the fertility study no differences were noted among the male reproductive parameters. Maternal parameters in the pre and post natal study were similar. For the dams lower body weights and body weight gains were seen during the pre-mating and gestation periods. For the IGS rats and there was a tendency to lower litter sizes and related parameters for each study type. Lower fetal and pup weights were noted in the IGS litters. Other parameters for the offspring including fetal abnormalities and pup survival were considered to be comparable. – Key words: Rat, Crl:CD(SD)IGS, Reproductive toxicology, ICH

CD (SD) IGS-1998: 244-247

INTRODUCTION

The rat is a required species in all 3 standard studies ("most probable option") of the International Conference on Harmonization (ICH, 1993[1]) guidelines for reproductive toxicology testing. It is generally recognized that an outbred strain, with a good fertility rate is required for reproductive toxicology screening studies. Further, because of the importance of historical control data to the interpretation of findings, particularly from the embryo-fetal development studies, most testing laboratories utilize only a single strain of rat for reproductive toxicology testing from which they generate in-house historical control data.

One of the most commonly used strains of rat has been the "CD" rat from Charles River Laboratories (CRL). Currently, CRL provide the International Genetic Standard (IGS) CD rats which are claimed to be similar between various facilities and stable across time (CRL, 1996[2]). Prior to the establishment of the IGS animals CRL had been modifying their breeding programs (CRL, 1993[3]) to attempt to alleviate problems with longevity, fertility and increasing litter size in the CD animals.

Also, researchers had noted differences in parameters such as growth rates between different CRL breeding facilities and temporal changes for parameters such as litter size. Therefore, the provision of animals that are similar from the various CRL sites and that are more stable across time has great advantages for toxicology testing laboratories, not only for applicability of results from studies performed with animals from different CRL facilities, but in terms of timely supply.

To allow for examination of the current status of the IGS rats, the CD and IGS animals were compared by evaluating compiled historical data. Due to the size of the data sets, the limited number of CRL sites utilized by our laboratory and the comparatively short time the IGS animals have been available stability across time and similarity between CRL breeding sites has not been investigated.

MATERIALS AND METHODS

Animals were supplied from CRL facilities at Kingston and Hollister in the USA, and St Constant, in Canada. The animals prior to the IGS[R] designation were identified as Crl:CD(SD)BR, subsequently described here as CD rats were supplied mainly from Kingston, while subsequently, for the IGS rats (Crl:CD(SD)IGS), primarily Kingston and Canada, but also Hollister, have been used. All animals were housed individually, except during mating, in stainless steel wire mesh-bottomed cages, equipped with an automatic watering valve. Mated females in the pre and postnatal studies were transferred to solid-bottomed cages (with similar automatic watering) and housed on corn-cob bedding (Bed'O-Cob[R]) on gestation Day 18. Each cage was clearly labelled with a colour-coded cage card indicating project, group, animal numbers, sex and dose level. Each animal was uniquely identified using a tail tattooing system.

The conditions for animal room environment and photoperiod were temperature 22 ± 3°C; humidity 50 ± 20% and light cycle 12 hours light and 12 hours dark. The room air was changed with fresh air 10-15 times per hour.

All animals had free access to a standard certified pelleted commercial laboratory diet (PMI Certified Rodent Chow 5002: PMI Feeds Inc.). Municipal tap water which had been softened, purified by reverse osmosis and sterilized by ultraviolet light was freely available.

One female was placed with 1 proven male/partner during the mating period. The females were examined daily for evidence of mating by examination of the vaginal lavage for spermatozoa. The day of positive identification of spermatozoa in the vaginal lavage was termed Day 0 of gestation.

Animals were examined twice daily for mortality and signs of ill health or reaction to treatment on all studies. A complete detailed examination was performed at least at the same frequency as body weight assessments.

Any animal found dead as well as those euthanized during the study or at termination were given a complete necropsy examination. The uterus of any euthanized animal judged to be nonpregnant was stained with 10% aq. ammonium sulfide solution and examined for implantation sites (Sal-ewski, 1964[4]).

ICH 1 - Fertility and Early Embryonic Development Study: At the start of dosing for these studies the males were 12 weeks of age and the females 10 weeks of age. Treatment was typically performed for 4 weeks for males and 2 weeks for females prior to placement for mating, so that the animals were 16 and 12 weeks of age at mating, for males and females,

respectively. Males were usually dosed throughout a 3 week mating period and for another 2 to 3 weeks before necropsy at approximately 21 weeks of age. The mated dams were dosed during the mating period and up to Day 7 of gestation. In-life observations included, individual body weights and food consumption measured weekly, commencing the day of randomization and extending through the treatment period. Mated females were weighed on Days 0, 3, 7, 10 and 13 of gestation. The estrous cycles of the females were determined for the 10 days prior to placement for mating by examination of the vaginal lavage. Following the premating treatment period, 1 female was placed with 1 male in the same dosage group for a maximum of 21 days. Females failing to show signs of mating were killed at the end of the mating period.

For each male, the epididymides, prostate, seminal vesicles and testes were dissected free of fat and weighed. The left cauda epididymis was used to provide samples for assessments of sperm motility (either manually or with a computer-assister sperm analyzer), sperm concentration (millions/gram of epididymis), and sperm morphology (the percent of abnormal sperm was assessed). The right testis was prepared for histological examination by embedding in glycol methacrylate, sectioned and stained with PAS hematoxylin and examined. On Day 13 of gestation the reproductive tract of the females was dissected out, the ovaries weighed and the corpora lutea counted. The uterine contents were examined and the number and position of live embryos, dead embryos and resorptions was recorded.

ICH 2 - Pre and Postnatal Study: Females for these studies started dosing at 12 weeks of age. The animals were treated from Day 6 of gestation until at least Day 21 *post partum*, inclusive. A complete detailed examination was performed on the days of body weight assessment. Mated females were weighed on Days 0, 3, 6, 9, 12, 15, 18 and 20 of gestation and on Days 0, 4, 7, 14, 17 and 21 *post partum*. Individual food consumption was measured at similar intervals up to Day 18 of gestation. Females were observed for signs of parturition and where possible, parturition was observed, the time of onset and completion of parturition was recorded and any sign of dystocia noted. The females' behavior immediately *post partum* was observed. The day of completion of littering was termed Day 0 *post partum*.

The F_1 Generation Pups: After parturition (Day 0 *post partum*) the pups were examined for malformations, sexed and the numbers of live and dead recorded. The live pups were weighed individually. Daily litter observations included general condition of the pups which was evaluated each day during the lactation period. In addition to the assessment of body weight at birth, the pups were weighed individually on Days 4, 7, 14 and 21 *post partum*. On Day 4 *post partum* the litter were culled to 8 pups, where necessary, to give a litter of 4 males and 4 females, where possible. The selected pups were identified by tattooing of the paws. Parameters of physical and reflexological development were assessed, but the data on these observations is not presented. At weaning, on Day 21 *post partum*, the F_1 generation were separated from their dams and 1 male and 1 female rat was randomly selected from each litter, where possible, to provide the F_1 adult generation.

Observations - F_1 adult generation: Observations of clinical condition, body weight and observations at parturition and during lactation were similar to those of the F_0 generation dams and for the F_2 pups, observations up to Day 4 *post partum* were similar to the F_1 generation. Visual Function, on Day 21 *post partum*, the pupillary closure and visual placing responses were tested. Vaginal opening for females and preputial separation for males was assessed. For behavioral performance locomotor activity, auditory startle habituation and water maze were examined. Data for these sensory, physical and behavioral observations is not presented.

Terminal Procedures for Adult Generations: At their termination, at times relevant to their reproductive status the uteri of all females were examined and, if appropriate, the numbers of implantation site scars recorded.

Terminal Procedures For Pups (F_0 and F_1 Generations): Pups found dead or dying on or before Day 7 *post partum* and any pups born malformed which were euthanized and placed in Bouin's fluid for subsequent examination using a modified Barrow and Taylor[5] technique. F_1 generation pups dying between Days 8 and 20 *post partum* and unselected weanlings were given a complete gross external examination.

ICH 3 - Embryo-Fetal Development Study: Females started dosing at 12 weeks of age. The animals were treated from Day 6 to Day 17 of gestation, inclusive. A complete detailed examination was performed on the days of body weight assessment. Individual body weights and food consumption were measured on Days 0, 3, 6, 9, 12, 15, 18 and 20 of gestation.

On gestation Day 20 the reproductive tract was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterine contents, including the placentas, were examined and the number and position of live fetuses, dead fetuses and early, middle and late resorptions was recorded.

For fetal examinations each fetus was weighed, given a detailed external examination, the external sex recorded and the fetus killed by subcutaneous injection of euthanasia solution. A detailed internal examination using a dissecting microscope was performed on approximately 1/2 of the fetuses in each litter which were then eviscerated. The heads of these fetuses were removed and placed in Bouin's fluid for examination by the Technique of Wilson[6]. The remaining 1/2 of the fetuses in each litter were eviscerated and were placed in 85% ethanol/15% methanol for subsequent staining with alizarin red S (using a modified Dawson technique[7]) and skeletal examination. Abnormalities were classified as major malformations, minor visceral or skeletal anomalies or common skeletal variants (Palmer, 1977 [8]).

Data is presented as group mean ranges, with calculation on a litter mean basis where appropriate. For sperm motility, comparable data was not available because the CD data was collected manually and most of the IGS data was collected using an automated system.

RESULTS

ICH 1 - Fertility and Early Embryonic Development Study: Males showed no clear differences for body weight or body weight gain, however, females had slightly lower weights and body weights gains in the pre-mating period. The fertility data (Table 1) were similar between the CD and IGS animals. The gestation Day 13 uterine examinations revealed a trend toward lower numbers of live embryos and associated parameters among the IGS animals (Table 2). Male reproductive assessments (Table 3), values for organ weights and sperm count were again similar; there were some differences for epididymal sperm morphology ranges, however, consideration of data from prior CD studies showed values comparable to the IGS animals.

