Biological Reference Data on CD(SD) IGS Rats - 2000

CD(SD)IGS Study Group

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PREFACE

It is my great pleasure to present the commemorative 2000's edition of "Biological Reference Data on CD(SD)IGS Rats" to our members.

Dr. Matsuzawa personally made monumental efforts as chief editor to issue two previous editions of this text. The editorial committee began work with six editors selected from three sub-groups of the IGS Study Group this year. They successfully completed the 2000's edition on schedule despite their busy schedules. Therefore, I would like to express our deepest gratitude to Dr. Matsuzawa and the editors.

This publication presents the results of numerous carcinogenicity studies conducted in Japan. Readers will find survival, body weight and pathological data of great interest.

In the 59th annual meeting of the Japanese Cancer Association this past October, a panel discussion entitled "Chemical carcinogenesis in new millennium" was held. It was reconfirmed that although oncogene and molecular biology are current concerns in recent chemical carcinogenesis research, animal experiments will continue to play a vital role in detection of various carcinogenic factors, particularly environmental carcinogens since numerous factors cannot be resolved with our current technology without the use of whole-body lifetime studies.

In this context, IGS rats will continue to be the most useful international standard utilized in long-term carcinogenicity studies.

Our previous publications contained numerous data on general toxicity, reproduction and carcinogenicity, but little data on other areas. More extensive data such as basic pharmacology and drug metabolism supplied by our members are needed and would be much appreciated to enrich the database.

Fall 2000

Hiroyuki INOUE, Ph. D., Chairman

PREFACE

I decided to retire from the board of the CD(SD)IGS Study Group on New Year's Day, 2000 and announced this decision at our official steering meeting in this past April. The rules of these committees were distributed to the members. I would like to resign the chief editor next year. So two associate editors were appointed.

The Study Group bureau intended to ask to write the articles of Chapter 1 (Review section) to associate editors or reputable western individuals, but this could not be realized. I took on this role with the collaboration of Dr. M. Christian of Primedica Argus, USA since I began writing in middle of September. The title of the Review is "Strain differences in behavior, nervous system and immune responses and male fertility in laboratory rats". Please refer to a similar article published on the 1998 edition of the Reference Data Book.

CD(SD) IGS rat data still differ between facilities. In the near future, it will be necessary to collect and evaluate the study data in accordance with integrated protocols and SOPs. I anticipate that a worldwide standard strain of rats, what is calls ICH rats, will be produced by the IGS method. I wish the continuing growth of the CD(SD) IGS Study Group through the dedication of the new staff. I would like to extend my appreciation to the readers and for the cooperation of the Study Group secretariat for the past three years.

In the fall of 2000
Toshiaki MATSUZAWA, Ph.D., Editor-in-chief

ACKNOWLEDGMENTS

We would like to acknowledge all those involved in the contribution of numerous papers and articles over the years and the secretaries and support staff assisting in this mammoth undertaking. The papers were revised by Dr. R. Harling of Huntingdon Life Science (HLS-U.K.) and Japanese members (Dr. S. Okazaki, Dr. M. Hashimoto, Dr. T. Inoue, Dr. N. Hoshino, Dr. H. Maeda and Dr. M. Takechi) of the editorial board of the CD(SD)IGS Study Group.

Numerous American and European individuals assisted from their branch and liaison offices in Japan. Special thanks to Mr. J. Hayakawa of HLS Japan, Mr. K. Hitokubi of Miki Sangyo and Ms. Y. Miyoshi of Itias.

This publication was made possible through the courtesy and support Charles River, Inc., U.S.A. and Japan. We are especially grateful to Mr.T. Kashiwagi, President of Charles River Japan, Inc., for his strong support in the organization of this society and Mr. J. C. Foster, President & CEO of Charles River Laboratories, U.S.A. for his deep understanding and appreciation of this activity in Japan. We also acknowledge the efforts made by Dr. Lee who served as a liaison between Charles River U.S.A. and Charles River, Japan. Drs. E. Morimura and Y. Chazono of the CD(SD) IGS Study Group secretariat made numerous efforts to promote and solicit contributions and gather information in Japan. Ms. E. Hattori assisted with liaison and compilation activities. Close cooperation with the secretariat and the printing office much lightened the work of chief editor. Ms. Y. Suzuki, a proof-reader and Mr. Y. Tsudome, Manager of Best Printing Inc. assisted with shipments and acceptance of publications of printing offices. Such assistance and cooperation lightened the work of the chief editor.

We wish to thank Ms. M. Kimishima of U-STAFF, Inc. for a copyright assistance and book number registration (ISBN).

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

Lastly we wish you, all concerned individuals and readers, prosperity and health. It is with deep felt thanks that this publication will be available for worldwide publication.

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

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CHAPTER 1

Introduction

Strain differences in behavior and nervous system, immune response, and male fertility in the laboratory rat

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ABSTRACT. The 1998 edition of the "Reference Data Book on CD(SD)®IGS® Rats" [49], documents the fact that biological reference data on laboratory rats vary due to a variety of factors and appear as inter-laboratory differences. The CD(SD)®IGS® Rat Study Group has been active in the collection and analyses of historical data of general toxicity, reproductive and developmental toxicity and carcinogenicity studies. Fischer, Wistar, and Sprague-Dawley rats have been routinely used as the animals of choice in such studies. When the results of research/studies of a test substance are reported, the relationship between the mechanism of the toxic effects and the strain of rats used should be discussed. Recently reviewed strain differences in behavior, nervous system responses, immune responses and male fertility are discussed in this review. Additional behavioral assessments are discussed in this volume. — Key words: behavior, nervous system, immune response, male fertility, strain difference, laboratory rat

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INTRODUCTION

The laboratory rat is one of the species that has proven to be extremely useful in pharmacokinetics, pharmacology and toxicology studies. The rat is a convenient size, is relatively docile, has a short life span and gestation period, is economic to maintain, and there is a large database of its characteristics. In interpreting the results of toxicology studies, it is essential to consider the age, sex, nutrition, and strain of the test animals [53]. Most studies are carried out using a single strain, however, strain-related differences in responsiveness to chemicals and drugs have been well documented. There are three main classes of rats used in studies; these include inbred strains, outbred stocks, and mutants. Inbred strains are produced by at least 20 generations of sib-mating, with all individuals being derived from a single breeding pair in the 20th or subsequent generation. Currently, there are over 200 inbred rat strains, e.g., AC1, LEW, SHR, and F344. Outbred stocks are usually maintained at closed colonies of rats of undefined genotype and sometimes known by generic names such as Wistar, Sprague-Dawley, or Long-Evans, which indicate their historical origin. Over 300 genetic loci associated with mutants and polymorphisms of various sorts have been described in the rat. Some of these, such as the polymorphisms associated with drug-metabolizing enzymes, and mutants such as acholuric jaundice and Rowertt athymic nude, are important in pharmacology and toxicology studies.

CD(SD)IGS rats have been used extensively in numerous laboratories in Japan and western countries. Consequently, the extensive reference database established with this strain has achieved international acceptance [49-51]. Many members of the CD(SD)®IGS® Study Group have reported that there are minimal differences between CD and IGS rats in body weight, feed consumption, clinical pathology data, organ weights, morphology of fetuses and types of tumors. Inter-laboratory differences as factors of biological variations were reviewed in Matsuzawa and Inoue's 1998 edition[49]. Strain differences will be discussed in this edition.

Different strains of rats have been used in the past depending on the purpose of the research/studies. Fischer, Wistar and Sprague-Dawley (CD) rats have been frequently used in general toxicity, reproductive and developmental toxicity and carcinogenicity studies. When the results of research/studies of a test substance are reported, the relationship between the mechanism of the toxic effects and the strain of rats used should be discussed, whenever possible. This is especially true for different types of cancers where strain differences have been reported in many carcinogenicity studies for quite some time [45, 62-63]. Strain differences in immune and nervous system responses, behavior and male fertility are reviewed in this current edition. The authors would be pleased if this overview were to serve as a reference and be found helpful for the collection and analyses of biological reference data.

The reviews by Kacew et al. [42-43] were helpful in the preparation of many parts of this overview, and are highly recommended as a reference source.

1. Immune Response

The following information was discussed in the 1998 edition [49]. Serum interleukin-6 and corticosterone levels were measured in four strains of rats after footshock exposure; differences in responses occurred among the strains. It is well known that genetically different strains of rats show significant inter-strain differences in immunological and neuroendocrine features [55-57, 78]. Dhabhar et al. [20] have reported differences in corticosterone secretion and activation of adrenal steroid receptors among SD, F344 and Lewis rat strains. A comparison study with CD(SD)®IGS® rats is anticipated. Derijk and Sternberg [19] have reported that there are inter-strain differences in neuroendocrine-immune interactions. These authors investigated ACTH and corticosterone responses in SD and F344 rats. Immunotoxicology studies in CD(SD)®IGS® rats are expected.

Updated, current strain references in immune responses in rats are reviewed in this current edition. It is well known that genetically different strains of rats have remarkable inter-strain differences in immunological and neuroendocrine responses [48].

Sprague-Dawley (SD), Fischer-344 (F344), and Lewis (LEW) rats are used in a wide variety of laboratory studies. Compared to SD and LEW rats, F344 rats demonstrate significantly greater corticosterone secretion in response to stress or immune challenge [20-21]. These strain differences in hypothalamic-pituitary-adre-

nal (HPA) axis responsivity have been the basis for many comparative studies investigating immunological and behavioral differences among the three strains. However, the effects of these strain differences in HPA axis responsivity have not been investigated at the level of adrenal steroid receptor activation in target tissues. The present study demonstrates that compared to SD and LEW rats, F344 rats had a greater magnitude of Type Ⅱ adrenal steroid receptor activation in brain tissues during stress. In contrast, Type Ⅱ receptor activation in immune tissues of F344 rats following stress was similar to that of SD rats. Importantly, LEW rats had the lowest magnitude of activation of Type Ⅱ receptors in immune tissues during stress. No differences were observed among strains in the extent of stress-induced Type Ⅰ adrenal steroid receptor activation. The observed differences among strains in corticosteroid-binding globulin (CBG) levels in plasma, pituitary, and immune tissue may mediate the differential access of corticosterone to neural versus immune tissues. These results indicate that strain differences in corticosterone secretion are manifested by differences in Type Ⅱ receptor activation in neural and immune tissues. Moreover, they suggest that increased access of corticosterone to adrenal steroid receptors in brain areas of F344 rats may contribute to behavioral differences among strains, whereas decreased access of hormone to receptors in immune tissues of LEW rats may contribute to strain differences in susceptibility to autoimmune disease.

Serum IgE concentrations were examined following topical exposure of Brown Norway (BN) and Wistar rats to each of four chemicals [trimellitic anhydride (TMA), dinitrochlorobenzene (DNCB), formaldehyde (FA), and methyl salicylate (MS)] with known diverse sensitization potential in humans [3-4]. Of the four tested chemicals, only exposure to TMA resulted in a significant increase in serum IgE concentration; this response was only evoked in the high-IgE-responding BN rat. The latter two chemicals were also tested for lymph node activation, in the ear-draining lymph nodes. FA caused a dose-dependent activation of the draining lymph nodes, whereas MS was inactive. The results obtained with TMA, DNCB, and MS in rats are in agreement with human data. However, the results with FA indicate the need for further studies of chemicals that have both irritant and sensitizing properties at about similar concentrations or may act through non-IgE-mediated immune mechanisms.

It is well known that high IgE antibody responses are observed in Brown-Norway (BN) rats after immunization with alum-precipitated egg albumin [1]. BN rats are regarded as a useful allergy model and are widely used in the study of allergic diseases such as those of the airways [68]. Peritoneal mast cells from BN rats were compared with those from Wistar and Sprague-Dawley (SD) rats [70]. Peritoneal mast cells from BN rats showed the smallest values in number, cell diameter and histamine contents compared with those from Wistar and SD rats. The histamine release from passively sensitized peritoneal mast cells was weaker in BN rats than in Wistar or SD rats.

Strain dependence of the induction of skin and lung lesions by hexachlorohenzene (HCB) in rats was studied to further the insight into the etiology of the lesions [54]. To this end, three- to four-week-old female Brown Norway, Lewis, and Wistar rats were provided diets supplemented with 150 mg (BN and LEW), 450 mg (BN, LEW, and Wistar) or 900 mg (BN and Wistar) HCB per kilogram diet for four weeks. Gross skin lesion development during exposure and pathologic changes in skin and lungs and various parameters of immunomodulation after exposure were assessed. General toxicity, evident as a slight increase in body weight gain and induction of liver cell hypertrophy, was similar in BN and Lewis rats exposed to 450 mg/kg HCB and in Wistar rats exposed to 900 mg/kg HCB. Skin lesions ranged from redness to large exudating crusted sores. With regard to dose, time of onset, incidence, and severity, skin lesions were very severe in BN rats, moderate in Lewis rats, and negligible in Wistar rats. Porphyrins could not be detected in the skin, whereas porphyrins in the liver were seen only in Lewis rats. Histology showed epidermal hyperplasia, deep dermal venules with activated endothelium, and deep dermal inflammatory infiltrates mainly consisting of eosinophilic granulocytes in BN rats. Dermal inflammatory infiltrates consisted mainly of mononuclear cells in Lewis and Wistar rats. Nonlesional skin of HCB-exposed rats showed very similar, though less prominent, changes. Lung pathology appeared negligibly strain-dependent. Histology revealed venules with an activated endothelium surrounded by a perivascular infiltrate as well as focal alveolar macrophage accumulations in all strains. Parameters of immunomodulation had moderate strain dependence; relative spleen weights were dose-dependently increased in BN and Wistar rats and in the 450 mg/kg group in Lewis rats. BN rats had a more marked splenomegaly than the other strains. Relative popliteal lymph node weights were increased significantly in BN and Lewis rats exposed to 450 mg/kg HCB. In all strains, HCB increased lymph node of the high endothelial venules (HEVs). Serum IgE and IgG levels were increased significantly in a dose-dependent way in BN rats only. Total serum IgM levels were elevated significantly in BN, Lewis, and Wistar rats provided 450 mg/kg and in Wistar rats provided 900 mg/kg HCB. Serum IgM levels against -single-stranded DNA (against ssDNA) were dose-dependently increased in all strains, being more marked in BN and Lewis rats than in Wistar rats. It is concluded that the HCB-induced inflammatory skin and lung pathologies have different etiologies. Pronounced strain differences in the skin lesions suggest a specific involvement of the immune system. Skin lesions correlated significantly with all assessed parameters of immunomodulation in BN rats, with some in Lewis rats and with none in Wistar rats.

Five rat strains (Fischer, Wistar, Brown Norway, Sprague-Dawley and Piebald Virol Glaxo) were compared for their performance in the local lymph node assay (LLNA), a promising test system for identification of the skin-sensitizing potential of chemicals in the mouse [3]. The results obtained for 2,4-dinitrochlorobenzene (DNCB) and trimellitic anhydride (TMA) are summarized in Table 1. Following application (3-time concentrations) of DNCB or TMA, minor systemic effects were observed, as indicated by slightly elevated spleen and liver weights in a few rat strains and treated mice. Skin effects, consisting of increased ear thickness and presence of mononuclear inflammatory cell infiltrates, were observed in all rat strains treated with DNCB or TMA. LLN weights and the proliferative activity in these nodes were increased. It was concluded that effects induced by DNCB and TMA in all five rat strains were comparable with those in mice.

Table 1. Strain-dependent effects of DNCB and TMA on spleen, liver and LLN weights and ear thickness (Arts et al. 1996).

Rat strain	Classes Bred	Treatment	Spleen weight g/kg, bw	Liver weight g/kg, bw	LLN weight, mg	Ear thickness x10E-2 mm
Fischer (F3449)	Inbred	AOO	2.51±0.03	30.6±0.4	12.4±1.2	50.5±1.5
		DNCB	2.74±0.08*	32.0±0.9	51.9±1.3**	56.7±1.9*
		TMA	2.67±0.04	31.0±0.6	35.7±3.2**	57.2±1.1*
Wistar	Random Bred	AOO	2.11±0.07	36.1±1.0	19.0±1.7	54.5±1.9
		DNCB	2.26±0.08	36.9±0.4	77.0±5.6**	59.9±2.3
		TMA	2.32±0.12	36.0±1.4	59.1±5.5**	58.7±1.3
Brown Norway (BN)	Inbred	AOO	2.11±0.06	31.3±0.5	29.6±2.9	47.9±0.9
		DNCB	2.18±0.06	32.0±0.5	63.3±4.1**	61.0±4.8*
		TMA	2.75±0.43	32.8±0.6	65.6±4.6**	55.4±2.4
Sprague Dawley (SD)	Outbred	AOO	2.61±0.09	30.3±0.8	20.7±2.6	53.4±1.6
		DNCB	2.72±0.11	32.2±0.4	82.6±4.1**	63.8±3.4*
		TMA	2.86±0.09	32.7±0.5*	79.0±5.5**	61.4±2.2
Piebald Virol Glaxo (PVG)	Inbred	AOO	2.46±0.04	35.5±0.8	13.6±1.5	52.4±1.0
		DNCB	2.40±0.03	38.1±0.6*	49.3±2.5**	56.7±0.9
		TMA	2.42±0.07	37.9±0.3*	38.7±2.0**	58.2±2.0*

AOO: Acetone＋olive oil, DNCB: 2,4-dinitrochorobrnzene, TMA: Trimellitic anhydydride
Groups of five or six rats given 1% DNCB or 50% TMA in AOO or AOO alone
Values are means±S.E., LLN: local lymph nodes,
*p<0.05; ANOVA followed by two-sided Dunnett's multiple comparison test

In order to study intestinal mucosal immune cells, with emphasis on single T lymphocytes, an inventory was made of single and organized lymphocytes in the epithelium and lamina propria of the small intestines of untreated Wistar, Fischer-344, and Lewis rats [8]. The single and organized lymphocytes were examined microscopically. In addition, the single lymphocytes in the epithelium (IEL) and lamina propria (LPL) were analyzed by flow cytometry. Next, the use of flow cytometry analysis was explored to detect changes in the IEL T-lymphocyte population in subacute oral studies with the immunomodulating agents azathioprine and hexachlorobenzene. Untreated random-bred Wistar rats had a large inter-individual variability in IEL composition, while the variabilty was small in inbred Fischer-344 and Lewis rats. The explorative study with the two model immunomodulating compounds demonstrated that hexachlorobenzene increased the number of intraepithelial T lymphocytes with CD8＋phenotype at the cost of T cells with CD4＋phenotype in Lewis rats. Azathioprine did not induce distinct effects on the percentages of IEL. The data indicate that the intraepithelial lymphocytes in the intestines are a potential target for orally administered immunomodulating compounds.

Sugimoto and Kamei [71] have reported that intestinal histamine content of the colon in BN male rats following active sensitization of egg albumin was greater than those in Wistar and SD male rats. Male BN, Wistar and SD rats, aged 8 weeks (Charles River Japan) were used. The animals were immunized with egg albumin in combination with *Bordetella pertussis* and aluminum hydroxide gel, which were injected into the four footpads on day 0. Five days later, they were boostered with egg albumin alone on the back. On day 42 after the first immunization, histamine contents of the duodenum, jejunum, ileum, cecum, colon and rectum in the rats were determined by fluorometric assay. High concentrations of histamine were observed in the duodenum, jejunum, ileum and cecum in sensitized BN rats, in comparison with levels present in non-sensitized rats. The results are shown in Table 2. Sensitized Wistar and SD rats had small changes in intestinal histamine contents compared with the changes in non-sensitized rats. Histamine contents of the duodenum, jejunum, ileum, and colon in sensitized BN rats were significantly greater than those in Wistar and SD rats.

Table 2. Histamine contents in the intestinal tissues of sensitized Brown-Norway, Wistar and Sprague-Dawley male rats by egg albumin (Sugimoto and Kamei 1999)

	Histamine contents (ug/g wet weight)		
Tissues	Brown-Norway	Wistar	Sprague-Dawley
Duodenum	14.1±0.8**##	8.8±0.6	10.3±0.5
Jejunum	17.1±2.1**##	9.5±0.6	11.8±0.4
Ileum	16.7±2.4**#	10.7±0.4	11.8±0.6
Cecum	11.4±0.5	9.5±0.9	13.4±0.8
Colon	8.1±0.6**##	5.8±0.4	5.9±0.3
Rectum	4.0±0.5*	6.0±0.5	5.9±0.4

Each values represents the mean±S.E. obtained from 5 animals
*, **: Significantly different from Wistar rats at p<0.005 and p<0.01, respectively.
#, ##: Significantly different from Sprague-Dawley rats at p<0.005 and p<0.01, respectively.

Although several *in vivo* antigenicity assays using parenteral immunization are available, no fully validated enteral models are available to study food allergy and allergenicity of food proteins. To further validate a developed enteral Brown Norway (BN) rat food allergy model, systemic and local immune-mediated reactions were studied upon oral challenges [46]. The animals were provided ovalbumin (OVA) by daily gavage intubation (1 mg OVA/rat/day) for six weeks without the use of an adjuvant, or by intraperitoneal injections with OVA together with aluminum hydroxide [$AL(OH)_3$]. Subsequently, effects on breathing frequency, blood pressure, and gastrointestinal permeability were investigated following an oral challenge with 10 to 100 mg OVA *in vivo*. In both parenterally and orally sensitized rats, an increase in gut permeability (increased passage of *p*-lactoglobulin as bystander protein) was determined between 0.5 and 1 hour after an oral OVA challenge was given. An oral challenge with OVA did not induce a clear effect on the respiratory system or blood pressure in the majority of the animals. However, some animals demonstrated a temporary decrease in breathing frequency or in systolic blood pressure. Upon oral challenge with OVA of orally and parenterally sensitized animals, local effects were observed in all animals, whereas systemic effects were observed at a low frequency, which reflects the situation in food allergic patients after an oral challenge. These studies show that the BN rat provides a suitable animal model to study oral sensitization to food proteins as well as immune-mediated effects after oral challenge with food proteins.

Yoshida et al. [84] tested different strains of rats for the development of experimental immune-mediated blepharoconjunctivitis (EC). Lewis and Brown Norway (BN) rats were immunized once with 100 mcg (μg) of ovalbumin (OVA) in complete Freund's adjuvant (CFA) or $Al(OH)_3$. EC, OVA-specific IgG, and cellular immunity were induced in Lewis rats by using either adjuvant, whereas IgE was not produced by either adjuvant. In contrast, IgE was produced in BN rats using either adjuvant, whereas cellular immunity was evoked only when CFA was used. Less cellular infiltration as well as cellular proliferation were detected in BN rats immunized with $Al(OH)_3$. In both strains, $Al(OH)_3$ induced a higher IgG1/IgG2a ratio than did CFA. More interferon-gamma subsequent to stimulation with OVA was noted in Lewis rats than in BN rats, whereas interleukin-4 was detected only in BN rats. Therefore, it was concluded that the severity of EC evaluated by cellular infiltration was dependent on OVA-specific cellular immunity, and that genetic background is more important than the adjuvants used in determining the nature of EC and immunity.

Although SD and F344 rats appeared to be resistant at low concentrations of solvent exposure, a four-fold increase in 2-methoxyethanol (ME) and its metabolite 2-methoxyacetic acid (MAA) produced immunosuppression in these strains in descending rank order of WF = LEW > SD > F344 [65]. The basis for the differences in sensitivity of the immune system of rat strains to various chemicals is not known; however, recent evidence indicates that there are strain-related differences in macrophage cytokine formation in response to the lipopolysaccharide conclavin, an index of fibrosis [43]. Furthermore, it is known that amiodarone increased the white blood cell count in Fischer-344 but not Wistar rats [82], supporting the view that strain is a factor to consider in chemical-induced effects on immune function[64].

The International Collaborative Immunotoxicity Study (ICICIS) was established in 1986 as a joint activity of the International Programme on Chemical Safety[39]. The objectives were to examine whether various experimental techniques could be used in rats to indicate toxic effects on the immune system, and so to suggest their possible value as general indicators of immunotoxicity. Although these indicators were used for Wistar and Fischer rats only, it would be desirable to employ other strains such as CD rats. Guidelines for immunotoxicity studies have been published by OECD 407[61], FDA[26], EPA[24] and CPMP[14]. Differences in opinion arose between regulatory authorities and scientists as to whether immune responses to the test substances should be studied as part of general toxicity studies or as independent immunotoxicity studies. A second issue concerned the main constituent in immunotoxicity studies, whether to use histopathology or flow cytometry methods. Therefore, an integrated guideline is needed[17, 44]. Strains of rats to be used should be a major issue of consideration in integration of guidelines. Further studies on immune responses of CD(SD)®IGS® rats could provide validated systems necessary in the elucidation of immunological insult and meaningful evaluation of immunotoxicity studies.

2. Male Fertility

Compilations of teratogenic/developmental toxicology reference data have been amassed in Japan and western countries. These data clearly indicate that differences exist among different strains and/or animal colonies [6, 27]. Historical data in reproductive and developmental toxicity studies for external, visceral and skeletal anomalies and variations were reviewed in the 1998 edition of the CD(SD)®IGS® Study Group[49]. There are few differences in these data between CD(SD)®IGS® and CD rats over the last three years [49-51]. Collaborate studies complying with integrated standard operational procedures (SOPs) will be desirable in the future. We also expect that data for CD(SD)®IGS® rats on effects of test materials administered at different embryonic stages will become evident [52].

In teratology/developmental toxicity studies, Wistar and CD (Sprague-Dawley) rats have been used most frequently. However, there are few papers in which strain differences in male fertility are discussed. The following are representative papers.

The effects of differential mating stimulation on fertility in rats were examined by mating pro-estrus females for one ejaculatory series in tests in which they could or could not self-regulate, or pace, the timing of intromissions received by males. Female rats were necropsied on days 7, 14 or 21 after mating, or on the expected day of parturition, to confirm pregnancy; the number of implantation sites or of viable fetuses or pups was determined [15]. Because of substantial behavioral variability within an ejaculatory series, data from paced and nonpaced females were categorized according to whether they had a low (<8) or high (>9) number of intromissions. The incidence of pregnancy was significantly reduced among paced females having few intromissions relative to that of any other group. Histological examination of

ovaries from female rats necropsied on day 7 after mating suggested that the reduced pregnancy rate among the paced, low intromission group resulted from a failure of activation of the corpora lutea, a possible consequence of the low number of intromissions. However, in paced, low intromission female rats that became pregnant, litter size was significantly greater than in nonpaced, low intromission female rats. These results suggest a compensatory effect of the temporal patterning of intromissive stimulation on fertility. This effect was not a consequence of differential mortality of conceptuses after implantation because litter sizes were comparable among female rats necropsied at any of the four times. The differences between paced and nonpaced females may be attributable to preimplantation effects such as differential release of ova, sperm transport or hormonal response to the stimulation of mating activities.

Fertility was reduced in the progeny of Wistar rats exposed to 0.5 μg TCDD/kg/day from Gestational Day (GD) 6 to GD 15[36]. In a three-generation reproduction study, TCDD reduced fertility of Sprague-Dawley rats in the Fl and F2 generations but not in the F0 generation (no exposure during *in utero* developmental) at 0.01 μg/kg/day in the diet. Furthermore, administration of TCDD on GD 15 (at 0.064 to l μg/kg/day) both demasculinized and feminized morphology and behavior of the male offspring of Holtzman rats. Long-Evans (LE) hooded rats were intubated by gavage with 1 μg TCDD/kg on GD 8 (during the period of major organogenesis) or GD 15. When LE rats were intubated on GD 15, puberty (preputial separation) was delayed by about three days, ejaculated sperm counts were reduced by at least 58%, and epididymal sperm storage was reduced by 38%. Testicular sperm production was less affected. The size of the accessory sex glands was reduced in LE rat male offspring treated on GD 15, despite the fact that serum testosterone (T), T production by the testis *in vitro*, and androgen receptor (AR) levels were unaffected. Some reproductive measures, such as anogenital distance and male sex behavior, were altered by TCDD treatment in the offspring of rats, but not those of hamsters. Because T and AR levels appeared normal in the accessory sex glands and the epididymis after perinatal TCDD exposure, the alterations in these tissues were not likely to have resulted from alteration of the androgenic status of the male offspring.

Routinely, Fischer-344 rats have been used less frequently in reproductive and developmental toxicity studies, although such does occur [7, 11, 22-23, 28, 41, 60, 66-67, 74]. The mean litter size of Fischer rats is approximately 10 pups, fewer than those of CD and Wistar rats. The fertility index of Fischer rats is as low as 60-70% [22, 41]. The following experiments were conducted to compare fertility of Fischer and CD male rats.

Experiments:

Extra CD (Crj:CD(SD) and Fischer (F344/DuCrj) rats purchased from Charles River Japan, Inc., for reproductive and developmental toxicity studies and repeated toxicity studies conducted between 1991 and 1994 were used for the experiments. All animals were naive, virgin animals between 13 and 19 weeks of age at initiation of the individual experiments. Vaginal smears were examined each day for determination of estrous stages and for the presence of sperm. Environmental conditions of feeding were maintained in accordance with the Animal Welfare Law [18]. Females were euthanized under ether anesthesia on Day 13 or 14 of gestation and their uteri subsequently examined. Statistical analyses were not conducted because the times of animal arrival, number of animals, animal lots, ages in weeks, times of experiment, etc., differed. Each male rat was cohabited with 1 to 3 female rats. Female rats were not limited to those in the proestrus stage and were cohabited randomly with male rats. As shown in Table 3, precoitus times were 2.9 days in CD rats and 3.8 to 5.3 days in Fischer rats. In historical control data of Fischer rats, the litter size was smaller than CD rats, as reported in the literature. Reproductive cycle parameters such as days in estrus, plasma estradiol and progesterone levels differed by the choice of strains and interpretations of the results.

Table 3. Male fertility parameters in CD (Sprague-Dawley) rats and Fischer (F344) rats

Rat strain	No. of ♂ & ♀ Cohabited	No. of rats examined		Copulation index %	Days of precoitus Mean±S.D.	Male Fertility index %	Female Fertility index %	On day 13 or 14 of pregnancy	
		♂	♀					Live fetuses Mean±S.D.	Dead fetuses Mean±S.D.
Crj:CD(SD)	♂1 & ♀1	36	36	94.4	2.9±1.5	97.2	100	13.5±3.8	1.1±1.2
	♂1 & ♀2	30	60	91.7	2.9±1.5	100	89.1	13.0±2.9	1.1±1.1
	♂1 & ♀3	31	93	89.2	2.9±1.5	100	85.6	13.2±3.8	1.1±1.3
F344/DuCrj	♂1 & ♀1	27	27	88.9	3.8±2.1	88.9	100	9.9±2.7	0.7±1.2
	♂1 & ♀2	20	40	68.0	5.3±3.0	85.0	96.3	8.9±3.5	0.6±1.2
	♂1 & ♀3	21	63	71.0	4.7±3.0	95.2	91.0	8.9±3.6	0.8±1.6

Male fertility=No. males with mating resulting in pregnancy divided by the No. males mated.
Female fertility=No. of pregnant females divided by the No. of females mated.
Copulation/Mating=No. females showing evidence of copulation divided by No. females mated.
Statistical analyses were not conducted since the times of animal arrival, number of animals, animal lots, ages in weeks, time of experime etc, differed.

3. Behavior and Nervous Response

The following matters as strain differences in behavior and nervous responses were reviewed in the 1998 edition [49]. Strain differences in conditioned avoidance responses in the open-field behavior test have been reported by Holland and Gupta using two strains of rats [38] and by Harrington using 12 inbred strains, including ACI, F344, IR, MNR, TSI, WAG and others [37]. Strain differences in responses to shock have also been investigated using the shuttle-box test [81]. Not only genetic and environmental correlation, but also human factors may affect the responses. In this edition, recent studies on strain differences in behavior and nervous responses in rats are discussed. Strain-related differences in CNS responses are summarized in Table 4. This table was developed by Wetzel et al. [79] , with some modifications.

Table 4. Reproductive cycle parameters in female Fischer (F344) and Sprague-Dawley rats (Wetzel, L.T. et al. 1994)

Rat Strain	Time month	Percent days in estrus±SD (n)	Percent days in proestrus±SD (n)	Plasma estradiol levels of females pg/ml±SD	Plasma progesterone levels of females pg/ml±SD	Plasma prolactin levels of females pg/ml±SD	Incidence of galactocele formation in females
Fischer (F344)	1	24.9±5.8 (10)	23.8±3.6 (10)	3.4±3.9 (10)	7.8±1.7 (10)	HBS	0/6
	3	24.2±5.8 (10)	26.3±5.2 (10)	9.8±5.7 (10)	11.8±4.6 (10)	11.7±5.5 (10)	0/10
	9	25.8±4.2 (10)	26.6±4.1 (10)	15.3±5.0 (10)	16.3±8.5 (10)	81.9±53.3(10)	0/10
	12	22.0±4.5 (10)	31.8±6.4 (10)	13.9±12.6(10)	41.6±25.9(10)	14.3±11.0(10)	1/9
Sprague-Dawley	1	19.0±3.9 (10)	30.0±5.0 (10)	9.0±9.4 (10)	19.4±6.0 (10)	HBS	0/9
	3	24.8±7.7 (10)	29.5±4.8 (10)	3.5±6.4 (10)	15.6±7.9 (10)	HBS	0/10
	9	24.2±7.6 (10)	30.9±5.7 (10)	22.8±20.6(10)	11.6±11.0(10)	17.8±12.4(10)	1/10
	12	42.9±10.1(10)	26.0±7.9 (10)	13.1±10.6(10)	4.0±1.5 (9)	13.2±2.9 (10)	5/9

HBS: hemolyzed blood sample

Male rats of the Long-Evans (LE), Fischer-344 (F344), and Sprague-Dawley (SD) strains were administered diisopropyl fluorophosphate (DFP) at dosages of 0 to 1.5 mg/kg (sc) [34]. The animals were placed 60 minutes later into one of two motor activity chambers and tested for 30 minutes. Motor activity was measured using either a Doppler-based system or a commercial photocell device. Following measurement of motor activity in the Doppler system, body temperature (Tb) was measured, and blood was then withdrawn by cardiac puncture and analyzed for serum cholinesterase activity (ChE). The remaining rats were retested in the photocell device one day after DFP administration. The results showed a significant influence of strain on the effects of DFP. Motor activity of LE rats was reduced by DFP at dosages of 1.0 and 1.5 mg/kg, whereas the activity of F344 rats was reduced only at 1.5 mg/kg. The relative sensitivity of SD rats depended on the device used to measure motor activity. The SD rats resembled F344 rats in their response to DFP when motor activity was measured in the photocell device, and LE rats when motor activity was measured in the Doppler system. The (Tb) of F344 rats was unaffected by DFP, while the LE and SD rats became hypothermic at 1.5 mg/kg. The DFP-induced inhibition of serum ChE activity was significantly less in F344 rats. At retest the day after DFP treatment, all three strains still showed significant decreases in motor activity. Overall, it appears that the F344 strain is relatively resistant to the behavioral and autonomic effects of DFP. This intraspecies variability should be considered in selecting appropriate experimental models for assessing the neurotoxicological hazards of cholinesterase-inhibiting pesticides.

In Wistar rats, the serotonergic 5-HT_2 receptor antagonists ketanserin and risperidone reduced the disruptive effects of the noncompetitive N-methyl-D-aspartate (NMDA) antagonist dizocilpine on prepulse inhibition (PPI), suggesting that there is an interaction between serotonin and glutamate in the modulation of PPI [76]. Studies using the noncompetitive NMDA antagonist phencyclidine (PCP) in Sprague-Dawley rats found no effect with 5-HT_2, antagonists. To test the hypothesis that strain differences might explain the discrepancy in these findings, risperidone was tested for its ability to reduce the PPI-disruptive effects of dizocilpine in Wistar and Sprague-Dawley rats. Furthermore, to determine which serotonergic receptor subtype may mediate this effect, the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SDZ SER 082 [59] were tested against dizocilpine. These studies also found that the PPI-disruptive effects of PCP are reduced by the α_1 adrenergic receptor antagonist prazosin [5]. Furthermore, the α_1 receptor agonist cirazoline disrupts PPI. As risperidone and M100907 have affinity at the α_1 receptor, a final study examined whether M100907 would block the effects of cirazoline on PPI. Risperidone partially, but non-significantly, reduced the effects of dizocilpine in Wistar rats, although this effect was smaller than reported by Varty and Higgins [75]. Consistent with risperidone did not alter the effects of dizocilpine in Sprague-Dawley rats. M100907 pretreatment fully blocked the effect of dizocilpine in both strains; whereas SDZ SER 082 had no effect. M100907 had no influence on PPI by itself and did not reduce the effects of cirazoline on PPI [9]. These studies confirm the suggestion that serotonin and glutamate interact in modulating PPI and indicate that the 5-HT_{2A} receptor subtype mediates this interaction. Furthermore, this interaction occurs in at least two rat strains (Wistar and Sprague-Dawley).

Lewis is an inbred strain of rats frequently used as an animal model of autoimmune diseases. The hypothalamus-pituitary-adrenal (HPA) system is involved in the pathophysiology of these diseases. Stöhr et al. [69] compared two LEW lines (SsNHsd and HANRijHsd) in their behavioral and neuroendocrine response to

stress. They studied the psychostimulant effects of acute and repeated amphetamine in these two LEW rat lines. HAN rats were less active in the open field test and showed faster habituation of novelty-induced locomotion. The acoustic startle response was lower in HAN than in SSN rats, whereas prepulse inhibition of the startle response was greater in the HAN than in the SSN LEW subline. Moreover, HAN rats showed impaired acquisition of the two-way active avoidance response relative to SSN rats. Basal concentrations of serum corticosterone did not differ between the two rat strains.

Chisari et al. [12] showed higher basal release of corticosterone in LEW than in F344 rats.

Swerdlow et al. [72] demonstrated that Sprague- Dawley rats are more sensitive to disruption of PPI by APO than are Wistar rats, and that clozapine can reverse this APO-induced attenuation at lower concentrations in Sprague-Dawley rats than in Wistar rats.

Kinney et al. [47] tested the effects of apomorphine, amphetamine, 8-hydroxy-2-(di-n-propylamino)tetralin 88-0H-DPAT9, and phencyclidine(PCP) on the prepulse inhibition of the acoustic startle response (PPI) in the Sprague-Dawley and Wistar rat strains. Because apomorphine disrupts PPI via activation of dopamine (DA) receptors in the nucleus' accumbens, apomorphine-induced hyperlocomotion, also a behavioral model of nucleus accumbens DA receptor activation, was measured in both rat strains. Administration of PCP or 8-0H-DPAT attenuated PPI in both strains, whereas apomorphine and amphetamine only attenuated PPI in Wistar rats. The ability of apomorphine to increase motor activity in the absence of a startle-eliciting stimulus was similar in the two strains, as was apomorphine-induced hyperlocomotion. A time course analysis of the effects of apomorphine on startle response in Sprague-Dawley rats found that changes in the magnitude of PPI followed changes in basic startle amplitude. Similarly, no apomorphine-induced attenuation of PPl was observed in Sprague-Dawley rats after 6-OHDA-induced DA receptor supersensitivity in the nucleus accumbens. These data suggest a dissociation between the effects of DA receptor agonists in PPI and other behavioral models of DA receptor activation.

Tamminga et al. [73] reported that vacuous chewing movements (VCMs) in three different rat strains developed at considerably different rates after 19 weeks of continual haloperidol treatment at an average daily dosage of 1.5 mg/kg. Sprague-Dawley rats displayed relatively high rates of VCMS with low variability, compared to Wistar and Long-Evans rats. Atropine decreased but did not abolish VCMS in two of the three strains (LE > SD). After haloperidol withdrawal, VCMS remitted gradually in all strains, but least rapidly in the SD rats.

Motor activity for four days after administration of 1.5 mg/kg diisopropyl fluorophosphate (DFP) (sc) was studied in four common rat strains: Sprague-Dawley. Long-Evans, Fischer-344, and Wistar [35]. The F344 rat was least susceptible to DFP in terms of both a minimal hypothermic response and recovery of the day-night difference in core temperature. The SD rat strain was unusual in that its heart rate was elevated relative to the other rat strains after DFP, in spite of a marked decrease in core temperature and motor activity. The LE rat strain had the largest reduction in core temperature and heart rate following DFP treatment. Serum and brain cholinesterase activity (ChE) measured 3 hours after administration of 1.0 mg/kg DFP also indicated strain effects. The F344 had less inhibition in these variables than the other strains, a response that may explain its attenuated thermoregulatory response to DFP. Overall, the inbred F344 rat demonstrated better resistance to DFP than outbred strains. Therefore, the impact of genetic differences on sensitivity to neurotoxicants such as DFP could be an important tool in understanding the mechanism of action of these agents.

A functional observational battery (FOB) was utilized to assess the effects of three-day exposure to the formamidine pesticide amitraz in outbred Sprague-Dawley-derived and inbred Fischer-344-derived (F344) rats (both from Charles River Laboratories) and in outbred Long-Evans rats obtained from two commercial suppliers (Charles River Breeding Laboratories and Blue Spruce Farms) [56]. Significant strain and stock differences were obtained in baseline values for one-third of the FOB measures. In most cases, F344 rats were different from the others. Characteristic signs of amitraz exposure consisting of increased excitability, hyperreactivity, and physiological and autonomic changes were evident in all treated rats (Table 5). These effects increased with repeated treatments, and many were still present six days after treatment. On individual measures, there were differences between the strains and stocks in terms of sensitivity and time course of amitraz effects. In general, Blue Spruce, Long-Evans rats displayed more effects of amitraz, and F344 rats recovered more quickly than other rat strains. Although Sprague-Dawley rats were the least affected overall, they had the largest increases in the sensorimotor responses to stimuli. These data indicate that although some behavioral and physiological parameters showed strain and supplier differences, in both baseline values and the effects of amitraz, conclusions could be reached concerning its neurotoxic potential in a screening context of rats.

Table 5. Measures of the FOB that showed significant differences in baseline values between strains and stocks of rats (Moser et al. 1991)

	Fischer (F344)	Sprague-Dawley (SD)	Charles River Long-Evans (CR)	Blue Spruce Long-Evans (BS)
	Inbred	Outbred	Outbred	Outbred
CNS excitability, activity				
Unsupported rearing (rears)	0a	0.3ab	0.9b	0.9b
Autonomic				
Urination (urinations)	0.1a	1.2bc	0.6b	1.7c
Defecation (boluses)	0a	0.5b	0.7b	0.3ab
Sensorimotor				
Tail pinch response(rank, 1-5)	2.0a	2.6b	2.3ab	2.3ab
Touch response(rank, 1-5)	2.2ab	2.0a	2.4b	1.8a
Finger snap response (rank, 1-5)	2.2b	2.1ab	2.3b	2.0a
Neuromuscular function				
Forelimb grip strength(kg)	0.746a	0.973b	1.022b	1.092b
Hindlimb grip strength(kg)	0.363a	0.556b	0.614b	0.582b
Landing foot splay (mm)	45.7a	81.3c	69.1b	93.2d
Physiologic				
Body weight (g)	239a	409c	345b	397c

Groups with the same letter are not significantly different ($p<0.05$). Mean values for each measure are indicated.
N＝24 for each group. Male animals used＝70-80 days of age.
a, b, c, d Groups with the same letter are not significantly different ($p<0.05$).

Tri-*o*-cresylphosphate (TOCP) is well known as the most notorious induce of a delayed neurotoxity [2]. The responsiveness of the central nervous system (CNS) to TOCP is highly dependent on the strain of rat examined. In a series of studies, Abou-Donia et al. [2] and Somkuti et al. [67] demonstrated that TOCP failed to induce neurobehavioral and neuropathologic alterations in Fischer-344 and Sprague-Dawley rats, indicating that these two strains were not sensitive to the delayed neurotoxic effects of this organophosphate ester, a finding opposite to the effect reported for Long-Evans rats [77]. Subsequently, Carrington and Abou-Donia [10] found that the TOCP-induced inhibition of brain neurotoxic esterase, believed to be involved in delayed neurotoxicity, was equivalent in LE and F344 rats, with a two-fold higher concentration of chemical needed to produce an effect in SD rats. A similar pattern was noted for brain AChE inhibition, with SD rats being the least sensitive. It is well established that in LE and W/SPF rats inhibition of neurotoxic esterase by organophosphorous compounds correlates with histopathologic lesions [40] and that a lack of enzymatic inhibition in SD rats is associated with no apparent neurotoxicity. However, the findings that TOCP-induced inhibition of neurotoxic esterase is associated with no evidence of neurotoxicity in F344 rats suggests that metabolic and pharmacokinetic differences between LE and F344 animals might account for the observed discrepancy [2, 10, 67]. Because a metabolite of TOCP, 2-*o*-cresyl-4H-1:3:2-benzyldioxaphoran-2-one, was found to induce delayed neurotoxicity of an unspecified rat strain [25], it is conceivable that F344 rats, in comparison with LE animals, may not generate sufficient quantities of metabolite to inhibit neurotoxic esterase, and even if the metabolite does reach the target tissue, the F344 rat seems resistant to any toxic manifestation [10]. Data thus show that an outbred (SD) stock is not sensitive to TOCP-induced delayed neurotoxicity. There is a marked difference in the neuronal responsiveness of rats to TOCP, with LE and Wistar displaying sensitivity whereas in F-344 rats there is a lack of response.

Within other stocks, there is a marked difference in the neuronal responsiveness of rats to TOCP, with LE and Wistar rats displaying sensitivity, while F344 rats have a lack of response. This is not surprising based on an extensive study of 46 different rat strains by Glowa and Hansen [29], who found a wide variation in responsiveness of about 9 to 1 in acoustic startle stimulus among strains. It is evident that there are phenotypic differences among strains, and that these genetic components contribute to variation in neuronal responsiveness.

The organophosphate insecticides bind to AChE in an irreversible fashion, and inhibition of this enzyme may reflect the adverse effects on behavior and CNS function including hypothermia and reduced motor activity [30, 32-33]. These disturbances in CNS function and marked inhibition of AChE activity are not a feature of organophosphorous triester toxicity described previously. In a recent study, Gordon and MacPhail [31] noted that rat strain was a factor in diisopropyl fiuorophosphate (DFP)-induced neurotoxicity. DFP inhibited the activity of serum ChE in LE, SD, and F344 rats; however, the inhibition observed in F344 animals was significantly less than in other rat strains. DFP produced hypothermia in LE and SD rats but no change in body temperature in F344 rats. Motor activity was reduced by DFP in LE rats at a low dose, whereas a high dose was required to produce this effect in F344 rats. The DFP-induced decrease in motor activity produced in SD rats was dependent on the methodology used to test this parameter, but this strain in general was either more sensitive or equal to F344 in responsiveness. In a subsequent study, Gordon and Watkinson [35] confirmed that DFP decreased body temperature and heart rate to the greatest extent in LE compared to F344 rats. Although DFP lowered body temperature in SD rats, this was associated with a rise in heart rate,

suggesting that autonomic function responses to this organophosphate are distinct from thermoregulatory and behavioral functions. These results taken together clearly demonstrate that the F344 rat strain is most resistant and the LE rat is most sensitive to DFP-induced neurotoxicity. Strain-related differences also exist in the ability to detoxify organophosphate pesticides. It was found that serum carboxylesterase activity, believed to inactivate organophosphate pesticides, was highest in SD rats, followed by LE and F344 rats in descending order, but that in the presence of an organophosphate compound this enzyme was inhibited to the greatest extent in F344 rats [13]. These findings would support the conclusion that F344 rats are more resistant to DFP-induced CNS changes and that this rat strain should be considered in the assessment of neurotoxicologic hazards of organophosphates. Tables 6 and 7 summarize strain-related differences in CNS responses

Table 6. Summary of strain-related differences in CNS responses (Kacew et al. 1995)

Chemicals	Responses	Strains			
		SD	F344	LE	Wistar
TOCP	Neuropathologic alterations	Absent	Absent	Present	Not done
	Brain neurotoxic estrase activity inhibition (ED50 in mg/kg)	458	209	288	Not done
	Brain AChE activity inhibition (ED50 in mg/kg)	1,007	408	420	Not done
DFP	Serum ChE activity inhibition	Serve#	Mild	Moderate	Moderate
	Brain AChE activity inhibition	Moderate	Mild	Moderate	Mild
	Heart rate	Increase	Decrease	Decrease	Decrease
	Decreased motor activity	Moderate	Moderate	Moderate	Moderate
	Decreased body temperature	Moderate	Mild	Mild	Mild
Methanol	Decreased body temperature within 1 hour and for 6 hours	Not done	Mild	Marked	Not done
TMT	Syndrome of tremor, hyperactivity, and elevated reactivity	Not done	Marked	Mild	Not done
DSP-4	Reduction in noradrenergic terminals in neocortex and cerebellum	Marked	Not done	Absent	Not done

Abbreviations of chemicals: TOCP＝tri-*o*-cresylphosphate, DFP＝diisopropyl fiuorophosphate, TMT＝trimethyltin,.
DSP-4＝N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine.
ED: effective dose
# Denotes severity in response, as adapted from Gordon and Watkinson 1995.

The neurotoxicant actions of methanol were evaluated on the thermoregulatory system of LE and F344 rats. In general, Mohler and Gordon [58] found that methanol induced a similar hypothermia in both strains without an effect on basal metabolic rate. However, there was a strain-related difference in the time course of methanol-induced hypothermia. In LE rats, methanol produced a sharper drop in colonic temperature within one hour, as compared to F344 rats. In addition, the colonic temperature remained lower throughout the six-hour duration in LE rats, suggesting that F344 rats may be less sensitive to the neurotoxicant action. This would be in accordance with the findings of DFP-induced neurotoxicity [31].

The neurotoxicant effects of trimethyltin (TMT) on CNS histopathology and temperature regulation are well documented [16]. In a recent study, Gordon and Fogelson [32] found that the colonic temperature of F344 rats was significantly less than in LE rats. Following intravenous administration of TMT, both rat strains became significantly hypothermic; however, five hours after administration of TMT, colonic temperature was significantly higher in F344 rats than in the respective control groups, whereas in LE rats, colonic temperature was equal between treated and respective control groups. The evaporation water loss was markedly higher in F344 rats as ambient temperature was increased, indicating that this strain of rat adapts more readily to transient hyperthermic conditions. Because a rise in body temperature is associated with enhanced toxicity to chemicals, the ability to reach a lower body temperature sooner, as seen in F344 rats, may be related to reduced neurotoxicity. It is thus conceivable that the decreased sensitivity of F344 rats to TMT in acute exposure (one day), compared to LE rats, is due to the increased ability for evaporative water loss [32]. However, chronic 42-day intravenous administration of TMT [57] demonstrated a characteristic syndrome of tremor, hyperactivity, and elevated reactivity in LE and F344 rats, although the magnitude of response was considerably greater in the F344 strain.

Guidelines for a functional observational battery (FOB) have been published by OECD 407 and EPA. An international harmonized guideline for FOB is expected in the near future.

Table 7. Summary of strain-related differences in CNS responses (Kacew and Festing 1996)

Responses	Strains							
	F344	LEW	SD	Long-Evans	Wistar	NBR	AC1	MR
SCW-induced hypothalamic CRF secretion	Resistant	Susceptible						
Acute startle stimulus increase in corticosterone	Susceptible	Resistant						
CRF-induced open-field behavior	Susceptible	Resistant						
Body temperature adaptation	Resistant		Susceptible	Susceptible				
Apomorphine disruption of prepulse inhibition			Resistant		Susceptible			
Morphine-induced antinoception	Susceptible		Resistant					
Footshock-induced analgesia	Susceptible		Resistant					
Imipramine-induced decrease in immobility (despair)	Susceptible		Resistant		Susceptible			
Naloxone attenuation of footshock analgesia			Susceptible		Resistant			
Cocaine-induced increase in locomotor activity	Susceptible Resistant	Resistant Susceptible				Resistant		
Amphetamine-induced increase in locomotor activity	Susceptible	Resistant						
Ethanol preference and behavior activation	Resistant	Susceptible					Resistant	Susceptible

Resistant does not imply a lack of effect but a significantly lower incidence than in susceptible strains
Blank: not done

4. Monitoring and Global Standards

Although this is repetition of the 1998 edition [49], we would like to include the following information.

The necessity of global standardization of strains, housing conditions, genetic control, or microbiological control of rats is of great concern to the authors of this publication as well as to many other investigators,. In order to closely monitor phenotypes in rats, 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 [18], statistical methods, pathological terms, teratological terms [83], clinical pathology examinations [80], neurotoxicological observation methods [61], etc. With this aspect in mind, some international conferences such as ICH and ICLAS 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.

Although the scale and extent of comparative studies of strain differences in rats have increased, the rewards are less, resulting in a smaller number of studies. Therefore, databases are extremely valuable. As described previously, there are many rat strains used, depending on the objectives of the research/studies. However, relatively few rat strains are used in general toxicity, reproductive and developmental toxicity and carcinogenicity studies conducted for regulatory use. Therefore, international integration of these stains is not only possible, but also highly desirable. Once this is realized, expenses and time will be reduced, and the value and reproducibility of the results of safety studies across laboratories will be increased.

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CHAPTER 2

General Toxicology Related To

Comparison of General Toxicological Parameters Between Crj:CD(SD)IGS and Slc:SD rats

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ABSTRACT. General toxicological parameters were compared between Sprague-Dawley rats from different breeders: Crj:CD(SD)IGS (hereafter, IGS) and Slc:SD (hereafter, Slc). IGS rats (20/sex/group) were obtained from Charles River Japan Inc, and Slc rats (20/sex/group) were from Japan Slc. Co., Ltd To imitate an oral toxicity study, the rats were given a 0.5% methylcellulose solution by oral gavage for 6 months beginning at 6 weeks of age. Hematology, serum chemistry, urinalysis and ophthalmology examinations were carried out before and 1, 3 and 6 months after beginning of dosing. Organ weight measurements and pathological examination were carried out at 6 months (32 weeks of age). When compared to male Slc rats, male IGS rats showed higher body weight, while in females the values were comparable. A similar tendency was observed in food consumption. In urinary examination, protein excretion was observed less frequently in IGS rats than in Slc rats. There were differences between the two groups in the following hematology and serum chemistry parameters: white blood cell counts and lymphocyte counts, IGS>Slc (Male and Female); ALP, IGS<Slc (M & F); globulin, IGS<Slc (M & F); 5'nucleotidase, IGS<Slc (M). Absolute and relative weights of the adrenal glands (M & F) and testes of Slc rats were higher than of IGS rats. Renal changes associated with chronic progressive nephropathy, though the degrees were slight, were more prominent in the Slc rats. The present data indicate that there are differences in body weight, hematology and serum chemistry parameters, organ weight and pathological findings between IGS and Slc Sprague-Dawley rats, indicating intra-strain differences. — Key words: Intra-strain difference, Intra-species difference

CD(SD)IGS-2000: 15-30

INTRODUCTION

Reviewing normal values presented in various booklets and communications, it appears that there are differences in general toxicological parameters between Sprague-Dawley rats from different breeders. However, published reports concerning the intra-strain differences are less available, except for comparisons between IGS and Crj:CD(SD) rats [8, 9, 10, 12, 14, 16, 17, 18, 19, 24]. In this study, we conducted a 6 month oral toxicity study to make a comparison of the toxicological parameters between two Sprague-Dawley rats: Crj:CD(SD)IGS (hereafter, IGS) and Slc:SD (hereafter, Slc).

MATERIALS AND METHODS

Animals:

Male and female IGS rats, 4 weeks age old, were purchased from Charles River Japan Inc. (Hino farm). Both sexes of Slc rats, also 4 weeks age, were from Japan SLC Co. Ltd. (Inasa farm).

Animal husbandry:

All animals were housed in one animal room under the following conditions: temperature at 23 ± 2℃, relative humidity at 55 ± 5%, air change at 10 to 15 times per hour and 12-hour illumination (06:00hr to 18:00hr). The animals were kept individually in metal mesh cages (260 × 200 × 180mm). Animals had free access to tap water and to a pelleted commercial laboratory animal food (CE-2, Clea Japan Inc.).

Dosing procedure:

In order to imitate typical toxicological studies, the animals were dosed orally. A 0.5% solution of methylcellulose, a commonly used vehicle solution, was administered five days per week.

Observations and examinations:

1) Mortality and clinical signs

All animals were observed five days per week in their cages for clinical signs of viability and any changes in appearance and behavior.

2) Body weight

Each animal was weighed using an electronic balance (Sartorius LC-4201, Zeiss) weekly.

3) Food consumption

The weight of animal diet given and that of the diet remaining were measured weekly for all animals with an electrical balance (Sartorius LC-4201, Zeiss). Food consumption (g) per day was calculated.

4) Ophthalmology

Ophthalmology examinations were performed on all animals once before the study (before beginning of dosing, 4 weeks of age) and 1, 3 and 6 months after the study (9-10 weeks of age, 17-18 w and 29-30 w, respectively). During the study period, the examinations were performed before dosing. The pupillary light reflex was evaluated, prior to instillation of the mydriatic (Mydrin®-P, Santen Pharmaceutical Co., Ltd.). After induction of mydriasis with the mydriatic, the external ocular part was examined with a slit lamp (SL-J, Neitz). The anterior and posterior (fundus) segments were examined with a hand-held camera (RC-2 model 621 or Genesis K9L22, Kowa Co., Ltd.).

5) Clinical pathology

(1) Urinalysis

Urinalysis was carried out before the study (5 weeks of age) and 1, 3 and 6 months after the beginning of study (8-9 weeks of age, 16-17 w and 30-31 w, respectively). Urine was collected individually in a metabolic cage for approximately 15 hours prior to blood collection. The rats were fasted during the urine collection, but were permitted free access to water. Volume of urine was measured using a calibrated cylinder. Urine was grossly examined for color and clarity. Specific gravity was measured with a refractometer (TS-SE, Atago Co., Ltd.). Creatinine and N-acetyl- β-D-glucosaminidase (NAG) were determined by the following procedures using a centrifugal analyzer (Cobas Fara, Baxter); the enzymatic assay (creatinine) and m-cresolsulfonphthaleinyl-N-acetyl- β -D-glucosaminide substrate method (NAG). Urine samples

were examined for pH, protein, ketones, bilirubin, occult blood, urobilinogen and glucose, using a reagent strip (Multistix test paper, Miles-Sankyo Co., Ltd.) and an automatic urine analyzer (Clinitec 200 +, Bayer-Medical Co., Ltd.). The urinary sediment was stained with Sternheimer-Malbin (Muto Pure Chemicals Ltd.). Microscopic examination was carried out for red blood cells (RBC), white blood cells (WBC), squamous cells, small round cells, round cells, transitional cells, bacteria, ammonium magnesium phosphate crystals, calcium oxalate crystals, calcium carbonate crystals, other crystals, granular casts, hyaline casts, waxy casts and other casts.

(2) Hematology

Blood samples for hematology were collected in the fasted state (approximately 15 hours) from the jugular vein at 1, 3 and 6 months (8-9 weeks of age, 16-17 w and 30-31 w, respectively). EDTA-2K was used as an anticoagulant. The following parameters were measured with the Technicon H･1 (Bayer-Medical Co., Ltd.); red blood cell counts (RBC), hemoglobin concentration, hematocrit, platelet counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell counts (WBC). White blood cell differential was also measured as absolute counts and % of WBC; neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells (LUC). Reticulocytes were microscopically estimated on smears stained the EDTA-2K-anticoagulated blood by the Brecher method, and expressed as % of RBC. Blood samples for fibrinogen were collected in the non-fasted state from the jugular vein in a ratio of 1 vol. of 3.8% sodium citrate and 9 vol. of blood at 1, 3 and 6 months (10 weeks of age, 18 w and 30 w, respectively). The plasma level of fibrinogen was determined from the thrombin time with a coagulometer (KC-10A, Baxter).

(3) Serum chemistry

Blood samples for serum chemistry determinations were collected in the fasted state from the jugular vein before (5 weeks of age) and 1, 3 and 6 months after the study (8-9 weeks of age, 16-17 w and 30-31 w, respectively). The following assays were performed using a centrifugal analyzer (Cobas Fara, Baxter); alanine aminotransferase (ALT) (the UV-rate method), aspartate aminotransferase (AST) (the UV-rate method), alkaline phosphatase (ALP) (the p-nitrophenyl phosphate substrate method), γ-glutamyl transferase (GGT) (Orlouski method), 5'nucleotidase (the UV-rate method), total bilirubin (the vanadic acid method), total protein (the Biuret method), albumin (the bromocresol green method), total cholesterol (the enzymatic assay), triglycerides (the α-glycerophosphoric oxidase method), glucose (the glucokinase-glucose 6-phosphate dehydrogenase method), blood urea nitrogen (BUN) (the urease UV method), creatinine (the enzymatic assay) and calcium (the cresolphthalein complexone method). Globulin and albumin/globulin ratio (A/G) were calculated from total protein and albumin values. Sodium, potassium and chloride were measured with the ion-selective electrode method using an electrolyte system (EL-ISE, Beckman)

6) Necropsy and organ weight

Necropsy and organ weight measurements were conducted for all surviving animals at 32 weeks. Before necropsy, all animals were fasted overnight (approximately 15 hours) and were weighed before they were exsanguinated under CO_2 inhalation. The weight of the heart, kidneys (combined), adrenals (combined), brain, liver and testes (combined) were measured with an electrical balance (Sartorius L-420S, Zeiss). Ratios of organ weight to terminal body weight (% of body weight) were calculated.

7) Histopathology

Eyes and Harderian glands were fixed in Davidson fixative. All other organs and tissues were fixed in 10% neutral buffered formalin: *i.e.*, the adrenal glands, aorta, bone (sternum, including bone marrow), brain (cerebrum, cerebellum and pons), cecum, colon, duodenum, esophagus, epididymides, heart, ileum, jejunum, kidneys, liver, lung, mesenteric lymph node, ovaries, pancreas, peripheral nerve, pituitary gland, prostate, salivary gland, seminal vesicle, skeletal muscle, skin (including mammary gland), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus and vagina. Tissues and organs were then trimmed, dehydrated, embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin. The processed tissues were examined by light microscopy.

8) Statistical analysis

Specific gravity and pH in urinalysis were analyzed with the Mann-Whiteney rank sum test. The cumulative chi-square test was used with respect to qualitative parameters in urinalysis. Incidences of histopathological findings were analyzed with the chi-square test or the Fisher's extract test. The unpaired *t*-test was applied for other parameters in body weight, food consumption, urinalysis, hematology, serum chemistry and organ weight. We considered a probability (P) of less than 0.05 as significant.

RESULTS

All animals survived except two male IGS rats and one female Slc rat due to gavage error. Broken incisors were observed in four male IGS rats during the experimental period.

Fig. 1 shows changes in body weight. When compared to male Slc rats, male IGS rats showed higher body weight. The difference was approximately 10% at the end of experiment (32 weeks of age). In females, the changes of body weight were similar to each other. There were temporary decreases corresponding to blood collection at 1, 3 and 6 months.

Food consumption in male IGS rats was higher than in male Slc rats (Fig. 2). In females, food consumption was comparable between IGS and Slc rats.

In ophthalmology, a lens opacity was observed in one male Slc rat at 6 months.

Tables 1 - 4 show results of urinalysis. The excretion of NAG in male IGS rats was greater than that in male Slc rats throughout the experimental period, and in females higher values were observed before and 1 month after the study stage. IGS rats excreted less creatinine than Slc rats before the study. Other differences were found in protein (IGS < Slc, in both sexes at 6 months), ketones (IGS > Slc, in females before the study), urobilinogen

(IGS > Slc, in females at 1 and 6 months) and ammonium magnesium phosphate crystals (IGS > Slc, in females before the study and at 1 month).

The results of hematology are summarized in Tables 5 and 6. Lymphocyte counts in IGS rats were higher than in Slc rats (in males at 1, 3 and 6 months and in females at 1 month). In contrast, neutrophil counts in female IGS rats were lower than that in female Slc rats. These changes affected WBC; *i.e.*, IGS rats had higher WBC than Slc rats (in males at 1, 3 and 6 months and in females at 1 month). The lymphocyte counts showed an age-dependent decrease in both sexes, while neutrophil counts showed an age-dependent increase in males.

The results of serum chemistry are summarized in Tables 7 and 8. Differences were found in ALP (IGS < Slc in both sexes), 5'nucleotidase (IGS < Slc in males), bilirubin (IGS > Slc in both sexes before the study) and creatinine (IGS < Slc in both sexes before the study). ALP decreased in an age-dependent manner. 5'Nucleotidase increased with aging. Concerning globulin, IGS rats showed a lower level than Slc rats, and thus a mirror image was obtained in the A/G ratio. In regard to lipid parameters, male IGS rats showed relatively higher levels in cholesterol and triglycerides than Slc rats before the study, and in turn showed a lower level of triglycerides than male Slc rats at 6 months. Female IGS rats had lower cholesterol levels than female Slc rats at 3 and 6 months.

The organ weights are shown in Table 9. As shown in the first line, male IGS rats weighed more than male Slc rats. The absolute weights in heart and kidney of male IGS rats were higher than male Slc rats, whereas their relative weights became comparable. The relative weights of brain and liver in male IGS rats were lower than those male Slc rats. The absolute and relative weights of adrenal and testes in IGS rats were lower than in Slc rats. On the other hand, in females the terminal body weights were close to each other (Lower part of Table 9). Female IGS rats showed lower values in the absolute and relative weights of kidney, adrenal and liver, and in absolute weight of brain.

Table 10 shows the histopathological findings. Noticeable findings, though the degrees were slight, were confined to the kidney from Slc rats. Some renal lesions associated with chronic progressive nephropathy were more prominent in the Slc rats. The renal lesions consisted of focal or multifocal areas of basophilic tubules in cortices and were often accompanied by intratubular proteinaceous cast formation.

Sporadic findings in histopathology were present in some organs for both IGS and Slc rats, and were incidental in nature. Other statistical differences including urinalysis, hematology and serum chemistry were biologically meaningless.

DISCUSSION

Many authors have reported that the body weight in IGS rats is easily changeable dependent on diet [1, 6, 8, 11, 15, 20]. The body weight of the IGS rats in the present study was within the range of these previous studies. The present comparison clearly indicated that male IGS rats had greater body weight in comparison with Slc rats, while in females the body weights were close to each other. These results confirmed our expectation. Temporary decreases in body weight were probably result of blood sampling, being well in accordance with the previous report [13].

There were no significant differences in clinical signs and ophthalmology between IGS and Slc rats.

The renal lesions which consisted of focal or multifocal areas of basophilic tubules in cortices were interpreted as an early manifestation of chronic progressive nephropathy [2]. The incidence was higher in Slc than in IGS rats. This was consistent with the result of protein excretion, but not with NAG excretion in urinalysis.

In urinalysis, IGS rats showed relatively lower excretion of creatinine than Slc rats. This difference is probably related with that in serum level of creatinine. Biological significances regarding the different incidences of ketones, urobilinogen and ammonium magnesium phosphate crystals are uncertain.

Worford et al. [22, 23] reported that rat WBC and lymphocyte counts decrease with aging, whereas neutrophil counts increase. The present results agreed well with these previous reports. IGS rats showed higher lymphocyte and less neutrophil counts in comparison with Slc rats. This can not be explained by a difference in body weight gain, since IGS rats showed a higher growth rate. It is noteworthy that Slc rats had greater adrenal weights. Dhabhar et al. [3, 4, 5] reported that corticosterone from the adrenal gland lowers the lymphocyte count in circulating blood and raises the neutrophil count in rats. Together with the result on lymphocytes described above, globulin, an immunological parameter, also showed a difference. This area involving hormonal control and immunological differences may be of future interest.

ALP decreased in an age-dependent manner, being in agreement with the previous report [21]. In this study, IGS rats showed a lower level of ALP than Slc rats. This may be partially caused by a higher body weight gain in male IGS rats, but is still unclear in females.

Inoue et al. [7] reported relatively low levels of serum triglycerides and cholesterol in IGS rats. In this study, again, IGS rats showed relative lower levels in triglycerides and cholesterol. However, this tendency was limited to the later stage of the present experiment. In the early stage, opposite results were obtained.

Biological significance in different levels of 5'nucleotidase and total bilirubin between these two rats is uncertain.

In organ weight, a marked difference was found in testes. This becomes important in evaluating the effects of test substance on the reproductive system.

In conclusion, the present data indicate biological differences between the groups of Sprague-Dawley rats, supporting previous data and expectations. These findings should be taken into consideration when assessing the effects of the test substance.

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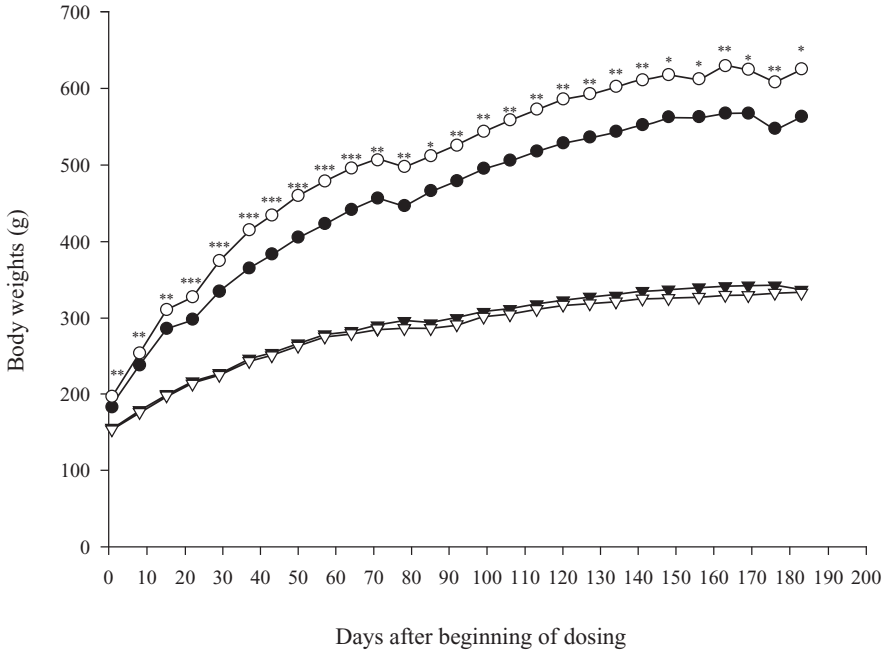

Figure 1. Comparison of body weight between IGS and Slc rats. Each symbol means male IGS (open circle ○), male Slc (filled in circle ●), female IGS (open triangle △) and female Slc (filled in triangle ▲). The data represent the mean. Statistical significant difference was as follows; *P<0.05, **P<0.01 and ***P<0.001 (t-test).

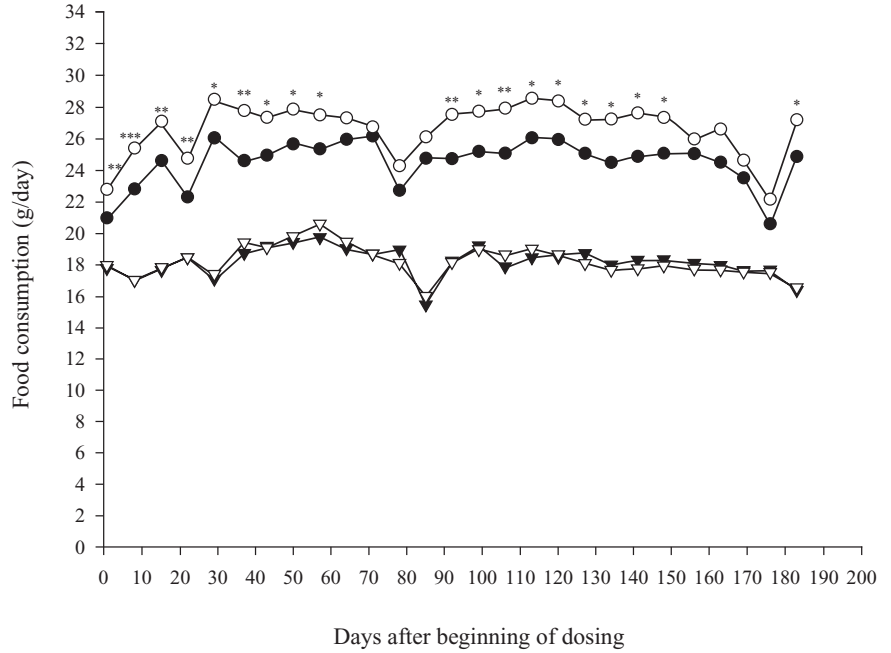

Figure 2. Comparison of food consumption between IGS and Slc rats. Each symbol means male IGS (open circle ○), male Slc (filled in circle ●), female IGS (open triangle △) and female Slc (filled in triangle ▲). The data represent the mean. Statistical significant difference was as follows; *P<0.05, **P<0.01 and ***P<0.001 (t-test).

Table 1. Comparison of urinalysis data between male IGS and Slc rats

Parameters	Before		1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=18)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
Specific gravity	1.016±0.015	1.019±0.014	1.018±0.009	1.026±0.018	1.034±0.009	1.038±0.017	1.049±0.017	1.051±0.020
Volume (ml)	16.990±9.043	13.980±7.922	18.380±11.028	20.600±15.650	10.870±3.715	12.570±7.838	7.160±3.198	8.878±4.941
pH	6.280±0.444	6.430±0.406	6.680±0.373	6.680±0.467	6.530±0.255	6.580±0.373	6.350±0.328	6.420±0.393
N-acetyl-β-D-glucosaminidase (NAG) (U/L)	5.985±5.792	12.380±9.507*	9.935±3.954	15.940±9.241*	14.600±3.368	20.325±8.939*	20.265±5.664	24.867±9.046
Creatinine (mg/dl)	20.140±18.792	21.145±16.288	41.800±18.566	51.625±33.644	89.765±24.147	105.515±47.748	145.490±56.412	152.872±67.681
NAG (overnight excretion) (U)	0.063±0.019	0.114±0.023***	0.151±0.041	0.221±0.072***	0.152±0.032	0.206±0.061**	0.133±0.044	0.192±0.064**
Creatinine (overnight excretion) (mg)	2.137±0.250	1.928±0.310*	6.053±0.737	6.515±1.087	9.132±1.387	10.321±1.575*	8.985±1.837	10.890±1.964**

*: p<0.05, **: p<0.01, ***: p<0.001 (t-test)
#: p<0.05, ##: p<0.01, ###: p<0.001 (Mann-Whitney rank sum test)

Table 2. Comparison of urinalysis data between female IGS and Slc rats

Parameters	Before		1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=19)	IGS (n=20)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
Specific gravity	1.015±0.013	1.022±0.012##	1.019±0.008	1.038±0.016###	1.041±0.022	1.033±0.019	1.034±0.018	1.057±0.015###
Volume (ml)	16.570±8.579	8.180±4.174***	20.570±22.947	7.440±4.021*	8.733±7.634	11.880±9.675	10.824±9.436	4.950±2.194*
pH	6.430±0.406	6.380±0.455	6.350±0.462	6.080±0.294#	6.220±0.392	6.250±0.596	6.290±0.470	5.800±0.441##
N-acetyl-β-D-glucosaminidase (NAG) (U/L)	5.195±4.551	12.150±6.318***	6.540±3.187	17.380±6.985***	16.094±6.823	13.460±8.444	14.182±6.876	25.935±8.110***
Creatinine (mg/dl)	19.195±16.454	26.125±12.519	32.780±14.821	63.525±25.591***	87.128±41.482	66.590±36.096	76.229±40.663	130.300±41.614***
NAG (overnight excretion) (U)	0.057±0.014	0.079±0.019***	0.083±0.019	0.109±0.030**	0.101±0.027	0.105±0.028	0.107±0.034	0.116±0.028
Creatinine (overnight excretion) (mg)	2.172±0.410	1.707±0.286***	4.230±0.475	3.884±0.769	5.349±0.833	5.398±0.979	5.512±0.750	5.749±0.910

*: p<0.05, **: p<0.01, ***: p<0.001 (t-test)
#: p<0.05, ##: p<0.01, ###: p<0.001 (Mann-Whitney rank sum test)

Table 3-1. Comparison of urinalysis data between male IGS and Slc rats

Parameters		Before							1 Month							3 Month							6 Month						
		N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+
Color	Slc	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	18	(18	0	0	0	0	0)
Clarity	Slc	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	18	(18	0	0	0	0	0)
Protein	Slc	20	(17	1	2	0	0	0)	20	(1	9	10	0	0	0)	20	(0	1	17	2	0	0)	20	(0	1	6	10	3	0)
	IGS	20	(16	2	2	0	0	0)	20	(5	5	8	2	0	0)	20	(2	2	13	3	0	0)	18	(0	5	8	5	0	0)**
Ketones	Slc	20	(17	3	0	0	0	0)	20	(11	9	0	0	0	0)	20	(0	9	11	0	0	0)	20	(0	11	9	0	0	0)
	IGS	20	(13	7	0	0	0	0)	20	(10	8	2	0	0	0)	20	(2	7	11	0	0	0)	18	(4	10	4	0	0	0)*
Bilirubin	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(19	−	1	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(17	−	1	0	0)	
Occult blood	Slc	20	(20	0	0	0	0)		20	(20	0	0	0	0)		20	(15	5	0	0	0)		20	(20	0	0	0	0)	
	IGS	20	(20	0	0	0	0)		20	(20	0	0	0	0)		20	(19	0	0	0	1)		18	(15	0	0	1	2)*	
Urobilinogen	Slc	20	(20	−	0	0	0	0)	20	(19	−	1	0	0	0)	20	(19	−	1	0	0	0)	20	(11	−	9	0	0	0)
	IGS	20	(19	−	1	0	0	0)	20	(15	−	5	0	0	0)	20	(16	−	4	0	0	0)	18	(9	−	9	0	0	0)
Glucose	Slc	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	18	(18	0	0	0	0	0)
RBC	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(19	−	0	1	0)		18	(16	−	1	0	1)	
WBC	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(16	−	2	0	0)	
Squamous cells	Slc	20	(0	−	20	0	0)		20	(0	−	20	0	0)		20	(0	−	15	5	0)		20	(0	−	20	0	0)	
	IGS	20	(0	−	20	0	0)		20	(0	−	20	0	0)		20	(0	−	20	0	0)*		18	(0	−	18	0	0)	
Small round cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)	

*p<0.05, **p<0.01 (cumulative chi-square test)

Table 3-2. Comparison of urinalysis data between male IGS and Slc rats

Parameters		Before							1 Month							3 Month							6 Month						
		N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+
Round cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0		20	(20	−	0	0	0)		18	(18	−	0	0	0)	
Transitional cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0		20	(20	−	0	0	0)		18	(18	−	0	0	0)	
Bacteria	Slc	20	(0	−	17	3	0)		20	(0	−	15	5	0)		20	(0	−	20	0	0)		20	(0	−	19	1	0)	
	IGS	20	(0	−	18	2	0)		20	(0	−	18	2	0)		20	(0	−	20	0	0)		18	(0	−	15	3	0)	
Amonium magnesium phosphate crystals	Slc	20	(13	−	7	0	0)		20	(5	−	14	1	0)		20	(0	−	13	7	0)		20	(1	−	15	3	1)	
	IGS	20	(8	−	11	1	0)		20	(3	−	12	5	0)		20	(0	−	11	9	0)		18	(0	−	13	5	0)	
Calcium oxalate crystal	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)	
Calcium carbonate crystals	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)	
Other crystals	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)	
Granular casts	Slc	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				18	(18	−	0)			
Hyaline casts	Slc	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				18	(18	−	0)			
Waxy casts	Slc	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				18	(18	−	0)			
Other casts	Slc	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				18	(18	−	0)			

*p<0.05, **p<0.01 (cumulative chi-square test)

Table 4-1. Comparison of urinalysis data between female IGS and Slc rats

Parameters		Before							1 M							3 M							6 M						
		N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+
Color	Slc	20	(20	0	0	0	0	0)	20	(18	2	0	0	0	0)	18	(18	0	0	0	0	0)	17	(15	2	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
Clarity	Slc	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	18	(18	0	0	0	0	0)	17	(17	0	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
Protein	Slc	20	(18	1	1	0	0	0)	20	(16	4	0	0	0	0)	18	(7	7	4	0	0	0)	17	(5	3	3	3	3	0)
	IGS	20	(18	1	1	0	0	0)	20	(11	5	4	0	0	0)*	20	(11	3	6	0	0	0)	20	(4	6	9	1	0	0)*
Ketones	Slc	20	(19	1	0	0	0	0)	20	(20	0	0	0	0	0)	18	(17	1	0	0	0	0)	17	(16	1	0	0	0	0)
	IGS	20	(13	7	0	0	0	0)*	20	(19	1	0	0	0	0)	20	(19	1	0	0	0	0)	20	(18	2	0	0	0	0)
Bilirubin	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(17	−	1	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(19	−	1	0	0)		20	(20	−	0	0	0)		20	(17	−	3	0	0)	
Occult blood	Slc	20	(20	0	0	0	0)		20	(20	0	0	0	0)		18	(18	0	0	0	0)		17	(15	1	0	0	1)	
	IGS	20	(20	0	0	0	0)		20	(20	0	0	0	0)		20	(20	0	0	0	0)		20	(20	0	0	0	0)	
Urobilinogen	Slc	20	(19	−	1	0	0	0)	20	(20	−	0	0	0	0)	18	(11	−	7	0	0	0)	17	(15	−	2	0	0	0)
	IGS	20	(18	−	2	0	0	0)	20	(11	−	9	0	0	0)**	20	(14	−	6	0	0	0)	20	(8	−	12	0	0	0)**
Glucose	Slc	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	18	(18	0	0	0	0	0)	17	(17	0	0	0	0	0)
	IGS	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)	20	(20	0	0	0	0	0)
RBC	Slc	20	(20	−	0	0	0)		20	(19	−	1	0	0)		18	(18	−	0	0	0)		17	(16	−	0	1	0)	
	IGS	20	(20	−	0	0	0)		20	(19	−	1	0	0)		20	(19	−	0	1	0)		20	(20	−	0	0	0)	
WBC	Slc	20	(19	−	1	0	0)		20	(18	−	1	1	0)		18	(18	−	0	0	0)		17	(15	−	2	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(19	−	0	1	0)		20	(20	−	0	0	0)	
Squamous cells	Slc	20	(0	−	20	0	0)		20	(0	−	20	0	0)		18	(0	−	18	0	0)		17	(0	−	17	0	0)	
	IGS	20	(0	−	20	0	0)		20	(0	−	20	0	0)		20	(0	−	20	0	0)		20	(0	−	20	0	0)	
Small round cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	

*p<0.05, **p<0.01 (cumulative chi-square test)

Table 4-2. Comparison of urinalysis data between female IGS and Slc rats

Parameters		Before							1 M							3 M							6 M						
		N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+	N	neg	tr	1+	2+	3+	4+
Round cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
Transitional cells	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
Bacteria	Slc	20	(0	−	16	4	0)		20	(0	−	15	5	0)		18	(0	−	18	0	0)		17	(0	−	14	3	0)	
	IGS	20	(0	−	16	4	0)		20	(0	−	20	0	0)*		20	(0	−	19	1	0)		20	(0	−	19	1	0)	
Amonium magnesium phosphate crystals	Slc	20	(10	−	9	1	0)		20	(11	−	9	0	0)		18	(3	−	15	0	0)		17	(1	−	16	0	0)	
	IGS	20	(3	−	17	0	0)*		20	(1	−	19	0	0)**		20	(8	−	11	1	0)		20	(0	−	20	0	0)	
Calcium oxalate crystals	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
Calcium carbonate crystals	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
Other crystals	Slc	20	(20	−	0	0	0)		20	(20	−	0	0	0)		18	(18	−	0	0	0)		17	(17	−	0	0	0)	
	IGS	20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)		20	(20	−	0	0	0)	
Granular casts	Slc	20	(20	−	0)				20	(20	−	0)				18	(18	−	0)				17	(17	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
Hyaline casts	Slc	20	(20	−	0)				20	(20	−	0)				18	(18	−	0)				17	(17	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
Waxy casts	Slc	20	(20	−	0)				20	(20	−	0)				18	(18	−	0)				17	(17	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			
Other casts	Slc	20	(20	−	0)				20	(20	−	0)				18	(18	−	0)				17	(17	−	0)			
	IGS	20	(20	−	0)				20	(20	−	0)				20	(20	−	0)				20	(20	−	0)			

*p<0.05, **p<0.01 (cumulative chi-square test)

Table 5. Comparison of hematological data between male IGS and Slc rats

Parameters	1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=18)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
WBC ($\times 10^3$/mm^3)	6.27±0.67	9.48±2.14***	5.93±1.23	8.48±2.35***	6.42±1.71	7.97±1.52**
Neutrophils (Count) (/mm^3)	775.4±191.8	907.5±303.9	1224.9±464.4	1296.2±680.9	1887.2±1482.9	1336.4±459.7
Lymphocytes (Count) (/mm^3)	5151.7±571.9	8040.9±1869.3***	4205.1±971.8	6578.0±2321.9***	3980.0±988.8	5906.2±1498.7***
Monocytes (Count) (/mm^3)	151.1±49.5	281.9±90.5***	244.8±124.5	292.1±102.1	233.9±97.1	353.2±105.4**
Eosinophils (Count) (/mm^3)	96.3±57.4	74.0±34.8	146.8±138.2	177.0±232.1	201.0±128.5	237.8±335.0
Basophils (Count) (/mm^3)	13.5±5.4	29.7±14.0***	14.8±8.4	27.5±14.9**	12.2±7.2	17.7±8.1*
Large Unstained Cells (Count) (/mm^3)	86.2±34.4	132.7±56.8**	96.1±41.3	99.0±41.9	101.3±36.1	117.9±32.2
Neutrophils (%)	12.33±2.68	9.71±2.59**	20.65±5.98	17.01±11.85	28.19±12.19	16.95±5.59**
Lymphocytes (%)	82.16±3.55	84.82±2.56*	71.14±8.81	76.03±12.67	63.40±13.22	73.39±10.11*
Monocytes (%)	2.40±0.70	2.97±0.61**	4.03±1.78	3.52±1.16	3.61±0.98	4.44±1.23*
Eosinophils (%)	1.54±0.88	0.83±0.44**	2.36±1.83	1.99±1.93	3.03±1.50	3.50±6.50
Basophils (%)	0.22±0.08	0.30±0.11**	0.25±0.10	0.31±0.11	0.18±0.09	0.22±0.08
Large Unstained Cells (%)	1.3±0.7	1.4±0.5	1.6±0.6	1.2±0.5*	1.6±0.5	1.4±0.6
RBC ($\times 10^6$/mm^3)	8.25±0.35	7.74±0.39***	9.78±0.35	9.50±0.31*	9.17±0.44	8.93±0.39
Hemoglobin (g/dl)	16.51±0.74	16.26±0.62	16.56±0.67	16.74±0.71	15.96±0.81	16.26±0.63
Hematocrit (%)	47.80±2.32	47.47±2.20	51.11±2.47	52.02±2.82	47.03±2.47	47.38±2.85
MCV (fl)	58.1±1.1	61.5±1.7***	52.5±1.6	54.8±1.9***	51.4±2.0	53.1±2.2*
MCH (pg)	20.01±0.40	21.04±0.66***	16.94±0.45	17.62±0.52***	17.41±0.63	18.21±0.78**
MCHC (%)	34.53±0.40	34.27±0.76	32.38±0.80	32.21±0.77	33.96±0.77	34.33±1.02
Reticulocyte (%)	0.76±0.40	1.18±0.38**	0.98±0.37	0.90±0.29	2.20±0.76	1.72±0.54*
Platelets ($\times 10^3$/mm^3)	931.2±85.1	1095.3±83.3***	869.8±99.2	823.2±301.6	954.3±102.1	1059.9±144.2*
Fibrinogen (g/L)	2.220±0.037	2.171±0.028***	2.267±0.052	2.220±0.053**	2.360±0.094	2.314±0.077

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (t-test)

Table 6. Comparison of hematological data between female IGS and Slc rats

Parameters	1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=19)	IGS (n=20)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
WBC ($\times 10^3$/mm^3)	5.19±0.90	7.39±1.53***	5.53±1.26	6.04±1.69	4.95±0.92	4.63±1.14
Neutrophils (Count) (/mm^3)	963.7±264.1	964.3±566.6	1556.1±501.3	1087.7±549.9**	1386.5±460.6	795.3±292.5***
Lymphocytes (Count) (/mm^3)	3869.2±812.5	5885.9±1391.4***	3539.4±1243.3	4387.6±1591.8	3108.1±686.7	3392.0±1020.2
Monocytes (Count) (/mm^3)	155.7±48.6	288.8±173.7**	191.4±71.8	266.9±191.7	207.7±113.6	235.1±53.0
Eosinophils (Count) (/mm^3)	105.7±85.8	123.9±79.6	155.7±177.4	175.8±208.1	144.0±132.1	107.8±38.2
Basophils (Count) (/mm^3)	9.6±4.8	15.8±6.4**	10.2±5.6	15.2±10.6	8.6±4.3	8.2±5.0
Large Unstained Cells (Count) (/mm^3)	71.9±23.1	107.7±65.3*	65.7±23.1	92.8±71.5	89.6±42.0	89.4±24.4
Neutrophils (%)	18.93±5.51	13.17±6.59**	29.12±9.88	19.05±10.21**	27.88±7.17	17.37±5.33***
Lymphocytes (%)	74.47±5.69	79.63±8.67*	63.06±10.61	72.02±12.18*	63.06±8.70	72.82±5.75***
Monocytes (%)	3.00±0.79	3.89±1.79*	3.56±1.27	4.47±3.03	4.14±2.08	5.31±1.40*
Eosinophils (%)	2.05±1.77	1.70±1.04	2.84±3.26	2.74±2.49	2.94±2.63	2.37±0.87
Basophils (%)	0.18±0.08	0.21±0.06	0.18±0.07	0.24±0.13	0.17±0.08	0.17±0.07
Large Unstained Cells (%)	1.4±0.5	1.4±0.7	1.3±0.5	1.5±0.8	1.8±0.8	2.0±0.6
RBC ($\times 10^6$/mm^3)	7.92±0.27	7.52±0.42***	8.53±0.48	7.79±1.53*	7.99±0.35	7.74±0.26*
Hemoglobin (g/dl)	16.36±0.53	16.03±0.96	15.70±0.85	15.32±0.91	15.64±0.68	15.72±0.63
Hematocrit (%)	44.95±1.09	44.93±2.49	46.54±2.61	44.02±8.64	44.16±2.02	44.57±1.99
MCV (fl)	56.8±1.2	59.8±1.6***	54.5±1.3	56.6±1.4***	55.4±1.2	57.6±1.5***
MCH (pg)	20.67±0.57	21.33±0.90**	18.42±0.47	22.52±15.68	19.60±0.47	20.33±0.50***
MCHC (%)	36.38±1.02	35.67±1.14*	33.77±0.51	33.60±0.58	35.43±0.51	35.30±0.43
Reticulocyte (%)	0.75±0.27	0.94±0.38	1.00±0.41	1.00±0.45	1.72±0.35	1.54±0.43
Platelets ($\times 10^3$/mm^3)	986.3±123.7	955.3±190.0	835.1±232.9	819.1±246.9	924.2±147.6	919.5±74.5
Fibrinogen (g/L)	2.141±0.022	2.117±0.044*	2.149±0.035	2.114±0.019***	2.144±0.031	2.099±0.020***

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (t-test)

Table 7. Comparison of serum chemistry data between male IGS and Slc rats

Parameters	Before		1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=18)
	Mean SD	Mean SD	Mean SD	Mean SD	MeanSD	Mean SD	Mean SD	Mean SD
ALT (IU/L)	34.6±3.7	34.7±5.1	33.8±6.8	35.0±6.9	41.7±9.7	38.1±6.9	44.8±14.8	42.1±24.8
AST (IU/L)	112.4±14.5	133.8±12.1***	94.1±14.1	107.0±14.0**	101.1±23.4	111.7±24.1	89.1±17.6	106.3±21.3*
ALP (IU/L)	1590.1±245.6	1052.8±160.1***	956.7±155.2	766.0±178.4***	476.1±106.3	385.6±93.5**	321.8±100.0	253.5±69.6*
GGT (IU/L)	ND	ND	ND	ND	0.35±0.49	0.31±0.76	0.20±0.29	0.10±0.00
5'Nucleotidase (U/L)	25.4±6.1	20.6±3.1**	23.7±3.9	20.6±4.7*	27.5±4.4	21.3±6.3***	34.9±7.2	31.3±9.5
Total Bilirubin (mg/dL)	0.026±0.015	0.052±0.015***	0.030±0.022	0.035±0.020	0.086±0.036	0.089±0.042	0.044±0.024	0.066±0.034*
Total Protein (g/dL)	5.38±0.24	5.24±0.17*	5.86±0.36	5.81±0.36	6.84±0.26	6.54±0.53*	7.02±0.58	6.92±0.41
Albumin (g/dL)	3.58±0.19	3.76±0.25*	3.84±0.25	3.97±0.28	4.28±0.25	4.18±0.31	4.46±0.43	4.51±0.25
Globulin (g/dL)	1.80±0.14	1.48±0.16***	2.03±0.24	1.84±0.26*	2.56±0.18	2.36±0.33*	2.56±0.23	2.41±0.32
A/G Ratio	2.01±0.19	2.60±0.43***	1.94±0.29	2.21±0.37*	1.68±0.18	1.82±0.24*	1.74±0.15	1.91±0.28*
Cholesterol (mg/dL)	77.6±16.4	92.9±17.7**	55.0±12.0	59.1±13.3	66.9±21.0	55.9±16.6	93.9±28.7	79.2±17.2
Triglycerides (mg/dL)	58.7±16.8	87.5±41.2**	39.6±12.2	44.7±16.2	77.4±35.7	46.3±18.5**	117.4±53.3	68.8±30.1**
Glucose (mg/dL)	85.7±7.0	70.3±8.5***	108.7±8.6	107.7±6.8	116.1±15.7	116.2±20.3	120.0±23.7	116.3±10.1
BUN (mg/dL)	12.50±1.67	10.28±1.65***	14.20±1.54	12.12±1.78***	15.98±1.66	15.79±2.06	14.72±1.42	14.34±1.74
Creatinine (mg/dL)	0.208±0.060	0.154±0.064**	0.307±0.049	0.316±0.078	0.442±0.075	0.420±0.061	0.446±0.066	0.427±0.075
Calcium (mg/dL)	9.68±0.40	9.79±0.49	9.92±0.48	9.42±0.47**	10.11±0.59	9.99±0.52	10.49±0.48	10.37±0.62
Sodium (mmol/L)	ND	ND	145.5±1.7	145.5±2.4	144.9±2.4	145.5±2.8	143.0±1.7	143.9±2.6
Potassium (mmol/L)	ND	ND	5.85±0.39	5.44±0.52**	5.31±0.58	5.51±0.78	5.13±0.28	5.40±0.85
Chloride (mmol/L)	ND	ND	104.1±1.3	103.7±2.3	103.7±1.3	104.3±1.8	101.8±1.6	102.3±1.1

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (t-test)

Table 8. Comparison of serum chemistry data between female IGS and Slc rats

Parameters	Before		1 Month		3 Month		6 Month	
	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=20)	IGS (n=20)	Slc (n=19)	IGS (n=20)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
ALT (IU/L)	29.0±5.4	27.6±6.1	26.4±5.2	25.8±4.9	37.8±12.2	28.8±6.1**	65.6±20.0	43.8±17.2***
AST (IU/L)	97.6±10.7	116.6±13.8***	86.2±6.7	99.5±11.1***	92.2±15.9	99.7±21.3	126.7±58.1	103.2±26.3
ALP (IU/L)	1288.7±271.1	817.4±118.2***	601.3±117.5	436.3±80.3***	271.6±99.1	165.9±38.6***	181.8±88.1	106.4±30.8***
GGT (IU/L)	ND	ND	ND	ND	0.91±1.17	0.49±0.91	1.00±2.20	0.34±0.52
5'Nucleotidase (U/L)	32.7±7.6	28.4±9.4	41.1±11.6	49.2±17.4	42.8±13.2	51.5±23.4	48.5±12.2	50.5±21.3
Total Bilirubin (mg/dL)	0.022±0.012	0.046±0.028**	0.037±0.022	0.048±0.028	0.096±0.028	0.105±0.035	0.092±0.039	0.104±0.035
Total Protein (g/dL)	5.96±0.36	5.88±0.28	6.30±0.35	6.29±0.36	7.32±0.45	6.97±0.38*	7.80±0.38	7.14±0.54***
Albumin (g/dL)	4.05±0.25	4.35±0.19***	4.02±0.22	4.20±0.35	4.74±0.35	4.79±0.37	5.15±0.35	5.02±0.53
Globulin (g/dL)	1.92±0.27	1.53±0.26***	2.28±0.22	2.10±0.21*	2.59±0.23	2.18±0.21***	2.65±0.25	2.12±0.29***
A/G Ratio	2.17±0.38	2.96±0.64***	1.79±0.18	2.05±0.31**	1.84±0.21	2.22±0.33***	1.97±0.24	2.43±0.47***
Cholesterol (mg/dL)	78.5±16.5	75.7±15.3	75.5±15.0	72.9±18.1	106.8±18.8	81.6±21.7***	144.9±25.3	94.9±21.4***
Triglycerides (mg/dL)	44.7±15.4	58.9±30.1	31.5±10.7	32.7±13.4	40.2±22.6	32.1±14.0	93.3±64.0	64.3±25.4
Glucose (mg/dL)	89.1±7.6	81.9±6.5**	114.3±8.9	114.8±11.6	106.9±13.6	106.9±13.7	106.6±7.8	104.0±8.7
BUN (mg/dL)	12.82±1.63	12.47±2.20	14.64±1.96	15.26±2.21	13.72±1.59	15.87±1.73***	14.50±1.78	15.61±2.20
Creatinine (mg/dL)	0.316±0.066	0.213±0.050***	0.378±0.071	0.368±0.093	0.383±0.092	0.456±0.086*	0.333±0.055	0.351±0.082
Calcium (mg/dL)	9.47±0.35	9.44±0.31	10.13±0.46	10.12±0.34	10.13±0.54	9.80±0.35*	10.83±0.44	10.47±0.42*
Sodium (mmol/L)	ND	ND	144.2±1.1	145.3±1.5*	142.2±1.2	142.7±1.6	142.3±0.9	143.7±1.3***
Potassium (mmol/L)	ND	ND	5.46±0.45	5.33±0.41	4.58±0.34	4.62±0.57	4.53±0.38	4.91±0.37**
Chloride (mmol/L)	ND	ND	105.9±1.3	106.2±1.6	104.3±1.8	105.3±1.6	102.6±1.2	104.9±1.5***

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (t-test)

Table 9. Comparison of organ weight data between IGS and Slc rats

Organ	Absolute (g)				Relative (%)			
	Slc		IGS		Slc		IGS	
	Mean SD	n	Mean SD	n	Mean SD	n	Mean SD	n
Male								
Terminal BW	530.53±54.38	20	592.19±81.33	18**				
Heart	1.558±0.168	20	1.792±0.236	18**	0.294±0.018	20	0.304±0.029	18
Kidney	3.327±0.332	20	3.885±0.505	18***	0.628±0.041	20	0.660±0.066	18
Adrenal	0.0720±0.0093	20	0.0622±0.0128	18*	0.0136±0.0018	20	0.0106±0.0025	18***
Brain	2.102±0.0721	20	2.119±0.0836	18	0.399±0.0329	20	0.364±0.0491	18*
Liver	14.979±2.473	20	15.040±2.664	18	2.808±0.202	20	2.533±0.227	18***
Testes	3.719±0.291	20	3.447±0.177	18**	0.707±0.085	20	0.590±0.065	18***
Female								
Terminal BW	318.311±28.18	19	312.825±27.07	20				
Heart	1.076±0.091	19	1.077±0.113	20	0.339±0.028	19	0.345±0.0365	20
Kidney	2.226±0.236	19	2.019±0.139	20**	0.700±0.047	19	0.649±0.0551	20**
Adrenal	0.0902±0.0108	19	0.0708±0.0094	20***	0.0284±0.0032	19	0.0228±0.0040	20***
Brain	1.986±0.076	19	1.905±0.068	20**	0.628±0.056	19	0.613±0.0553	20
Liver	8.905±1.127	19	7.663±0.834	20***	2.794±0.197	19	2.455±0.230	20***

*p<0.05, **p<0.01, ***: p<0.001 (t-test)

Table 10-1. Incidence summary of histopathology findings

	Male		Female	
Finding	Slc	IGS	Slc	IGS
No. of animals	20	18	19	20
Liver				
Infiltration, mononuclear cells	13	15	19	15*
Hyperplasia, bile duct	3	6	3	0
Focus, clear cell	0	1	0	0
Focus, eosinophilic	1	0	0	0
Fatty change	2	3	0	1
Inflammation	1	0	0	0
Necrosis	0	1	2	1
Kidney				
Nephropathy, chronic progressive	1	0	1	0
Cast	7	0**	6	0**
Basophilic tubules	15	5*	10	0***
Hyperplasia, transitional cell	0	1	0	1
Infiltration, mononuclear cell, interstitial	3	3	2	2
Inflammation, pelvis	0	0	2	0
Inflammation, capsular	0	0	0	1
Heart				
Infiltration, mononuclear cells	1	3	0	0
Thyroid				
Cyst, embryonal remnant	8	7	15	13
C-cell adenoma	0	1	1	0
Hyperplasia, C cell	0	1	0	0
Adrenal				
Accessory cortical tissue	1	0	0	0

*p<0.05, **p<0.01, ***p<0.001 (the one-sided Fisher's exact probability test or Chi-square test)

Table 10-2. Incidence summary of histopathology findings

Finding	Male		Female	
	Slc	IGS	Slc	IGS
No. of animals	20	18	19	20
Pituitary				
Angiectasis	0	0	1	0
Rathke's pouch	0	0	0	1
Lung				
Inflammation	2	0	2	0
Foam cell foci	2	4	6	2
Choresterol granuloma	0	0	0	1
Stomach				
Cyst, keratogenous, nonglandular	0	1	0	0
Cecum				
Inflammation	1	0	0	0
Pancreas				
Atrophy, acinar	0	2	3	2
Infiltration, mononuclear cells	1	2	4	7
Urinary bladder				
Infiltration, mononuclear cells	2	3	5	0*
Inflammation	0	0	1	0
Testes				
Atrophy, tubular	1	0	—	—
Prostate				
Inflammation	3	5	—	—
Uterus				
Deciduoma	—	—	0	1
Eye				
Atrophy, retina	1	0	0	1
Harderian gland				
Infiltration, mononuclear cells	2	0	0	0
Inflammation	0	0	0	1
Skin and adnexa				
Ectasia, mammary duct	0	0	1	0

*p<0.05, **p<0.01, ***p<0.001 (the one-sided Fisher's exact probability test or Chi-square test)

Background Control Data of General Toxicity Parameters in Crj:CD(SD)IGS Rats Fed Standard Diet or Low Protein Diet

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ABSTRACT. Two groups of Crj:CD(SD)IGS rats consisting of 60 males and 60 females each were fed either a low protein diet (CR-LPF, protein content 18.4%) or a diet with standard protein content (CRF-1, protein content 23.1%). Both groups were treated orally with 0.5% aqueous tragacanth gum solution every day, and after 4, 13 and 26 weeks of treatment, 20 males and 20 females each were euthanatized and necropsied. During or after the treatment period, a series of observations and examinations commonly conducted in a general toxicity study was carried out to collect background control data and to compare the effects of two diets on the general toxicity parameters.