Table 1. Fertility

Strain / Parameter	CD	IGS
Body Weight (g) Week 0		
Males	397-436	359-431
Females	223-274	214-235
Body Weight Gain (g)		
Males Week 0-4	86-109	58-128
Females Week 0-2	27-48	20-37
Mean Day to Mating	2.6-4.0	2.5-3.9
Mating Index %	100	95-100
Conception Rate %	75-100	82-96
Fertility Index %	75-100	83-96

Mating Index % = Number of animals mating/Number of animals placed for mating x 100
Conception Rate % = Number of females pregnant/Number of females mating x 100
Fertility Index % = Number of females pregnant/Number of females placed for mating x 100

Table 2. Gestation Day 13 Uterine Findings

Strain / Parameter	CD	IGS
Number of corpora lutea/dam	16.1-20.3	16.4-18.4
Number of implantations/dam	15.1-18.6	14.9-16.8
Number of live embryos/dam	14.3-17.3	13.8-15.8
Number of dead embryos/dam	0.0-0.2	0.0-0.2
Number of early resorptions/dam	0.8-1.4	0.7-1.0
Pre-implantation loss %	3.4-11.9	5.6-10.9
Post-implantation loss%	4.3-10.7	4.1-9.6

Pre-implantation loss % = No. of corpora lutea-no. of implants/ no. of corpora lutea x 100
Post-implantation loss% = No. of implants-no. of live embryos / no. of implants x 100

Table 3. Male Reproductive Assessments

Strain / Parameter	CD	IGS
Organ Weights -		
Epididymis (left)	0.64-0.71	0.66-0.71
Prostate	1.27-1.50	1.32-1.67
Testis (right)	1.78-1.98	1.74-1.86
Seminal Vesicles	0.97-1.30	0.95-1.12
Epididymal Sperm. -		
Count x	644-901	703-889
Morphology (% abnormal)	4.2-4.6	2.6-3.3

ICH 3 - Embryo-Fetal Development Study: The maternal body weights at term were clearly lower as shown by the corrected body weights, further the corrected body weight gains were also lower (Table 4). The remaining maternal performance data (Table 4) showed similar values between the CD and IGS animals. For the cesarean section (Table 5) data there is a clear trend toward higher numbers of corpora lutea, implantations and live fetuses for the CD animals.

The fetal findings data (Table 6) shows similar values for abnormalities between the CD and IGS fetuses, but a tendency to lower fetal weights in the IGS animals.

Table 4. Maternal Performance

Strain / Parameter	CD	IGS
Corrected Body Weights (g)	327-362	292-332
Corrected Body Weight gain (g)	38-67	15-39
No. of dams +ve with ammonium sulfide	0	0
No. of dams dying/euthanized during gestation	0	0-1
No. of dams with total resorption	0-2	0
No. of dams littering early	0	0
Pregnancy Rate %	84-100	88-100

Corrected Body Weight = Body weight on gestation day 20 - gravid uterine weight
Corrected Body Weight Gain = Body weight gain gestation days 6 to 20 - gravid uterine weight

Table 5. Gestation Day 20 Uterine Findings

Strain / Parameter	CD	IGS
Number of corpora lutea/dam	17.3-19.4	16.7-17.5
Number of implantations/dam	16.0-17.4	15.2-16.3
Number of live fetuses/dam	14.4-16.8	14.3-15.7
Sex Ratio (% males)	44.2-51.7	48.9-56.6
Number of dead fetuses/dam	0.0-0.1	0.0
Number of early resorptions/dam	0.6-1.7	0.4-1.3
Number of middle resorptions/dam	0.0	0.0
Number of late resorptions/dam	0.0	0.0
Pre-implantation loss %	7.0-16.3	5.8-10.0
Post-implantation loss%	3.6-17.2	2.7-9.1

Pre-implantation loss % = No. of corpora lutea-no. of implants/no. of corpora lutea x 100
Post-implantation loss% = No. of implants-no. of live fetuses/no. of implants x 100

Table 6. Fetal Findings

Strain / Parameter	CD	IGS
Fetal Weight (g)		
Male	3.9-4.3	3.7-4.2
Female	3.7-4.0	3.5-3.9
Combined	3.8-4.1	3.6-4.1
Major Malformations		
Litters Affected	0.0-12.0	0.0-12.0
Fetuses Affected	0.0-0.8	0.0-0.8
Minor External and Visceral Anomalies		
Litters Affected	0.0-20.8	0.0-16.0
Fetuses Affected	0.0-1.3	0.0-1.1
Minor Skeletal Anomalies		
Litters Affected	87.0-92.0	(27.5)+87.5-90.0
Fetuses Affected	13.3-24.7	6.6-26.5
Minor Skeletal Variants - % Fetuses/litter		
Thoracic centrum	30.6-46.5	9.7-46.4
Sternebrae 1 to 4	3.4-8.8	2.6-9.0
Sternebrae 5 and 6	47.4-82.0	59.6-82.6

+ Change in technical procedure, 1/2 of fetuses per litter only examined

ICH 2 - Pre and Postnatal Study: The maternal data (Table 7) of the littering studies showed a tendency to lower litter sizes at birth in the IGS rats, obviously this being related to the lower numbers of fetuses per litter noted in the other study designs. Pup data (Table 8) showed lower pups weights from birth to weaning, however, pup survival was similar.

Table 7. Dam Maternal Performance

Strain	CD	IGS
Parameter		
Pregnancy Rate %	91.7-93.3	95.2-100.0
Gestation Index %	92.9-100.0	95.3-100.0
Length of gestation (days)	21.4-21.9	21.5-21.9
Number of live pups/dam	13.5-16.1	13.5-15.0
Number of dead pups/dam	0.0-0.6	0.1-0.7
Number of implant. sites/dam	14.8-17.8	15.3-16.7
Live Birth Index %	87.1-92.6	88.2-93.9

Table 8. Litter Data

Strain	CD	IGS
Parameter		
Pup Survival		
Viability index %	97.8-99.8	96.7-100.0
Lactation Index %	99.2-100.0	99.0-100.0
Pup Weight (g)		
Day 0 - Male	6.4-6.8	6.2-6.4
Female	6.1-6.5	5.8-6.0
Day 4 - Male	10.0-11.0	9.3-10.0
Female	9.5-10.4	8.8-9.6
Day 7 - Male	16.6-17.1	15.3-16.2
Female	15.9-16.3	14.8-15.4
Day 14 - Male	34.5-36.9	32.6-32.8
Female	33.4-35.7	31.6-31.7
Day 21 - Male	55.9-61.6	53.0-53.9
Female	55.1-59.0	50.7-51.8

DISCUSSION

In each study type (ICH 1, 2 and 3), there was a trend toward lower litter sizes among the IGS animals. This appears to support CRL's contention that the revised breeding program would decrease the litter size (CRL, 1993). Also the fetal and pup weights suggest similar lower weights among the IGS animals. One might expect lower litter sizes to produce a slightly heavy litter, however this does not appear to be the case. For males in the fertility studies no clearly lower body weight was identified, although the lower pup weights at weaning might be expected to give lower weights among the adult males. For the females lower weights and body weights gains were evident in the pre-mating period. This lower growth is supported by the lower corrected body weight and corrected body weight gains seen during gestation. In our laboratory we had not seen any deterioration in fertility among the CD rats and so not surprisingly no differences for the male parameters were noted. Many maternal parameters were similar.

The differences noted here are between CD animals from the Kingston facility as compared to IGS animals, primarily, from the Kingston and Canadian facilities. Since there were considered to be differences between CD's various CRL facilities the conclusions cannot necessarily be extended CD rats from other CRL facilities.

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Comparison of Reproductive and Developmental Parameters in Crj:CD(SD)IGS rats with the historical control data of Crj:CD(SD) rats

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ABSTRACT. We examined the reproductive and developmental characteristics of the Crj:CD (SD)IGS rat, developed by the gold standard system, a new animal breeding system developed by Charles River, Inc. to obtain uniform quality in experimental animals, in comparison with the historical data for the Crj:CD (SD) rat.

Clear differences were found in some reproductive and developmental data between the Crj:CD(SD)IGS and Crj:CD(SD) rats. Some endpoints were different from lot to lot, such as fertility index and implant rate in the Crj:CD(SD)IGS females (F0). The presence of such differences should be taken into consideration when using the Crj:CD (SD) IGS strain in reproductive and developmental toxicity studies.

—Key words: Crj:CD (SD) IGS rat, Development, Historical data, Reproduction

CD (SD) IGS-1998: 248-260

INTRODUCTION

The gold standard system is a new animal breeding system developed by Charles River, Inc. to obtain uniform quality in experimental animals. The Crj:CD (SD) IGS rats (hereafter referred to as IGS rats) developed by this system, can be used for internationalization of scientific research and development of new chemicals. However, their biological parameters have not been characterized yet. We compared the reproductive and developmental data in IGS rats with those in the historical data in Crj:CD (SD) rats (hereafter referred to as SD rats).

MATERIALS AND METHODS

Animals and husbandry

Fifty male and 50 female specific pathogen free (SPF) IGS rats were supplied by Charles River Japan Inc. (Kanagawa Japan) at 7 weeks of age. After arrival, all animals were examined visually for diseases and were housed individually in stainless-steel wire mesh cages in a temperature- and humidity-controlled room. The temperature range was 24±2°C and relative humidity was maintained at 55±10%. The room air was changed 15 times per hour. A 12-hour light/12-hour dark cycle was maintained (Fluorescent light from 07:00 to 19:00 ; 150-300 lux). Commercial rodent diet and tap water were always available. The animals were allotted to the cesarean section and the delivery group, 25 males and 25 females per group.

Study design

The estrous cycle in females was observed for 14 days before mating. One female was placed together for mating with one male for a maximum of 2 weeks. Vaginal smears were observed microscopically during the mating period continuing to the day before copulation. The day on which sperm was present in the vaginal smear was defined as Day 0 of gestation. The stage of estrus was recorded before and during the mating period, and the mean estrous cycle was calculated in days (number of days between each estrus). The animals were examined until successful copulation after mating. Copulation index was calculated from the results of mating data for both sexes. The fertility index was also calculated.

The body weight of pregnant dams was measured on Days 0, 4, 7-20 of gestation during the gestation period, and on Days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 21 after delivery during the lactation period. Body weight gain was calculated from Day 0 to 20 of gestation, and from Day 0 to 21 of the lactation period. Daily food consumption of pregnant dams was measured on Days 1, 4, 7, 11, 14 and 20 of gestation during the gestation period, and on Days 4, 8, 12, 16, 18 and 21 after delivery during the lactation period.

Organ weight measurement and the clinical laboratory tests were conducted on all males at the necropsy after mating. Blood samples were collected from the abdominal aorta under ether anesthesia following 16 hours of fasting. In hematology, hematocrit (HCT), hemoglobin (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and white blood cell count (WBC) were determined by THMS H·1E (Miles, U.S.A.). Differential leucocyte ratios were determined by the flow cyto chemistry method. Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen (FIB) of plasma were determined by a blood clotting time automatic analyzer KC–40 (Amelung Co., Germany). In blood chemistry, we measured serum levels of total protein, albumin, A/G, glucose, triglyceride, total cholesterol, blood urea nitrogen (BUN), total bilirubin, calcium, inorganic phosphate, sodium, potassium and chloride by EKTACHEM 700N (EASTMAN KODAK Co., U.S.A.), creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and γ–glutamyl transpeptidase(γ –GTP) by CentrifiChem ENCORE II (Baker Instruments Corp., U.S.A.).