The body weight gain was smaller in the CR-LPF group than in the CRF-1 group though food consumption was greater in the CR-LPF group than in the CRF-1 group. There were differences in several parameters between the two groups, such as urinary output of urea nitrogen and calcium, plasma levels of total cholesterol, phospholipid, total protein and albumin. However, the differences were small and values of hematology and blood chemistry parameters in both groups largely fell within the historical control range in our laboratories on Jcl:SD strain rats fed a diet with standard protein content. Therefore, it is thought that the present findings do not raise any concern for using Crj:CD(SD)IGS rats and for feeding the animals with the low protein diet (CR-LPF) in the evaluation of the general toxicity of new drugs. — Key words: background control data, Crj:CD(SD)IGS rat, low protein diet

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INTRODUCTION

Charles River Inc. has established a new breeding system called the International Genetic Standard System to supply experimental animals with minimized genetic ramifications worldwide. The Crj:CD(SD)IGS rat is a new strain produced from CD strain of rats, utilizing this breeding system. So far a considerable amount of background data has been accumulated on the general toxicology, reproduction and developmental toxicology and carcinogenicity parameters and so on. The present study was undertaken to collect background control data of general toxicity parameters for 4-week, 13-week and 26-week toxicity studies.

In the past, we have used a diet with approximately 25% protein content for general toxicity studies in rodents. A diet with this protein level is appropriate for growth and reproduction, but could cause overnutrition and adversely affect health conditions of the animals in long-term studies. Recently, a laboratory animal diet with lower protein content has become available. The diet is expected to have beneficial effects on the animal's health status and life span. However, it is also possible that lower protein intake compromises validity of the study outcome. Thus, in the present study the effects of a low protein diet on the general toxicity parameters was compared with those of a diet with standard protein content.

Though most of the background control data have been collected from untreated animals, animals in the present study were given 0.5% aqueous tragacanth gum solution, a vehicle routinely used in our laboratories, by oral gavage. It is because we believe that the data obtained from the animals undergoing dosing stress every day is most appropriate for reference purpose since data from test article-treated groups were compared with those from the group treated with vehicle in actual toxicity studies.

MATERIALS AND METHODS

Animals

A total of 143 male and 137 female Crj:CD(SD)IGS rats were obtained at 5 weeks of age from Charles River Japan Inc. (Hino Breeding Center, Shiga, Japan) on August 26, 1997. After an acclimation period for about one week, 120 animals per sex were selected on the basis of clinical signs and body weight gain. They were allocated randomly to two groups each comprised of 60 males and 60 females. At the start of dosing, the animals were 6 weeks old and their body weights ranged from 160.8 to 191.7 g for males and from 117.8 to 155.7 g for females.

Animal husbandry

Animals were housed individually in stainless steel wire mesh cages and kept in a barrier-sustained animal room maintained at a temperature of 20-26℃ and relative humidity of 40-70% with a ventilation rate of 13 to 18 times/hour and a 12 hr lighting cycle (06:00 h-18:00 h). Before group assignment, all animals were allowed free access to a laboratory animal diet (CRF-1, Oriental Yeast Co., Ltd.) having a standard protein content (23.1%) and tap water (Suita municipal waterworks). After group assignment, one group (hereafter refer to as the CRF-1 group) continued to receive the standard diet, whereas for the other group (hereafter refer to as the CR-LPF group) the standard diet was replaced by a laboratory animal diet (CR-LPF, Oriental Yeast Co., Ltd.) having a lower protein content (18.4%). Both diets were sterilized by gamma-ray irradiation and the tap water was sterilized by autoclaving.

Negative control article and dosing method

Tragacanth gum (Nippon Funmatsu Yakuhin & Co., Ltd.) dissolved in water at a concentration of 0.5% was used as a negative control article. The dose volume was set at 5 ml/kg body weight, and volumes calculated based on the most recent body weight were administered orally by gavage, once daily, 7 days a week, for 4, 13 or 26 weeks.

Observations and examination

All animals were observed for clinical signs, mortality and morbidity once daily before dosing throughout the administration period. Body weights were measured for all animals 3 times

at week 1, twice a week from week 2 through 4 and once a week from week 5 through 26. Food consumption was measured for all animals twice a week from week 1 through 4 and once a week from week 5 through 26. Water intake was determined for all animals once a week from week 1 through 4 and once every 2 weeks from week 5 through 26.

Ophthalmoscopic examination was done for all animals at week 4, 13 and 26. The anterior part and transparent body were examined using a slit lamp biomicroscope (HSO-10, Carl Zeiss Co.) before and after mydriasis. Moreover, the fundus oculi as well as the anterior part and transparent body were examined using a head-worn binocular indirect ophthalmoscope (OMEGA 200, Heine Optotechnik) after mydriasis. Mydriasis was achieved by the application of phenylephrine hydrochloride (Mydrin P, Santen Pharmaceutical Co., Ltd.).

Urine samples were collected from 16 males and 16 females per group by forced urination at week 4, 12 and 26, and then pH, protein, glucose, occult blood and urobilinogen were determined with a reagent strip for urinalysis (Uro-Hema-Combistix, Bayer Medical Ltd.) and an automated urine analyzer (Clinitek 10, Bayer Medical Ltd.). Moreover, 18-hour urine was collected from the same animals with matabolic cages, and then other parameters shown in Table 6 were determined using an automated blood chemistry analyzer (Model 7170, Hitachi, Ltd.).

After 4, 13 and 26 weeks of administration, 20 males and 20 females each were subjected to laparotomy under sodium pentobarbital anesthesia, and blood samples were drawn from the abdominal aorta into heparinized syringes. Animals were fasted overnight (approximately 16 hours) prior to the collection of the blood samples. The collected samples were immediately transferred into blood collection tubes containing EDTA-2K. Parameters shown in Table 7 except for reticulocyte, PT, APTT and fibrinogen were determined using a hematological diagnostic device THMS H･1 (Bayer Medical Ltd.). For reticulocyte, Brilliant Cresyl Blue-Giemsa stained blood smears were prepared from the blood samples and reticulocyte counted manually using a light microscope. Blood samples were also drawn from the abdominal aorta into syringes containing 3.8% sodium citrate, and PT, APTT and fibrinogen were measured on the plasma using a Coagulometer KC 10A (Heinrich Amelung GmbH). However, PT, APTT and fibrinogen determined after 4 weeks of administration were not incorporated into the study because of abnormal values probably due to contamination of the samples. Further to the blood collection for the hematological examination, blood samples were collected from the abdominal aorta using heparinized syringes or unheparinized syringes, and the parameters shown in Table 8 were measured on plasma using an automated blood chemistry analyzer (Model 7170, Hitachi, Ltd.) except for serum protein fractions and A/G, which were determined on the serum using REP (Helena Laboratories).

At the completion of the above-mentioned blood sample collection, animals were euthanatized by exsanguination, and subjected to detailed macroscopic examination. The organs listed in Table 10 were weighed, and relative weights to the body weights on the day of necropsy were calculated.

Statistical Analysis

Group means and their standard deviations were calculated on data of body weights, food consumption, water intake, urinalysis (except for the data determined with a reagent strip), hematological examination, blood chemistry and organ weights. These data were then analyzed for homogeneity of variance using F-test. The difference in mean values between the CR-LPF and the CRF-1 groups was evaluated by Student's t-test where the variance was found to be homogeneous, or by the method of Aspin-Welch where it was found to be heterogeneous.

RESULTS AND DISCUSSION

No deaths were found in either group, nor were there any significant abnormal clinical signs during the study period. Abnormal signs included scab on the neck or dorsal region, alopecia on the neck, forelimb, thoracic region or antebrachial region, wound on the neck, loss of the upper incisor and ocular discharge found each in a small number of animals, and opacity of the eyeball found in one female fed CRF-1. There was no significant difference in the incidence of these signs between the two groups in both sexes.

Group mean body weights and group mean food consumption are listed in Table 1 and Table 2, respectively. The mean body weights of the CRF-1 group were higher than those of the CR-LPF group throughout the study in both sexes. Statistical significance was attained from day 7 through 91 for males and from day 18 through 28 for females. In contrast, group mean food consumption was higher in the CR-LPF group than in the CRF-1 group throughout the study. Statistical significance was attained on most of the time points during the first half of the study in males, and on most of the time points during the study in females.

Table 1. Body weight in males and females-Group mean value

		Male		Female	
Days	Weeks	CR-LPF	CRF-1	CR-LPF	CRF-1
		n=60	n=60	n=60	n=60
1	1	176.3±6.3	176.9±6.4	142.0±5.7	141.5±6.6
4	1	200.2±8.1	202.4±8.1	153.9±7.4	153.6±7.3
7	1	224.7±10.4	228.5±9.7*	164.4±7.7	165.3±8.6
11	2	255.6±13.2	261.2±12.0*	177.5±9.1	180.0±10.7
14	2	277.2±16.3	284.1±14.1*	186.5±10.7	190.5±12.7
18	3	300.8±19.8	310.0±16.2**	196.7±11.9	202.7±14.4*
21	3	317.8±22.1	328.7±18.1**	204.8±13.1	210.9±15.6*
25	4	335.4±25.8	347.4±21.0**	213.5±14.6	220.9±17.9*
28	4	347.7±28.3	360.3±22.4**	219.9±15.5	227.5±19.4*
		n=40	n=40	n=40	n=40
35	5	373.9±32.2	388.8±22.9#	233.3±18.8	238.9±20.1
42	6	399.6±35.8	416.6±25.2#	245.3±19.1	252.2±20.5
49	7	420.7±39.7	441.8±28.3##	255.5±20.0	263.0±22.7
56	8	439.6±43.9	460.2±31.0#	265.4±21.5	272.2±24.7
63	9	455.8±47.3	477.3±33.0#	273.7±22.5	279.8±24.8
70	10	470.1±51.9	495.1±36.1#	280.4±23.3	286.0±27.3
77	11	483.1±54.3	509.8±37.5#	285.3±23.5a)	290.9±26.4
84	12	492.8±55.5	519.7±40.6*	289.3±24.6	294.7±27.3
91	13	503.3±57.5	530.4±40.9#	294.3±24.4	301.2±27.7
		n=20	n=20	n=20	n=20
98	14	507.1±65.2	535.1±37.5	296.9±27.3	300.6±27.2
105	15	517.7±67.3	546.5±38.7	302.6±29.4	306.8±29.0
112	16	528.6±68.7	557.2±39.6	307.4±31.5	312.6±27.1
119	17	536.3±69.9	567.3±41.5	310.2±31.3	316.4±29.5
126	18	544.1±71.7	573.7±42.7	313.8±32.8	320.2±30.4
133	19	550.3±74.0	581.4±43.7	317.1±33.3	324.5±30.8
140	20	556.1±75.0	587.4±44.2	320.6±35.5	328.3±30.6
147	21	561.8±76.7	593.3±45.2	323.9±35.9	330.8±32.2
154	22	567.8±78.1	599.3±45.5	325.5±37.2	337.0±34.6
161	23	574.4±81.3	605.9±45.8	330.3±38.3	340.3±34.6
168	24	579.6±81.8	613.2±46.2	334.0±39.2	344.1±34.7
175	25	585.5±85.2	618.7±47.0	337.3±39.6	348.4±35.5
182	26	585.8±84.6	617.3±45.3	336.2±38.3	347.5±35.8

Mean±S.D. a):n=39 Unit:g
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)
##:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Table 2. Food consumption in males and females-Group mean value

Days	Weeks	Male		Female	
		CR-LPF	CRF-1	CR-LPF	CRF-1
		n=60	n=60	n=60	n=60
1～4	1	22.4±1.2	22.6±1.4	17.2±1.6	17.0±1.5
4～7	1	25.3±1.8	24.4±1.5**	18.4±1.5	17.9±1.2*
7～11	2	26.8±2.0	25.4±1.7**	19.3±1.6	18.2±1.5**
11～14	2	26.7±2.4	26.0±1.9	19.3±1.7	18.7±1.6*
14～18	3	27.7±2.6	26.9±2.1*	19.6±1.8	18.9±1.7*
18～21	3	28.2±2.8	27.1±2.3*	20.4±2.1	19.6±2.0*
21～25	4	27.2±2.3	26.8±2.1	19.7±2.0	19.4±1.8
25～28	4	27.1±2.6	26.5±2.5	20.1±2.1	19.4±2.0*
		n=40	n=40	n=40	n=40
32～35	5	28.7±2.9	27.0±2.0##	21.6±2.4	20.2±2.0**
39～42	6	28.2±3.0	26.7±2.0#	20.5±2.2	19.2±2.2**
46～49	7	28.4±3.0	26.6±2.1##	21.0±2.5	19.7±1.9**
53～56	8	28.3±3.5	26.4±2.1##	20.9±2.0	19.3±2.2**
60～63	9	28.2±3.3	26.2±2.5**	20.9±1.8	19.0±1.7**
67～70	10	27.8±3.9	26.3±2.7	20.0±2.1	18.6±2.3**
74～77	11	27.5±3.4	26.3±2.4	20.0±2.0	18.4±2.1**
81～84	12	27.1±3.4	25.5±2.5*	19.3±1.8	17.8±2.0**
88～91	13	27.4±3.5	25.6±2.0##	19.9±1.6	18.6±1.9**
		n=20	n=20	n=20	n=20
95～98	14	26.7±2.7	25.4±1.8	19.2±1.7	17.7±1.6**
102～105	15	26.2±3.1	25.1±2.1	18.8±2.0	17.6±2.0
109～112	16	26.8±3.3	25.6±2.3	19.7±2.3	18.5±1.7
116～119	17	25.8±2.8	25.2±2.0	18.6±1.5	17.7±1.9
123～126	18	26.1±2.9	24.6±2.4	18.9±2.0	17.7±2.1
130～133	19	25.8±3.0	24.8±2.2	19.5±2.0	16.9±2.4**
137～140	20	25.2±2.9	24.4±2.4	19.4±2.1	17.7±1.6**
144～147	21	25.6±3.0	24.6±2.5	18.7±1.7	17.6±2.0
151～154	22	25.6±3.2	24.5±2.4	18.7±2.4	17.7±3.0
158～161	23	25.7±3.2	24.6±2.3	19.4±2.0	17.9±1.8*
165～168	24	25.4±3.0	24.8±1.9	19.8±2.4	18.2±2.2*
172～175	25	26.1±4.2	24.9±2.5	19.4±2.8	17.9±2.3
179～182	26	24.9±3.1	24.4±2.2	18.1±2.0	17.0±1.9

Mean±S.D Unit:g/day
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)
##:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Group mean water intake are presented in Table 3. Mean water intake in males tended to be higher in the CR-LPF group than in the CRF-1 group during most of the study period, whereas that in females tended to be higher in the CRF-1 group than in the CR-LPF group throughout the study period. There was no consistent pattern between males and females.

Table 3. Water intake in males and females-group mean value

		Male		Female	
Days	Weeks	CR-LPF	CRF-1	CR-LPF	CRF-1
3～4	1	28.5±3.4 (59)	28.6±3.1 (60)	23.6±3.2 (60)	24.1±4.5 (60)
10～11	2	29.9±3.8 (56)	30.3±3.7 (57)	22.9±3.7 (60)	24.1±4.5 (59)
17～18	3	32.2±5.0 (60)	32.6±4.2 (60)	22.7±4.6 (60)	25.0±5.2 (59)*
24～25	4	31.9±4.5 (60)	32.2±5.0 (60)	22.9±4.7 (60)	25.3±5.1 (60)*
31～32	5	32.2±5.9 (40)	32.0±4.5 (39)	23.2±5.5 (39)	24.9±6.4 (40)
45～46	7	32.3±7.7 (40)	31.1±5.7 (40)	24.6±4.5 (40)	26.3±7.3 (40)
59～60	9	32.9±6.4 (40)	32.1±5.9 (40)	26.5±5.3 (39)	26.6±7.4 (40)
73～74	11	31.6±6.4 (39)	31.3±5.0 (40)	26.1±4.5 (40)	26.9±6.3 (39)
87～88	13	30.7±6.9 (39)	29.1±5.9 (39)	23.5±4.1 (38)	27.3±7.6 (39)##
101～102	15	33.2±9.3 (20)	28.7±5.2 (20)	26.0±6.4 (20)	29.6±7.0 (19)
115～116	17	32.5±8.7 (20)	28.6±4.4 (20)	25.5±6.9 (20)	29.3±8.0 (18)
129～130	19	30.3±8.2 (20)	27.6±5.6 (20)	26.3±8.0 (20)	28.2±8.3 (20)
143～144	21	32.1±9.8 (20)	26.3±5.3 (20)#	24.9±4.3 (20)	27.3±6.2 (20)
157～158	23	28.8±7.0 (19)	26.5±4.3 (19)	27.9±6.6 (20)	28.0±8.2 (19)
171～172	25	30.0±8.8 (20)	25.7±5.7 (20)	28.0±6.4 (20)	30.4±8.7 (20)

Mean±S.D. Unit:g/day
( ):Number of animals determined
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
# :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)
##:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Ophthalmological findings are summarized in Table 4 (slit lamp) and Table 5 (funduscopy). In the slit lamp examination, the incidence of punctate opacity of the cornea was increased with age in both sexes of both groups. There was no apparent difference in the incidence of the findings between the two groups in both sexes. In the funduscopy, the incidence of the persistent hyaloid artery was decreased with age in both sexes of both groups. Retinal atrophy was found in two males and three females fed CRF-1. The incidence fell within the historical control range in our laboratories on Jcl:SD strain rats fed a diet with standard protein content.

Table 4. Ophthalmology -slit lamp findings

Sex	Group	Findings	Week		
			4	13	26
Male	CR-LPF	No. of animals examined	60	40	20
		Punctate opacity of cornea	0	18	9
	CRF-1	No. of animals examined	60	40	20
		Punctate opacity of cornea	4	13	9
		Opacity of lens	1	1	0
		Anterior synechia	1	0	0
Female	CR-LPF	No. of animals examined	60	40	20
		Punctate opacity of cornea	5	13	9
		Opacity of lens	2	0	0
		Hemorrhage in iris	1	0	0
	CRF-1	No. of animals examined	60	40	20
		Punctate opacity of cornea	8	11	8
		Opacity of lens	1	1	1
		Hemorrhage in iris	1	0	0

Table 5. Ophthalmology - funduscopy findings

Sex	Group	Findings	Week		
			4	13	26
Male	CR-LPF	No. of animals examined	60	40	20
		Persistent hyaloid artery	6	2	1
	CRF-1	No. of animals examined	60	40	20
		Persistent hyaloid artery	3	1	0
		Retinal atrophy	2	1	1
Female	CR-LPF	No. of animals examined	60	40	20
		Persistent hyaloid artery	3	1	0
	CRF-1	No. of animals examined	60	40	20
		Persistent hyaloid artery	3	2	1
		Retinal hemorrhage	2	0	0
		Retinal atrophy	3	3	2

Group mean values of urinary parameters are shown in Table 6-1 (male) and Table 6-2 (female) together with results of semi-quantitative parameters. Apart from urea nitrogen and calcium, there were no apparent differences in urinary parameters between the two groups in both sexes. Group mean urea nitrogen values were lower for both sexes in the CR-LPF group than in the CRF-1 group. The difference in the protein intake was considered to be responsible for this difference. Group mean calcium values were also lower for both sexes in the CR-LPF group than in the CRF-1 group. It has been reported that dietary protein restriction results in a reduction in urinary calcium output [3].

Table 6-1. Urinalysis in males-Group mean value

	Male					
	CR-LPF			CRF-1		
	4W	12W	26W	4W	12W	26W
No. of animals examined	16	16	16	16	16	16
Urine volume (ml/18h)	10.3±2.5	13.0±5.7	14.5±6.3	10.8±2.8	11.7±3.0	11.3±2.9
Protein (mg/18h)	19.74±4.94	17.94±5.15	13.33±3.59	22.73±5.52	18.10±4.39	17.26±6.91
Na (mEq/18h)	1.63±0.28	1.29±0.33	1.28±0.42	1.97±0.45*	1.27±0.37	1.18±0.44
K (mEq/18h)	3.62±0.37	3.27±0.61	2.79±0.69	3.46±0.63	2.84±0.53*	2.23±0.52*
Na/K ratio	0.45±0.05	0.39±0.06	0.45±0.08	0.56±0.06**	0.44±0.08	0.52±0.11
Cl (mEq/18h)	2.66±0.30	1.76±0.47	1.66±0.53	2.89±0.62	1.66±0.46	1.47±0.62
IP (mg/18h)	12.19±5.11	15.66±5.00	9.65±3.75	10.05±4.00	12.62±5.13	8.24±3.46
Calcium (mg/18h)	0.80±0.20	0.78±0.23	1.23±0.56	1.03±0.30*	0.92±0.25	1.87±0.85*
Urea nitrogen (mg/18h)	175±21	233±40	231±36	273±57##	297±49**	301±76##
Creatinine (mg/18h)	6.93±0.68	12.77±1.35	13.89±1.21	8.03±1.26##	14.09±1.02**	15.01±2.01
pH	8.4±0.4	8.1±0.7	8.1±0.7	8.0±0.5	8.7±0.4	8.2±1.0
Protein	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
	1 1 6 7 1	2 1 10 3 0	1 2 8 3 2	1 0 6 7 2	0 2 7 6 1	0 2 4 8 2
Glucose	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
	16 0 0 0 0	14 1 1 0 0	15 1 0 0 0	16 0 0 0 0	15 0 1 0 0	16 0 0 0 0
Occult blood	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
	14 0 0 2 0	10 4 2 0 0	11 1 2 0 2	10 2 2 1 1	14 0 2 0 0	10 0 2 2 2
Urobilinogen (EU/dl)	0.2±0.3	0.1±0	0.3±0.4	0.4±0.5	0.2±0.2	0.4±0.4

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
##:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Table 6-2. Urinalysis in females-Group mean value

		Female					
		CR-LPF			CRF-1		
		4W	12W	26W	4W	12W	26W
No. of animals examined		16	16	16	16	16	16
Urine volume	(ml/18h)	8.7±3.1	9.9±2.8	12.0±4.0	11.0±4.3	13.0±4.0*	15.7±7.1
Protein	(mg/18h)	6.43±2.29	4.32±0.85	4.19±1.44	6.07±0.90	4.58±1.33	4.93±3.09
Na	(mEq/18h)	1.24±0.30	1.02±0.28	1.05±0.38	1.45±0.28*	1.13±0.24	1.24±0.34
K	(mEq/18h)	2.26±0.43	2.38±0.54	2.28±0.61	2.09±0.25	2.08±0.41	2.10±0.44
Na/K ratio		0.55±0.05	0.43±0.06	0.45±0.10	0.69±0.10##	0.55±0.06**	0.58±0.08**
Cl	(mEq/18h)	1.52±0.41	1.45±0.37	1.40±0.47	1.59±0.34	1.50±0.31	1.50±0.34
IP	(mg/18h)	7.19±3.18	11.55±4.67	10.52±4.12	6.40±3.33	8.98±3.53	8.86±3.03
Calcium	(mg/18h)	0.87±0.45	1.63±0.48	3.15±1.01	1.44±0.62**	2.64±0.79**	3.89±1.74
Urea nitrogen	(mg/18h)	168±32	190±31	187±46	225±29**	232±42**	238±46**
Creatinine	(mg/18h)	4.32±0.52	6.65±0.75	7.24±0.58	4.48±0.41	6.56±1.03	7.96±1.21#
pH		8.0±0.5	8.0±1.0	7.3±1.1	8.2±0.5	8.1±1.0	8.2±0.7
Protein		− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
		8 5 2 1 0	0 5 6 4 1	2 5 2 5 2	8 6 1 1 0	1 3 5 6 1	4 2 5 4 1
Glucose		− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
		15 1 0 0 0	16 0 0 0 0	16 0 0 0 0	14 2 0 0 0	16 0 0 0 0	16 0 0 0 0
Occult blood		− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+	− ± + 2+ 3+
		10 1 4 1 0	14 1 1 0 0	12 1 2 1 0	8 2 5 1 0	13 0 1 2 0	12 1 1 1 1
Urobilinogen	(EU/dl)	0.1±0.0	0.6±0.5	0.5±0.5	0.2±0.3	0.5±0.5	0.4±0.4

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
\# :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)
\#\#:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Group mean values of hematological parameters are listed in Table 7-1 (male) and Table 7-2 (female). There were no substantial differences in the parameters between the two groups in both sexes. In both groups, values largely fell within the historical control range (data not shown) in our laboratories on Jcl:SD strain rats fed a diet with standard protein content. There were several parameters that show age-related changes as follows. RBC values was increased in males of both groups. WBC was decreased in both sexes of both groups. This alteration was associated with increase in the absolute and relative counts of neutrophils and decrease in the absolute and relative counts of lymphocytes.

Table 7-1. Hematology in males-Group mean value

	Male					
	CR-LPF			CRF-1		
	4W	13W	26W	4W	13W	26W
No. of animals examined	20	20	19	20	20	20
RBC ($\times 10^6/\mu l$)	7.44±0.40	8.57±0.33	8.79±0.48	7.51±0.34	8.69±0.39	8.71±0.56
Hemoglobin (g/dl)	14.4±0.7	15.0±0.5	15.1±0.6	14.5±0.7	15.3±0.8	15.0±0.8
Hematocrit (%)	41.1±2.4	42.1±1.2	42.8±1.8	41.4±2.4	42.8±2.3	42.3±1.9
MCV (fl)	55.3±1.5	49.2±1.8	48.8±2.0	55.1±1.8	49.3±1.4	48.7±1.8
MCH (pg)	19.4±0.5	17.6±0.7	17.3±0.7	19.3±0.5	17.6±0.5	17.2±0.6
MCHC (g/dl)	35.2±0.5	35.7±0.6	35.4±0.4	35.1±0.6	35.7±0.6	35.3±0.4
Reticulocyte (%)	2.6±0.6	2.6±0.8	2.6±0.7	2.7±0.6	2.3±0.7	2.4±1.3
WBC ($\times 10^3/\mu l$)	7.66±2.77	7.47±1.70	6.03±1.01	7.05±2.00	7.57±1.46	6.36±1.14
Basophil (%)	0.2±0.1	0.2±0.1	0.2±0.1[a)]	0.2±0.1	0.2±0.1[b)]	0.2±0.1
Eosinophil (%)	1.3±0.4	1.3±0.4	1.6±0.5[a)]	1.0±0.4**	1.3±0.4[b)]	1.7±0.6
Neutrophil (%)	10.7±3.2	15.6±3.7	22.1±7.2[a)]	10.3±4.1	13.0±2.9*[b)]	19.2±3.9
Lymphocyte (%)	83.7±3.8	77.3±4.4	69.6±7.8[a)]	84.6±4.8	80.1±3.6*[b)]	71.9±4.6
Monocyte (%)	2.4±0.8	3.3±1.0	3.9±1.1[a)]	2.3±0.8	3.3±0.8[b)]	4.3±0.8
LUC (%)	1.7±0.5	2.4±0.6	2.5±0.8[a)]	1.6±0.3	2.1±0.5[b)]	2.8±0.8
Basophil ($\times 10^3/\mu l$)	0.02±0.01	0.01±0.01	0.01±0.01[a)]	0.01±0.01	0.02±0.01[b)]	0.01±0.00
Eosinophil ($\times 10^3/\mu l$)	0.10±0.04	0.10±0.03	0.10±0.04[a)]	0.06±0.02##	0.10±0.03[b)]	0.11±0.04
Neutrophil ($\times 10^3/\mu l$)	0.78±0.28	1.15±0.33	1.34±0.44[a)]	0.68±0.20	0.97±0.24[b)]	1.21±0.28
Lymphocyte ($\times 10^3/\mu l$)	6.46±2.43	5.80±1.48	4.26±0.93[a)]	6.03±1.90	6.09±1.33[b)]	4.59±0.93
Monocyte ($\times 10^3/\mu l$)	0.18±0.07	0.24±0.07	0.24±0.07[a)]	0.16±0.04	0.24±0.05[b)]	0.27±0.07
LUC ($\times 10^3/\mu l$)	0.13±0.06	0.17±0.05	0.15±0.05[a)]	0.11±0.04	0.16±0.04[b)]	0.17±0.04
Platelet ($\times 10^3/\mu l$)	1126±108	1091±133	1085±106	1079±67	1080±153	1121±126
PT (sec)	No data	14.4±0.6	14.7±0.4[a)]	No data	15.0±1.6	14.7±1.0
APTT (sec)	No data	22.5±1.4	21.8±1.0[a)]	No data	24.4±2.4##	23.1±2.3#
Fibrinogen (g/l)	No data	2.37±0.15	2.90±0.23[a)]	No data	2.39±0.12	2.83±0.26

Number of animals examined: a)=18, b)=19
Mean±S.D.
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)
##:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Aspin-Welch's t-test)

Table 7-2. Hematology in females-Group mean value

	Female					
	CR-LPF			CRF-1		
	4W	13W	26W	4W	13W	26W
No. of animals examined	20	20	20	20	20	20
RBC ($\times 10^6/\mu l$)	7.34±0.24	8.13±0.33	7.95±0.41	7.32±0.35	8.13±0.30	7.98±0.38
Hemoglobin (g/dl)	14.3±0.5	14.9±0.6	14.6±0.9	14.5±0.6	15.0±0.4	14.7±0.7
Hematocrit (%)	40.0±1.2	41.8±1.8	40.6±2.5	40.3±1.8	42.1±1.2	41.3±1.8
MCV (fl)	54.5±1.4	51.4±1.6	51.1±1.4	55.1±1.2	51.8±1.7	51.8±1.5
MCH (pg)	19.5±0.5	18.4±0.5	18.3±0.5	19.8±0.4*	18.4±0.5	18.5±0.5
MCHC (g/dl)	35.8±0.5	35.7±0.7	35.9±0.5	35.9±0.4	35.7±0.4	35.7±0.4
Reticulocyte (%)	2.2±0.7	2.1±0.6	2.1±0.6	2.4±0.4	2.1±0.8	2.6±0.8*
WBC ($\times 10^3/\mu l$)	6.85±2.26	4.29±1.48	3.72±0.80	6.03±1.74	4.16±0.98	3.79±1.12
Basophil (%)	0.2±0.1	0.1±0.1[a)]	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.1
Eosinophil (%)	1.0±0.3	1.4±0.6[a)]	2.6±1.1	1.2±0.3*	1.4±0.5	2.5±1.1
Neutrophil (%)	9.0±3.8	13.1±4.5[a)]	19.8±5.8	9.2±3.7	15.1±4.8	23.0±8.4
Lymphocyte (%)	86.3±4.2	80.5±5.0[a)]	69.4±7.7	86.2±4.0	78.5±5.4	67.2±9.4
Monocyte (%)	2.2±0.5	3.0±0.9[a)]	5.1±1.8	2.0±0.4	3.0±1.0	4.6±1.8
LUC (%)	1.4±0.4	1.9±0.6[a)]	2.9±0.7	1.3±0.2	1.9±0.6	2.6±0.7
Basophil ($\times 10^3/\mu l$)	0.01±0.01	0.01±0.01[a)]	0.01±0.01	0.01±0.01	0.01±0.01	0.00±0.01
Eosinophil ($\times 10^3/\mu l$)	0.06±0.02	0.06±0.03[a)]	0.09±0.04	0.07±0.02	0.06±0.02	0.09±0.04
Neutrophil ($\times 10^3/\mu l$)	0.60±0.29	0.53±0.15[a)]	0.74±0.31	0.55±0.28	0.62±0.23	0.91±0.58
Lymphocyte ($\times 10^3/\mu l$)	5.93±2.07	3.57±1.44[a)]	2.59±0.61	5.20±1.53	3.27±0.86	2.51±0.72
Monocyte ($\times 10^3/\mu l$)	0.15±0.06	0.13±0.06[a)]	0.19±0.08	0.12±0.04	0.12±0.05	0.17±0.08
LUC ($\times 10^3/\mu l$)	0.10±0.05	0.08±0.04[a)]	0.11±0.03	0.08±0.03	0.08±0.03	0.09±0.03
Platelet ($\times 10^3/\mu l$)	1080±114	993±108	1053±124	1047±106	1028±68	997±131
PT (sec)	No data	14.2±0.6	14.0±0.5	No data	13.8±0.5*	13.8±0.5
APTT (sec)	No data	18.0±1.6	18.3±1.7	No data	18.3±1.2	17.9±1.3
Fibrinogen (g/l)	No data	1.76±0.16	1.95±0.32	No data	1.74±0.13	2.06±0.35

Number of animals examined: a)=17
Mean±S.D.
*:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)

Group mean values of blood chemistry parameters are presented in Table 8-1 (male) and Table 8-2 (female). Values of total cholesterol, phospholipid and FFA were higher for females in the CR-LPF group than those in the CRF-1 group after 13 and 26 weeks. Though protein intake was lower in the CR-LPF group than in the CRF-1 group, total protein and albumin were higher for females in the CR-LPF group than in the CRF-1 group after 13 and 26 weeks. With regard to plasma protein fractions, albumin and A/G ratio were higher for females in the CR-LPF group than in the CRF-1 group after 4, 13 and 26 weeks. The difference became pronounced with age. These paradoxical changes in the plasma protein parameters could be a manifestation of overadaptation to lowered protein utilization. Similar to the present findings, high levels of plasma protein parameters in Crj:CD(SD)IGS rats fed a low protein diet as compared with those in the rats fed a diet with standard protein content were reported in the literature [1, 2, 4]. Total bilirubin was lower for both sexes in the CRF-1 group than in the CR-LPF group. There were many parameters showing age-related changes. Parameters that show increase with age included total cholesterol, phospholipid, triglyceride, FFA, glucose, total protein and calcium in both sexes of both groups, creatinine in males of both groups and albumin in females of both groups. Alteration of the lipid parameters was much more pronounced in females. Parameters that show decrease with age included ALP and IP in both sexes of both groups. With regard to plasma protein fractions, albumin and A/G ratio were decreased in males of both groups, β -globulin was increased in males of both groups, and γ -globulin was increased in both sexes of both groups. In general, values of blood chemistry parameters fell within the historical control range (data not shown) in our laboratories on Jcl:SD strain rats fed a diet with standard protein content.

Table 8-1. Blood chemistry in males-Group mean value

		Male					
		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No. of animals examined		20	20	19	20	20	20
AST	(IU/l)	64±7	79±56	64±15	66±9	66±10	65±15
ALT	(IU/l)	23±4	39±30	32±13	24±5	28±7	31±11
ALP	(IU/l)	534±82	254±52	189±33	561±109	252±45	192±29
LD	(IU/l)	95±36	105±64	115±66	93±34	95±32	114±84
Total bilirubin	(mg/dl)	0.05±0.01	0.07±0.02	0.08±0.02	0.04±0.01**	0.06±0.02*	0.06±0.01**
Total cholesterol	(mg/dl)	64±16	76±21	76±16	56±12	70±16	84±18
Phospholipid	(mg/dl)	99±18	114±22	116±20	91±15	109±19	125±21
Triglyceride	(mg/dl)	24±12	43±23	47±21	27±16	47±21	54±18
FFA	(mEq/l)	0.42±0.08	0.44±0.09	0.56±0.11	0.42±0.06	0.49±0.13	0.49±0.12
Glucose	(mg/dl)	116±16	147±15	163±19	126±17	162±18*	167±20
Total protein	(g/dl)	5.24±0.19	5.93±0.27	6.07±0.22	5.32±0.16	5.99±0.22	6.00±0.23
Albumin	(g/dl)	3.73±0.14	3.93±0.22	3.98±0.15	3.75±0.14	3.97±0.14	3.85±0.17*
Urea nitrogen	(mg/dl)	11.6±1.3	13.2±1.8	12.8±2.1	11.9±1.7	12.6±1.8	12.8±1.1
Creatinine	(mg/dl)	0.19±0.03	0.24±0.03	0.25±0.03	0.18±0.02	0.24±0.03	0.25±0.04
IP	(mg/dl)	8.03±0.68	6.35±0.52	5.66±0.56	8.01±0.46	6.52±0.46	5.82±0.57
Calcium	(mg/dl)	9.52±0.26	9.97±0.28	9.92±0.21	9.52±0.22	10.04±0.23	10.08±0.19*
Na	(mEq/l)	144±2	144±1	143±1	145±1	144±1	143±1
K	(mEq/l)	3.6±0.3	3.5±0.2	3.8±0.2	3.5±0.2	3.5±0.2	3.9±0.3
Cl	(mEq/l)	111±1	112±1	108±1	111±1	112±2	108±1
Albumin	(%)	50.14±1.81	46.05±1.19	44.34±1.84	49.42±1.69	45.84±1.44	43.14±1.88
α 1-globulin	(%)	24.70±1.35	25.56±1.51	26.27±1.20	25.37±2.11	26.55±1.63	26.42±1.87
α 2-globulin	(%)	5.44±0.51	4.61±0.49	4.41±0.69	5.31±0.66	4.53±0.58	4.81±0.71
β -globulin	(%)	16.44±0.74	18.71±1.16	18.62±1.45	16.45±0.82	17.82±0.96*	19.45±1.34
γ -globulin	(%)	3.28±0.68	5.07±0.83	6.36±1.42	3.45±0.87	5.26±1.02	6.18±1.01
A/G ratio		1.01±0.07	0.85±0.04	0.80±0.06	0.98±0.07	0.85±0.05	0.76±0.06

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)

Table 8-2. Blood chemistry in females-Group mean value

		Female					
		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No. of animals examined		20	20	20	20	20	20
AST	(IU/l)	64±16	64±13	102±84	63±20	63±12	72±31
ALT	(IU/l)	21±10	23±4	41±33	21±11	23±9	33±17
ALP	(IU/l)	300±51	121±30	64±19	315±69	121±37	79±30
LD	(IU/l)	146±120	128±94	118±70	109±63	118±88	92±39
Total bilirubin	(mg/dl)	0.08±0.01	0.08±0.02	0.10±0.03	0.07±0.01**	0.08±0.02	0.09±0.02
Total cholesterol	(mg/dl)	68±13	89±16	115±24	65±11	76±13*	88±22**
Phospholipid	(mg/dl)	119±20	160±26	212±40	113±16	141±21*	170±37**
Triglyceride	(mg/dl)	10±4	21±10	55±34	11±5	24±19	52±30
FFA	(mEq/l)	0.38±0.11	0.56±0.13	0.82±0.21	0.38±0.07	0.48±0.10*	0.69±0.21*
Glucose	(mg/dl)	109±21	135±14	139±8	122±15*	137±10	139±14
Total protein	(g/dl)	5.60±0.25	6.56±0.38	7.03±0.46	5.59±0.28	6.32±0.40	6.72±0.48*
Albumin	(g/dl)	4.25±0.26	4.99±0.44	5.41±0.46	4.14±0.26	4.68±0.40*	5.07±0.49*
Urea nitrogen	(mg/dl)	11.7±1.6	12.1±2.1	12.2±1.5	11.1±1.5	12.5±2.4	13.2±2.7
Creatinine	(mg/dl)	0.22±0.04	0.27±0.05	0.24±0.04	0.21±0.03	0.25±0.03	0.25±0.04
IP	(mg/dl)	7.14±0.75	5.05±1.01	4.69±0.67	7.16±0.83	5.53±0.67	5.03±0.86
Calcium	(mg/dl)	9.57±0.20	10.13±0.31	10.27±0.31	9.59±0.28	10.10±0.27	10.24±0.29
Na	(mEq/l)	144±1	143±1	141±1	143±1*	143±1	142±1
K	(mEq/l)	3.5±0.2	3.5±0.2	3.6±0.3	3.4±0.3	3.5±0.3	3.8±0.3
Cl	(mEq/l)	108±2	110±2	107±2	107±1	110±2	108±1
Albumin	(%)	53.32±2.38	53.08±1.84	52.34±1.61	51.81±1.75*	51.94±1.51*	51.08±1.87*
α 1-globulin	(%)	21.43±1.93	21.69±1.14	21.50±1.38	22.56±1.82	22.16±1.32	21.58±1.08
α 2-globulin	(%)	4.94±0.71	3.60±0.38	3.89±0.50	4.86±0.54	4.07±0.54**	4.03±0.78
β -globulin	(%)	15.87±1.23	15.91±1.16	15.71±1.50	16.38±1.04	16.05±0.80	16.47±1.23
γ -globulin	(%)	4.45±1.00	5.72±1.07	6.57±0.91	4.39±1.08	5.78±1.20	6.83±1.15
A/G ratio		1.15±0.11	1.13±0.09	1.10±0.07	1.08±0.08*	1.08±0.06*	1.05±0.08*

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)

Necropsy findings are summarized in Table 9-1 (male) and Table 9-2 (female). Dilatation of the renal pelvis with calculi was found in three females in the CRF-1 groups after 26 weeks. However, it could not be determined whether this finding had biological significance favoring a low protein diet because examined number was small and the incidence rate was low. Most of the other findings were not considered biologically significant.

Table 9-1. Necropsy findings in male rats

Findings		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No. of animals examined		20	20	20	20	20	20
Spleen	Accessory spleen	0	1	0	0	0	0
Lung	Dark-red dots	1	0	0	0	0	0
Liver	Diaphragmatic hernia	2	2	0	0	0	1
	Yellow-white focus	2	0	7	0	0	3
Abdomen	Nodule	0	0	0	0	1	0
Incisor	Loss	0	0	1	0	0	0
Kidney	Brown foci	0	0	1	1	0	0
	Cyst	2	0	0	1	0	0
	Dilatation of pelvis	0	0	0	1	1	0
Testis	Atrophy	1	0	0	1	1	2
	Friable	1	2	0	0	0	0
Epididymis	Atrophy	0	2	0	1	1	2
	Nodule	0	0	0	1	0	0
Skin	Hair loss in forelimb	0	0	1	0	2	3

Table 9-2. Necropsy findings in female rats

Findings		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No. of animals examined		20	20	20	20	20	20
Spleen	Accessory spleen	0	2	1	0	1	0
	Rough surface with adhesion	0	0	0	0	1	0
Thymus	Petechia	1	0	0	0	0	0
Lung	Dark-red	0	1	0	0	0	0
	Dark-red foci	1	1	0	1	0	0
Liver	Diaphragmatic hernia	0	0	1	0	0	1
	White focus	0	1	0	1	0	0
	Yellow-white focus	3	2	0	1	2	4
Kidney	Dilatation of pelvis with calculi	0	0	0	0	0	3
	Depressed foci	0	0	0	0	1	0
Mammary	Hypersecretion	0	0	1	0	0	0
Ovary	Cyst	0	1	1	0	1	1
Oviduct	Cyst	0	1	0	0	0	0
Uterus	Hydrometra	2	5	0	2	5	1
	Nodule	0	1	0	0	0	0
Pituitary	Cyst	0	0	0	0	0	1
	Enlargement	0	0	0	0	0	1
Eye	Opacity	0	0	0	0	0	1
Skin	Loss of hair	0	2	3	0	0	3

Group mean values of absolute and relative organ weights are listed in Table 10-1 (male) and Table 10-2 (female). Absolute weights of the liver and kidney were greater for males in CRF-1 group than in the CR-LPF group after 26 weeks. Relative weights of the testis and epididymis and absolute and relative weights of the prostate were smaller for males in the CRF-1 group than in the CR-LPF group after 26 weeks. There were no difference in the organ weights for females between the two groups. The thymus weight was decreased with age in both sexes of both groups.

Table 10-1. Organ weight in males-Group mean value

		Male					
		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No.of animals examined		20	20	20	20	20	20
Body weight	(g)	326.6±28.7	483.6±49.4	562.4±83.0	339.4±25.7	514.8±42.6*	596.5±44.4
Brain	(g)	1.87±0.07	2.03±0.10	2.09±0.08	1.91±0.07*	2.04±0.08	2.07±0.10
	(g%)	0.575±0.044	0.422±0.041	0.377±0.046	0.566±0.041	0.398±0.036	0.348±0.025#
Heart	(g)	1.08±0.09	1.35±0.15	1.42±0.15	1.14±0.09	1.41±0.10	1.47±0.12
	(g%)	0.332±0.025	0.280±0.019	0.254±0.020	0.336±0.019	0.275±0.022	0.246±0.015
Lung	(g)	1.19±0.12	1.40±0.10	1.46±0.11	1.22±0.08	1.43±0.10	1.51±0.11
	(g%)	0.365±0.025	0.290±0.017	0.263±0.028	0.360±0.024	0.280±0.021	0.253±0.016
Liver	(g)	9.2±1.0	12.0±1.8	12.7±2.2	9.9±1.2	12.9±1.7	14.2±1.8*
	(g%)	2.81±0.13	2.48±0.17	2.26±0.15	2.90±0.22	2.49±0.20	2.37±0.19
Kidney	(g)	2.42±0.20	2.92±0.36	2.87±0.32	2.54±0.20	2.99±0.30	3.14±0.31**
	(g%)	0.743±0.056	0.604±0.054	0.514±0.046	0.749±0.052	0.582±0.044	0.528±0.044
Spleen	(g)	0.68±0.06	0.79±0.13	0.78±0.11	0.68±0.09	0.77±0.11	0.81±0.14
	(g%)	0.208±0.020	0.165±0.024	0.140±0.020	0.202±0.025	0.150±0.020*	0.136±0.022
Salivary gland	(g)	0.60±0.05	0.67±0.07	0.69±0.09	0.61±0.06	0.68±0.07	0.72±0.07
	(g%)	0.183±0.017	0.140±0.014	0.124±0.014	0.180±0.019	0.133±0.016	0.120±0.011
Pituitary	(mg)	10±2	13±3	13±2	10±2	13±3	13±2
	(mg%)	3.0±0.5	2.7±0.6	2.3±0.4	2.8±0.5	2.6±0.5	2.1±0.4
Thyroid	(mg)	18±3	23±4	25±5	19±4	23±4	26±4
	(mg%)	5.6±0.9	4.8±0.8	4.4±1.0	5.7±1.2	4.4±0.7	4.3±0.8
Thymus	(g)	0.47±0.11	0.29±0.07	0.15±0.04	0.51±0.12	0.32±0.10	0.16±0.04
	(g%)	0.143±0.027	0.061±0.014	0.027±0.007	0.151±0.032	0.063±0.018	0.027±0.007
Adrenal	(mg)	53±10	51±8	46±7	49±6	54±8	52±5**
	(mg%)	16.1±2.7	10.6±1.6	8.2±1.1	14.6±2.6	10.5±1.3	8.8±1.0
Testis	(g)	2.97±0.31	3.12±0.28	3.45±0.26	2.91±0.23	3.20±0.21	3.01±0.83#
	(g%)	0.917±0.128	0.650±0.079	0.625±0.099	0.860±0.085	0.625±0.070	0.503±0.138**
Epididymis	(g)	0.76±0.08	1.19±0.13	1.40±0.14	0.75±0.07	1.26±0.12	1.27±0.26
	(g%)	0.234±0.039	0.249±0.039	0.253±0.039	0.221±0.024	0.246±0.031	0.213±0.043**
Seminal vesicle	(g)	1.38±0.19	1.80±0.32	2.09±0.31	1.27±0.19	1.74±0.20	2.08±0.28
	(g%)	0.428±0.084	0.372±0.048	0.380±0.079	0.377±0.060*	0.341±0.050	0.348±0.041
Prostate	(g)	0.44±0.11	0.58±0.17	0.69±0.15	0.43±0.12	0.60±0.14	0.59±0.14*
	(g%)	0.135±0.030	0.119±0.032	0.126±0.033	0.128±0.034	0.117±0.030	0.100±0.025**

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)

Table 10-2. Organ weight in females-Group mean value

		Female					
		CR-LPF			CRF-1		
		4W	13W	26W	4W	13W	26W
No.of animals examined		20	20	20	20	20	20
Body weight	(g)	205.1±15.6	279.3±18.8	319.7±37.4	219.8±20.2*	289.1±28.3	332.1±34.4
Brain	(g)	1.75±0.06	1.84±0.08	1.86±0.07	1.76±0.06	1.85±0.08	1.85±0.08
	(g%)	0.858±0.061	0.660±0.046	0.590±0.067	0.808±0.067*	0.644±0.067	0.563±0.057
Heart	(g)	0.74±0.06	0.88±0.06	0.98±0.09	0.80±0.09#	0.88±0.06	0.96±0.09
	(g%)	0.363±0.023	0.315±0.025	0.308±0.033	0.365±0.026	0.306±0.022	0.291±0.025
Lung	(g)	0.95±0.07	1.06±0.08	1.10±0.05	0.98±0.09	1.04±0.09	1.10±0.07
	(g%)	0.465±0.034	0.380±0.038	0.348±0.041	0.447±0.028	0.360±0.025	0.336±0.036
Liver	(g)	6.1±0.5	6.8±0.4	7.7±1.0	6.6±0.7*	7.1±0.8	7.7±0.9
	(g%)	2.97±0.22	2.45±0.17	2.41±0.21	3.01±0.15	2.45±0.13	2.33±0.19
Kidney	(g)	1.59±0.15	1.69±0.11	1.83±0.19	1.68±0.14*	1.79±0.11**	1.93±0.30
	(g%)	0.774±0.059	0.608±0.039	0.576±0.046	0.768±0.051	0.623±0.042	0.583±0.067
Spleen	(g)	0.46±0.07	0.48±0.08	0.49±0.06	0.47±0.08	0.49±0.10	0.52±0.09
	(g%)	0.223±0.032	0.171±0.031	0.156±0.022	0.212±0.031	0.169±0.027	0.157±0.030
Salivary gland	(g)	0.39±0.03	0.41±0.03	0.44±0.04	0.40±0.04	0.43±0.04	0.44±0.04
	(g%)	0.188±0.016	0.146±0.012	0.139±0.019	0.180±0.016	0.148±0.012	0.134±0.017
Pituitary	(mg)	13±2	15±3	18±5	15±3	16±3	16±5
	(mg%)	6.3±1.2	5.5±1.0	5.5±1.5	6.7±1.4	5.7±1.0	4.9±1.4
Thyroid	(mg)	14±3	17±4	21±5	16±4	18±3	19±5
	(mg%)	6.6±1.4	6.0±1.4	6.7±1.6	7.1±1.6	6.3±1.3	5.5±1.3*
Thymus	(g)	0.41±0.08	0.28±0.06	0.15±0.03	0.45±0.10	0.26±0.07	0.16±0.04
	(g%)	0.199±0.039	0.099±0.021	0.046±0.010	0.207±0.038	0.089±0.020	0.049±0.010
Adrenal	(mg)	65±12	62±7	61±7	64±7	66±7	64±13
	(mg%)	31.6±5.2	22.3±2.4	19.2±2.4	29.4±3.3	22.9±3.2	19.3±3.5
Ovary	(mg)	79±11	76±10	54±15	88±12*	80±14	67±18*
	(mg%)	38.9±5.4	27.2±3.7	17.2±5.4	40.0±4.6	27.7±4.7	20.5±5.3
Uterus	(g)	0.45±0.10	0.63±0.11	0.75±0.12	0.52±0.15	0.60±0.15	0.67±0.16
	(g%)	0.219±0.055	0.225±0.039	0.237±0.035	0.237±0.075	0.208±0.055	0.205±0.051*

Mean±S.D.
* :Significantly different from the CR-LPF group of the same week at $p<0.05$ (Student's t-test)
**:Significantly different from the CR-LPF group of the same week at $p<0.01$ (Student's t-test)
:Significantly different from the CR-LPF group of the same week at $p<0.05$ (Aspin-Welch's t-test)

In conclusion, there were differences in several parameters when values in the CR-LPF group were compared with those in the CRF-1 group. However, the differences were small and values of hematology and blood chemistry parameters in both groups largely fell within the historical control range in our laboratories on Jcl:SD strain rats fed a diet with standard protein content. Therefore, it is thought that the present findings do not raise any concern for using Crj:CD(SD)IGS rats and for feeding the animals with the low protein diet (CR-LPF) in the evaluation of the general toxicity of new drugs.

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Background Data of General Toxicological Parameters in Crj:CD(SD)IGS Rats at 9, 12 and 18 weeks of Age

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ABSTRACT. This study was performed to obtain general toxicological parameters of male and female Crj:CD(SD)IGS rats. Three different lots of animals at two or three different ages were used in the study to confirm variability of these parameters. It was concluded that the overall results in the present study did not indicate any obvious problems. Therefore, it would be able to use the results in the present study as the reference data in the general toxicity studies. — Key words: General toxicity study, Toxicological parameter, Crj:CD(SD)IGS rat

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INTRODUCTION

Crj:CD(SD)IGS strain rat was produced by International Genetic Standard system and supplied by Charles River Japan, Inc. To confirm variability of these parameters among different lots of Crj:CD(SD)IGS rats, clinical signs, body weights, food and water consumption, urinalysis, hematology, blood chemistry, ophthalmology, necropsy and organ weights were examined in the present study.

MATERIALS AND METHODS

Animals and housing

Three lots of male and female Crj:CD(SD)IGS rats aged 4 weeks were obtained from Charles River Japan, Inc. (Tsukuba, Ibaraki, Japan) on May 8, 1996, May 22, 1996, and June 5, 1996, and called Lot 1, 2 and 3, respectively. Each lot contained 21 males and 21 females. Animals of all lots were used after an acclimatization and quarantine period for 6 days. The animals of each lot were randomly allocated into 2 groups comprised 10 males and 10 females according to body weights. The 2 groups were used for 4-week (1M) and 13-week (3M) studies, respectively. At the termination of study, animals were 9 and 18 weeks of age. They were housed individually in wire-mesh cages and allowed to free access to pellet diet (CRF-1, Oriental Yeast Co., Ltd.) and chlorinated tap water. Water was supplied by the water bottles. The animal room was controlled at 23±1℃ with relative humidity of 55 ± 10%, ventilation of 10 times/hr or more, and a 12 hr light/dark cycle (light on 06:00-18:00).

Examinations and methods

1) Clinical signs

All animals were observed for mortality, morbidity and clinical signs once a day during the experimental period.

2) Body weights

Each animal was weighed using an electronic balance twice a week and before necropsy. Prior to necropsy, the animals were deprived of food overnight.

3) Food and water consumption

In 13-week studies, food and water consumption of animals were measured using an electronic balance twice a week.

4) Urinalysis

At 9 weeks of age, 16hr-urine samples of 4-week (1M) studies were collected using metabolic cages. At 12 and 18 weeks of age, urine samples of 13-week (3M) studies were collected in the same manner. Urine volume was measured individually. Protein, glucose, occult blood, ketones, bilirubin and urobilinogen (test paper method) were determined using an automated urine analyzer MA-4210 (Kyoto Daiichi Kagaku Co., Ltd.). After centrifugal separation, osmolarity (Cryoscopic method) was determined using an automated osmolarity analyzer Osmotic Pressure AUTO & STAT OM-6030 (Kyoto Daiichi Kagaku Co., Ltd.). Sodium (ISE method), potassium (ISE method) and chloride (coulometric titration method) were determined using an automated electrolyte analyzer PVA α Ⅱ (A & T corporation). Calcium (OCPC method), inorganic phosphate (Fiske-Subbarow method), creatinine (Jaffé method) and N-acetyl- β-D-glucosaminidase (NAG: CPR-NAG method) were determined using an automatic analyzer Model 7070 (Hitachi, Ltd.). Urinary pH was determined using a pH meter HM-70V (TOA Electronics Ltd.).

5) Hematology

At 9 and 18 weeks of age, before necropsy, blood samples were collected from the abdominal aorta under ethyl ether anesthesia following depriving of food overnight (16 hours or more). The following hematological tests were performed after the treatment of EDTA anticoagulant using an automated hematology analyzer K-1000 (Sysmex Co., Ltd.): erythrocytes (RBC: electric resistance detection method), hemoglobin (HGB: SLS-Hb method), hematocrit (PCV: cumulative pulse height detection method), platelets (electric resistance detection method), and leukocytes (WBC: electric resistance detection method). The following hematological testes were performed after blood smear preparation by the light microscopy: reticulocyte counts (Brecher method) and differential leukocyte counts (Giemsa stain). The following hematological tests were performed on the plasma after centrifugal separation according to the treatment of 3.2% sodium citrate anticoagulant (Blood: Sodium citrate = 9 : 1, v/v) using a semi-automated blood coagulation analyzer KC-10A (Heinrich Amelung GmbH): prothrombin time (PT: quick step method) and activated partial thromboplastin time (APTT: ellagic acid method).

6) Blood chemistry

At 9 and 18 weeks of age, before necropsy, blood samples were collected from the abdominal aorta under ethyl ether anesthesia following depriving of food overnight (16 hours or more). The following serum biochemistry tests were performed using an automatic analyzer Model 7070 (Hitachi, Ltd.) after centrifugal separation: glutamic oxaloacetic transaminase (GOT: UV rate method, IFCC), glutamic pyruvic transaminase (GPT: UV rate method, IFCC), alkaline phosphatase (ALP: p-NPP rate method, JSCC), amylase (BG5P method), glucose (GK・G-6-PDH method), total cholesterol (ChOD・DAOS method), free cholesterol (ChOD・DAOS method), triglyceride (GPO・DAOS method), phospholipid (COD・DAOS method), nonesterified fatty acid (NEFA: ASC・ASOD method), total bilirubin (Azobilirubin method), urea nitrogen (Urea N: Urease・GlDH method), creatinine (Jaffé method), total protein (Biuret method), albumin (BCG method), sodium (ISE method), potassium (ISE method), chloride (ISE method), calcium (OCPC method) and inorganic phosphate (Fiske-Subbarow method). Protein fractions (albumin, alpha1 globulin, alpha2 globulin, beta globulin, gamma globulin and A/G) were determined using an electrophoresis apparatus (Helena Laboratories) and cellulose acetate membranes.

7) Ophthalmology

Ophthalmoscopic examinations were performed for the uneven number animals in 13-week studies once before the commencement of the experiment and at 9, 12 and 18 weeks of age. The anterior portion of eyes was observed macroscopically using a Halogen ophthalmoscope BX (Neitz instruments Co., Ltd.). Then the pupil of eyes was dilated by application of mydriatica Midrin P (Santen Pharmaceutical Co., Ltd.) and ocular fundus was examined using a fundus camera RC-2 model-621 (Kowa Co., Ltd.) and photographed.

8) Necropsy and organ weights

At 9 weeks of age in 4-week studies and at 18 weeks of age in 13-week studies, each animal was killed by exanguination from the abdominal aorta under ethyl ether anesthesia following depriving of food for overnight (16 hours or more) and complete macroscopic examination of all organs was performed. Then the brain, pituitary gland, salivary gland, thymus, heart, lung, liver, kidney, spleen, adrenal gland, cecum, testis, epididymidis, ovary and uterus were dissected and weighed using an electronic balance.

RESULTS

1) Clinical signs

There were no deaths throughout the observation period. Crust in the neck, or shoulder or loss of hair was observed in each one male of Lot 1 in the 13-week studies.

2) Body weights (Table 1, 2)

Variation of mean body weights among three lots was almost within 10% for both sexes.

3) Food and water consumption (Table 3, 4)

There were no remarkable differences in the food and water consumption among three lots.

4) Urinalysis (Table 5, 6)

Creatinine in males and females in the 4-week studies at 9 weeks of age varied among three lots and the fluctuation was greater than that of the other parameters. In urine semi-quantitative data, occult blood was observed in one male each in Lot 2 or 3 in the 4-week studies at 9 weeks of age and one male of Lot 2 in the 13-week studies at 12 weeks of age.

5) Hematology (Table 7)

There were no remarkable differences in any parameters among three lots of animals at the same age. No age-related changes were also noted in any parameters.

6) Blood chemistry (Table 8)

There were no remarkable differences in any parameters among three lots at the same age. Values for amylase, glucose, total cholesterol and triglyceride were higher in males and females at 18 weeks of age than those of animals at 9 weeks of age. Values for ALP and inorganic phosphate were lower in males and females at 18 weeks of age than those of animals at 9 weeks of age.

7) Ophthalmology

There were no abnormalities in the anterior portion of the eyes. Hemorrhage from the optic disk of left eye was observed in one female of Lot 3 in the 13-week studies alone before the commencement of the experiment, at 9 and 12 weeks of age, however it disappeared by 18 weeks of age.

8) Necropsy and organ weights (Table 9, 10)

White nodule in the spleen was observed in one males of Lot 1 in the 4-week studies at 9 weeks of age. Yellow watery foam in the jejunum/ileum was observed in one females of Lot 3 in the 13-week studies at 18 weeks of age. The other animals did not show any abnormalities. There were no remarkable differences in the organ weights among lots at the same age. An age-related decrease in the thymus weights was evident in males and females.

DISCUSSION

It was concluded that the overall results in the present study did not indicate any obvious problems. Therefore, it would be able to use the results in the present study as the reference data in the general toxicity studies.

Table 1-1. Body weight (g) of male Crj:CD(SD)IGS rats in 4-week studies

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Day 1	131.5± 4.1	130.9± 4.8	132.5± 6.0
4	156.9± 6.5	154.9± 7.7	159.4± 7.5
8	189.2± 7.7	187.9±11.4	195.5±10.7
11	213.2± 9.3	212.7±13.7	222.1±11.7
15	248.1±12.8	243.6±17.9	255.9±13.1
18	271.5±16.4	265.6±21.2	280.6±15.6
22	301.3±19.5	293.6±23.4	313.2±18.1
25	320.6±23.2	313.4±26.2	335.6±20.8
29	345.2±25.8	332.7±28.0	359.0±22.5

Values represent mean±S. D.

Table 1-2. Body weight (g) of female Crj:CD(SD)IGS rats in 4-week studies

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Day 1	113.5± 4.3	117.5± 4.5	101.4± 3.0
4	129.0± 5.3	133.7± 5.8	123.6± 4.3
8	142.7± 6.3	152.9± 9.1	147.6± 4.6
11	151.5± 7.5	164.4±10.5	161.3± 6.0
15	162.6± 9.0	176.7±11.4	176.8± 8.2
18	170.4± 8.2	186.2±13.9	185.9±11.1
22	181.0± 9.0	198.9±16.8	197.0±14.2
25	187.3±10.2	207.2±17.1	207.2±16.2
29	193.8± 9.6	214.2±15.6	214.8±18.9

Values represent mean±S. D.

Table 1-3. Body weight (g) of male Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 1	131.9± 4.0	129.8± 3.3	132.4± 5.5
4	156.9± 5.0	153.2± 4.3	159.7± 5.8
8	190.0± 6.3	187.6± 5.8	196.3± 8.2
11	213.8± 7.6	212.1± 6.8	222.3± 9.4
15	245.9±12.1	243.9± 9.0	256.7±12.5
18	269.6±14.8	268.6±10.1	280.6±15.1
22	297.6±19.2	297.0±14.6	313.4±17.3
25	315.4±22.4	317.4±16.3	335.0±18.4
29	336.7±25.7	340.3±20.6	359.9±21.7
32	351.2±27.3	354.5±24.6	375.6±24.8
36	371.5±30.8	373.2±29.0	394.1±27.0
39	385.6±32.8	386.4±29.3	407.2±27.8
43	400.6±33.5	403.6±31.3	423.6±29.4
46	411.8±35.5	413.9±30.3	435.6±30.6
50	427.3±35.2	428.2±33.5	449.2±32.2
53	437.4±36.6	435.1±32.1	456.2±34.1
57	448.7±36.8	445.9±33.0	468.0±34.9
60	454.1±38.9	454.5±33.9	477.9±35.3
64	465.5±36.7	467.0±35.9	487.5±36.3
67	474.9±39.3	472.7±35.9	492.9±36.5
71	483.9±43.2	477.3±39.3	503.8±36.9
74	489.6±44.2	482.1±47.9	512.2±37.2
78	498.4±44.2	489.5±49.6	519.4±38.7
81	504.4±46.0	495.1±48.7	525.5±39.6
85	512.8±46.6	503.2±46.5	534.1±40.0
88	518.9±46.7	509.5±45.6	538.5±42.1
92	526.1±48.4	515.2±44.7	547.4±42.4

Values represent mean±S. D.

Table 1-4. Body weight (g) of female Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 1	110.7± 5.1	116.6± 4.2	102.3± 3.5
4	128.7± 8.3	134.1± 6.8	122.2± 4.9
8	146.0±11.0	153.5± 8.8	148.0± 5.8
11	157.3±12.4	166.3± 8.8	160.6± 5.5
15	169.2±15.0	181.3± 9.5	175.5± 8.2
18	176.5±14.5	192.2±10.2	185.6± 8.1
22	187.8±15.8	205.4±11.6	197.1±10.4
25	196.2±18.1	215.1±14.7	206.2±13.3
29	204.2±20.1	225.9±16.8	216.3±14.8
32	211.9±21.2	233.3±20.0	222.1±15.3
36	221.7±22.5	246.6±23.0	229.4±16.2
39	226.5±21.6	254.7±21.7	235.6±16.7
43	233.7±22.3	261.2±22.0	243.6±17.1
46	237.3±22.9	267.3±21.9	248.3±16.6
50	244.5±25.0	272.5±20.9	253.8±15.2
53	248.3±24.7	275.0±23.4	256.5±17.9
57	253.7±26.8	280.6±23.4	259.4±16.8
60	257.0±23.7	282.5±24.0	262.2±20.5
64	262.5±25.5	287.2±25.0	270.1±16.7
67	268.8±25.3	291.5±23.3	273.1±17.5
71	272.2±24.4	295.7±24.0	278.0±17.5
74	272.9±25.5	299.1±23.9	281.1±17.1
78	276.9±25.7	303.0±25.7	283.9±17.9
81	277.6±25.0	306.9±27.4	285.8±20.0
85	280.5±26.3	310.0±26.9	291.1±21.7
88	283.0±26.3	311.2±29.0	292.8±21.2
92	285.4±27.2	313.7±27.3	296.5±21.9

Values represent mean±S. D.

Table 2-1. Body weight gain (g/day) of male Crj:CD(SD)IGS rats in 4-week studies

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Day 4	8.5±1.1	8.0±1.3	9.0±0.9
8	8.1±0.8	8.3±1.1	9.0±1.0
11	8.0±0.9	8.3±1.0	8.9±0.9
15	8.7±1.2	7.8±1.4	8.5±0.9
18	7.8±1.6	7.3±2.0	8.2±1.2
22	7.5±1.4	7.0±0.8	8.2±1.3
25	6.5±1.5	6.6±1.2	7.5±1.4
29	6.2±1.3	4.8±1.3	5.9±1.4

Values represent mean±S. D.

Table 2-2. Body weight gain (g/day) of female Crj:CD(SD)IGS rats in 4-week studies

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Day 4	5.2±0.7	5.4±0.9	7.4±1.0
8	3.4±0.5	4.8±0.9	6.0±0.6
11	3.0±1.0	3.9±1.1	4.6±1.0
15	2.8±0.6	3.1±0.6	3.9±1.1
18	2.6±0.5	3.2±1.4	3.0±1.4
22	2.7±0.7	3.2±0.9	2.8±1.0
25	2.1±1.3	2.8±0.8	3.4±1.3
29	1.6±0.5	1.8±1.0	1.9±1.3

Values represent mean±S. D.

Table 2-3. Body weight gain (g/day) of male Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	8.3±0.8	7.8±0.7	9.1±0.6
8	8.3±0.5	8.6±0.7	9.2±1.0
11	7.9±1.1	8.2±0.7	8.6±1.1
15	8.0±1.2	8.0±0.8	8.6±1.2
18	7.9±1.2	8.3±0.7	8.0±1.2
22	7.0±1.3	7.1±1.5	8.2±0.9
25	5.9±1.5	6.8±1.3	7.2±1.0
29	5.4±1.2	5.7±1.6	6.2±1.2
32	4.8±1.0	4.7±1.5	5.2±1.7
36	5.1±1.3	4.7±1.5	4.6±0.8
39	4.7±2.1	4.4±0.5	4.4±0.7
43	3.8±0.8	4.3±0.7	4.1±0.8
46	3.7±1.2	3.5±1.0	4.0±0.9
50	3.9±1.3	3.6±1.4	3.4±1.0
53	3.3±1.3	2.3±1.6	2.3±1.7
57	2.8±0.7	2.7±0.9	3.0±0.6
60	1.8±0.9	2.9±0.9	3.3±1.0
64	2.9±1.4	3.1±0.6	2.4±0.7
67	3.2±1.8	1.9±1.0	1.8±3.1
71	2.3±1.5	1.2±2.4	2.7±2.0
74	1.9±1.2	1.6±4.8	2.8±0.8
78	2.2±1.0	1.9±0.8	1.8±1.1
81	2.0±1.4	1.9±1.1	2.0±1.2
85	2.1±0.9	2.1±1.6	2.1±0.6
88	2.0±0.7	2.1±1.8	1.5±1.3
92	1.8±1.5	1.4±1.4	2.2±0.6

Values represent mean±S. D.

Table 2-4. Body weight gain (g/day) of female Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	6.0±1.4	5.8±1.6	6.6±1.0
8	4.4±0.9	4.8±0.8	6.5±0.6
11	3.7±1.0	4.3±1.2	4.2±1.3
15	3.0±1.0	3.7±0.5	3.8±1.0
18	2.4±1.5	3.6±1.0	3.4±1.4
22	2.8±0.8	3.3±0.7	2.9±1.3
25	2.8±1.5	3.3±1.9	3.0±1.8
29	2.0±1.0	2.7±0.8	2.5±0.6
32	2.6±1.9	2.5±1.7	2.0±1.1
36	2.4±0.9	3.3±1.7	1.9±0.6
39	1.6±1.8	2.7±1.5	2.1±1.4
43	1.8±0.6	1.6±0.8	2.0±0.7
46	1.2±0.9	2.0±1.1	1.6±0.9
50	1.8±1.1	1.3±0.7	1.4±0.7
53	1.3±1.7	0.9±1.1	0.9±1.4
57	1.3±0.9	1.4±0.8	0.7±1.1
60	1.1±1.9	0.6±1.2	1.0±2.5
64	1.4±0.7	1.2±0.7	2.0±1.3
67	2.1±1.2	1.4±1.3	1.0±1.9
71	0.8±0.8	1.0±0.8	1.2±1.2
74	0.2±0.9	1.1±1.2	1.1±1.2
78	1.0±0.7	1.0±0.7	0.7±0.9
81	0.2±1.9	1.3±1.2	0.7±1.2
85	0.7±1.0	0.8±0.7	1.3±0.8
88	0.8±1.5	0.4±1.6	0.6±1.2
92	0.6±1.2	0.6±0.8	0.9±0.8

Values represent mean±S. D.

Table 3-1. Food consumption (g/day) of male Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	21.7±1.0	19.6±0.9	21.4±1.2
8	24.0±1.1	22.0±1.2	23.4±1.4
11	24.7±1.1	23.7±1.4	24.6±1.7
15	25.6±1.4	25.2±1.5	26.5±1.9
18	26.0±1.4	26.0±1.5	25.0±5.6
22	26.4±2.1	26.7±1.7	28.6±2.2
25	26.3±2.1	26.1±2.2	28.8±2.2
29	27.3±2.4	27.5±1.8	29.1±2.1
32	26.3±2.5	27.5±2.4	25.6±6.3
36	27.2±2.0	27.5±2.4	28.4±2.1
39	27.4±1.9	27.4±2.0	27.4±4.8
43	27.3±1.9	27.3±2.1	29.0±2.1
46	27.6±2.3	27.1±1.8	29.2±2.6
50	28.1±1.7	27.3±1.5	29.0±2.3
53	27.3±1.5	26.7±1.7	27.8±2.9
57	27.2±1.8	26.8±1.5	28.4±2.7
60	27.3±1.9	27.3±1.7	28.6±2.5
64	27.7±1.7	27.4±1.7	28.2±2.5
67	27.3±1.9	27.3±1.9	27.7±2.6
71	27.4±2.6	26.7±2.5	28.1±2.4
74	27.6±2.3	24.7±8.3	28.3±2.7
78	27.5±2.3	26.5±4.7	28.1±2.5
81	27.3±2.6	26.9±1.9	27.4±2.7
85	27.6±2.3	26.8±2.7	27.3±3.7
88	27.4±2.2	27.0±1.9	27.9±2.6
92	27.5±2.2	27.2±2.1	28.3±2.7

Values represent mean±S. D.

Table 3-2. Food consumption (g/day) of female Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	16.9±1.9	16.5±1.0	18.6±1.5
8	17.5±1.6	17.7±0.8	19.1±1.2
11	17.0±1.6	18.1±1.1	18.1±1.1
15	17.3±2.0	18.8±0.9	18.6±1.4
18	16.5±4.1	18.8±1.3	18.8±1.5
22	18.1±1.6	19.6±1.2	18.8±1.9
25	18.6±1.9	19.6±1.8	17.9±4.0
29	18.8±1.8	20.4±1.5	18.9±1.8
32	18.8±1.7	19.9±1.7	20.5±4.8
36	19.2±2.2	21.4±2.1	19.0±1.7
39	18.9±2.0	20.9±2.0	19.0±1.6
43	19.3±2.1	20.8±2.2	19.5±1.5
46	18.9±1.5	21.0±2.3	19.3±1.3
50	19.8±2.2	20.2±1.8	19.3±1.4
53	18.8±1.7	19.3±2.2	18.8±1.7
57	19.3±2.2	20.1±2.0	18.7±1.9
60	19.9±1.5	20.0±2.0	18.9±1.8
64	19.5±1.8	20.4±1.9	19.6±1.5
67	20.1±1.5	20.1±2.1	19.1±1.6
71	19.4±1.5	20.3±2.1	19.4±2.0
74	18.8±1.8	19.9±2.5	19.2±1.8
78	19.0±1.6	20.2±2.4	18.8±2.0
81	19.1±1.8	20.0±2.6	18.9±1.9
85	18.9±2.4	20.2±2.2	19.6±1.9
88	19.0±1.6	20.2±2.3	19.4±1.5
92	18.6±1.9	19.9±1.7	19.4±1.8

Values represent mean±S. D.

Table 4-1. Water consumption (g/day) of male Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	29.9± 2.8	27.3± 3.0	28.3± 3.2
8	31.9± 2.9	29.0± 2.9	30.1± 3.1
11	33.4± 3.6	30.5± 3.1	31.0± 2.8
15	33.8± 3.1	31.5± 2.4	31.9± 3.1
18	35.2± 4.3	33.7± 2.8	33.4± 4.1
22	35.2± 6.9	34.3± 2.9	34.0± 4.2
25	35.3± 4.1	35.2± 3.2	35.3± 4.4
29	36.1± 5.6	35.2± 3.1	36.1± 5.5
32	37.4± 5.4	35.9± 3.1	35.4± 5.0
36	36.9± 6.0	34.9± 3.7	37.3± 5.4
39	38.2± 6.7	36.3± 3.7	36.2± 6.2
43	40.4±12.1	36.6± 5.1	37.6± 7.0
46	40.2±11.2	36.9± 4.1	37.4± 7.6
50	39.2± 5.9	39.1± 4.8	40.3± 8.5
53	35.9± 6.1	36.5± 4.2	39.1±11.6
57	35.8± 5.3	36.5± 4.6	37.1± 8.8
60	40.8±10.7	36.3± 4.6	38.5± 8.6
64	40.5±12.6	36.9± 4.4	35.9± 4.9
67	39.8±11.1	35.5± 4.1	42.0±21.1
71	38.8±11.0	36.9± 6.4	36.3± 7.6
74	40.2±10.8	35.9± 7.9	38.2± 8.4
78	33.8± 4.9	36.2± 5.4	36.3± 8.0
81	37.7± 9.0	35.5± 4.9	35.0± 8.9
85	35.2± 4.7	35.1± 3.7	36.2± 7.7
88	38.3± 7.5	36.4± 4.6	33.6± 4.4
92	39.9± 7.3	37.3± 3.8	37.1±10.0

Values represent mean±S. D.

Table 4-2. Water consumption (g/day) of female Crj:CD(SD)IGS rats in 13-week studies

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Day 4	26.1± 3.0	28.2± 6.8	24.4± 2.4
8	25.6± 2.8	26.9± 2.0	25.7± 2.5
11	25.4± 3.1	28.3± 3.4	31.8±18.4
15	27.1± 4.6	27.1± 2.3	25.7± 4.2
18	27.4± 4.5	29.0± 3.7	26.3± 4.1
22	30.5± 7.0	30.5± 5.5	24.7± 4.8
25	32.6±12.1	29.8± 4.7	25.7± 6.1
29	31.2± 7.3	31.4± 6.7	26.5± 5.7
32	32.8± 8.3	31.2± 4.2	31.6±21.5
36	30.6± 5.6	33.3± 7.6	26.8± 5.2
39	32.6± 8.9	35.2± 9.0	26.2± 5.6
43	35.6±13.5	36.3±14.5	26.5± 4.2
46	33.0± 7.1	37.8±18.9	32.1±19.2
50	38.5±12.0	35.9± 7.1	28.7± 5.8
53	31.8± 5.8	31.6± 7.5	26.4± 5.6
57	36.7±14.6	33.5± 6.5	31.8±16.1
60	33.8±10.3	31.7± 6.2	38.1±23.5
64	35.1±13.4	32.6±11.0	32.6±15.3
67	41.4±17.5	34.1± 8.7	31.6±13.4
71	32.4± 7.2	34.9± 7.2	32.0±10.3
74	36.0±10.7	35.5± 6.1	31.4± 8.4
78	29.3± 8.8	36.7±12.9	30.9± 7.6
81	33.3±10.8	38.6±13.2	31.9± 7.2
85	33.8± 8.3	39.0±11.7	33.3±10.1
88	35.5± 8.9	37.0±11.3	33.1± 7.4
92	39.0±12.5	40.9±11.2	37.3±10.3

Values represent mean±S. D.

Table 5-1. Urinalysis values of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Urine volume (ml)	10.20±3.06	10.05±3.39	12.02±3.42
Osmolarity (mOsm/kg)	1797±243	1784±292	1777±338
Sodium (mEq/l)	179.4±30.0	177.6±29.3	169.0±31.2
Potassium (mEq/l)	282.1±47.4	288.6±52.6	283.3±61.7
Chloride (mEq/l)	236.1±38.8	236.5±45.5	223.0±39.8
Calcium (mg/dl)	9.00±2.31	8.69±4.25	7.86±2.43
I. phosphate (mg/dl)	40.5±22.2	41.8±33.7	59.2±27.1
Creatinine (mg/dl)	166.7±23.5	88.3±15.9	77.4±16.9
NAG (IU/l)	17.71±3.60	16.83±6.01	16.33±3.87
Sodium (mEq/16hr)	1.75±0.26	1.70±0.30	1.96±0.36
Potassium (mEq/16hr)	2.76±0.44	2.77±0.55	3.23±0.38
Chloride (mEq/16hr)	2.30±0.33	2.26±0.43	2.58±0.41
Calcium (mg/16hr)	0.93±0.38	0.83±0.45	0.93±0.37
I. phosphate (mg/16hr)	4.3±2.5	4.3±3.8	7.0±3.3
Creatinine (mg/16hr)	16.53±3.47	8.47±1.69	8.84±1.12
NAG (IU/16hr)	0.18±0.07	0.17±0.08	0.19±0.04
pH	7.80±0.33	7.81±0.30	7.52±0.36

Values represent mean±S. D.

Table 5-2. Urinalysis values of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Urine volume (ml)	9.28±4.03	12.11±4.61	6.06±1.96
Osmolarity (mOsm/kg)	1618±455	1364±479	1926±260
Sodium (mEq/l)	132.5±40.7	106.3±34.3	151.8±44.8
Potassium (mEq/l)	237.8±72.4	199.4±75.5	292.3±49.7
Chloride (mEq/l)	189.3±54.2	149.9±53.1	204.5±51.5
Calcium (mg/dl)	11.16±2.56	9.87±3.93	12.69±5.06
I. phosphate (mg/dl)	38.8±18.4	51.2±33.9	58.4±26.3
Creatinine (mg/dl)	126.0±38.0	54.7±22.8	76.5±12.1
NAG (IU/l)	13.22±4.48	10.32±3.67	14.54±3.27
Sodium (mEq/16hr)	1.09±0.18	1.15±0.17	0.93±0.33
Potassium (mEq/16hr)	1.95±0.29	2.12±0.20	1.74±0.47
Chloride (mEq/16hr)	1.57±0.27	1.61±0.20	1.24±0.40
Calcium (mg/16hr)	1.00±0.36	1.11±0.42	0.79±0.51
I. phosphate (mg/16hr)	3.3±1.3	5.3±2.7	3.4±1.5
Creatinine (mg/16hr)	10.36±1.60	5.77±0.84	4.49±1.00
NAG (IU/16hr)	0.11±0.04	0.11±0.03	0.08±0.02
pH	7.61±0.24	7.47±0.39	7.66±0.21

Values represent mean±S. D.

Table 5-3. Urinalysis values of male Crj:CD(SD)IGS rats at 12 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Urine volume (ml)	14.87±3.98	12.74±2.77	12.84±2.20
Osmolarity (mOsm/kg)	1600±295	1653±247	1685±159
Sodium (mEq/l)	148.3±32.5	132.6±28.3	133.3±16.0
Potassium (mEq/l)	235.7±42.8	257.4±40.7	272.3±32.1
Chloride (mEq/l)	209.4±76.7	178.7±30.2	183.1±17.1
Calcium (mg/dl)	5.98±1.89	6.30±2.25	5.77±2.50
I. phosphate (mg/dl)	46.1±27.8	41.0±17.9	61.4±30.6
Creatinine (mg/dl)	78.5±13.7	92.9±15.5	93.9±9.9
NAG (IU/l)	15.90±4.04	13.55±2.14	16.29±3.43
Sodium (mEq/16hr)	2.10±0.25	1.64±0.21	1.69±0.20
Potassium (mEq/16hr)	3.36±0.39	3.20±0.40	3.46±0.41
Chloride (mEq/16hr)	2.99±1.15	2.22±0.27	2.33±0.25
Calcium (mg/16hr)	0.87±0.33	0.82±0.41	0.73±0.32
I. phosphate (mg/16hr)	6.4±3.0	5.3±2.5	7.9±4.1
Creatinine (mg/16hr)	11.27±1.78	11.56±1.69	11.92±1.35
NAG (IU/16hr)	0.23±0.04	0.17±0.04	0.21±0.06
pH	7.70±0.13	7.78±0.23	7.74±0.30

Values represent mean±S. D.

Table 5-4. Urinalysis values of female Crj:CD(SD)IGS rats at 12 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Urine volume (ml)	13.90±6.07	10.97±2.83	8.86±3.37
Osmolarity (mOsm/kg)	1329±364	1529±304	1651±388
Sodium (mEq/l)	112.0±33.0	111.9±30.5	122.7±31.1
Potassium (mEq/l)	191.8±56.9	228.0±46.3	255.2±62.4
Chloride (mEq/l)	140.3±35.5	153.9±34.6	167.1±45.9
Calcium (mg/dl)	13.82±4.89	15.00±4.84	12.96±3.91
I. phosphate (mg/dl)	36.7±14.1	62.1±29.2	63.1±19.3
Creatinine (mg/dl)	53.1±17.2	68.3±16.8	79.1±18.3
NAG (IU/l)	10.40±3.29	10.04±2.07	14.36±3.08
Sodium (mEq/16hr)	1.41±0.23	1.17±0.22	1.00±0.15
Potassium (mEq/16hr)	2.40±0.43	2.40±0.32	2.08±0.25
Chloride (mEq/16hr)	1.78±0.37	1.61±0.25	1.35±0.21
Calcium (mg/16hr)	1.99±1.32	1.69±0.75	1.09±0.40
I. phosphate (mg/16hr)	4.9±2.5	6.5±3.0	5.5±2.4
Creatinine (mg/16hr)	6.56±0.89	7.11±0.89	6.50±1.01
NAG (IU/16hr)	0.13±0.04	0.10±0.01	0.12±0.04
pH	7.72±0.18	7.42±0.32	7.50±0.28

values represent mean±S. D.

Table 5-5. Urinalysis values of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Urine volume (ml)	15.61±4.31	14.15±3.36	13.06±4.36
Osmolarity (mOsm/kg)	1588±299	1626±302	1855±375
Sodium (mEq/l)	124.2±31.3	118.8±34.9	142.8±35.8
Potassium (mEq/l)	253.8±52.3	245.4±47.9	282.3±69.8
Chloride (mEq/l)	160.7±37.7	152.3±36.1	181.8±37.6
Calcium (mg/dl)	5.66±2.21	6.03±2.62	5.26±3.03
I. phosphate (mg/dl)	54.3±25.2	39.8±20.8	61.9±32.7
Creatinine (mg/dl)	94.0±17.7	99.5±20.8	112.2±25.3
NAG (IU/l)	13.79±3.32	12.40±3.27	14.11±2.45
Sodium (mEq/16hr)	1.84±0.24	1.60±0.19	1.74±0.27
Potassium (mEq/16hr)	3.77±0.35	3.35±0.32	3.44±0.42
Chloride (mEq/16hr)	2.39±0.34	2.06±0.16	2.24±0.35
Calcium (mg/16hr)	0.87±0.36	0.84±0.41	0.68±0.45
I. phosphate (mg/16hr)	7.8±2.5	5.9±3.6	7.3±2.8
Creatinine (mg/16hr)	14.06±1.85	13.51±1.17	13.71±1.64
NAG (IU/16hr)	0.21±0.05	0.18±0.08	0.18±0.04
pH	7.86±0.20	7.98±0.32	7.96±0.16

Values represent mean±S. D.

Table 5-6. Urinalysis values of female Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	9	10
Urine volume (ml)	15.06±6.97	13.40±6.18	12.69±5.89
Osmolarity (mOsm/kg)	1254±426	1386±504	1377±377
Sodium (mEq/l)	90.4±30.6	88.0±29.3	99.7±29.1
Potassium (mEq/l)	188.7±69.6	206.5±79.7	204.5±59.2
Chloride (mEq/l)	120.2±44.2	120.8±48.1	129.4±35.7
Calcium (mg/dl)	15.62±4.36	19.04±6.29	17.52±4.66
I. phosphate (mg/dl)	45.3±13.6	67.9±41.4	48.9±20.9
Creatinine (mg/dl)	60.2±21.0	68.3±24.4	68.8±23.9
NAG (IU/l)	10.44±3.40	10.63±3.29	11.01±2.91
Sodium (mEq/16hr)	1.21±0.24	1.04±0.18	1.12±0.27
Potassium (mEq/16hr)	2.50±0.45	2.40±0.34	2.30±0.53
Chloride (mEq/16hr)	1.60±0.32	1.41±0.29	1.46±0.35
Calcium (mg/16hr)	2.29±1.03	2.37±0.91	2.15±1.00
I. phosphate (mg/16hr)	6.4±2.4	7.3±2.2	5.4±1.8
Creatinine (mg/16hr)	7.91±1.05	8.00±1.23	7.57±1.47
NAG (IU/16hr)	0.14±0.03	0.13±0.03	0.13±0.04
pH	7.58±0.19	7.37±0.39	7.57±0.25

Values represent mean±S. D.

Table 6-1. Urinary semi-quantitative data of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M					Lot 2-1M					Lot 3-1M				
No. of animals	10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10				
Protein			10					9	1				9	1	
Ketones	2	8				1	9				3	6	1		
Occult blood	10					9			1		9			1	
Bilirubin	10					10					10				
Urobilinogen		2	5	3				6	3	1		3	4	3	

Table 6-2. Urinary semi-quantitative data of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M					Lot 2-1M					Lot 3-1M				
No. of animals	10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10				
Protein		4	6			3	3	4				1	9		
Ketones	8	2				7	3				3	7			
Occult blood	10					10					10				
Bilirubin	10					10					10				
Urobilinogen		8	2				8	2				6	4		

Table 6-3. Urinary semi-quantitative data of male Crj:CD(SD)IGS rats at 12 weeks of age

	Lot 1-3M					Lot 2-3M					Lot 3-3M				
No. of animals	10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10				
Protein		1	8	1				9	1				9	1	
Ketones	4	6				3	7				5	5			
Occult blood	10					9	1				10				
Bilirubin	10					10					10				
Urobilinogen		6	4				7	3				3	5	2	

Table 6-4. Urinary semi-quantitative data of female Crj:CD(SD)IGS rats at 12 weeks of age

	Lot 1-3M					Lot 2-3M					Lot 3-3M				
No. of animals	10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10				
Protein	1	6	3				5	5				1	9		
Ketones	9	1				7	3				9	1			
Occult blood	10					10					10				
Bilirubin	10					10					10				
Urobilinogen		9	1				9	1				8	2		

Table 6-5. Urinary semi-quantitative data of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M					Lot 2-3M					Lot 3-3M				
No. of animals	10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10				
Protein			10					7	3				6	4	
Ketones	6	4				7	3				5	5			
Occult blood	10					10					10				
Bilirubin	10					10					10				
Urobilinogen		8	2				8	2				4	4	2	

Table 7-1. Hematology values of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
RBC ($\times 10^4$/mm^3)	774±42	772±24	771±22
HGB (g/dl)	15.47±0.70	15.75±0.37	15.62±0.58
PCV (%)	45.88±2.11	46.49±1.33	45.70±1.27
MCV (fl)	59.4±2.6	60.2±1.9	59.3±1.7
MCH (pg)	20.03±0.89	20.39±0.50	20.26±0.73
MCHC (g/dl)	33.71±0.48	33.87±0.43	34.18±0.53
Platelet ($\times 10^4$/mm^3)	120.9± 9.7	119.6±12.7	123.3± 9.8
Reticulocyte (0/00)	19.9±5.7	19.6±7.9	17.9±6.4
WBC ($\times 10^2$/mm^3)	118.9±42.8	101.3±22.2	132.3±39.3
Neutrophil-band (%)	0.0±0.0	0.0±0.0	0.0±0.0
Neutrophil-segment (%)	7.7±3.5	6.9±3.0	8.3±4.4
Lymphocyte (%)	86.4±4.2	89.8±3.2	87.2±4.8
Monocyte (%)	4.9±2.6	2.9±1.4	4.0±2.2
Eosinophil (%)	1.0±0.8	0.4±0.5	0.5±1.3
Basophil (%)	0.0±0.0	0.0±0.0	0.0±0.0
PT (sec)	13.94±1.09	13.93±1.05	13.92±1.53
APTT (sec)	20.29±2.10	22.18±1.64	21.31±1.53

Values represent mean±S. D.

Table 7-2. Hematology values of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
RBC ($\times 10^4$/mm^3)	785±28	803±33	780±37
HGB (g/dl)	15.88±0.46	16.08±0.45	15.84±0.53
PCV (%)	45.37±1.61	46.07±1.30	44.90±1.70
MCV (fl)	57.8±0.8	57.4±1.6	57.6±2.1
MCH (pg)	20.25±0.39	20.03±0.71	20.33±0.76
MCHC (g/dl)	35.00±0.45	34.88±0.40	35.28±0.40
Platelet ($\times 10^4$/mm^3)	127.3±12.7	128.8±12.8	119.5± 7.4
Reticulocyte (0/00)	8.2±2.7	12.9±4.5	10.5±5.2
WBC ($\times 10^2$/mm^3)	69.1±16.4	88.4±19.7	73.5±13.7
Neutrophil-band (%)	0.0±0.0	0.0±0.0	0.0±0.0
Neutrophil-segment (%)	9.0±3.5	6.6±2.8	7.5±3.5
Lymphocyte (%)	86.3±4.6	90.3±3.1	88.1±3.9
Monocyte (%)	3.9±2.0	2.4±1.9	3.1±1.4
Eosinophil (%)	0.8±1.0	0.7±0.8	1.3±1.1
Basophil (%)	0.0±0.0	0.0±0.0	0.0±0.0
PT (sec)	13.11±0.36	13.00±0.52	12.63±0.62
APTT (sec)	16.81±1.11	16.51±1.31	16.74±1.82

Values represent mean±S. D.

Table 7-3. Hematology values of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
RBC ($\times 10^4$/mm^3)	872±30	885±40	903±33
HGB (g/dl)	15.94±0.56	15.75±0.40	16.09±0.37
PCV (%)	45.94±1.74	45.37±1.42	45.80±1.41
MCV (fl)	52.7±1.4	51.3±1.5	50.8±2.3
MCH (pg)	18.28±0.45	17.82±0.55	17.85±0.72
MCHC (g/dl)	34.70±0.26	34.72±0.33	35.14±0.51
Platelet ($\times 10^4$/mm^3)	108.3±14.4	103.7±10.2	102.8±12.7
Reticulocyte (0/00)	16.3±4.7	13.2±5.9	16.2±3.4
WBC ($\times 10^2$/mm^3)	114.3±18.7	112.7±24.5	132.3±11.7
Neutrophil-band (%)	0.0±0.0	0.0±0.0	0.0±0.0
Neutrophil-segment (%)	11.4±4.9	9.3±4.2	13.3±4.6
Lymphocyte (%)	85.0±5.4	87.0±3.8	82.9±6.3
Monocyte (%)	3.1±2.3	2.8±1.7	3.0±1.7
Eosinophil (%)	0.5±0.5	0.9±1.2	0.8±0.9
Basophil (%)	0.0±0.0	0.0±0.0	0.0±0.0
PT (sec)	13.52±1.30	13.12±0.77	13.34±1.64
APTT (sec)	19.40±1.62	20.79±1.18	20.45±1.55

Values represent mean±S. D.

Table 7-4. Hematology values of female Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
RBC ($\times 10^4$/mm^3)	816±40	803±32	807±37
HGB (g/dl)	16.11±0.72	15.59±0.48	15.81±0.53
PCV (%)	45.60±2.24	44.10±1.60	43.76±1.82
MCV (fl)	55.9±1.8	54.9±1.6	54.2±1.5
MCH (pg)	19.76±0.63	19.42±0.42	19.59±0.44
MCHC (g/dl)	35.34±0.44	35.36±0.60	36.15±0.55
Platelet ($\times 10^4$/mm^3)	100.3± 9.1	104.6±10.6	103.5± 6.8
Reticulocyte (0/00)	16.0±5.1	12.7±4.5	20.6±5.9
WBC ($\times 10^2$/mm^3)	80.0±30.2	75.0±23.0	64.4±15.8
Neutrophil-band (%)	0.0±0.0	0.0±0.0	0.0±0.0
Neutrophil-segment (%)	13.3±8.8	11.3±4.7	11.5±4.9
Lymphocyte (%)	82.6±7.8	85.6±4.7	85.1±5.2
Monocyte (%)	2.8±1.8	2.3±1.8	2.6±1.5
Eosinophil (%)	1.3±0.8	0.8±0.6	0.8±0.8
Basophil (%)	0.0±0.0	0.0±0.0	0.0±0.0
PT (sec)	12.48±0.60	12.45±0.29	11.85±0.63
APTT (sec)	16.70±1.32	16.32±1.53	16.37±0.96

Values represent mean±S. D.

Table 8-1. Blood chemistry values of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
GOT (IU/l)	120.5±29.0	141.5±40.8	115.4±22.9
GPT (IU/l)	18.7±4.1	21.9±3.8	23.1±5.5
ALP (IU/l)	618±96	656±126	597±151
Amylase (IU/l)	1195±172	1208±213	1455±110
Glucose (mg/dl)	128.1±16.2	111.5± 7.0	140.4±13.4
T. cholesterol (mg/dl)	48.5± 9.1	54.9± 8.6	54.1± 7.8
F. cholesterol (mg/dl)	7.6±1.8	8.4±2.4	9.6±2.1
Triglyceride (mg/dl)	43.6±15.0	30.3± 9.9	47.4±12.5
Phospholipid (mg/dl)	88.5±12.8	93.3±11.2	100.4±11.2
NEFA (mEq/l)	0.596±0.133	0.592±0.095	0.572±0.084
T. bilirubin (mg/dl)	0.05±0.01	0.05±0.01	0.04±0.01
Urea N (mg/dl)	16.7±2.5	16.4±1.7	16.2±1.2
Creatinine (mg/dl)	0.46±0.05	0.48±0.02	0.49±0.05
T. protein (mg/dl)	5.18±0.57	5.79±0.32	5.83±0.28
Albumin (mg/dl)	2.13±0.27	2.34±0.15	2.37±0.13
Sodium (mEq/l)	134.7±11.1	145.6± 2.4	141.8± 3.4
Potassium (mEq/l)	4.24±0.43	4.58±0.30	4.82±0.35
Chloride (mEq/l)	99.7±8.1	107.5±2.1	105.5±2.0
Calcium (mg/dl)	8.90±0.96	9.63±0.43	9.68±0.50
I. phosphate (mg/dl)	8.36±1.01	9.17±0.43	8.59±0.46
Albumin (%)	50.5±3.7	51.9±1.3	50.8±2.4
α 1-Globulin (%)	21.8±2.3	21.7±1.4	22.5±1.6
α 2-Globulin (%)	8.5±1.0	9.7±0.7	9.5±1.0
β -Globulin (%)	16.3±2.1	13.9±0.6	14.4±1.2
γ -Globulin (%)	2.9±0.9	2.9±0.5	2.9±0.6
A/G	1.03±0.14	1.08±0.05	1.04±0.10

Values represent mean±S. D.

Table 8-2. Blood chemistry values of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
GOT (IU/l)	80.3±23.9	87.6±21.8	84.4±27.2
GPT (IU/l)	17.0±2.3	16.0±1.6	17.6±2.8
ALP (IU/l)	405±113	378±52	312±29
Amylase (IU/l)	655±105	653±118	676±136
Glucose (mg/dl)	115.5±18.6	117.8±16.3	118.6±15.2
T. cholesterol (mg/dl)	63.9± 7.6	67.0± 7.1	72.8± 6.4
F. cholesterol (mg/dl)	12.8±4.2	14.4±3.9	14.3±1.9
Triglyceride (mg/dl)	14.3± 2.7	14.0± 5.3	19.5± 5.9
Phospholipid (mg/dl)	120.2±10.6	122.9± 8.1	134.4±11.3
NEFA (mEq/l)	0.644±0.054	0.599±0.082	0.610±0.139
T. bilirubin (mg/dl)	0.05±0.01	0.05±0.01	0.05±0.01
Urea N (mg/dl)	20.4±3.4	18.5±2.8	18.5±4.0
Creatinine (mg/dl)	0.50±0.05	0.48±0.04	0.47±0.02
T. protein (mg/dl)	6.22±0.37	6.17±0.22	5.95±0.18
Albumin (mg/dl)	2.68±0.18	2.67±0.22	2.55±0.16
Sodium (mEq/l)	147.1± 2.6	144.1± 2.0	141.1± 2.9
Potassium (mEq/l)	4.28±0.27	4.27±0.35	4.39±0.35
Chloride (mEq/l)	113.9±3.4	110.4±1.8	108.9±2.1
Calcium (mg/dl)	10.68±0.71	10.18±0.25	9.81±0.32
I. phosphate (mg/dl)	9.21±0.82	8.50±0.64	7.47±0.31
Albumin (%)	55.7±1.8	56.6±3.2	53.8± 2.9
α 1-Globulin (%)	16.7±1.8	17.0±1.6	18.8±1.6
α 2-Globulin (%)	8.1±0.9	8.8±1.0	9.5±1.2
β -Globulin (%)	15.7±0.9	14.3±1.8	15.1±1.6
γ -Globulin (%)	3.7±0.8	3.3±1.1	2.8±0.5
A/G	1.26±0.09	1.31±0.17	1.17±0.13

Values represent mean±S. D.

Table 8-3. Blood chemistry values of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
GOT (IU/l)	83.0±12.0	106.3±17.8	103.5±21.9
GPT (IU/l)	23.4±2.8	25.4±4.1	23.7±2.7
ALP (IU/l)	333±73	297±45	281±66
Amylase (IU/l)	1554±168	1469±128	1644±193
Glucose (mg/dl)	173.5±15.1	162.2±12.2	174.7±18.8
T. cholesterol (mg/dl)	65.7±11.1	70.6±11.5	69.8±14.8
F. cholesterol (mg/dl)	9.7±2.3	9.1±2.4	9.7±3.0
Triglyceride (mg/dl)	68.6±22.9	66.2±35.2	78.9±41.0
Phospholipid (mg/dl)	111.7±17.3	109.3±13.3	113.7±24.0
NEFA (mEq/l)	0.561±0.079	0.606±0.122	0.652±0.102
T. bilirubin (mg/dl)	0.06±0.01	0.05±0.01	0.05±0.01
Urea N (mg/dl)	15.5±1.3	15.8±2.9	15.7±1.8
Creatinine (mg/dl)	0.60±0.07	0.58±0.08	0.56±0.05
T. protein (mg/dl)	6.40±0.26	6.02±0.20	6.23±0.26
Albumin (mg/dl)	2.46±0.07	2.40±0.13	2.41±0.17
Sodium (mEq/l)	145.6± 0.8	142.9± 1.0	140.9± 1.1
Potassium (mEq/l)	4.83±0.69	4.18±0.20	4.30±0.24
Chloride (mEq/l)	103.9±2.0	102.8±2.5	102.2±1.8
Calcium (mg/dl)	10.76±0.34	9.66±0.39	9.65±0.25
I. phosphate (mg/dl)	7.32±0.92	6.32±0.52	6.36±0.35
Albumin (%)	47.6±2.2	46.2±2.4	47.0±2.2 [a)]
α 1-Globulin (%)	22.9±1.4	22.9±3.0	23.6±2.7 [a)]
α 2-Globulin (%)	9.7±0.6	9.6±0.8	8.4±0.4 [a)]
β -Globulin (%)	15.8±1.2	16.8±1.1	16.9±0.7 [a)]
γ -Globulin (%)	4.0±0.7	4.5±0.8	4.0±0.7 [a)]
A/G	0.91±0.08	0.86±0.08	0.89±0.08 [a)]

Values represent mean±S. D.
a): n=9

Table 8-4. Blood chemistry values of female Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
GOT (IU/l)	95.3±27.7	95.0±37.0	88.3±34.7
GPT (IU/l)	24.6±8.2	33.4±22.4	27.4±12.4
ALP (IU/l)	136±28	109±21	142±34
Amylase (IU/l)	874±125	888±110	847±129
Glucose (mg/dl)	133.4±17.4	152.3±15.4	165.0±22.5
T. cholesterol (mg/dl)	71.9±12.2	81.6±10.5	81.2±13.8
F. cholesterol (mg/dl)	12.2±3.2	12.3±3.0	12.5±3.0
Triglyceride (mg/dl)	33.9±20.3	26.1± 9.2	21.8±13.6
Phospholipid (mg/dl)	138.8±22.1	146.0±19.7	144.1±22.5
NEFA (mEq/l)	0.793±0.177	0.706±0.128	0.827±0.078
T. bilirubin (mg/dl)	0.09±0.02	0.06±0.02	0.07±0.02
Urea N (mg/dl)	18.8±4.1	17.6±2.5	17.1±2.8
Creatinine (mg/dl)	0.65±0.06	0.58±0.06	0.59±0.06
T. protein (mg/dl)	6.75±0.38	6.80±0.31	6.56±0.40
Albumin (mg/dl)	2.91±0.26	3.04±0.22	2.87±0.22
Sodium (mEq/l)	144.1± 1.4	141.3± 0.8	140.3± 1.1
Potassium (mEq/l)	4.09±0.31	3.85±0.34	3.61±0.32
Chloride (mEq/l)	105.7±1.6	104.4±1.3	104.4±1.1
Calcium (mg/dl)	10.70±0.43	10.97±0.16	10.08±0.36
I. phosphate (mg/dl)	6.18±0.96	5.52±0.68	5.57±0.62
Albumin (%)	55.2±1.9	55.9±1.9	54.3±2.0
α 1-Globulin (%)	15.8±1.5	17.0±1.3	17.9±1.4
α 2-Globulin (%)	8.8±1.1	8.5±0.7	8.5±1.0
β -Globulin (%)	15.1±1.4	14.2±0.6	14.7±1.6
γ -Globulin (%)	5.2±1.0	4.4±0.9	4.6±0.6
A/G	1.23±0.09	1.27±0.10	1.19±0.10

Values represent mean±S. D.