Copulated females in the cesarean section group were anesthetized with ether and killed by exsanguination from the abdominal aorta on Day 20 of gestation. Ovaries and uteri were removed and the rats with uterine implantations were

necropsied and intrauterine examinations were performed. The number of corpus lutea, number of implantations, number of live fetuses and number of resorptions and dead fetuses (number of early deaths, number of late deaths, number of dead fetuses) were recorded and preimplantation loss rate, implantation rate, live fetus rate and resorption and dead fetus rate were calculated. Also, placentae of live fetuses were weighed after grossly observed for abnormalities. External examinations were performed on all live fetuses. The sex of the fetuses was identified and sexual ratios were calculated. Individual body weights were also measured and mean body weights of males and females of a litter were calculated. Half of the animals in each litter were fixed with Bouin's fixing fluid. Heads and abdomens were microscopically observed according to the modification of Wilson's method [5] and breasts according to Nishimura's microscopic necropsy method [3] for visceral abnormalities. Remaining litters except for those fetuses which were used for visceral examinations, were fixed with 70% ethyl alcohol and Alizarin red S stained transparent skeletal specimens were prepared following the modified Dawson method [1]. Skeletal variation and ossification were examined on specimens.

All successfully copulated females in the delivery group were allowed to deliver naturally, and observed for abnormalities. The duration of gestation and gestation index were calculated. Live newborns received milk for 22 days (date of delivery = lactation Day 0), during which period lactation was observed. At necropsy of dams on Day 22 of lactation, organ weight measurement was performed and live birth index and delivery index were calculated by observing the number of implants. The numbers of live newborns and stillbirth were recorded at birth separately for males and females and external observations were performed and sex ratios of live pups were calculated. Live newborns were observed for deaths, cannibalism and general conditions daily for 22 days after birth and the viability index of live pups at 4 days after birth and weaning index were calculated. When the number of pups of a litter exceeded 8 at 4 days after birth, the number was adjusted to 8 (4 males and 4 females, as a rule). Male and female pups were weighed separately on a litter basis on Days 0, 4, 7, 14, 21 and 22 of lactation. Growth of live pups during the lactation period was examined by observing detachment of auricles, eruption of lower incisors and separation of eyelids until weaning, each litter was recorded as one unit. At weaning, 2 male and 2 female pups from each litter were necropsied, while the remaining pups were weaned. At the same time, 1 male and 1 female selected from each litter were examined for visual placing response, Preyer's reflex, pinna reflex, pupillary reflex, corneal reflex and grip tone. After weaning, the growth of offspring was examined by observing descent of testis and opening of vagina and body weight of offspring was measured once a week until the age of 9 weeks. The open field test [2] was performed to examine emotionality on 1 animal/sex/litter at the age of 4-5 weeks.

At the age of 9 weeks, the estrous cycle in F1 females was observed for 2 weeks. F1 females were assigned for mating with non-sibling males on a one-to-one basis at the age of 11 weeks. All successfully copulated females were weighed on Days 0, 4, 8, 12, 16 and 20 of gestation. At Day 20 of gestation, the cesarean section was performed in the same manner as in F0 generation. External examinations were performed on all live fetuses(F2). Sexual identification was performed and sexual ratios were calculated. The body weight of each animal was measured and the mean body weight was calculated for males and females of a litter.

Statistical analyses

For comparison between the data on IGS rats and the historical data on SD rats, statistical significance was tested using Dunnet's test, χ^2-test, the Aspin-Weltch t-test and Steel's test. A difference was considered statistically significant at $P < 0.05$ and $P < 0.01$ [4].

RESULTS AND DISCUSSION

The results of mating and estrus cycle in the F0 animals are presented in Table 1. No significant differences in copulation index were observed between IGS and SD rats. The fertility index in IGS rats was lower than that in SD rats. This difference was considered to have been due to the lot used this study, because the values in the historical control data using IGS rats in the other 2 studies performed at the An-pyo center ranged between 90 and 95%. No significant differences were observed in mean estrus cycle between IGS and SD rats.

At necropsy after mating, no abnormalities in gross findings were observed in IGS males. Absolute and relative organ weight of male animals(F0) are presented in Table 2. Absolute weights of heart and spleen, absolute and relative liver weights in SD males were significantly higher than those in IGS males. Relative weights of lungs, heart, spleen, kidneys, adrenals, testes and epididymides in SD males were significantly lower than those in IGS males.

Table 1. Copulation and fertility results in animals(F0)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
Copulation index (%) [1)]	47/50 (94.0)	20/20 (100.0)	22/22 (100.0)
Fertility index (%) [2)]	39/47 (83.0)	20/20 (100.0)	20/22 (90.9)
Estrus cycle (days)			
(Mean±S.D.)	4.2 ± 0.4 (50)	4.2 ± 0.4 (20)	4.9 ± 0.7** (21)

1):(No. of animals with successful copulation / no. of animals mated) x 100
2):(No. of pregnant animals / no. of animals with successful copulation) x 100
Values in parentheses are expressed no. of animals observed
**:P<0.01

Table 2. Absolute and relative organ weight of male animals(F0) after mating

Strain		Crj:CD(SD)IGS	Crj:CD(SD)
No. of animal examined		50	20
		Mean ± S.D.	Mean ± S.D.
Body weight	(g)	457 ± 29	534 ± 50
Lung	(g)	1.50 ± 0.15	1.47 ± 0.10
	(g%)	0.329 ± 0.037	0.276 ± 0.021**
Heart	(g)	1.44 ± 0.11	1.54 ± 0.11**
	(g%)	0.316 ± 0.025	0.288 ± 0.016**
Liver	(g)	12.18 ± 1.19	20.38 ± 2.59**
	(g%)	2.664 ± 0.154	3.813 ± 0.265**
Spleen	(g)	0.71 ± 0.10	0.77 ± 0.12*
	(g%)	0.157 ± 0.022	0.145 ± 0.019*
Kidneys	(g)	3.18 ± 0.32	3.34 ± 0.37
	(g%)	0.695 ± 0.059	0.627 ± 0.048**
Adrenals	(mg)	70 ± 10	64 ± 11
	(mg%)	15.296 ± 2.449	12.081 ± 2.325**
Testis-R	(g)	1.67 ± 0.23	1.70 ± 0.12
	(g%)	0.367 ± 0.055	0.320 ± 0.033**
Testis-L	(g)	1.64 ± 0.27	1.69 ± 0.12
	(g%)	0.361 ± 0.062	0.320 ± 0.033**
Epididymis-R	(mg)	619 ± 84	604 ± 47
	(mg%)	130.027 ±20.519	13.795 ± 11.113**
Epididymis-L	(mg)	617 ± 66	605 ± 38
	(mg%)	135.598 ±16.649	113.947 ± 10.469**

(%):(Organ weight / Body weight) x 100

*:P<0.05 **:P<0.01

The results of hematology and coagulation studies on the male animals are presented in Table 3-1 to 3-2. In the hematological examination, significant decreases in the values of HGB,RBC,MCHC and LUC and significant increases in the value of MCV and WBC were noted in SD males compared to those in IGS males. No strain-related differences were observed in the coagulation of blood. Blood chemistry of male animals are presented in Table 4. The levels of glucose, triglyceride, total cholesterol, total bilirubin, sodium and calcium were significantly higher in SD males, while BUN, creatinine and chloride levels were significantly lower in SD males than in IGS males.

The body weight changes of dams(F0) are presented in Figure 1. The body weight of IGS females(F0) was significantly lower than that of SD females(F0) during the gestation period. However, no significant differences were observed during the lactation period. Food consumption of dams(F0) is presented in Figure 2. No significant differences were observed between IGS and SD females(F0) either during the gestation or the lactation period.

Reproductive findings in dams(F0) and observations on their fetuses(F1) are presented in Table 5. The number of total corpora lutea, number of total implants, number of total live fetuses and number of total live male fetuses in SD rats were significantly higher and weights of live male and female SD fetuses were significantly lower than those in the IGS fetuses. The implant rate was lower in IGS rats than that in SD rats. This change was caused to have been due to the lot used this study, because historical control data using the IGS rats in the other 2 studies performed at the An-pyo center ranged between 94.4 and 94.5%.

Absolute and relative organ weights of the dams(F0) at the cesarean section are presented in Table 6. Absolute weights of kidneys, absolute and relative liver weights in SD rats were significantly higher than those in IGS rats. Relative weights of lungs, heart and adrenals in SD rats were significantly lower than those in the IGS strain. Cysts in the kidneys and thinning of hair were observed singly in IGS females at cesarean section.

External findings on live fetuses are presented in Table 7. Pes varus was observed in IGS rats. The results of visceral examination in live fetuses are presented in Table 8. The incidence of fetuses with visceral abnormalities was significantly higher in SD rats than in IGS rats. As abnormal findings, thymic remnant in the neck was observed in fetuses in both strains, and the incidence of this findings was significantly higher in SD rats than in IGS rats. As other findings, ventricular septal defect and left umbilical artery were observed in a few IGS fetuses. The results of skeletal examination in live fetuses are presented in Table 9. No significant differences in the incidence of fetuses with skeletal malformations were observed between the two strains. As abnormal findings, nodulation of lib, wavy rib, deformity of frontal bone, deformity of parietal bone and deformity of interparietal bone were observed in either IGS or SD rats. There were no significant differences in the incidence of these findings. As the skeletal variations, no significant differences were observed in the incidence of fetuses with skeletal variations between the two strains. As abnormal findings, splitting of thoracic vertebral body, 5th lumbar vertebra, splitting of lumbar vertebral body, lumbar rib, shortening of 13th rib and cervical rib were sporadically observed in each strain. No differences were observed in the degree of ossification between the two strains.

The findings of delivery are presented in Table 10. The number of implants, number of newborns and number of live newborns tended to be higher in SD rats than in IGS rats.

Absolute and relative organ weight of dams(F0) at weaning are presented in Table 11. Absolute and relative weights of lung and absolute weights of heart were significantly lower in SD rats than in IGS rats.

Viability of pups during the lactation are presented in Table 12. No differences in viability index on day 4 or weaning index were observed between the two strains.

External differentiations in F1 animals are presented in Table 13 and 14. The completion rate for detachment of auricle on day 3 was significantly higher in SD rats than in IGS rats. The completion rate for eruption of lower incisor on day 11 and 12 was higher in SD rats than in IGS rats. The completion rate for separation of eyelids on day 14 and 15 was higher in the SD rats than IGS rats. No differences were observed in the appearance day of testis or vaginal opening between the two strains.

Table 3-1. Hematology of male animals(F0)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of animals examined	50	12	12	12
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
HCT (%)	43.3 ± 1.3	43.4 ± 1.7	40.6 ± 1.5**	44.5 ± 1.4*
HGB (g/dl)	15.8 ± 0.5	14.5 ± 0.4**	14.6 ± 0.6**	15.4 ± 0.4
RBC (x10^6/mm^3)	8.69 ± 0.28	7.90 ± 0.45**	7.95 ± 0.35**	8.24 ± 0.26**
MCV (μm^6)	49.8 ± 1.7	55.0 ± 1.5**	51.1 ± 1.9*	54.0 ± 1.1**
MCH (pg)	18.2 ± 0.6	18.3 ± 0.6	18.4 ± 0.7	18.7 ± 0.4*
MCHC (%)	36.5 ± 0.5	33.4 ± 0.4**	36.0 ± 0.3*	34.7 ± 0.5**
PLT (x10^3/mm^3)	1024 ± 91	969 ± 98	983 ± 157	1177 ± 124**
WBC (x10^3/mm^3)	7.8 ± 2.3	11.3 ± 2.2**	12.1 ± 2.8**	10.8 ± 3.8**
Differential leukocyte counts (%)				
NEUT	16 ± 4	15 ± 4	13 ± 4	13 ± 5
LYMPH	79 ± 5	79 ± 4	82 ± 4	83 ± 6
MONO	2 ± 1	3 ± 1**	2 ± 1	2 ± 1
EOSN	1 ± 1	1 ± 1	1 ± 0*	1 ± 0
BASO	0 ± 0	0 ± 0	0 ± 0	0 ± 0
LUC	2 ± 1	1 ± 0**	1 ± 0**	1 ± 0*

NEUT : Neutrophil LYMPH : Lymphocyte MONO : Monocyte EOSN : Eosinophil
BASO : Basophil LUC : Large unstained cells
*:P<0.05 **:P<0.01

Table 3-2. Coagulation of male animals(F0)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
No. of animals examined	50	12	12
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
PT (sec.)	15.3 ± 1.9	13.1 ± 0.3**	14.6 ± 0.9
APTT (sec.)	25.9 ± 2.7	24.0 ± 1.8**	25.4 ± 1.7
Fibrinogen (mg/dl)	248 ± 19	260 ± 21	245 ± 26

**:P<0.01

Table 4. Blood chemistry of male animals(F0)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of animals examined	50	12	12	12
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
T.protein (g/dl)	5.90 ± 0.28	5.68 ± 0.22*	5.86 ± 0.28	6.20 ± 0.23**
Albumin (g/dl)	3.18 ± 0.14	3.24 ± 0.12	3.15 ± 0.12	3.19 ± 0.09
A/G	1.18 ± 0.06	1.33 ± 0.04**	1.17 ± 0.05	1.06 ± 0.05**
Glucose (mg/dl)	132 ± 16	164 ± 15**	153 ± 13**	148 ± 20**
Triglyceride (mg/dl)	40.5 ± 9.7	NE	79.2 ± 32.9**	59.3 ± 23.2**
T.cholesterol (mg/dl)	34 ± 7	54 ± 14**	58 ± 12**	53 ± 7**
BUN (mg/dl)	14.5 ± 2.3	12.4 ± 1.1**	11.4 ± 1.0**	14.2 ± 1.6
Creatinine (mg/dl)	0.65 ± 0.07	0.56 ± 0.07**	0.69 ± 0.06	0.58 ± 0.08**
T.bilirubin (mg/dl)	0.18 ± 0.02	0.07 ± 0.02**	0.41 ± 0.02**	0.21 ± 0.03**
GOT (U/l)	43 ± 6	41 ± 11	37 ± 6*	41 ± 11
GPT (U/l)	17 ± 3	16 ± 5	16 ± 3	17 ± 2
ALP (U/l)	110 ± 19	NE	126 ± 22*	85 ± 18**
Gamma-GTP (U/l)	0.3 ± 0.3	0.6 ± 0.3*	0.5 ± 0.3	0.2 ± 0.3
Sodium (mmol/l)	143.7 ± 1.2	NE	145.0 ± 1.1**	144.8 ± 1.4*
Potassium (mmol/l)	4.63 ± 0.24	4.88 ± 0.22*	4.61 ± 0.29	4.51 ± 0.31
Chloride (mmol/l)	110.8 ± 2.1	106.9 ± 1.5**	108.0 ± 1.3**	109.5 ± 2.1
Calcium (mg/dl)	9.69 ± 0.27	10.00 ± 0.18**	10.30 ± 0.29**	9.34 ± 0.25**
I.phosphorus (mg/dl)	6.49 ± 0.63	6.64 ± 0.63	6.29 ± 0.39	6.26 ± 0.69

*:P<0.05 **:P<0.01
NE:Not examined

Table 5. Reproductive findings in dams(F0) and observations on their fetuses(F1)

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of dams examined		20	23	21	19
No. of dams with live fetuses		20	23	21	19
No. of total corpora lutea	(Mean ± S.D.)	309(15.5 ± 3.4)	425(18.5 ± 3.4)**	379(18.0 ± 2.5)*	354(18.6 ± 2.1)**
No. of total implants	(Mean ± S.D.)	231(11.6 ± 5.6)	379(16.5 ± 2.3)**	314(15.0 ± 3.2)*	326(17.2 ± 2.8)**
No. of total live fetuses	(Mean ± S.D.)	220(11.0 ± 5.3)	355(15.4 ± 2.0)**	299(14.2± 3.5)*	292(15.4 ± 3.4)**
Male	(Mean ± S.D.)	97(4.9 ± 3.0)	175(7.6 ± 1.8)**	136(6.5 ± 2.6)	137(7.2 ± 2.9)*
Female	(Mean ± S.D.)	123(6.2 ± 3.3)	180(7.8 ± 2.2)	163(7.8 ± 2.3)	155(8.2 ± 2.7)
Sex ratio (Male/Female)		0.79(97/123)	0.97(175/180)	0.83(136/163)	0.88(137/155)
No. of total resorptions and dead fetuses		11	24	15	34
Early death	(%, Mean ± S.D.)	11(3.5 ± 6.8)	21(5.2 ± 5.7)	13(5.6 ± 8.6)	28(8.8 ± 10.1)*
Late death	(%, Mean ± S.D.)	0(0.0 ± 0.0)	3(0.7 ± 1.9)	1(0.3 ± 1.4)	5(1.5 ± 3.3)
Dead fetuses	(%, Mean ± S.D.)	0(0.0 ± 0.0)	0(0.0 ± 0.0)	1(0.3 ± 1.4)	1(0.3 ± 1.3)
Weight of live fetuses(g)					
Male	(Mean ± S.D.)	3.83 ± 0.28	3.35 ± 0.22**	3.37 ± 0.26**	3.45 ± 0.23**
Female	(Mean ± S.D.)	3.64 ± 0.29	3.20 ± 0.20**	3.19 ± 0.22**	3.28 ± 0.23**
Weight of placenta(mg)					
Male	(Mean ± S.D.)	486 ± 96	495 ± 70	508 ± 138	489 ± 58
Female	(Mean ± S.D.)	483 ± 113	476 ± 48	495 ± 112	471 ± 59
Implant rate	(%, Mean ± S.D.) [1)]	76.0 ± 32.0	91.2 ± 15.5	83.0 ± 18.5	92.5 ± 13.6
Pre-implant loss rate	(%, Mean ± S.D.) [2)]	24.0 ± 32.0	8.8 ± 15.5	17.0 ± 18.5	7.5 ± 13.6
Live fetus rate	(%, Mean ± S.D.) [3)]	96.5 ± 6.8	94.1 ± 6.3	93.8 ± 8.6	89.4 ± 12.5**
Resorption and dead fetus rate	(%, Mean ± S.D.) [4)]	3.5 ± 6.8	5.9 ± 6.3	6.2 ± 8.6	10.6 ± 12.5**

1):(No. of implants / no. of corpora lutea) x 100
2):{(No. of corpora lutea - no. of implants) / no. of corpora lutea} x 100
3):(No. of live fetuses / no. of implants) x100
4):(No. of resorptions and dead featuses / no. of implants) x 100
*:P<0.05 **:P<0.01

Table 6. Absolute and relative organ weight of dams(F0) at the cesarean section

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of dams examined		20	23	21	19
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Body weight (g)		376 ± 36	427 ± 22	413 ± 24	425 ± 35
Corrected body weight(g) [1)]		316 ± 22	347 ± 20	338 ± 15	342 ± 25
Lung	(g)	1.24 ± 0.19	1.24 ± 0.10	1.20 ± 0.07	1.23 ± 0.08
	(g%)	0.392 ± 0.045	0.359 ± 0.029**	0.354 ± 0.021**	0.360 ± 0.024**
Heart	(g)	1.05 ± 0.09	1.06 ± 0.08	1.07 ± 0.10	1.08 ± 0.12
	(g%)	0.335 ± 0.024	0.305 ± 0.018**	0.316 ± 0.028*	0.317 ± 0.023*
Liver	(g)	15.01 ± 1.82	17.71 ± 1.43**	17.02 ± 1.15**	17.27 ± 2.13**
	(g%)	4.753 ± 0.444	5.108 ± 0.267**	5.031 ± 0.275*	5.041 ± 0.276*
Spleen	(g)	0.62 ± 0.07	0.72 ± 0.21	0.70 ± 0.11	0.72 ± 0.12
	(g%)	0.198 ± 0.026	0.208 ± 0.061	0.206 ± 0.028	0.211 ± 0.024
Kidneys (g)		1.94 ± 0.17	2.13 ± 0.16**	2.06 ± 0.12*	2.11 ± 0.18**
	(g%)	0.616 ± 0.037	0.616 ± 0.043	0.610 ± 0.033	0.618 ± 0.043
Adrenals	(mg)	87 ± 11	80 ± 12	76 ± 7**	80 ± 15
	(mg%)	27.453 ± 3.513	23.176 ± 3.746**	22.408 ± 2.337**	23.432 ± 4.097**
Ovaries	(mg)	127 ± 19	132 ± 26	129 ± 21	148 ± 21**
	(mg%)	40.051 ± 5.268	38.032 ± 6.962	38.053 ± 5.409	43.530 ± 5.787

1):Body weight - gravid uterus weight
(%):{Organ weight /(body weight - gravid uterus weight)} x 100
*:P<0.05 **:P<0.01

Table 7. External observations on live fetuses(F1)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of litters	20	23	21	19
No. of live fetuses examined	220	355	299	292
No. of live fetuses with external anomalies (%,Mean±S.D.)	1 2.5 ± 11.2	3 0.8 ± 2.2	1 0.3 ± 1.1	1 0.3 ± 1.2
Type and incidence of external anomalies (%) 1)				
Pes varus	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Anophthalmia	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)
Rudiment tail	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
General edema	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Exencephalia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Nanofetus	0 (0.0)	2 (0.6)	1 (0.3)	1 (0.3)

1):(No. of fetuses with external anomalies / no. of live fetuses examined) x 100

Table 8. Visceral observations on live fetuses(F1)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of litters	19	23	21	19
No. of live fetuses examined	105	173	144	140
No. of live fetuses with visceral anomalies (%,Mean±S.D.)	10 7.3 ± 12.5	55 32.5 ± 22.7**	36 23.5 ± 21.6*	27 17.4 ± 17.9
Type and incidence of visceral anomalies (%) 1)				
Ventricular septal defect	2 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)
Left umbilical artery	1 (1.0)	2 (1.2)	1 (0.7)	1 (0.7)
Thymic remnant in the neck	7 (6.7)	44 (25.4)**	20 (13.9)	26 (18.6)*
Malformative nodule of the liver	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Dilatation of the renal pelvis	0 (0.0)	9 (5.2)	14 (9.7)**	2 (1.4)
Dilatation of ureter	0 (0.0)	8 (4.6)	10 (6.9)*	1 (0.7)
Anophthalmia	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Situs inversus totailis	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)