Table 9-1. Absolute organ weights of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Body weight (g)	313.6±24.2	306.8±25.1	326.4±21.2
Brain (g)	1.952±0.067	1.925±0.080	1.997±0.063
Heart (g)	1.130±0.085	1.137±0.111	1.201±0.172
Lung (g)	1.164±0.081	1.168±0.144	1.197±0.104
Liver (g)	10.22±1.16	9.26±0.65	10.80±0.82
Kidney (L, g)	1.225±0.147	1.195±0.082	1.275±0.112
Kidney (R, g)	1.233±0.137	1.205±0.102	1.282±0.118
Cecum (g)	2.957±0.507	3.498±0.880	2.892±0.921
Testis (L, g)	1.506±0.104	1.420±0.078	1.527±0.088
Testis (R, g)	1.511±0.093	1.437±0.100	1.529±0.084
Pituitary gland (mg)	11.1±1.8	10.2±0.8	10.3±1.3
Salivary gland (L, mg)	276±31	275±33	285±30
Salivary gland (R, mg)	271±32	274±36	281±36
Thymus (mg)	632± 77	551±112	608±168
Spleen (mg)	658±151	606± 82	636±147
Adrenal gland (L, mg)	27.2±4.1	24.1±2.9	29.3±6.1
Adrenal gland (R, mg)	25.6±4.4	23.9±3.4	27.2±5.6
Epididymis (L, mg)	325±14	347±45	353±25
Epididymis (R, mg)	335±14	341±35	354±23

Values represent mean±S. D.

Table 9-2. Absolute organ weights of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Body weight (g)	178.2±8.4	197.7±14.5	196.6±17.9
Brain (g)	1.789±0.075	1.822±0.058	1.851±0.069
Heart (g)	0.728±0.051	0.784±0.098	0.830±0.080
Lung (g)	0.810±0.047	0.932±0.071	0.908±0.070
Liver (g)	5.36±0.37	6.02±0.56	6.33±0.84
Kidney (L, g)	0.731±0.042	0.813±0.065	0.857±0.062
Kidney (R, g)	0.751±0.056	0.827±0.065	0.869±0.061
Cecum (g)	2.519±0.473	2.642±0.460	2.188±0.546
Pituitary gland (mg)	10.0±1.1	12.0±2.1	12.5±1.0
Salivary gland (L, mg)	189±12	198±20	211±22
Salivary gland (R, mg)	190±11	199±21	209±18
Thymus (mg)	419± 71	444± 36	472± 94
Spleen (mg)	369± 52	396± 72	429± 66
Adrenal gland (L, mg)	29.3±2.0	29.7±4.3	30.5±4.2
Adrenal gland (R, mg)	27.8±3.9	29.4±5.0	29.2±4.3
Ovary (L, mg)	39.4±4.3	38.1±5.1	39.8±4.9
Ovary (R, mg)	36.7±4.1	41.7±7.4	43.9±7.6
Uterus (mg)	390±137	390±102	385± 93

Values represent mean±S. D.

Table 9-3. Absolute organ weights of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Body weight (g)	495.1±46.2	484.7±44.2	514.4±39.8
Brain (g)	2.079±0.060	2.068±0.080	2.091±0.070
Heart (g)	1.517±0.163	1.384±0.122	1.432±0.118
Lung (g)	1.461±0.088	1.302±0.076	1.349±0.096
Liver (g)	13.79±1.91	13.26±1.39	14.20±1.51
Kidney (L, g)	1.570±0.144	1.480±0.144	1.541±0.078
Kidney (R, g)	1.576±0.126	1.470±0.150	1.571±0.095
Cecum (g)	3.999±1.122	3.712±0.922	3.877±0.931
Testis (L, g)	1.711±0.071	1.678±0.114	1.645±0.172
Testis (R, g)	1.707±0.088	1.669±0.094	1.652±0.158
Pituitary gland (mg)	13.0±1.3	12.1±2.7	11.6±1.2
Salivary gland (L, mg)	339±33	344±28	355±37
Salivary gland (R, mg)	342±27	337±32	355±36
Thymus (mg)	317± 54	338± 99	355± 89
Spleen (mg)	730±120	663± 92	706±107
Adrenal gland (L, mg)	30.4±6.7 a)	25.5±4.9	27.5±2.3
Adrenal gland (R, mg)	27.3±4.4	24.0±4.9	26.5±2.8
Epididymis (L, mg)	644±55	611±49	630±56
Epididymis (R, mg)	641±54	614±41	637±54

Values represent mean±S. D.
a): n=9

Table 9-4. Absolute organ weights of female Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Body weight (g)	264.4±24.4	292.2±25.6	277.2±20.2
Brain (g)	1.912±0.089	1.950±0.092	1.948±0.073
Heart (g)	0.897±0.094	0.952±0.075	0.913±0.060
Lung (g)	1.040±0.077	1.067±0.100	1.020±0.047
Liver (g)	6.63±0.97	7.48±0.89	7.09±0.34
Kidney (L, g)	0.850±0.111	0.920±0.103	0.855±0.065
Kidney (R, g)	0.858±0.114	0.933±0.113	0.910±0.077
Cecum (g)	2.759±0.278	3.192±0.909	2.795±0.688
Pituitary gland (mg)	16.7±3.0	14.5±3.9	14.0±1.8
Salivary gland (L, mg)	233±27	226±18	219±15
Salivary gland (R, mg)	234±27	224±17	216±17
Thymus (mg)	281± 63	303± 72	263± 48
Spleen (mg)	480± 87	488± 69	454± 20
Adrenal gland (L, mg)	35.2±6.3	33.0±5.0	32.1±3.7
Adrenal gland (R, mg)	32.2±5.8	30.5±4.6	31.3±3.4
Ovary (L, mg)	43.3±7.4	44.7±6.5	46.1±7.0
Ovary (R, mg)	42.5±5.4	43.7±7.3	42.3±6.3
Uterus (mg)	613±158	590±297	594±284

Values represent mean±S. D.

Table 10-1. Relative organ weights of male Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Body weight (g)	313.6±24.2	306.8±25.1	326.4±21.2
Brain (g%)	0.626±0.055	0.630±0.039	0.614±0.039
Heart (g%)	0.361±0.023	0.371±0.025	0.367±0.037
Lung (g%)	0.372±0.014	0.382±0.051	0.367±0.025
Liver (g%)	3.25±0.18	3.02±0.12	3.32±0.26
Kidney (L, g%)	0.390±0.032	0.390±0.013	0.391±0.029
Kidney (R, g%)	0.393±0.029	0.394±0.023	0.393±0.023
Cecum (g%)	0.942±0.130	1.146±0.313	0.881±0.251
Testis (L, g%)	0.484±0.064	0.465±0.033	0.469±0.033
Testis (R, g%)	0.485±0.059	0.470±0.033	0.470±0.036
Pituitary gland (mg%)	3.5±0.5	3.3±0.2	3.2±0.3
Salivary gland (L, mg%)	88± 9	90±10	87± 8
Salivary gland (R, mg%)	87± 9	90±10	86±10
Thymus (mg%)	202±27	179±26	185±45
Spleen (mg%)	211±53	198±22	195±44
Adrenal gland (L, mg%)	8.7±1.3	7.8±0.5	9.0±2.0
Adrenal gland (R, mg%)	8.2±1.4	7.8±0.7	8.4±1.8
Epididymis (L, mg%)	104±10	113±11	108± 9
Epididymis (R, mg%)	108±13	111± 9	109±10

Values represent mean±S. D.

Table 10-2. Relative organ weights of female Crj:CD(SD)IGS rats at 9 weeks of age

	Lot 1-1M	Lot 2-1M	Lot 3-1M
No. of animals	10	10	10
Body weight (g)	178.2± 8.4	197.7±14.5	196.6±17.9
Brain (g%)	1.006±0.057	0.926±0.068	0.947±0.074
Heart (g%)	0.410±0.039	0.396±0.036	0.424±0.041
Lung (g%)	0.455±0.022	0.472±0.026	0.463±0.031
Liver (g%)	3.01±0.19	3.05±0.20	3.21±0.21
Kidney (L, g%)	0.412±0.036	0.412±0.032	0.438±0.034
Kidney (R, g%)	0.423±0.042	0.419±0.032	0.444±0.036
Cecum (g%)	1.412±0.241	1.333±0.197	1.110±0.238
Pituitary gland (mg%)	5.6±0.6	6.1±1.0	6.4±0.8
Salivary gland (L, mg%)	106± 8	100± 9	107± 9
Salivary gland (R, mg%)	107± 7	101± 8	106± 7
Thymus (mg%)	235±38	226±19	239±37
Spleen (mg%)	208±33	200±33	218±27
Adrenal gland (L, mg%)	16.5±1.2	15.1±2.2	15.5±1.6
Adrenal gland (R, mg%)	15.6±2.0	14.9±2.5	14.8±1.7
Ovary (L, mg%)	22.1±2.1	19.3±2.4	20.3±1.8
Ovary (R, mg%)	20.6±2.6	21.2±3.9	22.3±3.2
Uterus (mg%)	218±70	199±55	197±49

Values represent mean±S. D.

Table 10-3. Relative organ weights of male Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Body weight (g)	495.1±46.2	484.7±44.2	514.4±39.8
Brain (g%)	0.423±0.034	0.429±0.030	0.409±0.035
Heart (g%)	0.306±0.015	0.286±0.021	0.279±0.019
Lung (g%)	0.297±0.023	0.270±0.025	0.263±0.018
Liver (g%)	2.79±0.35	2.74±0.16	2.76±0.16
Kidney (L, g%)	0.318±0.022	0.306±0.020	0.301±0.026
Kidney (R, g%)	0.319±0.018	0.304±0.026	0.307±0.025
Cecum (g%)	0.805±0.206	0.769±0.198	0.754±0.170
Testis (L, g%)	0.348±0.035	0.349±0.037	0.322±0.049
Testis (R, g%)	0.348±0.040	0.347±0.037	0.324±0.048
Pituitary gland (mg%)	2.6±0.3	2.5±0.5	2.3±0.3
Salivary gland (L, mg%)	69± 7	71± 6	69± 8
Salivary gland (R, mg%)	70± 7	70± 6	69± 8
Thymus (mg%)	64±11	69±17	69±16
Spleen (mg%)	148±26	137±16	138±25
Adrenal gland (L, mg%)	6.2±1.1 [a)]	5.3±1.1	5.4±0.6
Adrenal gland (R, mg%)	5.6±0.8	5.0±1.1	5.2±0.6
Epididymis (L, mg%)	131±16	127±16	123±14
Epididymis (R, mg%)	131±17	128±11	124±14

Values represent mean±S. D.
a): n=9

Table 10-4. Relative organ weights of female Crj:CD(SD)IGS rats at 18 weeks of age

	Lot 1-3M	Lot 2-3M	Lot 3-3M
No. of animals	10	10	10
Body weight (g)	264.4±24.4	292.2±25.6	277.2±20.2
Brain (g%)	0.727±0.049	0.671±0.059	0.706±0.051
Heart (g%)	0.340±0.034	0.327±0.020	0.331±0.030
Lung (g%)	0.394±0.023	0.365±0.017	0.369±0.024
Liver (g%)	2.50±0.19	2.56±0.14	2.56±0.15
Kidney (L, g%)	0.322±0.038	0.315±0.018	0.320±0.025
Kidney (R, g%)	0.325±0.032	0.319±0.020	0.329±0.028
Cecum (g%)	1.055±0.165	1.093±0.316	1.016±0.271
Pituitary gland (mg%)	6.3±0.8	4.9±1.2	5.1±0.8
Salivary gland (L, mg%)	89±10	78± 3	80± 8
Salivary gland (R, mg%)	89±10	77± 4	78± 8
Thymus (mg%)	107±24	103±20	94±13
Spleen (mg%)	181±24	167±16	164±11
Adrenal gland (L, mg%)	13.4±2.4	11.3±1.2	11.6±1.4
Adrenal gland (R, mg%)	12.2±2.0	10.4±1.2	11.3±1.2
Ovary (L, mg%)	16.3±2.3	15.4±2.7	16.8±3.2
Ovary (R, mg%)	16.0±1.1	14.9±1.9	15.4±3.0
Uterus (mg%)	233±61	201±97	213±98

Values represent mean±S. D.

Comparison of General Toxicological Parameters between Crj:CD(SD)IGS Rats Fed Irradiated Diet and Crj:CD(SD)IGS Rats Fed Autoclaved Diet at 10 and 19 weeks of Age

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ABSTRACT. This study was performed to compare Crj:CD(SD)IGS rats fed irradiated diet with those fed autoclaved diet. It was concluded that there were no obvious differences in the general toxicological parameters investigated between the two groups of rats. — Key words: General toxicity study, Toxicological parameter, Crj:CD(SD)IGS rat, Diet

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INTRODUCTION

Crj:CD(SD)IGS strain rat was produced by International Genetic Standard system and supplied by Charles River Japan, Inc. To confirm variability of these parameters among Crj:CD(SD)IGS rats fed diet sterilized by different methods, clinical signs, body weights, food and water consumption, urinalysis, hematology, blood chemistry, ophthalmology, necropsy, organ weights and histopathological examination were examined in the present study.

MATERIALS AND METHODS

Animals and housing

Forty five male and 45 female Crj:CD(SD)IGS rats aged 4 weeks were obtained from Charles River Japan, Inc.(Atsugi, Kanagawa, Japan) on June 25, 1997. Animals were used after an acclimatization and quarantine period for 6 days. The animals were randomly allocated to 2 groups, each comprising 20 males and 20 females, according to body weights. The group receiving autoclaved diet was referred to as the control group, whilst the group receiving irradiated diet was referred to as the γ-control group. The 2 groups were used a half of animals for 5- and 14-week studies, respectively. At the termination of study, animals were 10 and 19 weeks of age. They were housed individually in wire-mesh cages and allowed to free access to pellet diet (CRF-1, Oriental Yeast Co., Ltd.) sterilized by either high pressure steam or γ-ray radiation and chlorinated tap water. Water was supplied by the water bottles. The animal room was controlled at 23 ± 1 ℃ with relative humidity of 55 ± 10%, ventilation of 10 times/hr or more, and a 12 hr light/dark cycle (light on 06:00-18:00).

Examinations and methods

1) Clinical signs

All animals were observed for mortality, morbidity and clinical signs once a day during the experimental period.

2) Body weights

Each animal was weighed using an electronic balance once a week and before necropsy. Prior to necropsy, the animals were deprived of food overnight.

3) Food and water consumption

Food and water consumption of animals were measured using an electronic balance twice a week .

4) Urinalysis

At 10 and 19 weeks of age, 16hr-urine samples in a half of animals were collected using metabolic cages. Urine volume was measured individually. Protein, glucose, occult blood, ketones, bilirubin and urobilinogen (test paper method) were determined using an automated urine analyzer MA-4210 (Kyoto Daiichi Kagaku Co., Ltd.). After centrifugal separation, osmolarity (Cryoscopic method) was determined using an automated osmolarity analyzer Osmotic Pressure AUTO & STAT OM-6030 (Kyoto Daiichi Kagaku Co., Ltd.). Sodium (ISE method), potassium (ISE method) and chloride (coulometric titration method) were determined using an automated electrolyte analyzer PVA α II (A & T corporation). Calcium (OCPC method), inorganic phosphate (Fiske-Subbarow method), creatinine (Jaffé method) and N-acetyl- β-D-glucosaminidase (NAG: CPR-NAG method) were determined using an automatic analyzer Model 7070 (Hitachi, Ltd.). Urinary pH was determined using a pH meter HM-70V (TOA Electronics Ltd.).

5) Hematology

At 10 and 19 weeks of age, before necropsy, blood samples in a half of animals were collected from the abdominal aorta under ethyl ether anesthesia following depriving of food overnight (16 hours or more). The following hematological tests were performed after the treatment of EDTA anticoagulant using an automated hematology analyzer K-1000 (Sysmex Co., Ltd.): erythrocytes (RBC: electric resistance detection method), hemoglobin (HGB: SLS-Hb method), hematocrit (PCV: cumulative pulse height detection method), platelets (electric resistance detection method), and leukocytes (WBC: electric resistance detection method). The following hematological tests were performed after blood smear preparation by the light microscopy: reticulocyte counts (Brecher method) and differential leukocyte counts (Giemsa stain). The following hematological tests were performed on the plasma after centrifugal separation according to the treatment of 3.2% sodium citrate anticoagulant (Blood: Sodium citrate = 9 : 1, v/v) using a semi-automated blood coagulation analyzer KC-10A (Heinrich Amelung GmbH): prothrombin time (PT: quick step method) and activated partial thromboplastin time (APTT: ellagic acid method).

6) Blood chemistry

At 10 and 19 weeks of age, before necropsy, blood samples in a half of animals were collected from the abdominal aorta under ethyl ether anesthesia following depriving of food over-

night (16 hours or more). The following serum biochemistry tests were performed using an automatic analyzer Model 7070 (Hitachi, Ltd.) after centrifugal separation: glutamic oxaloacetic transaminase (GOT: UV rate method, IFCC), glutamic pyruvic transaminase (GPT: UV rate method, IFCC), alkaline phosphatase (ALP: p-NPP rate method, JSCC), amylase (BG5P method), glucose (GK・G-6-PDH method), total cholesterol (ChOD・DAOS method), free cholesterol (ChOD・DAOS method), triglyceride (GPO・DAOS method), phospholipid (COD・DAOS method), nonesterified fatty acid (NEFA: ASC・ASOD method), total bilirubin (Azobilirubin method), urea nitrogen (Urea N: Urease・GlDH method), creatinine (Jaffé method), total protein (Biuret method), albumin (BCG method), sodium (ISE method), potassium (ISE method), chloride (ISE method), calcium (OCPC method) and inorganic phosphate (Fiske-Subbarow method). Protein fractions (albumin, alpha1 globulin, alpha2 globulin, beta globulin, gamma globulin and A/G) determined using an electrophoresis apparatus (Helena Laboratories) and cellulose acetate membranes.

7) Ophthalmology

Ophthalmoscopic examinations were performed for a half of animals once before the commencement of the experiment and at 10 and 19 weeks of age. The anterior portion of eyes was observed macroscopically using a Halogen ophthalmoscope BX (Neitz instruments Co., Ltd.).

8) Necropsy, organ weights and histopathological examination

At 10 weeks of age in 5-week studies and at 19 weeks of age in 14-week studies, a half of animals were killed by exanguination from the abdominal aorta under ethyl ether anesthesia following depriving of food overnight (16 hours or more) and complete macroscopic examination of all organs was performed. Then the brain, pituitary gland, salivary gland, thymus, heart, lung, liver, kidney, spleen, adrenal gland, cecum, testis, epididymidis, ovary and uterus were dissected and weighed using an electronic balance.

For histopathlogical examination, the brain, pituitary gland, submandibular gland, sublingual salivary gland, thyroid gland, parathyroid gland, thymus, heart, trachea, lung, liver, kidney, spleen, adrenal gland, pancreas, esophagus, forestomach, glandular stomach, duodenum, jejunum/ileum, cecum, colon/rectum, mesenteric lymph node, urinary bladder, skin, mammary gland, skeletal muscle, lumber vertebra/bone marrow, spinal cord, testis, epididymidis, prostate, seminal vesicle, ovary, uterus and vagina were fixed with 10% neutral buffered formalin. The eye was fixed in Davidson's fixative. These organs and tissues were embedded in paraffin, sectioned and stained with haematoxylin and eosin. Histopathlogical examination was performed on the organs and tissues of all groups.

Statistical analysis

Statistical significance of differences between the control and γ-control groups was examined at 5% and 1% probability levels. The Student's t-test was used for those data with homogeneous variance among the groups as determined by F-test. The Welch test was used for those data without homogeneous variance among the groups as determined by F-test. The Wilcoxon rank sum test was used for urine semi-quantitative data.

RESULTS AND DISCCUSION

1) Clinical signs

There were no deaths and no abnormal clinical signs throughout the observation period either in the control group or γ-control group.

2) Body weights (Table 1, 2)

Body weights in males of the γ-control group were significantly higher than those of the control group from day 15 (7 weeks of age) to day 37 (10 weeks of age), body weights in females of the γ-control groups were significantly higher than those of the control group at day 78 (16 weeks of age) and from day 92 (18 weeks of age) to day 99 (19 weeks of age), respectively; however, the differences were not considered to be of toxicological significance.

3) Food and water consumption (Table 3, 4)

Food consumption in males of the γ-control group were significantly lower than those of the control group at day 8 (6 weeks of age) and were significantly higher than those of the control group from day 31 (9 weeks of age) to day 37 (10 weeks of age), food consumption in females of the γ-control groups were significantly higher than those of the control group at day 31 (9 weeks of age) and day 37 (10 weeks of age), respectively; however, the differences were not considered to be of toxicological significance. There were no remarkable differences in water consumption among the groups.

4) Urinalysis (Table 5, 6)

The concentration and excretion of calcium in males of the γ-control group at 10 weeks of age in the 5-week studies and females of the γ-control group at 19 weeks of age in the 14-week studies were significantly higher than those of the control group. The concentration and excretion of inorganic phosphate in males and females of the γ-control group at 10 weeks of age and males of the γ-control group at 19 weeks of age were significantly lower than those of the control group. High excretion of calcium and low excretion of inorganic phosphate were considered to be related to the dietary availability of these analytes in the γ-control diet, and these differences were considered to be of toxicological significance. A decrease in creatinine concentration was observed in females of the γ-control group at 10 weeks of age. A decrease in pH was observed in males and females of the γ-control group at 19 weeks. An increase in excretion of sodium was observed in males and females of the γ-control group at 19 weeks of age. In semi-quantitative data, negative change in urobilinogen was observed in males of the γ-control group at 10 and 19 weeks of age. Positive change in protein was observed in females of the γ-control group at 19 weeks of age. Except calcium and inorganic phosphate, inter-group differences from the control achieved statistical significance were generally minimal and considered to be of no toxicological significance.

5) Hematology (Table 7)

There were no remarkable differences in hematological parameters among the groups at 10 weeks of age in the 5-week studies. Prolongation of APTT was observed in males of the γ-control group at 19 weeks in the 14-week studies; however, despite attaining statistical significance the difference was generally minimal and considered to be of no toxicological significance.

6) Blood chemistry (Table 8)

Increases in ALP and inorganic phosphate were observed in males of the γ-control group at 10 weeks in the 5-week studies; however, the differences from the control achieved statistical significance were generally minimal and considered to be of no toxicological significance. There were no remarkable differences in blood chemical parameters among the groups at 19 weeks of age in the 14-week studies.

7) Ophthalmology

There were no abnormalities in the anterior portion of the eyes.

8) Necropsy

Atrophy of the testes was observed in one male of the control group at 10 weeks of age in 5-week studies.

9) Organ weights (Table 9, 10)

In males of the γ-control group, higher values for the absolute organ weights of the heart, lung, liver and adrenal gland (left) and lower values for the relative organ weight of the brain were observed at 10 weeks of age in the 5-week studies, lower value for the relative organ weight of the pituitary gland was observed at 19 week of age in the 14-week studies. In females of the γ-control group, higher value for the absolute organ weight of the adrenal gland and higher values for the relative organ weights of the liver, kidney (right) and adrenal gland were observed at 10 weeks of age, lower values for the relative organ weights of the heart, lung and pituitary gland were observed at 19 weeks of age. Inter-group differences from the control achieved statistical significance were generally minimal and considered to be of no toxicological significance.

10) Histopathological findings (Table 11)

Atrophy of the testes observed in one male with the atrophied testis of the control group at 10 weeks of age was considered to be incidental change. There were no remarkable differences in histopathological findings among the groups at 10 weeks of age in the 5-week studies and at 19 weeks of age in the 14-week studies.

In conclusion, there were no obvious differences in the general toxicological parameters between Crj:CD(SD)IGS rats fed irradiated diet and those fed autoclaved diet for 14 weeks.

Table 1. Body weight(g) of Crj:CD(SD)IGS rats fed diet sterilized by different methods

Sex	Male		Female	
Group	Control	γ-Control	Control	γ-Control
Day 1	140.3± 4.4(20)	140.4± 4.0(20)	118.1± 4.9(20)	117.8± 4.1(20)
8	198.5± 7.0(20)	202.5± 7.3(20)	153.7± 8.9(20)	154.5± 7.4(20)
15	252.9±15.8(20)	265.2±12.1(20)**	181.3±13.2(20)	182.5±12.4(20)
22	305.9±14.0(20)	318.9±17.4(20)*	204.2±17.0(20)	208.0±13.4(20)
29	342.6±19.9(20)	360.2±22.4(20)*	222.3±19.2(20)	229.3±15.9(20)
37	375.5±26.4(20)	398.8±29.1(20)*	236.9±21.4(20)	247.4±17.8(20)
43	402.0±34.7(10)	414.5±26.7(10)	241.8±23.0(10)	259.8±17.1(10)
50	422.7±35.8(10)	437.2±29.1(10)	252.9±26.4(10)	270.4±17.8(10)
57	443.8±41.2(10)	455.3±29.6(10)	262.0±28.2(10)	282.0±19.8(10)
64	460.3±43.5(10)	471.5±32.7(10)	267.5±28.4(10)	290.7±21.2(10)
71	476.1±45.9(10)	488.2±34.6(10)	274.2±30.3(10)	296.1±22.8(10)
78	492.4±49.1(10)	503.0±35.4(10)	276.9±31.5(10)	303.6±22.5(10)*
85	506.6±52.0(10)	516.3±38.3(10)	283.3±32.6(10)	309.3±23.0(10)
92	517.1±53.2(10)	526.6±39.6(10)	285.7±32.3(10)	312.6±24.3(10)*
99	525.8±54.4(10)	530.3±37.2(10)	286.2±33.0(10)	312.8±21.3(10)*

Values represent mean±S.D.
Number in parentheses indicates the number of animals examined
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 2. Body weight gain(g/day) of Crj:CD(SD)IGS rats fed diet sterilized by different methods

Sex	Male		Female	
Group	Control	γ -Control	Control	γ -Control
Day 8	8.3±0.6(20)	8.9±0.7(20)*	5.1±0.8(20)	5.2±0.7(20)
15	7.8±1.9(20)	8.9±0.9(20)*	3.9±0.8(20)	4.0±1.0(20)
22	7.6±1.4(20)	7.7±0.9(20)	3.3±0.8(20)	3.6±0.7(20)
29	5.2±1.5(20)	5.9±1.0(20)	2.6±0.5(20)	3.0±0.7(20)*
37	4.1±1.0(20)	4.8±1.0(20)*	1.8±0.5(20)	2.3±0.5(20)**
43	3.3±0.9(10)	3.5±0.8(10)	1.6±0.7(10)	1.9±0.5(10)
50	2.9±0.8(10)	3.2±0.8(10)	1.6±0.7(10)	1.5±0.5(10)
57	3.0±0.9(10)	2.6±0.6(10)	1.3±0.8(10)	1.7±0.5(10)
64	2.4±1.0(10)	2.3±0.7(10)	0.8±0.5(10)	1.2±0.5(10)*
71	2.3±0.7(10)	2.4±0.6(10)	0.9±0.5(10)	0.8±0.4(10)
78	2.3±1.2(10)	2.1±0.5(10)	0.4±0.6(10)	1.1±0.8(10)*
85	2.0±0.7(10)	1.9±0.7(10)	0.9±0.5(10)	0.8±0.9(10)
92	1.5±0.8(10)	1.5±0.4(10)	0.3±0.8(10)	0.5±0.6(10)
99	1.2±0.8(10)	0.5±0.8(10)	0.1±0.7(10)	0.0±0.7(10)

Values represent mean±S.D.
Number in parentheses indicates the number of animals examined
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 3. Food consumption(g/day) of Crj:CD(SD)IGS rats fed diet sterilized by different methods

Sex	Male		Female	
Group	Control	γ -Control	Control	γ -Control
Day 8	22.7±1.1(20)	22.0±1.2(20)*	17.8±1.1(20)	17.4±1.2(20)
13	24.7±1.2(20)	25.1±1.5(20)	18.3±1.2(20)	18.1±1.4(20)
16	24.6±4.3(20)	26.3±2.0(20)	18.5±1.5(20)	18.5±1.7(20)
21	26.7±1.8(20)	27.9±2.0(20)	19.2±1.5(20)	19.7±1.2(20)
23	26.9±2.2(20)	27.4±2.1(20)	18.7±1.9(20)	19.5±1.6(20)
27	26.8±2.3(20)	27.9±2.3(20)	19.2±1.8(20)	20.3±1.7(20)
31	26.1±2.2(20)	28.0±2.2(20)*	19.2±1.8(20)	20.3±1.7(20)*
34	26.5±2.5(20)	28.3±2.4(20)*	19.5±1.7(20)	20.8±2.3(20)
37	26.0±2.5(20)	27.9±2.5(20)*	18.9±1.9(20)	20.2±2.0(20)*
41	26.3±2.2(10)	26.7±2.0(10)	19.4±1.7(10)	20.2±1.9(10)
45	26.4±1.8(10)	27.4±2.2(10)	19.1±1.8(10)	19.9±1.9(10)
48	26.2±2.0(10)	27.0±1.8(10)	19.5±2.3(10)	20.4±1.8(10)
50	26.6±1.9(10)	27.2±1.6(10)	19.5±2.7(10)	19.9±2.0(10)
56	26.8±2.2(10)	26.4±1.8(10)	19.1±1.8(10)	19.9±2.1(10)
59	26.3±2.1(10)	26.4±1.5(10)	19.0±2.2(10)	19.5±1.7(10)
62	26.6±2.1(10)	26.4±1.9(10)	19.1±1.9(10)	20.5±1.9(10)
66	26.9±2.2(10)	26.9±2.2(10)	19.0±1.9(10)	19.3±2.0(10)
69	26.8±2.2(10)	26.6±2.0(10)	19.1±2.4(10)	19.7±2.3(10)
73	27.0±2.4(10)	27.0±1.8(10)	19.3±1.7(10)	19.4±1.8(10)
77	27.5±2.9(10)	27.2±2.3(10)	18.8±1.9(10)	19.7±1.7(10)
80	26.3±2.7(10)	26.7±1.8(10)	18.2±1.9(10)	18.6±1.4(10)
83	27.0±2.6(10)	26.2±1.8(10)	18.4±2.3(10)	19.3±1.8(10)
87	26.9±2.5(10)	26.6±2.1(10)	18.3±1.5(10)	18.4±2.1(10)
90	26.7±2.5(10)	26.4±2.1(10)	19.1±2.1(10)	18.7±2.5(10)
94	27.1±2.4(10)	26.3±2.6(10)	18.9±1.8(10)	18.7±1.7(10)
97	26.4±2.5(10)	25.1±2.4(10)	18.1±2.1(10)	18.2±1.6(10)
99	25.8±3.1(10)	25.2±1.9(10)	16.7±2.2(10)	17.2±1.3(10)

Values represent mean±S.D.
Number in parentheses indicates the number of animals examined
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 4. Water consumption(g/day) of Crj:CD(SD)IGS rats fed diet sterilized by different methods

Sex	Male		Female	
Group	Control	γ -Control	Control	γ -Control
Day 8	28.5± 2.7(20)	27.8± 2.1(20)	24.9± 3.8(20)	24.2± 2.1(20)
13	30.3± 3.0(20)	30.2± 2.4(20)	25.8± 4.9(20)	24.4± 2.5(20)
16	31.7± 4.6(20)	32.7± 2.9(20)	26.5± 4.4(20)	26.6± 2.7(20)
21	33.3± 4.0(20)	32.9± 2.3(20)	27.1± 4.2(20)	27.5± 5.7(20)
23	34.3± 3.6(20)	35.3± 3.3(20)	27.8± 4.8(20)	28.4± 4.8(20)
27	33.2± 4.3(20)	34.3± 3.7(20)	28.7± 6.1(20)	30.9± 9.4(20)
31	34.1± 4.3(20)	35.0± 4.0(20)	29.6± 5.6(20)	28.8± 3.3(20)
34	33.8± 4.9(20)	34.2± 3.3(20)	28.8± 5.2(20)	29.5± 6.4(20)
37	34.7± 5.4(20)	35.1± 3.8(20)	29.0± 4.9(20)	30.6± 5.2(20)
41	33.2± 4.6(10)	35.8± 7.2(10)	30.1± 5.8(10)	26.4± 5.8(10)
45	35.3± 5.3(10)	35.9± 5.7(10)	30.3± 7.6(10)	26.5± 4.6(10)
48	33.5± 4.4(10)	33.7± 4.6(10)	32.5± 8.7(10)	26.2± 5.0(10)
50	37.2± 8.9(10)	35.7± 5.8(10)	29.2± 6.3(10)	28.8±10.1(10)
56	39.3± 9.5(10)	49.2±17.7(10)	43.8±17.5(10)	38.6±20.4(10)
59	41.8± 8.0(10)	39.8± 6.9(10)	34.9±11.6(10)	34.2±10.0(10)
62	36.1± 5.3(10)	33.2± 5.8(10)	32.1± 6.5(10)	35.1± 7.6(10)
66	35.4± 3.6(10)	36.0± 9.4(10)	33.6±11.7(10)	32.2± 9.5(10)
69	34.8± 4.2(10)	35.3± 7.1(10)	33.9±11.4(10)	32.3±14.5(10)
73	35.0± 3.7(10)	33.7± 6.7(10)	35.5±12.5(10)	34.1±16.1(10)
77	35.6± 4.4(10)	31.8± 3.8(10)	32.2± 7.7(10)	32.4±13.0(10)
80	35.3± 3.8(10)	34.2± 8.2(10)	33.2± 8.6(10)	27.7± 4.2(10)
83	37.2± 7.5(10)	34.2± 7.6(10)	33.9± 9.6(10)	32.5±10.9(10)
87	36.4± 4.4(10)	33.0± 5.9(10)	32.0± 6.7(10)	35.6±20.6(10)
90	35.3± 4.7(10)	33.5± 5.5(10)	33.8± 9.8(10)	27.6± 4.5(10)
94	37.2± 8.8(10)	32.1± 4.1(10)	35.5±15.4(10)	28.8± 6.1(10)
97	36.7±11.0(10)	44.9±26.6(10)	38.9±22.0(10)	46.0±26.6(10)
99	36.3± 4.4(10)	33.5± 7.0(10)	32.9± 9.1(10)	29.6± 8.6(10)

Values represent mean±S.D.
Number in parentheses indicates the number of animals examined
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 5-1. Urinalysis values of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Urine volume (ml)	9.44±2.29	10.44±2.56	12.56±7.01	8.67±2.78
Osmolarity (mOsm/kg)	1943±264	1939±315	1989±419	2118±362
Sodium (mEq/l)	180.4±30.0	178.9±32.2	165.5±31.5	174.5±40.6
Potassium (mEq/l)	332.7±53.8	305.0±67.6	289.9±79.6	286.3±64.5
Chloride (mEq/l)	242.5±35.8	225.6±41.5	199.7±48.1	208.9±43.5
Calcium (mg/dl)	6.16±2.62	10.46±4.98*	5.82±1.87	9.06±4.68
I. phosphate (mg/dl)	80.3±36.3	36.4±31.4**	57.1±43.0	20.6±14.8*
Creatinine (mg/dl)	95.3±18.0	91.7±14.1	114.8±22.3	115.9±22.9
NAG (IU/l)	16.63±3.28	16.42±4.58	13.97±4.71	12.25±4.28
Sodium (mEq/16hr)	1.65±0.21	1.81±0.24	2.04±1.16	1.46±0.39
Potassium (mEq/16hr)	3.05±0.40	3.04±0.21	3.44±1.85	2.37±0.51
Chloride (mEq/16hr)	2.23±0.33	2.27±0.17	2.37±1.24	1.75±0.44
Calcium (mg/16hr)	0.57±0.25	1.04±0.42**	0.78±0.55	0.75±0.36
I. phosphate (mg/16hr)	7.7±4.5	3.7±3.2*	6.5±4.8	1.9±1.4*
Creatinine (mg/16hr)	8.70±1.29	9.27±0.81	14.04±7.83	9.71±2.35
NAG (IU/16hr)	0.16±0.04	0.17±0.06	0.18±0.12	0.11±0.05
pH	7.72±0.36	7.95±0.29	8.09±0.20	7.83±0.30*

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 5-2. Urinalysis values of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Urine volume (ml)	8.09±3.34	10.04±2.85	11.20±3.11	10.37±2.73
Osmolarity (mOsm/kg)	1917±544	1690±261	1437±497	1792±308
Sodium (mEq/l)	168.1±56.0	140.2±22.4	110.3±36.7	141.8±22.3*
Potassium (mEq/l)	309.2±99.9	240.3±44.1	204.3±70.9	237.7±47.2
Chloride (mEq/l)	218.3±62.3	181.8±26.4	137.2±52.7	164.7±28.0
Calcium (mg/dl)	12.40±8.95	16.35±4.34	16.05±6.14	24.63±8.69*
I. phosphate (mg/dl)	78.5±41.0	18.3±13.8**	36.9±19.8	28.5±17.6
Creatinine (mg/dl)	76.4±19.9	58.9±11.0*	69.3±27.7	79.3±16.6
NAG (IU/l)	12.26±4.71	10.91±5.15	9.75±2.80	12.55±3.33
Sodium (mEq/16hr)	1.20±0.25	1.38±0.31	1.18±0.34	1.45±0.39
Potassium (mEq/16hr)	2.23±0.47	2.30±0.29	2.18±0.65	2.40±0.56
Chloride (mEq/16hr)	1.59±0.36	1.76±0.28	1.46±0.46	1.68±0.43
Calcium (mg/16hr)	1.11±1.11	1.67±0.72	1.72±0.68	2.43±0.67*
I. phosphate (mg/16hr)	5.7±2.8	1.7±1.1**	4.0±2.2	3.0±1.9
Creatinine (mg/16hr)	5.60±1.26	5.64±0.75	7.26±2.30	7.91±1.61
NAG (IU/16hr)	0.09±0.04	0.10±0.03	0.10±0.03	0.13±0.04
pH	7.75±0.47	8.00±0.37	8.23±0.24	7.92±0.22**

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 6-1. Urinary semi-quantitative data of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks										19 weeks									
Group	Control					γ -Control					Control					γ -Control				
No. of animals	10					10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10					10				
Protein			6	4				3	7				4	5	1			4	6	
Ketones	3	7				5	5				6	4				7	3			
Occult blood	10					9	1				10					10				
Bilirubin	10					10					10					10				
Urobilinogen		3	3	4			10**					5	3	2			10*			

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

Table 6-2. Urinary semi-quantitative data of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks										19 weeks									
Group	Control					γ -Control					Control					γ -Control				
No. of animals	10					10					10					10				
Grade	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
Glucose	10					10					10					10				
Protein		2	5	3				8	2		1	4	5				10*			
Ketones	9	1				10					10					10				
Occult blood	10					10					10					10				
Bilirubin	10					10					10					10				
Urobilinogen		7	2	1			10					9	1				10			

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

Table 7-1. Hematology values of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
RBC ($\times 10^4$/mm^3)	800± 24	804± 11	873± 29	832±119
HGB (g/dl)	15.74±0.42	15.85±0.30	15.79±0.36	14.96±2.11
PCV (%)	45.48±1.26	45.40±1.04	45.43±1.13	42.56±5.71
MCV (fl)	56.9±2.2	56.5±1.6	52.1±2.0	51.3±1.4
MCH (pg)	19.69±0.65	19.72±0.41	18.10±0.52	18.00±0.53
MCHC (g/dl)	34.61±0.33	34.92±0.39	34.75±0.46	35.10±0.50
Platelet ($\times 10^4$/mm^3)	117.4±13.8	121.8±14.3	104.5±13.1	112.3±13.2
Reticulocyte (0/00)	21.4±5.9	19.7±7.4	15.9±4.6	15.5±8.3
WBC ($\times 10^2$/mm^3)	111.9±27.2	102.8±26.2	98.8±16.9	121.4±69.5
Neutrophil-band (%)	0.1±0.3	0.1±0.3	0.1±0.3	0.1±0.3
Neutrophil-segment (%)	8.4± 2.4	8.1± 4.2	11.3± 4.3	15.8±11.6
Lymphocyte (%)	88.1± 2.8	87.2± 5.8	82.6± 5.9	79.4±11.3
Monocyte (%)	2.8±1.1	4.2±3.0	5.0±2.4	4.0±2.6
Eosinophil (%)	0.6±0.7	0.5±0.5	1.0±0.8	0.7±0.8
Basophil (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PT (sec)	14.52±2.42	14.73±2.08	13.22±1.24	14.95±3.26
APTT (sec)	22.04±2.72	23.51±3.66	20.05±2.08	22.87±3.11*

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 7-2. Hematology values of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
RBC ($\times 10^4$/mm^3)	810± 26	811± 25	839± 26	814± 40
HGB (g/dl)	16.42±0.43	16.44±0.57	16.02±0.51	15.87±0.49
PCV (%)	45.71±1.49	46.24±1.62	45.19±1.98	44.36±1.82
MCV (fl)	56.4±1.8	57.0±1.5	53.9±2.0	54.5±2.0
MCH (pg)	20.27±0.45	20.26±0.42	19.08±0.56	19.51±0.61
MCHC (g/dl)	35.92±0.57	35.56±0.41	35.48±0.64	35.79±0.49
Platelet ($\times 10^4$/mm^3)	125.8±10.6	123.2±11.1	111.4±14.4	110.0±11.0
Reticulocyte (0/00)	12.6±3.9	14.0±5.1	15.9±6.0	16.0±4.8
WBC ($\times 10^2$/mm^3)	104.9±23.5	108.1±29.9	83.9±15.0	104.3±34.4
Neutrophil-band (%)	0.4±0.7	0.2±0.4	0.0±0.0	0.1±0.3
Neutrophil-segment (%)	6.8± 3.3	5.7± 3.5	8.3± 6.2	8.0± 5.3
Lymphocyte (%)	89.5± 4.0	89.7± 3.4	88.2± 7.6	88.2± 6.9
Monocyte (%)	2.9±1.6	3.2±2.1	3.0±1.9	3.0±2.4
Eosinophil (%)	0.4±1.0	1.2±0.8	0.4±1.0	0.7±0.7
Basophil (%)	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0
PT (sec)	13.09±0.80	12.71±0.76	13.21±0.88	12.99±0.60
APTT (sec)	16.30±1.84	16.48±1.00	15.90±0.90	15.68±0.50

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 8-1. Blood chemistry values of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
GOT (IU/l)	104.9±17.7	97.2±18.4	88.7±21.3	82.0±25.8
GPT (IU/l)	24.9± 3.2	26.1± 2.9	26.5± 4.1	27.9± 3.7
ALP (IU/l)	644± 65	556± 81*	251± 48	279± 45
Amylase (IU/l)	1242±129	1252±140	1285±149	1304±109
Glucose (mg/dl)	135.8±10.5	146.3±13.7	163.0±15.0	170.1±16.0
T. cholesterol (mg/dl)	59.8± 9.2	61.9±14.7	67.3±11.7	68.1±10.1
F. cholesterol (mg/dl)	12.0±2.2	12.1±3.3	14.8±2.9	14.4±2.8
Triglyceride (mg/dl)	52.7±24.8	61.5±32.5	63.1±18.2	63.7±16.1
Phospholipid (mg/dl)	100.3±16.0	101.5±16.8	108.3±14.3	108.1±16.0
NEFA (mEq/l)	0.590±0.113	0.586±0.094	0.532±0.094	0.464±0.059
T. bilirubin (mg/dl)	0.06±0.01	0.06±0.01	0.07±0.01	0.07±0.01
Urea N (mg/dl)	16.4±2.2	16.8±2.6	16.1±3.1	15.1±1.5
Creatinine (mg/dl)	0.43±0.03	0.46±0.03	0.53±0.05	0.51±0.03
T. protein (mg/dl)	5.63±0.23	5.80±0.17	5.89±0.17	6.00±0.24
Albumin (mg/dl)	2.36±0.12	2.38±0.06	2.38±0.09	2.39±0.10
Sodium (mEq/l)	142.9±1.4	142.9±1.0	142.4±0.8	143.2±0.9
Potassium (mEq/l)	4.63±0.15	4.54±0.27	4.41±0.38	4.31±0.47
Chloride (mEq/l)	101.0±0.9	100.7±0.5	104.1±1.2	105.0±1.2
Calcium (mg/dl)	9.69±0.29	9.78±0.32	9.85±0.22	10.10±0.35
I. phosphate (mg/dl)	7.89±0.39	8.25±0.37*	5.78±0.39	6.02±0.47
Albumin (%)	49.7±1.6	50.1±2.0	47.3±1.7	48.0±2.1
α 1-Globulin (%)	23.6±1.7	22.7±2.2	22.9±2.6	23.1±1.8
α 2-Globulin (%)	7.1±0.7	7.7±0.9	7.7±0.7	7.9±0.4
β -Globulin (%)	16.8±1.3	16.3±0.6	17.8±1.0	17.1±0.9
γ -Globulin (%)	2.9±0.6	3.2±0.5	4.4±0.8	3.9±0.6
A/G	0.99±0.06	1.01±0.08	0.90±0.06	0.92±0.08

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 8-2. Blood chemistry values of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
GOT (IU/l)	90.8±18.7	96.6±23.2	113.1±58.7	96.4±31.6
GPT (IU/l)	19.0± 3.1	19.1± 3.3	39.9±40.5	28.7±15.7
ALP (IU/l)	294± 74	363±118	119± 32	125± 24
Amylase (IU/l)	679±89	655±140	852±147	758±128
Glucose (mg/dl)	112.4±25.7	120.3±27.1	130.4±26.3	145.3±16.8
T. cholesterol (mg/dl)	73.0±10.4	67.3±13.4	79.8±19.6	81.0±11.8
F. cholesterol (mg/dl)	13.7±2.8	11.6±3.2	16.8±5.1	16.4±3.0
Triglyceride (mg/dl)	21.2±11.8	21.8±15.5	22.4±10.4	28.5±19.2
Phospholipid (mg/dl)	126.4±16.3	122.3±24.4	143.1±32.3	144.8±23.2
NEFA (mEq/l)	0.689±0.137	0.728±0.138	0.676±0.180	0.656±0.138
T. bilirubin (mg/dl)	0.09±0.02	0.08±0.01	0.11±0.02	0.11±0.02
Urea N (mg/dl)	20.3±4.0	18.5±1.9	19.2±5.0	20.2±6.7
Creatinine (mg/dl)	0.47±0.03	0.47±0.07	0.56±0.08	0.56±0.06
T. protein (mg/dl)	6.06±0.26	6.11±0.33	6.72±0.64	6.53±0.39
Albumin (mg/dl)	2.67±0.13	2.70±0.12	2.96±0.33	2.88±0.26
Sodium (mEq/l)	142.5±1.5	143.7±1.6	142.7±1.8	142.4±1.6
Potassium (mEq/l)	4.51±0.35	4.22±0.44	4.15±0.40	4.01±0.50
Chloride (mEq/l)	102.5±1.3	103.6±1.4	104.9±2.8	105.5±1.7
Calcium (mg/dl)	10.32±0.34	10.19±0.44	10.50±0.68	10.49±0.38
I. phosphate (mg/dl)	7.83±0.53	7.82±0.43	6.36±0.74	6.43±0.54
Albumin (%)	54.4±3.5	53.6±1.2	53.0±2.7	54.7±2.0
α 1-Globulin (%)	20.2±1.7	19.7±1.4	19.3±1.7	17.8±2.1
α 2-Globulin (%)	7.0±1.1	7.6±0.4	7.1±1.4	7.2±0.6
β -Globulin (%)	14.8±1.4	15.3±1.0	15.1±1.5	15.0±1.2
γ -Globulin (%)	3.7±1.0	4.0±0.9	5.6±0.6	5.4±0.6
A/G	1.21±0.18	1.16±0.06	1.13±0.12	1.21±0.10

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 9-1. Absolute organ weights of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Body weight (g)	338.1±18.1	372.0±31.7**	495.1±52.9	502.0±35.2
Brain (g)	2.037±0.067	1.993±0.104	2.093±0.114	2.113±0.080
Heart (g)	1.206±0.069	1.378±0.121	1.398±0.132	1.463±0.104
Lung (g)	1.163±0.102	1.265±0.108*	1.366±0.116	1.363±0.078
Liver (g)	9.97±0.81	11.41±1.66*	12.65±1.45	13.03±1.18
Kidney (L, g)	1.305±0.116	1.368±0.093	1.549±0.128	1.556±0.121
Kidney (R, g)	1.332±0.093	1.389±0.114	1.560±0.108	1.573±0.125
Cecum (g)	3.460±0.809	3.502±0.970	3.802±0.815	3.639±0.804
Testis (L, g)	1.460±0.366	1.511±0.125	1.660±0.109	1.659±0.092
Testis (R, g)	1.464±0.364	1.528±0.110	1.667±0.109	1.666±0.115
Pituitary gland (mg)	10.3±1.5	10.2±1.5	12.2±1.4	10.8±1.5
Salivary gland (L, mg)	291±42	298±42	361±47	366±36
Salivary gland (R, mg)	287±41	300±41	355±48	366±38
Thymus (mg)	480±110	526±129	317±110	338± 84
Spleen (mg)	636± 67	682±123	745±134	759±118
Adrenal gland (L, mg)	25.2±3.3	30.6±6.6*	26.2±3.5	27.0±3.7
Adrenal gland (R, mg)	24.5±2.8	28.7±6.0	24.9±2.8	25.4±3.2
Epididymis (L, mg)	397±63	414±31	636±60	652±50
Epididymis (R, mg)	405±65	424±36	639±68	667±53

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 9-2. Absolute organ weights of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Body weight (g)	221.7±18.4	226.7±19.7	267.0±32.2	294.5±20.3*
Brain (g)	1.844±0.049	1.878±0.068	1.911±0.088	1.912±0.062
Heart (g)	0.816±0.088	0.845±0.080	0.911±0.088	0.944±0.065
Lung (g)	0.952±0.077	0.944±0.077	0.993±0.081	0.999±0.087
Liver (g)	6.16±0.60	6.74±0.83	6.55±1.08	6.92±0.59
Kidney (L, g)	0.818±0.076	0.881±0.097	0.866±0.078	0.925±0.078
Kidney (R, g)	0.825±0.093	0.907±0.094	0.858±0.100	0.947±0.089
Cecum (g)	2.742±0.600	2.971±1.137	2.820±0.481	3.006±0.954
Pituitary gland (mg)	12.5±1.9	12.7±1.7	15.4±2.8	14.2±2.0
Salivary gland (L, mg)	210±24	205±21	211±21	233±29
Salivary gland (R, mg)	214±23	207±21	213±19	229±27
Thymus (mg)	486± 86	496± 84	257± 42	257± 65
Spleen (mg)	464± 50	483± 54	438± 70	477± 68
Adrenal gland (L, mg)	29.4±3.1	34.8±4.2**	32.2±4.9	32.4±5.0
Adrenal gland (R, mg)	28.1±3.4	33.1±3.1**	32.2±4.5	33.6±4.9 a)
Ovary (L, mg)	39.3±8.1	44.6±7.0	35.9±7.7	39.9±11.3
Ovary (R, mg)	38.5±8.4	44.5±6.0	36.1±4.9	41.2±10.4
Uterus (mg)	423± 89	529±240	583±243	548±289

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01
a): n=9

Table 10-1. Relative organ weights of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Body weight (g)	338.1±18.1	372.0±31.7**	495.1±52.9	502.0±35.2
Brain (g%)	0.603±0.026	0.539±0.047**	0.426±0.036	0.422±0.024
Heart (g%)	0.358±0.028	0.371±0.027	0.283±0.021	0.292±0.017
Lung (g%)	0.344±0.028	0.341±0.023	0.277±0.023	0.272±0.015
Liver (g%)	2.95±0.16	3.06±0.24	2.56±0.17	2.60±0.15
Kidney (L, g%)	0.386±0.031	0.369±0.017	0.315±0.025	0.310±0.016
Kidney (R, g%)	0.394±0.025	0.374±0.019	0.317±0.025	0.314±0.020
Cecum (g%)	1.022±0.229	0.942±0.251	0.779±0.189	0.726±0.155
Testis (L, g%)	0.434±0.111	0.409±0.048	0.339±0.040	0.332±0.027
Testis (R, g%)	0.435±0.110	0.413±0.042	0.341±0.044	0.333±0.028
Pituitary gland (mg%)	3.1±0.5	2.8±0.4	2.5±0.2	2.2±0.3*
Salivary gland (L, mg%)	86±14	80±11	74±12	74±10
Salivary gland (R, mg%)	85±13	81±12	73±12	74± 9
Thymus (mg%)	141±26	141±30	64±19	67±14
Spleen (mg%)	188±20	183±21	150±20	151±19
Adrenal gland (L, mg%)	7.5±1.2	8.2±1.5	5.3±0.7	5.4±0.7
Adrenal gland (R, mg%)	7.3±1.1	7.7±1.4	5.1±0.7	5.1±0.7
Epididymis (L, mg%)	118±21	112±10	129±15	131±15
Epididymis (R, mg%)	120±22	115±12	130±18	134±14

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01

Table 10-2. Relative organ weights of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age	10 weeks		19 weeks	
Group	Control	γ -Control	Control	γ -Control
No. of animals	10	10	10	10
Body weight (g)	221.7±18.4	226.7±19.7	267.0±32.2	294.5±20.3*
Brain (g%)	0.838±0.087	0.833±0.073	0.725±0.090	0.652±0.042
Heart (g%)	0.367±0.018	0.373±0.020	0.342±0.020	0.321±0.015*
Lung (g%)	0.430±0.024	0.418±0.037	0.375±0.043	0.340±0.024*
Liver (g%)	2.78±0.10	2.97±0.16**	2.45±0.25	2.35±0.14
Kidney (L, g%)	0.369±0.020	0.388±0.023	0.326±0.022	0.314±0.010
Kidney (R, g%)	0.372±0.025	0.401±0.030*	0.322±0.025	0.321±0.015
Cecum (g%)	1.234±0.223	1.289±0.386	1.071±0.242	1.012±0.279
Pituitary gland (mg%)	5.6±0.6	5.6±0.7	5.8±1.2	4.8±0.6*
Salivary gland (L, mg%)	95±11	91± 9	79± 6	79±10
Salivary gland (R, mg%)	97± 9	92± 8	81± 8	78± 9
Thymus (mg%)	219±35	219±31	97±15	87±18
Spleen (mg%)	210±24	213±13	165±24	162±20
Adrenal gland (L, mg%)	13.4±1.8	15.4±2.0*	12.2±2.1	11.0±1.3
Adrenal gland (R, mg%)	12.8±1.9	14.7±1.6*	12.1±1.7	11.5±1.3 a)
Ovary (L, mg%)	17.7±3.1	19.8±3.4	13.4±2.4	13.5±3.8
Ovary (R, mg%)	17.4±3.4	19.7±2.5	13.6±2.0	14.0±3.2
Uterus (mg%)	193± 52	234± 98	223±100	188±102

Values represent mean±S.D.
Significant difference from the control group, *: P<0.05, **: P<0.01
a): n=9

Table 11-1. Histopathological findings of male Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age		10 weeks								19 weeks							
Group		Control				γ-Control				Control				γ-Control			
No. of animals		10				10				10				10			
Organ/Tissue	Microscopic findings	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
Liver	Microgranuloma	7	3			9	1			9	1			10			
	Fatty change of hepatocytes	10				7	3			9	1			10			
	Focal necrosis of hepatocytes	10				9	1			10				10			
Kidney	Hyaline droplets in proximal tubular epithelium	9	1			4	6			1	5	4			5	4	1
	Eosinophilic body in proximal tubular epithelium	9	1			10				7	2	1		7	2	1	
	Atrophy/Regeneration of tubules	10				10				9	1			10			
Heart	Inflammatory cell infiltration	10				10				9	1			9	1		
Lung	Aggregation of foamy cells	10				10				9	1			9	1		
	Hemorrhage	10				9	1			10				10			
	Inflammatory cell infiltration	10				9	1			10				10			
Spleen	Deposition of hemosiderin	10				10				4	6			8	2		
Pancrea	Inflammatory cell infiltration	10				10				8	2			10			
	Lobular atrophy	10				10				10				9	1		
Jejunum/Ileum	Mineralization in lymphoid follicle	9	1			9	1			8	2			9	1		
Cecum	Mineralization in mucosa	10				9	1			10				10			
Pituitary gland	Cyst	10				10				10				9	1		
Thyroid gland	Ultimobrancial body	8	2			7	3			7	3			6	4		
Testis	Atrophy	9			1	10				10				10			
Epidydimis	Atrophy	9	1			10				10				10			

−: No remarkable changes, +: Slight, ++: Moderate, +++: Severe

Table 11-2. Histopathological findings of female Crj:CD(SD)IGS rats fed diet sterilized by different methods

Age		10 weeks								19 weeks							
Group		Control				γ-Control				Control				γ-Control			
No. of animals		10				10				10				10			
Organ/Tissue	Microscopic findings	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
Liver	Microgranuloma	10				8	1	a)		10				8	2		
	Focal necrosis of hepatocytes	10				8	1	a)		10				9	1		
Kidney	Mineralization	9	1			10				10				10			
Lung	Aggregation of foamy cells	10				10				9	1			10			
Spleen	Deposition of hemosiderin	10				10				1	9				10		
Thymus	Epitheloid structure	10				10				9	1			9	1		
Pancreas	Inflammatory cell infiltration	10				9	1			7	3			8	2		
Jejunum/Ileum	Mineralization in lymphoid follicle	8	2			10				10				7	3		
Cecum	Mineralization in mucosa	8	2			8	2			9	1			10			
Eye	Retinal atrophy	10				9	1			10				10			
Thyroid gland	Ultimobrancial body	6	4			4	5	a)		6	4			5	5		

−: No remarkable changes, +: Slight, ++: Moderate, +++: Severe
a): n=9

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

- Histopathological Findings -

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ABSTRACT. To collect background data in Crj:CD(SD)IGS rats, a toxicological study using the rats administered with distilled water orally for 4, 13 or 26 weeks were conducted in 1997 - 1998. In this paper, organs and tissues obtained from these rats at each terminal were evaluated morphologically. Histopathological changes were mainly observed in the heart, spleen, lungs, pancreas, kidneys, pituitary gland, thyroid glands, mammary gland and eyes. The incidence of some lesions were increased with age and had sexual differences. Retinal hypoplasia observed for a female in the 4-week group was considered to be developmental anomaly. The histopathological changes observed until 33 weeks of age for IGS rats may be comparable to spontaneous lesions that occurred in other strains of rats. – Key words: Crj:CD (SD) IGS rats, distilled water, Histopathological data

CD(SD)IGS-2000: 75-78

INTRODUCTION

Crj:CD(SD)IGS rats were established and used internationally in toxicology studies. We joined the Crj:CD (SD) IGS study group to collect background data for the IGS rat. A typical toxicological study using the rats administered orally with distilled water for 4, 13 or 26 weeks was done in our labs. Hematological, blood chemical and urinalytical evaluations for the rats have already been published separately [1]. In this study, organs and tissues from these rats were histopathologically evaluated.

MATERIALS AND METHODS

Test system and study groups: A total of 50 male and 50 female Crj:CD (SD) IGS rats was purchased from a commercial breeder (Charles River Japan Inc., Hino, Japan) at the age of 6 weeks. After the quarantine and acclimation period of 1 week, rats were weighed and randomly allocated to 3 groups. The 4-, 13- and 26-week groups comprised 15, 15 and 20 animals/sex, respectively. All rats of 7 weeks old at the start of administration were dosed with distilled water orally for 4, 13 or 26 weeks at 1.0 ml/100 g body weight.

Accommodation: 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-dri® bedding (Oriental Yeast Co., Ltd.) under controlled environmental conditions of temperature (19-24℃), and relative humidity (40-75%) with a light cycle of 12 hrs (6:00 to 18:00) in the barrier-sustained animal facility. Ventilation was 15 times/hr. Standardized dry pellet diet CRF-1 (γ-ray irradiated diet, Charles River Japan Inc.) and municipal tap water were available *ad libitum*.

Gross findings at necropsy: At the end of 4, 13 or 26 weeks, all survivors were anesthetized by intraperitoneal injection with Nembutal® (Abbott Lab., USA), weighed and bled through the aorta abdominalis. At necropsy, organs and tissues were observed macroscopically. Mandatory organs and tissues were removed and fixed in phosphate buffered 10 % formalin solution except for eyes in Davidsons's solution.

Histopathological evaluation: All fixed organs and tissues from each rat were embedded in paraffin, sectioned by a sliding microtome, mounted and stained with hematoxylin and eosin. Additional sections of testis were stained with PAS. Slides of the bone marrow were stained with Giemsa according to Walbach's modification.

The following organs and tissues were examined microscopically: liver, spleen, lungs, kidneys, heart, brain, adrenals, pituitary, thoracic aorta, sciatic nerve, thyroids, parathyroids, pancreas, skeletal muscle (quadriceps femoris), skin, mammary glands, esophagus, trachea, testes, epididymides, seminal vesicle, prostate, ovaries, uterus, vagina, stomach, duodenum, jejunum, ileum, colon, thymus, salivary glands (submaxillary, sublingual and parotid), urinary bladder, submaxillary lymph node, eyes with optic nerves, knee joint and bone marrow.

RESULTS

Histopathological findings

The incidence of spontaneous lesions and the number of tissues examined are summarized in Table 1. A female in the 26-week group showing swelling of the pituitary gland revealed histopathologically hyperplasia in the pars distalis, and a female in the same group showing rough surface in the right kidney revealed cyst and fibrosis at the cortex.

In some animals that no abnormal findings were recorded macroscopically, various histopathological changes were observed in the heart, spleen, lungs, pancreas, kidneys, pituitary gland, thyroid glands, mammary gland and eyes. Mononuclear cell infiltration, fibrosis and granuloma in the heart were observed in the 13- and/or 26-week groups. In the spleen, deposition of hemosiderin was seen in the 13- and 26-week groups, which incidence was increased with age, and more in females than males. In the lungs, calcification in the artery was shown in both sexes of all groups and foam cell accumulation was noticed in both sexes of all groups except males in the 4-week group. In the pancreas, increased incidence of pigmentation was observed and focal necrosis and atrophy of the acinus were seen for male rats in the 26-week group. In the kidneys, debris of neutrophils in the calyx was present for a male and three female rats in the 26-week group. Pelvic transitional epithelium for two female rats was slightly hyperplastic. In

Table 1. Histopathological findings in IGS rats

Sex	Male			Female		
Groups	4-week	13-week	26-week	4-week	13-week	26-week
Age (weeks)	11	20	33	11	20	33
Number of animals	15	15	19	15	15	20
Heart						
Cellular infiltration, mononuclear cell	0	1	0	0	0	0
Fibrosis	0	3	2	0	0	0
Granuloma	0	0	1	0	0	0
Inflammation, focal	0	0	1	0	0	0
Spleen						
Deposition, hemosiderin	0	3	4	0	6	12
Extramedullary hemopoiesis	0	0	1	0	0	1
Accessory spleen	0	0	0	0	2	0
Lung						
Calcification, artery	3	3	6	1	5	2
Granuloma	0	1	0	0	0	1
Foam cell accumulation	0	1	3	2	1	3
Osseous metaplasia	0	0	0	0	1	0
Cellular infiltration, mononuclear cell	0	1	0	0	0	0
Parotid						
Fatty change, interstitium	0	2	0	0	0	0
Esophagus						
Inflammatory cell infiltration	0	1	0	0	0	0
Liver						
Microgranuloma	1	0	0	1	0	2
Pancreas						
Fatty change, interstitium	0	3	0	0	0	0
Necrosis, acinus, focal	0	0	1	0	0	0
Pigmentation	0	0	4	0	0	0
Atrophy, acinus, focal	0	0	1	0	0	0
Cellular infiltration, mononuclear cell	1	0	3	0	2	1
Kidney						
Basophilic cell, tubule	0	6	2	1	0	0
Cellular infiltration, mononuclear cell	1	1	0	2	0	0
Hyperplasia, pelvic transitional cell	0	0	0	0	0	2
Vacuolation, tubule	0	1	0	0	0	0
Fibrosis	0	1	0	0	0	1
Cyst, cortex	1	0	0	0	0	1
Necrosis, tubule	0	0	0	1	0	0
Calcification, tubule	0	0	0	0	0	1
Hyaline cast	0	0	1	0	0	0
Neutrophilic debris, calyx	0	0	1	0	0	3

Table 1. -continued

Sex	Male			Female		
Groups	4-week	13-week	26-week	4-week	13-week	26-week
Age (weeks)	11	20	33	11	20	33
Number of animals	15	15	19	15	15	20
Urinary bladder						
Cellular infiltration, mononuclear cell	0	1	0	0	0	0
Pituitary						
Cyst, pars distalis	1	1	0	1	0	2
Cyst, pars intermedia	1	0	1	0	0	0
Hyperplasia, pars distalis	0	1	0	0	0	1
Thyroid						
Ultimobranchial body	1	2	3	2	3	3
Follicular cell hyperplasia	0	0	1	0	0	0
Adrenal						
Hypertrophy, cortex, focal	0	0	1	0	0	0
Epididymis						
Spermatic granuloma	1	0	1	-	-	-
Prostate						
Atrophy, acinus	0	1	0	-	-	-
Inflammation, focal	0	0	2	-	-	-
Eye						
Rosette, retina	1	1	0	0	0	0
Hypoplasia, retina	0	0	0	1	0	0
Skin						
Cellular infiltration, mononuclear cell	0	1	0	0	0	0
Mammary gland						
Hyperplasia, lobule	0	1	0	0	0	0

the endocrine system, the pituitary and thyroid glands showed hyperplastic lesions. That is, a male rat in the 13-week group and a female rat in the 26-week group showed hyperplasia in the pars distalis of the pituitary gland, the male rat also exhibited lobular hyperplasia in the mammary gland. In the thyroid glands, the follicular cell hyperplasia was seen for a male rat in the 26-week group. In the eye of a female rat in the 4-week group, hypoplasia of the inner plexiform and the inner granular layers was observed at almost all sides of the unilateral retina (Photo 1).

There were no lesions in the brain, thoracic aorta, sciatic nerve, parathyroid glands, skeletal muscle, trachea, testes, seminal vesicle, ovaries, uterus, vagina, stomach, duodenum, jejunum, ileum, colon, thymus, submaxillary and sublingual glands, knee joint, submaxillary lymph node and bone marrow.

A male rat in the 26-week group was euthanized at the age of 10 weeks because of the severe exhaustion. The rat suddenly showed ventral position, tachypnea, hematuria and serous diarrhea on Day 26 of administration. At necropsy, reddish urine in the dilated bladder, nodulation in the bladder epithelium, dilated ureter, edema in discolored kidneys (weight 5.61 g), hemorrhage in the gastric mucosa and tarry stool in the intestine were observed macroscopically. Histopathological examinations for the rat revealed focal inflammation in the heart, atrophy in the spleen, erosion in the glandular stomach, focal inflammation at the renal hilus and tubular dilatation in the kidney, cystitis and prostatis. Since these findings suggested that the severe lesion in the urinary tract occurred incidentally in the early period of the study, the rat was eliminated from the evaluation.

DISCUSSION

In the previous publication [1], age-related changes were reported in hematological, blood chemical and urinalytical examinations. Namely, minimal decrease of the leukocyte count, slightly increased values of total protein, total cholesterol and triglycerides, and slightly decreased values of creatinine, inorganic phosphorus and alkaline phosphatase were demonstrated with increasing age of rats. According to results of this study, no histopathological findings considered to be related to these changes of laboratory values were noticed. Hyperplasia of pars distalis in the pituitary gland and lobular hyperplasia in the mammary gland for a male in the 13-week group were considered to be incidental and the secondary hormonal effect. Regarding retinal hypoplasia observed for a female rat at the age of 11 weeks, the lesion was thought to be developmental anomaly because the finding occurred unilaterally and in relatively younger animal [3]. The incidence of deposition of hemosiderin in the spleen was apparently higher in females than males, and some findings including this changes showed sexual differences in the incidence.

Based on the present results, the histopathological changes observed until 33 weeks of age for IGS rats may be comparable to spontaneous lesions that occurred in other strains for rats. There were no IGS-specific pathological changes, when compared to Sprague-Dawley rats [4, 5] or Fischer -344 rats [2].

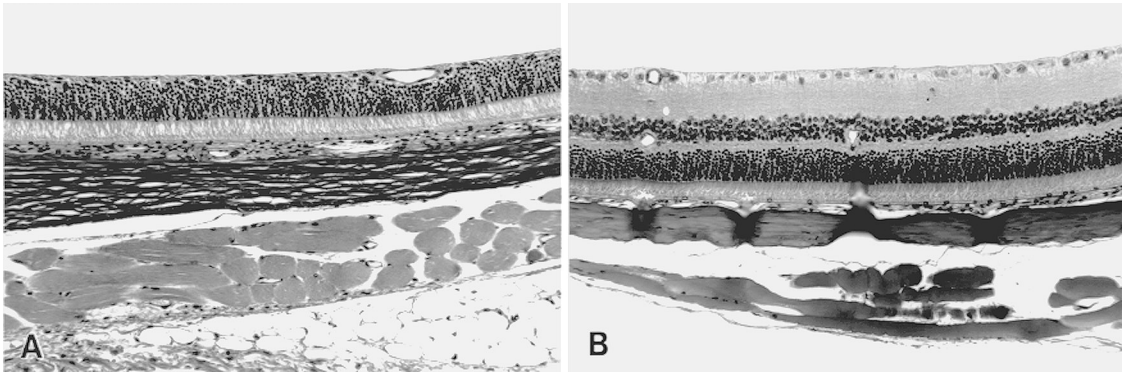

Photo 1. One of the eyes (A) in a female IGS rat at the age of 11 weeks shows hypoplasia of the inner plexiform and the inner granular layers in contrast to the other (B). H-E staining, × 100.

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Background Data on Organ Weights and Histopathological Lesions in Crj:CD(SD)IGS Rats for 4-, 13- and 26-week Repeated-Dose Toxicity Studies

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ABSTRACT. For the 4-, 13- and 26-week repeated-dose toxicity studies, background data on organ weights and spontaneous occurring lesions were obtained from Crj:CD(SD)IGS (IGS) rats fed a low protein commercial diet (CR-LPF). Thirty male and 30 female IGS rats at 5 weeks of age were used in each study. A few gross pathological lesions were observed in these studies but no age- or sex-related changes were seen. Histopathologically, in males vascular calcification and foam cell aggregation in the lungs, granuloma (microgranuloma) in the liver, basophilic tubular epithelium in the kidney, and ultimobranchial cyst in the thyroid occurred in each study at a high incidence (≧ 20%). Incidences of granuloma in the heart, vascular calcification and foam cell aggregation in the lungs, fibrosis of the pancreatic islet, pigment deposition in the pancreas, protein casts in the kidney, and corneal degeneration in the eye of males increased with age. In females, foam cell aggregation in the lungs, granuloma in the liver, basophilic tubular epithelium in the kidney and ultimobranchial cyst in the thyroid occurred in each study at a high incidence (≧ 20%). Incidences of calcification in the kidney and corneal degeneration in the eye of females increased with age. － Key words: Crj:CD(SD)IGS, Histopathological lesions, Organ weights, Rat.

CD(SD)IGS-2000: 79-87

INTRODUCTION

Crj:CD(SD)IGS (IGS) rats have been created by the international genetic standard system, which was organized to supply experimental animals with homogeneous characteristics, minimizing genetic diversification in Charles River, Inc. The animals are expected to meet internationalization of research and development of new drugs. However, background data on histopathological lesions of IGS rats have not yet been fully accumulated. We have obtained control data on organ weights and histopathological lesions in IGS rats of 9, 18, and 31 weeks of age, being useful in the 4-, 13- and 26-week toxicity studies.

MATERIALS AND METHODS

Ninety male and 90 female Crj:CD(SD)IGS rats were purchased from Charles River Japan, Inc. (Tsukuba Breeding Center, Ibaraki) at 4 weeks of age. The rats were divided into three study groups (4-, 13- and 26-week studies), each consisting of 30 males and 30 females. The animals were housed individually in a wire-mesh cage (21 x 35 x 20 cm) and were maintained in a barrier-sustained room controlled at 21 - 23℃ and 30 - 63% relative humidity, and ventilated 10 times per hr, with a 12-hr light-dark cycle. The animals had free access to a low protein commercial diet (18% protein content) for rats (CR-LRF with γ-ray irradiation, Oriental Yeast Co., Tokyo) and tap water. They cared for and were treated humanely in accordance with the *Guidelines for Animal Experimentation*, published by the Japanese Association for Laboratory Animal Science (Exp. Anim. 36: 285-288, 1987).

All rats were observed for clinical signs and mortality twice daily and weighed weekly. At 4, 13 and 26 weeks after the start of the studies, 30 males and 30 females in each study were bled from the abdominal aorta under deep anesthesia with ether following 17 hr of fasting and subjected to a complete necropsy. The following organs were weighed; liver, spleen, kidneys, heart, lungs, adrenal glands, thymus, thyroid, pituitary, salivary glands (submandibular and sublingual glands), testes, prostate gland, epididymides, seminal vesicle, ovaries, uterus, and brain. Ratios of organ weights to body weights were calculated. All organs and tissues were fixed in 10% neutral buffered formalin except for testes and eyes, which were fixed in Bouin's solution and Davidson's solution, respectively. The following organs and tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined histopathologically; heart, aorta (thoracic), bone marrow (sternum and femur), spleen, thymus, mesenteric lymph node, trachea, bronchus, lungs, tongue, submandibular gland, sublingual gland, parotid gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, kidneys, urinary gladder, pituitary, thyroids, parathyroids, adrenal glands, testes, epididymides, seminal vesicle, prostate gland, ovaries, uterus, vagina, brain, spinal cords (cervical and lumber), sciatic nerve, eyes, lacrimal gland, Harderian gland, diaphragm, skeletal muscle, sternum, femur, skin, mammary gland and any other tissues with gross lesions.

RESULTS

Gross pathology

At 9 weeks of age, no gross lesions were observed in any males and pelvic dilatation of the kidney was observed in two females. At 18 weeks of age, a supernumerary lobe of the liver and pelvic dilatation of the kidney were observed in one and 2 males, respectively, and no gross lesions were detected in any females. At 31 weeks of age, red foci of the liver, yellow foci of the kidney and swelling of the thyroid was observed in one male each and red foci and abscess of the liver, depressed foci of the kidney and loss of hair in the thorax were observed in one female each.

Organ weight and ratio of organ weight to body weight

Organ weights and ratios of organ weights to body weights are shown in Tables 1 and 2. The absolute weights of most organs increased with age. Whereas the absolute weights and the ratios of organ weights to body weights of the thymus in males and females decreased evidently with age.

Table 1. Organ weights in IGS rats

Sex	Organs		Age(weeks)	9	18	31
			No. of anmals	30	30	30
Male	Body weight	(g)		363 ± 19	506 ± 49	640 ± 65
	Liver	(g)		11.1 ± 1.1	13.3 ± 1.7	15.7 ± 1.9
	Spleen	(mg)		685 ± 126	734 ± 94	807 ± 143
	Kidneys	(g)		2.77 ± 0.22	3.07 ± 0.28	3.64 ± 0.36
	Heart	(g)		1.27 ± 0.09	1.52 ± 0.14	1.70 ± 0.14
	Lungs	(g)		1.35 ± 0.10	1.49 ± 0.17	1.66 ± 0.16
	Adrenal glands	(mg)		61.1 ± 10.3	56.7 ± 8.2	55.4 ± 7.7
	Thymus	(mg)		586 ± 108	299 ± 56	225 ± 58
	Thyroid	(mg)		26.7 ± 3.1	30.7 ± 5.3	37.6 ± 5.7
	Pituitary	(mg)		11.4 ± 1.4	12.8 ± 1.5	12.4 ± 1.4
	Salivary gland	(mg)		716 ± 77	804 ± 89	831 ± 71
	Testes	(g)		3.28 ± 0.24	3.46 ± 0.30	3.69 ± 0.32
	Prostate gland	(mg)		753 ± 121	1153 ± 195	1251 ± 238
	Epididymides	(mg)		918 ± 91	1480 ± 167	1621 ± 150
	Seminal vesicle	(mg)		1023 ± 160	1538 ± 269	1626 ± 444
	Brain	(g)		2.10 ± 0.09	2.15 ± 0.08	2.26 ± 0.07
Female	Body weight	(g)		205 ± 15	274 ± 29	313 ± 33
	Liver	(g)		6.2 ± 0.7	7.0 ± 0.8	7.4 ± 0.8
	Spleen	(mg)		438 ± 81	475 ± 60	478 ± 67
	Kidneys	(g)		1.69 ± 0.17	1.81 ± 0.12	1.93 ± 0.14
	Heart	(g)		0.82 ± 0.07	0.97 ± 0.09	1.02 ± 0.09
	Lungs	(g)		1.00 ± 0.08	1.10 ± 0.08	1.13 ± 0.09
	Adrenal glands	(mg)		65.4 ± 9.0	66.4 ± 8.4	64.5 ± 7.4
	Thymus	(mg)		482 ± 95	255 ± 48	159 ± 33
	Thyroid	(mg)		21.3 ± 3.5	23.3 ± 3.7	25.6 ± 4.6
	Pituitary	(mg)		12.5 ± 1.8	14.8 ± 2.0	15.4 ± 3.1
	Salivary gland	(mg)		442 ± 45	476 ± 40	502 ± 49
	Ovaries	(mg)		89.1 ± 11.2	96.5 ± 15.6	93.0 ± 15.1
	Uterus	(mg)		562 ± 177	689 ± 239	799 ± 239
	Brain	(g)		1.88 ± 0.10	1.99 ± 0.07	2.01 ± 0.08

Figures represent mean and SD.

Table 2. Ratios of organ weights to body weights in IGS rats (%)

Sex	Organs	Age(weeks)	9	18	31
		No. of animals	30	30	30
Male	Liver		3.05 ± 0.22	2.62 ± 0.22	2.45 ± 0.11
	Spleen		0.188 ± 0.032	0.146 ± 0.019	0.126 ± 0.018
	Kidneys		0.764 ± 0.052	0.609 ± 0.045	0.572 ± 0.054
	Heart		0.351 ± 0.022	0.302 ± 0.023	0.266 ± 0.017
	Lungs		0.372 ± 0.021	0.295 ± 0.025	0.261 ± 0.025
	Adrenal glands		0.0169 ± 0.0029	0.0112 ± 0.0015	0.0087 ± 0.0013
	Thymus		0.162 ± 0.030	0.060 ± 0.013	0.035 ± 0.007
	Thyroid		0.0074 ± 0.0008	0.0061 ± 0.0010	0.0059 ± 0.0007
	Pituitary		0.0031 ± 0.0004	0.0025 ± 0.0003	0.0019 ± 0.0003
	Salivary gland		0.197 ± 0.019	0.159 ± 0.015	0.131 ± 0.014
	Testes		0.906 ± 0.079	0.690 ± 0.083	0.584 ± 0.086
	Prostate gland		0.207 ± 0.032	0.228 ± 0.036	0.197 ± 0.040
	Epididymides		0.253 ± 0.024	0.294 ± 0.036	0.256 ± 0.031
	Seminal vesicle		0.282 ± 0.044	0.305 ± 0.048	0.256 ± 0.072
	Brain		0.579 ± 0.034	0.429 ± 0.037	0.358 ± 0.040
Female	Liver		3.00 ± 0.17	2.54 ± 0.15	2.36 ± 0.16
	Spleen		0.213 ± 0.031	0.174 ± 0.022	0.153 ± 0.020
	Kidneys		0.824 ± 0.065	0.667 ± 0.057	0.622 ± 0.056
	Heart		0.401 ± 0.022	0.355 ± 0.024	0.329 ± 0.024
	Lungs		0.489 ± 0.032	0.403 ± 0.038	0.364 ± 0.032
	Adrenal glands		0.0319 ± 0.0041	0.0245 ± 0.0037	0.0208 ± 0.0029
	Thymus		0.234 ± 0.042	0.094 ± 0.016	0.051 ± 0.008
	Thyroid		0.0104 ± 0.0015	0.0086 ± 0.0012	0.0082 ± 0.0014
	Pituitary		0.0061 ± 0.0007	0.0055 ± 0.0008	0.0050 ± 0.0010
	Salivary gland		0.215 ± 0.019	0.175 ± 0.018	0.161 ± 0.016
	Ovaries		0.0435 ± 0.0055	0.0354 ± 0.0056	0.0301 ± 0.0059
	Uterus		0.274 ± 0.085	0.254 ± 0.091	0.261 ± 0.094
	Brain		0.917 ± 0.079	0.734 ± 0.067	0.648 ± 0.074

Figures represent mean and SD.

Histopathology

The incidences of histopathological lesions are shown in Tables 3 and 4. No neoplastic lesions were observed in male and female IGS rats examined. In the heart, granuloma was characterized by small aggregations of histiocytic cells and the incidence of the lesion in males increased with age; 3.3%, 10.0%, and 33.3% at 9, 18, and 31 weeks of age, respectively. In the lungs, vascular calcification was found as basophilic amorphous deposits in the media of the pulmonary arteries. The incidence of the lesion in males was higher than that in females and increased with age; 26.7%, 33.3%, and 43.3% at 9, 18, and 31 weeks of age, respectively. Foam cell aggregation was observed in the lung of both sexes at a high incidence. The incidences of the lesion were 30.0%, 40.0%, and 66.7% in males and 20.0%, 56.7%, and 53.3% in females at 9, 18, and 31 weeks of age, respectively. In the liver, granuloma (microgranuloma) was detected frequently in both sexes and the incidences were 100%, 100%, and 96.7% in males and 96.7%, 100%, and 100% in females at 9, 18, and 31 weeks of age, respectively. In the pancreas, islet lesions, characterized by islet cell degeneration, focal hemorrhage, brown pigment deposition and

Table 3. Histopathology in male IGS rats

Organs	Findings	Age (weeks)	9	18	31
		No. of animals	30	30	30
Heart					
	Myocardial degeneration		0 (0.0)#	0 (0.0)	1 (3.3)
	Myocardial atrophy		0 (0.0)	1 (3.3)	0 (0.0)
	Fibrosis		0 (0.0)	1 (3.3)	0 (0.0)
	Lymphangiectasis		0 (0.0)	1 (3.3)	0 (0.0)
	Granuloma		1 (3.3)	3 (10.0)	10 (33.3)
	Hemangioma		0 (0.0)	1 (3.3)	0 (0.0)
Mesenteric LN					
	Granuloma		0 (0.0)	1 (3.3)	0 (0.0)
Trachea					
	Cell infiltration		0 (0.0)	0 (0.0)	2 (6.7)
Lung with bronchus					
	Vascular calcification		8 (26.7)	10 (33.3)	13 (43.3)
	Arteriosclerosis		1 (3.3)	0 (0.0)	0 (0.0)
	Intraalveolar osseous metaplasia		2 (6.7)	2 (6.7)	6 (20.0)
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	0 (0.0)
	Foam cell aggregation		9 (30.0)	12 (40.0)	20 (66.7)
	Cholesterine crystal		0 (0.0)	0 (0.0)	2 (6.7)
	Granuloma		0 (0.0)	1 (3.3)	0 (0.0)
Mandibular gland					
	Ectopic parotid gland		0 (0.0)	7 (23.3)	10 (33.3)
	Ectopic sublingual gland		0 (0.0)	1 (3.3)	0 (0.0)
Sublingual gland					
	Ectopic parotid gland		1 (3.3)	0 (0.0)	1 (3.3)
Parotid gland					
	Acinar cell degeneration		0 (0.0)	1 (3.4)a	3 (10.0)
	Acinar cell atrophy		1 (3.3)	1 (3.4)a	0 (0.0)
	Basophilic hypertrophy		0 (0.0)	1 (3.4)a	1 (3.3)
	Ductal epithelial degeneration		1 (3.3)	0 (0.0)a	0 (0.0)
	Duct hyperplasia		0 (0.0)	0 (0.0)a	1 (3.3)
	Cell infiltration		1 (3.3)	2 (6.9)a	0 (0.0)
	Lymphoid cell aggregation		1 (3.3)	1 (3.4)a	4 (13.3)
Duodenum					
	Cell infiltration		0 (0.0)	0 (0.0)	1 (3.3)
Jejunum					
	Calcification in Peyer's patch		0 (0.0)	1 (3.3)	3 (10.0)
	Lymphoid follicle hyperplasia in Peyer's patch		0 (0.0)	0 (0.0)	3 (10.0)
Cecum					
	Cell infiltration		0 (0.0)	1 (3.3)	0 (0.0)
Colon					
	Cell infiltration		0 (0.0)	1 (3.3)	0 (0.0)
Rectum					
	Submucosal edema		0 (0.0)	1 (3.3)	0 (0.0)
	Cell infiltration		0 (0.0)	1 (3.3)	0 (0.0)

#:Numbers in parentheses represent the precentages.
a:29 rats examined.

Table 3-1. Continued.

Organs	Findings	Age (weeks)	9	18	31
		No. of animals	30	30	30
Liver					
	Focal fatty change		2 (6.7)	2 (6.7)	3 (10.0)
	Periportal fatty change		0 (0.0)	0 (0.0)	1 (3.3)
	Focal necrosis		0 (0.0)	0 (0.0)	1 (3.3)
	Bile duct hyperplasia		0 (0.0)	0 (0.0)	1 (3.3)
	Atypical hypertrophy of biliary epithelium		0 (0.0)	2 (6.7)	0 (0.0)
	Fibrosis		0 (0.0)	1 (3.3)	0 (0.0)
	Hemorrhage		0 (0.0)	0 (0.0)	1 (3.3)
	Cell infiltration		0 (0.0)	1 (3.3)	1 (3.3)
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	0 (0.0)
	Granuloma		30 (100.0)	30 (100.0)	29 (96.7)
	Altered cell foci		0 (0.0)	2 (6.7)	1 (3.3)
Pancreas					
	Acinar cell degeneration		0 (0.0)	0 (0.0)	1 (3.3)
	Acinar cell atrophy		1 (3.3)	4 (13.3)	5 (16.7)
	Islet cell degeneration		0 (0.0)	4 (13.3)	0 (0.0)
	Pigment deposition		0 (0.0)	5 (16.7)	14 (46.7)
	Focal fibrosis		1 (3.3)	0 (0.0)	0 (0.0)
	Fibrosis of islet		0 (0.0)	7 (23.3)	13 (43.3)
	Hemorrhage		0 (0.0)	3 (10.0)	2 (6.7)
	Cell infiltration		0 (0.0)	6 (20.0)	4 (13.3)
	Lymphoid cell aggregation		0 (0.0)	3 (10.0)	4 (13.3)
	Fibrin exudation in islet		0 (0.0)	1 (3.3)	0 (0.0)
Kidney					
	Basophilic tubular epithelium		20 (66.7)	19 (63.3)	26 (86.7)
	Calcification		1 (3.3)	3 (10.0)	3 (10.0)
	Pelvic epithelial hyperplasia		0 (0.0)	0 (0.0)	4 (13.3)
	Epithelial hyperplasia of papilla		0 (0.0)	1 (3.3)	0 (0.0)
	Tubular dilatation		1 (3.3)	1 (3.3)	3 (10.0)
	Protein casts		2 (6.7)	5 (16.7)	9 (30.0)
	Pelvic dilatation		0 (0.0)	1 (3.3)	0 (0.0)
	Cyst		2 (6.7)	0 (0.0)	1 (3.3)
	Glomerulosclerosis		0 (0.0)	1 (3.3)	0 (0.0)
	Interstitial fibrosis		1 (3.3)	2 (6.7)	0 (0.0)
	Infarction		0 (0.0)	0 (0.0)	1 (3.3)
	Cell infiltration		0 (0.0)	4 (13.3)	1 (3.3)
	Lymphoid cell aggregation		7 (23.3)	4 (13.3)	11 (36.7)
Urinary bladder					
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	0 (0.0)
Testis					
	Tubular atrophy		0 (0.0)	0 (0.0)	6 (20.0)
	Papillary hyperplasia of rete testis		0 (0.0)	1 (3.3)	0 (0.0)
Epididymis					
	Calcification		0 (0.0)	0 (0.0)	1 (3.3)
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	1 (3.3)

Table 3-2. Continued.

Organs	Findings	Age (weeks)	9	18	31
		No. of animals	30	30	30
Prostate gland					
	Epithelial cell hyperplasia		0 (0.0)	1 (3.3)	0 (0.0)
	Cell infiltration		2 (6.7)	1 (3.3)	0 (0.0)
	Lymphoid cell aggregation		0 (0.0)	9 (30.0)	4 (13.3)
Brain					
	Calcification		0 (0.0)	1 (3.3)	0 (0.0)
	Dysplasia of parietal cortex		0 (0.0)	1 (3.3)	0 (0.0)
	Gliosis		0 (0.0)	1 (3.3)	0 (0.0)
Eye					
	Corneal degeneration		0 (0.0)	4 (13.3)	23 (76.7)
	Retinal atrophy		0 (0.0)	0 (0.0)	1 (3.3)
	Granuloma		0 (0.0)	0 (0.0)	1 (3.3)
	Persistant hyaloid artery		3 (10.0)	0 (0.0)	0 (0.0)
	Preretinal arteriolar loop		0 (0.0)	0 (0.0)	1 (3.3)
	Retinal dysplasia		0 (0.0)	3 (10.0)	2 (6.7)
	Retinal hypoplasia		0 (0.0)	0 (0.0)	2 (6.7)
Lacrimal gland					
	Acinar cell degeneration		0 (0.0)	0 (0.0)	1 (3.3)
	Acinar cell atrophy		0 (0.0)	0 (0.0)	1 (3.3)
	Lymphoid cell aggregation		6 (20.0)	3 (10.0)	9 (30.0)
Harderian gland					
	Acinar cell degeneration		0 (0.0)	0 (0.0)	2 (6.7)
	Cell infiltration		0 (0.0)	0 (0.0)	1 (3.3)
	Lymphoid cell aggregation		0 (0.0)	2 (6.7)	2 (6.7)
Pituitary					
	Hyperplastic foci		0 (0.0)a	0 (0.0)	1 (3.4)a
	Cyst		0 (0.0)a	1 (3.3)	3 (10.3)a
Thyroid					
	C-cell hyperplasia		0 (0.0)	0 (0.0)	2 (6.7)
	Ultimobranchial cyst		6 (20.0)	14 (46.7)	9 (30.0)
	Follicular cell hyperplasia		0 (0.0)	0 (0.0)	1 (3.3)
Parathyroid					
	Fibrosis		0 (0.0)c	1 (3.6)b	2 (7.4)c
Adrenal gland					
	Cortical fatty change		0 (0.0)	1 (3.3)	2 (6.7)
	Cortical nodular hyperplasia		1 (3.3)	1 (3.3)	2 (6.7)
Skeletal muscle					
	Muscular degeneration		0 (0.0)	0 (0.0)	1 (3.3)
	Muscular necrosis		0 (0.0)	2 (6.7)	0 (0.0)
	Cell infiltration		0 (0.0)	2 (6.7)	0 (0.0)
	Granuloma		0 (0.0)	0 (0.0)	1 (3.3)
Skin/subcutis					
	Cell infiltration		2 (6.7)	3 (10.0)	0 (0.0)

a:29 rats examined.
b:28 rats examined.
c:27 rats examined.

Table 4. Histopathology in female IGS rats

Organs	Findings	Age (weeks)	9	18	31
		No. of animals	30	30	30
Heart					
	Cell infiltration		0 (0.0)[#]	0 (0.0)	2 (6.7)
	Granuloma		3 (10.0)	3 (10.0)	0 (0.0)
Aorta					
	Cell infiltration		1 (3.3)	0 (0.0)	0 (0.0)
Thymus					
	Epithelial cell hyperplasia		0 (0.0)	0 (0.0)	4 (13.3)
Lung with bronchus					
	Vascular calcification		6 (20.0)	5 (16.7)	6 (20.0)
	Alveolar epithelial hyperplasia		0 (0.0)	0 (0.0)	1 (3.3)
	Intraalveolar osseous metaplasia		1 (3.3)	0 (0.0)	2 (6.7)
	Foam cell aggregation		6 (20.0)	17 (56.7)	16 (53.3)
	Granuloma		0 (0.0)	2 (6.7)	0 (0.0)
Mandibular gland					
	Acinar cell degeneration		0 (0.0)	1 (3.3)	0 (0.0)
	Ectopic parotid gland		1 (3.3)	7 (23.3)	4 (13.3)
Sublingual gland					
	Ectopic parotid gland		0 (0.0)	0 (0.0)	1 (3.3)
Parotid gland					
	Acinar cell degeneration		1 (3.3)	0 (0.0)	0 (0.0)
	Basophilic hypertrophy		0 (0.0)	1 (3.3)	0 (0.0)
	Ductal epithelial degeneration		0 (0.0)	0 (0.0)	1 (3.3)
	Calcification		0 (0.0)	0 (0.0)	1 (3.3)
	Cell infiltration		1 (3.3)	1 (3.3)	3 (10.0)
	Lymphoid cell aggregation		4 (13.3)	3 (10.0)	1 (3.3)
	Granuloma		0 (0.0)	2 (6.7)	0 (0.0)
Stomach					
	Cystic dilatation of gastric gland		0 (0.0)	1 (3.3)	0 (0.0)
	Hyperkeratosis		0 (0.0)	0 (0.0)	1 (3.3)
Jejunum					
	Calcification in Peyer's patch		0 (0.0)	0 (0.0)a	1 (3.3)
Ileum					
	Calcification in Peyer's patch		1 (3.3)	0 (0.0)	1 (3.3)
	Lymphoid follicle hyperplasia in Peyer's patch		1 (3.3)	0 (0.0)	0 (0.0)
Rectum					
	Lymphoid follicle hyperplasia in Peyer's patch		1 (3.3)	0 (0.0)	0 (0.0)
Liver					
	Focal fatty change		1 (3.3)	1 (3.3)	0 (0.0)
	Periportal fatty change		2 (6.7)	0 (0.0)	0 (0.0)
	Diffuse fatty change		1 (3.3)	0 (0.0)	0 (0.0)
	Focal necrosis		0 (0.0)	1 (3.3)	0 (0.0)
	Angiectasis		0 (0.0)	0 (0.0)	1 (3.3)
	Cell infiltration		0 (0.0)	1 (3.3)	1 (3.3)
	Granuloma		29 (96.7)	30 (100.0)	30 (100.0)
	Abscess		0 (0.0)	0 (0.0)	1 (3.3)
	Altered cell foci		0 (0.0)	0 (0.0)	2 (6.7)
Pancreas					
	Aciner cell atrophy		0 (0.0)	1 (3.3)	0 (0.0)
	Cell infiltration		0 (0.0)	3 (10.0)	0 (0.0)
	Lymphoid cell aggregation		0 (0.0)	0 (0.0)	7 (23.3)

#:Numbers in parentheses represent the precentages.
a:29 rats examined.

Table 4. Continued.

Organs	Findings	Age (weeks)	9	18	31
		No. of animals	30	30	30
Kidney					
	Basophilic tubular epithelium		15 (50.0)	14 (46.7)	7 (23.3)
	Calcification		5 (16.7)	7 (23.3)	18 (60.0)
	Calculus		0 (0.0)	1 (3.3)	3 (10.0)
	Vascular calcification		0 (0.0)	0 (0.0)	2 (6.7)
	Pelvic epithelial hyperplasia		0 (0.0)	0 (0.0)	3 (10.0)
	Tubular dilatation		3 (10.0)	1 (3.3)	0 (0.0)
	Protein casts		1 (3.3)	3 (10.0)	2 (6.7)
	Pelvic dilatation		2 (6.7)	0 (0.0)	0 (0.0)
	Cyst		2 (6.7)	0 (0.0)	0 (0.0)
	Interstitial fibrosis		1 (3.3)	1 (3.3)	0 (0.0)
	Infarction		0 (0.0)	1 (3.3)	1 (3.3)
	Cell infiltration		1 (3.3)	2 (6.7)	4 (13.3)
	Lymphoid cell aggregation		3 (10.0)	6 (20.0)	6 (20.0)
	Cell debris in pelvis		0 (0.0)	0 (0.0)	8 (26.7)
Urinary bladder					
	Epithelial desquamation		1 (3.3)	0 (0.0)	0 (0.0)
	Hemorrhage		1 (3.3)	0 (0.0)	0 (0.0)
	Edema		1 (3.3)	0 (0.0)	0 (0.0)
	Cell infiltration		1 (3.3)	0 (0.0)	0 (0.0)
	Lymphoid cell aggregation		0 (0.0)	3 (10.0)	0 (0.0)
Ovary					
	Cyst		1 (3.3)	0 (0.0)	4 (13.3)
Uterus					
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	0 (0.0)
Eye					
	Corneal degeneration		0 (0.0)	5 (16.7)	15 (50.0)
	Conjunctival calcification		0 (0.0)	0 (0.0)	2 (6.7)
	Persistant hyaloid artery		6 (20.0)	2 (6.7)	0 (0.0)
	Retinal dysplasia		5 (16.7)	0 (0.0)	0 (0.0)
Lacrimal gland					
	Lymphoid cell aggregation		1 (3.3)	3 (10.0)	4 (13.3)
Harderian gland					
	Acinar cell degeneration		0 (0.0)	0 (0.0)	2 (6.7)
	Cell infiltration		0 (0.0)	2 (6.7)	2 (6.7)
	Lymphoid cell aggregation		0 (0.0)	6 (20.0)	4 (13.3)
Pituitary					
	Dilatation of Rathke's cleft		0 (0.0)	2 (6.7)	0 (0.0)
	Cyst		1 (3.3)	1 (3.3)	2 (6.7)
Thyroid					
	C-cell hyperplasia		0 (0.0)	0 (0.0)	2 (6.7)
	Ultimobranchial cyst		18 (60.0)	18 (60.0)	11 (36.7)
	Lymphoid cell aggregation		1 (3.3)	0 (0.0)	0 (0.0)
Parathyroid					
	Fibrosis		2 (8.7)a	1 (3.8)b	10 (40.0)c
Adrenal gland					
	Cortical nodular hyperplasia		0 (0.0)	1 (3.3)	3 (10.0)
	Lymphoid cell aggregation		0 (0.0)	0 (0.0)	1 (3.3)
Skeletal muscle					
	Muscular degeneration		0 (0.0)	0 (0.0)	1 (3.3)
	Muscular necrosis		0 (0.0)	0 (0.0)	1 (3.3)
	Cell infiltration		0 (0.0)	0 (0.0)	1 (3.3)
Skin/subcutis					
	Cell infiltration		0 (0.0)	1 (3.3)	0 (0.0)
	Granuloma		1 (3.3)	1 (3.3)	0 (0.0)

a:23 rats examined.
b:26 rats examined.
c:25 rats examined.

cell infiltration around the islet, fibrin exudation in the islet, and fibrosis of the islet were detected in males at 18 and 31 weeks of age. The islet lesion revealed to progress from acute phase to chronic phase with age. The incidence of islet cell degeneration was 13.3% at 18 weeks of age. The incidences of hemorrhage, cell infiltration, pigment deposition and fibrosis of the islet were 10.0%, 20.0%, 16.7%, and 23.3% at 18 weeks of age, respectively, and were 6.7%, 13.3%, 46.7%, and 43.3% at 31 weeks of age, respectively. In the kidney, basophilic tubular epithelium was frequently observed in both sexes and the incidences were 66.7%, 63.3%, and 86.7% in males and 50.0%, 46.7%, and 23.3% in females at 9, 18, and 31 weeks of age, respectively. Ultimobranchial cyst in the thyroid occurred frequently in males and females and the incidences were 20.0%, 46.7%, and 30.0% in males and 60.0%, 60.0%, and 36.7% in females at 9, 18, and 31 weeks of age, respectively. The incidences of protein casts of the kidney in males, calcification in the medulla of the kidney in females, and corneal degeneration of the eye in both sexes increased with age. Other nonneoplastic lesions in any organs occurred infrequently and the incidences of the lesions showed no correlations with aging or sex.

DISCUSSION

The background data on organ weights and spontaneous occurring lesions were obtained from male and female IGS rats fed a low protein commercial diet (CRF-LP, 18%) for 4, 13, and 26 weeks.

A few gross lesions were observed in male and female IGS rats in the present studies, but no age- or sex- related changes were seen. Pelvic dilatation in the kidney, i.e. hydronephrosis, and supernumerary lobes in the liver have been described to be spontaneous occurring lesions in several strains of the rats including the Sprague-Dawley strain [1, 2].

Histopathologically, granuloma in the liver, foam cell aggregation in the lungs, basophilic tubular epithelium in the kidney, and ultimobranchial cyst in the thyroid of male and female IGS rats and vascular calcification in the lungs in male IGS rats were observed commonly at a high incidence ($\geq$20%) in the 4-, 13- and 26-week studies. These frequent spontaneous lesions are possible to indicate some of biological characteristics of the IGS rats. On the other hand, granuloma in the heart, vascular calcification and foam cell aggregation in the lungs, fibrosis of the pancreatic islet pigment deposition in the pancreas and protein casts in the kidney of male IGS rats, calcification in the kidney of female IGS rats, and corneal degeneration in the eye of both sexes were detected as the age-related lesions. Granuloma, i.e. microgranuloma, in the liver has been reported to occur at a high incidence in male and females IGS rats from 3 different breeding colonies of Charles River Japan, Inc. [3], being similar to the present results. Foam cell agregation in the lungs of rats has been also described as alveolar histiocytosis [2] and disclosed to be associated with serum lipid levels and aging [7, 8]. Basophilic tubular epithelium, i.e. tubular regeneration, in the kidney is considered as one of the initial changes of the nephropathy in the rats [1, 4], and protein casts and calcification are also described to develop with the progression of nephropathy [1, 4]. Ultimobranchial cyst of the thyroid is known as one of congenital lesions in the rats [1], while the reason of a high incidence in male and female IGS rats in the present studies remains to be solved. Vascular calcification, i.e. calcium deposition in subintimal and medial regions of the pulmonary artery, in the lungs is known to be one of spontaneous lesions in aging rats [4]. Corneal degeneration was characterized by deposits of basophilic fine granules and basophilic laminated plaques in the corneal epithelial basement membrane [6]. The incidence of the lesion has been reported to increase with age [9], coinciding with the present results. Granuloma in the heart is one of the spontaneous lesions detected frequently in rats regardless of the strains [1]. The lesions has been shown an age-related increase in incidence, which was higher in males than in females under the same conditions [3], coincided with the present results. Pigment deposition around the pancreatic islet has been observed as one of the spontaneous lesions in aged SD-JCL rats [5]. It is likely that islet cell degeneration, hemorrhage, pigment deposition, cell infiltration, and fibrosis of the pancreatic islet observed in the present studies may be a series of the degenerative lesions in the pancreas in male IGS rats.

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Background Data of General Toxicological Parameters in Crj:CD(SD)IGS Rats at 30 Weeks of Age

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ABSTRACT. Thirty male and thirty female Crj:CD(SD)IGS rats at 5 weeks of age were reared for 6 months to collect toxicological background data in our facility. Rats were observed for clinical signs and subjected to measurements of body weights and food consumption during the experimental period. At 30 weeks of age, rats were examined for urinalysis, hematology, and blood biochemistry, and necropsied. Organ weights were measured at necropsy. In comparison of data between males and females, a tendency of higher values in males than in females was observed on the incidence of positive urinary protein, urinary volume, urinary K and Cl excretions, RBC, PLT, WBC, PT, APTT, differential count of neutrophils, NEFA, TG, ALP, LDH, LAP, CPK, iP, and fractions of α_1-globulin and α_2-globulin. A tendency of lower values in males than in females was observed on the urinary pH, urinary Na excretion, MCV, MCH, TP, T-CHO, PL, GOT, GPT, T-BIL, Na, Cl, and fractions of albumin and γ-globulin. At necropsy, testes and epididymides were small bilaterally in 1 male in principle and absolute weights of the pituitary gland and adrenal tended to be lower in males than in females. － Key words: Crj:CD(SD)IGS, General toxicological parameters, Rats, 30 weeks of age.

CD(SD)IGS-2000: 88-94

INTRODUCTION

Crj:CD(SD)IGS rats are the experimental animals that have been developed by Charles River Inc. in response to the internationalization of new drug R & D. The authors have already reported the toxicological background data on this species and strain of animals at 9 and 13 weeks of age , which were reared under the housing condition of our facility [1]. An additional study was performed to obtain more information by rearing the animals for 6 months, and the present report describes the background data on male and female rats of this strain at 30 weeks of age.

MATERIALS AND METHODS

Animals and housing conditions: Thirty male and thirty female Crj:CD(SD)IGS rats at 4 weeks of age were supplied by Charles River Japan Inc. (Tsukuba, Japan) on March 17, 1999. Animals were quarantined and acclimated for 7 days, and were used for study when they were at 5 weeks of age. Animals were housed in metal cages (W 280 × D 370 × H 180 mm), 1 rat of either sex per cage, in a room maintained at temperature of 20 to 26℃, humidity of 35 to 75%, 10 to 15 times air-changes/hr, and a 12-hr lighting cycle (8:00 to 20:00). Pelleted food CE-2 (CLEA Japan, Inc) was given at all times, and drinking water (Ishikari-shi public water) was supplied using automatic watering nozzles. Animals were individually identified by the animal No. tattooed on the right and left ears.

Observations and examinations:

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

Table 1. Parameter, method and instrument for clinical examination

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

RESULTS

Neither deaths nor abnormal signs were found during the experimental period.

Changes in body weight are summarized in Table 2; mean body weight gain was 477 g for males and 210 g for females.

Food consumption data are summarized in Table 3; food consumption became plateau since 8 or 6 weeks of age for males or females, respectively.

Table 2. Body weight changes

Sex		Male	Female
Number of animals		30	30
5	Age (weeks)	146.9±7.0	133.0±7.3
6		208.2±11.8	168.0±10.6
7		268.3±17.9	191.1±15.2
8		328.3±22.9	216.6±20.9
9		362.1±28.0	222.9±23.6
10		394.4±33.7	238.0±30.2
11		424.6±37.5	254.0±32.5
12		443.0±40.6	260.3±31.3
13		456.0±43.8	263.4±31.0
14		478.9±45.1	272.2±33.3
15		499.7±49.0	279.7±34.3
16		514.8±49.8	285.9±34.5
17		521.9±51.7	289.4±34.3
18		532.5±54.4	293.0±33.8
19		540.7±55.6	298.0±33.2
21		566.8±60.3	310.7±34.7
23		585.2±61.7	322.3±35.4
25		600.6±63.8	327.4±36.4
27		611.7±66.5	334.4±35.9
29		623.1±68.8	340.7±38.4
30		623.4±70.2	342.7±40.0

Values are expressed as Mean±S.D., g

Table 3. Food consumption

Sex		Male	Female
Number of animals		30	30
5	Age (weeks)	17.6±2.3	15.2±1.7
6		26.1±2.2	20.9±2.3
7		24.1±3.3	16.3±2.5
8		30.1±3.1	20.6±3.2
9		27.9±3.0	16.2±2.8
10		30.2±3.7	19.7±4.9
11		28.3±3.5	20.7±4.2
12		31.4±3.6	23.6±3.6
13		31.7±3.9	22.8±3.3
14		28.0±3.5	21.1±5.4
15		27.5±3.6	19.5±3.3
16		29.7±3.7	21.0±2.7
17		29.0±4.0	20.2±3.2
18		27.6±5.1	19.3±3.0
19		29.2±3.8	20.1±3.3
21		26.4±3.5	18.6±3.0
23		26.7±3.4	19.3±2.9
25		28.4±3.2	19.6±3.2
27		26.3±2.9	18.3±2.5
29		27.4±3.2	19.1±3.2

Values are expressed as Mean±S.D., g

Results of urinalysis are summarized in Table 4. As for pH, the incidence of pH 6 was recorded in half of males (most frequent), with pH 7 and 8 less frequently in this order. For females, pH 8 was most frequently recorded in half of the animals, with pH 6 and 7 in almost the same number of animals. As for protein, positive (++) was most frequently recorded in males, with (+) and (±) less frequently in this order. For females, negative (−), positive (±), and positive (+) were found in similar incidences. Incidences of urinary positive protein tended to be higher in males than in females. As for glucose, ketone bodies, bilirubin, urobilinogen, and occult blood, there were no changes in either males or females. As for urinary volume and excretions of K and Cl, there was a tendency of being higher in males than in females; on the other hand, Na excretion tended to be higher in females than in males.

Results of hematological examinations are summarized in Table 5. Higher values in males than in females were recorded for RBC, PLT, WBC, differential count of neutrophils, PT, and APTT, and lower values in males than in females for MCV and MCH. Other parameters showed no clear differences between males and females.

Results of blood biochemical examinations are summarized in Table 6. Higher levels in males than in females were recorded for NEFA, TG, ALP, LDH, LAP, CPK, iP, and fractions of α_1-globulin and α_2-globulin, and lower levels in males than in females for TP, T-CHO, PL, GOT, GPT, T-BIL, Na, Cl, and fractions of albumin and γ-globulin. Other parameters showed no clear differences between males and females. It should be noted here that abnormally higher levels were recorded for a few parameters; 737 U/l of GOT and 439 U/l of GPT in 1 female, 1716U/l of LDH in 1 male, and 1779U/l of LDH and 877 IU/1 of CPK in 1 female.

As for necropsy findings, there were bilateral small epididymides (absolute weight: 0.36 g for right, 0.38 g for left) and testes (absolute weight: 0.40 g for right, 0.37 g for left), a vesicle in the pituitary gland, a cyst in the kidney and white mottles in the adrenal in 1 male each, and a black spot in the adrenal and hemorrhagic mottle in the mucosa of the glandular stomach in 1 female each.

Table 4. Urinalysis

Sex	Male	Female
Number of animals	30	30
pH		
6	15	8
7	11	7
8	4	15
Protein		
−(0 mg/dl)	0	10
±(10-20 mg/dl)	4	9
+(30 mg/dl)	10	10
++ (100 mg/dl)	15	1
+++ (300 mg/dl)	1	0
Glucose		
−(0 mg/dl)	30	30
Ketone bodies		
−(0 mg/dl)	30	30
Bilirubin		
−(0 mg/dl)	30	30
Urobilinogen		
±(normal)	30	30
Occult blood		
−(0 mg/dl)	30	30
Urinary volume (ml/16 hr)	9.2±4.0[a)]	6.7±3.9
Specific gravity	1.044±0.011	1.044±0.014
Electrolytes (mEq/16 hr)		
Na	0.213±0.093	0.300±0.181
K	1.408±0.207	0.803±0.187
Cl	0.418±0.144	0.312±0.134

a): Values are expressed as Mean±S.D.

Table 5. Hematology

Sex	Male	Female
Number of animals	30	30
RBC ($\times 10^6$/mm^3)	8.14±0.39	7.37±0.34
Hb (g/dl)	14.9±0.6	14.8±0.6
Ht (%)	43.7±1.9	43.8±2.0
MCV (μm^3)	53.8±2.1	59.5±1.7
MCH (pg)	18.3±1.0	20.2±0.7
MCHC (%)	34.0±0.7	33.9±0.6
PLT ($\times 10^3$/mm^3)	1015±135	964±122
Ret (‰)	15±5	14±4
WBC ($\times 10^3$/mm^3)	7.7±1.6	5.4±1.4
Differential leucocyte count (%)		
Neutropils	18.0±6.2	15.8±8.1
Basophils	0.0±0.2	0.0±0.2
Eosinophils	1.1±1.0	1.3±1.1
Monocytes	4.4±2.5	3.6±2.4
Lymphocytes	76.5±6.9	79.2±9.2
PT (sec)	14.9±1.7	11.6±0.9
APTT (sec)	21.8±2.0	17.3±2.4

a): Values are expressed as Mean±S.D.

Table 6. Blood biochemistry

Sex	Male	Female
Number of animals	30	30
TP (g/dl)	7.4±0.3	8.2±0.5
ALB (g/dl)	4.2±0.1	4.8±0.3
Protein fraction (%)		
Albumin	51.0±3.9	57.9±2.6
Globurin		
α_1	24.9±3.3	19.6±2.5
α_2	8.1±1.0	5.5±0.7
β	12.7±1.4	12.2±1.9
γ	3.3±1.5	4.9±1.1
A/G ratio	1.32±0.08	1.38±0.07
GLU (mg/dl)	110±15	111±15
T-CHO (mg/dl)	82±19	103±22
F-CHO (mg/dl)	10±4	13±5
E/T ratio	0.88±0.02	0.87±0.02
NEFA (μEq/l)	1004±268	986±160
TG (mg/dl)	76±37	49±17
PL (mg/dl)	107±23	171±37
GOT (U/l)	158±29	176±55 (29)
GPT (U/l)	42±8	62±48 (29)
ALP (IU/l)	153±29	71±24
LDH (U/l)	617±150 (29)	344±78 (29)
γ-GTP (U/l)	1.6±1.5	1.6±1.7
LAP (IU/l)	28±3	23±3
CPK (IU/l)	237±59	140±34 (29)
T-BIL (mg/dl)	0.04±0.02	0.11±0.03
BUN (mg/dl)	15.7±2.2	15.4±4.4
CRE (mg/dl)	0.67±0.05	0.68±0.05
Na (mEq/l)	147.5±1.5	162.9±2.5
K (mEq/l)	5.02±0.35	4.74±0.37
Cl (mEq/l)	109.2±1.5	117.0±1.5
Ca (mg/dl)	10.8±0.3	11.0±0.4
iP (mg/dl)	8.6±0.7	6.8±0.6

a): Values are expressed as Mean±S.D.
( ): Number of animals: An abnormally higher value statistically judged as an outlier by Smirnov's outlier test was excluded from the tabulation.

Organ weight data are summarized in Table 7 (absolute weights) and Table 8 (relative weights). Absolute and relative weights of the pituitary gland and adrenals tended to be lower in males than in females. As for other organs, absolute weights tended to be higher in males than in females, but relative weights tended to be lower in males than females.

Table 7. Absolute organ weight

Sex	Male	Female
Number of animals	30	30
Body weight at necropsy (g)	598.1±68.5	322.5±38.5
Brain (g)	2.21±0.10	2.05±0.06
Pituitary gland (mg)	14.8±2.4	20.3±5.0
Thyroids (mg)	25.1±5.6	18.6±3.7
Right salivary gland (mg)	376±49	240±26
Left salivary gland (mg)	380±47	232±24
Thymus (mg)	187±49	157±33
Heart (g)	1.57±0.17	1.04±0.10
Lungs (g)	1.48±0.16	1.10±0.10
Liver (g)	14.84±2.13	8.13±0.92
Spleen (g)	0.84±0.11	0.52±0.07
Right kidney (g)	1.74±0.20	1.01±0.11
Left kidney (g)	1.75±0.19	0.99±0.11
Right adrenal gland (mg)	26±3	33±6
Left adrenal gland (mg)	27±4	34±6
Right testis (g)	1.70±0.17 (29)	
Left testis (g)	1.70±0.12 (29)	
Right epididymis (g)	0.71±0.05 (29)	
Left epididymis (g)	0.69±0.06 (29)	
Seminal vesicle (g)	1.65±0.27	
Prostate (mg)	502±152	
Right ovary (mg)		33±10
Left ovary (mg)		35±9
Uterus (g)		0.69±0.15

Values are expressed as Mean±S.D.
( ): Number of animals: One animal with small testes and epididymides was excluded from tabulation.

Table 8. Relative organ weight

Sex	Male	Female
Number of animals	30	30
Brain (g%)	0.37±0.04	0.65±0.08
Pituitary gland (mg%)	2.5±0.4	6.3±1.5
Thyroids (mg%)	4.2±0.8	5.8±0.9
Right salivary gland (mg%)	63±8	75±10
Left salivary gland (mg%)	64±8	73±10
Thymus (mg%)	31±7	49±10
Heart (g%)	0.26±0.02	0.32±0.03
Lungs (g%)	0.25±0.02	0.34±0.03
Liver (g%)	2.48±0.18	2.53±0.19
Spleen (g%)	0.14±0.01	0.16±0.02
Right kidney (g%)	0.29±0.03	0.32±0.03
Left kidney (g%)	0.29±0.03	0.31±0.04
Right adrenal gland (mg%)	4±1	10±2
Left adrenal gland (mg%)	5±1	11±2
Right testis (g%)	0.29±0.04 (29)	
Left testis (g%)	0.29±0.03 (29)	
Right epididymis (g%)	0.12±0.02 (29)	
Left epididymis (g%)	0.12±0.02 (29)	
Seminal vesicle (g%)	0.28±0.06	
Prostate (mg%)	85±29	
Right ovary (mg%)		10±3
Left ovary (mg%)		11±3
Uterus (g%)		0.22±0.05

Values are expressed as Mean±S.D.
( ): Number of animals: One animal with small testes and epididymides was excluded from tabulation.

DISCUSSION

Male and female Crj:CD(SD)IGS rats were reared for 6 months until the animals were 30 weeks of age for collecting toxicological background data. There were neither abnormal clinical signs nor changes in body weight or food consumption. As for urinalysis, higher incidences of positive protein were found in males than in females. As for hematological parameters, PT and APTT tended to be lower in females than in males with a tendency similar to that observed in the results at 13 weeks of age [1]. As for blood biochemical parameters, abnormal increases in GOT and GPT were found in an animal. Elevation in these enzyme activities is reported not necessarily to accompany necrosis of the hepatocytes but to occur from unknown causes relating to aging of animals or effect of food [2]. Although there are some variations in blood biochemical data depending on the strain or age of rats, it is generally known that ALP, GLU, and TG are higher and PL is lower in males than in females [3]. In the present study, the same tendency was confirmed except for GLU. In addition, fractions of albumin and γ-globulin were higher and fractions of α_1-globulin and α_2-globulin were lower in females than in males. At necropsy, there were a vesicle in the pituitary gland, a cyst in the kidney, small testes and epididymides, and white mottles in the adrenal in 1 male each, and a black spot in the adrenal and hemorrhagic mottle in the stomach in 1 female each. Absolute organ weights tended to be higher in males than in females, but absolute weights of the pituitary gland and adrenals were higher in females than in males.

Results of the present study at 30 weeks of age were compared with those of the previous study at 13 weeks of age for assessing effects of aging. As for urinalysis, there were higher incidences of positive urinary protein in males and lower urinary volume with higher Cl excretion in males and femals. Urinary protein is generally known to increase with age and the positive incidence to increase with aging [4.5]. As for the hematological parameters, there were higher RBC in males, lower PL in females, higher Ht, differential count of neutrophils, and APTT in males and females, and lower Ret and differential count of lymphocytes in males and females. As for blood biochemical parameters, there were higher TG in males, higher TP and Na in females, higher T-CHO, F-CHO, NEFA, PL, GOT, GPT, LDH, γ-GTP, and CPK in males and females, and lower GLU, ALP, LAP, and albumin fraction in males and females. As for absolute organ weights, there were higher epididymides weight and lower seminal vesicle weight in males, higher liver weight and lower thymus weight in males and females, and lower ovary weight and higher uterus weight in females. Relative weights were lower in all organs for both males and females.

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A Significant Relationship between Glutathione-Peroxidase (GSH-PO) Localization and Biological Action of Testosterone in Rat Ventral Prostate

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ABSTRACT. In order to confirm the relationship between glutathione-peroxidase (GSH-PO) localization and biological testosterone action in the rat ventral prostate, immunocytochemical localization of GSH-PO in glandular epithelial cells of the rat ventral prostate was investigated. In the untreated group, GSH-PO was predominantly demonstrated in glandular epithelial cells of the ventral prostate. Intracellular localization of GSH-PO in the glandular epithelial cells was mainly observed in cytoplasmic matrix near the rough endoplasmic reticulum and was occasionally noted as a small granular structure (GSH-PO-positive granule) at the supranuclear region. In a castrated animal, the intensity of GSH-PO staining in the glandular epithelial cells was remarkably decreased. By testosterone administration to the castrated animal, GSH-PO was clearly detected in the glandular epithelial cells. Intracellular localization of GSH-PO was mainly observed in cytoplasmic matrix and the number of GSH-PO-positive granules increased remarkably. These findings suggest that immunostainable GSH-PO in the glandular epithelial cells of the rat ventral prostate is testosterone-dependent, and that its staining pattern is a useful marker for biological testosterone action. — Key words: Glutathione-peroxidase (GSH-PO), Prostate, Lipid peroxidation, Testosterone, Castration

CD(SD)IGS-2000: 95-98

INTRODUCTION

It has been well documented that lipid peroxidation is the reaction of oxidative deterioration of lipids and peroxidation involves the direct action of oxygen and lipid to form free radical intermediates and to produce semistable peroxides [7]. Thus, free radicals result in the lipid peroxidation and damage of cellular membrane with release of intracellular components, e.g., lysosomal enzymes, leading to further tissue damage and even tumor-promoting [6, 7, 10, 23]. GSH-PO has been well recognized to effectively reduce lipid peroxides. A large number of studies have been reported on the role fulfilled by GSH-PO in the protection of various tissues against lipid peroxidation [13-18, 29]. Furthermore, the GSH-PO staining was modified by lipoperoxidative change in the tissue or cells. Therefore, we proposed that the pattern of GSH-PO staining should be a sensitive and specific indicator of oxidative damage in tissues or cells [13-18, 29]. Thus, the staining pattern of GSH-PO is thought to be a useful marker for lipid peroxidation in the cells.

In the present study, in order to reconfirm the biological significance of GSH-PO in the prostate, immunocytochemical GSH-PO localization in the prostate of nornal, castrated and testosterone-administered rats was investigated.

MATERIALS AND METHODS

Animal and tissue preparation: Male Crj:CD (SD) IGS rats were purchased from Charles River Japan Inc. (Atsugi, Japan) at the age of seven weeks. The animals were kept in a barrier-sustained animal room, which was maintained at a temperature of 22±2℃ with a relative humidity of 55±15%. The room was ventilated twenty-one times per hr and provided with 12-hr of light (from 8:00 to 20:00). Pelleted diet (CE-2, CLEA Japan Inc.) and tap water were given *ad libitum*. The animals were kept for a one-week acclimation period under laboratory conditions.

Five animals served as controls (group 1). In group 2, five rats were sacrificed two days after castration. In groups 3 and 4, five rats were subcutaneously administered 1 mg/animal of testosterone-propionate (testosterone, Sigma Chemical Co., St. Louis, MO) daily for 3 or 7 days after two days of castration, respectively. Testosterone was dissolved in dimethyl sulfoxide. Each rat was killed by decapitation and the ventral prostates were removed immediately.

Histopathological examination: The ventral prostates were fixed in 0.1 M phosphate-buffered 10% formalin, embedded in paraffin, mounted and stained with hematoxylin and eosin (HE).

Immunocytochemical staining of GSH-PO: The ventral prostates were fixed in periodate-lysine-4% paraformaldehyde solution [11] for 4 to 6 hr at 4℃ under constant agitation. The fixed tissues were then washed in 0.01 M phosphate-buffered saline (PBS) containing sucrose from 10 to eventually 20% overnight at 4℃. Subsequently, 6μm-thick frozen sections were prepared from the washed tissues in a cryostat, and were placed on albumin-coated glass slides. The sections were washed in 0.01 M PBS and then were stained by the direct peroxidase-labeled antibody method using rabbit anti-rat GSH-PO polyclonal antibody IgG Fab fragment [19].

For light microscopic observations of GSH-PO, 6μm-thick frozen sections were incubated with the antibody labeled with horseradish peroxidase (HRPO, Sigma Chemical Co., St. Louis., MO) for l hr. After the incubation was completed, the sections were treated in Graham-Karnovsky's reaction medium [8], which contained 0.2% 3, 3'-diaminobenzidine (DAB, Wako Pure Chemical Industries, Osaka) and 0.05% hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6, for 5 to 10 min at room temperature. Then the sections were counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer, pH 4.2.

For electron microscopic observations of GSH-PO, 6μm-thick frozen sections were incubated with HRPO-labeled antibody for 6 hr. After the incubation was completed, the sections were incubated for 30 min in Graham-Karnovsky's reaction medium [8], from which the substrate hydrogen peroxide was omitted, and then they were incubated in the fully equipped reaction medium for 5 min. The sections were post-fixed in 2% OsO_4 in 0.1 M phosphate buffer, pH 7.4, for 90 min, dehydrated in graded ethanol series, and embedded in Quetol 812 by an inverted gelatin

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capsule method. Ultrathin sections were prepared with LKB ultra-microtome and were observed under a JOEL 1200 EX electron microscope.

As an immunologic negative control, normal rabbit serum (NRS) IgG Fab fragment labeled with HRPO was applied in both light and electron microscopic investigations instead of an anti-GSH-PO IgG Fab fragment labeled with HRPO.

RESULTS

Group 1 (Intact control): The glandular epithelium of the prostate consisted of single-layered, cylindrical cells which showed pronounced eosinophilic staining of the cytoplasm, and the basal location of nuclei. Infrequently, papillary projections of the glandular epithelial cells into the acinar lumen were also noted. Immunocytochemical localization of GSH-PO was predominantly observed in the glandular epithelial cells. No reaction products were seen in interstitial tissues. The control serum (NRS) was negative for immunocytochemical localization of GSH-PO in the rat ventral prostate. By immunoelectron microscopic investigations, GSH-PO was noted in cytoplasmic matrix near the rough endoplasmic reticulum with dilated cisternae and mitochondria (Photo 1A). Occasionally, GSH-PO was noted as small granular structure (GSH-PO-positive granule) at the supranuclear region (Photo 1A). No reaction products were seen in other cell organelles, including mitochondria or endoplasmic reticulum.

Group 2 (Castration): Two days after castration, the height of the glandular epithelial cells was slightly reduced. The intensity of GSH-PO staining was markedly decreased. In immunoelectron microscopic examinations, GSH-PO was noted in cytoplasmic matrix. Furthermore, GSH-PO-positive granules were rarely seen.

Group 3 (Castration plus Testosterone 3 days): By testosterone administration to the castrated animals, the height of the glandular epithelial cells was slightly increased. GSH-PO was intensely stained in the glandular epithelial cells. In immunoelectron microscopic examinations, GSH-PO was observed in cytoplasmic matrix near well-developed rough endoplasmic reticulum or mitochondria (Photo 1B). Furthermore, GSH-PO-positive granules were also noted (Photo 1B).

Group 4 (Castration plus Testosterone 7 days): The glandular epithelial cells were hypertrophic and showed an increased number of papillary projections extendng into acini. GSH-PO was intensely stained in the glandular epithelial cells. The intensity of GSH-PO staining was stronger than that of group 3. By immunoelectron microscopic examinations, GSH-PO-positive granules increased remarkably compared with those of other experimental groups (Photo 1C).

DISCUSSION

In the present study, the intensity of GSH-PO staining in the glandular epithelial cells remarkably decreased after castration, and recovered by administration of testosterone to the castrated rats.

It is well known that the metabolic and secretory activities of the prostate are regulated by testosterone [24]. The secretory acid phosphatase in the glandular epithelial cells of the prostate appears to be testosterone-dependent, since it disappears after castration and is restored after treatment with testosterone [24]. Therefore, an enzyme histochemical and immunohistochemical staining of acid phosphatase has been thought to be a good marker of andogen action in the rat prostate. The present data and the facts reported on the immunohistological features of GSH-PO in the rat prostate indicate a close relationship to the secretory or metabolic status of the glandular epithelial cells.

In the present study, intracellular localization of GSH-PO in the glandular epithelial cells of the rat ventral prostate was mainly observed in cytoplasmic matrix near the rough endoplasmic reticulum. Occasionally, GSH-PO was noted as small granular structures (GSH-PO-positive granule) at the supranuclear region. Furthermore, we found that GSH-PO-positive granules were clearly restored by the treatment with testosterone to the castrated rats after its loss by castration. In addition, the number of GSH-PO-positive granules remarkably increased. In our previous reports [14, 16, 28], immunocytochemical localization of GSH-PO in the rat testicular interstitial macrophages and peritoneal macrophages was demonstrated. As a result, the number of GSH-PO-positive granules was increased by testosterone stimulation, i.e. testicular interstitial macrophages with gonadotropin administration to the hypophysectomized rats and peritoneal macrophages in a culture medium containing testosterone. Thus, GSH-PO-positive granules suggests the very close relationship to the status of testosterone action to the cells. Therefore, it was strongly suggested that GSH-PO-positive granules in the glandular epithelial cells of the rat ventral prostate were considered to be testosterone-dependent.

Amuller *et al.* [1] reported that at the subcellular level a hyperfunction of the rat prostate was reflected by an increase in the amount of rough endoplasmic reticulum, the size of the Golgi apparatus, in the number of Golgi vesicles, and in the number, size, and electron density of secretory granules and, in addition to the high secretory activity of the glandular epithelial cells, the turnover of biomembrane was enhanced as deduced from the large number of lysosomal structures and immature residual bodies. In this regard, lipid peroxidation may occur in the microsomes including rough endoplasmic reticulum. In fact, the microsomal membranes contain a relatively large amount of polyunsaturated fatty acid in their phospholipid, and the microsomes are very liable to lipid peroxidation and concurrent damage [22, 27]. Therefore, prostate GSH-PO might play an important role in prevention of damage to the microsome including rough endoplasmic reticulum. In addition, we further speculated that lipid peroxidation could continue even during the process of degradation within the lysosome-like structure. Therefore, GSH-PO-positive granules are thought to be a degradation process of the peroxidized materials.

The significant increase of GSH-PO protein triggered by lipid peroxides has been demonstrated in an experimental system of inactivation and reactivation of the arachidonate cascade in rat peritoneal macrophages [28]. These findings indicate that increased levels of lipid peroxides (or peroxidation) enhance the expression of GSH-PO, or, in other words, a decrease in lipid peroxides likely reduces the expression of the enzyme.

Lowered lipid peroxidation in cancer cells has been detailed by several investigators [2, 25] and has been related to changes in fatty acid composition [3]; anti-oxidants such as vitamin E [3] and a related form of glutathione [5]; and enzyme activities of lipid peroxide-scavenging enzymes, including GSH-PO [4, 9]; catalase [4]; superoxide dismutase [4, 21] and glutathione-S-transferase [12, 20]. Therefore, the suppressed expression of GSH-PO in cancer may be related to the low amount of lipid peroxides within the cell. The relationship between GSH-PO expression and testosterone stimulation of the prostatic glandular epithelial cells requires further study.

Although the activities of numerous enzymes in the prostate are stimulated by androgen, immunocytochemical observations of GSH-PO have not been performed. In this regard, immunocytochemical localization of GSH-PO in the glandular epithelial cells of the rat ventral prostate may well reflect the functional state of the cells, especially biological testosterone action. Therefore, the GSH-PO staining pattern is thought to be a useful marker for androgen action such as testosterone on the prostate.

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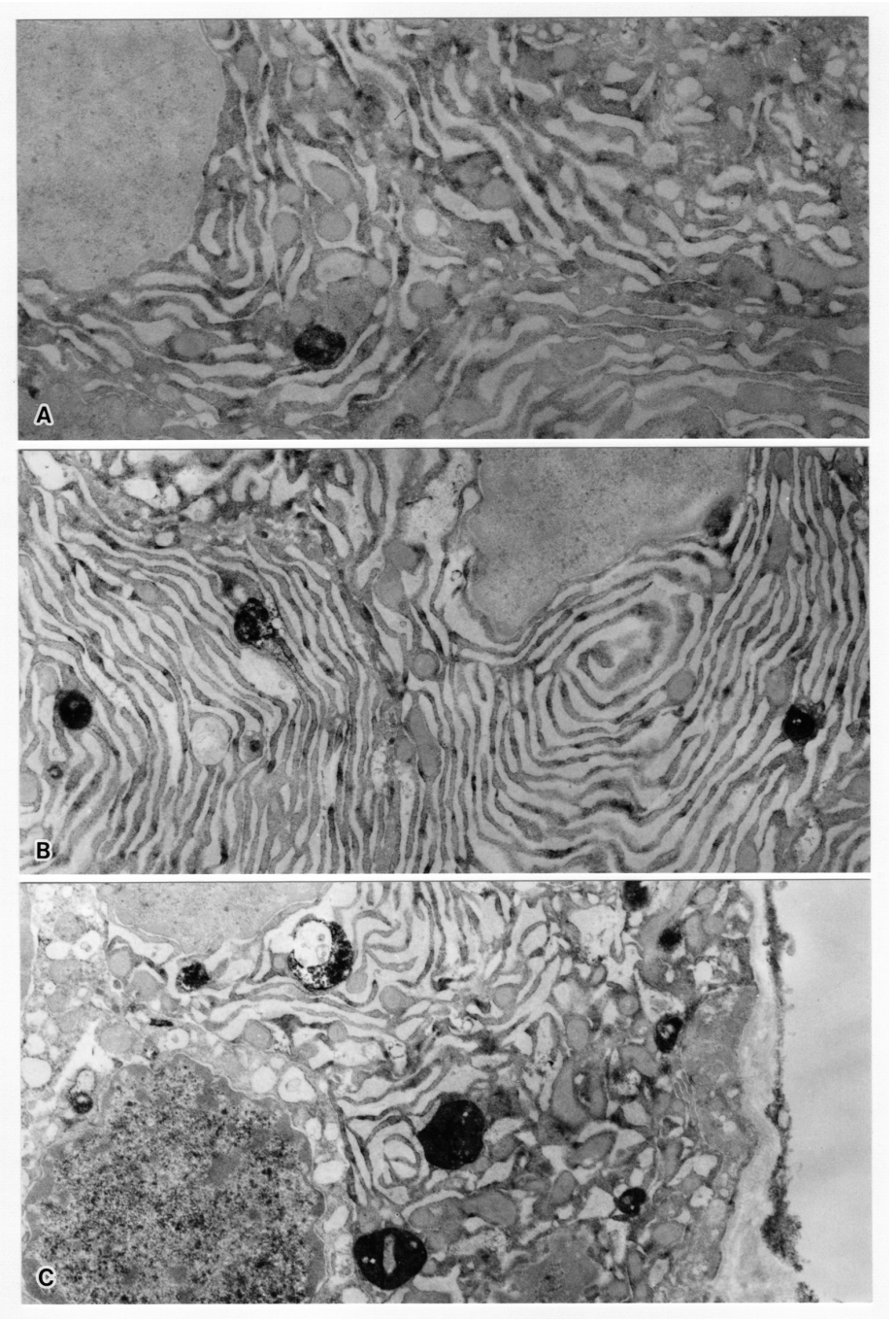

Photo 1. Immunocytochemical localization of GSH-PO in the glandular epithelial cells of control rat ventral prostate (A) and testosterone administration for 3 days (B) or 7 days (C) to the castrated rat. GSH-PO is observed in cytoplasmic matrix near the rough endoplasmic reticulum. Furthermore, GSH-PO-positive granules are also noted. x 15,000 (A-C).

Toxicological Data on Crj:CD(SD) IGS and Crj:CD(SD) Strain Rats-A Comparison of Clinical Pathology and Histopathology

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ABSTRACT. The historical data obtained from Crj:CD(SD)IGS and Crj:CD(SD) strain rats used in the control groups of toxicity studies were compared. In urinalysis, lower values of urinary volume, and of Na, K, and Cl concentrations (mEq/day) were obtained in male and female IGS rats than in SD rats. In blood chemistry, ALP values in male and female IGS rats were higher, and CRE values and A/G ratios tended to be lower than in SD rats. There were no apparent differences in hematological parameters, organ weights, or histopathological findings between IGS and SD rats. — Key words: Crj:CD(SD)IGS, Crj:CD(SD), Rats, Clinical parameters and histopathological findings

CD(SD)IGS-2000: 99-104

INTRODUCTION

Crj:CD(SD)IGS strain rats were developed by Charles River Inc. to eliminate international differences in the Crj:CD(SD) strain. We have been using Crj:CD(SD)IGS strain rats in toxicology studies. In this study, we compared the data from urinalysis parameters, hematological parameters, blood chemical parameters, organ weights, and histopathological findings between Crj:CD (SD) IGS strain rats and Crj:CD(SD) strain rats.

MATERIALS AND METHODS

Animals: Crj:CD(SD)IGS (IGS hereafter) strain rats, four-week-old, were obtained for separate toxicology studies conducted from 1997 to 1999 from Charles River Japan Inc. (Hino Breeding Center, Shiga, Japan). Crj:CD(SD)(SD hereafter) strain rats, four-week-old, were obtained from the same supplier for separate toxicology studies from 1994 to 1996. The animals were quarantined and acclimated for about 2 weeks. During this period, animals showing healthy and favorable growth were selected for the toxicology studies. Test animals were assigned to the control groups by stratified randomization so as to achieve similar mean body weights in each group. The animals were identified by ear punching.

Housing conditions: The animals were housed individually in suspended stainless steel cages (W:240 × D:380 × H:200 mm) in the SPF Animal Facility. Temperature and relative humidity in the animal rooms were set at 20 ～ 24℃ and 40 ～ 70%, respectively, with air changes of 12 times/hour (all fresh air) , and a 12hr artificial light cycle (6:00 to 18:00). The animal rooms were cleaned daily, and cages were replaced by sterilized ones at least once every 2 weeks. All the animals were allowed free access to tap water and the standard laboratory animal diet (CRF-1, Oriental Yeast Co., Tokyo, Japan).

Urinalysis: 24-hour urine collected using a metabolic cage under the usual conditions was used for the following examinations. Urinary volume was measured using a measuring cylinder, specific gravity was measured with a reflective index (specific gravity meter, Uripet- Ⅱ D, Nikon), and sodium (Na), potassium (K), and chlorine (Cl) were measured using an automated electrolyte analyzer (EA04, A&T Co., Ltd.).

Hematological examination: The rats were sacrificed at 9～11 weeks of age, respectively. Blood was collected from the abdominal aorta under sodium pentobarbital anesthesia. Red blood cell counts (RBC), white blood cell counts (WBC), platelet counts (PLT), hemoglobin concentration (HGB), hematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined or calculated using an automated hematology analyzer (Sysmex K-4500, Sysmex Co., Ltd.) with EDTA-2K as an anticoagulant. Differential white blood cell counts (May-Giemsa staining) and reticulocytes (RET, Brecher's method) were counted under a microscope with EDTA-2K as an anticoagulant. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration (FIB) were measured using an automated coagulation analyzer (Coagmaster ‡U, Sankyo Co., Ltd.) with citric acid sodium as an anticoagulant.

Blood chemical analysis: Blood samples were collected from the abdominal aorta at the time of blood collection for the hematological examination. Serum obtained from the blood by centrifugation (at about 4℃ and 3000 r.p.m. for 15 minutes) was divided into a part for measurement and a part for storage at −80 ℃ until analysis. Methods for the determination of serum biochemical parameters are shown in Table 1.

Organ weight measurement: After blood sampling, animals were sacrificed by exsanguination from the abdominal aorta. The following organs were weighed (paired organs were weighed together), and the relative organ weight, i.e., the ratio of each organ weight to the body weight measured before necropsy, was calculated:the brain (cerebrum, cerebellum, and medulla oblongata), pituitary, salivary glands (sublingual glands and submandibular glands), thyroids (parathyroids included), thymus, lungs, heart, liver, spleen, kidneys, adrenals, testes, prostate, ovaries, and uterus.

Histopathological examination: The following organs and tissues were fixed in 20% neutral buffered formalin (the eyeballs were fixed in glutaraldehyde-formalin for about 2 ～ 3 hours and then fixed again in 20% neutral buffered formalin): the heart, thoracic aorta, lungs, trachea, liver, pancreas, tongue, salivary glands (sublingual glands and submandibular glands), digestive tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum), thymus, spleen, mesenteric lymph nodes, kidneys, urinary bladder, testes, epididymides, prostate, ovaries, uterus, vagina, mammary glands, pituitary, adrenals, thyroid glands, parathyroid glands, brain (cerebrum, cerebellum, and medulla ob-

longata), spinal cord, sciatic nerve, skin, eyeballs, Harderian glands, bones with bone marrow (sternum and femur), and skeletal muscle. The organs and tissues were embedded in paraffin according to the usual methods for H.E. staining , and the specimens were examined histopathologically.

Statistical methods: Statistical analysis was not performed.

RESULTS AND DISCUSSION

Urinalysis: Lower values of urinary volume, and of Na, K, and Cl concentrations (mEq/day) were obtained in male and female IGS rats than in SD rats (Table 2-1 and 2-2). Similar tendencies have also been reported elsewhere [1]. There were no differences in other urinalysis parameters between IGS and SD rats.

Hematological examination: There were no apparent differences common to either sex or in measurement points between IGS and SD rats (Table 3-1 and 3-2). It has been reported that PT was shorter and APTT longer in male and female IGS rats than in SD rats [1, 2]. However, we did not see any such tendencies in our study.

Blood chemistry: ALP values in male and female IGS rats were higher, and CRE values and A/G ratios tended to be lower than in SD rats at measurement points (Table 4-1 and 4-2); this has not been reported by other authors. It is considered that these differences are due to differences in the measurement instruments used.

Organ weights: There were no apparent differences in organ weights between IGS and SD rats (Table 5-1 and 5-2), as has also been reported before [1].

Histopathological findings: There were no characteristic lesions in IGS rats when compared to SD rats (Table 6-1 and 6-2), as has also been reported by other authors [1, 3, 4].

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Table 1. Methods used for the blood chemical analysis

Parameters	Crj:CD(SD)IGS	Crj:CD(SD)
AST	JSCC standard method	UV rate
ALT	JSCC standard method	UV rate
ALP	JSCC standard method	p-Nitrophenylphosphate
T-Cho	Cholesterol oxidase・peroxidase	Cholesterol oxidase・peroxidase
TG	Glycerol-3-phosphate oxidase・peroxidase	Glycerol-3-phosphate oxidase・peroxidase
TP	Biuret	Biuret
Alb	Calculation ; TP×Alb ratio	Calculation ; TP×Alb ratio
Protein fraction	Electrophoresis	Electrophoresis
UN	Urease・glutamate dehydrogenase	Urease・glutamate dehydrogenase
CRE	Creatininase	Jaffe
T-Bil	Azobilirubin	Azobilirubin
Glu	Hexokinase・G-6-PDH	Glucose dehydrogenase
IP	Purine nucleotide phosphorylase・XDH	Molybdenum blue
Ca	o-Cresolphthalein complexone	o-Cresolphthalein complexone
Na	Ion selective electrode	Ion selective electrode
K	Ion selective electrode	Ion selective electrode
Cl	Ion selective electrode	Coulometric titration

JSCC:Japan Socitey of Clinical Chemistry, XDH:Xanthine dehydrogenase
G-6-PDH:Glucose-6-phosphate dehydrogenase

Table 2-1. Urinalysis of Crj:CD(SD)IGS (10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
U.V.	mL	33	11.3±6.33	7.9±3.71
S.G.		33	1.051±0.0149	1.051±0.0211
Na	mEq/L	33	136.8±57.4	101.6±62.6
K	mEq/L	33	258.5±80.8	228.2±109.7
Cl	mEq/L	33	185.9±72.6	141.8±76.06
Na	mEq/day	33	1.46±0.779	0.73±0.479
K	mEq/day	33	2.68±1.15	1.55±0.817
Cl	mEq/day	33	1.90±0.877	0.99±0.565

N : Number of animals

Table 2-2. Urinalysis of Crj:CD(SD) (8～10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
U.V.	mL	56	13.0±4.0	11.1±4.3
S.G.		56	1.052±0.011	1.046±0.014
Na	mEq/L	33	156.0±49.6	119.0±56.0
K	mEq/L	33	265.5±65.2	215.8±77.7
Cl	mEq/L	33	186.5±63.9	138.6±70.2
Na	mEq/day	33	2.00±0.61	1.13±0.56
K	mEq/day	33	3.42±0.82	2.08±0.83
Cl	mEq/day	33	2.36±0.73	1.30±0.67

N : Number of animals

Table 3-1. Hematological examination of Crj:CD(SD)IGS (10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
WBC	10^2/mm^3	44	78±22.5	54±18.5
RBC	10^4/mm^3	44	732±40.7	717±34.1
HGB	g/dl	44	14.7±1.31	14.4±0.68
HCT	%	44	43.2±2.10	41.7±1.93
MCV	fl	44	59.1±1.48	58.2±1.45
MCH	pg	44	20.1±1.29	20.2±0.54
MCHC	g/dl	44	34.1±2.11	34.6±0.57
PLT	10^4/mm^3	44	100.6±12.32	105.5±16.01
PT	sec	44	14.7±1.04	14.6±0.66
APTT	sec	44	25.4±2.73	23.1±1.58
FIB	mg/dl	44	249.6±27.30	192.7±14.4
RET	‰	44	34±7.5	27±6.0
Leucocytic Hemogram				
L	%	44	92±4.5	92±4.0
N(stab.)	%	44	7±4.0	7±4.1
E	%	44	0±0.6	0±0.6
B	%	44	0±0.0	0±0.0
M	%	44	1±0.6	0±0.6

N : Number of animals

Table 3-2. Hematological examination of Crj:CD(SD) (9～10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
WBC	10^2/mm^3	25	75±14.4	59±18.1
RBC	10^4/mm^3	25	758±28.6	748±35.3
HGB	g/dL	25	14.6±0.53	14.2±0.57
HCT	%	25	43.6±1.95	41.8±1.84
MCV	fL	25	57.6±1.73	55.9±1.30
MCH	pg	25	19.3±0.63	19.0±0.39
MCHC	g/dL	25	33.6±0.77	34.0±0.71
PLT	10^4/mm^3	25	105.0±11.80	99.8±9.47
PT	sec	25	14.8±0.88	15.0±1.00
APTT	sec	25	28.8±3.16	25.4±2.39
FIB	mg/dL	20	249.5±17.18	205.3±17.10
RET	‰	25	32±5.6	23±4.9
Leucocytic Hemogram				
L	%	25	93±3.4	94±4.1
N	%	25	6±3.0	6±3.7
E	%	25	0±0.6	0±0.6
B	%	25	0±0.0	0±0.0
M	%	25	0±0.7	0±0.6

N : Number of animals

Table 4-1. Blood chemical analysis of Crj:CD(SD)IGS (10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
AST	IU/ L	33	89.9±18.72	79.6±13.36
ALT	IU/ L	33	36.8±8.87	33.8±10.53
ALP	IU/ L	33	898.6±158.26	604.6±131.57
T-Cho	mg/dL	33	65.3±10.68	68.1±10.46
TG	mg/dL	33	87.3±29.12	35.4±19.67
T-Bil	mg/dL	33	0.07±0.027	0.09±0.021
UN	mg/dL	33	18.4±2.80	17.5±2.4
CRE	mg/dL	33	0.23±0.031	0.25±0.035
IP	mg/dL	33	7.2±1.39	6.1±0.99
Ca	mg/dL	33	9.8±0.29	9.8±0.27
Glu	mg/dL	33	150.4±12.47	150.8±14.86
Na	mEq/L	33	144.2±1.99	144.3±1.33
K	mEq/L	33	4.7±0.26	4.3±0.25
Cl	mEq/L	33	105.6±1.97	107.6±1.69
TP	g/dL	33	5.3±0.15	5.5±0.23
A/G		33	1.12±0.110	1.36±0.089
alb	%	33	52.6±2.61	57.5±1.60
α 1-glb	%	33	20.7±1.85	17.4±1.21
α 2-glb	%	33	7.6±0.64	6.7±0.51
β -glb	%	33	16.7±1.59	15.4±0.82
γ -glb	%	33	2.5±0.63	3.0±0.63
Alb	g/dL	33	2.81±0.164	3.14±0.145

N : Number of animals

Table 4-2. Blood chemical analysis of Crj:CD(SD) (9～10 Weeks)

			Male	Female
Exam.item	Unit	N	Mean±S.D.	Mean±S.D.
AST	IU/L	25	76.0±14.13	68.4±11.26
ALT	IU/L	25	28.3±5.57	25.2±8.15
ALP	IU/L	25	218.0±61.42	149.1±54.90
T-Cho	mg/dL	25	65.2±9.18	62.5±12.80
TG	mg/dL	25	152.2±47.12	59.0±27.56
T-Bil	mg/dL	25	0.05±0.010	0.06±0.011
UN	mg/dL	25	18.2±1.94	16.5±3.30
CRE	mg/dL	25	0.43±0.038	0.40±0.031
IP	mg/dL	25	7.0±0.80	6.9±1.08
Ca	mg/dL	25	10.1±0.31	10.3±0.33
Glu	mg/dL	25	149.5±10.75	143.0±8.15
Na	mEq/L	25	146.9±3.57	146.9±3.78
K	mEq/L	25	4.69±0.317	4.38±0.219
Cl	mEq/L	25	105.3±2.13	107.1±2.52
TP	g/dL	25	5.4±0.30	5.6±0.30
A/G		25	1.32±0.125	1.66±0.134
alb	%	25	56.8±2.30	62.2±1.85
α 1-glb	%	25	19.0±2.74	16.0±1.60
α 2-glb	%	25	5.1±0.88	3.3±0.97
α 3-glb	%	25	5.4±0.39	5.0±0.56
β -glb	%	25	11.6±0.67	11.0±0.86
γ -glb	%	25	2.0±0.61	2.4±0.65
ALB	g/dL	25	3.06±0.189	3.46±0.245

N : Number of animals

Table 5-1. Organ weights (Male)

		Crj:CD(SD)IGS (9～11Weeks)		Crj:CD(SD) (9～10Weeks)	
Organ	Unit	N	Mean±S.D.	N	Mean±S.D.
Body weight	(g)	50	356.2±30.9	53	361.0±37.6
Brain	(g)	43	1.949±0.080	53	1.938±0.078
	(g%)	43	0.551±0.047	53	0.541±0.049
Pituitary	(mg)	38	12.45±1.85	33	12.51±1.67
	(mg%)	38	3.49±0.42	33	3.36±0.33
Salivary glands	(g)	26	0.617±0.079	33	0.625±0.066
	(g%)	26	0.168±0.019	33	0.169±0.018
Thyroids	(mg)	38	19.69±4.36	33	17.83±2.91
	(mg%)	38	5.53±1.16	33	4.82±0.78
Thymus	(g)	45	0.514±0.107	53	0.603±0.137
	(g%)	45	0.146±0.036	53	0.167±0.033
Lungs	(g)	33	1.218±0.083	33	1.205±0.113
	(g%)	33	0.334±0.023	33	0.325±0.028
Heart	(g)	45	1.214±0.103	53	1.194±0.138
	(g%)	45	0.341±0.026	53	0.332±0.035
Liver	(g)	50	13.155±2.257	53	13.019±2.762
	(g%)	50	3.684±0.495	53	3.581±0.516
Spleen	(g)	45	0.699±0.090	53	0.694±0.109
	(g%)	45	0.196±0.022	53	0.192±0.024
Kidneys	(g)	50	2.590±0.212	53	2.605±0.225
	(g%)	50	0.729±0.044	53	0.725±0.054
Adrenals	(mg)	43	50.47±7.87	53	52.56±7.24
	(mg%)	43	14.19±1.93	53	14.66±2.18
Testes	(g)	43	2.943±0.248	53	3.074±0.196
	(g%)	43	0.831±0.090	53	0.859±0.086
Prostate	(g)	26	0.447±0.100	33	0.472±0.130
	(g%)	26	0.121±0.023	33	0.128±0.037

N : Number of animals
g, mg : Absolute organ weight
g%, mg% : Relative organ weight

Table 5-2. Organ weights (Female)

		Crj:CD(SD)IGS (9～11 Weeks)		Crj:CD(SD) (9～10 Weeks)	
	Unit	N	Mean±S.D.	N	Mean±S.D.
Body weight	(g)	44	231.8±29.3	45	219.1±18
Brain	(g)	37	1.819±0.073	45	1.811±0.06
	(g%)	37	0.795±0.089	45	0.832±0.06
Pituitary	(mg)	32	14.83±3.58	25	12.80±2.1
	(mg%)	32	6.34±1.08	25	5.83±0.9
Salivary glands	(g)	20	0.449±0.050	25	0.412±0.05
	(g%)	20	0.187±0.032	25	0.187±0.02
Thyroids	(mg)	32	14.57±2.93	25	13.38±3.1
	(mg%)	32	6.39±1.68	25	6.06±1.2
Thymus	(g)	39	0.443±0.074	45	0.486±0.08
	(g%)	39	0.193±0.038	45	0.222±0.03
Lungs	(g)	27	1.009±0.110	25	0.922±0.11
	(g%)	27	0.423±0.050	25	0.419±0.04
Heart	(g)	39	0.831±0.106	45	0.795±0.08
	(g%)	39	0.358±0.024	45	0.364±0.04
Liver	(g)	44	8.199±1.661	45	7.750±1.20
	(g%)	44	3.523±0.449	45	3.535±0.45
Spleen	(g)	39	0.535±0.110	45	0.504±0.07
	(g%)	39	0.231±0.036	45	0.230±0.03
Kidneys	(g)	44	1.740±0.184	45	1.673±0.13
	(g%)	44	0.756±0.082	45	0.766±0.06
Adrenals	(mg)	37	65.76±8.45	45	62.19±6.4
	(mg%)	37	28.68±4.33	45	28.49±3.0
Ovaries	(mg)	27	83.38±15.32	45	89.07±14.7
	(mg%)	27	38.66±6.71	45	40.70±6.1
Uterus	(g)	20	0.517±0.220	25	0.500±0.15
	(g%)	20	0.212±0.084	25	0.228±0.07

N : Number of animals
g, mg : Absolute organ weight
g%, mg% : Relative organ weight

Table 6-1. Histopathology (summary incidence of findings) of Crj:CD(SD)IGS (10～11 Weeks)

Sex	Male	Female
Number of animals	56	56
Findings		
Heart		
Cellular infiltration, histiocyte	4	
Fibrosis, focal	1	
Lung		
Cellular infiltration, neutrophil, focal	1	
Granulation tissue		1
Cellular infiltration, alveolar macrophage, focal		1
Liver		
Dilatation, sinusoid, focal	1	
Hematopoiesis, extramedullary	6	1
Microgranuloma	14	7
Necrosis, focal	4	2
Esophagus		
Cellular infiltration, muscular layer, focal		1
Kidney		
Basophilic change, tubular epithelium	9	3
Cellular infiltration, lymphoid cell	6	4
Cyst	3	
Hyaline droplet, proximal tubular epithelium	4	
Spleen		
Hematopoiesis, extramedullary	45	54
Testis		
Vacuolization, Sertori cell	6	
Epididymis		
Cellular infiltration, lymphoid cell	1	
Prostate		
Atrophy, lobular	1	
Cellular infiltration, lymphoid cell	9	
Pituitary		
Rathke's pouch		2
Thyroid		
Ectopic thymic tissue	5	10
Ultimobranchial remnant	3	3
Eyeball		
Dysplasia, retina	5	2
Degeneration, retina		1
Harderian gland		
Cellular infiltration, lymphoid cell, focal	2	3

Table 6-2. Histopathology (summary incidence of findings) of Crj:CD(SD) (9～11 Weeks)

Sex	Male	Female
Number of animals	68	68
Findings		
Heart		
Cellular infiltration		1
Granuloma	1	
Lung		
Cellular infiltration	1	
Aggregation, foamy cell	7	2
Granuloma		5
Pneumonia		1
Thymus		
Congestion	1	2
Hemorrhage		1
Liver		
Cellular infiltration		2
Fibrosis		1
Microgranuloma	8	8
Kidney		
Basophilic change, tubular epithelium	1	1
Cellular infiltration	2	1
Chronic nephropathy	7	2
Cyst	4	3
Dilatation,urinary tubules	1	
Hyaline droplet, tubular epithelium	10	
Pyelectasia	1	
Spleen		
Hematopoiesis, extramedullary	44	39
Stomach		
Dilatation, glands	2	
Pancreas		
Atrophy, acinus		1
Cellular infiltration	1	
Fibrosis, focal	1	
Hemorrhage, islet		1
Prostate		
Cellular infiltration	2	
Ovary		
Cyst		1
Thyroid		
Ectopic thymic tissue	1	
Skin		
Erosion		1
Cellular infiltration, corium		1
Harderian gland		
Cellular infiltration		1

Hormonal Parameters in Crj:CD(SD)IGS Rat from 3 to 23 weeks of Age

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ABSTRACT. The assay conditions for hormone measurement of rat samples were evaluated, and background data on hormones in Crj:CD(SD)IGS rats were collected. Under established hormone measurement conditions, developmental changes, differences of estrus-cycle stage, and changes in the dam's situation from lactation to weaning were clearly shown in this study. Hormone measurement might be an important means for the evaluation of toxicological effects derived from endocrine disrupters and chemical substances in the environment.

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INTRODUCTION

The need to define what tests should be used to assess endocrine disrupters has recently become a priority issue in the field of toxicology. It would be very useful to evaluate what endpoints are the most sensitive to detect endocrine-disrupting substances by toxicological protocols and the reliability among laboratories for the measurement of various parameters. One of the recommended parameters is hormone measurement.

In this study, the assay conditions of hormone measurement for rat samples were evaluated, and background data on hormones in Crj:CD(SD)IGS rats were collected.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats were supplied by Charles River Japan, Inc. In the reproductive study, animals were obtained at 5 weeks (male) and 10 weeks (female) of age , and the study was commenced at 6 weeks (male) and 13 weeks (female) of age. Maintenance conditions were consistent throughout the study, and the animals were housed individually in stainless steel cages with *ad libitum* access to a commercial diet (CE-2, CLEA Japan Inc., Tokyo, Japan) and tap water. The room temperature was maintained at 24 ± 1℃; and the humidity, at 50 to 65%. Lighting was controlled automatically to give a cycle of 12 hours of light (7:00 to 19:00) and 12 hours of darkness.

Clinical observations and mortality checks were performed on all animals daily. Body weight and food consumption were measured according to the same time intervals as mentioned previously [1].

All the females were examined daily for estrus-cycle stage by using the vaginal smear method from the age of 11 weeks. At the age of 11 (F_1) or 13 (F_0) 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 females assigned for delivery were housed in rat breeding cages from day 18 of gestation until day 10 of lactation (day 0 of lactation is the day on which delivery confirmed) with adequate paper pulp chip bedding. 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. Other study conditions and examinations were carried according to the former report [1]. At necropsy, animals were given an intraperitoneal injection of sodium pentobarbital followed by blood sampling from the abdominal caval vein.

In the general toxicological study, animals were obtained at 6 weeks of age, and the study was commenced one week later. All the females were examined for estrus-cycle stage by using the vaginal smear method daily from the age of 10 weeks. Other study conditions and examinations were carried according to former reports [2, 3]. Blood was drawn from the abdominal aorta of each animal under ether anesthesia.

In both studies, the serum was analyzed for hormones after the collected blood had been centrifuged.

Hormonal parameters reported in this article, thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), luteinizing hormone (LH), prolactin (PRL), testosterone (TES), estradiol (E2), corticosterone (COR), and follicle-stimulating hormone (FSH), are summarized in Table 1 with the list of reagents. These reagent kits were selected after assessing several points of applicability among a few reagent kits for each hormone.

RESULTS AND DISCUSSION

To demonstrate the application of these methods for rat serum samples, we spiked the samples with each hormone standard, and evaluated its recovery, range of linearity, etc. The reagent kit for rats used here, TSH, FSH, LH, COR, and PRL, showed good recovery and a wide range of linearity. These methods did not need further modifications for measurement when used carefully. However, the reagent kits developed for human, T3, T4, TES, and E2 showed some problems. Results obtained with them are shown in Table 2. The recoveries of these hormones (T3, T4, TES, and E2) were acceptable for toxicological comparison studies with careful measurements. Three of the hormones, T3, T4, and TES, showed an acceptable range of linearity, whereas E2 was out of the assay range. Furthermore, the values of E2 were higher than those of earlier studies. This method for E2 may be used for the comparison of effects among several dosages of chemical substance with control groups, or for evaluation of estrus cycle differences. However, differences in these values were not clear among groups. Pretreatment of samples is essential to obtain an accurate and precise value with this E2 method. In the E2 measurement, stage differences in the estrus cycle were clearly found, as shown in Table 3, after the pretreatment of samples by organic solvent extraction and concentration. In hormone measurement studies, many factors, such as the stage of the estrus cycle, time and age of sampling, sampling method, etc. affected the hormone

value [4 ～ 9]. In this study, the blood was taken under prefixed conditions, taken from the posterior vena cava under pentobarbital sodium anesthesia, at the diestrus stage for female rats, etc. If these conditions were changed, the hormone value was shifted. Each hormone has a suitable sampling and assay conditions. In particular, skillful, quick and less stressful blood sampling is necessary for PRL and COR measurements.

Table 4 shows developmental changes in hormonal parameters in Crj:CD(SD)IGS male rats; and Table 5, those in female rats. The range of each hormone value is shown in parentheses. These data are summarized from the data obtained in a few separate studies, and some of them showed clear developmental changes. In female rats (Table 5), the data at 19 ～ 21 weeks of age were collected from the mother under the day 21 of lactation, and the data at 21 ～ 23 weeks of age were collected from the mother under the day 22 of lactation (the first day of weaning). The value of PRL was distinctly different from point of sampling to the other.

In recent years, hormone measurement has become an important issue for the evaluation of effects derived from environmental contaminants or endocrine-disrupting substances, etc. Each animal has a specific individual hormonal condition for maintaining its homeostasis, indicating that the method of hormone measurement including sampling conditions needs to be assessed for its reliability before the animal experiment. We have evaluated at least 10 methods of measuring hormones with rat-specific reagent kits or reagent kits for human testing.

Animals having worldwide uniform quality are most suitable for this type of study. The Crj:CD(SD)IGS rat strain developed by Charles River Inc. is a candidate of these animal models.

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Table 1. Abbreviations and methods for hormonal parameters

Hormone	Code	Kit	Supplier
Thyroid-stimulating hormone	TSH	Rat thyroid stimulating hormone (rTSH) EIA system	Amersham Pharmacia Biotech
Triiodothyronine	T3	Active Total T3 EIA*	Diagnostic Systems Laboratories Inc.
Thyroxine	T4	Active Thyroxine (T4) EIA*	Diagnostic Systems Laboratories Inc.
Luteinizing hormone	LH	Rat luteinizing hormone (rLH) EIA system	Amersham Pharmacia Biotech
Follicle-stimulating hormone	FSH	Rat follicle stimulating hormone (rFSH) EIA system	Amersham Pharmacia Biotech
Prolactin	PRL	Rat prolactin (rPRL) EIA system	Amersham Pharmacia Biotech
Estradiol	E2	Active Estradiol EIA*	Diagnostic Systems Laboratories Inc.
Testosterone	TES	Active Testosterone EIA*	Diagnostic Systems Laboratories Inc.
Corticosterone	COR	Rat corticosterone [^{125}I] assay system	Amersham Pharmacia Biotech

* EIA for human use

Table 2. Measurement of rat serum hormones using reagent kits for human use

Hormone	Method	Recovery (%)	Range of linearity (fold)
T3	EIA	100～113	2
T4	EIA	117～137	2
TES	EIA	74～93	4
E2	EIA	103～119	＜2

Table 3. Evaluation of the method for rat serum estradiol

Estrus cycle	Number of animals	Estradiol (pg/mL)			
		After pretreatment		Before pretreatment	
		mean±S.D.	Min.～Max.	mean±S.D.	Min.～Max.
diestrus	5	34±3	29～38	123±20	98～144
estrus	5	ND		141±25	100～159
metestrus	5	ND		166±48	110～223
proestrus	9	77±21	47～106	224±50	145～280

ND : Lower than detection limit (6.7 pg/mL)

Table 4. Developmental changes in hormonal parameters in Crj:CD(SD)IGS male rats

Hormone (unit)	Age (Weeks)							
	3		11		18		23	
TSH (ng/mL)	10.2±1.7 (7.6～14.3)[3]	(48)[1]	NE[2]		13.4±2.6 (9.2～19.9)	(52)	13.1±2.9 (9.4～25.2)	(60)
T3 (ng/mL)	1.4±0.2 (1.0～2.0)	(48)	NE		0.8±0.1 (0.5～0.9)	(52)	1.0±0.2 (0.7～1.6)	(60)
T4 (ng/mL)	52±9 (34～73)	(48)	NE		72±12 (53～108)	(52)	73±11 (54～109)	(60)
LH (ng/mL)	5.9±1.8 (3.1～11.4)	(48)	10.1±2.9 (5.9～15.8)	(30)	13.2±2.9 (7.9～22.5)	(52)	12.1±4.8 (5.0～28.3)	(60)
FSH (ng/mL)	173±94 (84～564)	(48)	313±40 (135～424)	(10)	277±65 (163～395)	(52)	176±56 (101～460)	(60)
PRL (ng/mL)	NE		46.4±20.3 (18～95)	(30)	NE		NE	
E2 (pg/mL)	NE		10±3 (＜6.7～13)	(30)	NE		NE	
TES (ng/mL)	0.9±0.5 (＜0.1～2.2)	(48)	2.7±1.5 (0.5～7.7)	(30)	7.3±4.1 (1.8～22)	(51)	3.8±2.0 (0.8～11.5)	(60)
COR (ng/mL)	NE		100±117 (＜2.3～451)	(30)	NE		NE	

Values are expressed as mean±S.D.
Lower than detection limit (＜0.1 ng/mL for TES, ＜6.7 pg/mL for E2, ＜2.3 ng/mL for COR)
[1] : Number of animals examined
[2] : Not examined
[3] : Range (Min.～Max.)

Table 5. Developmental changes in hormonal parameters in Crj:CD(SD)IGS female rats

Parameter (unit)	Age (Weeks)							
	3		11[1]		19～21[2]		21～23[3]	
TSH (ng/mL)	10.1±2.3 (6.3～15.8)[6]	(51)[4]	NE[5]		16.2±3.4 (9.5～25.1)	(52)	16.8±3.3 (7.0～27.7)	(60)
T3 (ng/mL)	1.6±0.2 (1.0～2.1)	(51)	NE		0.8±0.2 (＜0.5～1.8)	(52)	1.1±0.3 (0.6～1.7)	(60)
T4 (ng/mL)	56±8 (37～87)	(51)	NE		51±11 (29～87)	(52)	63±13 (37～97)	(60)
LH (ng/mL)	6.8±2.3 (3.3～12.2)	(51)	9.8±3.8 (4.1～18.0)	(30)	13.0±2.4 (9.1～20.8)	(52)	10.0±2.7 (4.5～16.8)	(60)
FSH (ng/mL)	153±75 (94～497)	(51)	210±57 (118～343)	(10)	242±46 (166～342)	(52)	274±90 (122～525)	(60)
PRL (ng/mL)	19.4±9.4 (＜2.5～48.8)	(51)	39.4±25.6 (12～149)	(30)	169.9±219.5 (10.2～878.0)	(52)	65.7±60.3 (12.7～280.2)	(60)
E2 (pg/mL)	22±8 (＜6.7～37.4)	(50)	17±11 (＜6.7～48)	(30)	15±9 (＜6.7～36.8)	(52)	18±11 (＜6.7～41.8)	(60)
COR (ng/mL)	NE		235±235 (＜2.3～824)	(30)	NE		NE	

Values are expressed as mean±S.D.
Lower than detection limit (＜2.5 ng/mL for PRL, ＜6.7 pg/mL for E2, ＜2.3 ng/mL for COR, ＜0.5 ng/mL for T3)
[1] : At the day of diestrus stage
[2] : Day 21 of lactation
[3] : Day 22 of lactation
[4] : Number of animals examined
[5] : Not examined
[6] : Range (Min.～Max.)

Developmental Changes in Blood Biochemical Parameters and in Some Humoral Immunological Parameters in Crj:CD(SD)IGS Rats Fed a Normal Commercial Diet or Low-Protein Commercial Diet and in Crj:CD(SD) Rats Fed the Normal Commercial Diet

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ABSTRACT. The data presented in this paper are from the Crj:CD(SD)IGS rat strain fed a normal commercial diet in comparison to the same strain fed a low-protein commercial diet and to the original Crj:CD(SD) strain fed the normal commercial diet. Developmental changes in blood biochemical parameters and humoral immunological parameters were noted in all groups. More than 25 parameters of blood biochemical and humoral immunological parameters could be measured by using an automatic analyzer and micro volumes of samples. This method is capable of a serial measurement in an individual animal, and the specificity of these method is important for many studies using animal models.

CD(SD)IGS-2000: 109-115

INTRODUCTION

Crj:CD(SD)IGS rats were developed by Charles River Inc. as a new animal breeding system for the purpose of providing experimental animals having worldwide uniform quality.

In the present study, data on blood biochemical parameters and several humoral immunological parameters were collected repeatedly and evaluated for developmental changes. Those data from Crj;CD(SD)IGS fed normal commercial diet, Crj:CD(SD)IGS fed a low-protein commercial diet, and Crj:CD(SD) fed the normal commercial diet were compared with each other.

MATERIALS AND METHODS

Crj:CD(SD)IGS rats and Crj:CD(SD) rats were all supplied by Charles River Japan, Inc. All animals were obtained at 4 weeks of age, and the study was commenced one week later. Maintenance conditions were consistent throughout the study and the animals were housed individually in stainless steel cages with *ad libitum* access to a normal commercial diet (CE-2, 25% protein diet; CLEA Japan Inc., Tokyo, Japan), or low-protein commercial diet (CR-LPF, 18% protein diet; Oriental Yeast Co., Tokyo, Japan), and tap water. The room temperature was maintained at 24 ± 1℃; and the humidity, at 50 to 65%. Lighting was controlled automatically to give a cycle of 12 hours of light (7:00 to 19:00) and 12 hours of darkness.

Clinical observations and mortality checks were performed on all animals daily. Body weight and food consumption were measured according to the same time intervals as mentioned previously [1].

Blood samples were drawn repeatedly (about 500 μ L/sampling time) from a tail vein at 7, 9, 11, and 14 weeks of age after an overnight period without food. This blood was taken without anesthesia or anticoagulation reagent. At the end of the study period (18 weeks of age), all animals were weighed prior to necropsy. Another two lots of animals were used for the comparison of the humoral immunological parameters, one at 9 and 31 weeks and one at 18 and 31 weeks. At necropsy, animals were given an intraperitoneal injection of sodium pentobarbital, which was followed by blood sampling from the abdominal caval vein with heparin as an anticoagulants. Food was removed overnight prior to necropsy.

The blood biochemical parameters measured and the methods used are presented in Table 1. These parameters were measured with a COBAS-FARA apparatus using commercially available kits and/or reagents [1 ~ 3].

The humoral immunological parameters and albumin were measured according to the methods shown in Table 2. Immunonephelometrical assays were developed for the third component of complement (C3 [4 ~ 14]), immunoglobulin G (IgG [6 ~ 9, 11 ~ 14]), immunoglobulin M (IgM [7, 8, 12, 13]) and albumin (ALB_{im} [7 ~ 9, 11, 14, 15]) in our laboratory. The assay method for 50%-hemolysis units of complement (CH50) was also developed in our laboratory using homogeneous liposome-based assay [10, 12 ~ 14, 16]. This assay was originally developed by Wako Pure Chemical Industries, Ltd. for human use. This assay is acceptable for rat CH50 assay with some modifications. These assays were also performed by COBAS-FARA.

Statistical analyses were performed using the F test for homogeneity of variance followed by Student's t-test or the Aspin and Welch test.

RESULTS AND DISCUSSION

The blood biochemical parameters at 5 sampling points from each group are presented in Table 3-1 and 3-2. Each group had 10 male animals. Five hundred microliters of blood could not be easily obtained from some animals, especially at 7 weeks of age; and in some cases hemolysis occurred. Therefore some parameters could not be measured in the 7-week-old rats. However, many parameters of older ages were significantly different from the value at 7 weeks of age or the youngest age measured in each group, indicating developmental changes in the animals. The pattern of these changes depended on the parameter measured. Total and free cholesterol and phospholipids were significantly different between Crj:CD(SD)IGS fed CE-2 and Crj:CD(SD)IGS fed CR-LPF. These data showed a good reproducibility with respect to earlier studies [1, 2]. A larger number of parameters, such as alkaline phosphatase activity, triglyceride, phospholipids, calcium, etc., showed significant differences between Crj:CD(SD)IGS fed CE-2 and Crj:CD(SD) fed CE-2.

Table 4 shows the humoral immunological parameters and al-

bumin from 7 to 18 weeks of age of each strain fed CE-2 or CR-LPF. The humoral immunological parameters and albumin also showed developmental changes between sampling points. Many sampling points of ages showed the significant differences from the value at 7 weeks of age. But the significant differences between Crj:CD(SD)IGS fed CE-2 and Crj:CD(SD)IGS fed CR-LPF or Crj:CD(SD) fed CE-2 were few for these parameters. Table 5 and 6 show the humoral immunological parameters of other lots of rats. The blood biochemical data on the same rats shown in Tables 5 and 6 were already reported in the former editions of this book [1, 2], respectively. These humoral immunological parameters also showed a developmental difference between younger and older rats. But the differences were not clear in comparison with the data mentioned above. The lot difference of the rats and the limited point of measurement may have caused this difference.

Another important point is the difference in the albumin concentration between the dye-binding method (photometrical assay) and the immunonephelometry method. The latter method has high specificity for rat albumin [7 ~ 9, 11, 14, 15], and it showed a lower value than the former method. This means the photometrical assay measures albumin with other contaminants.

In this study, more than 25 parameters of blood biochemical parameters and humoral immunological parameters could be measured by using an automatic clinical chemistry analyzer to which was applied a micro volume of plasma or serum for each parameter and a small volume of reagent. These advantages allow serial measurement in an individual animal; and the specificity of these methods for animal samples, especially for humoral immunological parameters, are important for many studies using animal models.

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Table 1. Abbreviations and methods for blood biochemistry

Code	Parameter	Method
AST (SGOT)	Aspartate aminotransferase	IFCC[1)]
ALT (SGPT)	Alanine aminotransferase	IFCC
ALP	Alkaline phosphatase	GSCC[2)]
LDH	Lactate dehydrogenase	Wróblewski-La Due method
GTP	γ -Glutamyl transferase	(γ -L-Glutamyl)-3-carboxy-4-nitroanilide as substrate
CK	Creatine kinase	Kinetic
CHE	Cholinesterase	DTNB[3)] method (Acetylthiocholine iodide as substrate)
LAP	Leucine aminopeptidase	L-Leucine-p-nitroanilide as substrate
GLU	Glucose	Glucokinase and glucose-6-phosphate dehydrogenase reaction
IP	Inorganic phosphorus	No reduction of phosphomolybdate
Ca	Calcium	Spectrophotometric measurement of Ca^{2+} complexes
TCHO	Total cholesterol	Enzymatic endpoint (cholesterol esterase, cholesterol oxidase, and peroxidase)
FCHO	Free cholesterol	Enzymatic endpoint (cholesterol oxidase and peroxidase)
TG	Triglyceride	Enzymatic endpoint (lipase, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase)
NEFA	Nonesterified fatty acids	Enzymatic endpoint (acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase)
PL	Phospholipids	Enzymatic endpoint (phospholipase D, choline oxidase, and peroxidase)
TP	Total protein	Biuret method
ALB	Albumin	Bromcresol green binding
BUN	Blood urea nitrogen	Enzymatic endpoint (urease and glutamate dehydrogenase)
CRE	Creatinine	Jaffé method
UA	Uric acid	Enzymatic endpoint (uricase and peroxidase)
TBIL	Total bilirubin	Jendrassik-Grof method

[1)] International Federation of Clinical Chemistry
[2)] German Society for Clinical Chemistry
[3)] 5,5'-Dithiobis (2-nitrobenzoic acid)

Table 2. Abbreviations and methods for humoral immunological parameters and albumin

Code	Parameter	Method
CH50	50% Hemolytic unit of complement	Homogeneous liposome-based assay
C3	The third component of complement	Immunonephelometry, rat complement as standard
IgG	Immunoglobulin G	Immunonephelometry, rat IgG as standard
IgM	Immunoglobulin M	Immunonephelometry, rat IgM as standard
ALB_{im}	Albumin	Immunonephelometry, rat albumin as standard

Table 3-1. Comparison of blood biochemical parameters in male rats

Parameter	Group		Age(weeks)									
	Strain	Diet	7		9		11		14		18	
AST (U/L)	IGS	CE-2	199±46	(10)	156±28*	(10)	117±15**	(10)	147±26**	(10)	75±20	(10)
	IGS	CR-LPF	191±52	(8)	134±34*	(10)	118±23**	(10)	141±30*	(10)	81±35	(10)
	SD	CE-2	167±29	(7)	123±23** ##	(10)	107±23**	(10)	124±24**	(9)	66±10	(10)
ALT (U/L)	IGS	CE-2	41±7	(9)	38±3	(10)	35±3*	(10)	34±5*	(10)	35±12	(10)
	IGS	CR-LPF	40±7	(7)	35±5	(10)	36±5	(10)	37±7	(10)	37±17	(10)
	SD	CE-2	30±4##	(7)	32±3##	(10)	33±5	(10)	35±5	(9)	35± 8	(10)
ALP (U/L)	IGS	CE-2	643± 52	(10)	505±68**	(10)	394±72**	(10)	273±39**	(10)	191±27	(10)
	IGS	CR-LPF	617±117	(10)	508±92*	(10)	399±55**	(10)	284±48**	(10)	191±39	(10)
	SD	CE-2	488±151##	(8)	402±72##	(10)	310±52** ##	(10)	233±43** #	(9)	173±19	(10)
LDH (U/L)	IGS	CE-2	4840±1663	(10)	3969±1499	(10)	2332±892**	(10)	3633±1323	(10)	166± 95	(10)
	IGS	CR-LPF	5489±2342	(10)	3207±1651*	(10)	2151±818**	(10)	3154±1240*	(10)	224±176	(10)
	SD	CE-2	5286±2117	(10)	2896±1228**	(9)	1994±810**	(10)	2453± 951** #	(9)	220± 90	(10)
GTP (U/L)	IGS	CE-2	0.3±0.5	(4)	0.6±0.5	(5)	0.2±0.4	(10)	0.2±0.4	(9)	0.3±0.5	(10)
	IGS	CR-LPF	0.4±0.5	(5)	0.4±0.5	(5)	0.6±0.5	(10)	0.4±0.8	(7)	0.1±0.3	(10)
	SD	CE-2	0.0±0.0	(7)	0.2±0.4	(9)	0.1±0.3	(9)	0.4±0.5	(5)	0.1±0.3	(10)
CK (U/L)	IGS	CE-2	1230±340	(8)	985±298	(10)	640±190**	(10)	839±224**	(10)	461±222	(10)
	IGS	CR-LPF	1185±376	(6)	866±349	(10)	720±289*	(10)	820±273*	(10)	504±189	(10)
	SD	CE-2	1291±275	(8)	765±253**	(10)	598±231**	(10)	751±348**	(10)	400±162	(10)
CHE (U/L)	IGS	CE-2	568±143	(10)	492± 92	(10)	486±66	(10)	485±69	(10)	326±70	(10)
	IGS	CR-LPF	664±151	(10)	512± 85*	(10)	492±79**	(10)	514±94*	(10)	370±69	(10)
	SD	CE-2	647±143	(10)	550±123	(10)	527±78*	(10)	573±72#	(9)	390±60	(10)
LAP (U/L)	IGS	CE-2	45±5	(10)	45±5	(10)	42±4	(10)	41± 5	(10)	32±4	(10)
	IGS	CR-LPF	46±4	(8)	48±5	(10)	45±4	(10)	47± 5#	(10)	36±4#	(10)
	SD	CE-2	42±4	(7)	43±4	(10)	39±4	(10)	37±13	(10)	30±4	(10)
GLU (mg/dL)	IGS	CE-2	64±13	(10)	80±19*	(10)	90±17**	(10)	85±14**	(10)	163±11	(10)
	IGS	CR-LPF	73±14	(10)	80±13	(10)	92±12**	(10)	81±15	(10)	158±16	(10)
	SD	CE-2	78±10#	(10)	87± 6*	(10)	97± 8**	(10)	89±10*	(10)	182±15##	(10)
IP (mg/dL)	IGS	CE-2	10.1±1.0	(10)	8.4±0.7**	(10)	8.3±0.6**	(10)	7.4±0.4**	(10)	6.1±0.7	(10)
	IGS	CR-LPF	10.3±0.7	(10)	8.2±0.3**	(10)	8.3±0.3**	(10)	7.4±0.4**	(10)	6.2±0.7	(10)
	SD	CE-2	10.2±0.5	(10)	8.7±0.5**	(10)	8.5±0.2**	(10)	7.8±0.4** #	(10)	6.3±0.6	(10)
Ca (mg/dL)	IGS	CE-2	9.0±0.4	(10)	9.0±0.3	(10)	9.6±0.3**	(10)	9.3±0.2*	(10)	8.8±0.2	(10)
	IGS	CR-LPF	8.9±0.6	(10)	9.1±0.3	(10)	9.6±0.3**	(10)	9.4±0.2*	(10)	8.8±0.3	(10)
	SD	CE-2	9.1±0.8	(10)	9.3±0.3#	(10)	9.8±0.4*	(10)	9.6±0.4#	(10)	9.1±0.3#	(10)

Serum from the tail vein at 7, 9, 11, and 14 weeks, and plasma from the abdominal caval vein at 18 weeks
Values are expressed as mean±S.D.
Number of animals in the parentheses
*<0.05, **<0.01 : Significantly different from 7 weeks of age or youngest age in each group
#<0.05, ##<0.01 : Significantly different from parameter of IGS CE-2

Table 3-2. (continued)

Parameter	Group		Age(weeks)									
	Strain	Diet	7		9		11		14		18	
TCHO (mg/dL)	IGS	CE-2	60±13	(10)	58±11	(10)	57±10	(10)	58±10	(10)	50±11	(10)
	IGS	CR-LPF	79±18$^{\#}$	(10)	74±11$^{\#\#}$	(10)	72±12$^{\#\#}$	(10)	67±15	(10)	52±11	(10)
	SD	CE-2	66±16	(10)	60±14	(10)	60±11	(10)	60±13	(10)	48±11	(10)
FCHO (mg/dL)	IGS	CE-2	11±4	(10)	10±3	(10)	10±3	(10)	10± 2	(10)	14±4	(10)
	IGS	CR-LPF	19±4$^{\#\#}$	(10)	15±3* $^{\#\#}$	(10)	15±3* $^{\#\#}$	(10)	18±21	(10)	14±4	(10)
	SD	CE-2	16±5$^{\#}$	(10)	13±4	(10)	14±4$^{\#}$	(10)	12± 4	(9)	13±4	(10)
TG (mg/dL)	IGS	CE-2	64±15	(10)	71±12	(10)	80±19	(10)	84±22*	(10)	43±15	(10)
	IGS	CR-LPF	77±14	(10)	87±25	(10)	96±28	(10)	97±25*	(10)	47±15	(10)
	SD	CE-2	121±33$^{\#\#}$	(10)	138±40$^{\#\#}$	(10)	156±35* $^{\#\#}$	(10)	166±60$^{\#\#}$	(10)	92±23$^{\#\#}$	(10)
NEFA (mEq/L)	IGS	CE-2	1.57±0.21	(10)	1.40±0.26	(10)	1.13±0.18**	(10)	1.29±0.35*	(10)	0.50±0.13	(10)
	IGS	CR-LPF	1.42±0.24	(10)	1.35±0.29	(9)	1.18±0.25*	(10)	1.33±0.19	(10)	0.52±0.13	(10)
	SD	CE-2	1.33±0.31	(10)	1.36±0.18	(10)	1.13±0.15	(10)	1.28±0.15	(10)	0.46±0.11	(10)
PL (mg/dL)	IGS	CE-2	95±16	(10)	97±16	(10)	103±15	(10)	98±12	(10)	84±11	(10)
	IGS	CR-LPF	120±19$^{\#\#}$	(10)	116±16$^{\#}$	(10)	117±18	(10)	106±16	(10)	84±14	(10)
	SD	CE-2	112±18$^{\#}$	(10)	114±18$^{\#}$	(10)	117±17	(10)	117±21$^{\#}$	(10)	92±17	(10)
TP (g/dL)	IGS	CE-2	6.4±0.1	(10)	6.5±0.3	(10)	6.5±0.3	(10)	6.8±0.3**	(10)	5.8±0.2	(10)
	IGS	CR-LPF	6.8±0.5$^{\#}$	(10)	6.5±0.2	(10)	6.6±0.2	(10)	6.8±0.2	(10)	5.8±0.2	(10)
	SD	CE-2	6.6±0.5	(10)	6.5±0.2	(10)	6.7±0.2	(10)	7.2±0.3** $^{\#}$	(10)	5.8±0.3	(10)
ALB (g/dL)	IGS	CE-2	3.8±0.2	(10)	3.8±0.2	(10)	3.8±0.1	(10)	3.9±0.2	(10)	2.7±0.4	(10)
	IGS	CR-LPF	4.2±0.5$^{\#}$	(10)	3.9±0.2	(10)	3.9±0.2	(10)	3.9±0.2	(10)	2.8±0.5	(10)
	SD	CE-2	4.0±0.5	(10)	3.7±0.2	(10)	3.8±0.2	(10)	3.9±0.3	(10)	2.7±0.6	(10)
BUN (mg/dL)	IGS	CE-2	11±2	(10)	12±5	(10)	12±2	(10)	11±2	(10)	15±3	(10)
	IGS	CR-LPF	10±3	(10)	11±3	(10)	11±4	(10)	11±2	(10)	15±1	(10)
	SD	CE-2	11±2	(10)	10±2	(10)	13±3	(10)	12±2	(9)	15±2	(10)
CRE (mg/dL)	IGS	CE-2	0.6±0.1	(10)	0.6±0.1	(10)	0.6±0.0*	(10)	0.6±0.1	(10)	0.8±0.1	(10)
	IGS	CR-LPF	0.6±0.1	(10)	0.6±0.1	(10)	0.6±0.0	(10)	0.6±0.1	(10)	0.8±0.1	(10)
	SD	CE-2	0.6±0.1	(10)	0.6±0.0	(10)	0.7±0.1	(10)	0.6±0.1	(10)	0.8±0.1	(10)
UA (mg/dL)	IGS	CE-2	NE		0.6±0.2	(10)	0.3±0.2**	(10)	1.0±0.4**	(10)	0.3±0.1	(10)
	IGS	CR-LPF	NE		0.5±0.2	(9)	0.3±0.1**	(10)	0.9±0.3*	(10)	0.4±0.2	(10)
	SD	CE-2	NE		0.5±0.2	(10)	0.4±0.2	(10)	0.8±0.3*	(9)	0.7±1.0	(10)
TBIL (mg/dL)	IGS	CE-2	0.09	(2)	0.07±0.03	(8)	0.09±0.06	(8)	0.08±0.04	(8)	0.09±0.02	(10)
	IGS	CR-LPF	0.13	(2)	0.05±0.03*	(8)	0.08±0.04	(8)	0.08±0.06	(6)	0.10±0.02	(10)
	SD	CE-2	0.05±0.02	(4)	0.05±0.03	(9)	0.10±0.06	(9)	0.06±0.03	(7)	0.09±0.02	(10)

Serum from the tail vein at 7, 9, 11, and 14 weeks, and plasma from the abdominal caval vein at 18 weeks
Values are expressed as mean±S.D.
Number of animals in the parentheses
NE : Not examined
*<0.05, **<0.01 : Significantly different from 7 weeks of age or youngest age in each group
$^{\#}$<0.05, $^{\#\#}$<0.01 : Significantly different from parameter of IGS CE-2

Table 4. Comparison of humoral immunological parameters and albumin in male rats

Parameter (unit)	Group Strain	Diet	Age (weeks) 7	9	11	14	18
CH50 (U/mL)	IGS	CE-2	29.8±5.3	37.2±3.8**	40.7±4.3**	42.6±5.8**	41.1±3.6
	IGS	CR-LPF	30.0±4.5	38.5±2.9**	40.7±3.6**	42.2±5.1**	42.2±4.2
	SD	CE-2	27.1±5.4	37.2±2.4**	38.2±3.3**	40.1±4.5**	37.9±4.5
C3 (%)	IGS	CE-2	103 ±14.5	91.1±11.7	84.3±11.2**	79.8±13.4**	55.8± 4.5
	IGS	CR-LPF	94.8±14.6	83.7± 7.5*	75.5± 9.6**	72.6± 9.0**	63.7±11.4
	SD	CE-2	96.4±26.2	93.3± 6.7	81.4± 9.9	73.3± 6.1*	63.4± 6.2 ##
IgG (mg/mL)	IGS	CE-2	1.48±0.27	2.68±0.89**	3.28±1.23**	4.40±1.29**	4.25±1.34
	IGS	CR-LPF	1.65±0.40	2.72±1.04**	3.68±1.28**	5.65±1.94**	5.39±1.55
	SD	CE-2	1.22±0.27#	1.92±0.57** #	2.70±0.69**	4.21±0.89**	4.52±0.97
IgM (μg/mL)	IGS	CE-2	234±55	249±80	331±103*	375± 87**	354±97
	IGS	CR-LPF	238±45	253±53	331± 68**	388± 59**	339±43
	SD	CE-2	202±50	257±75	310± 94**	385±106**	313±87
ALB_{im} (g/dL)	IGS	CE-2	2.82±0.16	3.24±0.45*	3.29±0.19**	3.32±0.27**	2.57±0.32
	IGS	CR-LPF	2.92±0.19	3.30±0.20**	3.22±0.26**	3.42±0.20**	2.65±0.22
	SD	CE-2	2.64±0.23	3.03±0.27**	3.23±0.21**	3.14±0.29**	2.63±0.24

Serum from the tail vein at 7, 9, 11, and 14 weeks, and plasma from the abdominal caval vein at 18 weeks
Values are expressed as mean±S.D.
Number of animals : 10 male animals/each group
*＜0.05, **＜0.01 : Significantly different from 7 weeks of age
#＜0.05, ##＜0.01 : Significantly different from parameter of IGS CE-2

Table 5. Comparison of humoral immunological parameters and albumin in Crj:CD(SD)IGS

Group Sex	Diet	Age (weeks)	Parameter C3 (%)	IgG (mg/mL)	IgM (μg/mL)	ALB_{im} (g/dL)
Male	CE-2	18	79.6±7.2	3.35±0.88	159± 55	2.82±0.42
		31	68.4±8.2**	4.28±1.00*	328±122**	2.78±0.21
Male	CR-LPF	18	66.0± 6.4##	3.01±1.26	197±106	2.59±0.18
		31	71.2±10.5	4.64±1.11**	348±165*	2.63±0.25
Female	CE-2	18	78.0±9.9	6.20±1.30	245± 45	3.17±0.34
		31	72.7±8.3	7.31±1.57	396±148**	3.46±0.26*
Female	CR-LPF	18	73.9±4.5	6.12±1.20	260± 57	3.40±0.45
		31	66.5±7.8** #	6.79±1.04	381±114**	3.64±0.52

Values are expressed as mean±S.D.
Number of animals : 10 for 18 weeks, 20 for 31 weeks
*＜0.05, **＜0.01 : Significantly different from 18 weeks of age
#＜0.05, ##＜0.01 : Significantly different from corresponding CE-2 group

Table 6. Comparison of humoral immunological parameters and albumin between Crj:CD(SD)IGS and Crj:CD(SD) fed CE-2

Group		Age	Parameter				
Strain	Sex	(weeks)	CH50 (U/mL)	C3 (%)	IgG (mg/mL)	IgM (μ g/mL)	ALB_{im} (g/dL)
IGS	Male	9	36.4±3.0	54.5±4.2	1.77±0.38	139±63	2.14±0.23
		31	36.0±6.0	63.9±9.7**	4.29±0.88**	381±81**	1.98±0.15*
SD	Male	9	34.3±4.0	55.9±5.6	1.37±0.55	148± 63	2.10±0.08
		31	31.0±7.0$^{\#}$	67.4±7.0**	3.72±0.62** $^{\#}$	408±143**	1.94±0.11**
IGS	Female	9	36.4±3.0	55.3±3.7	2.98±0.90	185± 24	2.37±0.08
		31	27.7±4.0**	53.6±7.2	5.70±1.18**	423±136**	2.58±0.26*
SD	Female	9	34.1±3.1	61.5±8.2$^{\#}$	1.91±0.66$^{\#\#}$	145± 54$^{\#}$	2.35±0.13
		31	26.9±3.7**	56.3±5.1*	4.94±1.04** $^{\#}$	394±125**	2.82±0.29** $^{\#}$

Values are expressed as mean±S.D.
Number of animals : 10 for 9 weeks, 20 for 31 weeks except for SD females (19 animals)
*＜0.05, **＜0.01 : Significantly different from 9 weeks of age
$^{\#}$＜0.05, $^{\#\#}$＜0.01 : Significantly different from corresponding IGS group

CHAPTER 3

Reproduction Toxicology Related To

Comparison between Crj:CD (SD) and Crj:CD (SD) IGS in Reproductive Study:Pre- and Postnatal Development

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ABSTRACT. We conducted a study for effects on pre-and postnatal development, including maternal function to collect historical control data of the Crj:CD (SD) IGS rats, and compared with those of the Crj:CD (SD) rats. All pregnant rats were allowed to undergo natural parturition and the postnatal growth of the offspring was observed. As the results, there was a tendency of low value in body weight of the Crj:CD (SD) IGS rats in comparison with the Crj:CD (SD) rats. However, an increase in body weight gains was observed in the Crj:CD (SD) IGS rats during the lactational and developmental periods. At autopsy of dams, a decrease in implantation scars was observed in the Crj:CD (SD) IGS rats. The period of pregnancy, birth index, the number of newborn and body weight of newborns in the Crj:CD (SD) IGS rats did not differ from those in the Crj:CD (SD) rats. The results of the examination or the postnatal differentiation of offspring showed that cleavage of the balanopreputium in males was completed by 49 days after birth in the Crj:CD (SD) IGS rat. In females, a tendency of delay in opening of the vagina was observed in the Crj:CD (SD) IGS rats. As results of the exercise performance test and sensory function test, there were no significant difference between Crj:CD (SD) rats and Crj:CD (SD) rats. In the learning test, the Crj:CD (SD) IGS male rats had a higher ratio of avoidance response than the Crj:CD (SD) rats. At autopsy of mate offspring after the fertility test, a tendency of decrease in weights of the accessory glands was observed in the Crj:CD (SD) IGS rats. These results indicated that no clear difference in the reproductive parameter was observed between the Crj:CD (SD) IGS rats and the Crj:CD (SD) rats. It is considered that the Crj:CD (SD) IGS rats are useful animals in the study for effect on pre- and postnatal development, including maternal function. — Key words: Crj:CD (SD) IGS Rat, maternal function, postnatal development, differentiation

CD(SD)IGS-2000: 117-125

INTRODUCTION

The gold standard system, a new animal breeding system, has been developed by Charles River Inc. for supplying uniform experimental animals with minimal genetic variation. We performed a study for effects on pre- and postnatal development, including maternal function to collect historical control data of the Crj:CD (SD) IGS rats, and compared with those of the Crj:CD (SD) rats.

MATERIALS AND METHODS

Experimental Animals: Male and female Crj:CD (SD) IGS rats produced by Charles River Japan, Inc. were obtained at 10 weeks of age and 8 weeks of age respectively. After acclimatization, males at 11 to 12 weeks of age and females at 10 weeks of age were cohabited and the day when the vaginal plug was confirmed was designated as Day 0 of pregnancy. A total of 9 pregnant female Crj:CD (SD) IGS rats and 22 pregnant female Crj:CD (SD) rats were actually used in this study. Animals were housed in an animal room with a barrier system. The environmental conditions were controlled to a room temperature of 23 ± 2℃, a relative humidity of 55 ± 10%, an illumination period of 13 hours (6:00 to 19:00) at about 200 luces and a ventilation frequency of 10 — 13 times per hour.

During the acclimatization period, females were housed in groups of 4 in automatic water-washing bracket cages produced by Nippon Cage Co., Ltd., and males were individually housed in R-1 bracket cages produced by Shintoyo Seisakusho, Inc. During the mating period, male-female pairs were housed separately in R-1 type bracket cages. During the pregnancy period, females were housed individually in tapered bracket cages produced by ShinToyo Seisakusho Inc. Females were individually housed in Ekon PC cages with clean chip produced by Japan CLEA from Day 17 of pregnancy until Day 22 of post partum. After the 4th day of birth, neonates were identified by India ink (diluted twice with physiological saline) injected into the fore and hind limbs using a micro syringe. During the rearing period after weaning, offspring were separated into males and females of each litter and identified using an ear punch. For the fertility test, male-female offspring pairs were housed separately in R-1 type bracket cages. During the pregnancy period, females were housed individually in tapered bracket cages until Day 20 of pregnancy. 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*.

Observation and Examination: The clinical signs of dams were observed daily. In all dams, on Days 0, 3, 7, 9, 11, 13, 15, 19, and 20 of pregnancy, and further, in postpartal dams, on Days 0, 4, 7, 10, 14, 18, and 21 of post partum, body weights were measured with EB-3300DW electron even balance produced by Shimadzu Corporation. On days body weights were determined, except on day 0 of pregnancy, and on 1 day after parturition, food intake was weighed with EB-3300DW electronic even balance. Food intake was calculated by subtracting the weight of the remaining, uneaten food from the weight of the feed supplied on the previous day. All dams were allowed to undergo natural parturition and the postnatal growth of the offspring was observed. On Day 21 of post partum, the offspring were weaned and the weaning index was calculated. The following day, dams were euthanized and macroscopic examination on thoracic/peritoneal viscera was performed. Then, the number of implantation scars in uteri of dams was counted and the birth index (ratio of the number of live births to the number of implantation scars) was calculated.

On four days after birth, the number of neonates for each dams standardized, in principle, to 8 in total, 4 males and 4 females. At twenty-one days after birth, the offspring were weaned and the weaning index was calculated. The number of offspring for each dams was standardized, in principle, to 1 male and 1 female. Neonates were weighed 0, 4, 7, 10, 14, 18, 21, and offspring were weighed 28, 35, 42, 49, 56, 63, and 70 day after birth with EB-

3300DW electronic even balance. During the above period, clinical signs were observed, and the differentiations, such as cleavage of the balanopreputium in males and opening of vagina in females were observed from 40 days and 30 days after birth, respectively, until 70 days after birth. Furthermore, exercise performance test, sensory function tests, emotionality test, and learning ability test were conducted.

As the exercise performance test, righting reflex was conducted from 4 days to 10 days after birth. The animal was placed in the supine position on a veneer board, and the action of the animal to recover to the normal position was observed as an index. The observation time for one trial was limited to 30 seconds and the observation was performed once a day. Individuals who succeeded were judged as positive. In the sensory function tests, pupillary reflex test was conducted at 3 weeks old, and corneal reflex and Preyer's reflex tests were conducted at 5 weeks old.

In the pupillary reflex test, an animal was illuminated with a pencil-type lamp powered by 2 dry cells of unit-3, 1.5V after being kept in a darkness for about 5 minutes, and positive reaction for mitosis by the light was observed. In the corneal reflex test, the animal's cornea was touched with a tip of pig hair, and the eyelid closure reaction was observed.

In the Preyer's reflex test, a tone of 10-13 KHz with a Galton's whistle was given to animals 3 times at 2-second intervals, and the reflex was judged as normal when a wiggling of the auricle was observed.

In the emotionality test, the open field performance test was conducted at 4 weeks old according to Hall's method[1] with a video tracking system (Muromachi Kikai Co., Ltd.). An animal was placed in a round box of 80 cm in diameter and 25 cm in depth, and the latent time at the center, the number of sections crossed, rearing, preening, grooming, and frequency of defecation and urination were counted for 3 minutes.

In the learning test, the frequency of conditioned avoidance response was measured at 5 weeks old with the shuttle avoidance system (Muromachi Kikai Co., Ltd.). An animal was placed in a cage with with 2 rooms, and a conditioning stimulus of sound and light was given for 5 seconds repeatedly at 20-second intervals. When the animal did not move to another room during that time an electroshock was further given for 5 seconds from the floor grid as an unconditional stimulus. However, in the case that the animal moved to another room within 5 seconds after the start of the conditioning stimulus, the unconditional stimulus was not given. Under the above conditions, trials were performed 100 times daily for three consecutive days to examine the conditioned avoidance response.

At 10 to 11 week old, avoiding sib mating, males and females were cohabited at 1:1 for 14 days to examine the reproductive performance. During the mating period, the vaginal plugs or the presence of sperm in the smears was examined every morning. When either of them was observed, it was judged that mating was achieved and the day was assigned as Day 0 of pregnancy. Females that achieved mating were weighed on Days 0, 3, 7, 9, 11, 13, 15, 17, and 20 of pregnancy with EB-3300DW electron even balance.

On Day 20 of pregnancy, dams were autopsied, and thoracic/peritoneal organs were macroscopically observed. The numbers of corpora lutea, implantations, living fetuses and dead embryos/fetuses were counted, and placental conditions were observed. When no implantation sites were observed in the uterus, an implantation test, originally described by Salewski[2] was performed to identify implantation sites. Live fetuses were examined for external anomalies (including oral cavity) by stereoscopic microscopy produced by Nikon Corporation and their sex was identified. Their body weights were measured with EB-340DW electron even balance. After the copulation period, male offspring were autopsied and thoracic/peritoneal organs were macroscopically observed. The testis, epididymis, seminal vesicle and prostate were removed and measured with EB-340DW electron even balance. The left cauda epididymis was minced in Hank's solution (containing 0.5% BSA) and an aliquot of the sample was transferred to a sperm motility examination slide and examined for sperm motility under a microscope.

Statistical analysis: Results obtained in each test were expressed as the mean ± standard deviation or as a percentage. The data were compared with the data obtained from the study for effects on pre- and postnatal development, including maternal function using Crj:CD(SD) rats performed previously in our laboratory. Differences in values between them were analyzed according to the statistical program in the SAS system (SAS applications for preclinical study). The statistical results were shown with significance of 5% or 1%.

1) F-t analysis

Body weight, food intake, duration of pregnancy, number of implantations, number of implantation scars, number of newborns, number of still borns, body weight of living neonates, differentiation after birth (mean of day), open field test, shuttle avoidance test, organ weight, and sperm motility. The student t-test was used in the case that no difference was observed in the variance, while Aspin- Welch's test was used in the case that a difference was observed in the variance.

2) Wilcoxon's rank test

Birth index, index of external abnormality of newborns, living index of offspring, weaning index.

RESULT AND DISCUSSION

1. Clinical signs, Body weight and Food intake in Dams

There were no adverse effects noted in terms of clinical signs in the groups of Crj:CD (SD) IGS and Crj:CD (SD) rats. Body weight of dams during the pregnancy and lactational periods are shown in Figure 1. A tendency of low in body weight was observed in the group of Crj:CD(SD) IGS in comparison with the group of Crj:CD (SD) rats. There were no changes in food intake of dams during the pregnancy and lactational periods.

2. Observation at Parturition and Weaning

The results of observation during the parturition and weaning periods are shown in Table 1. In all dams, no abnormality was observed during the natural parturition in the groups of Crj:CD (SD) IGS and Crj:CD (SD) rats. A decrease in the number of implantation scars was observed in the Crj:CD(SD)IGS rats. There was no external abnormality of newborns. The survival rate of 4-day-old newborns and the weaning rate of 21-day-old neonates

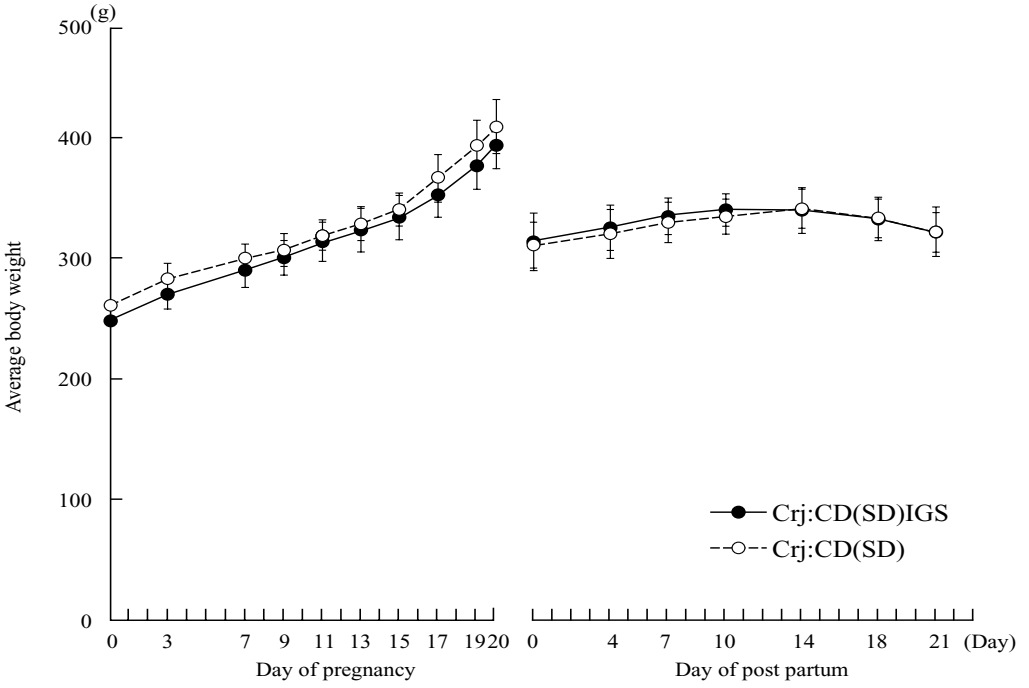

Figure 1. Body weight changes in dams

Table 1. Newborn observation

Strain			Crj:CD(SD)IGS	Crj:CD(SD)
No. of dams			9	22
No. of dams with live newborns		Total (%)	9 (100.0)	22 (100.0)
Gestation period (day)		Mean±S.D.	21.9±0.3	21.8±0.4
No. of implants		Total	128	367
		Mean±S.D.	14.2±2.6#	16.7±1.4
At birth (day 0)		Total	122	330
No. of live newborns		Mean±S.D.	13.6±2.9	15.0±2.6
Birth rate		%	94.9	89.6
No. of stillborns		Total	1	10
		Mean±S.D.	0.1±0.7	0.5±0.7
Sex ratio on live newborns		Male	44.3	51.2
		Male/Female	56/66	169/161
Body weight (g)	(Male)	Mean±S.D.	6.4±0.5	6.3±0.5
of live newborns	(Female)	Mean±S.D.	6.1±0.5	5.9±0.5
No. of live newborns with anomalies		Total (%)	0 (0.0)	0 (0.0)
At selection (day 4)				
Survival rate		%	98.4	97.2
Body weight (g)	(Male)	Mean±S.D.	10.7±1.6	9.5±1.7
of live offspring	(Female)	Mean±S.D.	10.2±1.4	9.1±1.7
At weaning (day 21)				
Weaning rate		%	100.0	100.0

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

were not different between Crj:CD(SD) IGS and Crj:CD(SD) rats. In the macroscopic examination at autopsy of dams during weaning, no abnormarity of thoracic / peritoneal organ was observed.3. Observation of Growth

There were no adverse effects on clinical signs during the lactational and developmental periods in the Crj:CD (SD) IGS rats. Body weights of offspring during the lactational period are shown in Figures 2 and 3. In comparison with the Crj:CD (SD) rats, a

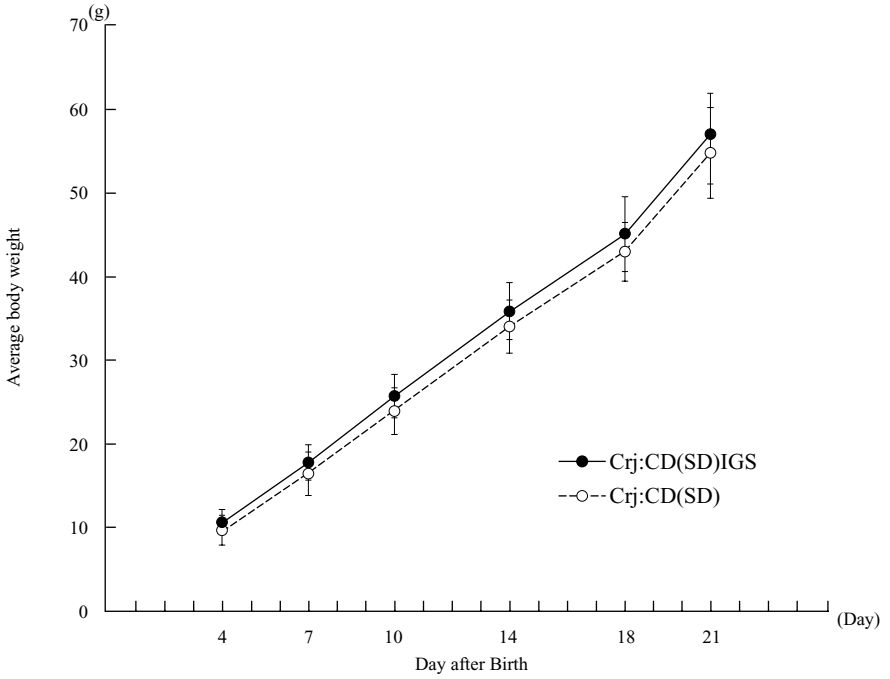

Figure 2. Body weight changes in male offspring

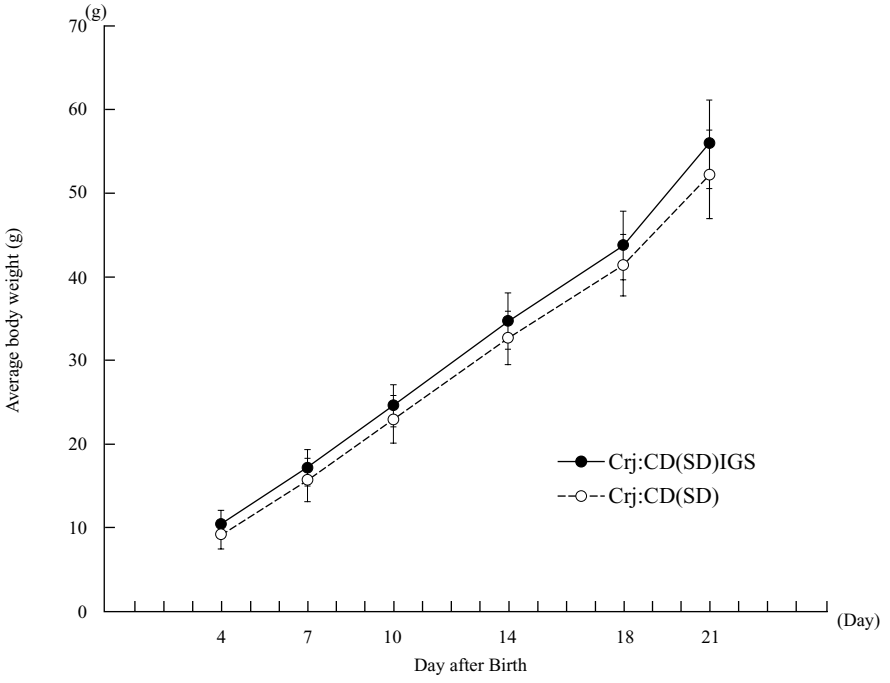

Figure 3. Body weight changes in female offspring

increase in body weight gains was observed in the Crj:CD (SD) IGS rats. Body weight of offspring during the developmental period are shown in Figures 4 and 5. In comparison with the Crj:CD (SD) rats, an increase in body weight gains was observed in the Crj:CD (SD) IGS rats.