1):(No. of live fetuses with visceral anomalies / no. of live fetuses examined) x 100
*:P<0.05 **:P<0.01

Table 9. Skeletal observations on live fetuses(F1)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD) 2	Crj:CD(SD)3
No. of litters	20	23	21	19
No. of live fetuses examined	115	182	155	152
No. of live fetuses with malformations (%,Mean±S.D.)	1 0.7 ± 3.2	0 -	0 -	1 0.5 ± 2.3
Type and incidence of malformations (%) 1)				
Nodulation of rib	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)
Wavy rib	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)
Deformity of frontal bone	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Deformity of parietal bone	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Deformity of interparietal bone	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
No. of live fetuses with variations (%,Mean±S.D.)	9 6.8 ± 12.4	9 4.9 ± 8.0	2 1.2 ± 3.8	10 5.7 ± 12.7
Type and incidence of variations (%) 2)				
Splitting of thoracic vertebral body	2 (1.7)	3 (1.6)	0 (0.0)	4 (2.6)
5 lumbar vertebra	0 (0.0)	1 (0.5)	0 (0.0)	5 (3.3)
Splitting of lumbar vertebral body	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Lumbar rib	7 (6.1)	5 (2.7)	1 (0.6)	0 (0.0)*
Shortening of 13th rib	0 (0.0)	1 (0.5)	0 (0.0)	2 (1.3)
Cervical rib	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.7)

1):(No. of live fetuses with malformations / no. of live fetuses examined) x 100
2):(No. of live fetuses with varietions / no. of live fetuses examined) x 100
*:P<0.05

Table 9 -continued. Skeletal observations on live fetuses(F1)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Cej:CD(SD)3
No. of litters	20	23	21	19
Type and incidence of incomplate ossifications (%)				
Parietal bone	2 (1.7)	2 (1.1)	0 (0.0)	0 (0.0)
Interparietal bone	1 (0.9)	10 (5.5)	0 (0.0)	0 (0.0)
Supraoccipital bone	2 (1.7)	17 (9.3)	4 (2.6)	1 (0.7)
Hyoid bone	35 (30.4)	55 (30.2)	29 (18.7)	26 (17.1)
Thoracic vertebral body	3 (2.6)	4 (2.2)	5 (3.2)	6 (3.9)
Cervical vertebral arch	0 (0.0)	8 (4.4)*	5 (3.2)	3 (2.0)
Lumbar vertebral arch	0 (0.0)	4 (2.2)	1 (0.6)	0 (0.0)
Lumbar vertebral body	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.7)
Cervical vertebral body	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Thoracic vertebral arch	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Ischium	2 (1.7)	3 (1.6)	1 (0.6)	2 (1.3)
Pubis	7 (6.1)	13 (7.1)	9 (5.8)	6 (3.9)

*:P<0.05

Table 10. Findings of delivery in dams(F0) and observations on their pups(F1)

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of dams examined		19	12	23	20
No. of dams observed		19	12	23	20
No. of dams delivered live newborns		19	12	23	20
Duration of gestation	(Mean ± S.D.)	21.8 ± 0.4	21.9 ± 0.3	22.0 ± 0.3	22.0 ± 0.0
No. of total implants	(Mean ± S.D.)	275 (14.5 ± 3.0)	190 (15.8 ± 1.5)	388 (16.9 ± 1.7)**	316 (15.8 ± 2.3)
No. of total newborns	(Mean ± S.D.)	259 (13.6 ± 2.7)	181 (15.1 ± 1.9)	362 (15.7 ± 1.6)*	287 (14.4 ± 3.6)
No. of total live newborns	(Mean ± S.D.)	259 (13.6 ± 2.7)	181 (15.1 ± 1.9)	361 (15.7 ± 1.5)*	287 (14.4 ± 3.6)
Male		115 (6.1 ± 2.1)	90 (7.5 ± 1.7) a)	177 (7.7 ± 2.2) a)	166 (8.3 ± 2.8)** a)
Female		144 (7.6 ± 2.3)	91 (7.6 ± 2.0)	184 (8.0 ± 1.9) a)	121 (6.1 ± 2.2) a)
Sex ratio (Male/Female)		0.80 (115/144)	0.99 (90/ 91)	0.96 (177/184)	1.37 (166/121)**
No. of total dead newborns	(Mean ± S.D.)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	1 (0.0 ± 0.2)	0 (0.0 ± 0.0)
stillbirth		0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)
cannibalism		0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	1 (0.0 ± 0.2)	0 (0.0 ± 0.0)
Gestation index	(%) 1)	100.0	100.0	100.0	100.0
Delivery index	(%, Mean ± S.D.) 2)	94.8 ± 6.6	95.2 ± 6.9	93.6 ± 6.9	90.6 ± 17.7
Birth index	(%, Mean ± S.D.) 3)	100.0 ± 0.0	100.0 ± 0.0	99.8 ±1.0	100.0 ± 0.0

1):(No. of females with live newborns / no. of pregnant females) x 100
2):(No. of newborns / no. of implants) x 100
3):(No. of live newborns / no. of newborns) x 100
Values in parentheses are expressed no. of dams observed
a):Includes live newborns died before observations
*:P<0.05 **:P<0.01

Table 11. Absolute and relative organ weight of dams(F0) at weaning

Strain .		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of dams examined		18	12	21	20
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Body weight	(g)	341 ± 22	329 ± 16	336 ± 17	347 ± 27
Lung	(g)	1.32 ± 0.14	1.21 ± 0.08**	1.19 ± 0.08**	1.24 ± 0.11*
	(g%)	0.389± 0.038	0.367 ± 0.021	0.353 ± 0.023**	0.357 ± 0.029**
Heart	(g)	1.25 ± 0.11	1.15 ± 0.10*	1.17 ± 0.08*	1.24 ± 0.09
	(g%)	0.367 ± 0.027	0.349 ± 0.031	0.347 ± 0.018	0.359 ± 0.035
Liver	(g)	17.17 ± 1.53	15.78 ± 1.20	16.28 ± 1.92	16.12 ± 1.53
	(g%)	5.034 ± 0.316	4.802 ± 0.341	4.832 ± 0.449	4.649 ± 0.318**
Spleen	(g)	0.63 ± 0.11	0.55 ± 0.05*	0.60 ± 0.08	0.60 ± 0.07
	(g%)	0.183 ± 0.027	0.167 ± 0.016	0.178 ± 0.024	0.173 ± 0.022
Kidneys	(g)	2.62 ± 0.27	2.57 ± 0.24	2.46 ± 0.18	2.66 ± 0.17
	(g%)	0.767 ± 0.063	0.782 ± 0.065	0.732 ± 0.047	0.771 ± 0.076
Adrenals	(mg)	79 ± 11	79 ± 12	84 ± 12	86 ± 14
	(mg%)	23.385 ± 2.523	24.090 ± 3.430	24.914 ± 3.716	25.110 ± 5.488
Ovaries	(mg)	102 ± 16	92 ± 13	90 ± 16	109± 21
	(mg%)	30.039 ± 4.718	28.009 ± 4.719	26.873 ± 4.334	31.794 ± 7.268

(%):(Organ weight / body weight) x 100
*:P<0.05 **:P<0.01

Table 12. Viability on pups(F1) during lactation period

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of dams examined	19	12	23	20
Viability index on day 4 [1)] (%,Mean±S.D.)				
Male	97.3 ± 5.5	90.8 ± 13.2	90.0 ± 21.3	95.4 ± 9.1
Female	92.9 ± 12.6	98.8 ± 4.1	87.8 ± 22.0	98.3 ± 4.1
Weaning index [2)] (%,Mean±S.D.)				
Male	98.6 ± 5.9	89.6 ± 19.8	97.7 ± 7.4	95.0 ± 10.3
Female	100.0 ± 0.0	83.3 ± 22.2**	97.7 ± 7.4	91.3 ± 18.6

1):(No. of live pups on day 4 / no. of live newborns) x 100
2):(No. of live pups at weaning days / no. of live pups on day 4 after adjustment) x 100
Values in parentheses expressed no. of litters observed
**:P<0.01

Table 13. External differentiations of pups(F1) during lactation period

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of litters examined	19	12	22	20
Detachment of auricle				
(day 2)	0.8 ± 2.5[a)] (19)	0.8 ± 2.6[a)] (12)	1.4 ± 6.7[a)](22)	0.9 ± 4.2[a)](20)
(day 3)	26.8 ± 33.3 (19)	65.4 ± 38.7 (12)*	57.5 ± 34.9 (22)*	37.9 ± 31.8 (20)
(day 4)	80.7 ± 33.7 (19)	NE	98.2 ± 4.7 (22)	85.5 ± 21.8 (20)
Eruption of lower incisor				
(day 10)	11.2 ± 14.8 (18)	15.6 ± 26.7 (12)	28.7 ± 23.3 (22)*	28.1 ± 25.3 (20)
(day 11)	24.5 ± 17.4 (18)	60.8 ± 36.9 (12)*	66.7 ± 25.2 (22)**	58.6 ± 25.6 (20)**
(day 12)	56.5 ± 28.0 (18)	95.3 ± 7.0 (12)**	91.6 ± 13.2 (22)**	83.1 ± 22.8 (20)**
Separation of eyelieds				
(day 14)	18.1 ± 29.1 (18)	23.0 ± 30.9 (12)	35.7 ± 25.1 (22)*	53.8 ± 34.6 (20)**
(day 15)	54.3 ± 41.7 (18)	77.9 ± 28.6 (12)	71.0 ± 23.5 (22)	89.5 ± 25.5 (20)**
(day 16)	92.4 ± 18.2 (18)	95.8 ± 14.4 (12)	98.3 ± 5.8 (22)	99.0 ± 4.5 (20)

a):No. of pups appeared / no. of pups observed (%,Mean ± S.D.)
Values in parentheses are expressed no. of litters observed
*:P<0.05 **:P<0.01
NE:Not examined

Table 14. External differentiations of offspring(F1) during growth period

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD) 2
Male			
No. of animals examined	35	24	44
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Descent of testes	23.3 ± 1.5 (35)	22.5 ± 1.4 (24)	22.6 ± 0.9 (44)*
Female			
No. of animals examined	33	23	44
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Opening of vagina	34.4 ± 2.7 (33)	34.1 ± 1.9 (23)	33.5 ± 2.2 (44)

*:P<0.05

Table 15-1. Sensory function test of male offspring(F1) at 22 days old

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of offspring examined	35	24	44	40
	a)	a)	a)	a)
Visual placing response	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	NE
Pain response (100g)	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	NE
Preyer's reflex4000Hz	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	40/40 (100.0)
Preyer's reflex8000Hz	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	40/40 (100.0)
Preyer's reflex 12000Hz	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	40/40 (100.0)
Pinna reflex	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	NE
Pupillary reflex	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	40/40 (100.0)
Corneal reflex	35/35 (100.0)	24/24 (100.0)	44/44 (100.0)	NE
Grip tone (g) Mean ± S.D. (N)	94.6 ± 8.2 (35)	93.4 ± 6.2 (24)	95.5 ± 7.0 (44)	NE

a):No. of positive responsive offspring / no. of offspring examined (%)
NE:Not examined