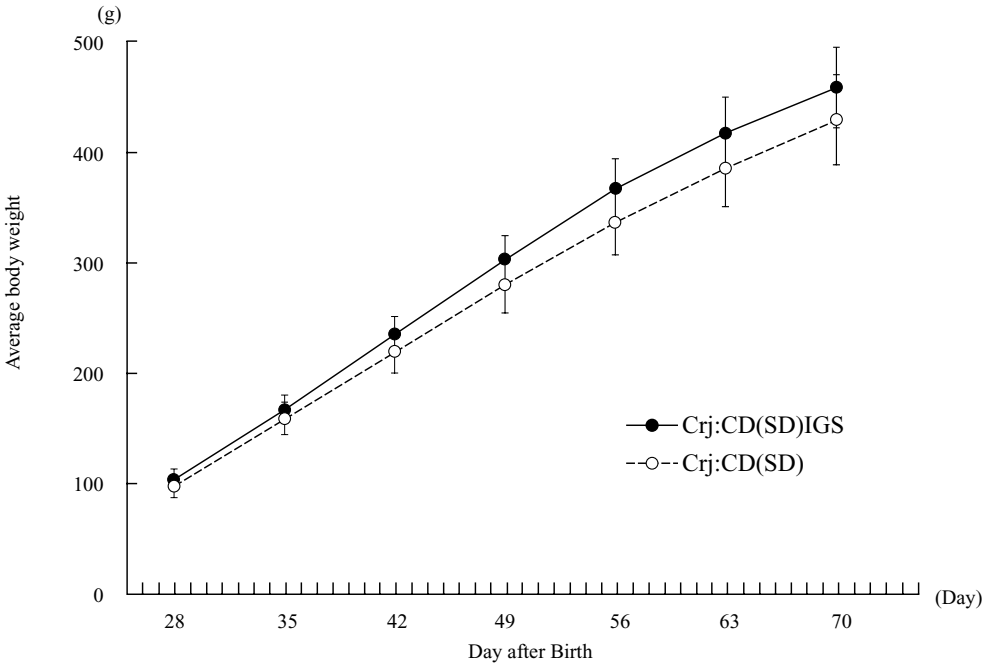

Figure 4. Body weight changes in male offspring

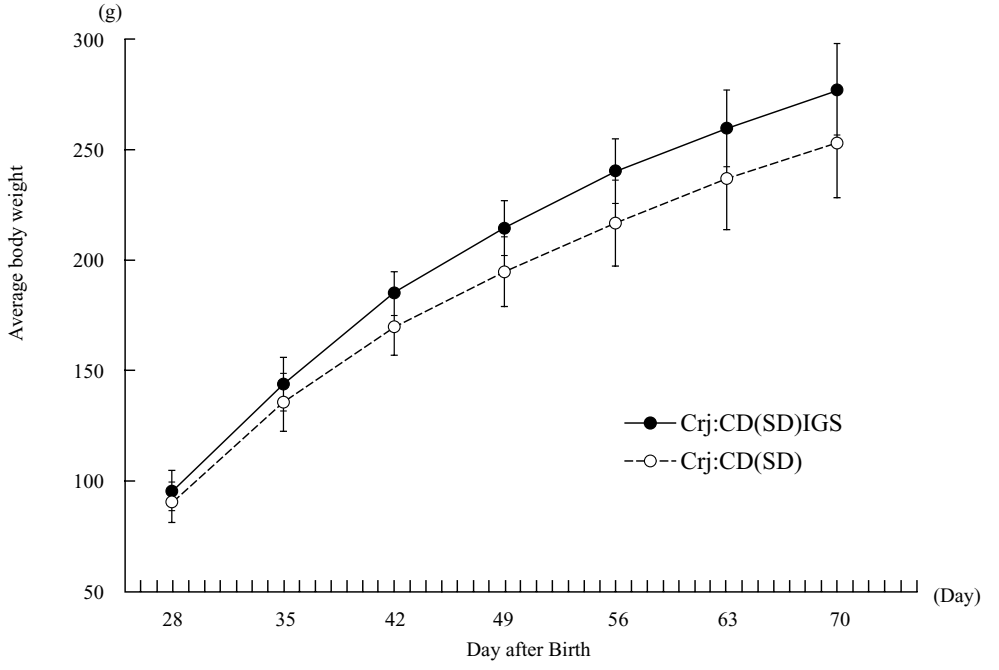

Figure 5. Body weight changes in female offspring

The results of the examination on the postnatal differentiation of neonates are shown in Table 2. Cleavage of the balanopreputium in males was observed from 41 day after birth and completed by 49 days after birth in the Crj:CD (SD) IGS rats. The opening of vagina in females was observed from 32 days after birth and completed by 35 days after birth in the Crj:CD (SD) IGS rats.

The results of the righting reflex are shown in Table 3. No significant difference was observed in the ratio of positive reaction between Crj:CD (SD) IGS and Crj:CD (SD) rats. The results of the sensory function tests are shown in Table 4. There were positive reactions in all male and female offspring for the pupillary, corneal and Preyer's reflex. The results of the emotional test in an open field are shown in Table 5. No significant differences in the latent time, ambulation, preening, grooming, number of defecation and urination were observed between Crj:CD (SD) IGS and Crj:CD (SD) rats.

Table 2. Postnatal differentiation of F1 Rats

Strain		Day after Birth	
		Crj:CD(SD)IGS	Crj:CD(SD)
		N=9	N=22
Male	Cleavage of the balanopreputial gland	44.6±2.79	44.3±4.59
Female	Opening of vagina	32.7±1.12*	31.5±1.54

(Mean±S.D.)

Significantly different from the Crj:CD(SD) group * $P<0.05$ (Student's t test)

Table 3. Righting reflex test of F1 Rats

Strain	Day after Birth			
	Male		Female	
	Crj:CD(SD)IGS	Crj:CD(SD)	Crj:CD(SD)IGS	Crj:CD(SD)
	N=33	N=88	N=38	N=88
Righting	4.0±0.0	4.0±0.0	4.1±24.0	4.0±0.0

(Mean±S.D.)

Table 4. Sensory function tests of F1 Rats

Strain	Male				Female			
	Crj:CD(SD)IGS N=9		Crj:CD(SD) N=10		Crj:CD(SD)IGS N=9		Crj:CD(SD) N=10	
Pupillary reflex	+	9	+	10	+	9	+	10
	−	0	−	0	−	0	−	0
Corneal reflex	+	9	+	10	+	9	+	10
	−	0	−	0	−	0	−	0
Preyer's reflex	+	9	+	10	+	9	+	10
	−	0	−	0	−	0	−	0

Table 5. Open-field test of F1 Rats

Strain	Male		Female	
	Crj:CD(SD)IGS N=9	Crj:CD(SD) N=10	Crj:CD(SD)IGS N=9	Crj:CD(SD) N=10
Latent time (second)	5.97±3.41	16.38±26.64	6.37±3.88	7.02±5.80
Ambulation (no. of sections crossed)	89.3±41.8	73.8±35.7	72.1±33.2	85.7±24.4
Rearing (times)	14.8±6.4	13.2±6.2	9.9±6.9※※	18.4±5.5
Preening (times)	1.3±1.3	1.0±1.2	1.4±1.9	1.2±1.1
Grooming (times)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Defecation (times)	1.2±1.0	1.7±1.9	1.6±1.0	2.0±1.6
Urination (times)	0.0±0.0	0.3±0.5	0.1±0.3	0.1±0.3

(Mean±S.D.)

Significantly different from the Crj:CD(SD) group ※※ P<0.05 (Student's t test)

The results of the learning test by the shuttle avoidance system are shown in Table 6. The avoidance response in the first, second and third blocks on the first day, in the first, second, fourth and fifth blocks on the second day and in the first, second, and fifth blocks on the third day significantly increased in males in the Crj:CD (SD) IGS rats. This indicated that the Crj:CD (SD) IGS male rats had a higher ratio of the avoidance response than the Crj:CD (SD) rats. At autopsy of male offspring after the fertility test, the results of organ weights are shown in Table 7. In the Crj:CD (SD) IGS male rats, a tendency of decrease in relative and absolute weights of the testis, epididymis, seminal vesicle and prostate was observed. The results of sperm motility are shown in Table 8. There was no abnormality in sperm motility in the Crj:CD (SD) IGS rats. At autopsy on Day 20 of pregnancy, the results of fetal observations are shown in Table 9. A tendency of increase in male fetal body weight was observed in Crj:CD (SD) IGS rats. There was no significant difference in the number of corpus lutem, implantations, dead embryos/fetuses and live fetuses between Crj:CD(SD) IGS and Crj:CD (SD) rats. With regard to the sex ratio of fetus and external abnormality , no significant difference was observed.

In conclusion, this study indicated that no clear difference

Table 6. Shuttle box avoidance test of F1 Rats

Strain		Male		Female	
		Crj:CD(SD)IGS N=9	Crj:CD(SD) N=10	Crj:CD(SD)IGS N=9	Crj:CD(SD) N=10
Avoidance response		%	%	%	%
Day 1	1st	20.00±19.53#	2.50±4.86	22.78±21.67	9.50±8.96
	2nd	61.67±30.92##	22.00±19.61	59.44±30.97	46.00±30.26
	3rd	83.33±23.72*	55.00±33.33	83.33±22.22	68.50±27.19
	4th	80.56±20.38	70.00±20.95	87.78±16.22	82.50±14.77
	5th	89.44±12.36	77.00±18.14	90.56±7.26	84.00±15.78
Day 2	1st	86.11±11.40##	44.00±35.81	92.22±9.39#	70.50±20.61
	2nd	93.33±6.61#	76.50±22.74	97.78±2.64	90.50±14.99
	3rd	97.22±5.07	88.50±11.56	97.22±3.63	90.50±17.23
	4th	96.11±4.17#	86.50±11.07	96.11±4.86	93.50±7.84
	5th	96.67±6.61*	85.50±9.85	100.00±0.00	93.00±7.89
Day 3	1st	83.33±15.41#	54.00±35.10	88.89±15.96	85.00±8.82
	2nd	96.11±6.97#	76.00±26.96	95.00±5.59	95.00±8.16
	3rd	95.56±6.35	84.00±18.38	94.44±7.26	94.50±6.85
	4th	95.00±7.07	85.50±16.74	97.78±3.63	97.50±2.64
	5th	97.78±2.64#	85.00±14.14	97.78±3.63	93.00±7.53

(Mean±S.D.)

Significantly different from the Crj:CD(SD) group # P<0.05, ## P<0.01 (Aspin-Welch's t test)
Significantly different from the Crj:CD(SD) group * P<0.05 (Student's t test)

Table 7. Organ weights of F1 male Rats

Strain		Crj:CD(SD)IGS N=9	Crj:CD(SD) N=10
Final body weight	(g)	532.68±34.55	503.00±52.69
Testis Right	(g)	1.681±0.106	1.724±0.138
	(g%)	0.317±0.023*	0.346±0.028
Testis Left	(g)	1.679±0.110	1.706±0.146
	(g%)	0.317±0.030	0.342±0.034
Epididymis Right	(g)	0.617±0.037*	0.658±0.046
	(g%)	0.114±0.009##	0.132±0.015
Epididymis Left	(g)	0.609±0.045	0.634±0.044
	(g%)	0.113±0.009#	0.127±0.016
Seminal vesicle	(g)	1.249±0.150	1.317±0.293
	(g%)	0.236±0.034	0.261±0.045
Prostate	(g)	0.470±0.084**	0.664±0.103
	(g%)	0.089±0.016**	0.133±0.017

(Mean±S.D.)

Significantly different from the Crj:CD(SD) group # P<0.05, ## P<0.01 (Aspin-Welch's t test)
Significantly different from the Crj:CD(SD) group * P<0.05, ** P<0.01 (Student's t test)

Table 8. Sperm examination of F1 male Rats

Strain	Day after Birth	
	Crj:CD(SD)IGS	Crj:CD(SD)
	N=9	N=10
Sperm motility (%)	83.3±2.50	84.0±2.11

(Mean±S.D.)

Table 9. Embryo and fetus observation

Strain			Crj:CD(SD)IGS	Crj:CD(SD)
No. of animals examined		Total	8	10
No. of dams with live fetuses		Total	8	10
No. of corpora lutea		Total	135	176
		Mean±S.D.	16.9±2.5	17.6±2.2
No. of implants		Total (%)	115 (84.4)	165 (94.3)
		Mean±S.D.	14.4±4.5	16.5±1.7
No. of dead fetuses		Total (%)	5 (4.5)	10 (5.8)
		Mean±S.D.	0.6±1.1	1.0±0.8
Dead		Total (%)	0 (0.0)	0 (0.0)
		Mean±S.D.	0.0±0.0	0.0±0.0
Late		Total (%)	2 (1.4)	1 (0.6)
		Mean±S.D.	0.3±0.7	0.1±0.3
Early		Total (%)	3 (3.1)	9 (5.2)
		Mean±S.D.	0.4±0.5	0.9±0.7
I. sites		Total (%)	0 (0.0)	0 (0.0)
		Mean±S.D.	0.0±0.0	0.0±0.0
No. of live fetuse		Total (%)	110 (95.5)	155 (94.2)
		Mean±S.D.	13.8±4.4	15.5±1.3
Sex ratio	Male:Female	Total	58:52	80:75
	male %	Mean±S.D.	52.6±11.9	52.2±15.4
Body weight (g)	Male	Mean±S.D.	3.87±0.21*	3.59±0.28
	Female	Mean±S.D.	3.61±0.20	3.43±0.26
No. of live fetuse with anomalies		Total (%)	0 (0.0)	1 (0.7)

Significantly different from the Crj:CD(SD) group * P<0.05 (Student's t test)

wasobserved in the reproductive parameter between the Crj:CD (SD) IGS rats and the Crj:CD (SD) rats. It is considered that the Crj:CD (SD) IGS rats are useful animals in the study for effect on pre- and postnatal development, including maternal function.

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Background Control Data of Reproductive and Developmental Parameters in Crj:CD(SD)IGS Rats

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ABSTRACT. Three studies were conducted to accumulate background control data of the maternal reproduction and fetal development in CD(SD)IGS rats fed a low-protein pelleted diet (CR-LPF, protein content 18.4%), and the parameters were compared to that of the dams fed a standard ordinary pelleted diet (CRF-1, protein content 23.1%). No prominent differences were seen among the studies on the reproductive and developmental parameters of the dams fed CR-LPF, such as maternal body weight and food consumption, numbers of corpora lutea, implantations and live fetuses, implantation rates, embryo-fetal mortality, sex ratio, and fetal body weights. Also, there were no apparent differences in the parameters between the dams fed CRF-1 and CR-LPF. The types and incidence of abnormalities and variations observed in the visceral and skeletal examination of the fetuses were similar to those reported in other SD rats. It is concluded from these results that CD(SD)IGS rats are useful in evaluating the potential toxicity of new drugs in embryo-fetal toxicity studies. — Key words: Crj:CD(SD)IGS rat, Development, Reproduction

CD(SD)IGS-2000: 126-130

INTRODUCTION

Crj:CD(SD)IGS rats are produced by Charles River Inc. using the international gold standard system, a new breeding system, developed for the purpose of supplying experimental animals with minimized genetic ramifications. However, there are not yet enough background control data available in these rats to evaluate the reproductive and developmental toxicity of new drugs. Therefore, we conducted 3 studies using Crj:CD(SD)IGS rats, which were accepted at 3 different times, and under the protocols of an embryo-fetal toxicity study applied when developing a new drug, we accumulated background control data regarding the maternal reproductive ability and fetal development of these rats. Low-protein pelleted diet (CR-LPF, protein content 18.4%) was developed recently by Oriental Yeast Co., Ltd. to prevent overfeeding of the animals which results in a shortened life span. However, the effects of the low-protein diet upon dams are unknown, therefore, in order to evaluate whether the difference in protein content of the diet affects maternal reproduction and fetal development, the parameters were compared by feeding the dams either standard pelleted diet (CRF-1, protein content 23.1%) or low-protein diet (CR-LPF) in one of the studies.

MATERIALS AND METHODS

Experimental animals

For the first study (Study A), 42 male and 63 female CD(SD)IGS rats were obtained at 9 weeks of age from Charles River Japan, Inc. Afterwards, for the second and third studies (Studies B and C), 32 females of the same strain were obtained at 9 weeks of age for each study with an interval of a week. The animals were acclimatized and quarantined for a week, and healthy animals were used for mating. Animals were housed in a barrier system animal room maintained at a temperature of 23 ± 2℃, relative humidity of 55 ± 10%, with ventilation frequency of 13 times/hour, and a 12hr lighting cycle (06:00h-18:00h). All the animals were given pelleted diet and tap water *ad libitum*. Regarding the diet, half of the females in Study A were given low-protein diet (CR-LPF, Oriental Yeast Co., Ltd.), whereas the remaining half were given standard diet (CRF-1, Oriental Yeast Co., Ltd.). The females in Studies B and C, and all the males were given low-protein diet. The females were mated with males at 10 weeks of age, and the day on which sperm was confirmed in the vaginal smear was designated as Day 0 of gestation. Twenty females confirmed of successful copulation were allocated to each experimental group. The weight range of the dams on Day 0 of gestation was 205.7-247.9g. The composition of the experimental groups was as follows.

Study	Experimental groups	Diet	No. of dams
A	CR-LPF-A	CR-LPF	20
	CRF-1-A	CRF-1	20
B	CR-LPF-B	CR-LPF	20
C	CR-LPF-C	CR-LPF	20

Negative control article and dosing method

Tragacanth gum (Lot no. BH7213, Nippon Funmatsu Yakuhin Co., Ltd.) was selected as the negative control article. The dams were administered 0.5% aqueous tragacanth gum solution orally using a stomach tube for 11 days, on Days 7-17 of gestation, which is the period of implantation to closure of the hard palate. The dosing volume was 5ml/kg body weight, based on the body weight of the dams on Day 7 of gestation.

Observations

The dams were observed daily during gestation for physical signs and mortality, and maternal body weight and food consumption were measured on Days 0 and 7-21 of gestation. On Day 21 of gestation, the dams were euthanized by carbon dioxide, and cesarean section was performed to record the numbers of corpora lutea, implantation sites, early embryo-fetal deaths, late embryo-fetal deaths, dead fetuses and live fetuses. Placentae of the live fetuses were observed for abnormalities. Necropsy was performed following the cesarean section. The live fetuses were sexed, examined for external abnormalities and weighed individually. Approximately half of the fetuses in each litter were fixed in 95% ethanol, stained with alizarin red S using modified Dawson's technique[1], and examined for skeletal abnormalities and variations, and ossification progress. The remaining half of the fetuses were fixed in Bouin's solution following the prefixation in 10% neutral formalin solution, and examined for visceral abnormalities by microdissection method[4].

Statistical Analysis

For the body weight, food consumption, number of corpora lutea, number of implantations, number of live fetuses and ossification progress of the fetuses, data from CR-LPF-A and CRF-1-A groups were analyzed for homogeneity of variance by *F*-test, and the Student's *t*-test was applied to the homogeneous data, and the Aspin-Welch's *t*-test to the non-homogeneous data. As for the rates of implantations and embryo-fetal mortality, and the incidence of fetuses with abnormality, variation or poor ossification, data from the above groups were analyzed by Wilcoxon rank sum test. The level of significance was set at 5% for all analyses.

RESULTS AND DISCUSSION

No death was found in any of the groups, nor were there any abnormalities in physical signs or progress in pregnancy observed. In the CRF-1-A group, body weight increased significantly on Day 21 of gestation (Table 1) and food consumption increased significantly on Days 20-21 of gestation (Table 2) compared to the CR-LPF-A group, although no differences between the CR-LPF-A and CRF-1-A groups were observed in either parameter until Day 20 of gestation. Comparing the 3 studies (the CR-LPF-A, CR-LPF-B and CR-LPF-C groups), changes in body weight and food consumption were similar throughout the gestation period. At necropsy, no abnormal findings were observed in any of the groups except for torsion of the uterine horn and ascites observed in 1 dam of the CRF-1-A group. Findings from the cesarean section and external examination of the fetuses are shown in Table 3. Total mortality of fetuses (2 fetuses) was observed in 1 dam of the CRF-1-A group. This change was not considered to occur spontaneously, but was provoked by the physical factor of excessive intrauterine pressure due to torsion of the uterine horn observed in this dam. The female fetal body weight in the CRF-1-A group was increased significantly compared to the CR-LPF-A group, although no differences were observed in the male fetal body weight or other developmental parameters. In the CR-LPF-A, CR-LPF-B and CR-LPF-C groups, the range of the number of corpora lutea was 13.9-15.7, and the number of implantations was 13.0-14.8. The range of the implantation rate was 92.1-98.4%, the embryo-fetal mortality rate was 2.1-6.1%, the number of live fetuses was 12.7-14.1, the sex ratio was 46.4-53.1%, and male body weight range was 5.29-5.41g, and female body weight range was 5.03-5.10g, indicating no prominent differences among the studies in any of the parameters. No external abnormality of the fetuses was observed in any of the groups. The outcome of the visceral observation of the fetuses is shown in Table 4. The number of fetuses with visceral abnormality was 3 (2.4%), 5 (4.3%), 5 (3.6%) and 4 (3.2%) in the CR-LPF-A, CRF-1-A, CR-LPF-B and CR-LPF-C groups, respectively. There were no significant differences in the incidence of abnormalities between the CR-LPF-A and CRF-1-A groups, and no prominent differences among the CR-LPF-A, CR-LPF-B and CR-LPF-C groups. The types of abnormality observed were thymic remnant in the neck, ventricular septal defect, quadricuspid pulmonic valve, and complication of dilatation of lateral cerebral ventricle and third cerebral ventricles and anophthalmia. These types of abnormalities were similar to those seen spontaneously in other SD rats, and the incidence of these abnormalities was also similar to those of the background control data in these rats[3]. The outcome of the skeletal observation of the fetuses is shown in Table 5. No skeletal abnormality was seen in any of the groups. The number of fetuses with skeletal variations was 9 (6.4%), 7 (5.0%), 11 (6.9%) and 21 (14.9%) in the CR-LPF-A, CRF-1-A, CR-LPF-B and CR-LPF-C groups, respectively. There were no significant differences in the incidence of variations between the CR-LPF-A and CRF-1-A groups, and no prominent differences among the CR-LPF-A, CR-LPF-B

Table 1. Body weights of Fo dams during gestation

Study	A		B	C
Group	CR-LPF-A	CRF-1-A	CR-LPF-B	CR-LPF-C
No. of dams	20	20	20	20
Days of pregnancy (g)				
0	222.2±7.8	226.0±7.6	232.7±8.7	222.7±6.5
7	259.3±10.8	264.3±9.9	267.2±9.5	256.9±9.7
8	264.8±11.2	268.5±10.3	271.6±9.8	260.7±9.8
9	268.7±12.0	273.9±10.7	276.3±10.4	265.4±9.9
10	274.8±12.4	279.8±11.1	281.6±10.8	270.1±10.2
11	281.3±12.0	285.4±10.6	287.5±10.6	275.4±10.3
12	284.0±15.0	291.5±12.1	292.4±11.3	279.6±10.9
13	290.3±13.4	295.9±11.5	297.4±11.5	282.9±12.5
14	295.9±14.2	301.7±12.2	303.3±11.5	287.9±15.3
15	302.2±16.2	307.8±12.7	310.8±12.5	296.9±10.9
16	311.3±18.5	318.2±13.1	320.6±13.2	305.6±12.5
17	323.4±21.0	330.8±13.1	333.7±13.6	317.5±14.3
18	337.6±24.6	345.4±15.8	349.2±15.2	331.5±15.7
19	350.1±27.0	359.2±17.4	363.0±16.4	345.9±17.5
20	362.1±30.6	372.8±20.0	376.0±15.5	358.3±17.8
21	368.7±34.3	391.4±25.2*	383.4±13.9	364.9±17.1

Mean±SD

*: Significantly different from Group CR-LPF-A at $p<0.05$ (Student's *t*-test)

Table 2. Food consumption of Fo dams during gestation

Study	A		B	C
Group	CR-LPF-A	CRF-1-A	CR-LPF-B	CR-LPF-C
No. of dams	20	20	20	20
Days of pregnancy (g/day)				
0-7	21.6±1.9	21.1±1.9	22.0±1.6	21.7±1.5
7-8	24.0±2.2	22.5±2.5	23.6±2.2	22.8±2.2
8-9	23.5±2.8	23.8±1.8	24.3±2.5	23.7±1.8
9-10	24.4±2.4	23.8±2.8	24.8±2.1	23.5±1.6
10-11	23.3±3.7	23.6±2.6	24.2±1.9	24.1±2.1
11-12	23.2±3.8	23.9±3.8	25.1±2.4	22.5±2.1
12-13	23.6±2.5	23.5±2.5	25.0±2.3	23.4±2.2
13-14	23.6±2.2	24.1±3.2	24.5±2.1	22.9±4.6
14-15	23.7±2.6	23.4±2.5	25.7±1.6	22.7±2.8
15-16	25.6±3.6	25.2±2.8	26.1±2.8	24.6±2.0
16-17	26.7±2.7	26.4±3.0	28.2±2.5	25.9±1.8
17-18	27.2±2.9	26.2±3.1	28.8±2.5	26.1±1.9
18-19	27.9±2.9	27.0±2.9	28.1±2.4	26.9±2.4
19-20	25.4±3.8	24.9±2.9	26.5±3.1	23.9±2.5
20-21	16.7±6.5	21.8±4.5**	16.4±5.3	14.6±3.7

Mean±SD
**: Significantly different from Group CR-LPF-A at $p<0.01$ (Student's *t*-test)

Table 3. Findings at cesarean section of Fo dams

Study	A		B	C
Group	CR-LPF-A	CRF-1-A	CR-LPF-B	CR-LPF-C
No. of animals examined	20	20	20	20
No. of dams with live fetuses	20	19	20	20
No. of corpora lutea	13.9±3.7	13.7±2.9	15.7±2.2	15.1±1.5
No. of implantations	13.0±4.3	12.8±4.1	14.7±1.8	14.8±1.5
Implantation rate[a)] (%)	92.1	90.6	94.1	98.4
Total no. of embryo-fetal deaths	6 (2.1)	9 (7.5)	11 (3.8)	18 (6.1)
Dead	0 (0.0)	2 (5.0)	0 (0.0)	0 (0.0)
Late	0 (0.0)	0 (0.0)	3 (1.1)	0 (0.0)
Early	6 (2.1)	7 (2.5)	8 (2.7)	18 (6.1)
Implantation sites	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. of live fetuses	12.7±4.3	12.4±4.3	14.1±1.9	13.9±1.8
Sex ratio[b)] (%)	49.1	54.7	53.1	46.4
Body weight (g)				
Male	5.41±0.38	5.49±0.36	5.36±0.25	5.29±0.26
Female	5.07±0.30	5.25±0.17*	5.10±0.29	5.03±0.23
Placental abnormality (%)	0.0	0.0	0.0	0.0
External abnormality (%)	0.0	0.0	0.0	0.0

Mean±SD
Values in parentheses indicate the average litter incidence.
*: Significantly different from Group CR-LPF-A at $p<0.05$ (Aspin-Welch's *t*-test)
a: (No. of implantations/ No. of corpora lutea)×100
b: (No. of males/ No. of live fetuses)×100

and CR-LPF-C groups. As for variations, extra 14th rib and cervical rib were observed in a few fetuses, on the other hand, the incidence of rudimentary 14th rib ranged from 4.3% to 14.9% in the CR-LPF-A, CRF-1-A, CR-LPF-B and CR-LPF-C groups. Background control data in other SD rats have shown that rudimentary 14th rib is a skeletal variation of which the incidence ranges widely among studies[3]. Axial variations have been reported to be caused by the homeotic transformation of vertebrae due to mutation of Hox gene in mice[2], therefore the incidence of variations should be carefully estimated when evaluating the teratogenic effect of a test article. The incidence of the fetuses with poor ossification and the number of ossified bones showed

Table 4. Visceral examination of F_1 fetuses

Study	A		B	C
Group	CR-LPF-A	CRF-1-A	CR-LPF-B	CR-LPF-C
No. of dams	18	18	20	20
No. of fetuses examined	122	119	134	134
No. of fetuses with anomalies	3 (2.4)	5 (4.3)	5 (3.6)	4 (3.2)
Dilatation of lateral cerebral ventricle	0 (0.0)	1 (0.7)[a]	0 (0.0)	0 (0.0)
Dilatation of third cerebral ventricles	0 (0.0)	1 (0.7)[a]	0 (0.0)	0 (0.0)
Anophthalmia	0 (0.0)	1 (0.7)[a]	0 (0.0)	0 (0.0)
Thymic remnant in neck	1 (0.8)	4 (3.3)	2 (1.4)	1 (0.8)
Ventricular septal defect	1 (0.7)	1 (0.9)	3 (2.2)	3 (2.4)
Quadricuspid pulmonic valve	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)

Values in parentheses indicate the average litter incidence.
a: Observed in a fetus as complication of abnormalities

Table 5. Skeletal examination of F_1 fetuses

Study	A		B	C
Group	CR-LPF-A	CRF-1-A	CR-LPF-B	CR-LPF-C
No. of dams	20	19	20	20
No. of fetuses examined	132	128	148	144
Malformations				
No. of fetuses with malformations	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Variations				
Total no. of fetuses with variations	9 (6.4)	7 (5.0)	11 (6.9)	21 (14.9)
No. of cervical rib	1 (0.7)	0 (0.0)	1 (0.8)	0 (0.0)
No. of extra 14th rib	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
No. of rudimentary 14th rib	8 (5.7)	6 (4.3)	10 (6.1)	21 (14.9)
Poor ossification				
Total no. of fetuses with poor ossification	27 (22.5)	24 (18.1)	35 (23.1)	33 (22.4)
No. of poorly ossified parietal bone	1 (0.7)	2 (1.6)	0 (0.0)	0 (0.0)
No. of poorly ossified hyoid bone	2 (1.4)	4 (2.8)	2 (1.4)	3 (2.1)
No. of poorly ossified sternebra	17 (11.3)	10 (7.6)	13 (8.5)	17 (11.7)
No. of unossified sternebra	2 (1.3)	2 (1.4)	0 (0.0)	3 (2.1)
No. of poorly ossified thoracic centrum	8 (9.9)	7 (5.4)	20 (13.4)	14 (9.2)
No. of poorly ossified lumbar centrum	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Ossification progress (Mean±SD)				
No. of cervical centrum	4.4±1.2	5.1±1.5	4.9±1.5	4.3±1.5
No. of caudal centrum	6.2±0.7	6.3±0.7	6.3±0.6	6.1±0.8
No. of metacarpal in fore limb	8.0±0.0	8.0±0.0	8.0±0.0	8.0±0.0
No. of proximal phalanx in fore limb	6.1±1.4	6.5±1.1	6.5±1.2	6.2±1.8
No. of distal phalanx in fore limb	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0
No. of metatarsal in hind limb	9.8±0.5	9.7±0.5	9.8±0.4	9.6±0.5
No. of proximal phalanx in hind limb	2.3±2.0	2.4±2.3	2.6±2.0	2.0±1.6
No. of distal phalanx in hind limb	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0

Values in parentheses indicate the average litter incidence.

no significant differences between the CR-LPF-A and CRF-1-A groups, and were similar among the CR-LPF-A, CR-LPF-B and CR-LPF-C groups. It can be concluded that abnormalities and variations observed in Crj:CD(SD)IGS rat fetuses were similar to those spontaneously observed in other SD rats, and that the incidence was also similar [3]. No apparent differences were seen on the reproductive and developmental parameters of the dams fed either low-protein or standard pelleted diet.

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Background Data of Reproductive Parameters in Crj:CD(SD)IGS Rats

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ABSTRACT. In order to collect background data on reproductive parameters using Crj:CD(SD)IGS rats which were developed Charles River Japan, Inc. We performed the same examination usually employed in the reproductive toxicity studies. It was concluded that there were no obvious differences in parameters between Crj:CD(SD)IGS rats and Crj:CD(SD) rats. It is considered that Crj:CD(SD)IGS rats can be used for the reproductive toxicity studies. — Key words: Reproductive toxicity study, Toxicological parameter, Crj:CD(SD)IGS rat

CD(SD)IGS-2000: 131-135

INTRODUCTION

Crj:CD(SD)IGS strain rat was produced by International Genetic Standard system and supplied by Charles River Japan, Inc. We performed the same examination usually employed in the reproductive toxicity studies to obtain background data on Crj:CD(SD)IGS rats, and compared with our Crj:CD(SD) rat data of the control groups.

MATERIALS AND METHODS

Thirty male Crj:CD(SD)IGS rats aged 11 weeks and 50 female Crj:CD(SD)IGS rats aged 10 weeks were obtained from Charles River Japan, Inc. (Tsukuba, Ibaraki, Japan) on January 17, 1996. Animals were used after an acclimatization and quarantine period for 12 days or more. They were housed individually in wire-mesh cages and allowed to free access to pellet diet (CRF-1, Oriental Yeast Co., Ltd.) and chlorinated tap water. The animal room was controlled at 23 ± 1℃ with relative humidity of 55 ± 10%, ventilation of 10 times/hr or more, and a 12 hr light/dark cycle (light on 06:00-18:00).

At 12 weeks of age, females were paired with 13-week-old males. The day on which the presence of sperm in the vaginal smear was confirmed, was designate as day 0 of gestation. There were 28 females with evidence of a positive mating. Throughout the experimental period, dams were observed for clinical signs once daily. All dams were weighed each day from day 0 to day 20 of gestation. Food consumption was also determined each day for the same time period.

On day 20 of gestation, all dams were sacrificed by exsanguination from the abdominal aorta under ethyl ether anesthesia. After complete macroscopic examination of all organs was performed, the numbers of corpora lutea, implantations, live/dead fetuses and resorptions were determined. Live fetuses were measured body weights and placental weights, and were sexed and observed for external anomalies including the oral cavity. Approximately half of the fetuses in each litter were fixed with Bouin's solution for visceral examination by Wilson's method. The remaining live fetuses in each litter were separated for preparation of skeletal specimens. They were stained by Alizarin red, and observed for abnormalities, variations and degree of ossification [1].

At 18 weeks of age, males having fertility were sacrificed by exsanguination from the abdominal aorta under ethyl ether anesthesia. After complete macroscopic examination of all organs was performed, the numbers of sperm in the caudal region of the epididymis were measured.

The results in the present study were compared with our Crj:CD(SD) rat data of the control groups.

RESULTS AND DISCUSSION

There were no notable clinical signs in dams during gestation period. Body weights and body weight gain of dams are shown in Table 1 and 2, respectively. Mean body weights of Crj:CD(SD)IGS rats were 274.9g at day 0 of gestation and 424.1g at day 20 of gestation, respectively. There were no remarkable differences between Crj:CD(SD)IGS and Crj:CD(SD) rats in body weight. Cumulative food consumption and food consumption of dams are shown in Table 3 and 4, respectively. There were no remarkable differences between Crj:CD(SD)IGS and Crj:CD(SD) rats in food consumption. There were no abnormalities in macroscopic examinations of pregnant animals and males having fertility. Mean sperm count of Crj:CD(SD)IGS rats was 81×10^6 /100mg caudal region of the epididymis, background data of sperm count in Crj:CD(SD) rats at same age was 71×10^6 /100mg caudal region of the epididymis, and there were no remarkable differences. Findings at cesarean section and external findings in fetuses are shown in Table 5. The mean number of corpora lutea, implantations, live fetuses, mean post-implantation loss and male ratio (male/live fetus) of Crj:CD(SD)IGS rats were 16.9%, 16.1%, 15.2%, 5.42% and 0.50%, respectively. There were no remarkable differences between Crj:CD(SD)IGS and Crj:CD(SD) rats. Visceral findings in fetuses are shown in Table 6. There were no visceral abnormalities in fetuses of Crj:CD(SD)IGS rats. Skeletal findings in fetuses are shown in Table 7, 8 and 9, respectively. As skeletal abnormalities, shortening of the rib or splitting of the sternebrae were observed each one fetus. As skeletal variations, lumber rib, number of the presacral vertebrae, deformation of the thoracic vertebral body, small thoracic vertebral body and asymmetry of the sternebrae were observed. There were no remarkable differences between Crj:CD(SD)IGS and Crj:CD(SD) rats in the incidence of skeletal abnormalities or skeletal variations and degree of ossification.

It was concluded that there were not obvious differences in parameters between Crj:CD(SD)IGS and Crj:CD(SD) rats. It is considered that Crj:CD(SD)IGS rats can be used for the reproductive toxicity studies.

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Table 1. Body weight (g) of dams during gestation period

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
Day 0	274.9±16.6	262.1±13.6	273.0±14.1
1	280.3±18.9	N.E.	N.E.
2	289.7±19.0	276.3±14.0	288.7±13.8
3	292.0±19.0	N.E.	N.E.
4	294.8±19.4	282.8±14.9	296.5±15.4
5	299.6±18.7	N.E.	N.E.
6	302.9±18.4	291.3±16.3	304.4±16.7
7	306.4±19.2	295.4±16.9	305.0±17.8
8	310.2±19.3	300.8±18.0	309.4±19.7
9	315.1±19.6	304.3±18.5	314.0±20.7
10	319.1±20.6	308.9±19.1	317.9±20.7
11	326.9±20.2	314.4±18.9	324.0±22.0
12	331.2±21.1	319.4±20.4	326.2±20.7
13	336.1±20.9	326.2±20.1	331.6±21.3
14	342.4±21.7	331.0±20.8	337.0±21.5
15	349.6±21.2	339.5±21.5	345.7±21.9
16	362.2±21.8	350.4±21.5	356.0±22.7
17	373.9±23.2	363.5±23.8	369.4±24.4
18	391.5±24.2	378.8±24.5	387.5±25.1
19	406.5±25.7	394.1±26.8	404.6±27.4
20	424.1±26.1	410.5±28.9	421.6±30.1

Values represent mean±S.D. N.E. : Not examined

Table 2. Body weight gain (g/day) of dams during gestation period

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
Day 1	5.5±6.8	N.E.	N.E.
2	9.4±3.8	7.2±2.3	8.0±3.2
3	2.3±4.9	N.E.	N.E.
4	2.8±4.2	3.4±2.1	3.9±2.5
5	4.9±4.6	N.E.	N.E.
6	3.4±3.5	4.3±2.6	3.8±1.9
7	3.4±3.6	4.2±4.4	0.6±3.3
8	3.9±3.3	5.4±3.8	4.5±4.5
9	5.0±3.9	3.5±3.1	4.5±4.9
10	4.0±4.7	4.6±3.4	4.1±6.1
11	7.9±3.4	5.5±4.4	6.0±5.8
12	4.3±3.1	5.1±3.7	2.3±4.9
13	4.9±3.8	6.8±4.8	5.6±3.9
14	6.3±3.8	4.9±4.2	5.4±3.2
15	7.2±4.5	8.4±3.4	8.9±3.3
16	12.6±2.6	11.0±4.6	10.3±4.4
17	11.7±4.0	13.1±5.1	13.6±4.7
18	17.6±3.9	15.5±4.6	18.1±2.3
19	15.0±4.5	15.2±4.8	17.2±4.6
20	17.6±4.5	16.5±5.1	17.1±5.5

Values represent mean±S.D. N.E. : Not examined

Table 3. Cumulative food consumption (g) of dams during gestation period

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
Day 1	21± 3	N.E.	N.E.
2	45± 5	45± 4	48± 5
3	70± 7	N.E.	N.E.
4	93± 9	95± 9	97± 9
5	118±11	N.E.	N.E.
6	142±13	146±14	151±15
7	167±14	173±16	179±18
8	192±17	199±19	205±21
9	217±18	226±22	230±24
10	242±20	253±24	256±27
11	268±22	281±26	282±30
12	295±24	308±29	309±33
13	322±26	337±31	337±36
14	348±28	364±33	363±39
15	376±30	390±34	390±42
16	404±32	418±36	419±44
17	434±33	447±38	448±48
18	463±35	476±40	478±51
19	493±38	505±43	507±54
20	521±39	531±44	534±57

Values represent mean±S.D. N.E. : Not examined

Table 4. Food consumption (g/day) of dams during gestation period

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
Day 1	21±3	N.E.	N.E.
2	24±2	23±2	24±2
3	24±3	N.E.	N.E.
4	24±3	25±2	25±3
5	25±3	N.E.	N.E.
6	25±3	25±3	27±3
7	25±2	27±3	28±4
8	25±3	26±4	26±4
9	25±2	27±4	26±5
10	25±3	27±3	26±3
11	26±2	27±3	26±4
12	27±3	28±3	27±4
13	27±3	29±3	28±4
14	26±3	27±3	27±3
15	27±3	26±3	27±4
16	29±3	28±3	28±3
17	30±2	29±3	29±4
18	30±3	30±3	30±3
19	29±3	28±3	29±4
20	28±3	26±3	27±3

Values represent mean±S.D. N.E. : Not examined

Table 5. Observation at cesarean section of dams

Strain		CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams		28	22	22
No. of corpora lutea	Total	473	374	383
	Mean±S.D.	16.9±1.3	17.0±2.4	17.4±2.6
No. of implantations	Total	450	341	347
	Mean±S.D.	16.1±1.5	15.5±3.4	15.8±3.3
Implantation rate		95.3	91.4	90.5
Post-implantation loss				
Total (%)		24 (5.42)	16 (4.49)	20 (5.64)
Implantation sites (%)		6 (1.39)	8 (2.03)	12 (3.34)
Placental remnants (%)		17 (3.77)	6 (1.85)	6 (1.75)
Resorptions (%)		1 (0.26)	2 (0.61)	2 (0.55)
Macerate fetuses (%)		0 (0.00)	0 (0.00)	0 (0.00)
Live fetuses	Total	426	325	327
	Mean±S.D.	15.2±1.7	14.8±3.2	14.9±3.3
Male ratio		0.50	0.52	0.50
(No. of live male fetuses / No. of live fetuses)		(209 / 209+217)	(170 / 170+155)	(169 / 169+158)
Fatal weight (g)	Mean±S.D.			
Male		3.83±0.19	3.55±0.36	3.52±0.16
Female		3.63±0.19	3.37±0.29	3.37±0.22
Placental weight (g)	Mean±S.D.			
Male		0.44±0.04	0.48±0.06	0.48±0.05
Female		0.42±0.05	0.46±0.05	0.46±0.06
External malformations	Total (%)	0 (0.00)	1 (0.45)[a)]	0 (0.00)

a) : Hematoma

Table 6. Visceral examination of fetuses

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
No. of fetuses	204	156	158
No. of fetuses with malformation (%)	0 (0.00)	1 (0.51)	2 (1.33)
Absence of eyeball (%)	0 (0.00)	1 (0.51)	0 (0.00)
Right aortic arch (%)	0 (0.00)	0 (0.00)	1 (0.57)
Aberrant of left subclavian artery (%)	0 (0.00)	0 (0.00)	1 (0.57)

Table 7. Skeletal abnormalities of fetuses

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
No. of fetuses	222	169	169
No. of fetuses with malformation (%)	2 (0.79)	3 (1.39)	3 (3.49)
Splitting of thoracic vertebral body (%)	0 (0.00)	3 (1.39)	2 (2.84)
Wavy rib (%)	0 (0.00)	0 (0.00)	1 (0.65)
Shortening of rib (%)	1 (0.40)	0 (0.00)	0 (0.00)
Splitting of sternebrae (%)	1 (0.40)	0 (0.00)	1 (0.57)
Fusion of sternebrae (%)	0 (0.00)	0 (0.00)	0 (0.00)

Table 8. Skeletal variations of fetuses

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
No. of dams with variation (%)	10 (35.71)	2 (9.09)	3 (13.64)
No. of fetuses	222	169	169
No. of fetuses with variation (%)	11 (4.68)	2 (0.92)	4 (2.44)
Deformation of thoracic vertebral body (%)	1 (0.40)	2 (0.92)	3 (1.87)
Small thoracic vertebral body (%)	1 (0.45)	0 (0.00)	0 (0.00)
Cervical rib (%)	0 (0.00)	0 (0.00)	0 (0.00)
Lumber rib (%)	6 (2.59)	0 (0.00)	0 (0.00)
Asymmetry of sternebrae (%)	1 (0.40)	0 (0.00)	1 (0.57)
Variation of number of presacral vertebrae (%)	2 (0.84)	0 (0.00)	0 (0.00)

Table 9. Skeletal ossification of fetuses

Strain	CD(SD)IGS	CD(SD)-1	CD(SD)-2
No. of dams	28	22	22
No. of fetuses	222	169	169
No. of sternebrae	5.6±0.3	5.0±0.6	4.9±0.8
No. of sacral and caudal vertebrae	7.8±0.3	7.3±0.4	7.1±0.4
No. of metacarpals of forepaw	3.6±0.3	3.2±0.3	3.2±0.3
No. of proximal phalanges of forepaw	0.0±0.0	0.0±0.0	0.0±0.0
No. of middle phalanges of forepaw	0.0±0.0	0.0±0.0	0.0±0.0
No. of metatarsals of hindpaw	4.0±0.0	4.0±0.0	4.0±0.0
No. of proximal phalanges of hindpaw	0.0±0.0	0.0±0.0	0.0±0.0
No. of middle phalanges of hindpaw	0.0±0.0	0.0±0.0	0.0±0.0

Values represent mean±S.D.

Background Control Data of Reproductive and Developmental Toxicity Study in Crj:CD(SD)IGS Rats - 2000

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ABSTRACT. The background control data in reproductive and developmental toxicity study were investigated using Crj:CD(SD)IGS rats under the inhalation testing condition throughout the last one year. These include body weights and food consumption of female rats for pregnant and lactation periods, fertility, reproductive and fetal parameters obtained by cesarean section, findings of fetal morphological observations, findings of delivery in dams and observations on pups, body weight of pups, findings of observations for behavioral and physical development in pups. These background data will contribute to evaluation of reproductive and developmental toxicity studies of chemical compounds in Crj:CD(SD)IGS rats. − Key words: CD(SD)IGS Rat, Development, Reproduction

CD(SD)IGS-2000: 136-140

INTRODUCTION

Recently Crj:CD(SD)IGS rats are being used, therefore, adequate historical control data are very important. As inhalation toxicity studies have been mainly conducted in our laboratory, we investigated the background control data of reproductive and developmental toxicity study of rats kept in the inhalation chamber for definite periods, as the continuation of our previous papers[4,5].

MATERIALS AND METHODS

Nine-week-old female and 10-week-old male Crj:CD(SD)IGS rats were purchased from Charles River Japan, Atsugi. A total of 222 females and 222 males in 12 studies performed during 12-month (March 1999 - February 2000) were used in this study. Following quarantine for 1 week, they were kept in the inhalation chamber (1.06m^3). They were individually housed in the suspended stainless-steel wire-mesh cage except for mating and lactation periods. Animals allowed to deliver were removed from the inhalation chamber on day 20 of gestation, and animals were housed in aluminium cages with paper pulp tip as nesting material (ALPHA-dri., Shepherd Specialty Paper, Inc., USA). Room temperature and humidity were maintained at 22 ± 2℃, 55 ± 10%, respectively, with a 12-hr light/ dark cycle (08:00-20:00/ 20:00-08:00). The chamber environment was maintained at 23 ± 2℃, 55 ± 10%, and 12 ± 1 times/h ventilation. Tap water and commercial pellet diet (CRF-1, γ-irradiated with 30 KGy, Oriental Yeast, Tokyo) were given *ad libitum*.

The animals were observed for clinical signs once a day. The females were weighed on days 0, 7, 14, 20 of gestation, and on days 0, 4, 7, 14, 21 of lactation. Food consumption was measured during days 0-7, 7-14, 14-20 of the gestation period, and during days 0-4, 4-7, 7-14 of the lactation period. At 10 weeks of age, they were paired 1:1 basis with males of 11 weeks of age for a maximum of 4 days. By the presence of a vaginal plug or sperms in the vaginal smear, day 0 of gestation was determined. The copulation index, fertility index, and days until copulation were determined.

For the cesarean section, 10-13 pregnant females on each study were autopsied 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 the modified Salewski's methods[5]. The embryo/fetal mortality was determined based on the number of implantations. After measurement of fetal body weights and placental weights, fetuses were observed for sex and external abnormality. Two-third of live fetuses in each litter were assigned for preparation of skeletal specimens by Dawson's methods[1], skeletal abnormality and the number of ossified sacral-caudal vertebrae were examined. The remaining live fetuses were observed for visceral abnormality by Wilson's and Nishimura's methods[3,7]. Terms for these observations mostly quoted from a previous report[2].

Six pregnant females on 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 abnormalities on the day of birth (day 0 of lactation), and the birth index and live birth index on day 0 of lactation were determined based on the litter size (total number of live and dead newborn). The number of pups counted daily after birth to determined the viability index on day 4 of lactation and weaning index. On day 4 of lactation, the number of pups per litter was adjusted to 4 animals of each sex. Male and female pups were weighed separately on a litter basis on days 0, 4, 7, 14 and 21 of lactation. They were weighted individually once a week from weaning until the age of 10 weeks. During the lactation period, 2 males and 2 females selected from each litter were examined for surface righting reflex, cliff drop aversion response and negative geotaxis as indices of early behavioral ontogeny and for eruption of upper incisors, ear opening and eyelid opening as indices of physical development until completion of each development.

RESULTS AND CONCLUSION

Body weights and food consumption of dams are shown in Table 1 and 2, respectively. Fertility in female rats is shown in Table 3. Reproductive and fetal parameters obtained by the cesarean sectioning are shown in Table 4. Morphological findings in fetuses are shown in Table 5. Most frequent skeletal variation was short supernumerary rib, and following dumbbell ossification of tho-

Table 1. Body Weight of Dams

Gestational period		
No. of dams		201
Body weight (g)		
	GD 0	241± 8 (228 - 259) [a)]
	7	282± 9 (268 - 300)
	14	315±11 (299 - 335)
	20	389±13 (367 - 413)
Lactational period		
No. of dams		66
Body weight (g)		
	LD 0	294±13 (282 - 317)
	4	306±17 (287 - 337)
	7	324±12 (306 - 340)
	14	337±13 (321 - 363)
	21	321±12 (300 - 341)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)
GD : gestational day LD : lactational day

Table 2. Food Consumption of Dams

Gestational period		
No. of dams		201
Food consumption (g/day)		
	GD 0 - 7	24±1 (22 - 26) [a)]
	7 - 14	25±1 (23 - 26)
	14 - 20	25±1 (23 - 28)
Lactational period		
No. of dams		65
Food consumption (g/day)		
	LD 0 - 4	29±2 (27 - 33)
	4 - 7	42±2 (37 - 46)
	7 - 14	57±6 (52 - 71)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)
GD : gestational day LD : lactational day

Table 3. Fertility in Female Rats

No. of mated pairs	222
Copulation index (%)	95.2±4.2 (89.5 - 100)[a)]
Fertility index (%)	96.4±5.4 (83.3 - 100)
Days until copulation	2.4±0.3 (2.1 - 3.0)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)
Copulation index (%) = (No. of animals copulated successfully / no. of mated animals) × 100
Fertility index (%) = (No. of pregnant animals / no. of animals copulated successfully) × 100

Table 4. Reproductive and Fetal Parameters obtained by Cesarean Section

No. of dams		136
No. of corpora lutea		16.1± 0.9 (14.8 - 17.5) [a)]
No. of implantation		14.7± 0.9 (13.1 - 16.3)
Implantation rate (%)		91.5± 3.5 (83.8 - 95.7)
Implantation loss (%)		6.5± 3.0 (3.3 - 14.7)
No. of live fetuses	Male	7.0± 0.6 (5.7 - 7.7)
	Female	6.8± 0.5 (6.0 - 7.6)
	Total	13.9± 0.9 (12.2 - 15.2)
Sex ratio		1.2± 0.2 (1.0 - 1.6)
Fetal weight (g)	Male	3.90±0.11 (3.77 - 4.15)
	Female	3.70± 0.1 (3.58 - 3.93)
Placental weight (g)	Male	0.51±0.05 (0.47 - 0.64)
	Female	0.48±0.02 (0.45 - 0.51)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)
Implantation rate (%) = (No. of corpora lutea / no. of implantations) × 100
Implantation loss (%) = (No. of intrauterine death / no. of implantations) × 100
Sex ratio = No. of male live fetuses / no. of female live fetuses

Table 5. Morphological Observations in Fetuses

	Number	%	(Min. - Max.) [b)]
External observation (1357) [a)]			
External abnormalities	1	0.07	(0 - 0.55)
Generalized edema	1	0.07	(0 - 0.55)
Skeletal observation (860)			
Skeletal abnormalities	1	0.12	(0 - 0.81)
Fused cervical arch	1	0.12	(0 - 0.81)
Supernumerary cervical vertebrae	1	0.12	(0 - 0.81)
Fused thoracic arch	1	0.12	(0 - 0.81)
Absent thoracic arch	1	0.12	(0 - 0.81)
Incomplete ossification of thoracic centrum	1	0.12	(0 - 0.81)
Fused lumber arch	1	0.12	(0 - 0.81)
Fused rib	1	0.12	(0 - 0.81)
Skeletal valiations	119	13.84	(8.33 - 21.77)
Dumbbell ossification of thoracic centrum	28	3.26	(0.96 - 7.43)
Bipartite ossification of thoracic centrum	7	0.81	(0 - 2.42)
Short supernumerary rib	85	9.88	(5.26 - 14.52)
Cervical rib	3	0.35	(0 - 1.92)
No. of ossified sacral-caudal vertebrae	7.9 ± 0.1 [c)]		
Visceral observation (497)			
Visceral abnormalities	15	3.02	(0 - 8.00)
Membranous ventricular septum defect	15	3.02	(0 - 8.00)
Abnormal origin of right common carotid artery	1	0.2	(0 - 1.75)
Diaphragmatic hernia	1	0.2	(0 - 2.08)
Visceral variations	26	5.23	(0 - 10.00)
Persistent left umbilical artery	2	0.40	(0 - 2.13)
Thymic remnant in neck	25	5.03	(0 - 10.00)
Supernumerary coronary ostium	1	0.2	(0 - 2.08)

( )[a)] No. of fetuses examined
( )[b)] Minumum and maximum values in incidence of 10 studies
[c)] Values represent mean ± S.D.

racic centrum. Most frequent visceral abnormality and variation were membranous ventricular septum defect and thymic remnant in neck, respectively. Although in comparison with our previous data [4,6], the numbers and/or most kinds of abnormalities and variations decreased.

The natural delivery parameter and viability of pups are shown in Table 6, and body weight of pups in Table 7. No abnormal clinical signs were observed in any pups. Observations for behavioral and physical development of pups are shown in Table 8. No remarkable differences were observed in comparison with our previous data [4,6].

These data will contribute to evaluate the results in reproductive and developmental toxicity study in Crj:CD(SD)IGS rats.

Table 6. Delivery Parameter and Viability of Pups

No. of dams		66
Gestation length (day)		22.0±0.1 (21.7 - 22.2) [a)]
No. of implantations		15.0±0.8 (14.0 - 16.3)
No. of pups delivered		13.8±1.3 (11.5 - 15.7)
No. of live pups		13.6±1.4 (11.2 - 15.7)
Birth index (%)		92.3±6.0 (77.5 - 99.0)
Sex ratio		1.1±0.3 (0.8 - 1.6)
Viability index (%)	at birth	98.3±2.7 (90.5 - 100)
	day 4	97.4±4.8 (83.3 - 100)
	day 21	98.3±3.3 (89.6 - 100)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)
Birth index (%) = (No. of live pups / no. of implantations) × 100
Sex ratio = No. of male live fetuses / no. of female live fetuses

Table 7. Body Weight of Pups

Before weaning				
No. of dams		66		
Body weight (g)		Male		Female
	at birth	6.6±0.1	(6.4 - 6.7) [a)]	6.2±0.1 (6.1 - 6.3)
	day 4	10.1±1.0	(8.5 - 11.4)	9.3±0.9 (8.1 - 10.7)
	day 7	16.3±1.6	(13.8 - 19.2)	15.5±1.5 (13.0 - 18.1)
	day 14	37.0±3.9	(28.9 - 41.6)	32.5±2.9 (26.8 - 35.8)
	day 21	55.7±4.9	(45.4 - 61.0)	52.7±4.8 (43.7 - 57.9)
After weaning				
		Male		Female
No. of pups		124		126
Body weight (g)				
	week 4	96.8± 9.1	(81.4 - 107.8)	89.2± 7.7 (73.8 - 99.5)
	week 5	155.2±13.4	(132.9 - 173.2)	133.1± 9.6 (114.0 - 148.7)
	week 6	217.9±16	(217.2 - 245.7)	168.8± 9.1 (152.7 - 186.4)
	week 7	298.4±16.9	(247.7 - 307.1)	195.5± 9.7 (180.8 - 211.8)
	week 8	342.1±21.6	(307.8 - 372.6)	220.6±10.0 (207.3 - 239.6)
	week 9	392.7±20.1	(356.9 - 423.8)	241.0±10.4 (225.5 - 257.1)
	week 10	433.9±17.7	(400.4 - 454.6)	257.3±10.0 (242.1 - 273.3)

[a)]mean ± S.D. (minimum and maximum values in average of 11 studies)
Body weights of pups before weaning were weighed separately on a litter basis.

Table 8. Behavial and Physical Development of Pups

Behavial development (Day of achievment)		
	Male	Female
No. of pups	128	126
Surface righting	2.0±0.5 (1.2 - 2.8)[a)]	2.4±0.7 (1.6 - 3.9)
Cliff aversion	6.4±1.2 (5.3 - 8.7)	6.5±1.0 (5.5 - 8.4)
Negative geotaxis	10.1±1.0 (8.3 - 11.5)	10.0±1.0 (8.3 - 11.5)
Physical development (Day of achievment)		
	Male	Female
No. of pups	128	126
Incisor eruption	10.0±0.3 (9.4 - 10.6)	10.2±0.4 (9.4 - 10.8)
Ear opening	12.5±0.3 (12.2 - 13.2)	12.3±0.4 (11.8 - 13.0)
Eye opening	14.5±0.2 (14.3 - 15.0)	14.4±0.2 (14.2 - 14.9)

[a)]mean ± S.D. (minimum and maximum values in average of 12 studies)

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Morphology and Incidence of Rudimentary Cervical Ribs in Crj:CD(SD) and Crj:CD(SD)IGS Rats

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ABSTRACT. We have examined 215 Crj:CD(SD) rats and 20 Crj:CD(SD)IGS rats, of the age arranging from 2 weeks to 8 months, supplied from 3 breeding centers (Atsugi, Hino and Tsukuba) of Charles River Japan, for the presence of ossicles, which were seemed to be rudimentary cervical ribs. The ossicles were classified into Type 1 (small ossicle) and Type 2 (large ossicle). These ossicles were observed in animals at 4 weeks of age and above. Both incidence and size increased according to age. The incidences of Type 2-ossicle in Tsukuba CD and Atsugi CD rats were significantly higher than those in Hino CD and Tsukuba IGS rats. The occurrence of Type 2-ossicle might have resulted from genetic factors. — Key words: Cervical ribs, Crj:CD(SD), Crj:CD(SD)IGS, Ossicle

CD(SD)IGS-2000: 141-145

INTRODUCTION

Cervical ribs are rudimentary abnormal ribs, which articulate with the transverse process of the seventh cervical vertebrae, and are considered as congenital anomalies classified as skeletal variations. In human, they occasionally compress the brachial plexus or the subclavian artery, and can cause 'thoracic outlet syndrome' clinically. This is characterized by pain, wasting of the thenar musculature along the inner side of the forearm [7]. In rats, cervical ribs could be induced by chemicals, such as nitrous oxide [5], tri-n-butylitin acetate [12], propylene oxide [6], dichloroacetonitrile [13], ofloxacin [15], methanol [11], sodium salicylate [2] and nefiracetam [17] *etc*. However, the spontaneous occurrence of cervical ribs is low (0.05-4.23 %) [10], and little detail of them has been reported.

We have observed the ossicles, which are considered to be cervical ribs, in Crj:CD(SD) rats and Crj:CD(SD)IGS rats. In this present study, we described their morphology and compared the incidences in four groups of rats from the three breeding centers in Charles River, Japan.

MATERIALS AND METHODS

One hundred forty-seven male and 68 female Crj:CD(SD) rats of the age between 2 weeks and 8 months were purchased from Hino Breeding Center (Shiga: Hino CD group), Atsugi Breeding Center (Kanagawa: Atsugi CD group) and Tsukuba Breeding Center (Ibaraki: Tsukuba CD group) (Table 1). Twenty male Crj:CD(SD)IGS rats at 8 months of age were purchased from Tsukuba Breeding Center (Ibaraki: Tsukuba IGS group) (Table 1). The animals were single-housed in wire-mesh cages and in an air conditioned room (temperature, 23 ± 2℃; humidity, 55 ± 15%; light cycle, 12 hr/day). Food and water were available *ad libitum*. Following the week long acclimatization, the animals were sacrificed under diethyl ether anesthesia and necropsied. The cervixes and thoraxes were excised, fixed in 99% ethanol, stained with alizarin, cleared in 1% KOH and preserved in glycerin [8]. All skeletal elements were examined under a dissecting microscope. The length and width of the ossicles were measured as indicated in Figure 1. Statistical differences of the data were determined using Fisher's Exact Test.

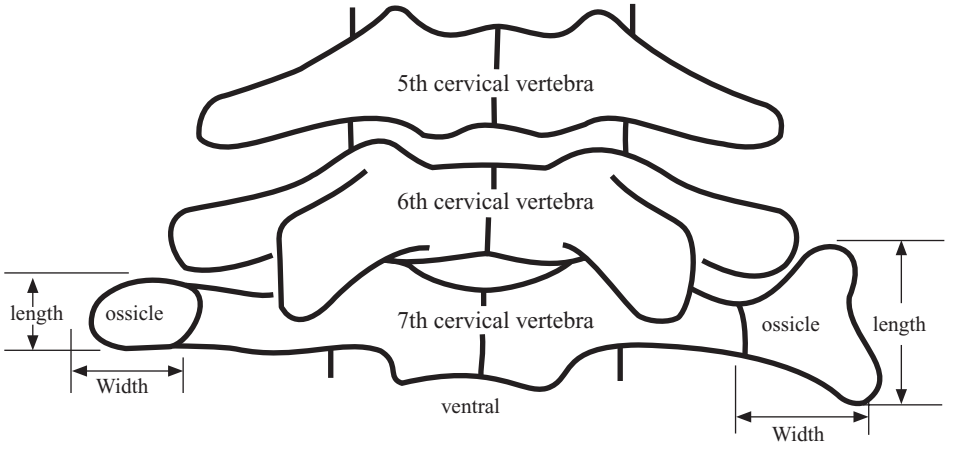

Figure 1. Measurements of ossicles

RESULTS

Signs: No abnormal general sign was noted in all rats.

Classification: The ossicles were observed next to the transverse process of the seventh cervical vertebrae. They were classified based on size as follows (Photo 1): Type 1 was defined as a small ossicle (less than 2mm × 2mm); Type 2 was defined as a large ossicle (more than 2mm × 2mm). Each type was divided into two subtypes based on separated or coalesced to the transverse process, "S" or "C", respectively. Type 2-ossicles occurred in various shapes, and they either reduced or deformed the transverse process of the seventh cervical vertebrae. Some affected the transverse process of the sixth cervical vertebrae or the first thoracic vertebrae.

Development of ossicles in Tsukuba CD group: The ossicles were observed after 4 weeks of age (Table 1, Photo 2). Both incidences and size increased according to age (Table 2). At 17 weeks of age, the ossicles were observed in all rats, and their size reached a plateau. At 8 months of age, they were coalesced to the transverse process (Type 1-C and Type 2-C) with bilateral occurrence incidence reaching 34.5%.

Incidence in each group: Incidences of each type of ossicles in all groups of rats were summarized in Table 1. There was no sex-difference and side-difference in the incidences in Tsukuba CD group. The incidences of Type 2-ossicle in Hino CD, Tsukuba IGS, Atsugi CD and Tsukuba CD groups at 8 month of age were 0 %, 10 %, 31.6 % and 41.4%, respectively (Fig. 2). The incidences in Tsukuba CD group were significantly higher than those in either Hino CD group ($p<0.01$) or Tsukuba IGS group ($p<0.05$). The incidences in Atsugi CD group were significantly higher than those in Hino CD group ($p<0.05$).

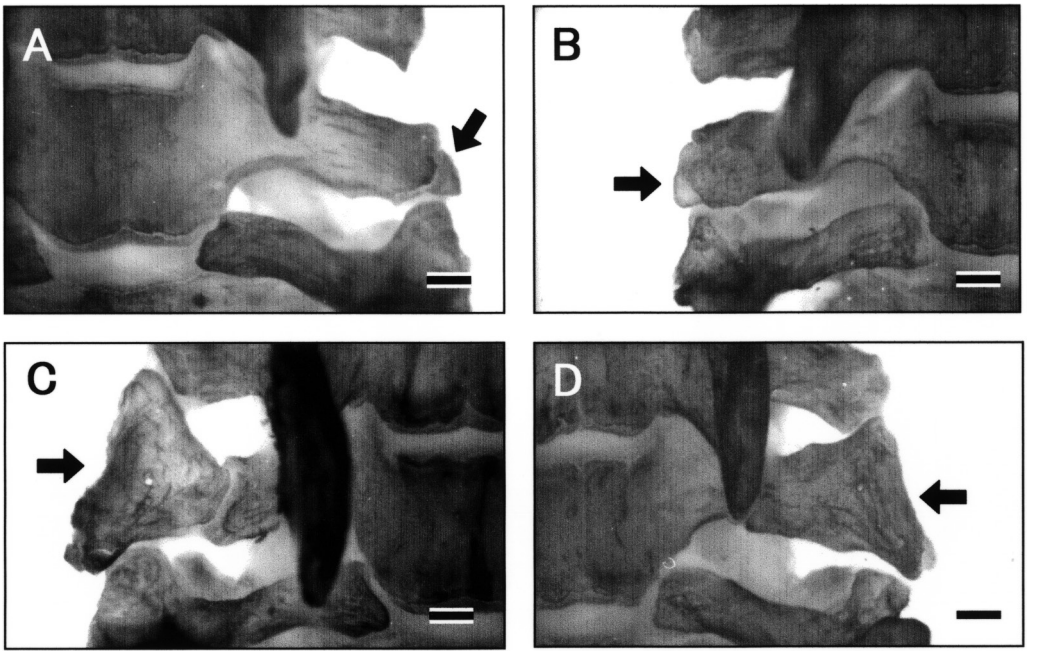

Photo 1. Each type of ossicles (arrow). (A): Type 1-S; Small ossicle separated to transverse process, (B): Type 1-C; Small ossicle coalesced to transverse process, (C): Type 2-S; Large ossicle separated to transverse process, (D): Type 2-C; Large ossicle coalesced to transverse process. Bar=1mm.

Table 1. Incidence of Ossicles in Crj:CD(SD) Rats

Group	Sex	Age	Number of animals	Incidence of ossicles (%)							
				Left				Right			
				Type 1		Type 2		Type 1		Type 2	
				Type 1-S	Type 1-C	Type 2-S	Type 2-C	Type 1-S	Type 1-C	Type 2-S	Type 2-C
Hino CD	Male	8M [1)]	19	73.7	26.3	0.0	0.0	70.0	30.0	0.0	0.0
Tsukuba IGS	Male	8M	20	70.0	20.0	5.0	5.0	80.0	20.0	0.0	0.0
Atsugi CD	Male	8M	20	15.8	57.9	26.3	0.0	26.3	42.1	21.1	10.5
Tsukuba CD	Male	2W [2)]	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		4W	22	4.5	0.0	0.0	0.0	13.6	0.0	0.0	0.0
		6W	6	33.3	0.0	0.0	0.0	66.6	0.0	0.0	0.0
		8W	24	75.0	0.0	0.0	0.0	75.0	0.0	0.0	0.0
		17W	7	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
		8M	29	55.2	27.6	10.3	6.9	44.8	20.7	20.7	13.8
	FeMale	2W	19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		4W	20	5.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
		6W	6	83.3	0.0	0.0	0.0	50.0	0.0	0.0	0.0
		8W	23	95.7	0.0	0.0	0.0	78.3	0.0	0.0	0.0

1) M: month-old, 2) W: week-old

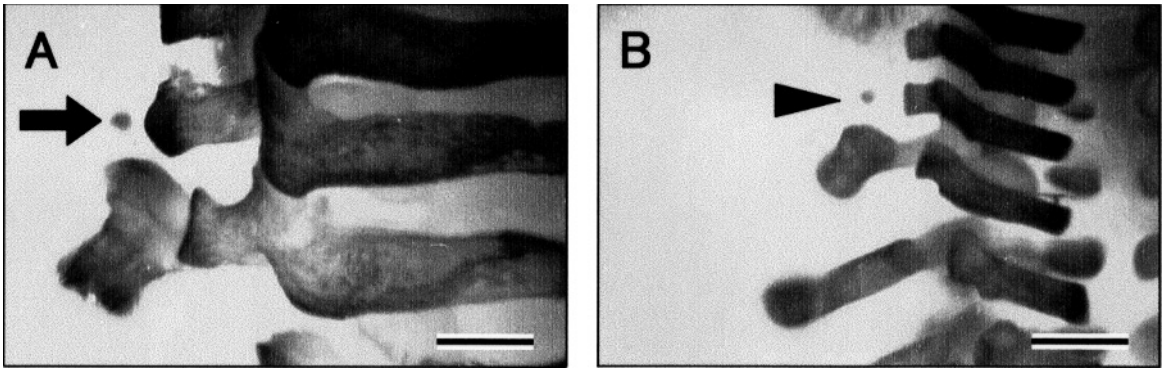

Photo 2. (A): Ossicle (arrow) at 4 weeks of age, (B): Cervical rib (arrow head) in neonatal rats. Bar=1mm.

Table 2. Length and Width of Type S Ossicles in Tsukuba CD Group

Sex	Age	Type 1-S			Type 2-S		
		Number of ossicles	Length Mean±SD	Width Mean±SD	Number of ossicles	Length Mean±SD	Width Mean±SD
Male	2W [1)]	0	ND	ND	0	ND	ND
	4W	4	0.20±0.08	0.20±0.08	0	ND	ND
	6W	4	0.40±0.17	0.50±0.23	0	ND	ND
	8W	36	0.42±0.21	0.36±0.17	0	ND	ND
	17W	14	1.19±0.31	1.01±0.22	0	ND	ND
	8M [2)]	97	1.20±0.30	1.14±0.30	19	3.05±0.65	2.16±0.39
FeMale	2W	0	ND	ND	0	ND	ND
	4W	3	0.37±0.38	0.27±0.21	0	ND	ND
	6W	5	0.29±0.04	0.33±0.07	0	ND	ND
	8W	40	0.44±0.16	0.34±0.18	0	ND	ND

1) W: week-old, 2) M: month-old
ND: Not detected

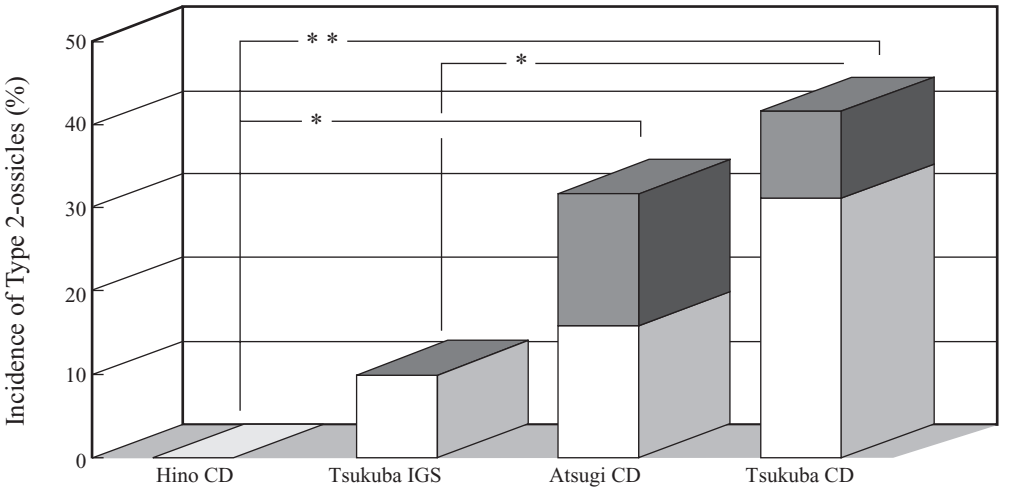

Figure 2. Incidence of Type 2-ossicles in each group
■: Bilateral, □: Unilateral.
*,**; Significantly different at the $p<0.05$, $p<0.01$, respectively (Fisher's Exact Test).

DISCUSSION

In the present study, we have observed the ossicles next to the transverse process of seventh cervical vertebrae in rats. The incidences increased according to age, and were observed in all rats at 17 weeks of age. After that, the ossicles were coalescent to the transverse process. In rats, it is known cervical ribs, as the ossicles, situated at the transverse process of seventh cervical vertebrae. In human, McNally *et al*. reported that cervical ribs were found in 63% of stillborn fetus [9]. Todd observed that the costal elements of the seventh vertebrae were normally present in fetus [16]. However, it has been reported that the incidence of cervical ribs in adults was between 0.03 - 1% [3, 4, 14, 18]. The differences between fetus and adult may be due to the coalescence of the cervical ribs to the transverse process of the seventh cervical vertebrae after birth. Although the rat ossicles, as shown in our study, and human cervical ribs appear to occur at different time point, they appear to be undergoing the same progression. Furthermore, the morphology of the ossicles at 4 weeks of age was very similar to the cervical ribs found in neonates (Photo 2). From our findings, we suppose that the observed ossicles are cervical ribs in rats. However, although the Type 2-ossicles were large and induced structural changes in cervical vertebrae in rats, they did not form a vertebrarterial foramen [1] and no clinical sign similar to the 'thoracic outlet syndrome' in human was observed. Thus, the clinical significance of cervical ribs in rats may have been different from that of human.

The ossicles were divided into two main types according to size. There were differences in incidence of each type among the groups at 8 months of age. The incidences of Type 2-ossicle in Tsukuba CD and Atsugi CD groups were higher than those in Hino CD and Tsukuba IGS groups. It is known that Tsukuba CD rats derived from Atsugi CD rats, therefore their hereditary characteristic may be similar. In addition, it is also known that Tsukuba IGS rats derived from some breeding colonies, including that of Hino CD rats. Therefore, the occurrence of Type 2-ossicle may be resulted from pre-deposited genetic factors specific to the individual rat groups.

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Effects of an Agent Inducing Dominant Lethals on Rat Sperm -Examination With Ethyl Methanesulfonate-

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ABSTRACT. Ethyl methanesulfonate (EMS), an alkylating agent which induces dominant lethals, was administered in oral doses of 100 mg/kg to Crj:CD(SD)IGS male rats for 5 consecutive days. At the termination of treatment and after a 28-day withdrawal, mating with untreated females and sperm analysis (motion, number, and morphology) were performed. The copulated females were sacrificed at 20 days of gestation.
At the termination of treatment, no clinical signs related to EMS were observed except for a decrease in body weight. Gross pathology and sperm analysis revealed no abnormalities in treated males. However, females mated at the termination of treatment had a clearly higher fetal mortality.
Females mated after the 28-day withdrawal exhibited lower fetal mortality than females mated at the termination of treatment. On the other hand, emales mated after the 28-day withdrawal exhibited a lower implantation rate that was not observed in females mated at the termination of treatment.
For males after a 28-day withdrawal, sperm analysis revealed both a decrease in sperm motion and number and an increase in morphological change.
These findings indicate that two types of male reproductive toxicity induced by EMS can be distinguished. One induces a low implantation rate that can be detected by sperm analysis, while the other induces fetal lethals that could not be detected by sperm analysis in this study. — Key words: Ethyl methanesulfonate, Dominant lethal, Sperm analysis, IGS rats, CASA, CellSoft-4000

CD(SD)IGS-2000: 146-151

INTRODUCTION

When drugs or other kinds of chemicals were assessed for their effects on male fertility, the importance of sperm analysis attracted a growing interest. Based on the ICH agreement, Japanese guidelines for Reproductive and Developmental Toxicity Studies were amended on September 11 in 1997. The new guidelines included sperm analysis for a study of Fertility and Early Embryonic Development to Implantation. However, appropriate indices of sperm analysis for a study of male fertility are not fully understood. So far, by means of sperm analysis, we have examined the antifertility effects of α-chlorohydrin, which induces a decrease in sperm motion without abnormalities in both histopathological examination of the testes and observation of sperm morphology (Yamada *et al*., 1995). In the present study, we selected ethyl methanesulfonate (EMS), an alkylating agent which induces dominant lethals in rodents. EMS is a mutagen which induces positive reaction in Ames assay (Klopman *et al*., 1990), UDS assay (van Erp *et al*., 1992), and Micronucleus assay (Kondo *et al*., 1989). The dominant lethal effect was reported to continue for 4 weeks in rats at both oral doses of 100 mg/kg for 5 consecutive days (McGregor *et al*., 1983) and at an intraperitoneal single dose of 300 mg/kg (Jackson *et al*., 1961). It was also reported that the agent induced dominant lethals in rats at an intraperitoneal single dose of 120 m/kg, but did not induce a decrease in testis weight or histopathological changes in testes for 10 weeks (Murao *et al*., 1980). The aim of the present study was to confirm the characteristics of EMS-inducing dominant lethals in Crj:CD(SD)IGS rats and to examine the sperm conditions inducing dominant lethals.

MATERIALS AND METHODS

Chemicals

EMS and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Tokyo, Japan). Dulbecco's phosphate-buffered saline (D-PBS) containing glucose was obtained from Gibco BRL (Tokyo, Japan). All other reagents were purchased from commercial sources and were of at least analytical grade.

Animals

Crj:CD(SD)IGS rats were purchased from Charles River Japan, Inc. (Tsukuba, Japan). After quarantine and acclimatization, healthy males (12 weeks old) and females (11 weeks old) were chosen for experiments. The animal room was maintained at 23 ± 3℃, with 50 ± 20% relative humidity and a 12 hr light - dark cycle (lights on 7:15 to 19:15). Pelleted diet (MF, Oriental Yeast Inc., Tokyo) and tap water were available *ad libitum.*

Experimental design

EMS was prepared freshly each day as a solution in distilled water. Male rats were treated with EMS (100 mg/kg/day, *p.o.*, 0.5 ml/100 g body) once daily for 5 consecutive days. Males in the control group received the solvent alone at the same volume-dosage during the same treatment period. On the day after final administration and after a 28-day withdrawal, the effects of EMS were assessed. The 28-day period is about two cycles of spermatogenesis in testes and enough for spermatozoa to pass through an epididymis. Nine males were mated with untreated virgin females, and 8 other males were autopsied to examine sperm condition. The experimental design is shown in Fig. 1.

Copulation

Treated males were paired on a one-to-one basis with untreated virgin pro-oestrus females overnight. The following morning, females with sperm detected in the vaginal smear were considered to be at day 0 of gestation. At day 20 of gestation, dams were exsanguinated under ether anesthesia and sacrificed. The numbers of implantations and corpora lutea were counted, and the implantation rate was calculated. Dead fetuses were classified as Resorbed, Early Death, Middle Death, or Late Death according to their size and shape, and the fetal death rate was calculated. Live fetuses were weighed and then examined for sex ratio and external anomalies.

Note: This paper is reprinted from original paper (J. of Toxicological Sciences; 25 (1), 25-31, 2000)

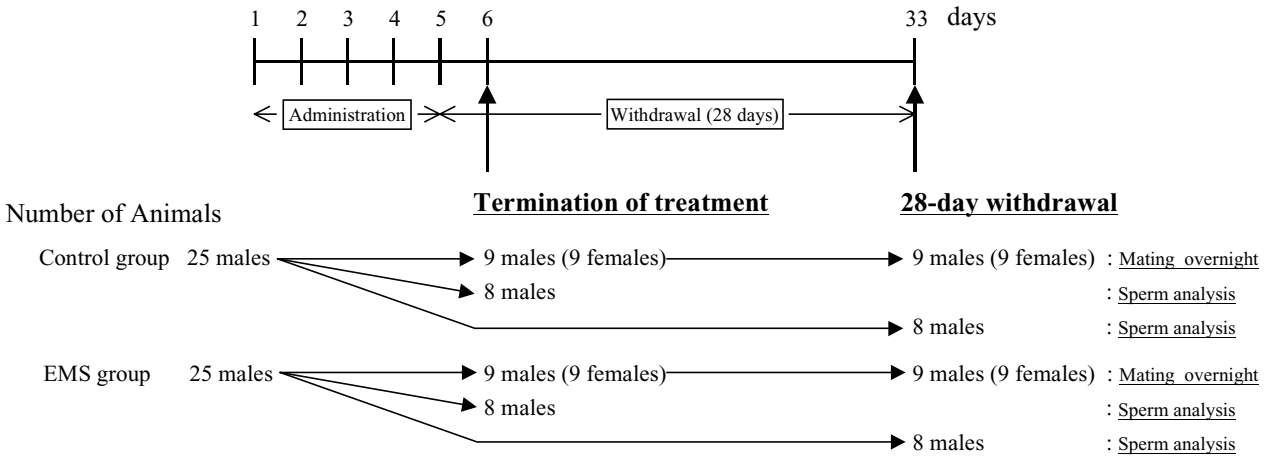

Figure 1. Experimental design.
Male rats were treated with distilled water or EMS once daily for 5 consecutive days. On the day after final dministration (Termination of treatment) and after a 28-day withdrawal (28-day withdrawal), mating and sperm analysis were performed.

Sperm analysis

The epididymides were dissected out and weighed. A small part of a caudal epididymis was cut and placed into Petri dishes containing 10 ml of medium (37℃, D-PBS containing 0.5% BSA). After 15 min, the motion of 100 or more sperm was analyzed with the CellSoft-4000 computer-assisted sperm analysis system (CASA, CRYO Resources Ltd., NY). The indices calculated by the CASA were percent motile, velocity, amplitude of lateral head displacement (A.L.H.), beat/cross frequency, radius, and percentage of circularly swimming sperm. The other caudal epididymis was weighed and minced in saline (37℃). fter removal of the pieces of organ by filtration, the sperm solution was used for microscopical examination of sperm number and morphology. Sperm number was calculated per weight of caudal epididymis. Sperm morphology was classified as tailless or anomalous.

Statistical analysis

Data were statistically analyzed using the tests listed below. All quantitative data were tested for equal variance ($p<0.05$). If equal variance was found, the data were analyzed by the Student's *t-test* (Yoshimura, 1988). If not, the data were analyzed by Welch's *t-test* (Yoshimura, 1988). Copulation rate, impregnation rate, and sex ratio were analyzed by Fisher's exact probability test (Yoshimura, 1988). Implantation rate and fetal death rate were analyzed by the Wilcoxon test (Yoshimura, 1988). For statistical analysis, fetal parameters served as the experimental units of a dam. All data in the EMS group were compared with data in the corresponding control group. In addition, the fertility data and the findings in dams and fetuses at caesarean section were compared between the day after final administration and after a 28-day withdrawal (Table 1, 2). All statistical tests were performed at the 0.05 or 0.01 levels of significance.

RESULTS

Clinical signs

No clinical signs related to EMS treatment were observed for either males or females, except for a decrease in body weight. Male body weight decreased soon after EMS treatment (Fig. 2). After the treatment period, body weight in the EMS group recovered in parallel with the trend of body weight in the control group, though the final weight in the EMS group was lower than the weight the control group.

Table 1. Fertility data.

	Termination of treatment		28-day withdrawal	
	Control	EMS	Control	EMS
No. of male animals	9	9	9	9
No. of copulate males [% a)]	8 [88.9]	8 [88.9]	9 [100]	9 [100]
No. of males impregnating [% b)]	8 [100]	8 [100]	9 [100]	9 [100]

a): (No. of copulate males/ No. of males mated)×100
b): (No. of males impregnating/ No. of copulate males)×100

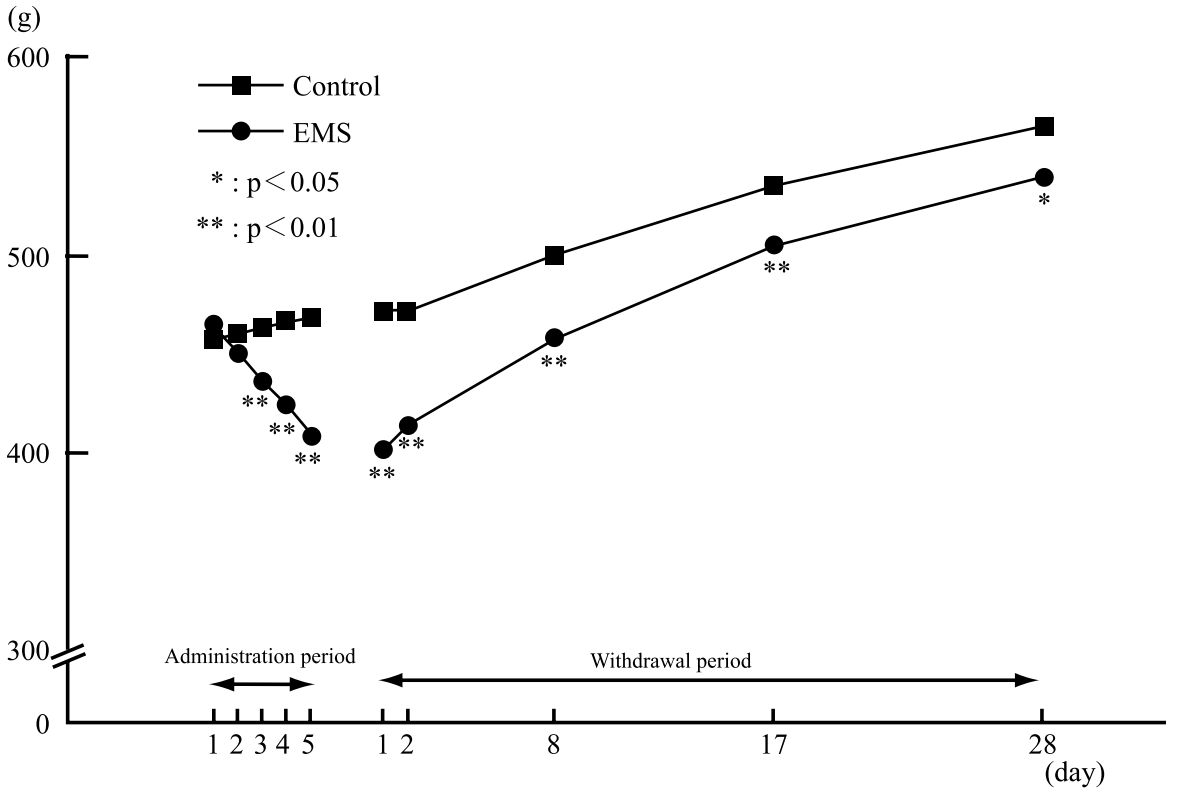

Figure 2. Body weight chages in males during administration period and withdrawal period.

Table 2. Findings in dams and fetuses at caesarean section.

	Termination of treatment		28-day withdrawal	
	Control	EMS	Control	EMS
No. of dams	8	8	9	9
No. of females aborted	0	0	0	1
No. of corpora lutea	17.0±2.0	15.4±3.1	17.9±3.4	16.0±2.7
No. of implantations	15.1±2.5	13.6±2.8	15.3±2.1	9.2±4.8** #
Implantation rate (%)	89.3	88.9	87.7	56.7** #
No. of fetal deaths				
Total [% a)]	11 [9.2]	85 [90.1**]	7 [5.1]	16 [22.2** ##]
Resorbed [%]	11 [9.2]	84 [88.9**]	6 [4.4]	12 [16.2##]
Early death [%]	0 [0.0]	1 [1.2]	1 [0.7]	3 [5.2]
Middle death [%]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]
Late death [%]	0 [0.0]	0 [0.0]	0 [0.0]	1 [0.9]
Live fetuses				
No. of live fetuses	13.8±2.9	1.4±1.3**	14.6±2.1	7.4±4.3** ##
Sex ratio	1.3	1.0	0.9	0.7
Male/Female	63/47	5/5	62/69	27/40
Body weight (g)				
Male	3.56±0.19	3.84±0.08*	3.48±0.46	3.85±0.38
Female	3.39±0.26	3.85±0.18*	3.33±0.42	3.60±0.29
No. of fetuses with external anomalies [%]	0 [0.0]	0 [0.0]	0 [0.0]	0 [0.0]

Mean±S.D.,
a): (No. of dead fetus/ No. of implantations)×100,
Significantly different from the corresponding control value (* p<0.05, ** p<0.01),
Significantly different from the EMS group at the termination of treatment (# p<0.05, ## p<0.01)

Copulation

Both numbers of copulate males and males impregnating were not influenced by EMS treatment (Table 1).

One of the dams copulated with EMS-treated males after a 28-day withdrawal was aborted (Table 2). Decrease in implantation rate was observed only in dams copulating with EMS-treated males after a 28-day withdrawal. Dams that copulated with EMS-treated males at the termination of treatment exhibited both a higher rate of fetal death and lower number of live fetuses. Dams copulating with EMS-treated males after a 28-day withdrawal also exhibited both a higher rate of fetal death and lower number of live fetuses, but the fetal death rate was decreased and the number of live fetuses was increased by comparison with dams that copulated with EMS-treated males at the termination of treatment.

There was a lower number of live fetuses accompanied with a higher body weight of live fetuses in dams copulating with EMS-treated males at the termination of treatment.

There were no fetuses with external anomalies in any groups.

Sperm analysis

At the termination of treatment, weights of testes and of epididymides in the EMS group were not significantly different from weights of the organs in the control group (Table 3). After a 28-day withdrawal, absolute weights of testes and of epididymides in the EMS group were lower than the control value, but relative weights of the organs were not significantly different from the control values.

At the termination of treatment, the indices of sperm motion in the EMS group were not significantly different from the control values except for the percentage of circularly swimming sperm (Table 4). Sperm number and morphology were not influenced by EMS treatment. After a 28-day withdrawal, some indices of sperm motion were changed by EMS treatment. The percent motile, velocity, A.L.H. max, and A.L.H. mean were lower than those of the control group. Sperm number and morphology were also influenced by EMS treatment. The sperm number was lower and the rate of morphological change was higher than in the control values.

DISCUSSION

In the examination at the termination of treatment, a high fetal death rate was observed in the EMS-treated group, but sperm analysis did not reveal abnormalities except for the percentage of circularly swimming sperm. The percentage of circularly swimming sperm, a sperm motion parameter, was lower than the value of the control group. Concerning this parameter, a report suggests that it may not be appropriate for use to detect alternations in sperm motion because of its wide range of distribution (Kaneto *et al*., 1999). Our finding about the parameter in this study, moreover, was not reproduced in a subsequent test (not reported). Judging from these facts, we considered that the difference in the percentage of circularly swimming sperm between the control and the EMS-treated group was unrelated to EMS treatment.

In the examination after a 28-day withdrawal, females that copulated with EMS-treated males exhibited a reduced fetal death rate by comparison with females that copulated at the termination of treatment. On the other hand, a decrease in implantation rate was observed, while it was not observed in females that copulated at the termination of treatment. For males treated with EMS, after a 28-day withdrawal, the sperm motion (percent motile, velocity, A.L.H. max and A.L.H. mean) and sperm number were decreased and the rate of morphological change of sperm was increased. Following withdrawal, the implantation rate and sperm condition were worse, while the fetal death rate was lower. Those findings led to a speculation that the decrease in implantation rate was due to the abnormal sperm condition. Decrease in motion and number of sperm may affect fertilization of gametes. However, it is unclear whether the abnormal sperm condition after a 28-day withdrawal affected only the fertilization of gametes or cleavage until implantation. Concerning fertilized gametes, an experiment in which male mice subcutaneously treated with EMS were mated with virgin females and fertilized ova were examined for chromosomal aberrations has been reported (Hashimoto *et al*., 1987). In this study, fertilized ova taken from females mated with males at 4 weeks or later did not exhibit chromosomal aberrations, while fertilized ova taken from females mated with males within 3 weeks after the treatment did. The results indicate that sperm fertilizing

Table 3. Reproductive organ weights in males.

	Termination of treatment		28-day withdrawal	
	Control	EMS	Control	EMS
No. of male animals	8	8	8	8
Final body weight (g)	469.6±28.6	401.9±21.0**	585.3±33.5	545.0±25.3*
Testis				
Absolute (g)	3.25±0.24	3.19±0.38	3.66±0.26	3.10±0.24**
Relative (%)	0.69±0.05	0.80±0.12	0.63±0.05	0.57±0.05
Epididymis				
Absolute (g)	1.27±0.10	1.14±0.10	1.40±0.10	1.24±0.12*
Relative (%)	0.27±0.04	0.29±0.04	0.24±0.01	0.23±0.03

Mean±S.D., Significantly different from the corresponding control value (* $p<0.05$, ** $p<0.01$)

Table 4. Data from sperm analysis.

	Termination of treatment		28-day withdrawal	
	Control	EMS	Control	EMS
No. of male animals	8	8	8	8
Sperm motion				
Motile (%)	56.0±13.1	54.9±13.6	7.5±10.7	37.8±17.9*
Velocity (μ m/s)	491.1±35.8	478.9±62.0	513.0±44.2	451.8±46.5*
Linearity	2.7±0.4	2.6±0.3	2.9±0.3	2.7±0.4
A.L.H. max (μ m)	29.5±4.2	25.6±4.2	29.1±4.5	21.1±5.0**
mean (μ m)	25.6±2.7	22.9±3.8	26.3±4.1	19.4±5.2*
Beat/Cross Freq. (Hz)	8.1±0.5	8.2±0.6	8.4±1.5	9.0±3.5
Average Radius (μ m)	26.9±5.8	22.5±4.8	31.9±14.1	22.8±9.1
Circ. Swim. Cells[per Motile Cells] (%)	10.8±2.1	6.6±2.9**	6.1±2.8	6.5±2.6
[per All Cells] (%)	6.0±1.9	3.6±1.8*	3.5±2.1	2.4±1.3
Sperm number ($\times 10^8$/1g distal cauda of epididymis)	6.42±1.01	6.89±1.22	6.39±0.95	3.50±1.11**
Sperm morphology				
Tailless (%)	2.53±1.72	4.49±6.27	3.01±2.77	22.10±16.83*
Anomalous (%)	0.36±0.49	0.31±0.45	0.31±0.60	5.37±1.63**

Mean±S.D., Significantly different from the corresponding control value (* $p<0.05$, ** $p<0.01$)

ova at 4 weeks or later after the treatment did not have chromosomal aberrations. Taking this report into consideration, sperm that fertilized ova after 28-day withdrawal in our study did not have chromosomal aberrations. The 28-day withdrawal sperm, however, induced the decrease in implantation rate. Judging from the length of the withdrawal period, the 28-day withdrawal sperm were affected by EMS in the period of spermatids in testes; the length of the spermatid period is about 22 days (Takahashi *et al.*, 1994) and spermatozoa need about 11 days to pass through an epididymis (Suzuki, 1989). In the spermatid period, the cells are not matured. As a result of this immatureness, the exposure to EMS in this period probably prevents the cells from growing normally. Sperm abnormalities observed in our sperm analysis, namely decreased number, decreased motion and morphological change, mean death, dysfunction and malformation of sperm, respectively. We consider that sperm having dysfunction and malformation are difficult to fertilize; as a result, fertilized ova do not have chromosomal aberrations.

No abnormality was detected in sperm by our analyses at the termination of EMS treatment, yet the sperm induced high fetal death. It is said that rat sperm stays in a caudal epididymis for 5 days before ejaculation (Suzuki, 1989). Judging from the length of the administration period, the sperm that induced a high fetal death rate at the termination of treatment was exposed by EMS in caudal epididymides. In caudal epididymides, sperm nuclei are condensed and covered with acrosome; as a result, nuclei in this period of spermatogenesis are more tolerant than those in another periods. The sperm of this period, however, induced a high fetal death in our study. In order to investigate the effects of EMS on sperm nuclei, Sega and Owens administered a single dose of tritium-labeled EMS to male mice and counted tritium level in sperm head for 2 weeks (Sega et al., 1978 and 1983). In the studies, the tritium level in sperm head exhibited approximately parallel changes with fetal mortality on dominant lethal tests, and the tritium of sperm head was detected not in DNA but in protamine. The results suggest that an analytical method detecting protamine alkylation is useful to assess the potential of chemicals inducing dominant lethals.

With the use of CASA and other methods, we could detect the EMS-inducing inhibition of spermatogenesis. Those findings indicate that sperm analysis using ejaculated sperm without killing of animals can probably detect abnormalities in testes. However, we could not detect EMS-inducing alkylation of sperm head that caused dominant lethals. In order to predict dominant lethals, we have examined several techniques detecting sperm protamine-alkylation.

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FLow Cytometric Detection and Analysis of Tailless Sperm Caused by Sonication or a Chemical Agent

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ABSTRACT. Flow cytometric analysis has been developed to detect tailless sperm with heads detached from the tails at the neck position. When isolated tailless sperm suspension was subjected to flow cytometry, a second sperm population appeared alongside the normal sperm population on light scatter-histogram. The percentage of this second sperm population (85.2%) was in good agreement with that for the tailless sperm (88.7%) determined microscopically, indicating that the second sperm population would correspond to tailless sperm population in the light scatter-histogram. Rates for tailless sperm determined by flow cytometry significantly correlated with those estimated microscopically following exposure of sperm to either sonication ($r = 0.94$, $P<0.01$), or nitrobenzene ($r = 0.80$, $P<0.01$). The results indicated the utility of the light scatter-histogram in flow cytometry as a simple and convenient procedure for the detection of tailless sperm induced by chemical compounds. — Key words: Flow cytometry, Analysis of tailless sperm, Rats

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INTRODUCTION

There have been a number of reports of sperm heads with tail detachment in mice or rats. Such tailless sperm can be experimentally induced by a variety of chemical compounds such as nitrobenzene, boric acid, 4-tert-octylphenol, tri-*o*-cresyl phosphate, gossypol, methyl chloride, Co60 and chlormadinone acetate (Kawashima *et al*., 1995; Linder *et al*., 1990; Bookfor *et al*., 1997; Somkuti *et al*., 1991; White *et al*., 1988; Working *et al*., 1985; Lock and Soares, 1980; Suzuki *et al*., 1978). Some of these compounds are known to decrease male fertility. In infertile men and bulls as in rodents, there appears to be a relation between tailless sperm and infertility (Baccetti *et al*., 1989; Soderquist *et al*., 1991; Toyama and Itoh, 1996). Therefore, tailless sperm can used be as one parameter for detecting effects of medical products on male fertility.

Sperm analyses such as counts, viability and motility testing and assessment of morphology are recommended for characterizing effects of medical products on male rat fertility (Japanese Ministry of Health and Welfare Ordinance No.21, 1997). Various procedures such as direct microscopic observation (Fukazawa and Kotosai, 1987; Suzuki, 1994), computer-assisted sperm analysis (Chapin *et al*., 1992; Toth *et al*., 1992; Hara *et al*., 1995) and flow cytometric analysis (Takizawa *et al*., 1995; Yamamoto *et al*., 1998) have been extensively developed for this purpose. For morphological analysis of rats or mice sperm, microscopic examination has been the standardized method (Wyrobek and Bruce, 1975; Filler *et al*., 1993). However, this is very time-consuming and allows evaluation of only relatively few sperm. In the past two decades, flow cytometry (Benaron *et. al*., 1982; Halamka *et al*., 1984; Evenson *et al*., 1989) and image analysis (Young *et al*., 1982; Moruzzi *et al*., 1988; Davis *et al*., 1994; Pasteur *et al*., 1988) have been developed to study morphological changes in sperm. Zucker *et al*. (1992) have demonstrated that light-scatter-parameters with flow cytometry might be useful for the morphological analysis of chemically isolated sperm heads. Takizawa *et al*. (1998) also reported that distribution patterns of sperm in the light scatter-histograms reflect morphological changes induced by pyridoxine. Flow cytometry can discriminate morphologically different cells because light scatter-parameters reflecting forward and side scatter are dependent on cell-size and granularity. Moreover, flow cytometry can be applied to precisely examine thousands of cells in a very short time without the extensive procedures necessary for preparation of dried sperm smears. However, there are many doubts regarding the reliability of detection of tailless sperm by flow cytometry and data are limited regarding effects of chemical compounds on male fertility.

Nitrobenzene has been well investigated for testicular toxicity and reproductive toxicity (*e.g.* Levin *et al*., 1988 ; Linder *et al*., 1922). Kawashima *et al*. (1995) reported that it may increase tailless sperm in the caudal epididymis of treated rats. Thus, nitrobenzene was here selected as a positive control to assess the reliability of detection of flow cytometry for tailless sperm caused by different agents.

In the present study, we have evaluated the flow cytometric analysis to detect tailless sperm in rats.

MATERIALS AND METHODS

Animals and animal husbandry: Sexually mature male Sprague-Dawley IGS rats (Charles River Japan, Inc., Kanagawa, Japan) were housed in suspended metal cages and given pelleted diet (CRF-1, Oriental Yeast Inc., Tokyo) and tap water *ad libitum.* The animal room was kept at 24 ± 1℃ and 55 ± 10% (relative humidity) with a 12-hr light/dark cycle (light, 6:00-18:00).

Preparation of non-treated sperm suspensions: Sperm suspensions were prepared from sexually mature rats as previously described (Yamamoto *et al*., 1998). Briefly, the left caudal epididymis was partially cut with a razor blade and 3 to 8 mg aliquots of epididymal fluid were collected using a small stainless steel spatula and carefully immersed into 8 ml of D-PBS supplemented with BSA (5 mg/ml), sodium pyruvate (1.65 μl/ml of 1.1% solution) and DL-sodium lactate (2 μl/ml of 60% solution) in a tube. All the above mentioned reagents were purchased from Wako Pure Chemical industries, Ltd. (Osaka, Japan).

Sperm analysis by flow cytometry: Approximately 10,000 sperm were analyzed in each sperm suspension with a COULTER EPICS XL flow cytometry system (BECKMAN COULTER Corpo-

Note: This paper is reprinted from original paper (J. of Toxicological Sciences; 25 (1), 41-48, 2000)

ration, USA, light source: argon-ion laser). The forward light scatter gate and side light scatter gate were set to contain the entire individual sperm population without cell debris or aggregates.

Morphological sperm analysis by microscopy: One-hundred μl of 1% eosin-Y solution was added to 1.5 ml of non-treated sperm suspension for staining for 20 min at 37℃. A 30-40 μl aliquot of the resulting sperm suspension was then placed on a clean glass slide. A total of two-hundred sperm were examined under a light microscope to count tailless sperm and total sperm to generate percentage values for tailless sperm. Morphological analysis by the method of Filler (1993) was also performed for 200 sperm in each sample.

Induction of tailless sperm by sonication and isolation of tailless sperm: The sperm were sonicated by a modification of the method of Zucker *et al*. (1992). Briefly, samples of about 16 mg of epididymal fluid in an 8 ml suspension of D-PBS were sonicated on ice with an ultrasonic generator, MODEL US150 (Nihonseiki Kaisha Ltd.) for six 15-second bursts with at least 30 second intervals. Sonically induced tailless sperm were isolated from the cell debris and aggregates according to the simplified Percoll-gradient stirring method (Takahashi *et al*., 1989). Aliquots of sonicated sperm suspensions (2 ml) were layered over 6 ml of 80% (w/v) Percoll solution (Pharmacia Biotech, USA) containing D-PBS in a tube, and centrifuged at 400 × g for 5, 10, 20 or 30 min to determine the optimal centrifugation-time for isolation of tailless sperm. The fraction which contained tailless sperm was microscopically located in the annular pellet at the bottom of the tube. This fraction (0.5 ml) was taken, washed twice with 2 ml of D-PBS and centrifuged at 400 × g for 30 min, and the resulting precipitate was resuspended in 2 ml of D-PBS. The suspension was subjected to microscopic observation to determine optimal conditions for the isolation of tailless sperm. The samples were also analyzed by flow cytometry to confirm the distribution pattern of tailless sperm on the light-scatter histograms.

Sperm suspensions containing various proportions of tailless sperm: The non-treated sperm suspensions were sonicated for two, four or six 15-sec bursts with at least 30 sec intervals as described above and subjected to flow cytometric and microscopic analysis.

Sperm suspensions from nitrobenzene-treated rats: Sixteen male rats at 7 week-olds were divided into 2 groups, each comprising 8 rats after a one-week acclimation period. The males at 8 weeks-old were administered orally once the dose of 100 mg/kg or 300 mg/kg of nitrobenzene (Wako Pure Chemical Industries, Ltd., Osaka, Japan) dissolved in corn oil (Nacalai Tesque, Ltd., Kyoto, Japan) (0.1 ml/100 g body weight). They were anesthetized with ether and sacrificed 28 days thereafter and sperm suspensions were prepared as described in "Preparation of non-treated sperm suspension", followed by flow cytometric and microscopic analyses. Analytic data obtained with both procedures were compared to confirm the reliability of flow cytometric detection of tailless sperm caused by a chemical compound.

Statistical analysis: Linear correlation and linear regression analyses were used to assess the relationship between data from flow cytometry and results of microscopy.

RESULTS

Distribution-patterns of rat sperm in light scatter-histograms: Fig. 1 shows representative light scatter-histograms for non-treated and sonicated sperm suspensions. In both cases, the sperm are distributed into two populations, gated with rectangular style-boxes, and termed sperm populations A and B.

Table 1 summarizes results of flow cytometric and microscopic analyses of non-treated and sonicated sperm suspensions. In the non-treated case, sperm population A constituted more than 95% of the total, in line with the rate (95.5%) for normal sperm obtained by direct microscopic observation. In contrast, with sperm suspensions sonicated for 90 sec, rates for both sperm population B (34.2%) and tailless sperm (57.2%) markedly increased and tended to yield a relatively similar result.

Table 2 shows the condition to isolate tailless sperm from the sonicated sperm suspension and analytic data for isolated tailless sperm by flow cytometry. After 5 min, the collected sperm were insufficient for analysis. With 10 min, the rate for tailless sperm was 88.7%. Thereafter, the rate exhibited a time dependent decrease, declining to 57.4% after 30 min. Therefore, in subsequent studies, 10 min-centrifugation was employed.

Fig. 2 shows the distribution pattern of the isolated tailless sperm in light scatter-histograms, after 90-sec sonication and isolation under optimal centrifugation conditions. Sperm population B ap-

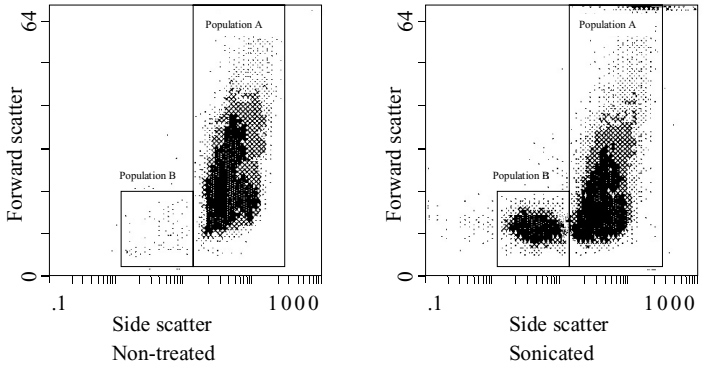

Figure 1. Light scatter-histograms for a non-treated sperm suspension containing morphologically normal sperm (left side) and a sonicated sperm suspensions containing high rates of tailless sperm (right side).

Table 1. Results of flow cytometric and microscopic analyses of non-treated and sonicated sperm suspensions.

	Non-treated	Sonicated
Number of animals	8	8
Sperm population A by flow cytometry (%)	96.7±2.4	65.8±6.7
Sperm population B by flow cytometry (%)	3.3±2.4	34.2±6.7
Normal sperm by microscopy (%)	95.5±2.8	42.8±11.3
Tailless sperm by microscopy (%)	4.5±2.8	57.2±11.3

Values are the mean±S.D. "Sonicated sperm suspension" was prepared by sonication of the non-treated sperm suspensions for 90 sec as described in MATERIALS AND METHODS.

peared at a rate of 85.2%, in good agreement with the rate of tailless sperm determined microscopically as shown in Table 2.

Validation of flow cytometry for detecting tailless sperm: Fig. 3 shows the correlation between flow cytometric data and microscopic data for sperm suspension sonicated for 0, 30, 60 or 90 sec. Microscopic observation of sonicated sperm suspension showed a sonically treated time-dependent increase of tailless sperm. The rate of sperm population B was also shown to increase similarly in the same sample. Moreover, both values correlated significantly in the range from 1.5% to 75.5% of tailless sperm ($r = 0.94$, $P<0.01$), indicating that the present flow cytometry would be suitable for monitoring the increase of tailless sperm rates induced sonically in a wide range. However, tailless sperm rates were not coincident with sperm population B rates perfectly. In particular, the correlation for the sperm suspension sonicated for 90 sec, which included high rates of tailless sperm, was not high ($r = 0.57$) in contrast to the sperm suspension sonicated for 30 sec ($r = 0.93$) which included low rates of tailless sperm.

Fig. 4 shows the distribution pattern for sperm of rats receiving 300 mg/kg of nitrobenzene on the light scatter-histogram. The distribution patterns of sperm were the same patterns as sonicated sperm in light scatter-histogram. Namely, sperm was distributed into sperm populations A and B. Sperm population B was increased to 38.5% in line with the incidence of tailless sperm (56.8%) (see photo 1). In addition to tailless sperm, incidences of sperm with abnormalities such as no-hook, banana and pin shapes (data not shown) was slightly increased. But the incidences of sperm with abnormalities except for tailless sperm was only a few percentage points.

Fig. 5 shows the correlation between analytic data from flow cytometry and microscopy for sperm collected from rats treated with nitrobenzene. The sperm population B rate was correlated significantly with the tailless sperm rate determined microscopically ($r = 0.80$, $P<0.01$). The results showed flow cytometry can also be applied to detect tailless sperm induced with chemical compounds.

DISCUSSION

The flow cytometric and microscopic data generated in the present study strongly indicate that sperm populations B corresponds to tailless sperm. This notion also indicates that tailless sperm could be divided from normal sperm by flow cytometry. Zucker and colleagues (1992) earlier showed that flow cytometry can discriminate chemically isolated sperm heads of various species such as cattle, rat *et al.* using only light scatter-histograms. However, there are no reports for flow cytometric analysis detect-

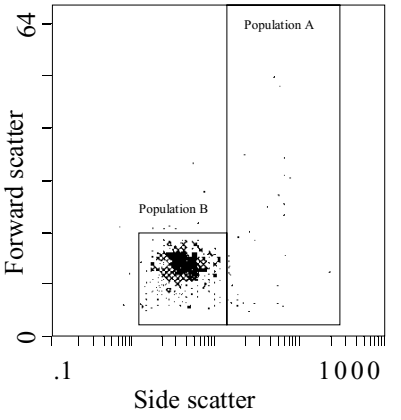

Figure 2. Flow cytometric patterns for isolated tailless sperm. Sperm population B constituted 85.2% of the total.

Table 2. Optimization of centrifugation period for isolation of tailless sperm.

Time of centrifugation (min)	5	10	20	30
Number of animals	8	8	8	8
Tailless sperm by microscopy (%)	ND	88.7±8.0	73.1±26.9	57.4±4.7
Sperm population B by flow cytometry (%)	NE	85.2±3.1	NE	NE

Values are the mean±S.D. Tailless sperm were isolated from the sonicated sperm suspensions as described in MATERIALS AND METHODS.
ND: not detectable, NE: not examined.

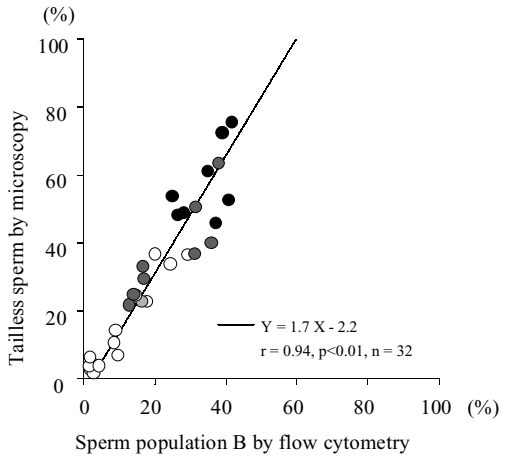

Figure 3. Correlation between sperm population B rate determined by flow cytometry and tailless sperm rate assessed by microscopy in the sonicated sperm suspensions. Flow cytometric and microscopic analyses were performed for non-treated sperm suspensions (◯), and sperm suspensions after sonication for 30 sec (◯), 60 sec (◉) or 90 sec (●), as described in MATERIALS AND METHODS. The correlation between the paired data sets was calculated by linear regression analysis.

ing tailless sperm. Consequently, it has not been elucidated what categories of morphological change in rat sperm were detectable by flow cytometry. Our data presented herewith have provided concrete evidence that flow cytometry can detect the tailless sperm.

The relatively good correlation between flow cytometric and microscopic data for both sonicated and chemical treatment cases (Fig. 3 and Fig. 5) suggest that the present light scatter-histogram approach may have sufficient reliability for detection of tailless sperm under various conditions. In spite of such a good relation between flow cytometric and microscopic analyses, flow cytometry tended to underestimate the percentage of tailless sperm in sperm suspension containing high rates of tailless sperm. We don't have any experimental evidence to explain the reason but there are some possibilities involved with the underestimation of tailless sperm rate, e.g. the increase of sperm present at the boundary of both sperm populations, the distribution patterns of sperm with no head (headless sperm) in the light scatter-histogram, contamination of cell debris and so on. In this study, it was elucidated that normal and tailless sperm were included in sperm populations A and B, respectively, but the location of headless sperm in the light scatter-histogram could not be clarified. Headless sperm would be much different from tailless sperm in those sizes or granularity. In other words, headless sperm might be involved in sperm population A rather than sperm population B. If headless sperm are involved in sperm populations A, the tailless sperm rate may be underestimated by flow cytometry. Thus, on the assumption that every headless sperm is involved in sperm population A, the flow cytometric (population A) and microscopic (headless sperm and normal sperm) analytic data of sonicated sperm shown in Fig. 3 was re-analyzed. The re-analysis produced the equation Y = 1.02X-2.2 and relation (r = 0.99) so that headless sperm might be involved in sperm population A. Herein, the one of reasons why flow cytometry tended to underestimate the percentages of tailless sperm might be the distribution of normal sperm and headless sperm into sperm population A. In the nitrobenzene-dosed group, sperm with abnormal heads such as the no-hook, banana and pin shape were slightly increased in addition to tailless sperm, as reported for several other compounds such as boric acid and tri-*o*-cresylphosphate (Linder *et al*., 1990; Somkuti *et al*., 1987). In the present study, there was no evidence whether these may be detected by flow cytometry or not. Taken together with analysis of the sonicated and chemical treated sperm, the results indicate that detailed microscopic evaluation should be performed to determine exact rates of tailless sperm and characterize categories of abnormality after increase in sperm population B is found.

In the present study, increased incidence of tailless sperm induced with nitrobenzene was detectable by this flow cytometry. Flow cytometry can discriminate morphologically different cells

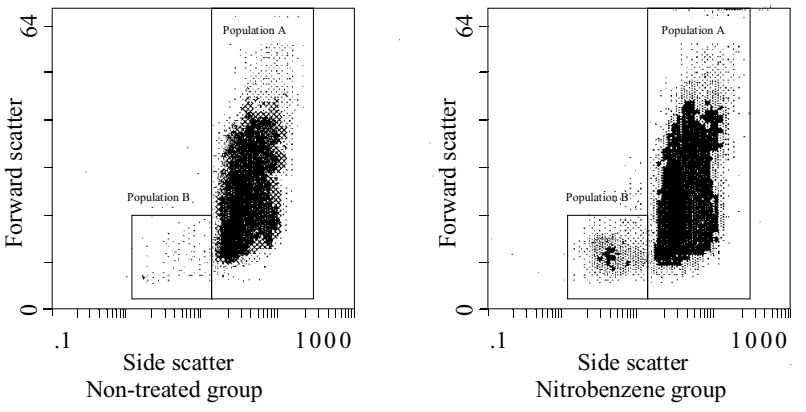

Figure 4. Light scatter-histograms for non-treated sperm (left side) and sperm (right side) from a rat treated with nitrobenzene.

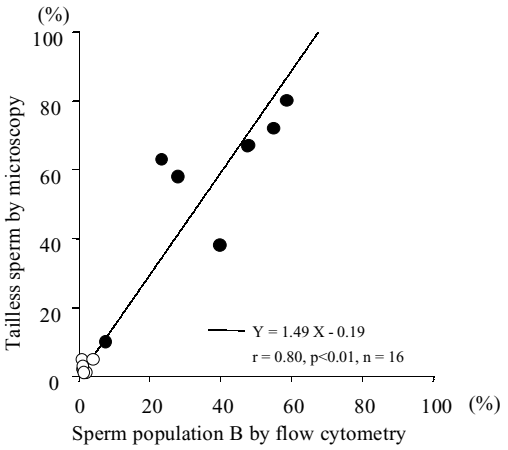

Figure 5. Correlation between sperm population B rate determined by flow cytometry and tailless sperm rate assessed by microscopy for sperm taken from rats treated with nitrobenzene. Flow cytometric and microscopic analyses were performed for 100 mg/kg (○) and 300 mg/kg groups (●) as described in MATERIALS AND METHODS. The correlation between the paired data sets was calculated by linear regression analysis.

because light scatter-parameters reflecting forward and side scatter are dependent on cell size and granularity. The cell size and granularity of tailless sperm would be different from those of morphological normal sperm. Thus, we can apply to flow cytometry to detect tailless sperm induced with a variety of chemical compounds.

Recently, due to increased concern that male infertility may result from exogenous or toxicant-induced anomalies in spermatogenesis, considerable efforts have been directed at establishing rapid and sensitive methods for detection of abnormal sperm. The currently applied microscopic procedures for evaluating populations of sperm cells are hindered by their time-consuming preparation; consequently, sample sizes are generally small. Sperm counts and viability can be analyzed, and approximate rates of tailless sperm can be estimated in a few min simultaneously using the present method in combination with those documented in our previous report (Yamamoto *et al*. 1998). Consequently, this approach would appear to be efficient for detecting effects of medical products on rat sperm.

In conclusion, light scatter-histogram on flow cytometry is useful for the detection of tailless sperm induced by sonication or chemical compounds.

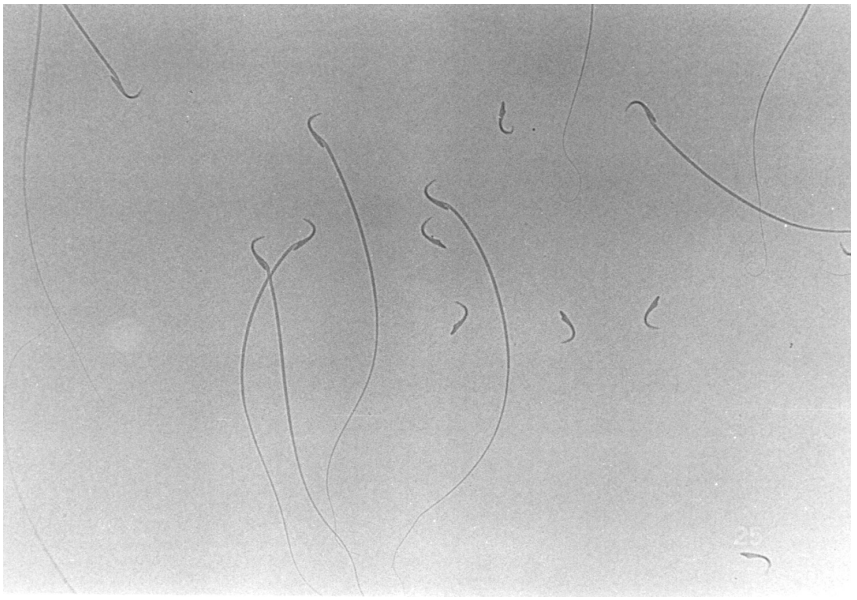

Photo 1. Sperm sample prepared from an epididymis of a rat treated with 300 mg/kg of nitrobenzene. Abnormal sperm are mainly tailless.

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Reproductive Indices, Fetal Gross, Visceral and Skeletal Alterations, Sexual Maturation, Passive Avoidance and Water Maze Data, a Comparison of Results in CD(SD)IGS Rats and CD(SD)Rats

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ABSTRACT. Data from over 150 studies using either CD(SD) rats or CD(SD)IGS rats were compared for selected parameters including reproductive indices, gross, visceral and skeletal alterations, sexual maturation, and behavioral tests of learning and memory. Rats were obtained from several breeding facilities in the United States. Most of the studies evaluated were conducted during the same time period (previous two to five years). Results of these comparisons demonstrated a very good correlation of the mean values between two rats. The range of mean values were usually slightly tighter for the CD(SD)IGS rats than the CD(SD) rats. The CD(SD)IGS rat appears to be a more homogeneous for the parameters evaluated. The breeder appears to be achieving its goal of a more uniform rat regardless of the breeding facility from which the rats are obtained. — Key words: CD(SD) rats, CD(SD)IGS rats, reproductive indices, sexual maturation, learning and memory

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INTRODUCTION

Over the past two years our laboratory has been collecting data from Caesarean-Derived Sprague-Dawley [CD(SD)] rats and Caesarean-Derived Sprague-Dawley International Genetic Standard [CD(SD)IGS] rats. CD(SD) rats and the CD(SD)IGS rats are outbred rat strains. Outbred strains are expected to have genetic change or drift over time. However, when the CD(SD) rat was only bred from stock within one breeding facility, genetic drift was occurring at different rates at each production facility. The CD(SD)IGS rat was derived to be more uniform regardless of the specific facility from which the rats were obtained. Breeding stock is provided from a central facility to all breeding facilities. The rat has a genetic background from breeding stock representative of all of the facilities that produce the CD(SD)IGS rat.

The introduction of a new stocking and breeding procedure to produce the CD(SD)IGS rat introduced concern among many users of the CD(SD)IGS rat that this newly derived rat would differ from the CD(SD) rat making comparison of historical control data sets from the two rats inappropriate. Also it was important to determine if the CD(SD)IGS rat was truly more uniform, with less variation for the parameters evaluated.

This paper presents the largest data base ever reported for CD(SD)IGS rats. All of the data presented were collected over a two to five year period. Outbred rats are considered to change or drift approximately every two years, making comparisons to databases covering five or greater years of data questionable. We presented our data in comparison with data from CD(SD) rats tested at our facility over this same period.

The parameters of reproductive indices and fetal alterations were presented because these parameters are known to be sensitive to genetic drift. Sexual maturation and measures of learning and memory were presented because these are currently of great concern to researchers. Our laboratory has extensive experience in the evaluation of these parameters, having used the same personnel to evaluate fetal alteration data for over 20 years and the same procedures for the behavioral evaluations of learning and memory for over 15 years.

MATERIALS AND METHODS

All data reported are from reproductive and developmental toxicity studies conducted for regulatory submission. Each study was conducted in compliance with the Good Laboratory Practices Regulations of the United States Food and Drug Administration or United States Environmental Protection Agency.

All rats were obtained from Charles River Breeding Laboratories in the United States at Kingston, New York; Raleigh, North Carolina; or Portage, Michigan. Environmental conditions at the testing facility were the same for all rats.

All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[1]. The animal room was independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature was maintained at 64°F to 79°F (18℃ to 26℃) and monitored constantly. Room humidity was also monitored constantly and maintained at 30% to 70%. Light was controlled at 12-hour light:12-hour dark with fluorescent lights. Rats were given Certified Rodent Diet® #5002 (PMI Nutrition International) available *ad libitum* from individual feeders. Water was available *ad libitum*. All water was from a local source and passed through a reverse osmosis membrane before use. Chlorine was added to the processed water as a bacteriostat; processed water contained no more than 1.2 ppm chlorine at the time of analysis. Water was analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

The day of mating was considered Day 0 of gestation. Mating was confirmed by the presence of sperm in a vaginal lavage and/or a sperm plug present *in situ*. Clinical observations, body weight and feed consumption were evaluated during gestation, typically on a daily basis or at least every three days.

In the developmental toxicity studies, rats were Caesarean-sectioned on day 20 of presumed gestation and examined for the number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions. Each fetus was examined for gross external alterations and sex. The weight of each live fetus was taken.

Approximately one-half of the live fetuses in each litter were examined for soft tissue alterations by using a variation of the micro-dissection technique of Staples[2] or using an adaptation of Wilson's[3] sectioning technique. The heads of these fetuses were fixed in Bouin's solution and subsequently examined by free-hand sectioning. The remaining live fetuses (approximately one-half of the fetuses in each litter) were examined for skeletal alterations after staining with alizarin red S[4].

Rats from delivered litters were maintained with their litter until weaning (usually 21 days postpartum). Litters were observed and counted daily. Body weights were taken at least weekly. The following describes the procedures followed for the postweaning evaluations presented in this paper.

SEXUAL MATURATION

Female rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum.

PASSIVE AVOIDANCE TESTING

Beginning at day 24 ± 1 postpartum, one male rat and one female rat from each litter, when possible, were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. The passive avoidance apparatus consisted of a two-compartment chamber with hinged Plexiglas® lids. One compartment was fitted with a bright light and Plexiglas® floor. The other compartment was fitted with a grid floor to which a brief (1 sec) pulse of mild electric current (1 mA) could be delivered. The two compartments were separated by a sliding door. On each test trial, the rat was placed into the "bright" compartment, the sliding door was opened and the light was turned on. The rat was allowed to explore the apparatus until it entered the "dark" compartment. The sliding door was then immediately closed, the light was turned off and the brief pulse of current was delivered to the grid floor. The rat was then removed from the apparatus and placed into a holding cage for a 30-second inter-trial interval prior to the start of the next trial. Trials were repeated until the rat remained in the "bright" compartment for 60 seconds on two consecutive trials (the criterion for learning) or until 15 trials had been completed. The latency to enter the dark compartment or the maximum 60-second interval was recorded for each trial. Each rat was tested twice. The first test session (acquisition phase) and the second test session (retention phase) were separated by a one-week interval, and the criterion was the same for both days of testing.

The following dependent measures were evaluated:

The number of trials to the criterion in the first session--this measure was used to compare groups for overall learning performance.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session--this measure was used to compare groups for activity levels and exploratory tendencies in a novel environment.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session--this measure was used to compare groups for short-term retention.

The number of trials to the criterion in the second test session--this measure was used to compare groups for long-term retention.

The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session--this value was another indication of long-term retention.

WATERMAZE TESTING

Beginning at approximately 60 to 70 days postpartum, one male rat and one female rat from each litter was evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested in a watertight 16-gauge stainless steel modified M-maze. The maze was filled with water to a depth of approximately nine inches, and the water was monitored for temperature (range of 21℃ ± 1℃).

On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial 1 was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal and removed from the water. A 15-second inter-trial interval separated each trial. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial. Each rat was tested twice. The first test session (acquisition phase) and the second test session (retention phase) was separated by a one-week interval, and the criterion was the same for both days of testing.

The following dependent measures were evaluated:

The number of trials to criterion on the first day of testing--this measure was used to compare groups for overall learning performance.

The average number of errors (incorrect turns in the maze) foreach trial on the first day of testing--this measure was also used to compare groups for overall learning performance.

The latency (in seconds) to reach the correct goal on trial 2 of the first day of testing--this measure was used to compare groups for short-term retention.

The number of trials to criterion on the second day of testing--this measure was used to compare groups for long-term retention.

The average number of errors for each trial on the second day of testing--this measure was also used to compare groups for long-term retention.

The latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing--this was another indicator of long-term retention.

RESULTS

This paper reports results from more than 150 studies with an almost equal distribution of CD(SD) and CD(SD)IGS rats. Gross fetal evaluations were performed on over 32,000 fetuses. Reproductive indices are presented in Figure 1 and Table 1. Tables 2 to 4 present the summary of fetal gross external, visceral and skeletal alterations. Table 5 presents the summary of fetal ossification sites skeletal averages. Tables 6 through 8 present the summary of sexual maturation, passive avoidance and watermaze data, respectively.

Previous publications have compared 10 or less studies using CD(SD)IGS rats to large databases collected over many years for CD(SD) rats[5]. These earlier publications noted differences between the two rats including numbers of 14th ribs, number of ossified caudal vertebrae and other changes in ossification or behavioral measurements[5 - 8]. Many studies did find most parameters evaluated to be similar between the CD(SD) and CD(SD)IGS rats[5 - 9].

Our data, on the largest number of CD(SD)IGS rats ever published, compared to an equivalent number of CD(SD) rats and for studies conducted over a similar time period, demonstrates a remarkable similarity in the average values with a definite decrease in the range of means for the CD(SD)IGS rats compared to the range of means for a particular parameter in the CD(SD) rats.

DISCUSSION AND CONCLUSION

It does appear that for the parameters presented in this paper, the CD(SD)IGS rat provides more homogeneous data with less variability then the CD(SD) rat. This is exactly what the breeders tried to achieve. It is presumed that as the outbred CD(SD)IGS rat changes (genetic drift) the changes that take place will be evident in all CD(SD)IGS rats irregardless of the specific breeding facility. However, drift will occur and the historical control databases must be updated and evaluated to see these trends. The larger variability in the CD(SD) rat is presumably due to the different breeding facilities from which the rats were obtained. It would be of interest to compare the data presented in this paper with that from CD(SD)IGS throughout the world to see if these rats are truly similar. Rats that continue to be bred within a single production facility should be expected to also change over time but these changes would be different than those from the CD(SD)IGS rat.

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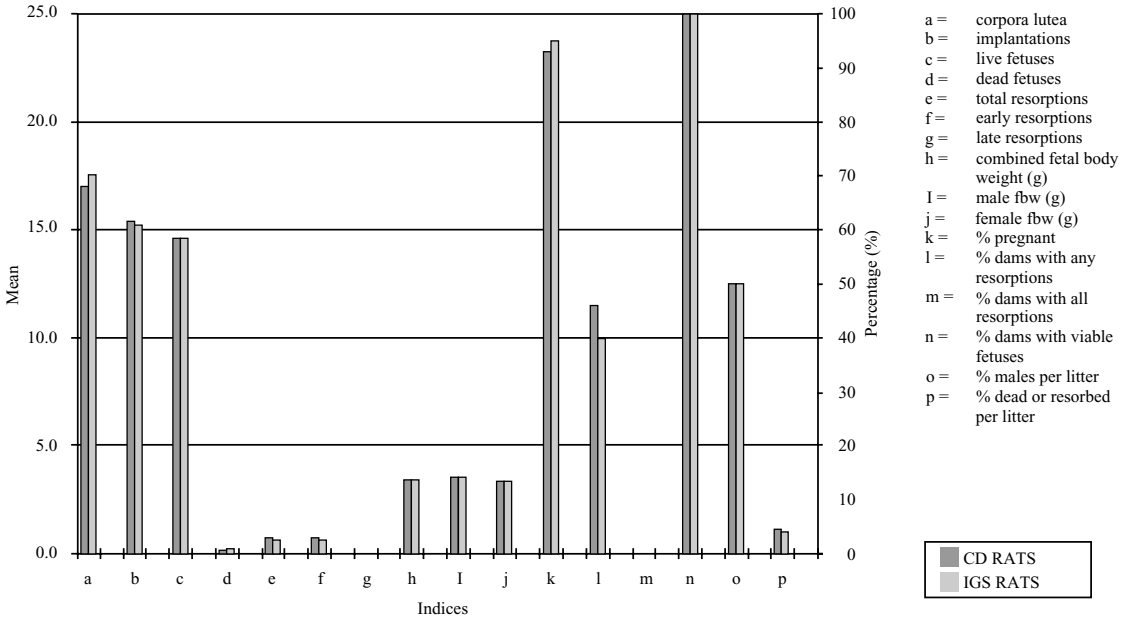

Figure 1. Reprodactive lndices

Table 1. Summary Of Reproductive Indices

	CD RATS		Crl:CD(SD)IGS BR RATS	
PERIOD	6/1996 - 6/1999		1/1998 - 1/2000	
NUMBER OF STUDIES	78		80	
NUMBER OF RATS:				
TESTED	1740		1594	
PREGNANT	1599		1500	
FOUND DEAD	3		3	
ABORTED	0		0	
DELIVERED	0		1	
NUMBER OF RATS PREGNANT AT CAESAREAN-SECTIONING	1597		1492	
NUMBER OF RATS WITH SINGLE CONCEPTUS LITTER:				
LIVE	4		2	
RESORBED	0		1	
ABORTED	0		0	
	MEAN or %	RANGE/STUDY MEAN or %	MEAN or %	RANGE/STUDY MEAN or %
% PREGNANT	93.0	(64.0-100)	94.5	(75.0-100)
AVERAGE # CORPORA LUTEA	17.0	(14.7-21.0)	17.5	(14.2-22.5)
AVERAGE # IMPLANTATIONS	15.4	(12.9-18.0)	15.2	(13.1-17.1)
AVERAGE LITTER SIZE				
AVERAGE # LIVE FETUSES	14.6	(11.8-16.9)	14.6	(12.3-16.6)
AVERAGE # DEAD FETUSES	0.1	(0-1.4)	0.2	(0-1.2)
AVERAGE # RESORPTIONS	0.7	(0-2.0)	0.6	(0-1.2)
AVERAGE # EARLY RESORPTIONS	0.7	(0-1.9)	0.6	(0-1.2)
AVERAGE # LATE RESORPTIONS	0.0	(0-0.1)	0.0	(0-0.1)
AVERAGE % DAMS WITH ANY RESORPTIONS	46.1	(16.7-87.5)	40.2	(0-79.2)
AVERAGE % DAMS WITH ALL CONCEPTUSES RESORBED	0.0	—	0.2	(0-12.5)
AVERAGE % DAMS WITH ONE OR MORE LIVE FETUSES	99.9	(95.6-100)	99.8	(95.4-100)
AVERAGE SEX RATIO, (% MALES/LITTER)	50.4	(44.5-56.7)	49.8	(42.8-67.7)
AVERAGE FETAL BODY WEIGHT (G)	3.49	(3.10-3.78)	3.47	(3.14-3.73)
AVERAGE FOR MALES (G)	3.59	(3.17-3.90)	3.57	(3.22-3.86)
AVERAGE FOR FEMALES (G)	3.40	(2.98-3.66)	3.38	(3.06-3.62)
AVERAGE % DEAD OR RESORBED CONCEPTUSES/LITTER	4.6	(0-12.4)	4.1	(0-8.2)

Table 2-1. Summary Of Fetal Gross External Alterations

		CD RATS				Crl:CD(SD)IGS BR RATS			
PERIOD		6/1996 - 6/1999				1/1998 - 1/2000			
# STUDIES INCLUDED		62				54			
# LITTERS EXAMINED		1310				921			
# LIVE FETUSES EXAMINED		18850				13247			
ALTERATION		N	%	RANGE/STUDY N	RANGE/STUDY %	N	%	RANGE/STUDY N	RANGE/STUDY %
HEAD									
	Exencephaly	L 2	0.15	0-1	(0-4.2)	L 3	0.33	0-1	(0-4.8)
		F 2	0.01	0-1	(0-0.3)	F 5	0.04	0-3	(0-1.0)
	All facial papilla absent	L 0	—	—	—	L 1	0.11	0-1	(0-4.0)
		F 0	—	—	—	F 1	0.01	0-1	(0-0.3)
	Hematoma	L 1	0.08	0-1	(0-2.2)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.2)	F 0	—	—	—
	Microcephaly	L 1	0.08	0-1	(0-4.0)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.3)	F 0	—	—	—
EYE(S)									
	Eye bulges depressed	L 7	0.53	0-2	(0-12.5)	L 3	0.33	0-1	(0-4.3)
		F 7	0.04	0-2	(0-0.9)	F 3	0.02	0-1	(0-0.3)
	Eyelids open	L 1	0.08	0-1	(0-4.2)	L 3	0.33	0-1	(0-4.8)
		F 1	0.01	0-1	(0-0.3)	F 5	0.04	0-3	(0-1.0)
	Microphthalmia	L 1	0.08	0-1	(0-4.0)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.3)	F 0	—	—	—
EARS									
	Low set	L 1	0.08	0-1	(0-12.5)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.9)	F 0	—	—	—
SNOUT									
	Short	L 1	0.08	0-1	(0-4.2)	L 1	0.11	0-1	(0-4.8)
		F 1	0.01	0-1	(0-0.3)	F 2	0.02	0-2	(0-0.6)
	Cleft	L 0	—	—	—	L 2	0.22	0-1	(0-4.8)
		F 0	—	—	—	F 2	0.02	0-1	(0-0.3)
PALATE									
	Cleft	L 0	—	—	—	L 1	0.11	0-1	(0-4.8)
		F 0	—	—	—	F 1	0.01	0-1	(0-0.3)
TONGUE									
	Protrudes	L 0	—	—	—	L 2	0.22	0-1	(0-4.8)
		F 0	—	—	—	F 4	0.03	0-3	(0-1.0)
	Absent	L 1	0.08	0-1	(0-4.0)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.3)	F 0	—	—	—

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 2-2. Summary Of Fetal Gross External Alterations

ALTERATION		CD RATS				Crl:CD(SD)IGS BR RATS			
		N	%	RANGE/STUDY N	%	N	%	RANGE/STUDY N	%
JAWS									
	Micrognathia	L 2	0.15	0-1	(0-4.5)	L 0	—	—	—
		F 2	0.01	0-1	(0-0.3)	F 0	—	—	—
	Agnathia	L 1	0.08	0-1	(0-12.5)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.9)	F 0	—	—	—
BODY									
	Edema	L 2	0.15	0-1	(0-4.3)	L 3	0.33	0-1	(0-4.5)
		F 2	0.01	0-1	(0-0.3)	F 3	0.02	0-1	(0-0.3)
	Umbilical hernia	L 4	0.30	0-1	(0-4.3)	L 5	0.54	0-1	(0-4.8)
		F 4	0.02	0-1	(0-0.3)	F 5	0.04	0-1	(0-0.3)
	Spina bifida	L 0	—	—	—	L 2	0.22	0-1	(0-4.3)
		F 0	—	—	—	F 2	0.02	0-1	(0-0.3)
	Trunk short	L 0	—	—	—	L 1	0.11	0-1	(0-4.5)
		F 0	—	—	—	F 1	0.01	0-1	(0-0.3)
	Hematoma	L 2	0.15	0-1	(0-4.0)	L 0	—	—	—
		F 2	0.01	0-1	(0-0.3)	F 0	—	—	—
	Conjoined twins	L 1	0.08	0-1	(0-4.0)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.3)	F 0	—	—	—
HINDLIMBS									
	Third limb present	L 1	0.08	0-1	(0-14.3)	L 0	—	—	—
		F 1	0.01	0-1	(0-0.9)	F 0	—	—	—
ANUS									
	No opening present	L 1	0.08	0-1	(0-2.6)	L 1	0.11	0-1	(0-4.2)
		F 1	0.01	0-1	(0-0.2)	F 1	0.01	0-1	(0-0.3)
TAIL									
	Threadlike	L 3	0.23	0-1	(0-12.5)	L 1	0.11	0-1	(0-4.0)
		F 3	0.02	0-1	(0-0.9)	F 1	0.01	0-1	(0-0.3)
	Agenesis	L 1	0.08	0-1	(0-4.5)	L 2	0.22	0-1	(0-4.5)
		F 1	0.01	0-1	(0-0.3)	F 2	0.02	0-1	(0-0.3)
	Constricted	L 1	0.08	0-1	(0-2.4)	L 1	0.11	0-1	(0-4.2)
		F 1	0.01	0-1	(0-0.2)	F 1	0.01	0-1	(0-0.3)
	Kinked	L 0	—	—	—	L 1	0.11	0-1	(0-4.3)
		F 0	—	—	—	F 1	0.01	0-1	(0-0.3)
	Short	L 1	0.08	0-1	(0-2.4)	L 1	0.11	0-1	(0-4.3)
		F 1	0.01	0-1	(0-0.2)	F 1	0.01	0-1	(0-0.3)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 3-1. Summary Of Fetal Soft Tissue Alterations

		CD RATS				Crl:CD(SD)IGS BR RATS			
PERIOD		6/1996 - 6/1999				1/1998 - 1/2000			
# STUDIES INCLUDED		28				19			
# LITTERS EXAMINED		660				452			
# FETUSES EXAMINED		4614				3115			
			RANGE / STUDY				RANGE / STUDY		
ALTERATION		N	%	N	%	N	%	N	%
BRAIN									
Lateral ventricles, moderate dilation	L	0	—	—	—	2	0.44	0-1	(0-4.3)
	F	0	—	—	—	2	0.06	0-1	(0-0.6)
Lateral ventricles, marked dilation	L	1	0.15	0-1	(0-4.2)	1	0.22	0-1	(0-4.3)
	F	1	0.02	0-1	(0-0.6)	1	0.03	0-1	(0-0.6)
Brain, irregularly shaped	L	0	—	—	—	1	0.22	0-1	(0-4.2)
	F	0	—	—	—	1	0.03	0-1	(0-0.6)
Third ventricle, marked dilation	L	1	0.15	0-1	(0-4.2)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
Lateral and third ventricles, irregularly shaped	L	1	0.15	0-1	(0-4.2)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
EYES									
Microphthalmia	L	1	0.15	0-1	(0-4.3)	3	0.66	0-1	(0-4.8)
	F	1	0.02	0-1	(0-0.7)	3	0.10	0-1	(0-0.7)
PALATE									
Cleft	L	0	—	—	—	1	0.22	0-1	(0-4.8)
	F	0	—	—	—	1	0.03	0-1	(0-0.7)
TONGUE									
Absent	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
JAW									
Micrognathia	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
HEART									
Septal defect	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 3-2. Summary Of Fetal Soft Tissue Alterations

		CD RATS				Crl:CD(SD)IGS BR RATS			
				RANGE / STUDY				RANGE / STUDY	
ALTERATION		N	%	N	%	N	%	N	%
VESSELS									
Innominate, absent	L	6	0.91	0-2	(0-8.3)	1	0.22	0-1	(0-4.2)
	F	6	0.13	0-2	(0-1.2)	1	0.03	0-1	(0-0.6)
Innominate arises on left	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
Umbilical artery, descends to left of urinary bladder	L	12	1.82	0-2	(0-8.7)	16	3.54	0-3	(0-12.5)
	F	13	0.28	0-2	(0-1.4)	16	0.51	0-3	(0-1.8)
Situs inversus	L	1	0.15	0-1	(0-4.2)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
Aorta, descends to right	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
Pulmonary artery, descends to right behind aorta	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
LUNGS									
Lobe(s), absent	L	1	0.15	0-1	(0-4.0)	6	1.33	0-3	(0-12.0)
	F	1	0.02	0-1	(0-0.6)	6	0.19	0-3	(0-1.8)
Right apical, cardiac and diaphragmatic lobes appear as one	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
KIDNEY(S)									
Pelvis, slight dilation	L	0	—	—	—	7	1.55	0-2	(0-9.5)
	F	0	—	—	—	8	0.26	0-2	(0-1.4)
Pelvis, moderate dilation	L	0	—	—	—	4	0.88	0-2	(0-8.7)
	F	0	—	—	—	4	0.13	0-2	(0-1.2)
Pelvis, marked dilation	L	0	—	—	—	1	0.22	0-1	(0-4.3)
	F	0	—	—	—	1	0.03	0-1	(0-0.6)
Small	L	0	—	—	—	1	0.22	0-1	(0-4.0)
	F	0	—	—	—	3	0.10	0-3	(0-1.8)
Left, displaced	L	0	—	—	—	1	0.22	0-1	(0-4.2)
	F	0	—	—	—	1	0.03	0-1	(0-0.6)
BODY									
Edema	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
ABDOMINAL CAVITY									
Situs inversus of liver, intestines, stomach, spleen, pancreas and kidneys	L	1	0.15	0-1	(0-4.0)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—
ADRENAL GLAND(S)									
Dark red	L	1	0.15	0-1	(0-4.2)	0	—	—	—
	F	1	0.02	0-1	(0-0.6)	0	—	—	—

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 4-1. Summary Of Fetal Skeletal Alterations

	CD RATS				Crl:CD(SD)IGS BR RATS			
PERIOD	6/1996 - 6/1999				1/1998 - 1/2000			
# STUDIES INCLUDED	28				31			
# LITTERS EXAMINED	645				721			
# FETUSES EXAMINED	4911				5311			
		RANGE / STUDY				RANGE / STUDY		
ALTERATION	N	%	N	%	N	%	N	%
SKULL								
Frontal(s): incompletely or	L 1	0.16	0-1	(0-4.2)	L 0	—	—	—
not ossified	F 1	0.02	0-1	(0-0.6)	F 0	—	—	—
Parietal(s): incompletely	L 1	0.16	0-1	(0-4.2)	L 0	—	—	—
or not ossified	F 1	0.02	0-1	(0-0.6)	F 0	—	—	—
Nasal(s): short	L 2	0.31	0-1	(0-4.2)	L 0	—	—	—
	F 2	0.04	0-1	(0-0.6)	F 0	—	—	—
Nasal - Frontal: suture	L 0	—	—	—	L 1	0.14	0-1	(0-4.0)
large	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
Eye socket: small	L 0	—	—	—	L 1	0.14	0-1	(0-4.3)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
Maxillae and Premaxillae:	L 2	0.31	0-1	(0-4.2)	L 0	—	—	—
short	F 2	0.04	0-1	(0-0.6)	F 0	—	—	—
Skull: incompletely or not	L 1	0.16	0-1	(0-4.2)	L 0	—	—	—
ossified	F 2	0.04	0-2	(0-1.2)	F 0	—	—	—
VERTEBRAE								
Cervical: Arches, fused	L 0	—	—	—	L 1	0.14	0-1	(0-4.2)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
Thoracic: Centrum, bifid	L 51	7.91	0-5	(0-20.8)	L 36	4.99	0-4	(0-17.4)
	F 56	1.14	0-6	(0-3.4)	F 41	0.77	0-5	(0-2.9)
: Centra, unilateral	L 5	0.78	0-2	(0-8.0)	L 2	0.28	0-1	(0-4.3)
ossification	F 5	0.10	0-2	(0-1.0)	F 2	0.04	0-1	(0-0.6)
: Centrum, incompletely	L 3	0.46	0-2	(0-8.3)	L 2	0.28	0-1	(0-4.2)
or not ossified	F 4	0.08	0-3	(0-1.8)	F 2	0.04	0-1	(0-0.6)
: 12 present	L 0	—	—	—	L 1	0.14	0-1	(0-4.0)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
: Centra, fused	L 1	0.16	0-1	(0-4.2)	L 0	—	—	—
	F 1	0.02	0-1	(0-0.5)	F 0	—	—	—
: Arch, small	L 1	0.16	0-1	(0-4.0)	L 0	—	—	—
	F 1	0.02	0-1	(0-0.6)	F 0	—	—	—
Lumbar: Centrum, bifid	L 0	—	—	—	L 1	0.14	0-1	(0-4.3)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 4-2. Summary Of Fetal Skeletal Alterations

	CD RATS				Crl:CD(SD)IGS BR RATS			
			RANGE / STUDY				RANGE / STUDY	
ALTERATION	N	%	N	%	N	%	N	%
VERTEBRAE (CONT.)								
Lumbar (Cont.)								
: Centrum, incompletely	L 2	0.31	0-2	(0-8.3)	L 1	0.14	0-1	(0-4.2)
or not ossified	F 3	0.06	0-3	(0-1.8)	F 1	0.02	0-1	(0-0.6)
: Centra, unilateral	L 2	0.31	0-1	(0-4.2)	L 0	—	—	—
ossification	F 2	0.04	0-1	(0-0.6)	F 0	—	—	—
: Arches, incompletely	L 11	1.70	0-3	(0-12.0)	L 1	0.14	0-1	(0-4.2)
or not ossified	F 16	0.32	0-4	(0-2.1)	F 1	0.02	0-1	(0-0.6)
: 4 present	L 0	—	—	—	L 1	0.14	0-1	(0-4.2)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
: 0 present	L 0	—	—	—	L 1	0.14	0-1	(0-4.0)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
Sacral: 0 present	L 0	—	—	—	L 2	0.28	0-1	(0-4.2)
	F 0	—	—	—	F 2	0.04	0-1	(0-0.6)
Caudal: 2 present	L 0	—	—	—	L 1	0.14	0-1	(0-4.2)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.5)
: 1 present	L 0	—	—	—	L 1	0.14	0-1	(0-4.3)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.5)
: 0 present	L 0	—	—	—	L 2	0.28	0-1	(0-4.2)
	F 0	—	—	—	F 2	0.04	0-1	(0-0.6)
RIBS								
Cervical Rib(s) present	L 14	2.17	0-2	(0-8.3)	L 16	2.22	0-4	(0-17.4)
	F 15	0.30	0-3	(0-1.6)	F 19	0.36	0-5	(0-2.9)
One or more, wavy	L 24	3.72	0-4	(0-16.0)	L 9	1.25	0-2	(0-8.3)
	F 41	0.83	0-8	(0-4.2)	F 9	0.17	0-2	(0-1.2)
One or more, incompletely								
ossified (hypoplastic), or	L 18	2.79	0-2	(0-9.1)	L 8	1.11	0-2	(0-8.3)
not ossified	F 29	0.59	0-5	(0-2.6)	F 8	0.15	0-2	(0-1.2)
Fused	L 0	—	—	—	L 1	0.14	0-1	(0-4.0)
	F 0	—	—	—	F 1	0.02	0-1	(0-0.6)
STERNEBRAE								
One or more incompletely	L 79	12.25	0-9	(0-36.0)	L 63	8.74	1-7	(4.2-29.2)
ossified or not ossified	F 115	2.34	0-12	(0-6.7)	F 79	1.49	1-9	(0.6-5.3)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 4-3. Summary Of Fetal Skeletal Alterations

		CD RATS				Crl:CD(SD)IGS BR RATS			
				RANGE / STUDY				RANGE / STUDY	
ALTERATION		N	%	N	%	N	%	N	%
STERNEBRAE (CONT.)									
Fused	L	1	0.16	0-1	(0-4.2)	1	0.14	0-1	(0-4.0)
	F	1	0.02	0-1	(0-0.6)	1	0.02	0-1	(0-0.6)
Asymmetric	L	1	0.16	0-1	(0-4.2)	1	0.14	0-1	(0-4.8)
	F	1	0.02	0-1	(0-0.5)	1	0.02	0-1	(0-0.6)
SCAPULAE									
Bent	L	1	0.16	0-1	(0-4.2)	0	—	—	—
	F	1	0.02	0-1	(0-0.5)	0	—	—	—
PELVIS									
Pubis(es) and/or									
Ischium(a): incompletely	L	92	14.26	0-7	(0-30.4)	42	5.82	0-7	(0-29.2)
or not ossified	F	140	2.85	0-16	(0-5.5)	68	1.28	0-16	(0-9.5)
Pubis(es): incompletely	L	72	11.16	0-7	(0-30.4)	43	5.96	0-7	(0-29.2)
ossified	F	107	2.18	0-10	(0-5.3)	68	1.28	0-16	(0-9.5)
Pubis(es): not ossified	L	3	0.46	0-1	(0-4.2)	0	—	—	—
	F	3	0.06	0-1	(0-0.6)	0	—	—	—
Ischium(a): incompletely	L	30	4.65	0-4	(0-16.7)	17	2.36	0-2	(0-8.3)
or not ossified	F	47	0.96	0-9	(0-4.7)	20	0.38	0-4	(0-2.4)

L: LITTER INCIDENCE
F: FETAL INCIDENCE

Table 5. Summary Of Fetal Ossification Sites Skeletal Averages

	CD RATS		Crl:CD(SD)IGS BR RATS	
PERIOD	6/1996 - 6/1999		1/1998 - 1/2000	
# STUDIES INCLUDED	27		19	
# LITTERS EXAMINED	622		453	
# FETUSES EXAMINED	4721		3366	
	FETUS/LITTER		FETUS/LITTER	
SKELETAL AVERAGES	MEAN	RANGE/STUDY	MEAN	RANGE/STUDY
HYOID	0.86	(0.69-0.93)	0.94	(0.83-1.00)
VERTEBRAE				
CERVICAL	7.00	—	7.00	—
THORACIC	13.04	(13.00-13.15)	13.06	(13.02-13.12)
LUMBAR	5.95	(5.85-5.99)	5.93	(5.87-5.98)
SACRAL	3.00	(2.96-3.01)	3.00	—
CAUDAL	4.84	(4.35-5.16)	4.84	(4.66-4.99)
RIBS (pairs)	13.03	(13.00-13.09)	13.04	(13.01-13.08)
STERNUM				
MANUBRIUM	1.00	(0.98-1.01)	1.00	(0.99-1.00)
STERNAL CENTERS	3.60	(3.26-3.75)	3.72	(3.61-3.80)
XIPHOID	0.99	(0.94-1.00)	0.99	(0.98-1.00)
FOREPAWS (Calculated as average per limb)				
CARPALS	0.00	—	0.00	—
METACARPALS	3.52	(3.33-3.71)	3.67	(3.50-3.76)
DIGITS	5.00	—	5.00	—
PHALANGES	5.04	(4.90-5.27)	5.07	(5.00-5.21)
HINDPAWS (Calculated as average per limb)				
TARSALS	0.00	—	0.00	—
METATARSALS	3.99	(3.93-4.03)	3.99	(3.99-4.00)
DIGITS	5.00	—	5.00	—
PHALANGES	4.98	(4.82-5.08)	4.97	(4.82-5.01)

Table 6. Summary Of Sexual Maturation Data

	CD RATS	Crl:CD® (SD)IGS BR RATS
PERIOD	3/1995 - 4/2000	8/1998 - 5/2000
NUMBER OF STUDIES	27	15
MALES		
Avg. Day Postpartum	45.9	46.5
range	(43.9-47.6)	(45.0-47.7)
FEMALES		
Avg. Day Postpartum	32.1	32.2
range	(30.1-34.0)	(30.8-33.9)

Table 7. Summary Of Passive Avoidance Data

	CD RATS	Crl:CD® (SD)IGS BR RATS
PERIOD	9/1995 - 4/2000	4/1999 - 5/2000
NUMBER OF STUDIES	17	16
MALES		
SESSION 1		
Trials to Criterion	5.0	5.2
range	(3.6-6.5)	(4.1-6.5)
Latency Trial 1	7.4	8.0
range	(4.2-11.7)	(5.9-10.2)
Latency Trial 2	25.1	21.3
range	(15.0-50.4)	(8.5-33.4)
SESSION 2		
Trials to Criterion	2.9	3.3
range	(2.1-3.6)	(2.8-4.0)
Latency Trial 1	31.9	24.9
range	(13.6-56.9)	(12.0-38.9)
FEMALES		
SESSION 1		
Trials to Criterion	4.8	5.1
range	(3.6-5.3)	(4.0-6.4)
Latency Trial 1	7.4	8.4
range	(5.07-12.8)	(5.9-11.9)
Latency Trial 2	24	22.6
range	(11.4-40.8)	(12.2-31.3)
SESSION 2		
Trials to Criterion	2.9	3.4
range	(2.2-3.8)	(2.9-4.0)
Latency Trial 1	29.2	20.7
range	(15.2-54.3)	(11.2-36.3)

Table 8. Summary Of Watermaze Data

	CD RATS	Crl:CD® (SD)IGS BR RATS
PERIOD	9/1995 - 4/2000	4/1999 - 5/2000
NUMBER OF STUDIES	18	12
MALES		
SESSION 1		
Trials to Criterion	8.9	9.4
range	(7.8-10.3)	(8.4-10.7)
Errors	0.4	0.4
range	(0.3-0.5)	(0.4-0.5)
Latency Trial 2	14.7	13.4
range	(11.4-20.0)	(11.7-16.4)
SESSION 2		
Trials to Criterion	6.5	6.4
range	(5.7-7.8)	(5.5-9.9)
Errors	0.1	0.1
range	(0.0-0.2)	(0.0-0.5)
Latency Trial 1	9.5	11.2
range	(6.7-12.6)	(7.9-23.5)
FEMALES		
SESSION 1		
Trials to Criterion	9	9.1
range	(7.7-10.2)	(7.4-11.2)
Errors	0.4	0.4
range	(0.4-0.5)	(0.3-0.5)
Latency Trial 2	14.4	14.1
range	(9.5-19.5)	(10.6-17.0)
SESSION 2		
Trials to Criterion	7.0	7.0
range	(6.0-9.0)	(6.0-9.0)
Errors	0.1	0.0
range	(0.1-0.25)	(0.0-0.0)
Latency Trial 1	11.0	12.0
range	(8.2-15.0)	(9.0-20.0)

Effect of Propylthiouracil (PTU) on Developmental Neurotoxicity Endpoints in Crl:CD® (SD)IGS BR Rats

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ABSTRACT. In humans, pre- and periatal hypothyroidism causes irreversible mental retardation and various neuromotor disabilities. Propylthiouracil (PTU) administration to pregnant rats is a commonly used experimental model of human congenital hypothyroidism. The objective of this study was to evaluate various endpoints within the U.S. EPA OPPTS Guideline 870.6300 (Developmental Neurotoxicity). PTU was administered at dosage levels of 3.8, 19 and 38 mg/kg/day by oral gavage once daily from gestation day 6 through lactation day 10. Offspring were evaluated by functional observational battery, motor activity, acoustic startle response, and learning and memory in the Biel water maze. Live litter size was reduced in the 38 mg/kg/day group. Pup viability from birth to PND 4 was reduced in the 19 and 38 mg/kg/day groups. Pup weights were reduced by 10% at birth for all dosage levels, and remained reduced for the duration of the study. The functional observational battery demonstrated delays in pupillary response, startle reactivity, mobility and neuromuscular development on postnatal day (PND) 20. Neuromuscular measures continued to be impaired at the PND 35 and 45 observations. Motor activity was not affected. Acoustic startle response was reduced at all dosage levels when evaluated on PND 22. Latency to escape the Biel maze was increased for all dosage levels at the PND 22 evaluation; however, swimming ability was markedly reduced for all treatment groups. When assessed at PND 62 swimming ability was comparable between all groups. However, time to escape the Biel maze was increased for males and females at all dosage levels by approximately 60% although there was no dose response relationship. The experimental design used in this study demonstrated that Sprague-Dawley Crl:CD® (SD)IGS BR rats are sensitive to the effects of PTU, a known developmental neurotoxicant, and can be considered to be an appropriate rodent model for OPPTS Guideline 870.6300 developmental neurotoxicity studies. — Key Words: Propylthiouracil, behavior, developmental landmarks

CD(SD)IGS-2000: 174-182

INTRODUCTION

In 1998, the United States Environmental Protection Agency (EPA) released the Developmental Neurotoxicity Study (OPPTS 870.6300) guideline[1]. The guideline required that various neurobehavioral endpoints such as motor activity, auditory startle, and learning and memory along with appearance of developmental landmarks and other standard toxicologic indices be tested to support product registration under FIFRA and TSCA. As such, whether there are differences in these endpoints in the Sprague-Dawley Crl:CD® (SD)IGS BR strain of rat needed to be examined. We chose to evaluate potential differences in control and treated animals using an agent which produces hypothyroidism, propylthiouracil (PTU).

PTU is commonly used as an experimental model of human congenital hypothyroidism. In humans, congenital hypothyroidism can produce irreversible mental retardation and various neuromotor disabilities[2-6]. In rats, pre- and peri-natal hypothyroidism produces behavioral deficits[7-9] and altered electrophysiology[7,10,11] along with changes in gross and microscopic measures of brain morphology[12-17]. It is quite apparent that early hypothyroidism whether chemically- or surgically-induced results in altered brain development and function and should alter developmental neurotoxicity endpoints in the Crl:CD® (SD)IGS BR rat.

MATERIALS and METHODS

Test Species and Husbandry

Female Sprague-Dawley Crl:CD® (SD)IGS BR rats were obtained from Charles River Laboratories (Raleigh, NC, USA). The animals were individually housed in clean suspended wire-mesh cages in an environmentally controlled room during acclimation. During cohabitation, the females were paired (1 male:1 female) with resident males in a suspended wire-mesh cage. Mating was evidenced by vaginal plug and/or the presence of sperm in a vaginal smear. Following successful mating, the females were individually housed in plastic maternity cages with nesting material (Bed-O-Cobs®). On postnatal day (PND) 4, pups were culled to reduce variability among litter size (3:5, 4:4 or 5:3 M:F) and dams and pups remained in these cages until the dam was euthanized on PND 21. Each litter remained housed together in plastic cages with nesting material through PND 28 whereupon they were housed 2-3/cage by sex in wire mesh cages. On PND 35 until scheduled euthanasia all animals were individually housed. Environmental controls were set to maintain temperature at 72 ± 4 ° F and relative humidity between 30-70%. Air handling units provided a minimum of 10 fresh air changes per hour. Fluorescent lighting, controlled by timers, provided illumination for a 12-hour (6:00 a.m. - 6:00 p.m.) light/dark photoperiod. Temperature and relative humidity were monitored continuously. Reverse osmosis-purified water was available *ad libitum.* PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 was offered *ad libitum* during the study.

Test Material and Preparation

6-N-Propyl-2-thiouracil (PTU) was purchased from Sigma Aldrich, St. Louis, MO, (lot #76H2500). An appropriate amount of PTU was mixed with 0.5% methylcellulose (Sigma Chemical Co., St. Louis, MO, lot #97H0980) to produce dosing solutions of 0.304, 1.52 and 3.04 mg/ml. Dosing solutions were prepared weekly and daily aliquots were dispensed for dosing.

Experimental Design

Four groups of 25 bred rats received a daily oral gavage of 0, 3.8, 19 or 38 mg/kg PTU daily from gestation day (GD) 6 through lactation day (LD) 10. Dams were examined daily for mortality, morbundity and for clinical signs of toxicity. Maternal body weight and food consumption were recorded twice weekly from GD 0 through LD 21. Pups were randomized into treatment groups and then assigned to testing groups within each treatment group. The following table summarizes the testing paradigm:

Number of Animals	Age	Evaluation
10/sex/group	PND 20, 35, 45 and 60	Functional Observational Battery (FOB)
10/sex/group	PND 22	Auditory startle
10/sex/group	PND 13, 17, 21 and 60	Locomotor Activity
10/sex/group	PND 21 and 62	Learning and Memory (Biel Maze)
3-10/sex/group	PND 4 and 28	T_3, T_4 and TSH levels

Developmental Landmarks: Anogenital distance was measured on PND 1 for all live pups. Balanopreputial separation was observed for all male pups beginning on PND 35 and continued daily until balanopreputial separation was present. The body weight of each male was recorded on the day of acquisition of balanopreputial separation. Vaginal patency was observed for all female pups beginning on PND 25 and continued daily until vaginal perforation was present. The body weight of each female was recorded on the day of acquisition of vaginal patency.

Behavioral Testing

Ten pups/sex/group were assigned to one of the following tests: functional observational battery (FOB), locomotor activity, auditory startle or learning and memory. The FOB utilized was based on previously developed protocols[18-21]. Testing was performed by the same trained technicians, whenever possible, who did not know the animal's group assignment. Locomotor activity was monitored on postnatal days 13, 17, 21 and 60 ($\pm$ 2 days). The same animals were monitored at each interval and testing of treatment groups was balanced across test times. Locomotor activity was measured using the Digiscan Micro Animal Activity System (Omnitech Electronics Inc., Columbus, Ohio) and the test session was 41 minutes duration with 1 minute test intervals for each animal. The auditory startle response test was performed on PND 22 using an automated Auditory Startle Response System (Coulbourn Instruments, Inc., Lehigh Valley, Pennsylvania). The test session consisted of a 5 minute acclimation period with a background noise level of 70 db, followed by 50 presentations of a 50-ms 120 db noise burst given at 8 second intervals. Mean amplitude on each block (1-5) of 10 trials was recorded for each animal. Learning and memory was tested on postnatal day 21 ($\pm$ 4 days) and 62 ($\pm$ 4 days) using a Biel maze[22]. Different animals were tested at each interval. The Biel maze is a water-filled multiple T-maze with an escape ladder. The time to escape and number of errors (animal deviates from the correct channel with all four feet) was recorded and animals were allowed three minutes to escape from the maze after which they were removed. The minimum intertrial interval was one hour and animals were tested for seven consecutive days as follows:

Day 1: Each animal given 4 trials to escape from a straight channel.
Days 2 and 3: Each animal given 2 trials per day in Path A.
Days 4, 5 and 6: Each animal given 2 trials per day in Path B (reverse of Path A).
Day 7: Each animal given 2 trials in Path A.

Serum T_3, T_4 and TSH analysis

Total T_3 total T_4 and TSH concentrations were determined for the F_1 offspring (N=3-10) on PND 4 and 28. Hormone levels were determined from samples combined within litters on PND 4 and for individual animals on PND 28. Serum total T_3 and T_4 levels were quantitatively measured by a commercially available solid-phase, chemiluminescent enzyme immunoassay (Immulite, Diagnostics Products Corporation, Los Angeles, CA). Serum TSH levels were quantitatively measured by a commercially available solid-phase, two site chemiluminescent enzyme immunoassay (Immulite, Diagnostics Products Corporation, Los Angeles, CA).

Statistical Analyses

All analyses were two-tailed for a significance level of 1% and 5% by a one-way analysis of variance (ANOVA [23]. If significant differences were indicated by the ANOVA, Dunnett's test [24] was used to compare the control and treated groups. Functional observational battery parameters which yield scalar and descriptive data were analyzed by Fisher's Exact Test [25]. Body weights, hormone concentrations, survival, developmental landmarks, litter size, locomotor activity, startle response and learning and memory were analyzed using a one-way ANOVA. If significant treatment effects were observed at a given time-point, then Dunnett's test was conducted to determine significant treatment differences from the control group ($p<0.05$).

RESULTS and DISCUSSION

Treatment with PTU significantly decreased maternal body weights in the 38 mg/kg/day group late in gestation (data not shown). Pup weights on PND 1 were significantly decreased in all treated groups. After the end of treatment on PND 10, off-

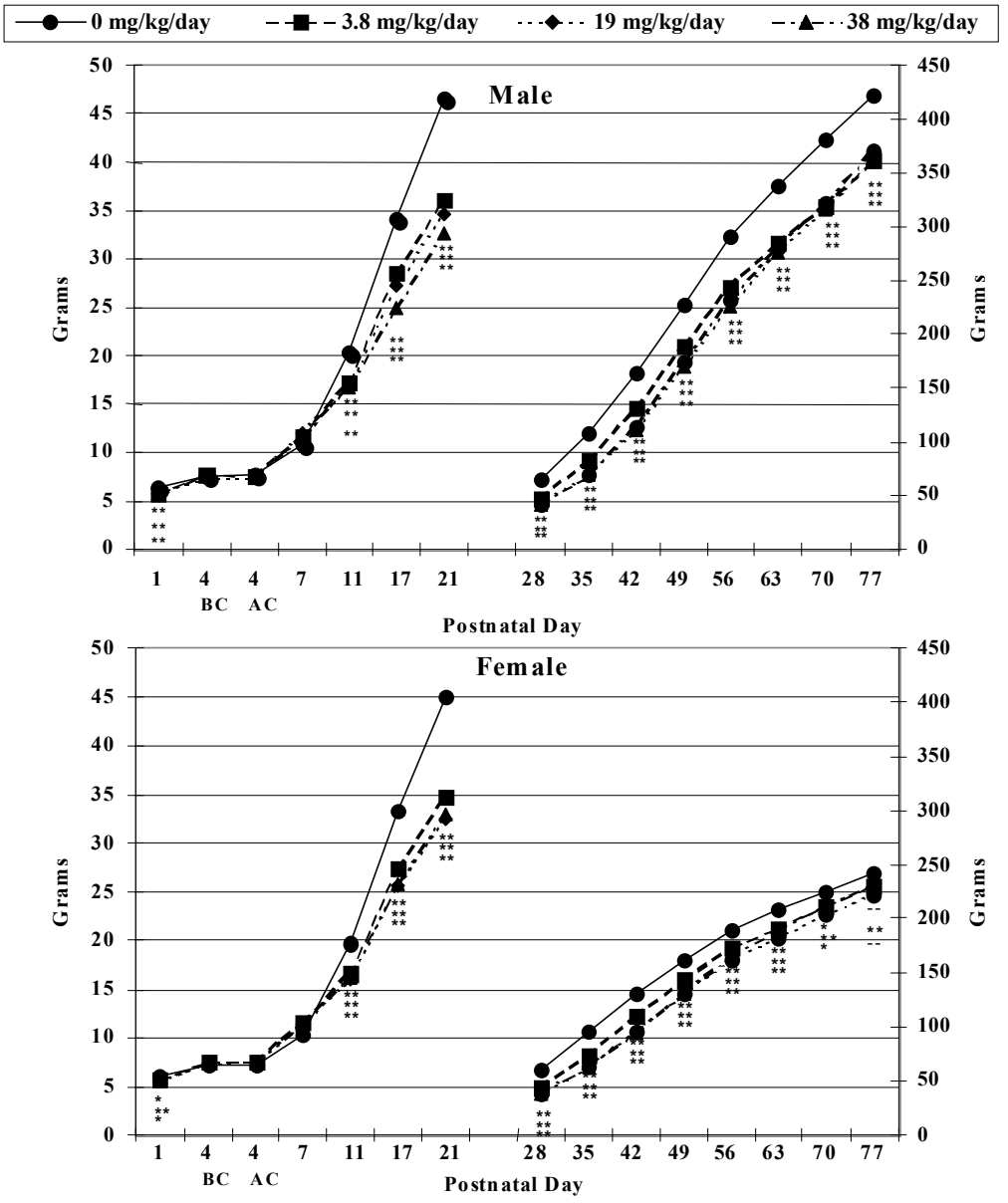

Figure 1. Mean ± SD body weight of Control and PTU-treated rat pups. Male pups are in the upper graph and female pups in the lower graph. Significance between the control and treated groups is indicated by * ($p<0.05$) and ** ($p<0.01$). The row of */** indicates which groups is different from control with top, middle and bottoms rows corresponding to 3.8, 19 and 38 mg/kg/day, respectively.

spring body weights in all PTU-treated groups remained significantly decreased at each day measured (Figure 1). Pup weights were reduced by approximately 10% at birth for all dosage levels and remained reduced throughout the study (Figure 1). Interestingly, in this study, the control body weights between PND 4-21 were about 3-10 g less, respectively than reported by other authors [26-27]. After weaning until 11 weeks of age, this difference in control body weights appears to hold true as the control animals in this study weighed about 25-40 g less than other reported weights for this age range [28-29]. The only apparent difference between these studies is the source of the rats: Charles River, USA versus Charles River, Japan.

Live litter size was significantly reduced in the 38 mg/kg/day group, and pup viability was significantly reduced from birth to PND 4 in the 19 and 38 mg/kg/day groups (Table 1). Unlike pup body weights, this study is in agreement with other published reports on the mean number of live pups delivered by the IGS rat [27, 30]. The appearance of balanopreputial separation was significantly delayed (3-4 days) in the 19 and 38 mg/kg/day dose groups. Similarly, vaginal patency was also significantly delayed

Table 1. Pup Viability and Physical Development

	Control	3.8 mg/kg/day	19 mg/kg/day	38 mg/kg/day
Number Born	15.1 ± 1.5	15.0 ± 1.4	14.1 ± 3.3	13.5 ± 2.3
Live Litter Size	14.2 ± 3.4	14.6 ± 2.3	12.9 ± 4.4	11.4 ± 5.2*
Males (Percent Per Litter)	52.2 ± 12.6	53.0 ± 11.5	50.1 ± 16.7	44.3 ± 15.1
PND 1 Body Weight (grams)				
-Males	6.5 ± 0.7	5.8 ± 0.6**	5.8 ± 0.5**	5.7 ± 0.6**
-Females	6.1 ± 0.7	5.7 ± 0.5*	5.5 ± 0.4**	5.6 ± 0.5*
Survival (Percent Per Litter)				
-Birth-PND4	88.1 ± 24.8	91.8 ± 20.2	77.4 ± 28.8**	69.4 ± 35.9**
-PND4-weaning	98.2 ± 4.8	96.3 ± 9.8	95.4 ± 12.6	89.9 ± 24.6
Developmental Landmarks				
-Balanopreputial separation (PND)	45.1 ± 3.0	46.6 ± 2.8	49.5 ± 4.0**	48.1 ± 2.4*
Body weight (grams)	212 ± 16.2	188 ± 21.9**	194 ± 14.3**	190 ± 14.7**
-Vaginal patency (PND)	33.4 ± 2.8	39.8 ± 2.5**	41.5 ± 3.0**	41.4 ± 2.4**
Body weight (grams)	101 ± 9.0	113 ± 15.4**	111 ± 11.6*	110 ± 15.6
Anogenital Distance Relative to Cube-Root of Body Weight (PND 1)				
-Male	2.71 ± 0.6	2.58 ± 0.5	2.59 ± 0.7	2.78 ± 0.8
-Female	1.63 ± 0.5	1.54 ± 0.4	1.58 ± 0.4	1.75 ± 0.6
Swimming ability (Seconds) PND 21				
-Male	15.8 ± 10.7	52.6 ± 55.5	66.3 ± 65.6	56.1 ± 68.9
-Female	14.3 ± 9.2	46.3 ± 46.8	86.0 ± 72.3*	60.2 ± 71.5
Swimming ability (Seconds) PND 61				
-Male	7.0 ± 2.3	8.9 ± 2.7	8.6 ± 3.9	9.6 ± 5.1
-Female	8.3 ± 3.4	7.8 ± 1.5	7.5 ± 1.9	8.4 ± 2.4

Data presented as mean ±SD (n).
* : Significantly different from the control group at P<0.05.
** : Significantly different from the control group at P<0.01.

Table 2. F_1 Offspring Thyroid Hormone Levels

		T3 (ng/dL)	T4 (µg/dL)	TSH (µIU/mL)
PND 4 Sexes combined	Control	31.2 ± 9.2 (7)	1.44 ± 0.48 (5)	0.11 ± 0.20 (3)
	3.8 mg/kg/day	23.2 ± 7.9 (8)	0.06 ± 0.70 (8)**	0.64 ± 0.21 (8)**
	19 mg/kg/day	22.4 ± 6.4 (8)	0.04 ± 0.53 (8)**	0.64 ± 0.073 (8)**
	38 mg/kg/day	21.3 ± 5.8 (8)	0.05 ± 0.077 (8)**	0.72 ± 0.23 (8)**
PND 28 Males	Control	108 ± 29.3 (10)	4.2 ± 1.48 (10)	0.25 ± 0.16 (8)
	3.8 mg/kg/day	82.9 ± 17.7 (8)*	3.1 ± 0.43 (8)**	0.21 ± 0.10 (8)
	19 mg/kg/day	77.1 ± 16.2 (9)**	2.8 ± 0.80 (9)**	0.21 ± 0.090 (9)
	38 mg/kg/day	81.4 ± 11.7 (7)*	2.0 ± 0.77 (7)**	0.32 ± 0.13 (6)
PND 28 Females	Control	87.9 ± 13.6 (9)	4.2 ± 0.63 (9)	0.20 ± 0.16 (10)
	3.8 mg/kg/day	78.4 ± 14.1 (9)	3.1 ± 0.75 (9)**	0.28 ± 0.082 (8)
	19 mg/kg/day	76.8 ± 13.1 (7)	2.3 ± 0.74 (6)**	0.26 ± 0.11 (6)
	38 mg/kg/day	82.4 ± 10.5 (5)	2.6 ± 0.42 (5)**	0.21 ± 0.033 (5)

Data presented as mean ±SD (n).
* : Significantly different from the control group at P<0.05.
** : Significantly different from the control group at P<0.01.

by 6-8 days (Table 1). These developmental landmarks are body weight dependent in the rodent and in this case are probably indicative of a body weight and not an endocrine effect. Comparing these control rats to published data, it appears that this study is in agreement in terms of the occurrence of vaginal patency[29, 30], but balanopreputial separation has been reported to occur 5-13 day earlier[29, 30] than reported herein.

Serum T_4 levels were reduced for each dose group on PND 4 for both sexes combined and on PND 28 for both sexes separately. Serum TSH levels were reduced at all dosage levels on PND 4 and serum T_3 levels were reduced for each male dose group on PND 28, but not for females (Table 2).

The functional observational battery demonstrated deficits in autonomic (pupillary response, papebral closure), neuromuscular (gait, mobility, foot splay, grip strength, righting reflex, rotorod), sensorimotor (startle), CNS activity (catalepsy, rearing, grooming) and physiologic (body weight, temperature) endpoints on PND 20 in both sexes and in all dose groups. Most of these findings were no longer evident by PND 35 although deficits in grip strength and body weight were sustained through PND 60 (Table 3).

Motor activity was increased in all male dose groups on PND 21 and in females motor activity was increased, albeit not statistically nor in a dose response, on PND 17 and 21 (Figure 2). The

peak auditory startle response was significantly reduced and the latency to peak response was significantly increased for both sexes at all dosage levels on PND 22 (Figure 3). Swimming ability was strikingly reduced for both sexes in all treatment groups at PND 21 although only the female 19 mg/kg/day group reached statistical significance (Table 1). By PND 61 there was no difference in swimming ability across all treatment groups (Table 1). Also, while on PND 62 swimming ability was comparable between all groups, latency to escape the Biel maze was significantly increased for both sexes at all dosage levels. However, on PND 22 when swimming ability was significantly reduced, latency to escape the Biel maze was also significantly increased at all dosage levels. The mean number of errors for all was not different on PND 22, but was numerically increased across all groups on PND 62.

In conclusion, Sprague Dawley Crl:CD®(SD)IGS BR rats have been shown to be sensitive to the effects of pre- and peri-natal PTU exposure as assessed by developmental and behavioral assessments described by the US Environmental Protection Agency Developmental Neurotoxicity Study (OPPTS 870.6300) guidelines. However, there may be developmental differences between the Crl:CD(SD)IGS pup and the Crj:CD(SD)IGS pups as indicated by differences in body weights and balanopreputial separation.

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Table 3. Summary of Pup Functional Observational Battery Findings

Evaluation		**PND 20**		**PND 35**		**PND 45**		**PND 60**	
		♂	♀	♂	♀	♂	♀	♂	♀
Autonomic	Lacrimation								
	Salivation								
	Pupil Response	NR,2,3,4	NR,2,3,4						
	Palpebral Closure	D,2,3,4	D,2,3,4						
	Defecation								
	Urination								
Neuromuscular	Time to First Step								
	Gait Score		IM,2,3,4						
	Mobility Score		IM,2,3,4						
	Foot Splay	D,2,3,4	D,2,3,4						
	Forelimb Grip Strength	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4		D,4
	Hindlimb Grip Strength	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4		D,4
	Air Righting	IM,2,3,4	IM,2,3,4						
	Rotorod	D,2,3,4	D,2,3,4						
Sensorimotor	Tail Pinch								
	Startle Response	D,2,3,4	D,2,3,4						
	Touch Response								
	Approach Response								
	Olfactory Response								
CNS Excitability	Ease of Removal								
	Handling Reactivity								
	Clonic Movements								
	Tonic Movements								
	Arousal								
	Vocalization								
CNS Activity	Posture								
	Catalepsy	I,4	I,4						
	Rearing		D,2,3,4			D,2,3,4		D,3,4	
	Grooming		D,2,3,4						
	Backing								
Physiological	Body Weight	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4	D,2,3,4
	Body Temperature	D,2,3,4	D,2,3,4						
	Appearance								

D= Decreased I= Increased IM= Impaired NR= No Response

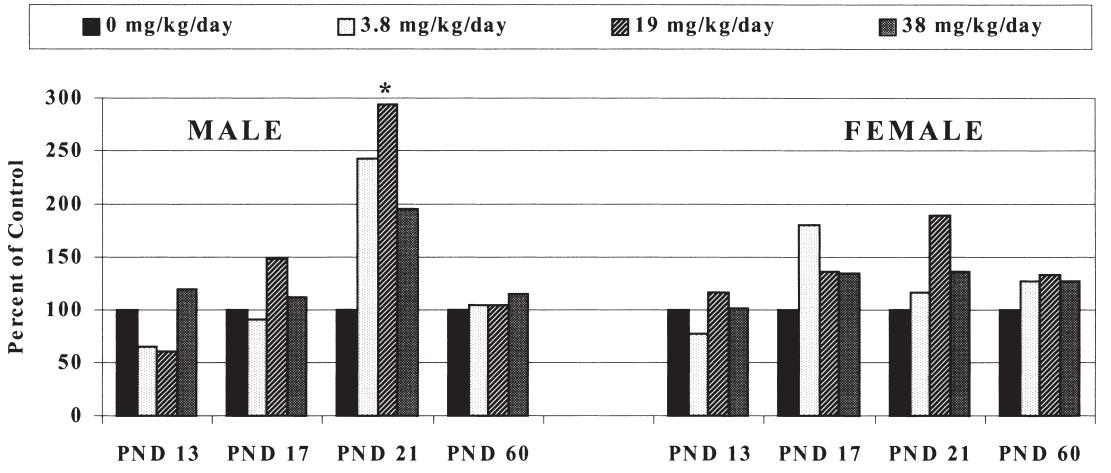

Figure 2. Locomotor activity expressed as a percentage of control in male (left) and female (right) pups. Significance is indicated by an * ($p<0.05$).

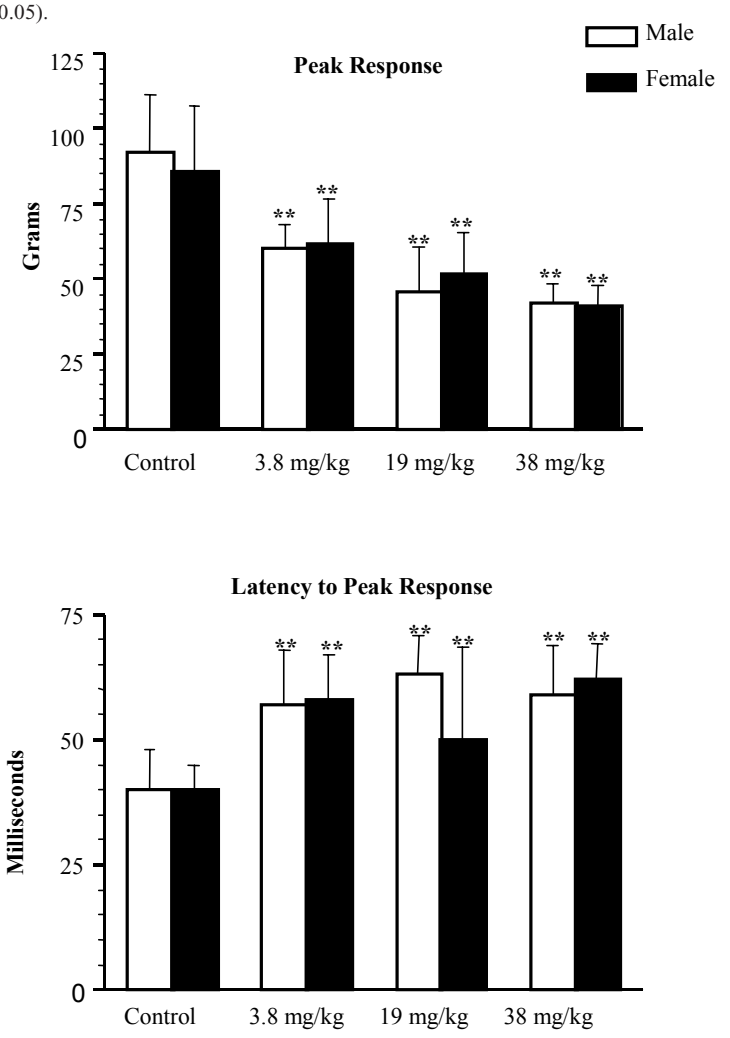

Figure 3. Mean ± SD auditory startle response on PND 22. Significance between the control and treated groups is indicated by * ($p<0.05$) and ** ($p<0.01$).

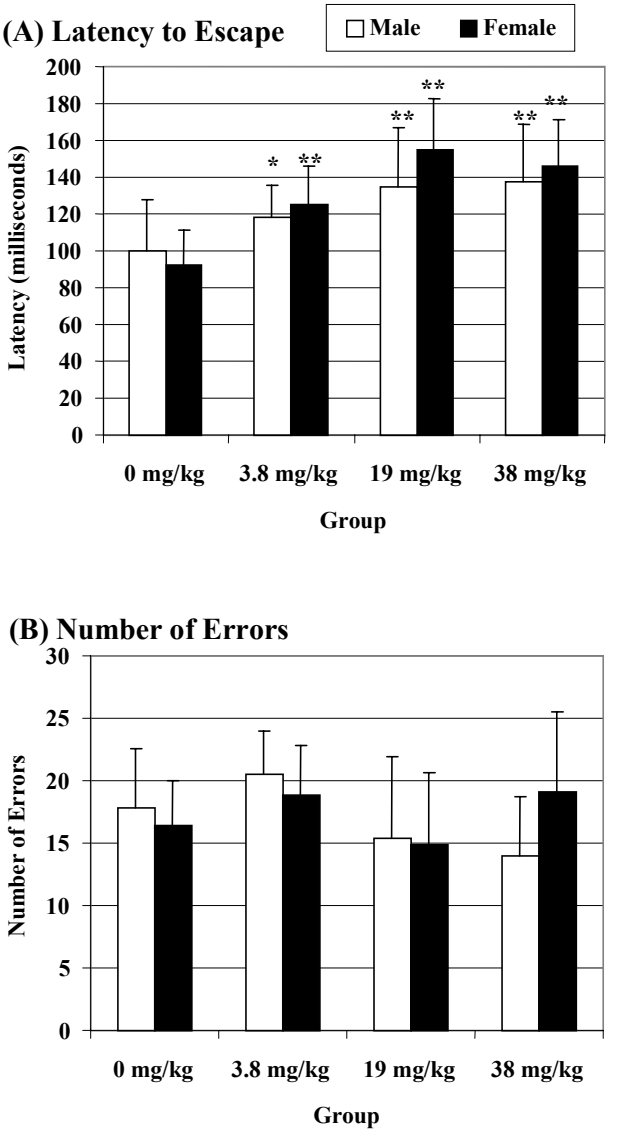

Figure 4. Mean ± SD Biel maze response on PND 22. Significance between the control and treated groups is indicated by * ($p<0.05$) and ** ($p<0.01$). No statistical analysis was performed on the mean total number of errors.

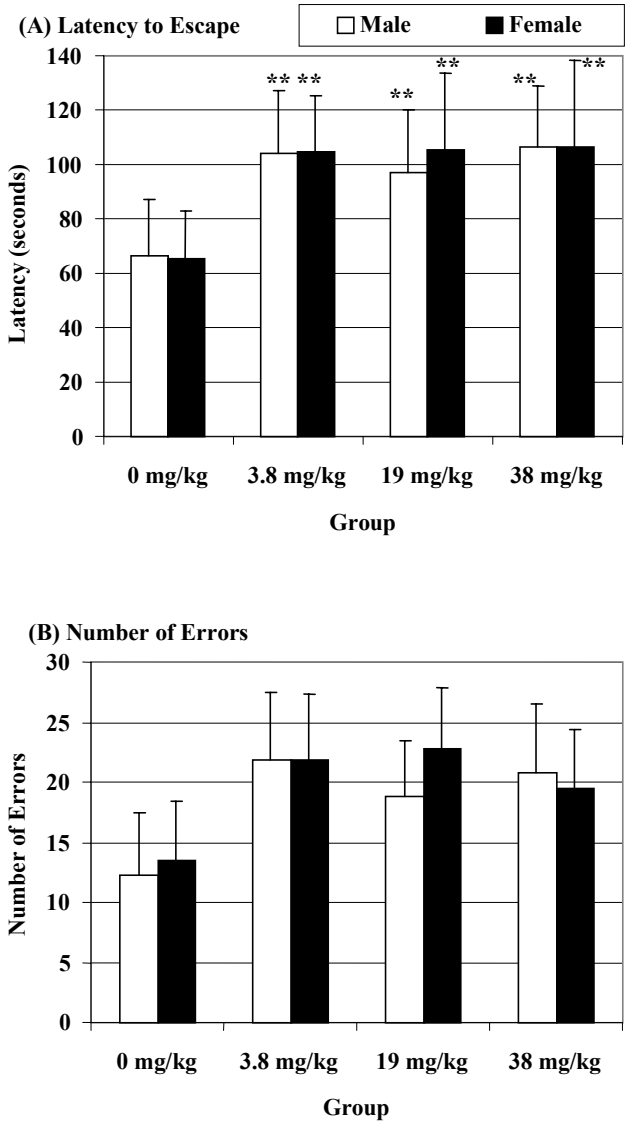

Figure 5. Mean ± SD Biel maze response on PND 62. Significance between the control and treated groups is indicated by * ($p<0.05$) and ** ($p<0.01$). No statistical analysis was performed on the mean total number of errors.

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Sperm Analysis in Crj:CD(SD)IGS Strain Rats

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ABSTRACT. Results of mating and sperm analysis in male Crj:CD(SD)IGS strain rats were compared among 10 reproductive studies performed at the testing facility during the 3 years from 1997 to 1999. The comparison was done using the data obtained in the control group for each of the studies. No differences were seen in male copulation or fertilizability among the studies examined. No differences were seen in the absolute or relative weight of the testes or epididymides among the studies examined. No differences were seen in the values of sperm motility, number of sperms, sperm viability, or sperm morphology among the studies. Thus the males of this strain rat were found to have no problems in copulation, nor were any abnormalities noted in the results of the sperm analysis. Accordingly, this strain rat is considered be a strain appropriate for use in reproductive studies.
— Key words: Crj:CD(SD)IGS, Rat, Fertility and Sperm Analysis

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INTRODUCTION

The results of mating and sperm analysis in male Crj:CD(SD)IGS strain rats were compared among 10 reproductive studies performed at the testing facility during the 3 years from 1997 to 1999 (Study Nos. 1-3, which were performed in 1997, Study Nos. 4-9, which were performed in 1998, and Study No. 10, which was performed in 1999). The comparison was done using the data obtained in the control group for each of the studies.

MATERIALS AND METHODS

Animals: Crj:CD(SD)IGS (abbreviated as "IGS" hereafter) strain rats aged about 6 weeks were purchased from Charles River Japan Inc. (Hino Breeding Center, Shiga, Japan) during the period from 1997 to 1999. The animals were quarantined and acclimatized for about 2 weeks. Animals in which no abnormalities had been found in general signs or body weight changes were grouped and used for the studies.

Housing Conditions: The animals were kept in animal rooms on a 12-hour light and dark cycle (lighting: 6:00 a.m. -6:00 p.m.) with a temperature range of 20-26℃, a relative humidity range of 40-70%, and air changes of 12 times per hour. The animals were housed in same-sex groups of up to 5 per cage in stainless steel cages during the quarantine and acclimatization period. After being quarantined and acclimatized, the animals were housed individually in stainless steel cages. The animals were given free access to feeders filled with solid feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and to tap water.

Mating: Male rats aged 12-13 weeks were paired with similarly-aged female IGS strain rats of control groups on a one-to-one basis for 14 days at the longest. Copulation was confirmed every morning. Females which had sperm in the vaginal smear or a vaginal plug were regarded as having copulated.

Necropsy: Male rats were sacrificed at the age of 17-18 weeks by exsanguination from the abdominal aorta under ether anesthesia and necropsied. The testes, epididymides, and caudae epididymidis were weighed.

Sperm Motion Analysis: Sperm motion analysis using a Hamilton-Thorne sperm analyzer (HTM-IVOS, Hamilton Thorne Research, MA, U.S.A.) was performed as follows. The right cauda epididymis was cut into 4 pieces with a scalpel in warmed medium (Medium 199, GIBCO BRL, NY, U.S.A.) containing 0.5% bovine serum albumin. The prepared sperm suspension was diluted with the warmed medium containing 0.5% bovine serum albumin, and the diluted sperm suspension was incubated in a CO_2 incubator (5% CO_2 and 95% air at 37℃) for about 30 minutes. After incubation, the diluted sperm suspension was subjected to measurement of the percentage of motile sperm (% motile sperm), percentage of progressive sperm (% progressive sperm), average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), and linearity (LIN). Procedures for sample preparation and sperm motion analysis using the Hamilton-Thorne sperm analyzer are shown in Tables 1 and 2. Conditions under which the sperm motion analysis was performed are shown in Table 2. The HTM-IVOS settings used for the sperm motion analysis are shown in Table 3.

Sperm Viability Analysis: Method of sperm viability analysis [1] was examined as follows. Calcein acetoxy methyl ester (Molecular Probes Inc., OR, U.S.A.) and ethidium homodimer-1 (Molecular Probes Inc., OR, U.S.A.) were added to the sperm suspension, and the mixture was incubated in a CO_2 incubator (5% CO_2 and 95% air at 37℃) for about 120 minutes. After incubation, a drop of the stained sperm sample was smeared on a slide glass, and 300 spermatozoa in each sample were examined by fluorescence microscopy (excitation wavelength: 490 nm; emission wavelength: 530 nm). The spermatozoa were classified as follows: (1) live sperm (green fluorescence on head and in midpiece-tail), (2) sperm dying during incubation (sperm which died during the 120-minute incubation; red fluorescence on head and green fluorescence in midpiece-tail), and (3) dead sperm (red fluorescence on head).

Sperm Morphology Analysis: Method of sperm morphology analysis was examined as follows. Three or four drops of the sperm suspension were smeared on a slide glass. The smear samples were fixed in 10% neutral buffered formalin and stained with 1% eosin Y, and 300 spermatozoa in each sample were examined by light microscopy.

Sperm Count Analysis: Sperm count analysis using HTM-IDENT was performed as follows. The left cauda epididymis was homogenized for 2 minutes in 10 mL of a 0.9% NaCl solution containing 0.1% Triton X-100, and the homogenate was di-

luted with 0.9% NaCl solution containing 0.1% TritonX-100 to 20 mL. After vortex mixing, 200 μL of the homogenate was put into a stain reaction vial (Supra Vital IDENT Stain Kit, Hamiton Thorne Research, MA, U.S.A.) containing bis-benzimide trihydrocholoride and stained for 2 minutes. After vortex mixing of the stained sample, the number of sperms was counted to obtain the numbers of sperms per cauda epididymis and sperms per gram of cauda epididymis. Procedures for sample preparation and sperm count analysis using the Hamilton-Thorne sperm analyzer are shown in Table 1. The HTM-IDENT settings used for sperm count analysis are shown in Table 3.

Statistical Analysis: Mean values and standard deviations were calculated for each study. No statistical analysis was performed.

RESULTS AND DISCUSSION

Mating: Only 1 male each in Study Nos. 2, 4, 5, and 7 did not copulate. Male IGS strain rats are not considered to have problems with copulation. Only 1 male each in Study Nos. 4 and 10 failed to fertilize. Male IGS strain rats are not considered to have problems with fertilizability. No differences were seen in the number of days before copulation among the studies examined (Tables 4-1 and 4-2).

Necropsy: Malacic testes and malacic epididymides were noted in 1 male in Study No. 3, and small, malacic testes and small epididymides were noted in 1 male in Study No. 5 (Tables 4-1 and 4-2).

Sperm Analysis: In the sperm motion analysis, no differences were seen in % motile sperm, % progressive sperm, VAP, VSL, VCL, ALH, BCF, STR, or LIN among the studies examined. In the sperm count analysis, no differences were seen in the number of sperms per cauda epididymis or the number of sperms per gram of cauda epididymis among the studies examined. Since the number of sperms per cauda epididymis was markedly small in a male whose testes and epididymides were found to be small in Study No. 5, the values from this male were excluded from calculation of the mean values. Regarding the sperm viability analysis, no differences were seen in the viability index or survivability index among the studies examined. Since the number of observable sperms was small in a male whose testes and epididymides were found to be malacic in Study No. 3, the values from this male were excluded from calculation of the mean values. Regarding the sperm morphology analysis, no differences were seen in the abnormal sperm ratio among the studies examined (Tables 6-1 and 6-2). As described above, the male IGS strain rats were found to have no problems in copulation, nor were any abnormalities noted in the results of sperm analysis. Accordingly, the IGS strain rat is considered to be a strain appropriate for use in reproductive studies.

LITERATURE

1. Kato, M., *et al*. 1995: *Cong. Anom*., 35, 394.

Table 1. Procedures for sample preparation by HTM-IVOS

Analysis conditions	Sperm motion analysis	Sperm count analysis
Sampling site	Right cauda epididymis	Left cauda epididymis
Sampling method	Diffusion	Homogenization
Culture medium	M199＋0.5% BSA	0.1% Triton X－100 in saline
Incubation time	30 minutes	－
Incubation condition	37℃ and 5% CO_2	－
Used chamber	Cannula (Depth: 100mm)	Cell-VU (Depth: 20mm)
Staining	－	Supra Vital IDENT Stain Kit
Number of sperms analyzed per sample	More than 200 sperms	－

M199: medium 199 (Earle's modified salts).
BSA: bovine serum albumin (cohn fraction V powder, pH 7).
Supra Vital IDENT Stain Kit: containing bis benzimide trihydrocholoride.

Table 2. Procedures for sperm motion analysis by HTM-IVOS

Parameters	Abbreviation		Definitions
Percentage of motile sperm	% motile sperm	(%)	The ratio of number of motile sperms to total number of sperms.
Percentage of progressive motile sperm	% progressive sperm	(%)	The ratio of number of motile sperms with both path velocity (VAP) > V0 and straightness (STR) > S0 to total number of sperms.
Average path velocity	VAP	(μm/s)	The average velocity of the smoothed sperm path.
Straight line velocity	VSL	(μm/s)	The average velocity measured in a straight line from the beginning to the end of track.
Curvilinear velocity	VCL	(μm/s)	The average velocity measured over the actual point to point track followed by the sperm.
Amplitude of lateral head displacement	ALH	(μm)	The mean over all sperm tracks of twice the maximum displacement between each sperm track and its average path.
Beat cross frequency	BCF	(Hz)	Frequency of sperm head crossing the sperm average path in either direction.
Straightness	STR	(%)	The departure of the sperm path from a straight line. Average value of the ratio VSL/VAP (VSL/VAP × 100).
Linearity	LIN	(%)	The departure of the sperm track from a straight line. Average value of the ratio VSL/VCL (VSL/VCL × 100).

Table 3. Conditions of sperm motion analysis and sperm count analysis by HTM-IVOS

Setup parameters	Sperm motion analysis	Sperm count analysis
Frames acquired	30	5
Frame rate (Hz)	60	60
Minimum contrast	117	100
Minimum cell size (pixel)	2	5
Minimum static contrast	28	20
Straightness (STR), threshold (S_0) (%)	55	60
Low VAP cutoff (μm/s)	30	30
Medium VAP cutoff, threshold (V_0) (μm/s)	150	150
Low VSL cutoff (μm/s)	12.0	12.0
Head size, non-motile (pixel)	25	15
Head intensity, non-motile	75	55
Static head size	0.45 to 2.50	0.25 to 10.00
Static head intensity	0.10 to 2.60	0.20 to 2.35
Static elongation	1 to 37	5 to 66
Temperature set (℃)	37	—
Apply sort (points in track)	16 to 30	—
Magnification	0.82	1.95
Brightness for ident	—	3750

Table 4-1. Necropsy findings and fertilization ratio in male Crj:CD(SD)IGS

Study No.	1997			1998	
	1	2	3	4	5
Number of animals	20	20	20	20	20
Age at examination	17 weeks	17 weeks	18 weeks	17 weeks	17 weeks
Necropsy findings					
Testis					
Normal	20	20	19	20	19
Small size	0	0	0	0	1 (left)
Malacia	0	0	1 (right)	0	1 (left)
Epididymis					
Normal	20	20	19	20	19
Small size	0	0	0	0	1 (left)
Malacia	0	0	1 (right)	0	0
Number of pairs	20	20	20	20	20
Number of pairs with successful copulation	20	19	20	19	19
Copulation index (%)	100.0	95.0	100.0	95.0	95.0
Number of days before copulation					
Mean±S.D.	3.0±1.1	2.2±1.1	2.5±0.9	2.9±1.2	2.8±2.2
Days 1-5	20	19	20	19	18
Days≧6	0	0	0	0	1
Number of fertilizable males	20	19	20	18	19
Fertilization ratio (%)	100.0	100.0	100.0	94.7	100.0

Table 4-2. Necropsy findings and fertilization ratio in male Crj:CD(SD)IGS

Study No.	1998				1999
	6	7	8	9	10
Number of animals	20	12	12	12	20
Age at examination	17 weeks	17 weeks	17 weeks	17 weeks	17 weeks
Necropsy findings					
Testis					
Normal	20	12	12	12	20
Small size	0	0	0	0	0
Malacia	0	0	0	0	0
Epididymis					
Normal	20	12	12	12	20
Small size	0	0	0	0	0
Malacia	0	0	0	0	0
Number of pairs	20	12	12	12	20
Number of pairs with successful copulation	20	11	12	12	20
Copulation index (%)	100.0	91.7	100.0	100.0	100.0
Number of days before copulation					
Mean±S.D.	2.7±1.3	1.8±0.9	2.6±1.1	2.4±1.0	3.2±0.9
Days 1-5	20	11	12	12	20
Days≧6	0	0	0	0	0
Number of fertilizable males	20	11	12	12	19
Fertilization ratio (%)	100.0	100.0	100.0	100.0	95.0

Table 5-1. Organ weights in male Crj:CD(SD)IGS

Study No.		1997			1998	
		1	2	3	4	5
Number of animals		20	20	20	20	20
Age at examination		17 weeks	17 weeks	18 weeks	17 weeks	17 weeks
Final body weight	(g)	490.6±44.4	505.0±36.5	493.6±39.8	412.7±24.4	510.3±35.2
Testicular weight						
Absolute	(g)	3.37±0.26	3.50±0.27	3.34±0.26	3.09±0.19	3.23±0.27
Relative	(g%)	0.69±0.07	0.69±0.06	0.68±0.08	0.75±0.07	0.64±0.07
Epididymal weight						
Absolute	(g)	1.20±0.09	1.28±0.07	1.26±0.11	1.13±0.07	1.19±0.11
Relative	(g%)	0.25±0.02	0.25±0.02	0.26±0.03	0.27±0.03	0.24±0.03

Table 5-2. Organ weights in male Crj:CD(SD)IGS

Study No.		1998				1999
		6	7	8	9	10
Number of animals		20	12	12	12	20
Age at examination		17 weeks	17 weeks	17 weeks	17 weeks	17 weeks
Final body weight	(g)	501.0±39.5	502.3±39.9	521.0±22.8	518.7±40.7	531.5±39.5
Testicular weight						
Absolute	(g)	3.30±0.32	3.57±0.34	3.36±0.27	3.40±0.25	3.30±0.27
Relative	(g%)	0.66±0.07	0.71±0.10	0.64±0.05	0.66±0.09	0.62±0.06
Epididymal weight						
Absolute	(g)	1.24±0.11	1.25±0.07	1.25±0.12	1.25±0.16	1.18±0.09
Relative	(g%)	0.25±0.03	0.25±0.02	0.24±0.02	0.24±0.04	0.22±0.02

Table 6-1. Sperm analysis in male Crj:CD(SD)IGS

Study No.		1997			1998	
		1	2	3	4	5
Number of animals		20	20	20	20	20
Age at examination		17 weeks	17 weeks	18 weeks	17 weeks	17 weeks
Sperm motion						
% motile sperm	(%)	82.9±4.2	83.6±4.0	77.2±18.8	90.7±3.1	88.9±3.9
% progressive sperm	(%)	51.3±5.0	52.0±9.4	42.5±17.1	53.7±9.2	54.0±7.7
Average path velocity (VAP)	(μm/s)	174.9±7.3	175.3±9.3	162.7±20.5	169.0±9.4	168.2±8.7
Straight line velocity (VSL)	(μm/s)	119.0±4.3	119.3±9.3	113.5±18.6	118.4±7.8	119.1±8.4
Curvilinear velocity (VCL)	(μm/s)	402.0±21.4	401.6±16.2	376.6±38.6	383.5±19.4	378.1±16.8
Amplitude of lateral head displacement (ALH)	(μm)	21.8±0.9	22.0±0.9	20.8±1.4	20.7±0.8	20.7±0.9
Beat cross frequency (BCF)	(Hz)	29.3±1.3	28.9±1.8	32.5±3.9	32.5±1.2	31.3±1.0
Straightness (STR)	(%)	68.7±2.4	68.6±2.7	69.5±5.2	70.2±1.8	70.6±2.7
Linearity (LIN)	(%)	30.2±1.8	30.3±1.8	30.7±3.1	31.5±1.5	31.8±1.8
Number of sperms						
per cauda epididymis	($x10^6$)	268±39	340±55	253±63	236±28	297±58[c]
per gram of cauda epididymis	($x10^6$/g)	960±122	1118±157	825±170	892±89	1022±148[c]
Sperm viability						
Viability index	(%)	99.2±0.6	98.6±0.7	99.6±0.3[c]	99.3±0.5	97.0±2.9
Survivability index	(%)	83.2±5.3	83.4±4.9	84.4±6.1[c]	88.7±3.4	85.1±5.3
Sperm morphology						
Abnormal sperm ratio	(%)	0.2±0.2	0.3±0.4	5.7±20.2	3.6±1.7	3.8±3.9

a) ((Number of live sperms+number of sperms dying during incubation)/number of sperms analized)×100.
b) Number of live sperms/number of sperms analized)×100.
c) One animal was excluded from analysis.