Table 15-2. Sensory function test of female offspring(F1) at 22 days old

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2	Crj:CD(SD)3
No. of offspring examined	36	23	44	40
	a)	a)	a)	a)
Visual placing response	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	NE
Pain response (100g)	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	NE
Preyer's reflex 4000Hz	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	40/40 (100.0)
Preyer's reflex 8000Hz	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	40/40 (100.0)
Preyer's reflex 12000Hz	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	40/40 (100.0)
Pinna reflex	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	NE
Pupillary reflex	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	40/40 (100.0)
Corneal reflex	36/36 (100.0)	23/23 (100.0)	44/44 (100.0)	NE
Grip tone (g) Mean ± S.D. (N)	91.6 ± 10.2 (36)	89.7 ± 8.0 (23)	95.0 ± 7.3 (44)	NE

a):No. of positive responsive offspring / no. of offspring examined (%)
NE:Not examined

Table 15-3. Open field test of male offspring(F1) at 4-5 weeks old

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
No. of offspring examined		17	10	22
		Mean ± S.D. (N)	Mean ± S.D. (N)	Mean ± S.D. (N)
ambulation	1	73.4 ± 31.6 (17)	47.6 ± 15.1 (10)*	62.3 ± 25.4 (22)
	2	96.1 ± 38.8 (17)	61.4 ± 25.6 (10)*	78.0 ± 22.5 (22)
	Mean	84.7 ± 30.1 (17)	54.5 ± 16.7 (10)**	70.1 ± 21.7 (22)
rearing	1	10.9 ± 4.3 (17)	6.2 ± 3.5 (10)*	8.5 ± 5.6 (22)
	2	9.2 ± 6.5 (17)	5.5 ± 4.4 (10)	6.2 ± 3.8 (22)
	Mean	10.1 ± 4.0 (17)	5.9 ± 2.9 (10)**	7.4 ± 4.4 (22)*
preening	1	0.0 ± 0.0 (17)	0.0 ± 0.0 (10)	0.0 ± 0.2 (22)
	2	0.1 ± 0.2 (17)	0.0 ± 0.0 (10)	0.0 ± 0.2 (22)
	Mean	0.0 ± 0.1 (17)	0.0 ± 0.0 (10)	0.0 ± 0.1 (22)
grooming	1	0.6 ± 1.0 (17)	0.6 ± 0.7 (10)	0.4 ± 0.7 (22)
	2	0.3 ± 0.5 (17)	0.6 ± 0.7 (10)	0.7 ± 0.9 (22)
	Mean	0.4 ± 0.6 (17)	0.6 ± 0.4 (10)	0.5 ± 0.6 (22)
defecation	1	1.0 ± 1.3 (17)	1.2 ± 0.9 (10)	1.6 ± 1.9 (22)
	2	1.9 ± 2.0 (17)	2.3 ± 1.6 (10)	2.1 ± 1.6 (22)
	Mean	1.4 ± 1.1 (17)	1.8 ± 1.1 (10)	1.9 ± 1.3 (22)

*:P<0.05**:P<0.01

Table 15-4. Open field test of female offspring(F1) at 4-5 weeks old

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
No. of offspring examined		18	11	22
		Mean ± S.D. (N)	Mean ± S.D. (N)	Mean ± S.D. (N)
ambulation	1	92.3 ± 39.4 (18)	47.7 ± 21.3 (11)**	61.7 ± 24.0 (22)*
	2	105.7 ± 32.4 (18)	54.5 ± 25.1 (11)**	90.2 ± 29.3 (22)
	Mean	99.0 ± 32.2 (18)	51.1 ± 19.4 (11)**	76.0 ± 24.3 (22)*
rearing	1	11.1 ± 5.4 (18)	7.5 ± 6.9 (11)	10.5 ± 5.7 (22)
	2	8.8 ± 5.2 (18)	5.0 ± 5.2 (11)	9.4 ± 4.8 (22)
	Mean	9.9 ± 4.4 (18)	6.2 ± 5.8 (11)*	10.0 ± 5.0 (22)
preening	1	0.1 ± 0.3 (18)	0.1 ± 0.3 (11)	0.0 ± 0.0 (22)
	2	0.0 ± 0.0 (18)	0.1 ± 0.3 (11)	0.0 ± 0.0 (22)
	Mean	0.1 ± 0.2 (18)	0.1 ± 0.2 (11)	0.0 ± 0.0 (22)
grooming	1	0.4 ± 0.9 (18)	1.1 ± 0.9 (11)	0.7 ± 0.9 (22)
	2	0.5 ± 0.9 (18)	0.6 ± 1.0 (11)	1.0 ± 1.2 (22)
	Mean	0.5 ± 0.8 (18)	0.9 ± 0.8 (11)	0.8 ± 0.9 (22)
defecation	1	1.9 ± 2.3 (18)	1.7 ± 1.8 (11)	1.9 ± 1.7 (22)
	2	1.4 ± 1.9 (18)	1.9 ± 1.4 (11)	1.6 ± 1.7 (22)
	Mean	1.7 ± 1.8 (18)	1.8 ± 1.0 (11)	1.8 ± 1.4 (22)

*:P<0.05 **:P<0.01

Table 16. Copulation and fertility results in animals(F1)

Strain	Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
Copulation index (%) [1)]			
Male	18/18 (100.0)	12/12 (100.0)	22/22 (100.0)
Female	15/15 (100.0)	12/12 (100.0)	21/21 (100.0)
Fertility index (%) [2)]			
Male	18/18 (100.0)	11/12 (91.7)	21/22 (95.5)
Female	15/15 (100.0)	11/12 (91.7)	20/21 (95.2)
Estrus cycle (days) (Mean±S.D.)	4.1 ± 0.3 (15)	4.3 ± 0.7 (12)	4.4 ± 0.6 (21)

1):(No. of animals with successful copulation / no. of animals mated) x 100
2):(No. of pregnant animals / no. of animals with successful copulation) x 100
Values in parentheses are expressed no. of animals observed

Table 17. Reproductive findings in dams(F1) and observations on their fetuses(F2)

Strain		Crj:CD(SD)IGS	Crj:CD(SD)1	Crj:CD(SD)2
No. of dams examined		15	11	20
No. of dams with live fetuses		15	11	20
No. of total corpora lutea		240 (16.0 ± 2.2)	180 (16.4 ± 2.5)	347 (17.4 ± 3.4)
No. of total implants	(Mean ± S.D.)	192 (12.8 ± 3.7)	166 (15.1 ± 2.0)	274 (13.7 ± 4.6)
No. of total live fetuses	(Mean ± S.D.)	182 (12.1 ± 3.7)	159 (14.5 ± 2.0)	257 (12.9 ± 4.7)
Male	(Mean ± S.D.)	97 (6.5 ± 2.4)	78 (7.1 ± 2.4)	128 (6.4 ± 2.8)
Female	(Mean ± S.D.)	85 (5.7 ± 2.6)	81 (7.4 ± 1.9)	129 (6.5 ± 3.3)
Sex ratio (Male/Female)		1.14 (97/ 85)	0.96 (78/ 81)	0.99 (128/129)
No. of total resorptions and dead fetuses		10	7	17
Early death	(%, Mean ± S.D.)	10 (5.3 ± 6.8)	6 (3.5 ± 4.3)	12 (4.6 ± 6.3)
Late death	(%, Mean ± S.D.)	0 (0.0 ± 0.0)	1 (0.6 ± 2.2)	4 (1.9 ± 4.3)
Dead fetuses	(%, Mean ± S.D.)	0 (0.0 ± 0.0)	0 (0.0 ± 0.0)	1 (0.3 ± 1.4)
Weight of live fetuses (g)				
Male	(Mean ± S.D.)	3.69 ± 0.27	3.31 ± 0.30**	3.58 ± 0.30
Female	(Mean ± S.D.)	3.55 ± 0.26	3.16 ± 0.26**	3.33 ± 0.25*
Weight of placenta (mg)				
Male	(Mean ± S.D.)	476 ± 56	474 ± 35	512 ± 72
Female	(Mean ± S.D.)	440 ± 45	461 ± 34	472 ± 54
Implant rate	(%, Mean ± S.D.) 1)	82.7 ± 26.7	92.8 ± 8.4	78.2 ± 25.5
Pre-implant loss rate	(%, Mean ± S.D.) 2)	17.3 ± 26.7	7.2 ± 8.4	21.8 ± 25.5
Live fetus rate	(%, Mean ± S.D.) 3)	94.7 ± 6.8	95.8 ± 4.3	93.2 ± 7.5
Resorption and dead fetus rate	(%, Mean ± S.D.) 4)	5.3 ± 6.8	4.2 ± 4.3	6.8 ± 7.5

1):(No. of implants / no. of corpora lutea) x 100
2):{(No. of corpora lutea - no. of implants) / no. of corpora lutea} x 100
3):(No. of live fetuses / no. of implants) x100
4):(No. of resorptions and dead featuses / no. of implants) x 100
*:P<0.05 **:P<0.01

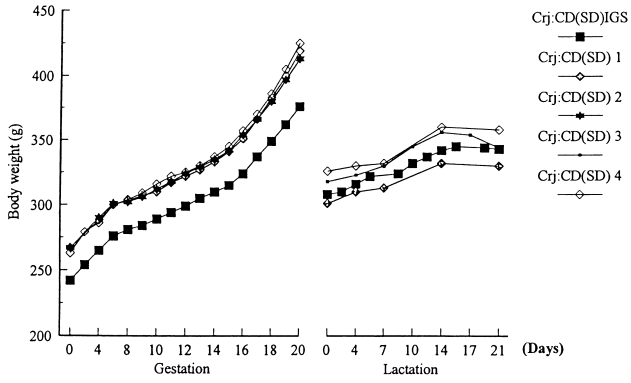

Fig. 1. Body weight change of dams(F0)

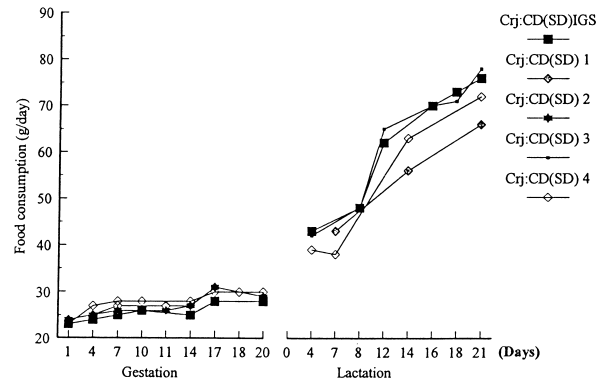

Fig. 2. Food consumption of dams(F0)