Table 6-2. Sperm analysis in male Crj:CD(SD)IGS

Study No.		1998				1999
		6	7	8	9	10
Number of animals		20	12	12	12	20
Age at examination		17 weeks	17 weeks	17 weeks	17 weeks	17 weeks
Sperm motion						
% motile sperm	(%)	91.1±3.3	88.5±7.5	89.8±2.8	78.0±22.6	88.1±3.2
% progressive sperm	(%)	56.3±9.7	51.8±17.5	48.1±10.7	36.0±22.1	40.5±10.9
Average path velocity (VAP)	(μm/s)	164.6±10.5	161.0±16.5	158.3±8.7	145.6±23.5	152.6±7.8
Straight line velocity (VSL)	(μm/s)	119.6±9.0	115.9±15.9	113.1±6.9	100.3±22.0	109.0±8.4
Curvilinear velocity (VCL)	(μm/s)	369.9±21.9	367.7±31.6	361.6±20.1	335.6±49.0	358.6±17.9
Amplitude of lateral head displacement (ALH)	(μm)	20.3±0.5	20.5±0.5	20.3±0.8	20.6±0.9	20.4±1.2
Beat cross frequency (BCF)	(Hz)	30.9±1.2	31.1±1.3	31.9±0.9	31.9±3.6	30.0±1.0
Straightness (STR)	(%)	72.1±2.3	71.5±3.8	71.0±1.7	67.2±5.7	71.3±3.4
Linearity (LIN)	(%)	32.6±1.4	31.7±2.5	31.8±1.9	29.5±3.1	30.9±2.5
Number of sperms						
per cauda epididymis	($x10^6$)	319±62	266±53	299±56	271±56	303±72
per gram of cauda epididymis	($x10^6$/g)	1036±122	884±132	1013±136	915±132	1079±200
Sperm viability						
Viability index	(%)	97.6±2.0	99.2±1.2	98.3±2.0	92.4±23.4	99.2±0.8
Survivability index	(%)	86.1±4.5	87.1±3.9	84.0±3.6	79.2±24.0	80.3±6.7
Sperm morphology						
Abnormal sperm ratio	(%)	4.0±3.1	3.7±2.8	5.7±5.2	10.9±23.4	5.5±3.0

a) ((Number of live sperms+number of sperms dying during incubation) / number of sperms analized)×100.
b) (Number of live sperms / number of sperms analized)×100.
c) One animal was excluded from analysis.

Strain Difference of Spermatogenic Cycle Stage and Incidence of Sperm Retention between Crj:CD(SD) and Crj:CD(SD)IGS Rats

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ABSTRACT. To clarify, the strain difference in the spermatogenesis between Crj:CD(SD) and Crj:CD(SD)IGS rats, histopathological examination of the testes in both strains of rats at 16 and 20 weeks of age and the classification of spermatogenic cycle stage of each seminiferous tubule were performed. Crj:CD(SD)IGS rats at 16 weeks of age showed a significant decrease in the number of seminiferous tubules at the stage Ⅱ and Ⅸ and increase in the number of seminiferous tubules at the stage Ⅲ and Ⅳ as compared with Crj:CD(SD) rats. However, there were no differences between strains in ratios of each germ cell stage to Sertoli cells. No strain differences were noticed in the number of seminiferous tubules or ratios of each germ cell stage to Sertoli cells in rats at 20 weeks of age. The seminiferous tubules at the stage Ⅸ showing the retention of Step 19 spermatids was not frequent in both strains of rats, and strain difference was not observed between the two strains. The Step 19 spermatids did not observed in the seminiferous tubules at the stage Ⅹ in the either strains of rat.
It was concluded that there were no strain differences in the testicular parameters between Crj:CD(SD) and Crj:CD(SD)IGS rats. — Key words: Spermatogenic cycle analysis, Retention of Step 19 spermatids, IGS rats

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INTRODUCTION

The International Genetic Standard system (IGS system), a breeding system, was developed by Charles River Inc. in order to apply the internationalization of the development of new drugs to minimize the genetic divergence and to harmonize the range and the variations of phenotypes[1]. The Crj:CD(SD)IGS rats had been established from the original Crj:CD(SD) rats by use of this breeding system. In this study, we conducted the classification of spermatogenic cycle stage of the testes in rats of both strains to compare the development of spermatogenesis.

MATERIALS AND METHODS

Animals

Ten male each from Crj:CD(SD) rats (abbreviated as SD) and Crj:CD(SD)IGS rats (abbreviated as IGS) at the age of 16 and 20 weeks respectively, were received from Charles River Japan Inc. (Tsukuba or Hino Breeding Center, Japan). All rats were killed by an ether overdose after measurement of body weight on the day of arrival, and then necropsied.

Histopathologic examination of the testes

The testes were fixed in Bouin's solution. Paraffin sections were made and stained with periodic acid Schiff method (PAS). The spermatogenic cycle stage was classified and counted into Ⅰ to ⅩⅣ phase in the 100 seminiferous tubules selected from the tubules vertically sectioned and excluded peripheral part of each section of testis under a light microscope. In addition, seminiferous tubules at the stage Ⅰ to ⅩⅣ were divided into 4 groups of Ⅰ to Ⅵ, Ⅶ to Ⅷ, Ⅸ to Ⅺ and Ⅻ to ⅩⅣ, and the population of the 4 groups was also counted according to the method previously reported[2].

In case where a significant difference was recognized in the number of seminiferous tubules at each stage, one seminiferous tubule at the same stage with almost the same diameter in vertical cross section was chosen from one testis in both strains, and the number of spermatogoniums, spermatocytes, spermatids except mature sperms and a Sertoli cell were counted and the ratio of each cells to Sertoli cells were calculated.

Incidences of retention of Step 19 spermatids in seminiferous tubules

The seminiferous tubules at the stage Ⅸ and Ⅹ were selected among the seminiferous tubules in each testis, and counted the number of seminiferous tubules with Step 19 spermatids. The incidence of seminiferous tubules showing retention of Step 19 spermatids was determined.

Statistical analysis

The measured values of each stage, four stages and incidences of retention of Step 19 spermatids were presented as the mean ± standard deviation (S.D.) in each group. The variance of the data was analyzed by the F-test. Student's t-test was conducted on the data between SD and IGS rats at the same age when the variance by F-test was homogeneous, whereas the Aspin-Welch t-test was used when it was not homogeneous. A p value of less than 0.05 or 0.01 was chosen as the level of statistical significance.

RESULTS

In the SD rats at 20 weeks old, testes of one rat was excluded from the further analysis because of marked unilateral testicular atrophy characterized by the severe loss of germ cells and hyperplasia of Leydig cells.

Table 1 shows the number of seminiferous tubules that spermatogenic cycle was classified. In comparison with SD rat, IGS rats at 16 weeks of age showed a significant decrease in the number of seminiferous tubules at the stage Ⅱ and Ⅸ and increase in the number of seminiferous tubules at the stage Ⅲ and Ⅵ. Table 2 shows the number of seminiferous tubules that divided into four groups according to the spermatogenic cycle. IGS rats showed a significant increase in the number of seminiferous tubules classified to group Ⅰ (stage Ⅰ to Ⅵ), whereas the number of seminiferous tubules classified to groups Ⅱ (stage Ⅶ to Ⅷ) and Ⅲ (stage Ⅸ to Ⅺ) decreased. However, as shown in Table 3, no signifi-

Table 1. Number of seminiferous tubles with classified spermatogenic cycle stage in the testes of Crj:CD(SD) and Crj:CD(SD)IGS strain of rats

Age (weeks)	Strain	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
16	SD	18.5 ± 3.4 (20)	6.7 ± 2.2 (20)	6.7 ± 3.0 (20)	4.8 ± 1.9 (20)	3.4 ± 1.7 (20)	6.1 ± 1.8 (20)	12.5 ± 2.5 (20)	8.9 ± 2.6 (20)	5.5 ± 2.7 (20)	3.3 ± 1.7 (20)	4.2 ± 1.5 (20)	8.6 ± 3.6 (20)	7.7 ± 3.7 (20)	3.4 ± 2.0 (20)
	IGS	19.1 ± 4.3 (20)	5.2* ± 2.2 (20)	8.7* ± 3.2 (20)	4.4 ± 3.3 (20)	3.7 ± 2.7 (20)	11.6** ± 5.3 (20)	10.8 ± 4.4 (20)	7.3 ± 4.2 (20)	3.6** ± 2.1 (20)	2.8 ± 1.8 (20)	3.8 ± 1.8 (20)	7.8 ± 3.0 (20)	7.9 ± 2.8 (20)	3.7 ± 1.5 (20)
20	SD	19.6 ± 3.3 (19)	5.7 ± 2.7 (19)	6.7 ± 2.5 (19)	3.9 ± 1.8 (19)	5.5 ± 2.1 (19)	12.0 ± 3.0 (19)	9.4 ± 2.9 (19)	6.6 ± 3.0 (19)	4.2 ± 1.8 (19)	2.5 ± 1.7 (19)	4.6 ± 1.8 (19)	8.2 ± 2.6 (19)	7.1 ± 2.0 (19)	3.9 ± 2.0 (19)
	IGS	20.7 ± 5.0 (20)	5.8 ± 3.2 (20)	7.0 ± 2.7 (20)	4.3 ± 2.4 (20)	4.4 ± 2.3 (20)	12.5 ± 3.9 (20)	8.9 ± 4.0 (20)	7.0 ± 3.1 (20)	4.7 ± 1.8 (20)	3.3 ± 2.0 (20)	3.1 ± 2.7 (20)	7.6 ± 3 (20)	7.8 ± 3.3 (20)	3.2 ± 1.8 (20)

*:Significantly different from Crj:CD(SD) rats, $p<0.05$
**:Significantly different from Crj:CD(SD) rats, $p<0.01$
Parameter : Mean±S.D.(%)
( ) : N

Table 2. Number of seminiferous tubles divided into four groups classified spermatogenic cycle stage in the testes of Crj:CD(SD) and Crj:CD(SD)IGS strain of rats

Age (weeks)	Strain	Group I (Stage I－VI)	Group II (StageVII－VIII)	GroupIII (StageIX－XI)	GroupIV (StageXII－XIV)
16	SD	46.1 ± 2.5 (20)	21.4 ± 2.5 (20)	13.0 ± 3.5 (20)	19.6 ± 3.9 (20)
	IGS	52.5** ± 6.6 (20)	18.1* ± 6.3 (20)	10.2* ± 2.6 (20)	19.3 ± 2.8 (20)
20	SD	53.4 ± 3.5 (19)	16.1 ± 3.7 (19)	11.4 ± 3.4 (19)	19.2 ± 4.5 (19)
	IGS	54.6 ± 5.2 (20)	15.9 ± 2.8 (20)	11.0 ± 3.8 (20)	18.6 ± 5.2 (20)

*:Significantly different from Crj:CD(SD) rats, $p<0.05$
**:Significantly different from Crj:CD(SD) rats, $p<0.01$
Parameter : Mean±S.D.(%)
( ) : N

Table 3. The ratio of the number of germ cells at the various stages to the number of Sertoli cells in each stage of seminiferous tubules of Crj:CD(SD) and Crj:CD(SD)IGS strains of rats

Age (weeks)	Strain	Stage II			Stage III			Stage VI			Stage IX		
		Sg	Sc	St	Sg	Sc	St	Sg	Sc	St	Sg	Sc	St
16	SD	0.79 ±0.09 (20)	2.52 ±0.47 (20)	6.77 ±0.94 (20)	1.17 ±0.34 (20)	2.68 ±0.50 (20)	6.88 ±1.28 (20)	2.22 ±0.54 (20)	3.10 ±0.74 (20)	7.46 ±1.36 (20)	0.35 ±0.06 (20)	5.53 ±1.19 (20)	7.19 ±1.41 (20)
	IGS	0.81 ±0.13 (20)	2.43 ±0.41 (20)	6.96 ±0.90 (20)	1.11 ±0.33 (20)	2.56 ±0.41 (20)	7.24 ±1.35 (20)	2.25 ±0.52 (20)	2.99 ±0.65 (20)	7.64 ±1.47 (20)	0.32 ±0.08 (20)	5.50 ±1.11 (20)	7.52 ±1.74 (20)

Sg : Spermatogonia
Sc : Spermatocytes
St : Spermatids

Parameter : Mean±S.D.
( ) : N

Table 4. Incidence of retention of step 19 spermatids in stage IX and X seminiferous tubules of Crj:CD(SD) and Crj:CD(SD)IGS strains of rats

Age (Weeks)	Strain	Stages					
		IX			X		
		Normal	Retention	Incidence(%)	Normal	Retention	Incidence(%)
16	Crj:CD(SD)	36.7 ±7.8 (10)	2.3 ±2.3 (10)	6.8 ±6.9 (10)	23.8 ±5.7 (10)	0 0 (10)	0 0 (10)
	Crj:CD(SD)IGS	33.5 ±6.8 (9)	1.4 ±1.8 (9)	4.4 ±5.7 (9)	26.6 ±7.8 (9)	0 0 (9)	0 0 (9)
20	Crj:CD(SD)	40.3 ±12.4 (10)	1.0 ±1.1 (10)	3.0 ±3.4 (10)	26.9 ±6.5 (10)	0 0 (10)	0 0 (10)
	Crj:CD(SD)IGS	41.9 ±13.5 (10)	0.4 ±0.7 (10)	1.2 ±2.0 (10)	28.4 ±6.6 (10)	0 0 (10)	0 0 (10)

Parameter : Mean ± S.D.
( ): N

cant differences between SD and IGS rats were observed in the ratio of germ cells to Sertoli cells in the spermatogenic cycles showing significant differences in the number of seminiferous tubules. No differences were observed in any parameter between IGS and SD rats at 20 weeks old.

Table 4 shows the incidence of Step 19 spermatids in stage IX and X. The retention of Step19 spermatids at the stage IX seminiferous tubule were observed in 7 of 10 animals of both strains at 16 weeks of age, 5 of 9 SD rats and 3 of 10 IGS rats at 20 weeks of age, respectively. The frequencies were 23 of 367 seminiferous tubules in SD rats (6.8 ± 6.9%), 14 of 335 seminiferous tubules in IGS rats (4.4 ± 5.7%) at 16 weeks of age, respectively. At 20 weeks of age, the frequencies were 9 of 363 seminiferous tubules in SD rats (3.0 ± 3.4%), and 4 of 419 seminiferous tubules in IGS rat (1.2 ± 2.0%). The frequency in IGS rats with stage IX showed lower than that in SD rats. However, no statistically significant differences were observed between strains. The spermatid retention was limited to small part of the tubular lumen in animals of both strains and most findings were thought to be just before release, and the strain specificities were not recognized in each age group (Photo 1, 2). On the other hand, the frequency and degree of retention of spermatids decreased in both strains at 20 weeks of age. In the stage X seminiferous tubules, the retention of Step19 spermatids was not recognized in either age of both strains (Photo 3).

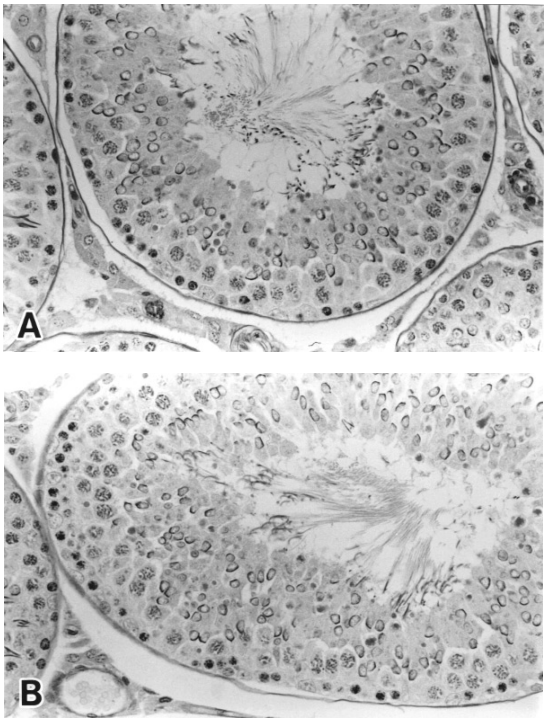

Photo 1. Microphotographs of stage IX seminiferous tubule in rats at 16 weeks of age. Both photos show slight retention of step 19 spermatids.
A) Crj:CD(SD) rat, B) Crj:CD(SD)IGS rat.
× 340, PAS stain.

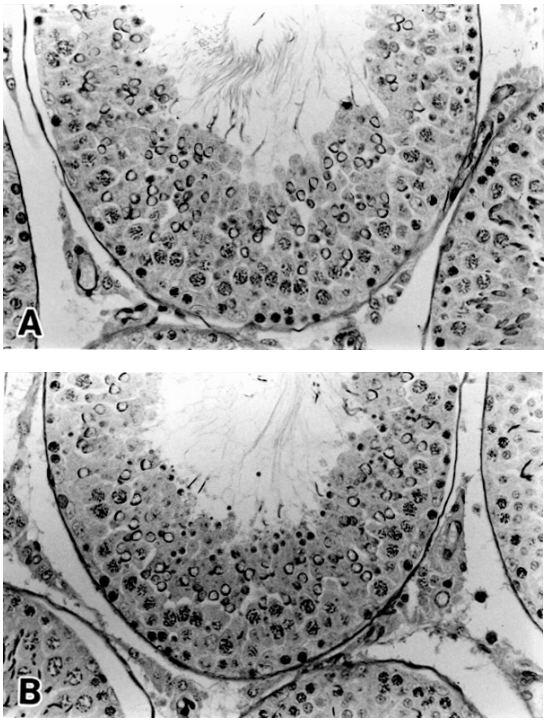

Photo 2. Microphotographs of stage IX seminiferous tubule in rats at 20 weeks of age. Both photos show slight retention of step 19 spermatids.
A) Crj:CD(SD) rat, B) Crj:CD(SD)IGS rat.
× 340, PAS stain.

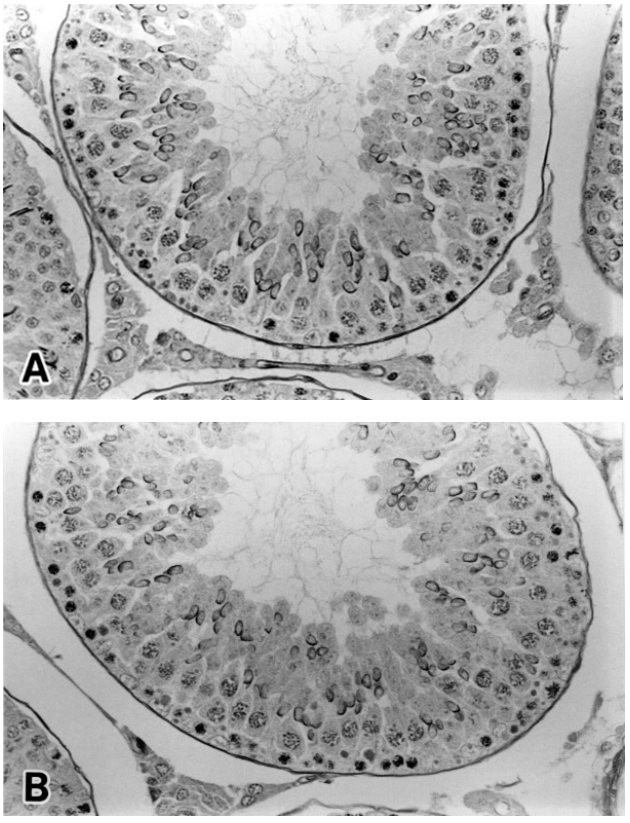

Photo 3. Microphotographs of stage X seminiferous tubule in rats at 16 weeks of age. Both photos show no sperm in the lumen.
A) Crj:CD(SD) rat, B) Crj:CD(SD)IGS rat.
× 340, PAS stain.

DISCUSSION

A stage analysis of the testes was performed in SD and IGS rats at 16 and 20 weeks of age to clarify the strain difference on the spermatogenic activity specifical reference to the retention of Step 19 spermatids.

Statistically significant differences were observed in the population of seminiferous tubules having stage Ⅱ, Ⅲ, Ⅵ and Ⅸ in IGS rats at 16 weeks of age, but no differences were observed in the ratios of germ cells to Sertoli cell in these stages. On the other hand, the retention of Step 19 spermatid was observed in the small area of stage Ⅸ seminiferous tubules in both strains at 16 and 20 weeks of age. The retention of spermatid was not observed in the seminiferous tubules at stage Ⅹ. Thus, these histopathological findings indicated that the some of tubules at the stage Ⅸ might have been under the spermiation phase (just before release of sperms into the lumen of seminiferous tubule).

Matsumoto et al.[3] reported that the retention of Step19 spermatid was recognized in the stages Ⅸ, Ⅹ and Ⅺ seminiferous tubules in the testis of IGS rats with a high frequency and morphological disorders observed in these animal sperm. However, the results of this study revealed that frequencies of the spermatid retention in seminiferous tubules were far less as compared with those reported in the previous study[3]. Furthermore, we showed that there were no indications of the strain differences on the histological findings of the testes as well as sperm mobilities, counts, and morphological abnormalities using sperm obtained from the caudal epididymis on the 16 and 20 age groups[4].

From these results, it was concluded that there were no indications of significant biologically differences in the spermatogenic cycle stages of the seminiferous tubules between SD and IGS rats.

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A Study of the Effects on Reproductive Performance in Crl:CD(SD)IGS Rats fed a variety of Rodent Diets.

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ABSTRACT. In this laboratory, the Crl:CD(SD)IGS rat, supplied by Charles River UK and fed on a high protein breeding diet (LAD2) has shown reduced litter survival in multigeneration studies paired at 16 weeks of age, as compared to the predecessor Crl:CD(SD) rat fed on the same diet. As an expedient the starting group size in regulatory studies was increased so that study integrity was maintained into the second generation. Meanwhile, in-house studies were performed to explore possible ways of mitigating the problem. A comparison of the performance of rats fed either a low protein maintenance diet (RM1) or one of three different higher protein breeder diets (LAD2, VRF1 or Teklad 2018) has been made. This has confirmed the importance of correct dietary protein levels to ensure reproductive success and has demonstrated the superiority of VRF1 diet (as used by Charles River UK) in reducing maternal weight loss during parturition, increasing litter size and offspring birth weight and supporting subsequent litter survival. VRF1 has now been adopted as our standard breeder diet and will allow for a reduction in starting group size and improve our ability to discriminate between treatment related and random effects on litter survival. — Key words: reproductive performance; diet; Crl:CD(SD)IGS rat

CD(SD)IGS-2000: 194-201

INTRODUCTION

We have previously demonstrated [1] a marked difference in offspring bodyweight at birth, and subsequent survival to Day 4 of age, between Crl:CD(SD)IGS rats paired at 16 weeks of age (multigeneration studies) as compared to our previous experience with the Crl:CD(SD) strain and with the IGS rat paired at 11 weeks of age (pre- and post-natal studies to ICH guidelines). Between 1997 and 1999 the mean incidence of total litter loss from 12 untreated control groups of our first generation (F_0) IGS multigeneration studies was 7.5% with study ranges of 0.0 to 30.8%. This compares to the previous means in the "old" strain of 0.7 to 2.0% and study ranges of 0.0 to 9.0%. Similar results were obtained with the second generations of these studies (F_1), with a mean of 7.6% and a study range of 0.0 to 27.3%. That this was not an isolated observation was confirmed by a review of the performance of animals in similar studies performed by other laboratories within the United Kingdom [2]. However, no such difference was seen between our previous Crl:CD(SD) data and current Crl:CD(SD)IGS data for animals paired at 11 weeks of age. A decline in fertility of virgin females with increasing age [3] and fertility problems have previously been encountered with Crl:CD(SD) rats in multigeneration studies where females were first paired at 20 weeks of age. Consultation with the animal supplier (Charles River UK, Margate, Kent England) indicated that they were not experiencing any decline in breeding output, even with significantly older females (up to 37 weeks). It was suggested that the problem might lie in nutrient differences between the Charles River diet and the LAD breeder diet that we used, either our diet was deficient in some way for prolonged feeding to the IGS rat strain, or this rat was much more susceptible to variations between commercial diets.

In this report we present the results of studies to test the effects of five different diets on reproductive performance in the IGS rat. We have used a low protein pelleted maintenance diet (RM1, 15% protein), two intermediate protein level breeder diets (VRF1, 18% protein, in both pelleted and powdered form, and Teklad 19% protein) and our standard high protein diet LAD2, 21% protein. RM1 diet is used for our long-term oncology studies, where it lengthens the life span of animals by suppressing excessive bodyweight gains without any nutritional problems, but it is not recommended by the supplier for use in breeding studies.

MATERIALS AND METHODS

Regulations: The studies reported here were conducted in compliance with the general principles of Good Laboratory Practice Standards and were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986.

Animals: All animals were purchased from Charles River UK, Margate, Kent, England and were of the re-derived International Genetic Standard strain of albino rat (Crl:CD®(SD)IGS BR).

Treatment groups: In the first study two types of pelleted diet (RM1 and VRF1) were compared. In the second study three types of powdered diet were assessed. As there were no major differences in bodyweight between animals at breeding stages the data for the two studies is combined. Animals were allocated randomly to one of five treatment groups as follows, within each treatment group each animal was uniquely identified by a tail tattoo.

Group	Treatment	Number of Animals	Female Animal Numbering
1	RM1 pelleted diet	20	43 to 62
2	VRF1 pelleted diet	20	63 to 82
3	LAD2 powdered diet	24	1073 to 1096
4	VRF1 powdered diet	24	1097 to 1120
5	Teklad powdered diet	24	1121 to 1144

Husbandry conditions: Animals were housed in a fully barriered rodent facility, maintained at a temperature of 19-25℃ and 40-70% relative humidity with at least 15 air changes per hour (filtered, not recirculated). The lighting was controlled to provide 12 hours light: 12 hours dark. In the maturation period preceding pairing, and for males after mating, animals were group housed (up to 4 animals per cage) in large stainless steel cages with stainless steel grid floors and lids. For mating, polypropylene cages with stainless steel grid floors and lids were used and the same cage type was used through

to Day 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 the larger stainless steel cages. Cages with grid floors were suspended above absorbent paper. The absorbent paper and wood flake bedding were changed at least twice weekly.

Diet in pelleted form was provided in food hoppers whilst powdered diet was presented 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. An analysis of the main constituents of the four diets tested is given in Table 1. RM1 low protein maintenance diet and LAD2 high protein breeder diet were obtained from Special Diet Services Ltd. UK. VRF1 intermediate level protein diet was obtained from Charles River UK and Teklad 9600 high protein diet was obtained from Harlan UK Ltd.

Mating procedures: One male was paired with one female when they were approximately 16 weeks of age. The day on which mating evidence (ejected copulation plugs or sperm in the vaginal smear), was detected was designated Day 0 of gestation.

Observations: All animals were monitored at least twice daily for ill health. Female bodyweights were recorded weekly until pairing then on Days 0, 6, 13 and 20 after mating and Days 1, 4, 7, 14 and 21 of lactation. Data recorded for the males used as part of this study, and data for females prior to pairing, is not included in this report. Food consumption was recorded for weekly until pairing then on the weigh days shown above. 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 ½ 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, this was designated Day 1 of lactation. Litter size was monitored daily and on Day 4 of age litter size was reduced to 8, where possible 4 males and 4 females per litter, using a non-selective random culling procedure. Offspring were sexed on Days 1, 4 and 21 of age and weighed on Days 1, 4, 7, 14 and 21.

Necropsy procedures: All animals were killed by inhaled carbon dioxide. Females failing to mate or produce a litter were killed on Day 25 after pairing/mating. Females whose litters died before weaning were killed on or after the day the last offspring died. Females that littered successfully, and their litters, were

Table 1. Typical dietary analyses supplied by manufacturers

Diet Type	RM1	VRF1	LAD2	Teklad 9605/8	Recommended Values $
Nutrients %					
Crude Protein	14.7	18.0	21.3	18.6	15
Crude Fat/Oil	2.6	4.3	3.3	6.9	5
Crude Fibre	5.3	4.5	2.9	4.3	#
Ash	5.9	6.0	4.2	5.7	#
Carbohydrates	61.5	50.0	58.3	53.4	#
Energy levels MJ/kg					
Digestible energy	12.1	11.4	14.0	13.6	15ⓐ
Major minerals %					
Calcium	0.71	0.90	0.84	1.0	0.63
Phosphorus	0.50	0.65	0.67	0.65	0.37
Calcium/phosphorus ratio	1.42	1.38	1.25	1.54	1.70
Sodium	0.25	0.30	0.30	0.27	0.05
Chlorine	0.40	#	0.37	0.46	0.05
Potassium	0.66	0.56	0.56	0.86	0.36
Magnesium	0.22	0.27	0.18	0.21	0.06
Trace minerals mg/kg					
Iron	114	250	138	190	75
Copper	11	15	13	13	8
Manganese	66	118	73	117	10
Zinc	18	129	47	65	25
Selenium	0.15	#	0.24	0.18	#
Vitamins					
Vitamin A (iu/kg)	6303	20000	32000	29500	0.7mg
Vitamin E (mg/kg)	75.9	80	115	105	18 mg
Vitamin C (mg/kg)	11.0	0.0	50	#	#

$ Data derived from Nutrient Requirements for Laboratory Animals (4)
#=data not supplied
ⓐ Based on food intake of 15 g/day : CD rats consume at least 25 g/day

killed after weaning. All animals were subject to a brief macroscopic examination for evidence of disease or reaction to treatment. For females killed at termination or whose litters died before weaning, the number of implantation sites was recorded.

Data Processing: Four offspring survival indices were calculated

1) Post-implantation survival index=
$$\frac{\text{Total number of offspring born x100}}{\text{Total number of uterine implantation sites.}}$$

2) Live-birth index=
$$\frac{\text{Number of live offspring on Day 1 after littering x 100}}{\text{Total number of offspring born.}}$$

3) Viability index=
$$\frac{\text{Number of live offspring at Day 4 (before cull) x100}}{\text{Number of live offspring on Day 1 after littering.}}$$

4) Lactation index=
$$\frac{\text{Number of live offspring at weaning x100}}{\text{Number of live offspring at Day 4 (after cull).}}$$

Net maternal bodyweight loss at parturition was defined as:
Maternal weight at Day 20 of gestation − (Maternal weight at Day 1 of lactation + litter weight)

RESULTS

Food consumption (data not shown).
There were no significant differences in food consumption between the five groups of animals at any stage of the study.

Maternal bodyweight (Figures 1 and 3; Table 2)
Group mean bodyweights at the start of gestation were similar in all groups, but bodyweight gain during gestation was greatest in females on VRF1 pelleted diet and least in females on RM1 pelleted diet. The weight gain of females on the three powered diets, LAD2, VRF1 and Teklad, were similar during gestation.

At parturition, females on the VRF1 diets showed low net bodyweight loss (approximately 10g per female), compared with LAD2 and Teklad at 34 – 40 g and RM1 at 57g

During lactation maternal weights were highest on the VRF1 diet, intermediate on LAD2 and Teklad and lowest on the RM1 diet.

Parturition parameters (Figures 2 and 3, Tables 2 and 3).
Low protein (non-breeder specification) RM1 diet was associated with three cases where the female had to be killed *in extremis* during the last two days of pregnancy and there were six further cases where the female was unable to support the litter in the first few days *post partum.* Gestation length was increased, with only about 30% of females in this group having a gestation length of 22 days (compared to approximately 60 – 80 %s of females on VRF1 diet) and there was an increased frequency of pregnancies lasting 23 or 23.5 days. Females receiving LAD 2 diet or Teklad diet also had longer gestation lengths than seen in with VRF1 diets and one female on LAD2 diet had to be killed during parturition.

Net maternal weight loss during the parturition period increased as gestation length increased; with the effect being most marked in the RM1 group.

Offspring bodyweight, litter size and survival (Figures 4 and 5, Table 2).
Group mean bodyweight of the offspring at Day 1 of age could be ranked in the sequence RM1<LAD2<VRF1 pelleted<Teklad<VRF1 powder, whilst litter size at birth ranked in the sequence RM1<LAD2=Teklad<VRF1 powder<VRF1 pellets. Although intergroup differences were relatively small, the combination of low bodyweight and low litter size confirms that RM1 diet was not suitable as a breeding diet for this strain of rat and that the best littering performance could be achieved on the VRF1 diets.

Bodyweight gain of the offspring was greatest in the high protein diets (Teklad and LAD2) with the intergroup differences becoming more marked after animals reached 14 days of age and started to consume the diet independently. Weight gain of offspring on the powdered VRF1 diet was marginally superior to that on the pelleted diet, whilst weight gain on RM1 diet was unsatisfactory, representing only approximately 66% of that achieved on the Teklad diet.

Post-implantation survival was slightly low for the RM1 group and offspring viablity to Day 4 of age was markedly low in litters receiving RM1 diet and slightly low in the LAD2 group. Where the whole litter died before Day 4 of age, the mother was found to show little evidence of milk production.

DISCUSSION

This study was undertaken to investigate problems with early post-partum litter survival which appeared to show overall reduction and much greater variability in the older females used in multigeneration studies within these laboratories following the introduction of the IGS rat. Findings which had also been seen within other laboratories in the United Kingdom, but had not been seen elsewhere using other diets. We had also noted that gestation length was marginally increased and that birth weight was low in affected litters [1]. The original diet used for these studies (LAD2) had been used for many years without problem prior to the redevelopment of the CD rat as the IGS rat.

Varying the diet type in these experiments demonstrated that even more extreme effects on maternal health status at end of pregnancy, gestation length and post-partum litter survival could occur when females where fed RM1 diet – a low (15%) protein diet designed for maintenance of animals rather than for breeding. In contrast, using the diet supplied by the animal breeder (VRF1) resulted in females which promoted higher birth weight and earlier parturition with better survival rates. All these are characteristics of a younger female rat.

A number of studies have examined the effect of diet on rat reproduction, and there is an extended review of dietary requirements in the Nutrient Requirements of Laboratory Animals [4], but none of the studies appear to have worked with a rat as sensi-

tive as the modern CD IGS rat with its very high reproductive capacity and a presumed highly specific dietary requirement.

An early study by Clapp [5] showed that protein levels of between 12.9% and 26.8% would all adequately support reproduction in the ICI Wistar rat: even when maternal bodyweight gain was restricted by 30%, although litter size and weaning weights were slightly low at protein levels of 15% and lower, there was no apparent effect on litter survival. Group mean litter sizes (maximum of 8 litters per group) varied between 9.2 and 13.6 at birth. The restriction of bodyweight gain during gestation for RM1 pelleted diet versus VRF1 pelleted diet is also approximately 30%, but the IGS CD rat appears less tolerant of dietary restriction than the rats commonly used in earlier papers and there was a high incidence of maternal/litter losses. The modern Sprague-Dawley rat has a far greater reproductive capacity, with an average litter size of approximately 14 in our laboratories, compared with data quoted in earlier work, and conclusions drawn on dietary requirements from early reports may no longer be valid.

Beck et al [6] compared a standard stock diet (22% protein) with a low protein diet (7.5% protein) when fed *ad libitum* to two groups of Wistar rats for 8 weeks. At the very low protein level, an 8% reduction in fecundity was reported but there was no significant effect on either litter size or the percentage of pups dead at birth. However, the postnatal mortality of the pups from dams on the low protein diet was higher than those on the normal diet (11.2% compared to 0.9%). Dams on the low protein diet lost 20% of their bodyweight during lactation whereas the bodyweight of dams on the normal diet was stable. Offspring bodyweight at birth from the low protein diet group was about 10% less than that from the normal diet group, rising to 50% less at weaning. In the current study, a greater range of postnatal pup mortality was seen at Day 4, from 40.5% on the RM1 diet to 1.4% on the VRF1 pelleted diet. The greatest differences in offspring bodyweight were less, however, being 12.5% at birth and 28.8% at Day 21 for RM1 diet versus the Teklad diet.

In a recent study assessing the Crj:CD(SD)IGS rat in Japan, Kato et al [7] examined the effect of an 18.4% protein diet (CR-LPF) as compared to a 23.1% protein diet (CRF-1) on reproductive function. They found no effects on the maintenance of pregnancy, parturition or lactation. Gestation length, number of implantations, viability indices, mortality and postnatal development were all unaffected by this small difference in dietary protein. They did, however, report increased food consumption and decreased bodyweight gain of females fed the lower protein diet and also a decreased bodyweight gain of their offspring. Our observations on the effect of reducing protein levels to 18 % are consistent with their published results but in the absence of information about the energy levels of the diets used, a detailed comparison of food consumption is not possible.

Rats provided with diet *ad lib* generally consume sufficient diet to satisfy their energy requirements but effective reproductive performance places a greater requirement on suitable protein levels in the diet [1]. On the basis of the recommendations of the National Research Council [4], all of the diets used would appear to have adequate protein levels, and examination of the quoted levels of essential amino acids, fats, vitamins and minerals in each of the diets used also closely approximate to, or exceed the nominal dietary requirements for breeding rats. The diets appear to be slightly deficient in energy level but it is difficult to define dietary energy levels in terms of what is actually available to the rat and data quoted in the NRC suggests that their figure were based on an expected intake of 15 g/day for growing rats and 15-20 g during gestation, whereas the normal figures for this strain of rat in our laboratories are 25 g and 30 g respectively.

CONCLUSION

Using a range of dietary protein concentrations from 21% down to 14.7%, we have extended the observations of Kato *et al* [7], and have found marked deleterious effects, both upon all aspects of female reproductive performance and upon offspring mortality and development, at the lowest protein level. We have also found subtle, but clear, differences between the other three protein levels with respect to all measured parameters, with the best maternal performance and offspring viability on a 18% protein powdered diet.

The 15% protein diet resulted in lower weight gain during maturation and pregnancy, especially in the last week of gestation, an increase in gestation length and a reduction in offspring birth weight, litter size and viability. Especially notable was the increase in parturition failure, the increased bodyweight loss at parturition and the increased incidence of litter loss by Day 4 of age. Whilst the correlation of low birth weight and reduced viability in the rat is not a novel observation, these findings are similar to our worst previous experience with the IGS rat and the LAD2 diet. This might predict that an increased reproductive success required an increased protein concentration over that in LAD2. However, the results presented here clearly indicate that the best reproductive performance was at a protein concentration lower than that of LAD2, so either other dietary factors are involved or the optimum protein concentration in a breeder diet for the IGS rat is about 18%.

For each animal, the net bodyweight loss at parturition was calculated as the difference between the Day 20 of gestation bodyweight and the Day 1 of lactation bodyweight plus the total litter weight. This is not a precise measure but we believe it to be useful to assess reproductive fitness, or response to the stress of parturition, and it clearly increases with increasing gestation length. By using this measure, together with the increased offspring birth weight, litter size and viability we conclude that the VRF1 diet is superior to the LAD2 diet we have previously used.

The Charles River UK IGS rat appears to be less tolerant of different dietary formulas than its predecessor. Standard LAD 2 diet which used to adequately support reproduction in the pre-IGS rat has now been found to be less satisfactory in the rederived animals. The full breeding potential of the IGS rat can, however, be restored by maintaining dietary continuity with that used by the animal supplier. As yet the critical components in the diet have not been identified and we have adopted the pragmatic approach to achieve optimum breeding performance in which the female progresses smoothly through the later stages of pregnancy to deliver a healthy litter of well-grown pups after a short gestation period and to rear the pups to maturity.

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Table 2. Parturition Data

Treatment		Gestation length (days)	Dam weight (g) Day 20 of gestation	Dam weight (g) Day 1 of lactation	Loss at birth (g) A	Litter weight (g) Day 1	Net Dam weight loss (g) at parturition B	Litter size Day 1	Mean pup weight (g) Day 1
RM1	Mean	22.6	429.2	301.3	131.2	72.7	56.9	12.9	5.7
Pelleted	SD	0.5	37.9	26.1	18.1	16.8	21.6	2.5	1.0
	Number	17	17	16	16	17	16	17	17
VRF1	Mean	22.1	475.9	367.6	108.3	95.6	12.7	15.4	6.2
Pelleted	SD	0.3	38	34.7	14.5	14.9	8.8	1.9	0.4
	Number	19	19	19	19	19	19	19	19
LAD2	Mean	22.6	449.7	327.3	120.8	81.0	39.8	13.9	5.9
Powdered	SD	0.4	37.6	44.2	26.2	12.7	23.9	2.3	0.7
	Number	24	24	23	23	23	23	23	23
VRF1	Mean	22.3	447.3	350.3	98.3	92.4	7.2	14.3	6.5
Powdered	SD	0.4	30.8	24.8	16.6	14.2	9.3	2.4	0.7
	Number	19	19	20	19	20	19	20	20
Teklad	Mean	22.5	445.7	327.4	121.2	88.3	33.5	13.9	6.4
Powdered	SD	0.4	35.1	35.6	18.9	21.2	26.3	3.6	0.5
	Number	21	21	22	21	22	21	22	22

Key: A=dam weight at Day 20 of gestation-dam weight at Day 1 of lactation. B=loss at birth-litter weight

Table 3. Reproductive failures

Treatment	Animal Number	Fate	Gestation bodyweight (g)			Gestation Length (days)	Mean pup Weight (g)	Litter size	Bodyweight post partum (g)			Necropsy	
			Day 0	Day 20	Day 0-20				Day1	Loss at birth	Net Dam loss	Day	Finding
RM1	51	KIE	321	387	66							Day 21 pc	PFP
Pelleted	52	KIE	272	350	78							Day 21 pc	PFP
	47	KIE	266	352	86							Day 22 pc	PFP
	45	TLL	306	419	113	22.5	5	12	294	125	65.7	Day 2 pp	MPI
	50	TLL	281	381	100	23	5.2	14	250	131	58	Day 2 pp	MPI
	57	TLL	289	376	87	22.5	4.2	11				Day 2 pp	MPI
	58	TLL	348	504	156	23	5	11	340	164	108.3	Day 2 pp	MPI
	62	TLL	291	396	105	23	5.2	8	270	126	84.8	Day 2 pp	MPI
	43	TLL	360	496	136	23	6	16	339	157	62	Day 3 pp	MPI
VRF1 Pelleted	80	TLL	307	474	167	22	6.3	14	377	97	10.9	Day 2 pp	MPI
LAD2	1081	KIE	339	485	146	23.5						Day 23.5 pp	PFP
Powdered	1085	TLL	296	457	161	23	4.9	12	291	166	107.7	Day 2 pp	MPI
VRF1 powdered	1112	HK	272	331	59							Day 20 pc	NL
Teklad powdered	1137	TLL	292	460	168	23.5	6	2	311	149	137.1	Day 2 pp	MPI

Key: HK=Humane Kill, KIE=Killed *in extremis*, TLL=Total litter loss, pc=*post coitum*, pp=*post partum.*
PFP=Pregnant, failure of parturition, MPI=Mammary tissue pale and inactive. NL: No live fetuses at Day 20 pc.

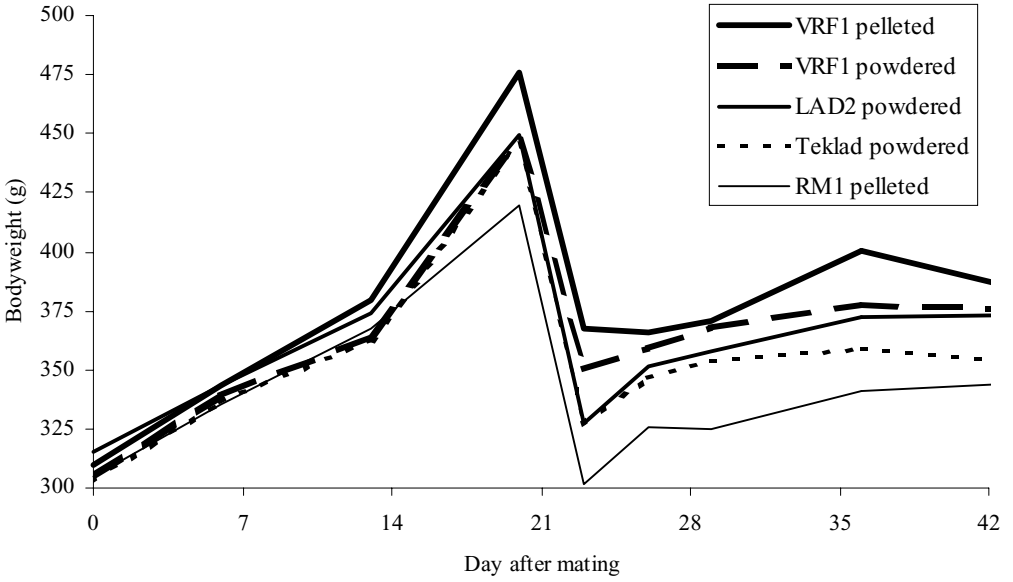

Figure 1. Maternal bodyweight changes (g)

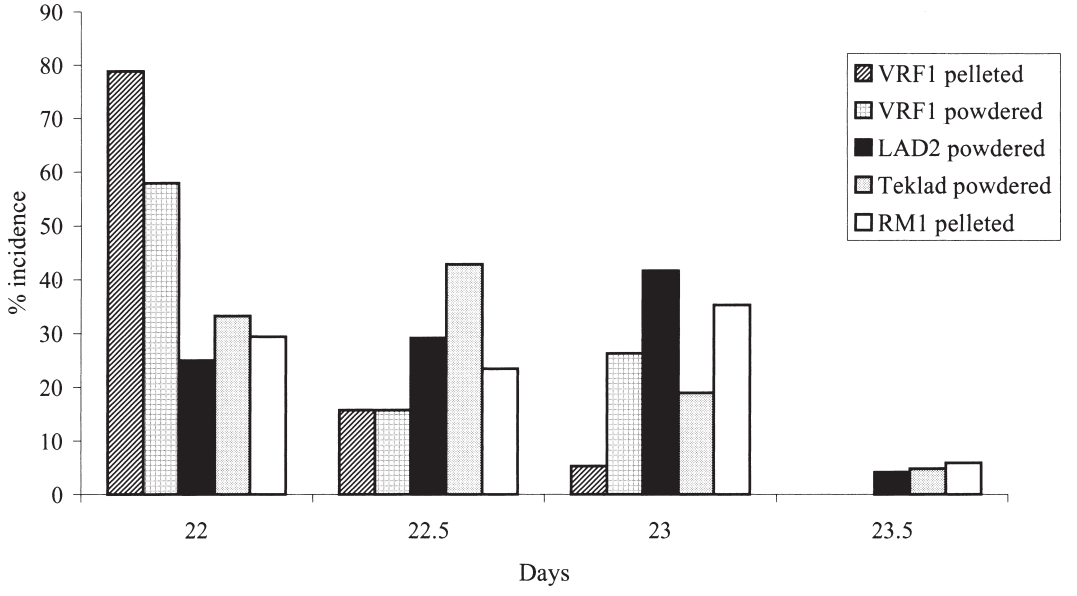

Figure 2. Gestation length (days)

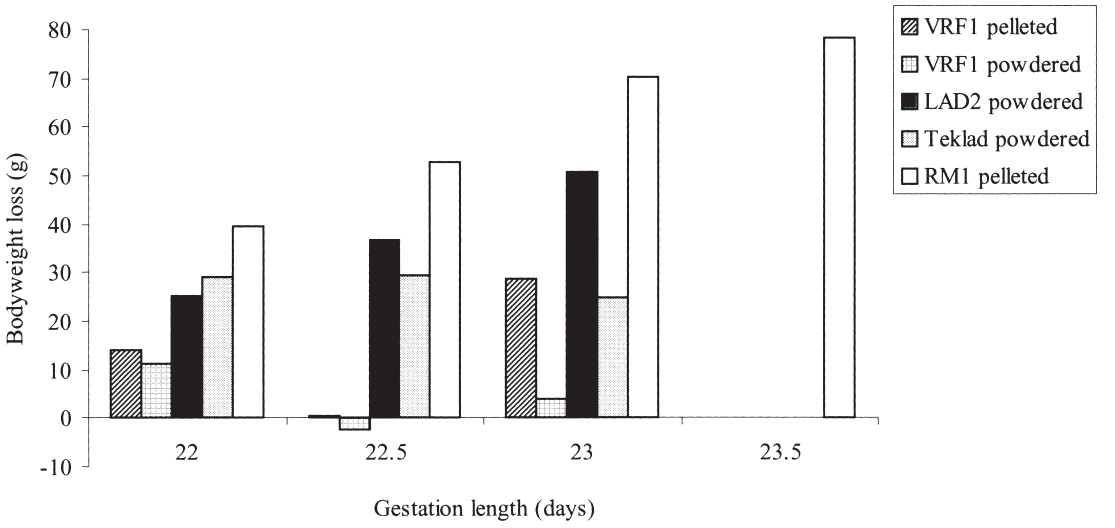

Figure 3. Maternal bodyweight loss (g) at parturition, by gestation length

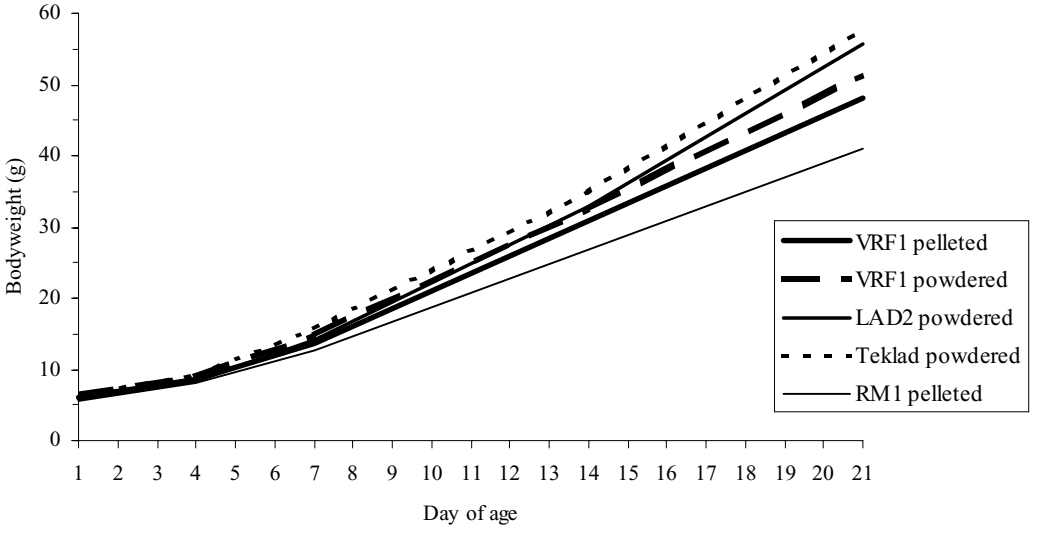

Figure 4. Offspring bodyweight changes (g)

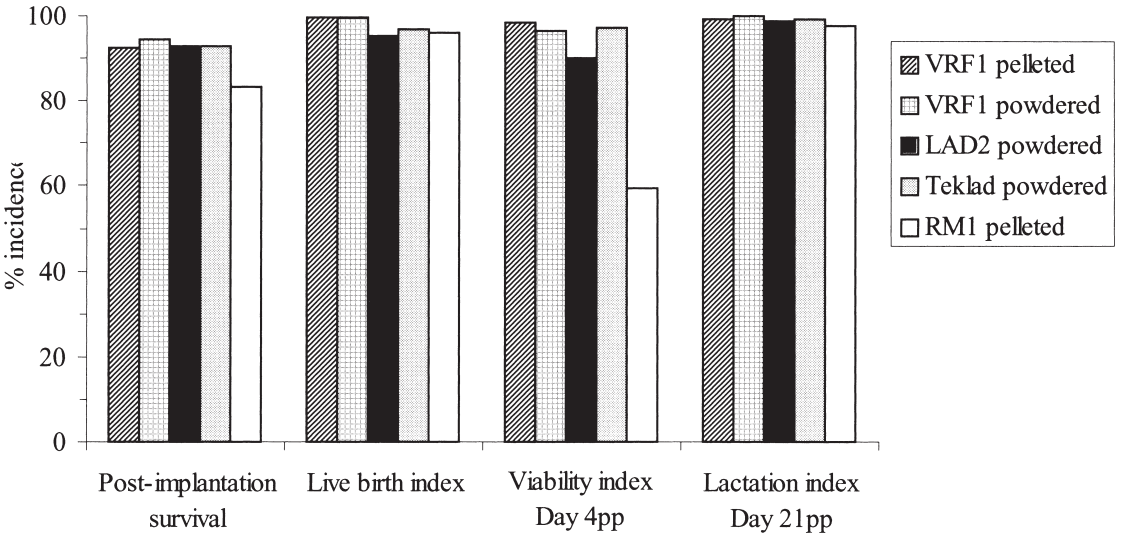

Figure 5. Offspring survival indices

Changes with Sexual Maturation of the Sperm Analytical Parameters in Crj:CD(SD)IGS rats.

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ABSTRACT. Sperm analysis was conducted in rats to assess quantitative and qualitative changes with sexual maturation of sperm. Sperm counts and motility parameters in the caudal epididymis of International Genetic Standard (IGS) rats or SD rats were analyzed using a CASA system (HTM-IVOS Motility Analyzer) at 7, 11, 15 and 26 weeks of age. The sperm motility parameters included the percentage of motile sperm (motile sperm), the percentage of progressively motile sperm (progressive sperm), average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN). A dramatic increase with age was observed in the sperm count in IGS rats at 11 weeks of age, and thereafter the sperm counts remained at a steady level up to 26 weeks of age. The pattern of maturation was nearly identical in SD rats. The age-related changes in sperm motility parameters were similar to that in sperm count in both IGS and SD rats. From these results, it is considered that sperm count and sperm motility parameters reach steady levels at 11 weeks of age and that no biological significant differences exist in the parameters between SD and IGS rats. － Key words: Crj:CD(SD)IGS rat, Reproduction, Fertility, Male rats, Sperm analysis, HTM-IVOS,CASA

CD(SD)IGS-2000: 202-207

INTRODUCTION

Sperm analysis is an essential approach for the evaluation of adverse effects to male rat fertility. Recently, a computer-assisted sperm analysis (CASA) system which can provide multiple parameters of sperm motion and can improve the objectivity of evaluation of sperm motion has been increasingly used. Several reports have described the methods of sample preparation and analysis for assessing sperm motility in adult rats using a CASA system [1-6], however, few studies was involved developing animals. In the present study, sperm motility and count were examined by CASA system at 7, 11, 15 or 26 weeks of age. SD rats were also examined as a control.

MATERIALS AND METHODS

IGS rats and SD rats were obtained form Charles River Japan, Inc. (Kanagawa, Japan) and Charles River Japan, Inc. (Shiga, Japan), respectively. Animal room temperature and relative humidity were set at 23±2℃ and 55± 10%, respectively. Lighting was controlled to give a light (8 a.m. to 9 p.m.) and dark cycle. The animals were housed individually in suspended stainless steel wire cages except for the mating period, during which one male and one female were placed together. Animals had free access to tap water and to a pelleted commercial laboratory and animal chow (CRF-1, Oriental Yeast Co., Ltd. Japan). Thirty pairs of ten-week old rats of each strain were mated. The mated female rats were allowed to deliver. Following weaning, all male offspring were selected and were reared to 7, 11, 15 or 26 weeks of age, during which time sperm analysis was conducted 9 or 10 male offspring in each. At each phase, male rats were euthanized by exsanguination under ether anesthesia. The testes (right, left), epididymides (right, left) and cauda epididymides (right, left) were removed and weighed. The right cauda epididymis was used for sperm motion analysis. The cauda epididymis was held with forceps and three stabs were made with scissors. Then it was incubated in Medium 199 with Hank's salts and L-glutamine (Gibco, Grand Island, NY), containing bovine serum albumin. The sperm suspensions were incubated for 5 to 30 minutes at 37℃. After incubation, the sperm suspensions were analyzed by HTM-IVOS Motility Analyzer (Hamilton Thorne Research, Beverly, MA) using a cannula (100 μm depth, 2 mm width: Vitro Com Inc., Mt Lakes, NJ). The percentage of motile sperm (% motile sperm), the percentage of progressively motile sperm (% progressive sperm), average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) were determined by HTM-IVOS. The left cauda epididymis was homogenized and placed into a stain reaction vial (Supra Vital IDENT Stain Kit, Hamilton Thorne Research, Beverly, MA) and then sperm were counted using the HTM-IVOS. Setup values for motility analysis in this study are shown in Table 1. Student's t-tests were carried out; with the limit for significance defined as p=0.05 or 0.01

RESULTS AND DISCCUSSION

No significant difference between IGS and SD rats in the weighs of reproductive organs was seen at any ages (Table 2). The results of the sperm count are summarized in Figure 1 and Table 3. Sperm count in the IGS rats at 7 weeks of age was 334.0×10^6/g. However, a dramatic increase with age was observed in the sperm count at 11 weeks of age, and thereafter the sperm counts were maintained at a steady level up to 26 weeks of age. The pattern of maturation was nearly identical in SD rats.

Sperm motility parameters are shown in Figure 2 and Table 3. The age-related changes in the percentage of motile sperm, the percentage of progressively motile sperm, VAP, VSL, VCL and ALH values were similar to that in the sperm count. These motility parameters were considered to be related to the maturation of the gonadal organs in male rats. On the other hand, BCF, STR and LIN values showed no clear changes with aging, suggesting the parameters showed no age-dependent changes. In comparison to the motility parameters of SD rats, the percentages of motile sperm and progressively motile sperm were slightly but significantly lower in IGS rats at the ages of 11 and 15 weeks. Essentially no biological significance was considered, however, be-

cause no significant difference in the parameters between SD rats and IGS rats was observed at the age of 26 weeks

From the results, it is considered that sperm count and sperm motility parameters reach steady levels from 11 weeks of age and that no differences exist in the parameters between SD and IGS rats.

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Table 1. Set up values for motile analysis

FRAMES ACQUIRED	30
DATA POINTS	16 to 30
MINIMUM CONTRAST	86
MINIMUM CELL SIZE	2
STRAIGHTNESS THRESHOLD	50.0
MEDIUM VAP CUTOFF	70.0
LOW VAP CUTOFF	40.0
LOW VSL CUTOFF	14.0
NON-MOTILE HEAD SIZE	10
NON-MOTILE HEAD INTENSITY	90
NON-MOTILE MINIMUM CONTRAST	20
STATIC HEAD SIZE	0.25 to 2.80
STATIC HEAD INTENSITY	0.35 to 1.00
STATIC ERONGATION	1 to 35
SLOW CELL MOTILE	NO

VAP: Path velocity
VSL: Straight line velocity

Table 2. Reproductive organ weights

Strain		Crj:CD(SD)IGS	Crj:CD(SD)
Age (weeks)		7	7
No.of Male		10	10
Organ weights (g)			
Testis	Right	1.460±0.10	1.460±0.08
	Left	1.430±0.10	1.461±0.10
Epididymis	Right	0.255±0.03	0.251±0.22
	Left	0.251±0.03	0.239±0.03
Cauda epididymis	Right	0.078±0.01	0.071±0.01
	Left	0.077±0.01	0.070±0.01
Age (weeks)		11	11
No.of Male		9	10
Organ weights (g)			
Testis	Right	1.761±0.17	1.742±0.11
	Left	1.746±0.20	1.698±0.11
Epididymis	Right	0.555±0.05	0.565±0.05
	Left	0.528±0.06	0.539±0.06
Cauda epididymis	Right	0.227±0.03	0.229±0.02
	Left	0.227±0.03	0.218±0.03
Age (weeks)		15	15
No.of Male		10	10
Organ weights (g)			
Testis	Right	1.912±0.15	1.801±0.13
	Left	1.862±0.13	1.781±0.11
Epididymis	Right	0.640±0.06	0.616±0.04
	Left	0.638±0.06	0.595±0.05
Cauda epididymis	Right	0.281±0.02	0.281±0.02
	Left	0.281±0.02	0.276±0.03
Age (weeks)		26	26
No.of Male		10	10
Organ weights (g)			
Testis	Right	1.980±0.31	1.937±0.21
	Left	2.014±0.46	1.920±0.18
Epididymis	Right	0.719±0.08	0.724±0.05
	Left	0.722±0.05	0.724±0.07
Cauda epididymis	Right	0.312±0.04	0.339±0.05
	Left	0.327±0.04	0.333±0.05

Parameters presented as the mean±S.D.

Table 3. Result of the epididymal sperm motion analysis

Strain	Crj:CD(SD)IGS	Crj:CD(SD)
Age (weeks)	7	7
No.of Male	10	10
Motility (%)	50.2±16.4	48.9±9.7
Progressive Motility (%)	34.0±14.0	29.4±9.9
VAP (μm/sec)	125.3±17.8	119.5±13.8
VSL (μm/sec)	77.7±12.2	70.5±12.7
VCL (μm/sec)	292.4±42.6	273.5±34.7
ALH (μm)	17.6±2.0	17.2±1.7
BCF (Hz)	30.6±3.3	32.7±3.5
STR (%)	61.3±4.3	58.7±7.6
LIN (%)	26.9±2.4	26.5±3.9
Count ($\times 10^6$ Sperm/gram)	334.0±128.1	320.6±111.8
Age (weeks)	11	11
No.of Male	9	10
Motility (%)	62.9±9.7	71.4±6.7 *
Progressive Motility (%)	52.7±8.6	62.1±6.2 *
VAP (μm/sec)	145.9±12.2	152.6±12.1
VSL (μm/sec)	101.8±8.3	110.6±12.1
VCL (μm/sec)	339.7±33.8	360.5±27.9
ALH (μm)	18.8±2.7	18.6±1.0
BCF (Hz)	27.9±2.6	28.9±2.5
STR (%)	69.9±5.9	67.8±13.2
LIN (%)	30.4±3.3	34.4±11.9
Count ($\times 10^6$ Sperm/gram)	1475.3±232.4	1419.5±213.7
Age (weeks)	15	15
No.of Male	10	10
Motility (%)	69.5±8.7	76.8±3.3 *
Progressive Motility (%)	59.4±7.4	64.4±4.4
VAP (μm/sec)	143.6±13.0	148.0±8.0
VSL (μm/sec)	102.2±10.6	100.4±7.3
VCL (μm/sec)	335.0±34.2	340.4±22.7
ALH (μm)	18.0±1.5	19.3±1.3
BCF (Hz)	29.1±2.0	26.7±2.1 *
STR (%)	71.1±2.5	68.1±3.2 *
LIN (%)	30.7±1.9	29.7±1.5
Count ($\times 10^6$ Sperm/gram)	1532.9±148.4	1567.6±165.9
Age (weeks)	26	26
No.of Male	10	10
Motility (%)	76.3±7.5	76.6±4.4
Progressive Motility (%)	59.0±7.9	59.6±4.0
VAP (μm/sec)	157.3±9.7	151.5±7.8
VSL (μm/sec)	103.0±11.1	100.0±5.6
VCL (μm/sec)	361.4±18.2	356.1±19.4
ALH (μm)	19.9±1.5	19.1±0.9
BCF (Hz)	29.6±2.0	30.7±1.3
STR (%)	65.7±5.4	66.5±2.7
LIN (%)	28.6±2.2	28.4±1.3
Count ($\times 10^6$ Sperm/gram)	1319.4±254.5	1429.2±144.3

Parameters presented as the mean±S.D. (N).
*: Significantly different between Crj:CD(SD) and Crj:CD(SD)IGS, $p<0.05$.

VAP: Path velocity
VSL: Straight line velocity
VCL: Curvilinear velocity
ALH: Amplitude of lateral head displacement
BCF: Beat cross frequency
STR: Straightness
LIN: Linearity

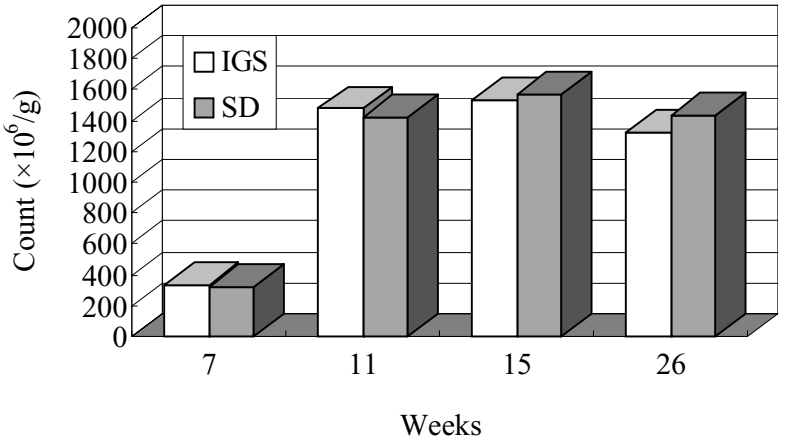

Figure 1. Sperm counts in the cauda epididymis in IGS and SD rats

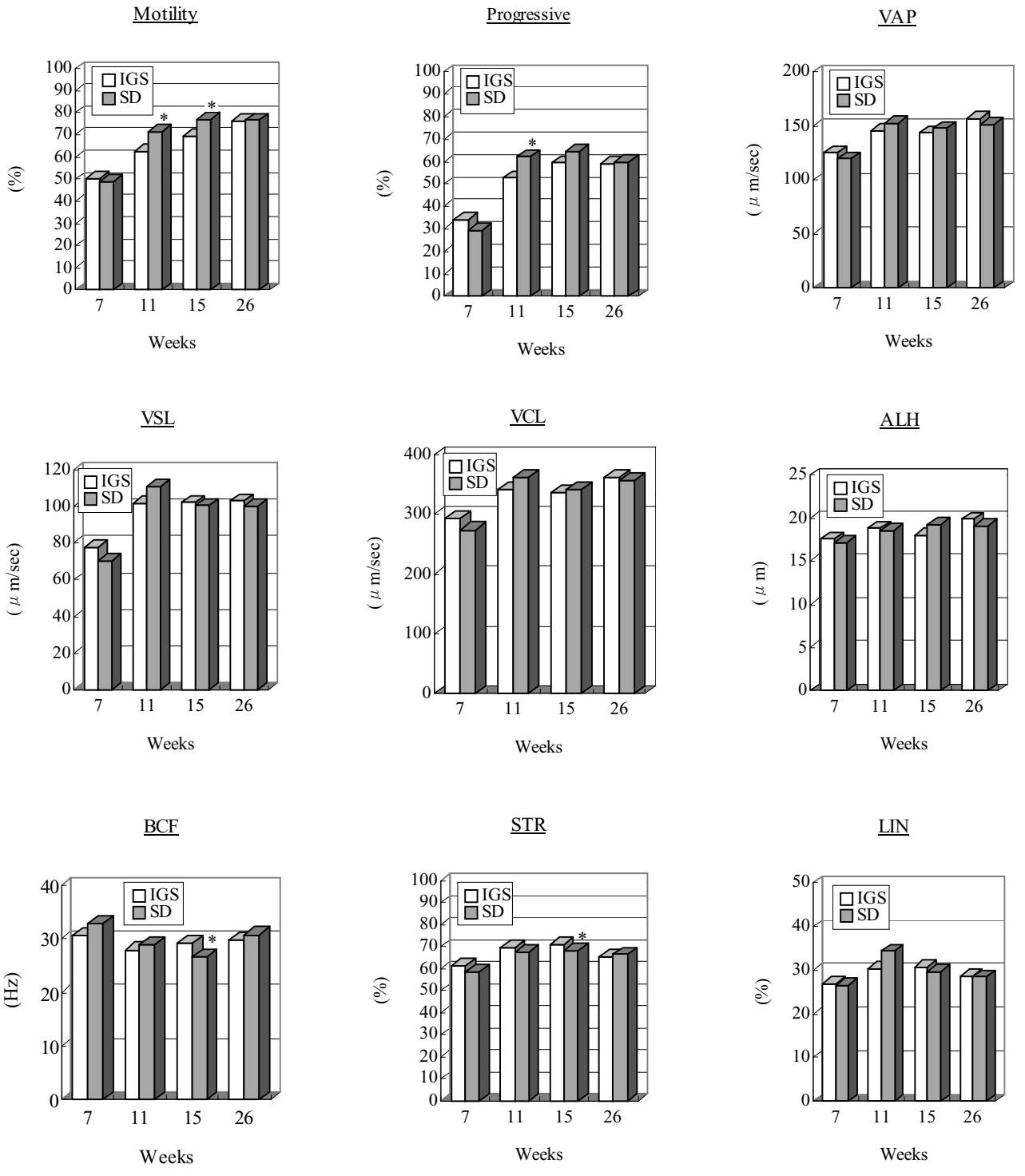

Figure 2. Sperm motility parameters in IGS and SD rats
*: Significantly different from SD rats ($p<0.05$)

VAP: Path velocity
VCL: Curvilinear velocity
BCF: Beat cross frequency
LIN: Linearity
VSL: Straight line velocity
ALH: Amplitude of lateral head displacement
STR: Straightness

CHAPTER 4

Carcinogenicity Related To

Histopathology Data in Crj:CD(SD)IGS Rats Fed Low Protein Commercial Diet CR-LPF

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ABSTRACT. To clarify the histopathological characteristics of Crj:CD(SD)IGS rats (IGS rats), 100 male and 100 female IGS rats were reared for 104 weeks fed low protein commercial diet (CR-LPF, protein content 18%) and were subjected to histopathological examination. The survival rate after a 104-week observation period was around 50% in both sexes. The major cause of deaths was anterior pituitary tumors in both sexes, which occurred for 31.9% of deaths in males and 84.3% of deaths in females. The most dominant tumor was anterior pituitary tumors in both sexes, and mammary gland tumor was the secondary dominant tumor in females. On the other hand, the incidence of leukemia was very low (less than 2%). The profile of tumors in the present study was essentially the same as that of CD and IGS rats reported by other investigators. —Key words: Crj:CD(SD)IGS rats, low protein diet, histopathology data, 104-week observation period

CD(SD)IGS-2000: 209-220

INTRODUCTION

Crj:CD(SD)IGS rats have been produced by Charles River Inc., and are originally derived from Crj:CD(SD) rats. Prior to introducing this strain into carcinogenicity studies, it is necessary to know their histopathological nature. One hundred (100) animals of each sex of Crj:CD(SD)IGS rats were reared for 104 weeks and were examined histopathologically. The mortality, body weights and food consumption were reported in the previous paper [1]. In the present paper, tumor and non-tumor lesions are described. In addition, in order to understand more easily the biological nature of IGS rats, comparison with F344 rats, which are frequently used in carcinogenicity studies, was made.

MATERIALS AND METHODS

Animals and Husbandry: Fifty (50) male and female Crj:CD(SD)IGS rats, at 4 weeks of age, were obtained twice on different dates (Lot 1: delivered on October 2, 1996, Lot 2: delivered on October 9, 1996) from Charles River Japan Inc. (Hino Breeding Center, Japan). In total, 100 males and 100 females were used in the present study. For each lot, the animals were acclimatized for 2 weeks and healthy animals were used at 6 weeks of age. The animals were housed individually in hanging stainless-steel wire mesh cages in an animal room under the following conditions: temperature at 23 ± 3 ℃, relative humidity at 50 ± 20%, air ventilation at 10 to 15 times per hour and 12-hour illumination (07:00 to 19:00). The animals were supplied commercial low protein feed (approximately 18% crude protein), CR-LPF (Oriental Yeast Co., Ltd., Japan), and tap water *ad libitum*.

Histopathology: The animals were sacrificed at the termination of a 104-week observation period. The animals found dead or sacrificed moribund were necropsied soon after discovery. After necropsy, all organs and tissues of all animals were dissected and fixed in phosphate buffered 10 vol% formalin. The eyeballs, Harderian glands and optic nerves were fixed with phosphate buffered fixative containing 3 w/v% glutaraldehyde and 2.5 vol% formalin, and then preserved in phosphate buffered 10 vol% formalin. All organs/tissues (approximately 50 per animal) were embedded in paraffin, stained with hematoxylin and eosin (H.E.) by a routine procedure and examined histopathologically.

In-house data on F344 rats: The data from the control animals in the carcinogenicity studied carried out in our laboratory was used. F344 rats were housed individually in hanging stainless-steel wire mesh cages and fed CRF-1 (approximately 23% crude protein, Oriental Yeast Co., Ltd., Japan) *ad libitum*.

RESULTS AND DISCUSSION

Cause of death: During the observation period, deaths (including the animals that were sacrificed moribund) occurred in 21/50 males in Lot 1 and 25/50 males in Lot 2 and in 25/50 females in Lot 1 and 26/50 females in Lot 2. Therefore, combined survival rate of the 2 lots was 54% in males and 49% in females. The cause of death was assigned based on the clinical signs and gross/histopathology. The cause of death in the present study is shown in Table 1. In the animals that died, 68.0% of males and 98.0% of females died from tumor. The most dominant cause of death was pituitary tumor in both sexes. The proportion of deaths of pituitary tumor was 31.9% for males and that for females was 84.3%, and was conspicuously high in females. All other tumor deaths were very low, not more than 5%, except leukemia in males (8.5%). Therefore, it was estimated that majority of deaths in IGS rats would be pituitary tumor. In the case of F344 rats, most dominant cause of death was leukemia (28% for males and 36% for females) and the second most dominant cause of death was pituitary tumor (21% for males and 36% for females).

Tumors: The tumors observed in the present study are shown in Table 2. Eighty-four (84)% of males and 98% of females had tumors in IGS rats. In case of F344 rats, 99.7% of males and 84.2% of females had tumors in our historical control data. The number of tumor-bearers in males was higher in F344 rats than in IGS rats. The high number of tumor-bearers in male F344 rats was attributable to an extremely high incidence of Leydig cell tumor, which has occurred in 95% of F344 male rats in our historical control data. On the other hand, the number of tumor-bearers in females was higher in IGS rats than in F344 rats. A high number of tumor-bearers in female IGS rats was attributable to an extremely high incidence of pituitary tumor and mammary tumor as described below. The tumors observed at relatively high incidence (more than 10%) in IGS rats are shown in the following.

Table 1. Cause of death in IGS rats fed CR-LPF.

	Sex	Male	Female
Cause of demise	No. of deaths	47[a)]	51
Tumor			
Pituitary tumor		15(31.9)[b)]	43(84.3)
Leukemia		4(8.5)	1(1.9)
Histiocytic sarcoma		2(4.2)	2(3.9)
Liposarcoma		2(4.2)	0(0.0)
Zymbal gland tumor		2(4.2)	0(0.0)
Malignant mesothelioma		2(4.2)	0(0.0)
Mammary gland tumor		0(0.0)	2(2.0)
Islet cell carcinoma		2(4.2)	0(0.0)
Pheochromocytoma		1(2.1)	0(0.0)
Keratoacanthoma, oral cavity		1(2.1)	0(0.0)
Hemangiosarcoma		1(2.1)	0(0.0)
Fibrosarcoma		1(2.1)	0(0.0)
Thymic lymphoma		0(0.0)	1(1.9)
Squamous cell carcinoma, stomach		0(0.0)	1(1.9)
Total deaths of tumor		32(68.0)	50(98.0)
Non-tumor			
Hemorrhage, trauma		2(4.2)	0(0.0)
Urination disturbance		2(4.2)	0(0.0)
Subcutaneous hematoma		1(2.1)	0(0.0)
Chronic nephropathy		1(2.1)	0(0.0)
Cyst, abdominal cavity		1(2.1)	0(0.0)
Circulatory disturbance		3(6.3)	1(1.9)
Total deaths of non-tumor lesions		10(21.3)	1(1.9)
Unclear		4(8.5)	0(0.0)

a) : One male that died during the necropsy period was excluded from calculation of the survival rate.
b) : Number in parenthesis indicates the percentage (%).

Table 2-1. Tumors in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Abdominal cavity							
Number examined		50	50	50	50	100	100
LIPOSARCOMA		0	1	0	0	1	0
MESOTHELIOMA, MALIGNANT		3	0	0	0	3	0
Adrenal							
Number examined		50	49	49	50	99	99
ADENOMA, CORTICAL CELL		0	2	1	1	2	2
PHEOCHROMOCYTOMA		5	9	0	1	14	1
CARCINOMA, CORTICAL CELL		1	0	0	0	1	0
Brain							
Number examined		50	50	50	50	100	100
GRANULAR CELL TUMOR		1	0	0	0	1	0
OLIGODENDROGLIOMA		1	0	0	0	1	0
Ear							
Number examined		50	50	50	50	100	100
NEURAL CREST TUMOR		0	1	0	0	1	0
Hemolymphoreticular							
Number examined		50	50	50	50	100	100
SARCOMA, HISTIOCYTIC		2	1	1	1	3	2
LEUKEMIA, GRANULOCYTIC		1	1	0	0	2	0
LEUKEMIA, LYMPHOCYTIC		1	1	1	0	2	1
Small intestine, jejunum							
Number examined		48	48	47	46	96	93
LEIOMYOMA		1	0	0	1	1	1
ADENOCARCINOMA		1	0	0	0	1	0
Kidney							
Number examined		48	48	47	48	96	95
LIPOMA		1	0	0	0	1	0
LIPOSARCOMA		1	0	0	0	1	0
Liver							
Number examined		50	50	50	49	100	99
ADENOMA, HEPATOCELLULAR		1	1	0	0	2	0
CYSTADENOMA		0	0	3	3	0	6
Mammary gland							
Number examined		50	50	49	49	100	98
ADENOMA, ACINAR CELL		0	0	3	0	0	3
FIBROADENOMA		0	0	21	18	0	39
Cholangioma? or Adenama, cholangiocellalar		0	0	13	16	0	29
Nasal cavity							
Number examined		50	50	50	50	100	100
SCHWANNOMA, MALIGNANT		0	1	0	0	1	0
Oral cavity							
Number examined		1	0	1	0	1	1
KERATOACANTHOMA		1	0	0	0	1	0
CARCINOMA, SQUAMOUS CELL		0	0	1	0	0	1
Ovary							
Number examined		-	-	50	49	-	99
GRANULOSA-THECA CELL TUMOR, BENIGN		-	-	1	0	-	1
CYSTADENOCARCINOMA		-	-	0	1	-	1

Table 2-2. Tumors in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Pancreas							
Number examined		49	49	48	50	98	98
ADENOMA, ACINAR CELL		1	3	0	0	4	0
ADENOMA, ISLET CELL		8	5	1	3	13	4
CARCINOMA, ACINAR CELL		0	1	0	0	1	0
CARCINOMA, ISLET CELL		4	7	0	2	11	2
Pituitary							
Number examined		47	49	50	49	96	99
ADENOMA, ANTERIOR		25	23	30	34	48	64
CARCINOMA, ANTERIOR		1	2	11	8	3	19
Prostate							
Number examined		50	49	-	-	99	-
ADENOMA, ACINAR CELL		0	1	-	-	1	-
Skin/subcutis							
Number examined		50	50	49	49	100	98
ADENOMA, SEBACEOUS		1	1	0	0	2	0
FIBROMA		1	0	1	1	1	2
KERATOACANTHOMA		1	3	0	0	4	0
LIPOMA		1	0	1	0	1	1
HEMANGIOSARCOMA		1	0	0	0	1	0
LIPOSARCOMA		0	1	0	0	1	0
Stomach							
Number examined		50	49	49	50	99	99
CARCINOMA, SQUAMOUS CELL		1	0	0	0	1	0
TERATOMA, MALIGNANT		0	0	1	0	0	1
Testis							
Number examined		50	50	-	-	100	-
LEYDIG CELL TUMOR		3	0	-	-	3	-
Thymus							
Number examined		41	45	47	48	86	95
THYMIC LYMPHOMA		0	0	0	1	0	1
Tongue							
Number examined		50	50	50	49	100	99
GRANULAR CELL TUMOR		0	0	0	1	0	1
Thyroid							
Number examined		48	50	50	50	98	100
ADENOMA, C CELL		1	3	3	2	4	5
ADENOMA, FOLLICULAR CELL		1	1	1	0	2	1
CARCINOMA, FOLLICULAR CELL		0	0	1	0	0	1
Uterus							
Number examined		-	-	50	50	-	100
POLYP, ENDOMETRIAL STROMAL		-	-	1	9	-	10
ADENOCARCINOMA		-	-	0	1	-	1
SARCOMA, NOS		-	-	1	0	-	1
Zymbal gland							
Number examined		50	50	50	50	100	100
ADENOMA		1	0	0	0	1	0
CARCINOMA		2	1	0	0	3	0

Males
anterior pituitary tumors (adenoma: 50% and carcinoma: 3%),
islet cell tumor in pancreas (adenoma: 13% and carcinoma: 11%),
pheochromocytoma in adrenal gland (14%)
Females
anterior pituitary tumors (adenoma: 65% and carcinoma: 19%)
mammary gland tumors (fibroadenoma: 40% and adenocarcinoma: 30%)
endometrial stromal polyp in uterus (10%)

On the other hand, in F344 rats, the tumors observed at relatively high incidence in our historical control data are shown in the following.

Males
Leydig cell tumor in testis (95%)
anterior pituitary tumors (29%)
C cell tumor in thyroid (20%)
pheochromocytoma in adrenal gland (17%)
islet cell tumor in pancreas (12%)
leukemia (10%)
Females
anterior pituitary tumors (39%)
endometrial stromal polyp in uterus (29%)
leukemia (15%)
C cell adenoma in thyroid (12%)
mammary gland tumors (10%)

As shown above, anterior pituitary tumor was the most dominant tumor in male IGS rats, and the incidence was approximately 2 times that in F344 rats. On the contrary, Leydig cell tumor in the testis was the most dominant tumor in male F344 rats (only 3% in IGS rats). In females, the most dominant tumor was anterior pituitary tumor in both IGS and F344 rats; however, the incidence was much higher in IGS rats (more than 80%) than in F344 rats (39%). The second most dominant tumor in IGS female rats was mammary gland tumor and the incidence was nearly 7 times that in F344 rats. In addition, leukemia was observed at very low incidence in IGS rat (1 or 2 %) as compared to that in both sexes in F344 rats (around 10%).

Non-tumors: The non-tumor lesions observed in the present study are shown in Table 3. Major non-tumor lesions observed in IGS rats are shown in the following.

Males
adrenal : focal medullary hyperplasia (37%)
eye : mineralization of cornea (72%)
heart : myocardial fibrosis (92%)
liver : bile ductular proliferation (83%), altered cell focus (35%)
lung : mineralization of arterial wall (56%)
pituitary : focal hyperplasia in anterior lobe (22%)
prostate : prostatitis (64%)
parathyroid : focal hyperplasia (22%)
stomach : dilated gland (68%)
Females
adrenal : focal medullary hyperplasia (34%), peliosis (97%)
eye : corneal mineralization (72%)
heart : myocardial fibrosis (81%)
kidney : mineralization in papilla (85%)
liver : bile ductular proliferation (65%), altered cell focus (44%)
stomach : dilated gland (54%)

As shown above, in male IGS rats, bile ductular proliferation in the liver, myocardial fibrosis and corneal mineralization were the most dominant non-tumor lesions and they were observed in more than 70% of males. Major non-tumor lesions in male F344 rats were bile ductular proliferation (94%), altered cell focus (82%), chronic progressive nephropathy (93%), myocardial fibrosis (62%) and retinal atrophy (62%). The incidence of altered cell focus in the liver was less in male IGS rats (35%) than in male F344 rats (82%). The incidence of chronic progressive nephropathy in male IGS rats was less than 40%, and was half that in F344 rats, and this was beyond our expectations. In female IGS rats, peliosis adrenalis, corneal mineralization, myocardial fibrosis, mineralization in the renal papilla and bile ductular proliferation in the liver were the most dominant non-tumor lesions and were observed in more than 60% of females. Major non-tumor lesions in female F344 rats were retinal atrophy (70%) and chronic progressive nephropathy (67%). Similar to males, chronic progressive nephropathy was observed less frequently in female IGS rats (14%) than in F344 rats (67%) and retinal atrophy was also observed less frequently in female IGS rats (5%) than in female F344 rats (70%). On the other hand, adenosis in the mammary gland was observed more frequently in female IGS rats (approximately 30%) than in F344 rats (less than 10%), and was thought to be associated with a high incidence of mammary tumors in female IGS rats. The characteristics noted in the present study, that is, extremely high incidence of anterior pituitary tumor and mammary gland tumor and relatively low incidence of leukemia, were common to those of Sprague-Dawley rats [2, 3]. In addition, the profile of the tumors in the present study was essentially the same as other investigations on CD rats and IGS rats [4, 5].

The survival rate after a 104-week observation period was around 50% in both sexes when low protein feed was supplied. Therefore, the number of carcinogenicity studies using IGS rats might be increased in future. However, it should be mentioned that we do not have ample back ground data on this strain, including differences from lot to lot, and therefore, it is needed to accumulate more data to clarify the biological nature of aged IGS rats.

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Table 3-1. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Abdominal cavity							
Number examined		50	50	50	50	100	100
Cyst		0	1	0	0	1	0
Adrenal							
Number examined		50	49	49	50	99	99
Tumor infiltration/metastasis		2	2	2	0	4	2
Hyperplasia, medullary, focal		11	26	14	20	37	34
Peliosis adrenalis		13	11	47	49	24	96
Extramedullary hematopoiesis		1	0	3	0	1	3
Altered cell focus		7	11	1	6	18	7
Aorta							
Number examined		50	50	50	48	100	98
Mineralization		1	1	0	1	2	1
Brain							
Number examined		50	50	50	50	100	100
Tumor infiltration/metastasis		1	2	7	1	3	8
Dilatation, ventricular		3	5	15	15	8	30
Hemorrhage, focal		1	0	0	0	1	0
Coagulating gland							
Number examined		50	50	-	-	100	-
Tumor infiltration/metastasis		2	0	-	-	2	-
Cell infiltration, inflammatory		0	1	-	-	1	-
Ear							
Number examined		50	50	50	50	100	100
Granuloma		0	0	0	1	0	1
Epididymis							
Number examined		50	50	-	-	100	-
Tumor infiltration/metastasis		1	2	-	-	3	-
Atrophy		1	1	-	-	2	-
Granuloma, spermatic		1	0	-	-	1	-
Cell debris, duct		4	8	-	-	12	-
Decreased sperm, duct		2	3	-	-	5	-
Esophagus							
Number examined		50	48	49	49	98	98
Tumor infiltration/metastasis		1	0	0	0	1	0
Fibrosis		0	1	0	0	1	0
Extraorbital lacrimal gland							
Number examined		49	50	50	49	99	99
Tumor infiltration/metastasis		1	1	0	0	2	0
Atrophy, focal		0	1	1	0	1	1
Ectopic Harderian gland		17	29	0	0	46	0
Eye							
Number examined		50	50	50	50	100	100
Tumor infiltration/metastasis		1	1	0	0	2	0
Atrophy, retinal		7	10	13	14	17	27
Mineralization, corneal		37	35	36	36	72	72
Phthisis bulbi		0	0	0	1	0	1
Keratitis		4	3	2	3	7	5
Mineralization, conjunctiva		2	2	4	1	4	5
Hemorrhage, vitreous body		1	0	0	0	1	0
Iritis		1	2	1	2	3	3
Cell infiltration, lens		1	0	0	0	1	0
Degeneration, lens		4	4	1	1	8	2

Table 3-2. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Femur + marrow							
Number examined		46	48	49	50	94	99
Tumor infiltration/metastasis		1	2	2	1	3	3
Fat infiltration		2	0	0	1	2	1
Osteosclerosis		0	2	1	0	2	1
Hematopoiesis, increased		9	6	13	6	15	19
Granulopoiesis, increased		2	2	3	0	4	3
Megakaryocytosis		0	0	1	0	0	1
Harderian gland							
Number examined		50	50	49	50	100	99
Tumor infiltration/metastasis		1	1	0	0	2	0
Cell infiltration		17	14	16	22	31	38
Hyperpigmentation		42	43	43	42	85	85
Regeneration, acinar cell		0	0	0	1	0	1
Hyperplasia, acinar cell, focal		14	4	0	3	18	3
Heart							
Number examined		50	50	50	48	100	98
Tumor infiltration/metastasis		1	1	0	1	2	1
Mineralization, myocardial		1	0	2	1	1	3
Cell infiltration		2	0	0	0	2	0
Fibrosis, myocardial		47	45	39	40	92	79
Myocarditis		5	6	4	6	11	10
Fibrosis, epicardial		0	0	0	1	0	1
Kidney							
Number examined		48	48	47	48	96	95
Tumor infiltration/metastasis		1	1	0	0	2	0
Cyst		0	3	1	1	3	2
Dilatation, tubular		5	2	5	3	7	8
Hydronephrosis		3	3	5	0	6	5
Vacuolation, tubular epithelial		1	2	1	0	3	1
Necrosis, tubular epithelial		1	0	7	5	1	12
Necrosis, papillary		1	0	0	0	1	0
Regeneration, tubular cell		13	6	11	8	19	19
Chronic progressive nephropathy		24	14	7	6	38	13
Hyaline droplet, tubular cell		1	1	0	0	2	0
Pigmentation, tubular epithelial		0	1	0	0	1	0
Urinary cast, hyaline		3	1	3	5	4	8
Mineralization, papillary		0	1	0	2	1	2
Fibrosis		0	1	0	0	1	0
Pyelitis		19	12	9	19	31	28
Pyelonephritis		2	2	1	0	4	1
Abscess		3	0	0	0	3	0
Mineralization, pelvic		6	6	40	41	12	81
Hyperplasia, tubular, focal		4	2	3	2	6	5
Hyperplasia, transitional, focal		1	3	0	0	4	0
Metaplasia, squamous		1	0	0	0	1	0
Large intestine, cecum							
Number examined		45	48	45	46	93	91
Tumor infiltration/metastasis		1	1	0	0	2	0
Hemorrhage		1	0	0	0	1	0
Cell infiltration		7	4	3	1	11	4
Erosion		2	0	0	0	2	0
Arteritis		1	0	0	0	1	0
Fibrosis, lamina propria		0	2	2	1	2	3

Table 3-3. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Large intestine, colon							
Number examined		46	48	48	47	94	95
Tumor infiltration/metastasis		1	1	1	0	2	1
Cell infiltration		2	3	1	1	5	2
Granuloma		0	0	1	0	0	1
Fibrosis		0	0	1	0	0	1
Serositis		1	0	0	0	1	0
Large intestine, rectum							
Number examined		47	48	48	47	95	95
Tumor infiltration/metastasis		2	0	1	0	2	1
Cell infiltration		0	0	1	0	0	1
Arteritis		0	1	0	0	1	0
Limb							
Number examined		9	10	2	2	19	4
Arthritis		1	0	0	1	1	1
Callosity		8	9	0	1	17	1
Hyperplasia, osteal		0	1	2	2	1	4
Liver							
Number examined		50	50	50	49	100	99
Tumor infiltration/metastasis		4	3	2	0	7	2
Hepatodiaphragmatic nodule		0	1	0	0	1	0
Cyst		1	0	0	0	1	0
Cyst, biliary		0	1	0	0	1	0
Vacuolation, hepatocyte, central		0	3	0	1	3	1
Vacuolation, hepatocyte, periportal		5	5	5	7	10	12
Necrosis, focal		7	1	1	1	8	2
Necrosis, centrilobular		0	0	1	1	0	2
Necrosis, massive		0	1	0	0	1	0
Hematopoiesis, increased		1	0	8	0	1	8
Spongiosis hepatis		4	8	1	0	12	1
Proliferation, bile ductular		39	44	36	28	83	64
Peliosis hepatis		2	1	10	7	3	17
Vacuolation, hepatocyte, focal		7	4	2	4	11	6
Altered cell focus		17	18	22	22	35	44
Lung							
Number examined		50	50	49	50	100	99
Tumor infiltration/metastasis		6	3	1	2	9	3
Mineralization, arterial wall		18	38	20	13	56	33
Congestion		2	5	6	4	7	10
Hemorrhage, focal		2	3	0	0	5	0
Appearance, foamy histiocytic		13	19	17	17	32	34
Edema		2	0	2	1	2	3
Fibrosis, capsular		1	2	0	0	3	0
Pleuritis		1	0	0	0	1	0
Pneumonia		0	0	2	0	0	2
Multinucleated giant cell		0	1	1	1	1	2
Hyperplasia, bronchiolo-alveolar		2	0	0	0	2	0
Metaplasia, osseous		4	1	1	1	5	2
Lymph node, cervical							
Number examined		47	48	46	46	95	92
Tumor infiltration/metastasis		2	4	2	0	6	2
Lymph node, NOS							
Number examined		0	3	0	0	3	0
Tumor infiltration/metastasis		0	2	0	0	2	0
Hyperplasia, plasma cell		0	1	0	0	1	0

Table 3-4. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Lymph node, mesenteric							
Number examined		50	48	46	49	98	95
Tumor infiltration/metastasis		2	2	1	2	4	3
Pigment larden macrophage		27	41	31	31	68	62
Hyperplasia, lymphoid		0	0	2	0	0	2
Mammary gland							
Number examined		50	50	49	49	100	98
Tumor infiltration/metastasis		0	0	1	1	0	2
Galactocele		13	15	2	2	28	4
Adenosis		0	0	21	7	0	28
Hyperplasia, acinar cell, focal		0	0	0	2	0	2
Nasal cavity							
Number examined		50	50	50	50	100	100
Tumor infiltration/metastasis		1	1	1	0	2	1
Cell infiltration		10	13	6	12	23	18
Hyperplasia, respiratory, focal		0	1	0	0	1	0
Ovary							
Number examined		-	-	50	49	-	99
Tumor infiltration/metastasis		-	-	1	0	-	1
Cyst, ovarian		-	-	7	4	-	11
Cyst, ovarian bursa		-	-	8	9	-	17
Pancreas							
Number examined		49	49	48	50	98	98
Tumor infiltration/metastasis		0	1	1	0	1	1
Atrophy, acinar, focal		6	2	1	1	8	2
Hyperplasia, islet cell		4	5	1	0	9	1
Hyperplasia, acinar cell, focal		0	1	0	0	1	0
Hyperplasia, ductal, focal		0	0	1	0	0	1
Basophilic cell focus		0	2	1	0	2	1
Parathyroid							
Number examined		48	46	42	42	94	84
Tumor infiltration/metastasis		0	1	0	0	1	0
Hyperplasia, diffuse		5	9	1	2	14	3
Hyperplasia, focal		13	8	5	1	21	6
Pituitary							
Number examined		47	49	50	49	96	99
Hyperplasia, anterior, focal		13	8	5	2	21	7
Hyperplasia, intermediate, focal		1	0	0	0	1	0
Prostate							
Number examined		50	49	-	-	99	-
Tumor infiltration/metastasis		1	2	-	-	3	-
Mineralization		38	36	-	-	74	-
Cell infiltration		7	4	-	-	11	-
Prostatitis		30	33	-	-	63	-
Salivary gland, parotid							
Number examined		48	49	45	42	97	87
Tumor infiltration/metastasis		1	1	0	0	2	0
Atrophy, acinar, focal		0	1	0	0	1	0
Mineralization		1	2	0	0	3	0
Salivary gland, submandibular							
Number examined		48	47	46	49	95	95
Tumor infiltration/metastasis		1	1	0	0	2	0
Cell infiltration		1	0	0	0	1	0

Table 3-5. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Salivary gland, sublingual							
Number examined		50	46	47	49	96	96
Tumor infiltration/metastasis		0	1	0	0	1	0
Sciatic nerve							
Number examined		48	50	50	49	98	99
Mineralization, arterial wall		5	4	0	0	9	0
Degeneration, nerve fiber		6	8	0	0	14	0
Seminal vesicle							
Number examined		50	48	-	-	98	-
Tumor infiltration/metastasis		1	0	-	-	1	-
Cell infiltration		0	1	-	-	1	-
Vasculitis		0	2	-	-	2	-
Skeletal muscle							
Number examined		50	50	50	49	100	99
Tumor infiltration/metastasis		2	2	0	0	4	0
Degeneration, muscular		1	2	2	1	3	3
Mineralization, muscular		0	0	1	1	0	2
Atrophy, muscle fiber		4	6	1	0	10	1
Skin/subcutis							
Number examined		50	50	49	49	100	98
Tumor infiltration/metastasis		3	3	0	1	6	1
Necrosis, fat		0	0	0	1	0	1
Hematoma		1	1	0	0	2	0
Ulcer		0	0	0	1	0	1
Inflammatory change		0	0	1	0	0	1
Small intestine, duodenum							
Number examined		48	49	46	46	97	92
Tumor infiltration/metastasis		0	1	0	0	1	0
Arteriosclerosis		0	1	0	0	1	0
Ulcer		1	0	0	0	1	0
Erosion		1	2	0	0	3	0
Arteritis		0	1	0	0	1	0
Small intestine, ileum							
Number examined		42	47	46	47	89	93
Tumor infiltration/metastasis		1	1	0	0	2	0
Erosion		0	1	0	0	1	0
Ulcer		1	0	0	0	1	0
Histiocytic nodule		0	1	0	0	1	0
Hyalinosis, vascular		9	3	11	9	12	20
Hyperplasia, lymphoid		1	1	0	0	2	0
Small intestine, jejunum							
Number examined		48	48	47	46	96	93
Tumor infiltration/metastasis		1	0	0	0	1	0
Spinal cord							
Number examined		50	50	50	50	100	100
Tumor infiltration/metastasis		1	1	0	0	2	0
Radiculoneuropathy		8	9	13	10	17	23

Table 3-6. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Spleen							
Number examined		50	48	49	48	98	97
Tumor infiltration/metastasis		2	2	2	1	4	3
Atrophy		4	1	2	4	5	6
Hematopoiesis, increased		19	16	23	15	35	38
Infarction		1	0	0	0	1	0
Fibrosis, capsular		0	0	0	1	0	1
Hyperplasia, nodular, stromal		0	2	0	0	2	0
Megakaryocytosis		0	0	1	0	0	1
Hyperplasia, marginal		1	1	0	0	2	0
Sternum + marrow							
Number examined		46	48	49	49	94	98
Tumor infiltration/metastasis		1	2	2	1	3	3
Fat infiltration		0	0	0	1	0	1
Osteosclerosis		0	1	1	0	1	1
Hematopoiesis, increased		8	7	13	6	15	19
Granulopoiesis, increased		1	2	2	0	3	2
Megakaryocytosis		0	0	1	0	0	1
Stomach							
Number examined		50	49	49	50	99	99
Tumor infiltration/metastasis		1	1	1	0	2	1
Ectopic forestomach mucosa		1	0	0	0	1	0
Cyst, epidermal		1	1	0	0	2	0
Dilatation, glandular		32	35	26	27	67	53
Necrosis, single cell		0	1	0	0	1	0
Cell infiltration		2	2	0	0	4	0
Edema		2	2	1	1	4	2
Arteritis		1	2	0	1	3	1
Thickened, limiting ridge		1	1	1	0	2	1
Ectopic intestinal tissue		1	0	0	0	1	0
Thickening, forestomach mucosa		2	1	0	2	3	2
Erosion, glandular stomach		5	8	2	4	13	6
Fibrosis, lamina propria		47	44	10	11	91	21
Proliferation, cardiac gland		1	0	0	0	1	0
Erosion/ulcer, forestomach		2	2	0	3	4	3
Ulcer, glandular stomach		0	1	1	0	1	1
Hyalinaization, vascular		0	0	1	0	0	1
Hyperplasia, squamous, focal		1	0	0	0	1	0
Hyperplasia, basal cell, focal		0	0	1	0	0	1
Hyperplasia, glandular, focal		1	0	0	1	1	1
Tail							
Number examined		0	1	0	0	1	0
Abscess		0	1	0	0	1	0
Testis							
Number examined		50	50	-	-	100	-
Tumor infiltration/metastasis		2	0	-	-	2	-
Atrophy, seminiferous tubular		8	7	-	-	15	-
Mineralization		0	2	-	-	2	-
Arteritis		0	1	-	-	1	-
Stasis, spermatid		1	0	-	-	1	-
Necrosis, seminiferous tubule		0	1	-	-	1	-
Cell infiltration, mastocyte		0	1	-	-	1	-
Thoracic cavity							
Number examined		50	50	50	50	100	100
Tumor infiltration/metastasis		0	1	0	0	1	0

Table 3-7. Non-tumor lesions in IGS rats fed CR-LPF.

Lot.		1	2	1	2	TOTAL	
Tissue	Sex:	Male	Male	Female	Female	Male	Female
Observation	Number:	50	50	50	50	100	100
Thymus							
Number examined		41	45	47	48	86	95
Tumor infiltration/metastasis		0	0	2	0	0	2
Thyroid							
Number examined		48	50	50	50	98	100
Tumor infiltration/metastasis		0	1	0	0	1	0
Hyperplasia, C cell, focal		6	7	6	4	13	10
Hyperplasia, follicular, focal		0	2	0	1	2	1
Tongue							
Number examined		50	50	50	49	100	99
Tumor infiltration/metastasis		1	2	1	0	3	1
Mineralization, arterial wall		1	6	0	0	7	0
Cell infiltration		1	0	0	0	1	0
Granuloma		0	0	1	0	0	1
Arteritis		0	3	0	0	3	0
Trachea							
Number examined		49	48	48	48	97	96
Tumor infiltration/metastasis		0	1	0	0	1	0
Cell infiltration		1	4	4	1	5	5
Fibrosis		0	1	0	0	1	0
Globule leukocyte, increased		3	1	0	0	4	0
Urinary bladder							
Number examined		46	47	49	49	93	98
Tumor infiltration/metastasis		2	1	0	0	3	0
Cell infiltration		3	1	0	1	4	1
Cystitis		1	1	0	1	2	1
Hyperplasia, mucosal, focal		0	0	0	1	0	1
Hyperplasia, mucosal, diffuse		1	1	0	0	2	0
Uterus							
Number examined		-	-	50	50	-	100
Dilatation, glandular, cystic		-	-	8	7	-	15
Dilatation, lumina		-	-	4	8	-	12
Pyometra		-	-	0	1	-	1
Hyperplasia, fibromuscular		-	-	12	11	-	23
Cyst, squamous epithelium		-	-	1	1	-	2
Hyperplasia, epithelial		-	-	1	1	-	2
Hyperplasia, endometrial, cystic		-	-	3	1	-	4

Crj:CD(SD)IGS Rats and the Effects of a Commercial Low Protein Diet in Carcinogenicity Studies at the Drug Safety Research Laboratories in Takeda Chemical Industries, Ltd.