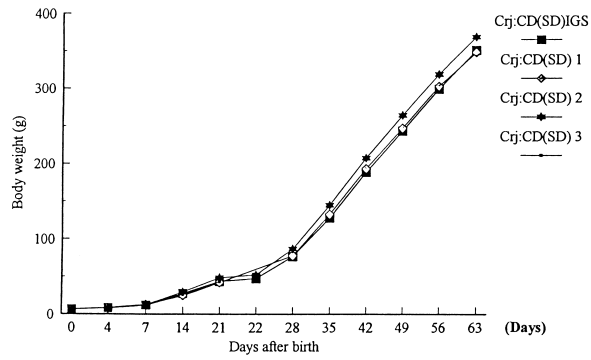

Fig. 3. Body weight change of male animals(F1)

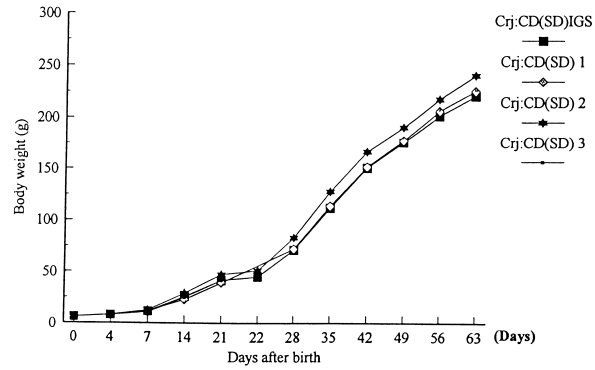

Fig. 4. Body weight change of female animals(F1)

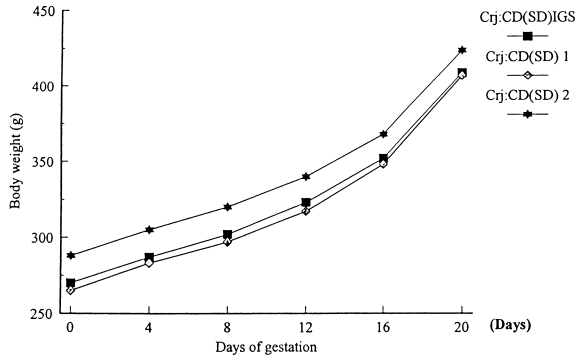

Fig. 5. Body weight change of dams(F1) during gestation period

The results of sensory function test and open field test are presented in Table 15-1 to 15-4. In the sensory function test, all parameters in the SD offspring were comparable to those in the IGS offspring. In the open field test, the mean number reared was significantly lower in SD males than in IGS males. The number of ambulation in the first trial and mean number of ambulation in females were significantly lower in SD rats than in IGS rats.

The body weight change of male and female rats(F1) is presented in Figure 3 and 4, respectively. Also, the body weight change of dams(F1) during the gestation period is presented in Figure 5. No strain-related differences were observed in them.

The results of mating and estrus cycle in F1 animals are presented in Table 16. No significant differences were observed between the two strains. The reproductive findings in dams(F1) and observations on their fetuses(F2) are presented in Table 17. The weights of live male and female fetuses were significantly lower in SD rats than in IGS rats.

As described above, differences were found between the two strains in the reproductive and developmental data. There were some endpoints that were different from lot to lot such as fertility index or implant rate in IGS females (F0). The presence of such differences should be taken into consideration when using IGS rats in the reproductive and developmental toxicity studies.

ACKNOWLEDGMENTS. The authors are grateful to the staff of the Developmental Toxicology Group at the An-pyo Center.

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The potential for confounding effects of pup body weight on interpreting the significance of anogenital distance in Crl:CD®(SD)IGS BR and Crl:CD®(SD)BR rats.

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DOW CORNING CORPORATION, MIDLAND, MICHIGAN and
WIL RESEARCH LABORATORIES, ASHLAND, OHIO, USA

Anogenital distance (AGD) is an endpoint in reproductive and developmental toxicity studies that is sensitive to estrogenic effects of chemicals. Decreases in AGD are associated with estrogenic action by a chemical.

In rats, it is known that pup body weight may vary widely as a function of litter size or effects on maternal health. Regardless of the cause of pup body weight changes, it is reasonable to suggest that AGD is likely to vary with pup body weight. Therefore, agents with no estrogenic activity might still induce apparent AGD alterations if they affect pup size.

Conversely, agents with hormonal effects might go undetected if they also induced an offsetting change in pup body weight.

We investigated whether there were any statistical correlations between pup body weight and AGD in control litters. Data from 743 Crl:CD® (SD) IGS BR pups derived from 56 untreated dams and 766 Crl:CD® (SD) BR pups derived from 57 untreated dams were collected over the course of two reproductive toxicity studies. Regression analysis indicated that mean AGD can be expected to increase approximately 0.19 mm for each 1 gram increase in pup body weight when the body weight ranges from 5.0 to 8.1g.

Analysis of covariance is indicated for studies in which AGD is an endpoint in order to estimate test article effects on AGD independently of potentially confounding effects of body weight.

This abstract was presented at the Teratology Society Meeting, June 20-25, 1998.

APPENDIX

Nutrients and Contaminants Levels of Diets for Laboratory Rodents

Special Diets Services, UK
PMI Feeds, Inc., USA
Oriental Yeast Co., Ltd., Japan
Funabashi Farms Co., Ltd., Japan

Special Diets Services (SDS), Product: RMI(E), Special Quality Control(SQC), UK

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	11.2	%	Fluoride	12	mg/kg	1.0 mg/kg
Crude Fat	3.2	%	Nitrate as NaNO3	14	mg/kg	1.0 mg/kg
Crude Protein	14.8	%	Nitrite as NaNO2	2.1	mg/kg	1.0 mg/kg
Crude Fibber	4.4	%	Lead	Non Detected	mg/kg	0.25 mg/kg
Ash	4.6	%	Arsenic	Non Detected	mg/kg	0.2 mg/kg
Calcium	0.70	%	Cadmium	0.07	mg/kg	0.05 mg/kg
Phosphorus	0.50	%	Mercury	0.01	mg/kg	0.01 mg/kg
Sodium	0.22	%	Selenium	0.05	mg/kg	0.05 mg/kg
Chloride	0.34	%				
Potassium	0.71	%				
Magnesium	0.19	%	Total Aflatoxins	Non Detected	mg/kg	1 mcg/kg each of B1, B2, G1, G2
Iron	118	mg/kg	Total P.C.B	Non Detected	mg/kg	10.0 mcg/kg
Copper	12	mg/kg	Total D.D.T	Non Detected	mg/kg	10.0 mcg/kg
Manganese	50	mg/kg	Dieldrin	Non Detected	mg/kg	10.0 mcg/kg
Zinc	46	mg/kg	Lindane	Non Detected	mg/kg	10.0 mcg/kg
			Heptachlor	Non Detected	mg/kg	10.0 mcg/kg
			Malathion	Non Detected	mg/kg	20.0 mcg/kg
Vitamin A	4.3	iu/g				
Vitamin E	41	mg/kg	Total Viable			
Vitamin C		mg/kg	Organisms ×1000	Non Detected	per grm	1000/g
			Mesophilic Spores ×100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	Non Detected	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

CRF-1, Oriental Yeast Co., Ltd. Japan

GENERAL ANALYSIS (per 100g)		
Moisture	g	8.1
Crude Protein	g	22.6
Crude Fat	g	5.6
Crude Ash	g	6.6
Crude Fiber	g	3.3
Nitrogen Free Extract	g	53.8
Calorie	kcal	356

Vitamins (per 100g)		
A	IU	3,783
D_{3*}	IU	503
E	mg	21.2
K_{3*}	mg	0.16
B_1	mg	4.44
B_2	mg	3.06
B_6	mg	1.26
Nicotinic acid	mg	14.60
Pantothenic acid	mg	7.07
Biotin	μg	27.8
Folic acid	mg	0.25
Inositol	mg	431
Choline	mg	0.31
B_{12}	μg	12.2
C	mg	14

*Additive quantity

Minerals (per 100g)		
Ca	g	1.27
P	g	0.84
Mg	g	0.25
Na	g	0.32
K	g	0.85
Fe	mg	16.6
Al	mg	4.2
Cu	mg	0.92
Zn	mg	6.94
Co	mg	0.28
Mn	mg	7.78
Ca/P		1.51
Ca/Mg		5.08
K/Na		2.66

Contaminant		
Mercury	ppm	0.01
Cadmium	ppm	0.07
Lead	ppm	0.12
Arsenic (as As)	ppm	0.3
Selenium	ppm	0.45
γ-BHC	ppm	ND*(MLD☆0.005)
DDT	ppm	ND (MLD 0.05)
Aldrin	ppm	ND (MLD 0.01)
Dieladrin	ppm	ND (MLD 0.01)
Endrin	ppm	ND (MLD 0.01)
Heptachlor	ppm	ND (MLD 0.01)
Malathion	ppm	0.09
Parathion	ppm	ND (MLD 0.05)
Aflatoxin B_1	ppb	ND (MLD 5)
Aflatoxin B_2	ppb	ND (MLD 5)
Aflatoxin G_1	ppb	ND (MLD 5)
Aflatoxin G_2	ppb	ND (MLD 5)
Polychlorobiphenyl	ppm	ND (MLD 0.01)
Estradiol	ppb	ND (MLD 10)
Dimethylnitrosamine	ppm	ND (MLD 0.01)
Diethylnitrosamine	ppm	ND (MLD 0.01)

* ND : none detected

☆MLD : Minimum limit of determination

Amino acid (per 100g)		
Isoleucine	g	0.86
Leucine	g	1.70
Lysine	g	1.22
Methionine	g	0.48
Cystine	g	0.35
Phenylalanine	g	0.97
Tyrosine	g	0.66
Threonine	g	0.88
Tryptophan	g	0.28
Valine	g	1.08
Arginine	g	1.36
Histidine	g	0.57
Alanine	g	1.20
Aspartic acid	g	1.97
Glutamic acid	g	3.67
Glycine	g	1.12
Proline	g	1.26
Serine	g	1.06

MF, Oriental Yeast Co., Ltd. Japan

GENERAL ANALYSIS (per 100g)		
Moisture	g	7.8
Crude Protein	g	23.8
Crude Fat	g	5.1
Crude Ash	g	6.1
Crude Fiber	g	3.2
Nitrogen Free Extract	g	54.0
Calorie	kcal	357

Vitamins (per 100g)		
A	IU	2,117
D_{3*}	IU	108
E	mg	9.4
K_{3*}	mg	0.04
B_1	mg	1.93
B_2	mg	1.16
B_6	mg	0.82
Nicotinic acid	mg	9.59
Pantothenic acid	mg	3.18
Biotin	μg	25.3
Folic acid	mg	0.14
Inositol	mg	424
Choline	mg	0.21
B_{12}	μg	5.1
C	mg	4

*Additive quantity

Minerals (per 100g)		
Ca	g	1.11
P	g	0.83
Mg	g	0.24
Na	g	0.24
K	g	0.87
Fe	mg	14.5
Al	mg	6.3
Cu	mg	0.75
Zn	mg	5.10
Co	mg	0.09
Mn	mg	5.32
Ca/P		1.34
Ca/Mg		4.63
K/Na		3.63

Contaminant		
Mercury	ppm	0.01
Cadmium	ppm	0.07
Lead	ppm	0.12
Arsenic (as As)	ppm	0.3
Selenium	ppm	0.45
γ-BHC	ppm	ND*(MLD☆0.005)
DDT	ppm	ND (MLD 0.05)
Aldrin	ppm	ND (MLD 0.01)
Dieladrin	ppm	ND (MLD 0.01)
Endrin	ppm	ND (MLD 0.01)
Heptachlor	ppm	ND (MLD 0.01)
Malathion	ppm	0.08
Parathion	ppm	ND (MLD 0.05)
Aflatoxin B_1	ppb	ND (MLD 5)
Aflatoxin B_2	ppb	ND (MLD 5)
Aflatoxin G_1	ppb	ND (MLD 5)
Aflatoxin G_2	ppb	ND (MLD 5)
Polychlorobiphenyl	ppm	ND (MLD 0.01)
Estradiol	ppb	ND (MLD 10)
Dimethylnitrosamine	ppm	ND (MLD 0.01)
Diethylnitrosamine	ppm	ND (MLD 0.01)

*ND : none detected

☆MLD : Minimum limit of determination

Amino acid (per 100g)		
Isoleucine	g	0.94
Leucine	g	1.84
Lysine	g	1.34
Methionine	g	0.49
Cystine	g	0.37
Phenylalanine	g	1.05
Tyrosine	g	0.70
Threonine	g	0.93
Tryptophan	g	0.29
Valine	g	1.15
Arginine	g	1.47
Histidine	g	0.61
Alanine	g	1.25
Aspartic acid	g	2.17
Glutamic acid	g	3.93
Glycine	g	1.20
Proline	g	1.33
Serine	g	1.14

CRF-LPF, Oriental Yeast Co., Ltd. Japan

GENERAL ANALYSIS (per 100g)		
Moisture	g	7.5
Crude Protein	g	18.2
Crude Fat	g	4.8
Crude Ash	g	6.6
Crude Fiber	g	5.0
Nitrogen Free Extract	g	57.9
Calorie	kcal	348

Vitamins (per 100g)		
A	IU	2,600
D_{3*}	IU	450
E	mg	24.3
K_{3*}	mg	0.16
B_1	mg	4.39
B_2	mg	3.04
B_6	mg	1.66
Nicotinic acid	mg	20.10
Pantothenic acid	mg	8.14
Biotin	μg	32.4
Folic acid	mg	0.23
Inositol	mg	581
Choline	mg	0.26
B_{12}	μg	12.0
C	mg	18

*Additive quantity

Minerals (per 100g)		
Ca	g	1.08
P	g	0.91
Mg	g	0.35
Na	g	0.28
K	g	0.95
Fe	mg	20.0
Al	mg	4.8
Cu	mg	1.11
Zn	mg	8.57
Co	mg	0.31
Mn	mg	11.00
Ca/P		1.19
Ca/Mg		3.09
K/Na		3.39

Contaminant		
Mercury	ppm	ND*(MLD☆0.01)
Cadmium	ppm	0.08
Lead	ppm	0.21
Arsenic (as As)	ppm	0.3
Selenium	ppm	0.41
γ-BHC	ppm	ND*(MLD 0.005)
DDT	ppm	ND (MLD 0.05)
Aldrin	ppm	ND (MLD 0.01)
Dieladrin	ppm	ND (MLD 0.01)
Endrin	ppm	ND (MLD 0.01)
Heptachlor	ppm	ND (MLD 0.01)
Malathion	ppm	0.12
Parathion	ppm	ND (MLD 0.05)
Aflatoxin B_1	ppb	ND (MLD 5)
Aflatoxin B_2	ppb	ND (MLD 5)
Aflatoxin G_1	ppb	ND (MLD 5)
Aflatoxin G_2	ppb	ND (MLD 5)
Polychlorobiphenyl	ppm	ND (MLD 0.01)
Estradiol	ppb	ND (MLD 10)
Dimethylnitrosamine	ppm	ND (MLD 0.01)
Diethylnitrosamine	ppm	ND (MLD 0.01)

* ND : none detected

☆MLD : Minimum limit of determination

Amino acid (per 100g)		
Isoleucine	g	0.61
Leucine	g	1.28
Lysine	g	0.85
Methionine	g	0.42
Cystine	g	0.30
Phenylalanine	g	0.75
Tyrosine	g	0.48
Threonine	g	0.65
Tryptophan	g	0.23
Valine	g	0.85
Arginine	g	1.07
Histidine	g	0.44
Alanine	g	0.96
Aspartic acid	g	1.44
Glutamic acid	g	2.82
Glycine	g	0.88
Proline	g	1.03
Serine	g	0.79

PMI Feeds, Inc., Certified Rodent Diet™ #5002* , USA

*=product code

Guaranteed Analysis	
Crude protein not less than	20.0%
Crude far not less than	4.5%
Crude fiber not more than	5.5%
Ash not more than	7.0%
Added minerals not more than	2.5%

Chemical Composition

Nutrients**		Iron, ppm	168.0
Protein %	20.1	Zinc, ppm	76.1
Arginine %	1.13	Manganese, ppm	74.5
Cystine %	.27	Copper, ppm	16.9
Glycine %	.86	Cobalt, ppm	.6
Histidine %	.49	Iodine, ppm	.53
Isoleucine %	1.03	Chromium, ppm	2.0
Leucine %	1.58	Selenium, ppm	.2
Lysine %	1.18		
Methionine %	.43	Vitamins	
Phenylalanine %	.88	Carotene, ppm	5.6
Tyrosine %	.59	Thiamin, ppm	13.3
Threonine %	.78	Riboflavin, ppm	8.0
Tryptophan %	.24	Niacin, ppm	60.0
Valine %	1.05	Pantothenic Acid, ppm	17.0
Fat %	4.5	Choline, ppmÅ~100	18.0
Cholesterol, ppm	250.0	Folic Acid, ppm	4.0
Fiber (Crude), %	4.6	Pyridoxine, ppm	6.0
Neutral Detergent Fiber %	13.8	Biotin, ppm	.13
Acid Detergent Fiber%	5.9	B12 mcg/kg	19.8
Total Digestible Nutrient %	77.0	Vitamin A, IU/gm	17.6
Nitrogen-Free Extract (by difference) %	55.1	Vitamin D, IU/gm	2.2
Gross Energy, KCal/gm	4.1	Vitamin E, IU/gm	66.1
Physiological Fuel Value KCal/gm	3.41	Ascorbic acid, mg/gm	–
Ash %	5.8		
Calcium %	.80	**=Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purposed of calculations.	
Phosphorus %	.60		
Potassium %	.86		
Magnesium %	.21		
Sodium %	.30		
Chlorine %	.47		

PMI Feeds, Inc., Laboratory Rodent DietTM #5001* , USA

*=product code

Guaranteed Analysis	
Crude protein not less than	23.0%
Crude far not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	8.0%
Added minerals not more than	2.5%

Chemical Composition

Nutrients**			
		Fluorine, ppm	35.0
		Iron, ppm	198.0
Protein %	23.5	Zinc, ppm	70.0
Arginine %	1.38	Manganese, ppm	64.3
Cystine %	.32	Copper, ppm	18.0
Glycine %	1.20	Cobalt, ppm	.6
Histidine %	.55	Iodine, ppm	.7
Isoleucine %	1.18	Chromium, ppm	1.83
Leucine %	1.70	Selenium, ppm	.20
Lysine %	1.42		
Methionine %	.43	Vitamins	
Phenylalanine %	1.03	Carotene, ppm	4.5
Tyrosine %	.88	Thiamin, ppm	15.0
Threonine %	.91	Riboflavin, ppm	8.0
Tryptophan %	.29	Niacin, ppm	95.0
Valine %	1.21	Pantothenic Acid, ppm	24.0
Fat %	4.5	Choline, ppm × 100	22.5
Cholesterol, ppm	270.0	Folic Acid, ppm	5.9
Fiber (Crude), %	5.8	Pyridoxine, ppm	6.0
Neutral Detergent Fiber %	16.0	Biotin, ppm	.07
Acid Detergent Fiber %	8.2	B_{12} mcg/kg	22.0
Total Digestible Nutrient %	76.0	Vitamin A, IU/gm	15.0
Nitrogen-Free Extract (by difference) %	49.0	Vitamin D, IU/gm	4.5
Gross Energy, KCal/gm	4.25	Vitamin E, IU/gm	40.0
Physiological Fuel Value, KCal/gm	3.30	Ascorbic acid, mg/mg	–
Ash %	7.3	Menadione(added), ppm	–
Calcium %	1.00		
Phosphorus %	.61	**=Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purposed of calculations.	
Potassium %	1.10		
Magnesium %	.21		
Sodium %	.40		
Chlorine %	.56		

F-2, Funabashi Farms Co., Ltd., Japan

GENERAL ANALYSIS	
Moisture	8.0%
Crude Protein	20.8
Crude Fat	4.8
Crude Ash	5.0
Crude Fiber	3.2
Nitrogen Free Extract	58.2
Calorie	4.14 kcal/g

Vitamins (per 100g)	
A	1000IU
D3*	450IU
E	5mg
K3*	1mg
B1	1.5mg
B2	1mg
B6	1mg
Nicotinic amide	10mg
Pantothenic Ca	3mg
Biotin	0.05mg
Folic acid	0.1mg
Inositol	10mg
Choline	230mg
B12	0.001mg
C	

*Additive quantity

Minerals (per 100g)	
Ca	0.74g
P	0.65g
Mg	0.21g
Na	0.19g
K	0.75g
Fe	0.02g
I	0.40mg
Cu	1.10mg
Zn	4.40mg
Co	0.20mg
Mn	10.00mg
Ca/P	1.14
Ca/Mg	3.52
K/Na	3.95

Amino acid (per 100g)	
Serine	0.71%
Isoleucine	0.88%
Leucine	1.55%
Lysine	1.08%
Methionine	0.39%
Cystine	0.32%
Phenylalanine	0.89%
Tyrosine	0.66%
Threonine	0.70%
Tryptophan	0.23%
Valine	0.96%
Arginine	1.22%
Histidine	0.54%
Glycine	0.98%

Amino acid (per 100g)	
Isoleucine	0.88%
Leucine	1.55%
Lysine	1.08%
Methionine	0.39%
Cystine	0.32%
Phenylalanine	0.89%
Tyrosine	0.66%
Threonine	0.70%
Tryptophan	0.23%
Valine	0.96%
Arginine	1.22%
Histidine	0.54%
Glycine	0.98%
Serine	0.71%