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ABSTRACT. Crj:CD(SD)IGS rats were fed either a commercial low protein (CR-LPF, 18%) or a normal protein (CRF-1, 24%) diet for 2 years starting at 6 weeks of age, and the effects of the 2 different dietary protein contents on carcinogenicity studies were examined. Body weights were lower and food consumption was higher in the CR-LPF (LPF) group than in the CRF-1 (F-1) group during the experimental period. However, there were no differences in the survival rate. No significant biological differences were observed between the LPF and F-1 groups in clinical signs, hematology, gross pathology, nor in neoplastic or non-neoplastic lesions. In conclusion, there were no major differences in the biological parameters used in carcinogenicity study between Crj:CD(SD)IGS rats fed commercial low and normal protein diets. — Key words: Carcinogenicity studies, CD(SD)IGS rat, Low protein diet

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INTRODUCTION

We have used a 23-24% protein diet for repeated-dose toxicity and carcinogenicity studies in rats. However, it has been suggested that lowering dietary protein levels would reduce the incidence of spontaneous lesions and prolong the animals' life span [1, 2]. In the present study, an 18% protein diet was fed to rats for up to 110 weeks of age and the effects of the diet on the survival rate and the other parameters were investigated. Crj:CD(SD)IGS rats are produced by Charles River Inc. under a new breeding system to help endure uniform experimental animals. This study was also designed to collect background data on Crj:CD(SD)IGS rats for carcinogenicity studies.

MATERIALS AND METHODS

1. Animals

One hundred and fifty male and 150 female Crj:CD(SD)IGS rats (SPF animals) aged 4 weeks were obtained from Charles River Japan Inc. on January 23, 1996 and acclimatized to the environmental conditions of our laboratories for 2 weeks. Two hundred animals, 100 per sex, in good conditions were selected for this study on the basis of clinical signs and body weights. They were allocated randomly to 2 groups each comprised of 50 males and 50 females on the basis of body weight stratification. At the start of the experiment, the animals were 6 weeks old and ranged in body weight from 198 to 229g (males) and from 141 to 168g (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 of a rack in a clean laminar airflow booth. The booth was placed in an animal room with a room temperature of 20-26 ℃, a relative humidity of 40-70%, air exchange 8-25 times/hr and a 12-hr light/dark cycle (lights on from 07:00 to 19:00). Before grouping, all the animals were allowed free access to tap water and the standard powdered laboratory animal diet (CRF-1, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source). After grouping, one group continued to receive the standard diet, and the other group was switched to a powdered laboratory animal diet having a lower protein content (CR-LPF, Oriental Yeast Co., γ-ray irradiated at 25-30 kGy from a ^{60}Co source).

3. Grouping and diet components

The experimental groups were as follows.

Group	Diet	Protein content (%)	No. of animals	
			Male	Female
1	CRF-1	24	50	50
2	CR-LPF	18	50	50

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

The diet components were as follows.

Diet Type	CRF-1 Normal protein diet	CR-LPF Low protein diet
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

4. Examinations and methods

1) Survival

All animals were observed daily for survival, once a day until week 70, and twice a day, once in the morning and once in the afternoon (once in the morning on holidays and weekends), during the experimental period.

2) Clinical signs and mass observations

All animals were observed once daily for clinical signs. In addition, detailed examinations including palpation for clinical signs were conducted for all animals once a week during the experimental period. From week 39, the existence and location of masses were examined weekly for all animals.

3) Body weight

Each animal was weighed using an electronic balance (PM4600, Mettler Toledo AG) once a week from weeks 1 to 14, once every 4 weeks from weeks 15 to 70, once a week thereafter and once in week 52.

4) Food consumption

Food consumption for each animal was calculated as the difference between the weight of diet given and that of the diet remaining one week later, and the measurement was carried out with an electronic balance (PM4600, Mettler Toledo AG) every week from weeks 1 to 14, once every 4 weeks thereafter and in weeks 52 and 104.

5) Hematology

At necropsy, blood samples were withdrawn from the abdominal aorta of all surviving animals with a vacuum blood collecting tube containing EDTA2K under ether anesthesia. The values of the following were determined or calculated with an automated hematology analyzer (E-5000, Sysmex Corp.) and an automated reticulocyte analyzer (R-2000, Sysmex Corp.). The differential leukocyte count was determined by microscopy after May-Giemsa staining.

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

6) Necropsy

Gross pathological examination was performed on all animals that were found dead, sacrificed *in extremis* or sacrificed at the end of the study. All surviving animals and animals killed *in extremis* were exsanguinated under ether anesthesia. For each animal, the visceral organs were examined visually.

7) Histopathology

The following organs/tissues from all animals in each group were fixed in 10 vol% neutral buffered formalin; adrenal glands, aorta, bone marrow, brain, cecum, cervical spinal cord, colon, duodenum, epididymides, esophagus, femurs, Harderian glands, heart, ileum, jejunum, kidneys, liver, lumbar spinal cord, lungs, mammary glands, mesenteric lymph nodes, ovaries, pancreas, parathyroid glands, pituitary gland, sciatic nerves, seminal vesicles, skeletal muscles, skin, spleen, sternum, stomach, sublingual glands, submandibular glands, submandibular lymph nodes, thymus, thyroidglands, tongue, trachea, urinary bladder, uterus, vagina, ventral prostate and gross abnormal sites. The eyes were pre-fixed in Davidson's solution containing 3 w/v% glutaraldehyde and post-fixed in 10 vol% 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 interval scale data on body weight, food consumption and hematology 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. Pairwise comparisons of survival rates between the F-1 and LPF groups were performed using a log rank type method [4]. All statistical tests were conducted at the two tailed significance level of 5%.

RESULTS

1. Mortality (Fig. 1)

The numbers of deaths and animals killed *in extremis* and survival curves for the entire study period are shown below and in Figure 1, respectively.

Sex	Male		Female	
Diet	F-1	LPF	F-1	LPF
Animals examined	50	50	50	50
Found dead	26	22	27	27
Killed *in extremis*	3	4	4	6
Total	29	26	31	33
Survival rete (%)	42	48	38	34

Deaths occurred in both sexes beginning week 39 (45 weeks old), but were not frequent until week 76. In the final phase of the experimental period (after week 80), deaths occurred frequently, especially in females. There were no statistically significant differences in survival rates between the F-1 and LPF groups.

2. Clinical signs (Table 1)

The types and incidences of clinical signs observed during the study are summarized in Table 1. Hair loss and hemorrhage in males and females and swelling in males were frequently observed in each group compared with the other signs; however, no significant differences were observed between the 2 groups. Decreases in feces and locomotor activity, bradypnea, stained fur around the eyes or by urine and wasting were observed when the general condition of the animals started to deteriorate prior to death in the final phase of the experimental period.

3. Mass observations (Table 2)

Sites and incidences of masses found or palpated during the experimental period are summarized in Tables 2. Masses were mainly observed in the axilla, chest and abdominal region in females in each group.

4. Body weight (Table 3, Fig. 2)

Body weights in males in the LPF group were decreased or

tended to be decreased compared with those in the F-1 group throughout the experimental period. Statistically significant decreases were noted until week 30 except for week 8. In females, body weights in the LPF group were decreased compared with those in the F-1 group except for weeks 34 and 62, and statistically significant decreases were noted from weeks 81 to 99.

5. Food consumption (Table 4, Fig. 3)

Food consumption in the LPF group was increased significantly or tended to be increased when compared with that in the F-1 group throughout the experimental period except for week 78 in males and until week 70 in females.

6. Hematology (Table 5)

There were no significant differences for any parameter between the 2 groups.

7. Gross pathology (Table 6)

There were no significant differences between the 2 groups in males. In the female LPF group, the numbers of animals with enlarged adrenals and with rough surface of the kidneys were smaller, and the number of animals with enlarged spleen was larger than those in the female F-1 group. Additionally, the number of animals with masses, nodules, swelling or enlargement of the hindlimbs was 4 in the male F-1 group and 9 in the male LPF group; this was observed in only one female in the F-1 group.

8. Histopathology

1) Cause of death

Fifty-five males and 64 females were found dead or killed *in extremis* during the study period. Half or more than half of the causes of death for these animals were adenoma or carcinoma in the pituitary. This result was similar to the data for the original SD rats as shown in previous reports [5, 6]. The number of animals found dead or killed *in extremis* and major causes of death are summarized in the following table.

Sex	Male		Female	
Diet	F-1	LPF	F-1	LPF
Number of animals	50	50	50	50
Found dead	26	22	27	27
Killed *in extremis*	3	4	4	6
Total	29	26	31	33
Major cause of death:				
Pituitary: Carcinoma, Pars distalis	10	8	16	16
Pituitary: Adenoma, Pars distalis	6	5	5	8
Kidney: Nephropathy	1	4	1	0
Heart: Cardiomyopathy	3	2	0	0

2) Neoplastic lesions (Table 7)

The number of tumor-bearing animals and total primary neoplasms including benign, malignant and hematopoietic neoplasms were not significantly different between the two groups in either sex (Table 12). In males, there were no differences between the two groups for the incidence of any tumor types. In females, the incidence of cortical adenoma in the adrenals was less in the LPF group (6%) than in the F-1 group (34%). Major tumors observed in both sexes were pituitary adenoma in the pars distalis (male; 35%, female; 29%) and pituitary carcinoma in the pars distalis (male; 31%, female; 55%). The incidence of pituitary carcinoma in the pars distalis was higher than that in a previous study (male; 0-3.6%, female; 1.7-13.6%) using the same strain of rats [5]. This difference is probably due to the method of preparation for histopathology sections. In this study, the pituitaries were cut transversally with surrounding tissues including the sphenoid bone, therefore, infiltration of tumor cells into the surrounding tissues, which is considered as a major diagnostic criteria for tumor malignancy, was more easily observed than when examining pituitary sections prepared as usual. In females, adenoma (16%), fibroadenoma (46%) and adenocarcinoma (26%) in the mammary gland were also observed in high incidence. Incidences of the other tumor types were almost similar to the data of the original SD rats as shown in the previous reports [5, 6].

3) Non-neoplastic lesions (Table 8)

The incidence and severity of any of the non-neoplastic lesions including nephropathy were not different between the two groups for either sex. Incidences of the non-neoplastic findings were almost similar to the data of the original SD rats as shown in the previous report [7].

DISCUSSION

Crj:CD(SD)IGS rats were fed either a commercial low protein or a normal protein diet for 2 years, and the effects of the different dietary protein contents on carcinogenicity studies were examined. No significant biological differences were observed between the CR-LPF (LPF) and CRF-1 (F-1) groups in clinical signs, hematology or gross pathology. Body weights were lower and food consumption was higher in the LPF group during the experimental period. It has been reported that food consumption levels for the low protein diet were higher than for the normal protein diet, and this may be due to the higher fiber content of the low protein diet [8, 9]. It has been suggested that lowering dietary protein level reduces the incidence of spontaneous lesions and prolongs their life span [1, 2]. However, no differences were observed in the survival rate and the incidence of neoplastic or non-neoplastic lesions between the LPF group and the F-1 group in this study. It has been reported that a low protein diet decreases the severity of cardiomyopathy, prevents nephrocalcinosis and decreases the incidence and severity of nephropathy in F344 rats [9]. In other reports, however, dietary restriction lowered the progression of cardiomyopathy and nephropathy in female SD rats, whereas protein restriction did not [5, 10, 11]. In this study, cardiomyopathy was observed more frequently in males than in females in both the F-1 and LPF groups, and no significant differences in the in-

cidences were observed between the two groups. The low protein diet was not considered to have a tendency to prevent the incidence of cardiomyopathy in Crj:CD(SD)IGS rats this being the same as in the original SD rats. In a previous study for repeated dose toxicity studies[12], urinary protein levels in males increased with age up to 32 weeks in the F-1 group, but not in the LPF group; however, nephropathy was not observed in either group. In this study with feeding the low protein diet for 2 years, nephropathy was observed in both sexes in both groups with a similar incidence. Calcification in the pelvis in the kidney was also observed more frequently in females and the incidences and severity were not different between the F-1 group and the LPF group. It was considered that the low protein diet did not affect incidence or severity of spontaneous renal disease in Crj:CD(SD)IGS rats. In conclusion, there were no toxicological significant differences in the biological parameters used in carcinogenicity studies between Crj:CD(SD)IGS rats fed commercial low and normal protein diets.

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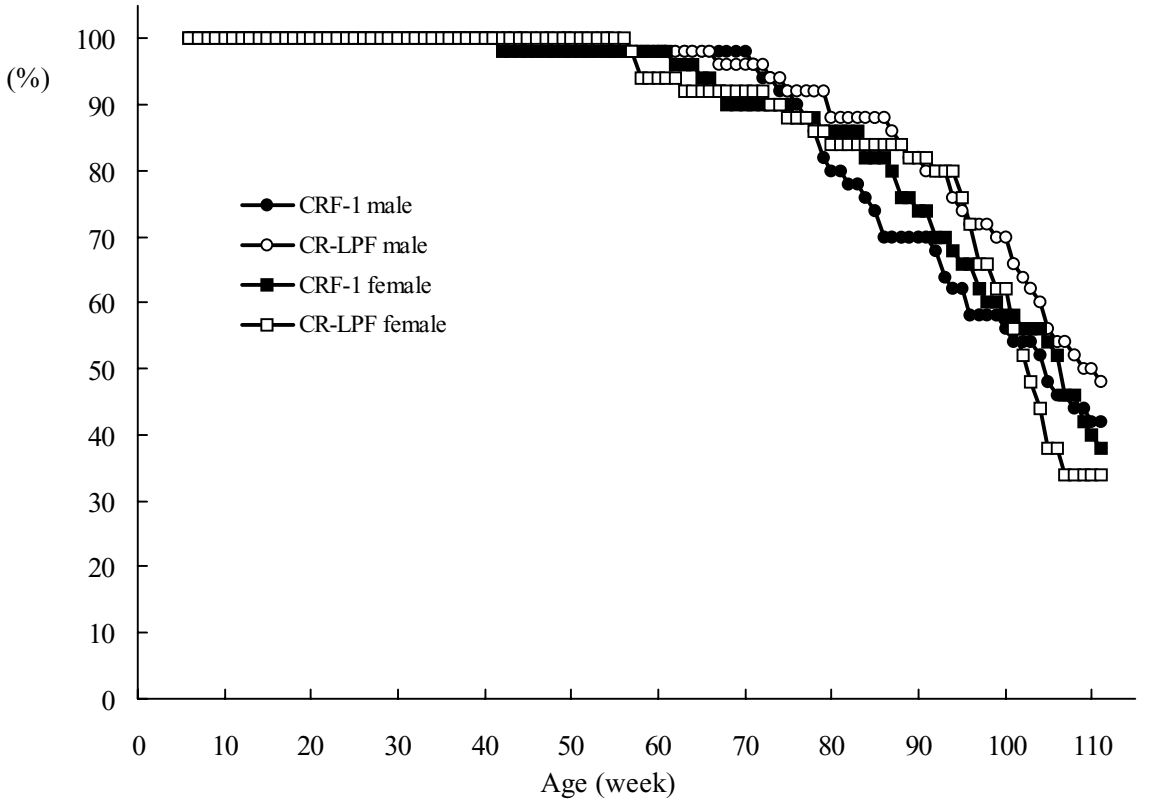

Figure 1. Survival curves of Crj:CD(SD)IGS rats fed a low protein or a normal protein diet

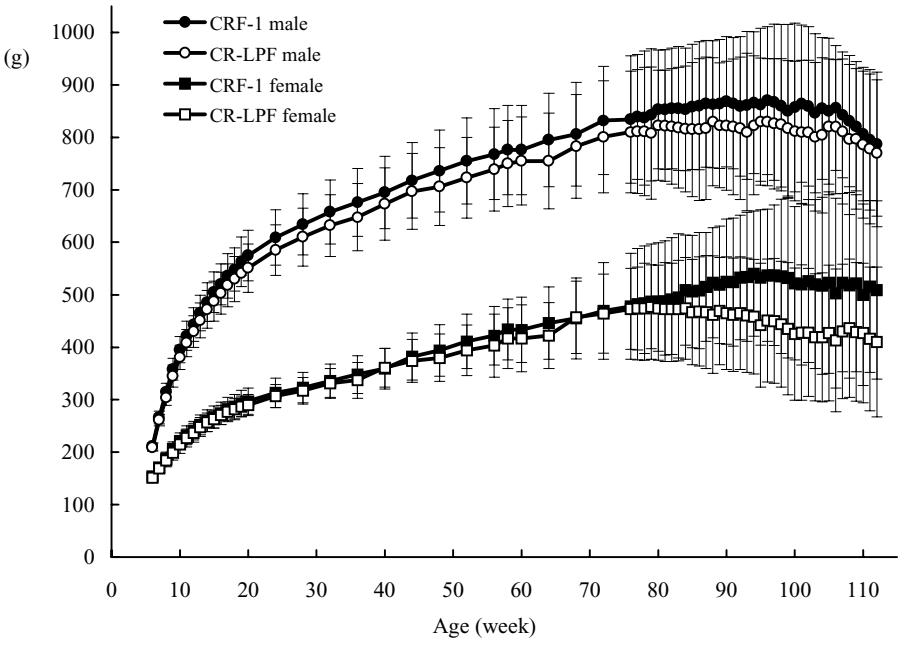

Figure 2. Mean body weight in Crj:CD(SD)IGS rats fed a low protein or a normal protein diet

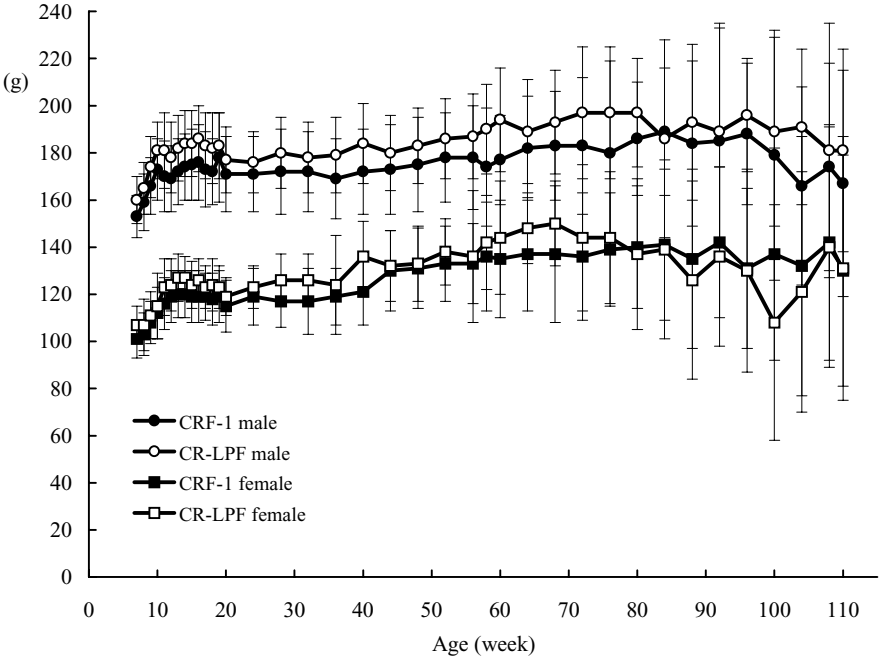

Figure 3. Mean food consumption in Crj:CD(SD)IGS rats fed a low protein or a normal protein diet

Table 1. Summary incidence of clinical signs

Observation	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
General appearance, Posture				
Emaciation	2	6	23	23
Lateral position	8	11	16	17
Prone position	21	14	14	10
Supine position	3	1	1	3
Wryneck	0	0	2	3
Cousciousness, Behavior				
Decreased locomotor activity	7	10	20	26
Irritability	1	0	1	0
Abnormal gait	2	0	0	0
Ataxic gait	0	0	6	3
Paralytic gait	1	0	0	0
Nervos system				
Rigidity	0	0	0	1
Loss of righting reflex	1	0	0	0
Tremor	1	1	0	0
Breathing				
Bradypnea	4	6	10	13
Wheezing	1	2	1	1
Body temperature, Fur, Skin				
Hypothermia	2	2	5	4
Soiled fur, around eye	8	11	22	26
Soiled fur, around nose	0	1	1	2
Soiled fur by urine	7	7	8	10
Soiled fur by feces	3	2	1	1
Soiled fur by saliva	1	2	1	0
Loss of fur	16	16	13	19
Hair, rough	3	2	0	2
Papule	1	3	6	7
Paleness	1	2	0	3
Wound	5	2	0	0
Hemorrhage	11	11	11	17
Crust formation	4	4	5	9
Swelling	13	19	5	2
Ulcer	1	1	2	7
Necrosis	0	1	1	3
Eye				
Ptosis	0	0	1	2
Discolored eye ball	7	8	5	19
Lacrimation	0	1	13	13
Reddish tear	0	0	1	0
Exophthalmos	0	0	3	1
Dry eye	0	0	1	0
Cornea, opacity	1	2	0	2
Cataract	1	3	1	1
Mydriasis	1	0	0	0
Mouth				
Loss of teeth	0	1	1	4
Discoloration of teeth	2	0	5	3
Malocclusion	0	3	4	7
Salivation	1	0	1	0
Anogenital region, Excretion				
Small testis	4	8	—	—
Decreased feces	25	26	38	43
Loose stool	1	1	0	0
Hematuria	2	1	1	2

Table 2. Summary incidence of palpable masses

Observation	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Pinna	0	1(102)	0	2(94)
Nose	1(75)	0	0	0
Face	0	0	0	1(69)
Oral region	0	0	1(48)	0
Lower jaw	0	0	0	1(42)
Neck	2(92)	1(97)	2(56)	2(42)
Axilla (L)	1(86)	0	9(39)	6(42)
Axilla (R)	2(55)	0	14(46)	9(46)
Chest	1(73)	1(106)	11(61)	12(39)
Abdominal region	1(83)	1(76)	23(39)	26(45)
Back	6(84)	8(64)	1(84)	2(59)
Around anus	0	0	5(39)	4(52)
External genitalia	0	0	5(51)	4(50)
Abdominal cavity	4(61)	3(70)	1(56)	4(65)
Hindlimb (L)	3(76)	3(77)	0	0
Hindlimb (R)	3(95)	2(76)	0	0
Tail	2(76)	2(98)	0	2(82)

Number in parentheses indicates the week of onset

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

Age	Male				Female			
(weeks)	CRF-1		CR-LPF		CRF-1		CR-LPF	
6	211±7	(50)	209±7	(50)	154±6	(50)	151±6*	(50)
7	266±12	(50)	261±11*	(50)	171±8	(50)	169±8	(50)
8	315±16	(50)	304±15**	(50)	188±12	(50)	184±12	(50)
9	358±21	(50)	345±21**	(50)	206±14	(50)	199±14*	(50)
10	395±25	(50)	381±23**	(50)	221±16	(50)	215±17	(50)
11	422±28	(50)	408±26*	(50)	232±18	(50)	227±17	(50)
12	443±32	(50)	430±30*	(50)	241±18	(50)	237±18	(50)
13	465±34	(50)	451±33*	(50)	252±19	(50)	248±18	(50)
14	486±37	(50)	472±36	(50)	260±21	(50)	257±18	(50)
15	505±39	(50)	488±38*	(50)	267±21	(50)	263±17	(50)
16	520±39	(50)	503±40*	(50)	274±21	(50)	271±18	(50)
17	536±42	(50)	518±41*	(50)	282±22	(50)	277±19	(50)
18	548±41	(50)	530±43*	(50)	287±23	(50)	282±20	(50)
19	563±47	(50)	541±44*	(50)	293±25	(50)	287±20	(50)
20	575±48	(50)	551±46*	(50)	297±25	(50)	290±20	(50)
24	609±53	(50)	585±48*	(50)	313±28	(50)	307±22	(50)
28	634±58	(50)	610±55*	(50)	323±29	(50)	317±25	(50)
32	658±61	(50)	632±59*	(50)	336±32	(50)	331±28	(50)
36	676±65	(50)	648±64*	(50)	348±36	(50)	337±34	(50)
40	695±69	(50)	673±69	(50)	359±39	(50)	361±37	(50)
44	718±72	(50)	697±72	(50)	382±45	(49)	374±41	(50)
48	736±78	(50)	706±74	(49)	394±49	(49)	380±45	(50)
52	755±82	(50)	723±77	(49)	411±52	(49)	394±48	(50)
56	768±87	(49)	739±80	(49)	422±56	(49)	403±60	(50)
58	776±85	(49)	750±82	(49)	434±58	(49)	418±58	(47)
60	776±85	(49)	755±84	(49)	433±62	(49)	417±64	(47)
64	795±89	(49)	755±91	(49)	446±69	(48)	422±63	(46)
68	806±99	(49)	783±99	(48)	455±77	(45)	457±69	(46)
72	832±103	(47)	801±107	(48)	469±92	(45)	464±75	(46)

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

Table 3. Body weight in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Age	Male				Female			
(weeks)	CRF-1		CR-LPF		CRF-1		CR-LPF	
76	834±121	(45)	810±116	(46)	478±101	(44)	473±77	(44)
77	839±119	(44)	811±118	(46)	482±106	(44)	474±79	(44)
78	837±128	(43)	811±122	(46)	484±109	(44)	474±86	(43)
79	843±126	(41)	808±126	(46)	487±110	(43)	477±80	(43)
80	853±113	(40)	823±105	(44)	487±112	(43)	474±82	(42)
81	853±114	(40)	822±106	(44)	488±115	(43)	473±88	(42)
82	855±115	(39)	821±110	(44)	491±118	(43)	473±87	(42)
83	855±118	(39)	818±115	(44)	495±121	(43)	473±93	(42)
84	853±117	(38)	816±120	(44)	509±105	(41)	474±97	(42)
85	858±119	(37)	815±128	(44)	506±107	(41)	467±100	(42)
86	860±122	(35)	815±135	(44)	508±111	(41)	468±104	(42)
87	864±121	(35)	817±136	(43)	515±104	(40)	467±108*	(42)
88	863±121	(35)	830±122	(42)	523±105	(38)	462±112*	(42)
89	864±123	(35)	823±119	(41)	519±112	(38)	469±102*	(41)
90	868±124	(35)	822±123	(41)	525±119	(37)	465±107*	(41)
91	865±125	(35)	820±129	(40)	524±122	(37)	462±108*	(41)
92	859±133	(34)	817±136	(40)	533±119	(35)	465±107*	(40)
93	861±141	(32)	810±150	(40)	534±123	(35)	461±109**	(40)
94	866±135	(31)	823±132	(38)	540±119	(34)	458±109**	(40)
95	862±146	(31)	830±121	(37)	533±129	(33)	442±118**	(38)
96	870±141	(29)	829±121	(36)	537±126	(33)	451±117**	(36)
97	867±149	(29)	826±124	(36)	537±134	(31)	450±119**	(33)
98	860±155	(29)	824±126	(36)	535±147	(30)	444±120**	(33)
99	850±165	(29)	817±129	(35)	532±152	(30)	435±126**	(31)
100	857±161	(28)	812±133	(35)	522±146	(29)	425±126**	(31)
101	864±150	(27)	810±137	(33)	520±154	(29)	428±129*	(29)
102	859±152	(27)	809±138	(32)	526±155	(28)	428±122*	(26)
103	846±152	(27)	801±142	(31)	520±161	(28)	419±121*	(24)
104	855±135	(26)	804±148	(31)	517±169	(28)	419±123*	(23)
105	850±139	(24)	820±129	(28)	523±173	(27)	427±129*	(19)
106	856±119	(23)	820±128	(27)	503±181	(26)	413±136	(19)
107	843±120	(23)	811±138	(27)	523±170	(23)	430±128	(17)
108	831±125	(22)	796±143	(26)	518±177	(23)	436±132	(17)
109	821±123	(22)	797±134	(25)	522±169	(22)	429±132	(17)
110	806±127	(21)	786±140	(25)	500±173	(20)	427±133	(17)
111	795±134	(21)	778±143	(25)	516±164	(19)	416±137	(17)
112	787±137	(21)	770±140	(24)	509±170	(19)	410±143	(17)

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

Table 4. Food consumption in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Age	Male				Female			
(weeks)	CRF-1		CR-LPF		CRF-1		CR-LPF	
7	153±9	(50)	160±10**	(50)	101±8	(50)	107±8**	(50)
8	159±12	(50)	165±11**	(50)	103±9	(50)	107±11**	(50)
9	166±12	(50)	174±13**	(50)	108±9	(50)	111±10**	(50)
10	173±13	(50)	181±12*	(50)	112±11	(50)	115±14*	(50)
11	170±15	(50)	181±16*	(50)	116±11	(50)	123±12**	(50)
12	169±14	(50)	178±15**	(50)	119±11	(50)	124±11**	(50)
13	172±14	(50)	182±14**	(50)	120±10	(50)	127±10**	(50)
14	174±14	(50)	184±14*	(50)	120±10	(50)	127±9**	(50)
15	175±15	(50)	184±14*	(50)	119±11	(50)	124±10**	(50)
16	176±16	(50)	186±14	(50)	119±11	(50)	126±9**	(50)
17	173±16	(50)	183±14	(50)	119±10	(50)	123±9**	(50)
18	172±14	(50)	182±15*	(50)	118±11	(50)	124±11**	(50)
19	178±19	(50)	183±14*	(50)	119±11	(50)	123±9**	(50)
20	171±16	(50)	177±14**	(50)	115±11	(50)	119±8**	(50)
24	171±16	(50)	176±13*	(50)	119±12	(50)	123±9*	(50)
28	172±18	(50)	180±15**	(50)	117±11	(50)	126±11*	(50)
32	172±17	(50)	178±15	(50)	117±14	(50)	126±11	(50)
36	169±17	(50)	179±16	(50)	119±12	(50)	124±21	(50)
40	172±18	(50)	184±17	(50)	121±14	(50)	136±15	(50)
44	173±19	(50)	180±16	(50)	130±17	(49)	132±15	(50)
48	175±20	(50)	183±16	(49)	131±17	(49)	133±16	(50)
52	178±19	(50)	186±17	(49)	133±16	(49)	138±14	(50)
56	178±22	(49)	187±18	(49)	133±17	(49)	136±28	(50)
58	174±25	(49)	190±19	(49)	136±23	(49)	142±20	(47)
60	177±19	(49)	194±22	(49)	135±25	(49)	144±24	(47)
64	182±22	(49)	189±22	(49)	137±24	(48)	148±15	(46)
68	183±23	(49)	193±22	(48)	137±29	(45)	150±18	(46)
72	183±29	(47)	197±28	(48)	136±27	(45)	144±31	(46)
76	180±39	(45)	197±28	(46)	139±24	(44)	144±28	(44)
80	186±24	(40)	197±23	(44)	140±26	(43)	137±32	(42)
84	189±27	(38)	186±42	(44)	141±32	(41)	139±38	(42)
88	184±35	(35)	193±33	(42)	135±38	(38)	126±42	(42)
92	185±48	(34)	189±46	(40)	142±32	(35)	136±38	(40)
96	188±30	(29)	196±24	(36)	131±34	(33)	130±43	(36)
100	179±53	(28)	189±40	(35)	137±45	(29)	108±50	(31)
104	166±42	(26)	191±33	(30)	132±55	(28)	121±51	(22)
108	174±44	(22)	181±54	(26)	142±50	(23)	140±51	(17)
110	167±48	(21)	181±43	(25)	130±49	(20)	131±56	(17)

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

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

Sex	Diet	Number of animals	Erythrocytes (×104/μL)	Hematocrit (%)	Hemoglobin (g%)	MCH (pg)	MCHC (%)
Male	CRF-1	21	748±88	41.4±4.8	13.4±1.8	17.8±1.1	32.2±1.1
	CR-LPF	24	771±100	43.5±5.5	14.0±2.1	18.2±1.3	32.2±1.6
Female	CRF-1	19	665±87	39.4±4.3	13.0±1.4	19.5±0.8	32.9±1.0
	CR-LPF	16	655±143	39.0±7.2	12.6±2.5	19.4±1.5	32.0±1.5

Sex	Diet	Number of animals	MCV (cμ)	Platelets (×10^4/μL)	Leukocytes (x10^2/μL)	Reticulocytes (%)
Male	CRF-1	21	55±3	112.9±22.8	108±44	3.6±1.9
	CR-LPF	24	56±4	111.5±29.9	91±37	3.4±3.5
Female	CRF-1	19	59±3	99.0±24.5	101±38	3.1±1.4
	CR-LPF	16	61±7	109.5±35.5	89±35	3.5±4.3

Data are expressed as mean±S.D.
No significantly different from the corresponding value in the CRF-1 group

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

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Alimentary system				
Cecum				
Discoloration, Contents, Black, Red	0	0	0	1
Discoloration, Contents, Yellow	1	0	0	0
Distension	0	0	0	2
Colon				
Discoloration, Contents, Black, Red	0	0	0	1
Discoloration, Contents, Yellow	1	0	0	0
Distension	0	0	0	2
Duodenum				
Distension	0	0	1	2
Ulcer	1	0	0	0
Ileum				
Discoloration, Contents, Black, Red	0	0	0	1
Discoloration, Contents, Yellow	1	0	0	0
Distension	0	0	0	2
Nodule	0	0	1	0
Jejunum				
Discoloration, Contents, Yellow	1	0	0	0
Distension	0	0	0	2

Table 6-1. Gross patholoty in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Liver				
Adhesion	1	1	0	0
Cyst	0	0	1	0
Deformity	0	0	1	0
Dilatation, Common bile duct	0	0	1	0
Discoloration, Pale	1	1	1	4
Discoloration, Red	0	0	1	0
Discoloration, Yellow	1	0	0	1
Enlarged	1	0	3	1
Focus	2	2	3	3
Focus, Multiple	6	3	0	1
Hernia, Median lobe	0	0	0	1
Mass	1	1	0	0
Nodule	3	2	1	0
Nodule, Multiple	0	0	0	1
Rough surface	2	2	0	2
Small	0	0	0	1
Pancreas				
Mass	1	0	0	0
Nodule	2	2	0	0
Nodule, Multiple	0	1	0	0
Rectum				
Thick				
	0	0	0	1
Stomach				
Distention	0	0	1	2
Focus, Forestomach	1	1	1	3
Focus, Forestomach, Multiple	1	0	0	0
Focus, Glandular stomach	2	1	0	2
Focus, Glandular stomach, Multiple	1	0	5	3
Nodule, Limiting ridge	0	1	0	0
Thick, Mucosa, Forestomach	0	0	0	1
Ulcer, Glandular stomach	1	0	0	0
Submandibular gland				
Mass Unilateral	0	0	0	1
Tooth				
Mass	0	0	0	1
Cardiovascular system				
Heart				
Dilatation, Ventricle	0	0	0	1
Endocrine system				
Adrenal gland				
Enlarged, Bilateral	1	1	8	2
Enlarged, Unilateral	1	4	6	1
Focus, Bilateral	0	0	1	0
Focus, Bilateral, Multiple	0	0	2	0
Focus, Unilateral	0	0	1	0
Mass, Unilateral	1	0	0	0
Rough surface, Bilateral	0	0	3	3

Table 6-2. Gross patholoty in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Parathyroid gland				
Enlarged, Bilateral	4	4	0	0
Enlarged, Unilateral	0	2	0	0
Pituitary gland				
Cyst	1	1	0	0
Enlarged	24	23	38	43
Focus	6	8	6	3
Focus, Multiple	0	0	1	0
Nodule	4	4	0	0
Thyroid gland				
Enlarged, Bilateral	1	0	0	0
Enlarged, Unilateral	0	1	0	1
General body system				
Adipose tissue				
Hindlimb				
Enlarged, Unilateral	0	2	0	0
Mass, Bilateral	1	0	0	0
Mass, Unilateral	2	1	0	0
Nodule Bilateral	0	1	0	0
Nodule, Unilateral	2	2	1	0
Swelling, Bilateral	0	1	0	0
Swelling, Unilateral	0	3	0	0
Serosa				
Mass, Abdominal, Cavity	1	0	0	0
Nodule, Abdominal, Cavity, Multiple	1	0	0	0
Tissue NOS				
Ascites	0	0	2	2
Hemorrhage, Pelvic, Cavity	1	0	0	0
Hydrothorax	2	3	1	4
Nodule, Cranium, Cavity	1	0	0	0
Nodule, Pelvis, Cavity	0	1	0	0
Genital system				
Epididymis				
Focus Unilateral	1	0	—	—
Focus Unilateral, Multiple	0	1	—	—
Nodule, Unilateral	0	1	—	—
Small, Bilateral	0	2	—	—
Prostate				
Discoloration, Yellow	2	0	—	—
Enlarged	1	0	—	—
Mass	0	1	—	—
Small	0	2	—	—
Seminal vesicle				
Discoloration, Bilateral, Yellow	0	1	—	—
Distension, Bilateral	1	0	—	—
Small, Bilateral	0	4	—	—
Testis				
Discoloration, Unilateral, Dark, Red	0	1	—	—
Small, Bilateral	5	8	—	—
Small, Unilateral	1	1	—	—

Table 6-3. Gross patholoty in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Clitoral gland				
Mass	—	—	0	1
Nodule	—	—	0	1
Ovary				
Cyst, Capsule, Unilateral	—	—	5	1
Enlarged, Bilateral	—	—	0	1
Uterus				
Cyst, Horn, Unilateral	—	—	0	1
Mass, Horn, Unilateral	—	—	1	0
Nodule, Horn, Unilateral	—	—	0	1
Vagina				
Nodule	—	—	1	0
Hematopoietic System				
Lymph node				
Cyst, Iliac	1	0	0	0
Enlarged, Axillary	0	0	1	0
Enlarged, Iliac	1	0	0	1
Enlarged, Pancreatic	1	0	0	0
Enlarged, Popliteal	0	1	0	1
Enlarged, Renal	1	0	0	1
Enlarged, Thoracic	1	0	0	1
Spleen				
Enlarged	1	1	0	8
Nodule	1	0	0	0
Small	1	0	0	2
Submandibular lymph node				
Enlarged	0	0	1	0
Thymus				
Focus	0	0	0	1
Mass	0	0	0	1
Small	0	1	0	0
Integumentary system				
Mammary gland				
Mass, Abdominal	0	0	10	12
Mass, Around anus	0	0	1	0
Mass, Axillary	0	0	18	11
Mass, Back	0	0	0	2
Mass, Inguinal	0	0	8	11
Mass, Neck	1	0	1	1
Mass, Thoracic	0	1	6	9
Nodule, Abdominal	0	0	0	2
Nodule, Axillary	0	0	0	1
Nodule, Cervical	0	0	1	0
Nodule, Cervical, Multiple	1	0	0	0
Nodule, Inguinal	0	0	3	3
Nodule, Inguinal, Multiple	0	0	0	2
Nodule, Thoracic	0	0	0	2

Table 6-4. Gross patholoty in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Skin				
Alopecia	0	1	0	0
Focus, Tail	0	1	0	0
Mass, Axillary	1	0	1	0
Mass, Back	2	1	0	0
Mass, Head	0	0	1	0
Mass, Tail	1	0	0	0
Nodule, Back	2	2	0	0
Nodule, Face	1	0	0	0
Nodule, Tail	0	0	1	1
Nodule, Tail, Multiple	0	0	0	1
Nodule, Thoracic	0	0	1	0
Ulcer, Neck	1	0	0	0
Subcutaneous tissue				
Discoloration, Pinna, Unilateral, Dark, Red	0	0	0	1
Discoloration, Yellow	2	0	0	0
Enlarged, Pinna, Unilateral	0	0	0	1
Mass, Abdominal	1	0	1	1
Mass, Axillary	1	0	1	0
Mass, Back	2	3	0	0
Mass, Head	1	0	0	0
Mass, Hindlimb	0	1	0	0
Mass, Inguinal	0	1	0	0
Mass, Neck	0	1	0	0
Mass, Tail	1	1	0	1
Nodule, Abdominal	2	0	1	0
Nodule, Anus	0	0	0	1
Nodule, Axillary	1	0	0	0
Nodule, Pinna, Unilateral	0	1	0	1
Thick, Fat	1	0	0	0
Thin	0	0	0	4
Tail				
Musculoskeletal system				
Femur				
Nodule	1	0	0	0
Sternum				
Nodule	1	0	0	0
Vertebra				
Nodule	1	0	0	0
Nervous system				
Brain				
Adhesion	0	0	1	0
Focus, Cerebellum	1	0	1	1
Focus, Multiple	0	0	0	1
Hemorrhage	0	0	1	0
Indentation, Hypothalamus	16	12	24	33

Table 6-5. Gross patholoty in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Respiratory system				
Lung				
Edema	0	1	0	0
Focus	1	0	1	3
Focus, Multiple	2	3	5	0
Nodule, Multiple	1	0	0	1
Nose				
Trachea				
Nodule	0	1	0	0
Special sense organs				
Ear				
Eye				
Discoloration, Bilateral, White	1	1	0	0
Discoloration, Unilateral, White	0	0	1	1
Rupture, Unilateral	0	0	1	0
Zymbal's gland				
Mass, Unilateral	0	0	0	1
Urinary system				
Kidney				
Abscess, Bilateral	0	1	0	0
Adhesion, Unilateral	0	1	0	0
Cyst, Unilateral	3	1	1	2
Dilatation, Pelvis, Bilateral	1	2	2	1
Dilatation, Pelvis, Unilateral	2	0	0	1
Discoloration, Bilateral, Brown	0	1	0	0
Discoloration, Bilateral, Pale	2	2	0	0
Discoloration, Pelvis, Bilateral	0	0	0	1
Discoloration, Unilateral, Dark	0	1	0	0
Discoloration, Unilateral, Yellow	1	0	0	0
Enlarged, Bilateral	1	1	0	0
Enlarged, Unilateral	1	0	0	0
Focus, Unilateral	0	0	1	1
Nodule, Pelvis, Unilateral	0	0	0	1
Nodule, Unilateral	0	0	0	1
Rough surface, Bilateral	3	4	4	0
Rough surface, Unilateral	0	1	0	0
Urethra				
Focus	1	0	0	0
Urinary bladder				
Abscess, Cervix	0	1	0	0
Distension	1	3	0	1
Hematuria	1	0	0	0

Table 7. Incidence of neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Alimentary system				
Ileum	(43)	(39)	(40)	(34)
Leiomyosarcoma	0	0	1	0
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	4	3	0	0
Pancreas	(50)	(50)	(50)	(50)
Adenoma	0	1	0	0
Islet cell adenoma	4	4	0	1
Adenocarcinoma	1	0	0	0
Islet cell carcinoma	0	1	0	0
Submandibuar gland	(50)	(50)	(50)	(50)
Adenocarcinoma	0	0	0	1
Tooth	(0)	(0)	(0)	(1)
Ameloblastoma	0	0	0	1
Endocrine system				
Adrenal gland	(50)	(50)	(50)	(50)
Cortical adenoma	3	1	17	3
Pheochromocytoma	4	7	2	0
Cortical carcinoma	0	0	1	0
Malignant pheochromocytoma	2	1	0	0
Parathyroid gland	(50)	(49)	(50)	(50)
Adenoma	1	0	0	1
Pituitary gland	(48)	(50)	(50)	(50)
Adenoma, Pars distalis	14	21	13	16
Adenocarcinoma, Pars distalis	18	13	26	29
Thyroid gland	(50)	(50)	(50)	(50)
C-cell adenoma	5	3	5	2
Follicular adenoma	1	2	0	0
C-cell carcinoma	0	1	0	0
General body system				
Tissue NOS	(1)	(1)	(0)	(0)
Squamous cell carcinoma, Cranium, Cavity	1	0	0	0
Transitional cell carcinoma, Pelvic, Cavity	0	1	0	0
Genital system				
Prostate	(50)	(50)	(−)	(−)
Adenocarcinoma	0	1	−	−
Clitoral gland	(−)	(−)	(0)	(2)
Adenoma	−	−	0	2
Ovary	(−)	(−)	(50)	(50)
Malignant granulosa cell tumor	−	−	0	1

( ): Number of organs/tissues examined

Table 7-1. Incidence of neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Uterus	(−)	(−)	(50)	(50)
Endometrial stromal polyp	−	−	5	5
Granular cell tumor	−	−	1	0
Leiomyoma	−	−	1	0
Vagina	(−)	(−)	(50)	(50)
Leiomyoma	−	−	1	0
Hematopoietic system				
Bone marrow	(50)	(50)	(50)	(50)
Granulocytic leukemia	0	1	0	0
Lymph node	(4)	(1)	(1)	(3)
Histiocytic sarcoma, Popliteal	0	0	0	1
Mesenteric lymph node	(50)	(50)	(50)	(49)
Malignant lymphoma	0	1	0	1
Spleen	(49)	(50)	(50)	(49)
Mononuclear cell leukemia	1	0	0	0
Thymus	(45)	(47)	(47)	(45)
Thymoma	1	0	0	0
Integumentary system				
Mammary gland	(49)	(50)	(50)	(50)
Adenoma	1	0	8	8
Fibroadenoma	0	1	23	23
Adenocarcinoma	0	0	11	15
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma	2	1	1	0
Squamous cell papilloma	3	1	0	0
Sebaceous adenoma	0	0	1	0
Squamous cell carcinoma	2	1	1	0
Subcutaneous tissue	(7)	(9)	(3)	(5)
Fibroma	3	1	0	1
Lipoma	1	0	1	0
Schwannoma	0	0	1	0
Chondrosarcoma	0	0	0	1
Fibrosarcoma	0	0	0	1
Sarcoma, Head	1	0	0	0
Malignant schwannoma	0	1	0	1
Histiocytic sarcoma	1	3	1	0
Musculoskeletal system				
Bone	(5)	(9)	(1)	(0)
Chondrosarcoma, Costochondral junction	1	0	0	0
Femur	(50)	(50)	(50)	(50)
Osteosarcoma	1	0	0	0
Sternum	(50)	(50)	(50)	(50)
Hemangiosarcoma	1	0	0	0

( ): Number of organs/tissues examined

Table 7-2. Incidence of neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Nervous system				
Brain	(50)	(50)	(50)	(50)
Glioma	1	0	0	0
Granular cell tumor, Cerebrum	1	0	0	0
Malignant meningioma, Cerebrum	0	0	1	0
Respiratory system				
Lung	(50)	(50)	(50)	(50)
Alveolar brounchiolar adenoma	1	0	0	0
Trachea	(50)	(50)	(50)	(50)
Undifferentiated carcinoma	0	1	0	0
Special sense organs				
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	0	1	0	0
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma adenosquamous	0	0	0	1
Urinary system				
Kidney	(50)	(50)	(50)	(50)
Lipoma	0	0	0	1
Carcinoma, Renal tubule	1	0	0	0
Transitional cell carcinoma	0	0	0	1

( ): Number of organs/tissues examined

Table 8. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Alimentary system				
Cecum	(40)	(37)	(30)	(30)
Dilatation, Crypt	0	0	0	1
Inflammation, Arteritis	0	1	0	0
Duodenum	(50)	(50)	(49)	(49)
Hyperplasia, Lamina propria, Plasma cell	0	1	0	0
Inflammation, Arteritis, Serosa	1	0	0	0
Ulcer	1	0	0	0
Esophagus	(50)	(50)	(50)	(50)
Hyperkeratosis	0	1	0	0
Jejunum	(46)	(42)	(44)	(43)
Calcification, Lymphoid follicle	1	0	0	0
Inflammation, Arteritis, Serosa	1	0	0	0
Liver	(50)	(50)	(50)	(50)
Basophilic focus	6	2	9	11
Clear cell focus	13	13	1	3
Degeneration, Cystic	4	9	0	0
Dilatation, Artery	0	1	0	0
Dilatation, Bile duct	0	0	2	0
Dilatation, Common bile duct	0	0	1	0
Dilatation, Sinusoid, Focal	0	0	0	3
Eosinophilic focus	10	10	4	3
Fibrosis, Bile duct	29	32	20	19
Fibrosis, Capsule, Focal	1	0	0	0
Hematopoietic cell proliferation	0	2	1	4
Hemorrhage	0	0	1	0
Hernia, Median lobe	0	0	0	1
Hyperplasia, Hepatocyte, Reactive	0	2	0	1
Infiltrative cell, Inflammatory cell	1	0	0	0
Infiltrative cell, Mononuclear cell	33	35	31	28
Necrosis, Hepatocyte, Focal	3	2	4	3
Necrosis, Hepatocyte, Multifocal	3	3	2	7
Pigmentation, Sinusoidal cell	1	0	0	0
Proliferation, Bile duct	39	42	31	31
Proliferation, Sinusoidal cell	0	1	3	2
Vacuolated cell focus	1	1	1	0
Vacuolization intracytoplasmic, Hepatocyte, Diffuse	13	15	17	9
Vacuolization intracytoplasmic, Hepatocyte, Focal	2	5	1	3
Vacuolization intracytoplasmic, Hepatocyte, Multifocal	4	4	0	0
Pancreas	(50)	(50)	(50)	(50)
Arteritis	2	2	1	0
Atrophy, Acinus, Diffuse	7	6	6	9
Atrophy, Acinus, Focal	5	10	1	5
Basophilic focus	2	3	0	0
Hyperplasia, Islet cell	2	0	0	0
Infiltrative cell, Fatty	11	11	7	5
Infiltrative cell, Mononuclear cell	2	0	0	0
Pigmentation	1	0	0	0
Vacuolization intracytoplasmic, Acinus, Diffuse	0	0	1	0

( ): Number of organs/tissues examined

Table 8-1. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Stomach	(50)	(50)	(50)	(50)
Calcification, Artery	0	1	0	0
Calcification, Mucosa, Forestomach	0	2	0	0
Calcification, Mucosa, Glandular stomach	0	2	0	0
Dilatation, Glands	3	7	4	1
Edema, Submucosa, Forestomach	0	0	1	0
Erosion, Forestomach	0	0	0	1
Erosion, Glandular stomach	1	1	5	1
Hyperkeratosis, Forestomach	1	1	0	0
Hyperkeratosis, Limiting ridge	0	1	0	0
Hyperplasia, Basal cell, Forestomach, Focal	0	1	0	0
Inflammation, Arteritis, Serosa	1	0	0	0
Metaplasia, Glandular stomach, Intestinal	0	1	0	1
Metaplasia, Glandular stomach, Squamous	1	0	0	0
Regeneration, Mucosa, Glandular stomach	0	1	0	0
Ulcer, Forestomach	2	6	1	5
Ulcer, Glandular stomach	3	1	1	2
Sublingual gland	(50)	(50)	(50)	(50)
Atrophy, Acinus, Diffuse	1	1	1	0
Hyperplasia, Duct, Focal	1	0	0	0
Submandibular gland	(50)	(50)	(50)	(50)
Atrophy, Acinus, Diffuse	3	6	4	9
Tongue	(50)	(50)	(50)	(50)
Arteritis	2	1	0	0
Calcification, Artery	0	2	0	0
Cardiovascular system				
Aorta	(50)	(50)	(50)	(50)
Calcification	1	4	0	0
Heart	(50)	(50)	(50)	(50)
Calcification, Aorta	0	1	0	0
Calcification, Artery	0	2	0	0
Calcification, Myocardium	0	4	0	0
Cardiomyopathy	46	44	17	13
Inflammation, Focal	0	0	1	0
Thrombus, Atrium	0	1	0	1
Endocrine system				
Adrenal gland	(50)	(50)	(50)	(50)
Calcification, Corticomedullary junction, Focal	0	1	0	0
Cyst	0	1	0	1
Dilatation, Sinus, Cortex	0	0	17	18
Fibrosis, Focal	0	1	2	2
Hematopoietic cell proliferation	1	0	0	5
Hyperplasia, Cortex, Focal	9	15	6	8
Hyperplasia, Medulla, Focal	16	11	9	10
Hypertrophy, Cortex, Focal	10	6	0	1
Necrosis, Cortex, Focal	0	0	1	1
Thrombus, Cortex	0	0	1	0
Vacuolization intracytoplasmic, Cortex, Diffuse	2	3	1	2
Vacuolization intracytoplasmic, Cortex, Focal	12	12	4	10

( ): Number of organs/tissues examined

Table 8-2. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Parathyroid gland	(50)	(49)	(50)	(50)
Cyst	0	0	0	1
Hyperplasia, Diffuse	6	9	4	3
Hyperplasia, Focal	0	5	2	6
Pituitary gland	(48)	(50)	(50)	(50)
Cyst, Pars distalis	6	0	1	1
Dilatation, Sinusoid	0	1	1	0
Hemorrhage	0	0	1	0
Hyperplasia, Pars distalis, Focal	9	6	3	3
Pigmentation	0	0	1	0
Thyroid gland	(50)	(50)	(50)	(50)
Dilatation, Follicle	2	0	0	0
Hyperplasia, C-cell, Diffuse	17	28	26	23
Hyperplasia, C-cell, Focal	4	1	4	2
Hyperplasia, Follicular cell, Focal	2	2	0	0
Ultimobranchial cyst	2	3	0	1
Genital system				
Epididymis	(50)	(50)	(−)	(−)
Atrophy	5	13	−	−
Cell debris, Lumen	1	0	−	−
Granuloma spermatic	1	2	−	−
Infiltrative cell, Mononuclear cell	5	5	−	−
Prostate	(50)	(50)	(−)	(−)
Atrophy	0	2	−	−
Hyperplasia, Focal	2	1	−	−
Inflammation	38	37	−	−
Seminal vesicle	(50)	(50)	(−)	(−)
Atrophy	6	6	−	−
Inflammation	1	2	−	−
Testis	(50)	(50)	(−)	(−)
Arteriris	4	8	−	−
Atrophy, Seminiferous tubule	7	12	−	−
Calcification, Seminiferous tubule	0	1	−	−
Granuloma spermatic	0	1	−	−
Hyperplasia, Interstitial cell	1	0	−	−
Ovary	(−)	(−)	(50)	(50)
Atrophy	−	−	39	42
Cyst	−	−	9	10
Uterus	(−)	(−)	(50)	(50)
Atrophy	−	−	12	8
Hyperplasia, Endometrium, Cystic	−	−	4	5
Inflammation, Endometrium	−	−	1	0
Vagina	(−)	(−)	(50)	(50)
Atrophy	−	−	13	10
Cornification	−	−	1	0
Cyst, Serosa	−	−	1	0
Mucification	−	−	3	1

( ): Number of organs/tissues examined

Table 8-3. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Hematopoietic system				
Bone marrow	(50)	(50)	(50)	(50)
Hypercellularity	10	8	14	20
Lymph node	(4)	(1)	(1)	(3)
Dilatation, Sinus, Iliac	2	0	0	0
Dilatation, Sinus, Renal	1	0	0	0
Hyperplasia, Iliac, Plasma cell	0	0	0	1
Hyperplasia, Popliteal, Plasma cell	0	1	0	0
Inflammation, Pancreatic	1	0	0	0
Mesenteric lymph node	(50)	(50)	(50)	(49)
Dilatation, Sinus	0	0	0	1
Inflammation, Arteritis, Serosa	1	0	0	0
Spleen	(49)	(50)	(50)	(49)
Atrophy, Lymphoid follicle	0	1	3	3
Fibrosis, Focal	0	1	0	0
Hematopoietic cell proliferation	37	39	39	28
Inflammation, Arteritis	0	1	0	0
Pigmentation	19	32	44	34
Stromal Hyperplasia	1	0	0	0
Submandibular lymph node	(50)	(50)	(50)	(50)
Cyst, Lymphoid follicle	1	0	0	0
Dilatation, Sinus	1	2	0	0
Hyperplasia, Plasma cell	20	17	15	13
Thymus	(45)	(47)	(47)	(45)
Cyst	0	3	6	5
Hyperplasia, Epithelial cell	2	5	5	9
Inflammation	0	0	0	1
Involution	39	40	43	41
Integumentary system				
Mammary gland	(49)	(50)	(50)	(50)
Dilatation, Duct	11	12	11	1
Hyperplasia, Diffuse	0	0	2	0
Hyperplasia, Focal	2	4	16	7
Inflammation	3	2	12	5
Skin	(50)	(50)	(50)	(50)
Cyst epidermal inclusion	1	0	0	1
Necrosis	1	0	0	0
Ulcer, Tail	1	1	1	1
Subcutaneous tissue	(7)	(9)	(3)	(5)
Abscess	0	1	0	0
Cyst epidermal inclusion	1	0	0	0
Fibrosis	0	0	0	1
Hemorrhage	0	0	0	1
Inflammation	1	1	0	1

( ): Number of organs/tissues examined

Table 8-4. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Musculoskeletal system				
Bone	(5)	(9)	(1)	(0)
Inflammation, Periosteum, Tarsal	4	9	1	0
Femur	(50)	(50)	(50)	(50)
Hyperosteosis	1	0	0	1
Skeletal muscle	(50)	(50)	(50)	(50)
Atrophy	5	8	6	6
Calcification	0	1	0	0
Fibrosis	0	1	0	1
Granuloma	1	0	0	0
Infiltrative cell, Mononuclear cell	3	3	0	0
Inflammation	0	0	1	0
Nervous system				
Brain	(50)	(50)	(50)	(50)
Calcification, Cerebrum	1	1	0	0
Dilatation, Ventricle, Cerebrum	10	14	26	26
Gliosis Cerebrum	0	1	0	0
Hemorrhage, Cerebellum	0	0	0	2
Hemorrhage, Cerebrum	0	0	0	2
Indentation, Hypothalamus	16	17	28	38
Thrombus, Hypothalamus	0	0	0	1
Respiratory system				
Lung	(50)	(50)	(50)	(50)
Calcification, Alveolus	0	2	0	0
Calcification, Artery	0	2	0	0
Exudate, Alveolus	0	1	0	0
Fibrosis, Pleura, Focal	0	1	0	0
Granuloma	1	0	3	0
Hemorrhage, Focal	0	1	0	0
Hyperplasia, Alveolar epithelium, Focal	1	3	0	0
Infiltrative cell, Alveolus, Diffuse, Macrophage	0	5	0	1
Infiltrative cell, Alveolus, Focal, Macrophage	0	1	0	0
Infiltrative cell, Focal, Foam cell	6	7	3	4
Inflammation, Multifocal	0	0	2	4
Metaplasia, Osseous	1	0	0	0
Trachea	(50)	(50)	(50)	(50)
Infiltrative cell, Submucosa, Mononuclear cell	1	0	0	0
Ulcer	0	0	0	1
Special sense organs				
Eye	(50)	(50)	(50)	(50)
Atrophy, Retinal, Diffuse	0	2	0	0
Atrophy, Retinal, Focal	0	2	1	1
Atrophy, Retinal, Peripheral	0	2	11	15
Calcification, Cornea	0	1	0	0
Cataract	4	3	1	1
Inflammation, Anterior chamber	2	2	3	0
Inflammation, Cornea	2	0	0	0
Ulcer, Cornea	0	2	0	0

( ): Number of organs/tissues examined

Table 8-5. Incidence of non-neoplastic lesions in Crj:CD(SD)IGS rats fed a low protein or normal protein diet (continued)

Sex	Male		Female	
Diet	CRF-1	CR-LPF	CRF-1	CR-LPF
Number of animals	50	50	50	50
Harderian gland	(50)	(50)	(50)	(50)
Dilatation, Duct, Focal	0	1	0	0
Granuloma	1	0	0	0
Hyperplasia, Focal	5	3	1	1
Infiltrative cell, Mononuclear cell	14	15	8	7
Inflammation	4	4	16	13
Urinary system				
Kidney	(50)	(50)	(50)	(50)
Calcification, Cortex	1	0	0	0
Calcification, Outer medulla	1	2	1	0
Calcification, Pelvis	2	5	37	33
Cyst	5	3	1	5
Dilatation, Pelvis	3	3	4	5
Granuloma	0	0	0	1
Hyaline droplet, Renal tubule	0	2	3	2
Hyperplasia, Transitional epithelium, Diffuse	8	18	39	33
Hyperplasia, Transitional epithelium, Focal	7	7	4	5
Inflammation, Arteritis	1	0	0	0
Inflammation, Medulla	0	0	0	1
Inflammation, Pelvis	1	1	0	0
Necrosis, Papilla	0	1	0	0
Nephropathy	47	44	30	24
Vacuolization intracytoplasmic, Renal tubule, Diffuse	3	0	0	0
Urethra	(1)	(0)	(0)	(0)
Hemorrhage	1	0	0	0
Urinary bladder	(50)	(50)	(50)	(50)
Dilatation, Lumen	0	0	0	1
Hyperplasia, Transitional epithelium, Diffuse	1	0	0	0
Hyperplasia, Transitional epithelium, Focal	0	0	0	1
Infiltrative cell, Mononuclear cell	3	1	0	0

( ): Number of organs/tissues examined

Data Collection of Crj:CD(SD)IGS Rats Given a Low Protein Diet

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ABSTRACT. This study was performed to collect the basic background data for carcinogenicity study using Crj:CD(SD)IGS rats fed low protein diet (CR-LPF). Mortality of IGS rats at the end of the 104-week observation period was 41.7% in males and 63.3% in females. No clear differences from the NIH open formula diet group in the body weight were observed in the low protein diet group. The organ weight measurement revealed large individual differences in the weight of the spleen and the adrenal gland. As neoplastic lesions, endocrine gland tumors such as pituitary tumors (both sexes) and islet tumors (males) appeared at a high incidence. Mammary gland tumors also appeared at a high incidence. — Key words: Crj:CD(SD)IGS rats, background data, low protein diet, carcinogenicity studies

CD(SD)IGS-2000: 246-251

INTRODUCTION

We previously reported the results of 2-year feeding of Crj:CD(SD)IGS rats using Modified NIH Open Formula Rat and Mouse Ration[1]. Recently, there is a problem of excessive caloric intake of animals due to diet overfeeding in general toxicity studies and carcinogenicity studies[2, 3]. Therefore, low protein diets have been used.

This time, we report the results of 2-year feeding of Crj:CD(SD)IGS rats using a low protein diet CR-LPF (approximately 18% crude protein).

MATERIALS AND METHODS

Animals and housing conditions:

Sixty male and 60 female 4-week-old Crj:CD(SD)IGS rats were purchased from Charles River Japan Inc. After one-week acclimation to the housing environments, they were reared for 2 years starting from 5 weeks of age. The animals were housed in an animal room with a barrier system under the following conditions: temperature of 23 ± 2℃, relative humidity of 55 ± 10%, 20 times per hour ventilation and 12 hours illumination at intensity of 150-300 lux. The animals were housed individually in aluminum cages with stainless steel wire mesh at the front and the floor (W 20.0 × D 28.2 × H 18.0 cm) and given free access to the diet sterilized by radiation CR-LPF (Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

Observations and examinations:

All animals were observed daily for the general condition and weekly palpated. The dead animals were necropsied immediately after they were found and the moribund animals were necropsied after the blood smear specimens were prepared.

The body weight was measured once a week from the start to Week 26 of the experiment and thereafter once every other week until the end of the experiment.

The food consumption was measured once a week.

At the end of the experiment, the animals were fasted for 16 hours and blood was collected under ether anesthesia via the abdominal aorta. EDTA-2K-added blood was analyzed for the hematocrit value (Ht: calculated from RBC and MCV), hemoglobin value (Hb: cyanmethemoglobin method), red blood cell count (RBC: dark-field disk method), mean corpuscular volume (MCV: dark-field disk method), mean corpuscular hemoglobin (MCH: calculated from HGB and RBC), mean corpuscular hemoglobin concentration (MCHC: calculated from HGB and HCT), platelet count (Plt: dark-field disk method), white blood cell count (WBC: flow cytometry method), and differential leukocyte count (flow cytometry method) with THMS H･1E (Miles Laboratories, USA).

Animals killed as scheduled and moribund animals were necropsied after euthanasia by exsanguination under ether anesthesia. The dead animals were necropsied immediately after they were found. The brain, heart, liver, kidneys, spleen, adrenal glands, testes, and ovaries were weighed and the organ weight to body weight ratios were calculated. At necropsy, the body surface, body cavities, etc. were observed and organs and tissues such as peritoneal cavity, thoracic cavity and cranial cavity were checked, and all of the gross findings were recorded along with the site, size and rigidity. The following organs and tissues removed were fixed in an adequate amount of 10% neutral buffered formalin solution: skin, mammary gland, lymph nodes (mesenteric and mandibular), salivary gland (sublingual gland, mandibular gland), sternum, femur, bone marrow, thymus, trachea, lungs and bronchi, heart, thyroid glands, parathyroid glands, tongue, esophagus, stomach, duodenum, small intestine, large intestine, liver, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicle, prostate, testes, ovaries, uterus, vagina, brain, pituitary gland, spinal cord, eyeballs, Harderian gland, and other organs and tissues with macroscopic abnormalities. The tissue preparations were made following the routine method and were stained with hematoxylin and eosin (H.E.). The histopathological examination was performed and all findings were recorded along with the kind and grade.

RESULTS

1. Mortality

No male animals died up to Week 53 of the experiment and no females died up to Week 22 of the experiment. The mortality in males at Week 78 and Week 104 (end) of the experiment was 1.7% and 41.7%, respectively. The mortality in females at Week 52, Week 78 and Week 104 (end) of the experiment was 1.7%, 15.0% and 63.3%, respectively.

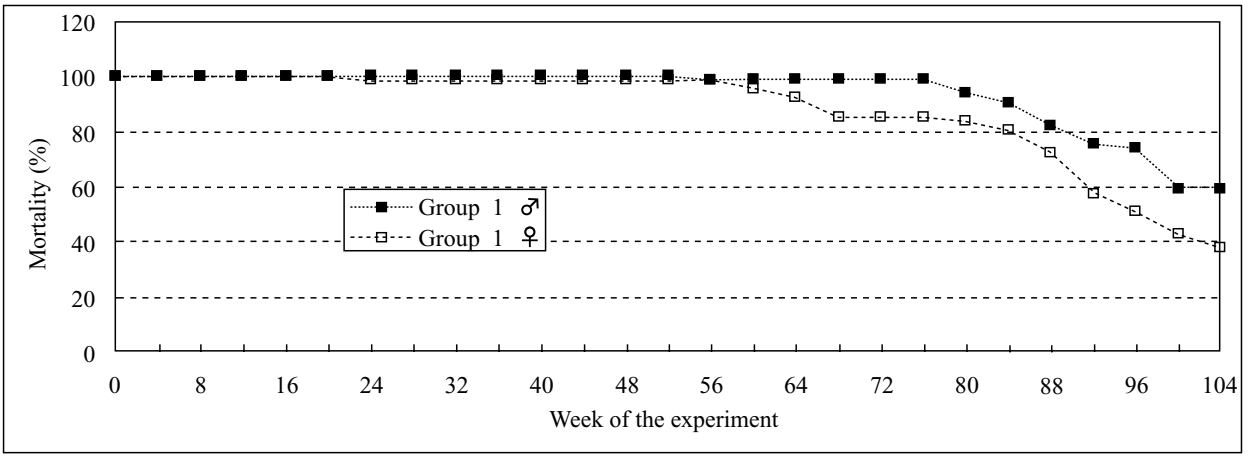

Figure 1. Mortality

2. Observation of general condition

Throughout the experiment, pallor (auricles, etc.) and plantar ulcer in both sexes and subcutaneous tissue masses, wasting, piloerection, eye discharge, decreased spontaneous motor activity and tachypnea in females appeared at a relatively high incidence. Until Week 26 of the experiment, teeth abnormalities and opacity of the eyeballs were observed in males and wasting, and intraabdominal tissue masses were observed in females. From Week 27 to 52 of the experiment, pallor (auricles, etc.), subcutaneous tissue masses and teeth abnormalities were observed in both sexes, opacity of the eyeballs in males, and thickening of the auricles in females. From Week 53 to 78 of the experiment, wasting, piloerection, trauma, opacity of the eyeballs, nodules of the auricles and plantar ulcer were observed in both sexes, tissue masses on the body surface and loose stool in males, and loss of hair, eye discharge, wryneck, staggering gait, decreased spontaneous motor activity, tachypnea, intra-ocular hemorrhage and irregular respiration in females, in addition to the signs observed from Week 27 to 52 of the experiment. From Week 79 to 104 of the experiment, dirty hair, subnormal temperature, eye discharge, loose stool, wryneck, irregular respiration, staggering gait, decreased spontaneous motor activity, tachypnea and dirty nose were observed in both sexes, loss of hair, crust, nodules of the nasal site, prone position, lateral position, induration of the plantar, nodules at the angulus oris, corneal leukoma and corneal erosion in males, and intraabdominal tissue masses, intraoral tissue masses, vaginal prolapse and salivation in females, in addition to the signs observed from Week 53 to 78 of the experiment (excluding loss of hair in females).

3. Body weight

In males, the body weight increased during Week 1-76 of the experiment, tended to increase with repeated increases and decreases during Week 78-98 of the experiment, and decreased after Week 100 of the experiment. In females, the body weight increased until Week 16 of the experiment, tended to increase with

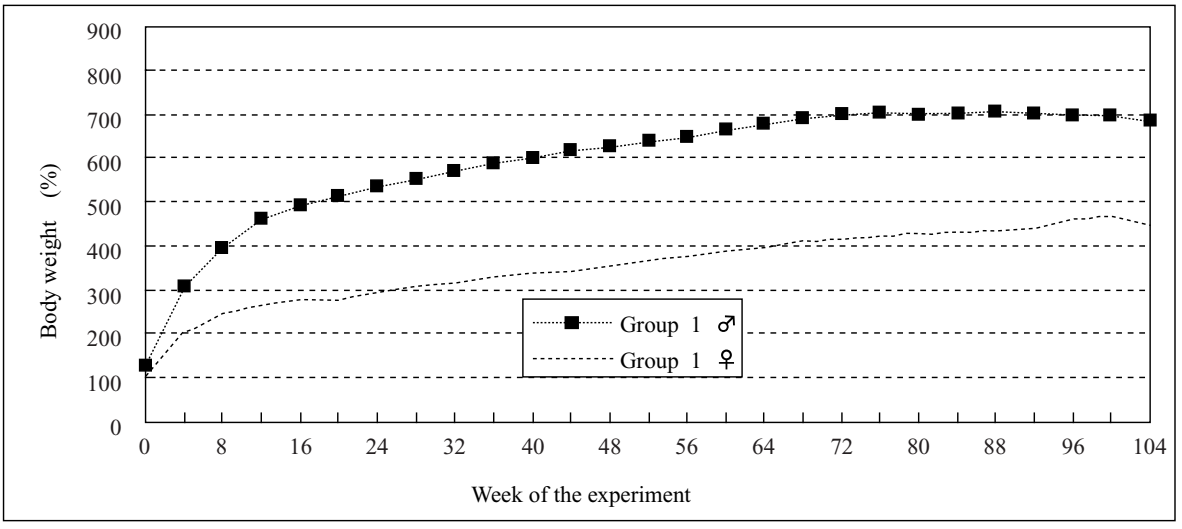

Figure 2. Body weight

repeated increases and decreases during Week 17-100 of the experiment, and decreased after Week 102 of the experiment.

4. Food consumption

In males, the food consumption rose during Week 1-7 of the experiment, fell during Week 8-13 of the experiment, and rose and fell around 150-200 g/week during Week 14-104 of the experiment. In females, the food consumption fell at Week 2 of the experiment, rose during Week 3-7 of the experiment, fell during Week 8-19 of the experiment, and rose and fell around 110-170 g/week during Week 20-104 of the experiment.

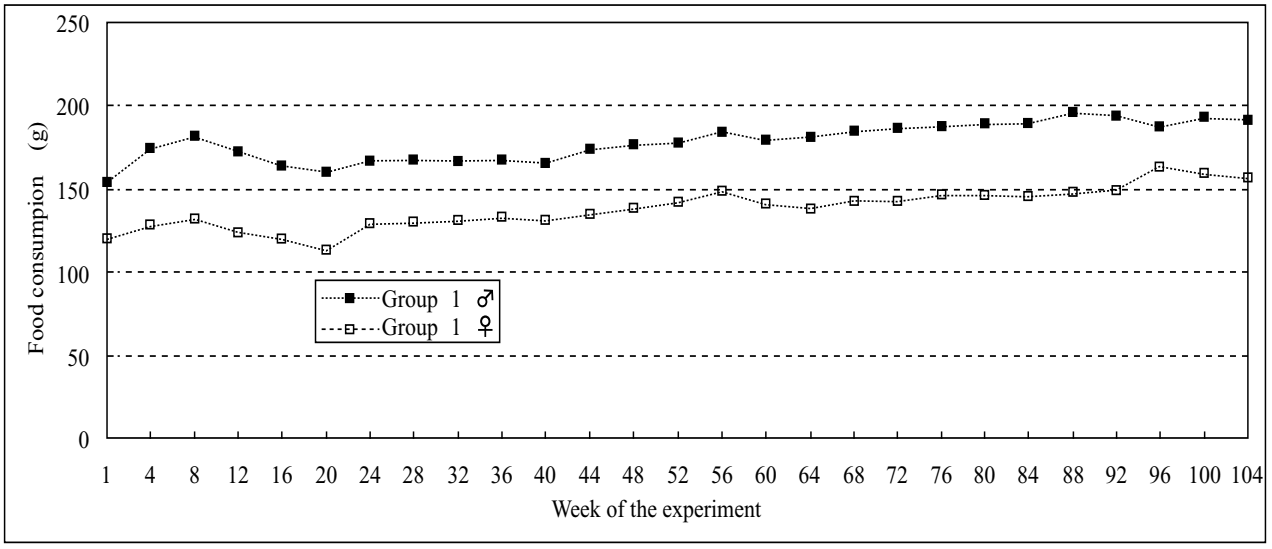

Figure 3. Food consumption

5. Organ weight

The variance was relatively small in the absolute and relative weights of the brain, heart, liver and kidneys in both sexes, testes in males, and ovaries and adrenal glands in females. There were individual differences in the spleen weight in both sexes and the adrenal weight in males.

6. Hematology and pathology

Hematological values are presented in Table 1.

Comparison of the hematological values at 104 revealed significant higher values in MCH and MCHC in female LP rats than in female NIH rats. Differential leukocyte counts revealed significant higher Large unstained cell ratio in both male and female LP rats than in both male and female NIH rats. Futhermore, eosinophil ratio in male LP rats showed sugnificant lower than in male NIH rats, but it was very slight difference.

As gross findings, atrophic thymus appeared at a high incidence in both sexes, nodules in the spleen and hind limbs in males, and hypertrophy of the pituitary and adrenal glands and subcutaneous tissue masses in females. In addition, red patches in the liver and nodules in the pituitary gland were observed in many males and females, white patches in the heart, hypertrophic pituitary and subcutaneous tissue masses in many males, and cysts in the ovaries in many females.

As neoplastic lesions, pituitary tumors showed a high incidence in both sexes, islet tumors in males, and mammary gland tumors in females. The incidence of pituitary adenoma was 48% and 68% and that of pituitary adenocarcinoma was 5% and 13% in males and females, respectively; the incidence of islet adenoma was 33% in males; and the incidence of mammary gland adenocarcinoma was 15%, that of mammary gland fibroadenoma was 37%, and that of mammary gland adenocarcinoma was 30% in females.

As nonneoplastic lesions, cardiomyopathy, increased hematopoiesis in the bone marrow, extramedullary hematopoiesis and deposit of pigment in the spleen, atrophic thymus, accumulation of foamy cells, dilated gland of the stomach, fatty change of liver cells, lymphocytic cellular infiltration, hyperplasia of the bile duct and foci in the liver, deposit of pigment and hypertrophy of zona glomerulosa in the adrenal glands, and ostecartilaginous degeneration in the sternum were observed at a high incidence in both sexes; spongiosis in the liver, chronic nephropathic changes such as glomerulosclerosis, basophilic tubules and hyaline cast in the kidneys, cavitation of the adrenal cortex, hyaline body in the brain and spinal cord, hyperplasia of the Harderian gland and subcutaneous inflammation (hind limbs) in males; and tubular cell hyperplasia in the thymus, concretion and transitional cell hyperplasia in the kidneys, dilatation of the tubules and hyperplasia of the mammary gland, decreased corpus luteum in the ovaries and angiectasis in the adrenal glands in females. In addition, as pro-

liferative lesions, local hyperplasia of the cortex and medullary hyperplasia in the adrenal glands were relatively high in incidence in both sexes and hyperplasia of the pituitary glands and islet in males.

In the animals that died in the course of study, pituitary tumors showed a high incidence in both sexes and mammary gland tumors showed a relatively high incidence in females. These lesions were considered to be the cause of death.

Table 1. Hematological data in CD(SD)IGS rats feeded by NIH Open Formula (NIH) and Low Protein formula (LP)

Item		NIH		LP	
		Male	Female	Male	Female
No. of animals		19	29	35	22
Ht	(%)	40.1±6.7	39.8±4.7	38.3±7.3	39.7±4.3
Hb	(g/dL)	13.4±2.6	13.6±2.1	12.7±3.2	14.0±2.0
RBC	($x10^6/mm^3$)	7.24±1.52	6.96±1.26	7.08±1.55	6.76±1.11
MCV	(μm^3)	56.2±4.7	58.5±8.8	54.7±4.1	59.5±5.9
MCH	(pg)	18.6±1.0	19.7±1.6	17.9±1.8	20.9±1.4**
MCHC	(%)	33.1±1.6	33.9±1.9	32.8±2.9	35.3±1.6
Plt	($x10^3/mm^3$)	1351±297	986±200	1260±292	1080±241
WBC	($x10^3/mm^3$)	11.5±4.2	8.5±4.7	12.3±4.7	10.2±4.1
Differential leukocyte counts(%)					
Neutrophils		39±10	48±14	40±12	46±14
Lymphocytes		51±10	42±14	50±11	44±14
Monocytes		6±2	7±2	6±2	6±2
Eosinophils		1±1	1±1	1±1*	1±1
Basophils		0±0	0±0	0±0	0±0
LUC		2±2	2±1	4±1***	3±1***

LUC:Large unstained cell Values are expressed as Mean±S.D.
Significant difference from control group; *:P≦0.05 **:P≦0.01 ***:P≦0.001

DISCUSSION

The survival rate in males was 58.3%, suggesting a higher value than that in the normal diet (commercially available Modified NIH Open Formula Rat and Mouse Ration with sterilization by radiation) group[1]. The survival rate in females was 36.7%, suggesting no differences from that in the normal diet group[1].

No clear differences from the normal diet group were observed in the body weight or survival rate during the experiment period[1]. As the changes in the general condition, subcutaneous tissue mass appeared at a high incidence (50%) in females. Plantar ulcer also showed a high incidence, i.e., 42% in males and 15% in females, but the incidence was within the range of our historical data.

The organ weight measurement revealed large individual differences in the weights of the spleen in both sexes and the adrenal gland in males. The large variance in the spleen was due to hypertrophy of the concerned organ attributable to reactive extramedullary hematopoiesis related to inflammation, hemorrhage or increase in blood requirement, because inflammation in the hind limbs and mammary gland tumors in males and mammary gland tumors in females were frequently observed. The large variance in the adrenal gland in males was due to hypertrophy of the concerned organ accompanying the cortical or medullary tumor. On the other hand, individual differences were relatively small in the absolute and relative weights of the brain, heart, liver and kidneys in both sexes, the testes in males, and the ovaries and adrenal glands in females. These suggested that there was little difference in the incidence of the lesions that appeared as organ weight changes.

As neoplastic lesions, endocrine gland tumors such as pituitary tumors (both sexes) and islet tumors (males) appeared at a high incidence. Mammary gland tumors, which were endocrinologically subordinate to the pituitary gland, also appeared at a high incidence. As nonneoplastic lesions, adrenal cortical and/or medullary hyperplasia in both sexes and hyperplasia of the pituitary gland and islet in males were observed at a relatively high incidence. All of these suggested endocrine system abnormalities. Pituitary tumor was suspected as the cause of death in this study, even in the animals that died before Week 78 of the experiment. Furthermore, sexual differences were suggested from the higher incidence of pituitary and mammary gland tumors in females and islet tumor in males.

Nonneoplastic lesions suggestive of hematopoietic hyperactivity such as increased hematopoiesis in the bone marrow and extramedullary hematopoiesis in the spleen were observed in many males and females. Most of the males had accompanying inflammation in the hind limbs and most of the females had accompanying large mammary gland tumors, suggesting responsive hematopoietic hyperfunction. Nonneoplastic lesions such as cardiomyopathy, spongiosis and lymphocytic cellular infiltration in the liver, chronic nephropathic changes, islet hyperplasia and inflammation in the hind limbs were more frequently observed in males, and deposit of pigment in the spleen, microgranuloma in the liver, concretion and transitional cell hyperplasia in the kidneys, dilatation of the tubules and hyperplasia of the mammary gland, and angiectasis, cortical cystic degeneration and local hypertrophy in the adrenal glands were more frequently observed in females, suggesting sexual differences.

Table 2. Summary of neoplastic findings

		Male	Female
No. of animals examined		60	60
Organ	Findings		
HEMATOPOIETIC SYSTEM			
spleen			
	LGL leukemia	1	0
	histiocytic sarcoma	0	1
	liposarcoma	1	0
lymph node			
	malignant lymphoma	1	0
DIGESTIVE SYSTEM			
stomach			
	squamous cell papilloma	0	1
exocrine pancreas			
	adenoma	2	0
	histiocytic sarcoma	0	1
liver			
	hepatocellular adenoma	3	1
	hepatocellular carcinoma	3	1
	histiocytic sarcoma	0	1
URINARY SYSTEM			
kidney			
	lipoma	1	1
	nephroblastoma	0	1
REPRODUCTIVE SYSTEM			
mammary gland			
	adenoma	0	9
	fibroadenoma	1	22
	fibroma	1	0
	adenocarcinoma	0	18
testis			
	interstitial cell tumor	1	-
prostate			
	adenoma	1	-
coagulation gland			
	adenoma	1	-
ovary			
	papilloma	-	1
uterus			
	endometrial stromal polyp	-	2
	fibroma	-	1
	malignant schwannoma	-	1
ENDOCRINE SYSTEM			
pituitary gland			
	adenoma	29	41
	adenocarcinoma	3	8

Table 3. –continued Summary of neoplastic findings

Organ	Findings	Male	Female
No. of animals examined		60	60
ENDOCRINE SYSTEM			
thyroid gland			
	C-cell adenoma	3	2
	C-cell carcinoma	1	1
adrenal gland			
	adenoma	4	2
	pheochromocytoma	8	0
	adenocarcinoma	1	0
	malignant pheochromocytoma	3	0
pancreatic islet			
	adenoma	20	1
	adenocarcinoma	1	1
NERVOUS SYSTEM			
brain			
	astrocytoma	0	1
SPECIAL SENSE SYSTEM			
ear			
	amelanotic melanoma	0	2
	melanoma, malignant	1	0
Zymbal's gland			
	adenoma	1	-
INTEGUMENTARY SYSTEM			
skin			
	basal cell epithelioma	2	0
	keratoacanthoma	1	0
	squamous cell papilloma	3	0
	squamous cell carcinoma	0	1
subcutaneous tissue			
	fibroma	1	0
	lipoma	2	0
	fibrosarcoma	2	0
	histiocytic sarcoma	1	0
	osteosarcoma	1	0
	malignant schwannoma	1	0

The appearance of lesions in this study in which a low protein diet was used was similar to that in the study in which a standard diet was used.

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A Comparison of Survival Rates and Body Weights in Crj:CD(SD)IGS Rats among Carcinogenicity Studies in Five Different Facilities

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ABSTRACT. Survival rates and body weights of Crj:CD(SD)IGS (IGS) rats were compared among ten 104-week studies for background data of carcinogenicity tests in 5 different facilities. In seven out of 10 studies, the rats were allowed free access to the diets. In the remaining 3 studies, the rats were restricted to access the diets. All rats in these studies were maintained under the barrier-sustained animal rooms. At weeks 28, 52, 80, and 104, no apparent differences of survival rates in male and female IGS rats were detected among 7 studies without diet restriction and among 3 studies with diet restriction. At weeks 4, 28, 52, 80, and 104, no apparent differences of body weights in male and female IGS rats were detected among the studies without diet restriction and among the studies with diet restriction. Consequently, cage size, number of animals in a cage (one or two per cage), or brands of commercial diets (different protein contents or calories) did not influence remarkably the survival rates and body weights of IGS rats in the104-week carcinogenicity studies. However, the diet restriction had a great advantage in the survival rates and suppressed markedly the body weight gain in male and female IGS rats in the 104-week carcinogenicity studies. — Key words: Background data, Body weight, Carcinogenicity study, IGS rat, Survival rate.

CD(SD)IGS-2000: 252-256

INTRODUCTION

The international genetic standard system, which has been developed by Charles River, Inc., is a new breeding procedure of laboratory rats. The system makes it possible to produce uniform laboratory rats owing to the genetic ramification control. Crj:CD(SD) IGS (IGS) rats have been induced by the international genetic standard system and are expected to meet internationalization of research and development of new drugs. Background data on survival rate and body weight gain are essential for the 104-week carcinogenicity tests in the rats [1-9], however, those in IGS rats have not been fully accumulated yet. In addition, interlaboratory heterogeneities may concern any biological data of laboratory animals from different facilities. Therefore, we compared the survival rates and body weights of IGS rats among ten 104-week studies for background data of carcinogenicity tests in 5 different facilities.

MATERIALS AND METHODS

Male and female Crj:CD(SD)IGS rats were purchased from Charles River Japan, Inc. at 4 weeks of age. The rats were maintained in barrier-sustained animal rooms controlled at 21 - 25℃ and 30 - 70% relative humidity, and ventilated 10 times per hr, with a 12-hr light-dark cycle at 5 different facilities in Japan (Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo), Shizuoka, Bozo Research Center Inc. (Bozo), Shizuoka, Mitsubishi Chemical Safety Institute Ltd. (MSI), Ibaraki, Nippon Institute for Biological Science (NIBS), Tokyo, and Shin Nippon Biomedical Laboratories, Ltd. (SNBL), Kagoshima). All rats were used as control animals in 10 different 104-week studies for background data of carcinogenicity tests. The experimental conditions, such as the number of animals used, caging, brands of diet (protein contents and calories), feeding methods, and ages at the start of the studies in each study are shown in Table 1. Commercial diets used in these studies were Modified NIH Open Formula Rat and Mouse Ration (NIH) (Oriental Yeast Co., Ltd., Tokyo, Japan), CR-LPF with γ-ray irradiation or autoclaved (CR-LPF) (Oriental Yeast Co., Ltd.), Certified Rodent Chow #5002 with γ-ray irradiation (#5002) (Purina Mills, MO, U.S.A.), and CE-2 with γ-ray irradiation (Clea Japan, Inc., Tokyo, Japan). In seven out of 10 studies, the rats were allowed free access to the diets. The remaining 3 studies were performed as the diet restriction studies, in which male rats were fed 22 g of the diet daily and female rats were fed 16 g of the diet daily throughout the studies according to the previously reported method [5].

All rats were observed for clinical signs and mortality once or twice daily. Body weight was measured once weekly or monthly. Survival rates and body weights were compared among different studies at 28, 52, 80, and 104 weeks and 4, 28, 52, 80, and 104 weeks, respectively.

RESULTS AND DISCUSSION

Survival rate

Survival rates of IGS rats in ten 104-week studies from 5 different facilities are shown in Fig. 1. The mean survival rates of male IGS rats in 7 studies without diet restriction were 98.9 ± 1.95% (ranging from 96 to 100%) at week 28, 97.3 ± 2.63% (94 to 100%) at week 52, 82.1 ± 4.91% (78 to 90%) at week 80, and 40.1 ± 14.03% (15 to 56%) at week 104. Those of male IGS rats in 3 studies with diet restriction were 100 ± 0% at week 28, 97.3 ± 4.62% (92 to 100%) at week 52, 92.0 ± 3.46% (88 to 94%) at week 80, and 79.0 ± 5.57% (73 to 84%) at week 104. The mean survival rates of female IGS rats in 7 studies without diet restriction were 99.4 ± 0.98% (98 to 100%) at week 28, 98.6 ± 1.51%

(96 to 100%) at week 52, 85.3 ± 7.59% (78 to 96%) at week 80, and 53.1 ± 7.54% (42 to 65%) at week 104. Those of female IGS rats in 3 studies with diet restriction were 100 ± 0% at week 28, 99.7±0.58% (99 to 100%) at week 52, 94.3±3.79% (90 to 97%) at week 80, and 80.3 ± 5.69% (74 to 85%) at week 104. In seven studies without diet restriction, no apparent differences of the survival rates in male and female IGS rats were observed among the studies at each observation period. No apparent differences of the survival rates in male and female IGS rats were also observed among 3 diet restriction studies at each observation period. These results suggest that the cage size, numbers of rats in a cage (one or two per a cage), diet protein contents (ranging from 18 to 25%) or calories of the diets (ranging from 345 to 410 kcal/100g) may not be effective factors on the survival rates of male and female IGS rats. Sprague-Dawley rats fed a modified diet containing lower protein, fat, metabolizable energy and increased fiber by *ad libitum* did not have significantly improved survival over the rats fed the standard diet *ad libitum* at week 106 [6]. However, the survival rates of male and female IGS rats were markedly higher in the diets restriction studies than in the studies without diet restriction at week 104. Therefore, the diet restriction may have a great advantage in the survival rates of male and female IGS rats.

Body weight

Body weights of IGS rats in ten 104-week studies from 5 different facilities are shown in Fig. 2. The mean body weights of male IGS rats in 7 studies without diet restriction were 336.3 ± 14.2 g (ranging from 322 to 360 g) at week 4, 613.0 ± 30.5 g (573 to 655 g) at week 28, 702.6±40.3 g (657 to 759 g) at week 52, 743.3 ± 48.2 g (680 to 800 g) at week 80, and 736.0 ± 39.4 g (689 to 793 g) at week 104. Those of male IGS rats in 3 studies with diet restriction were 274.3±9.5 g (265 to 284 g) at week 4, 464.3 ± 49.7 g (407 to 494 g) at week 28, 504.0 ± 33.2 g (466 to 527 g) at week 52, 497.0 ± 16.1 g (479 to 510 g) at week 80, and 427.0 ± 16.7 g (409 to 442 g) at week 104. The mean body weights of female IGS rats in 7 studies without diet restriction were 220.9 ± 9.0 g (210 to 233 g) at week 4, 336.9 ± 18.9 g (312 to 362 g) at week 28, 402.3±30.0 g (361 to 436 g) at week 52, 460.7 ± 26.3 g (424 to 499 g) at week 80, and 483.9 ± 38.2 g (439 to 547 g) at week 104. Those of female IGS rats in 3 studies with diet restriction were 182.7 ± 1.2 g (182 to 184 g) at week 4, 256.3 ± 18.0 g (244 to 277 g) at week 28, 271.7 ± 4.5 g (267 to 276 g) at week 52, 275.3±22.2 g (262 to 301 g) at week 80, and 262.7 ± 23.8 g (247 to 290) at week 104. In seven studies without diet restriction, no marked differences were observed in the body weights of male and female IGS rats among the studies at each observation period. No apparent differences of the body weights in male and female IGS rats were also detected among 3 diet restriction studies at each observation period. These results suggest that differences in the cage size, numbers of rats in a cage, diet protein contents or calories of the diets may not be reflected in the body weights of male and female IGS rats. However, the body weights of male and female IGS rats were markedly lower in the diets restriction studies than those in the studies without diet restriction during the study period.

To investigate the interlaboratory heterogeneities in 104-week carcinogenicity studies of IGS rats, survival rates and body weights of male and female IGS rats were compared among 10 studies performed in 5 different facilities. There were no apparent differences of the survival rates and body weights in male and female IGS rats among 7 studies without diet restriction and among 3 diet restriction studies. Consequently, caging and brands of commercial diets are not so important factors affecting the survival rates and body weights of male and female IGS rats in the 104-week carcinogenicity study.

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Table 1. Summary of study conditions in five different facilities

Facility name	An-Pyo[a]	Bozo[b]		MSI[c]	
Study name	A	B	M1	M2	M3
Number of rats (male, female)	50, 50	100, 100	50, 50	50, 50	50, 50
Cage size (W, D, H cm)	20.0, 28.2, 18.0	25.4, 35.0, 17.0	26.5, 42.6, 20.0	26.5, 42.6, 20.0	26.5, 42.6, 20.0
Number of rats in a cage	1	1	2	2	2
Brand of diets	NIH[f]	CR-LPF[g]	CR-LPF[h]	CR-LPF[h]	CR-LPF[h]
Protein contents (%)	20	18	18	18	18
Calorie of diets (kcal/100 g)	352	349	349	349	349
Feeding method	Free access	Free access	Free access	Free access	Free access
Age at start (weeks)	5	6	5	5	5

Facility name	NIBS[d]	SNBL[e]			
Study name	N	S1	S2	S3	S4
Number of rats (male, female)	50, 50	20, 20	100, 100	50, 50	50, 50
Cage size (W, D, H cm)	21.0, 35.0, 20.0	19.5, 32.5, 18.0	19.5, 32.5, 18.0	19.5, 32.5, 18.0	19.5, 32.5, 18.0
Number of rats in a cage	1	1	1	1	1
Brand of diets	CR-LPF[g]	CE-2[i]	#5002[j]	CE-2	#5002
Protein contents (%)	18	25	20	25	20
Calorie of diets (kcal/100 g)	349	345	410	345	410
Feeding method	Free access	Free access	Restriction	Restriction	Restriction
Age at start (weeks)	5	5	5	5	5

[a]Biosafety Research Center, Foods, Drugs and Pesticides.
[b]Gotenba Laboratory, Bozo Research Center, Inc.
[c]Mitsubishi Chemical Safety Institute Ltd.
[d]Nippon Institute for Biological Science.
[e]Shin Nippon Biomedical Laboratories, Ltd.
[f]Modified NIH Open Formula Rat and Mouse Ration (Oriental Yeast Co., Ltd.).
[g]CR-LPF with γ-ray irradiation (Oriental Yeast Co., Ltd.).
[h]Autoclaved CR-LPF (Oriental Yeast Co., Ltd.).
[i]CE-2 with γ-ray irradiation (Clea Japan, Inc.).
[j]Certified Rodent Chow #5002 with γ-ray irradiation (Purina Mills).

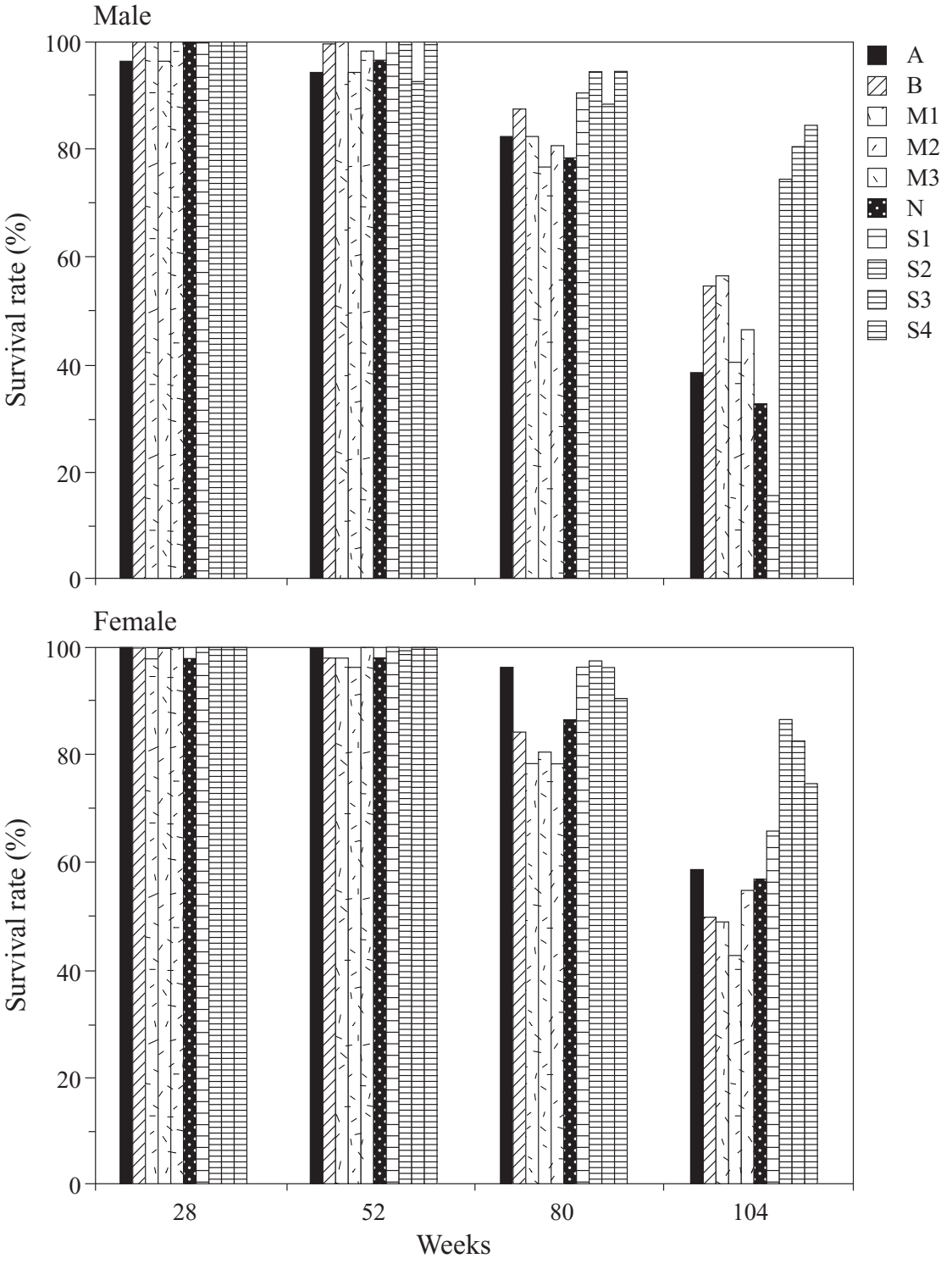

Figure 1. Survival rate of IGS rats in 5 different facilities. Ten studies in 5 different facilities showing as follows: A (■); An-Pyo, B (▨); Bozo, M1-3 (▽); MSI, N (▩); NIBS, and S1-4 (▭and▤); SNBL. Rats were allowed free access to the diets in the A, B, M1-3, N, and S1 studies. Rats were restricted to ingest the diet in the S2-4 studies.

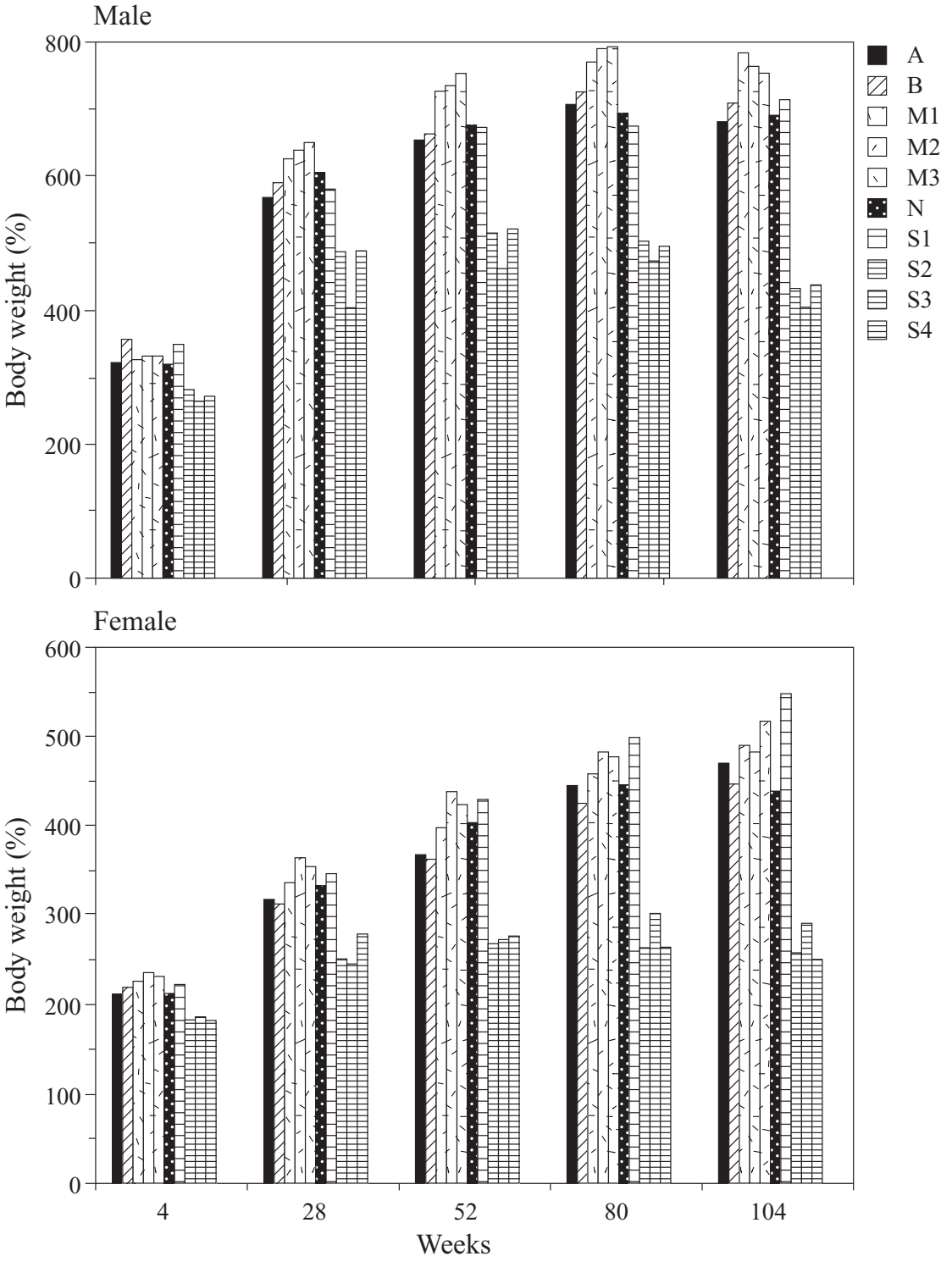

Figure 2. Body weight of IGS rats in 5 different facilities. Ten studies in 5 different facilities showing as follows: A (■); An-Pyo, B (▨); Bozo, M1-3 (▽); MSI, N (▩); NIBS, and S1-4 (▭and▤); SNBL. Rats were allowed free access to the diets in the A, B, M1-3, N, and S1 studies. Rats were restricted to ingest the diet in the S2-4 studies.

Different Survival Rates in Crj:CD(SD)IGS Rats Fed Two Types of Commercial Diets with Different Protein Content, CR-LPF or CRF-1, for 104 Weeks

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ABSTRACT. To examine the survival rates in Sprague-Dawley rats when two types of commercial diets with different protein content were supplied, Crj:CD(SD)IGS rats were fed CRF-1 (crude protein: 23.1%) or low protein diet CR-LPF (crude protein: 18.4%) for 104 weeks. In addition, the survival rates were compared to those in Crj:CD(SD) rats fed CRF-1. The terminal survival rate in Crj:CD(SD)IGS rats fed CR-LPF was 54.0% in males and 49.0% in females and considerably higher than that in Crj:CD(SD)IGS or Crj:CD(SD) rats fed CRF-1. A high survival rate in Crj:CD(SD)IGS rats fed CR-LPF corresponded to low body weight gains. — Key words: Crj:CD(SD)IGS rats, survival rate, body weight, 104-week observation period

CD(SD)IGS-2000: 257-262

INTRODUCTION

It is known that overfeeding or overnutrition in laboratory rats and mice causes early deaths, increased incidence of neoplasms, and lesions of the kidney and endocrine system [1] and that calorie-restriction prolongs life, and reduces the incidences of ageing-related non-neoplastic and neoplastic diseases [2]. Therefore, it is easy to expect that longevity would be improved by reducing body weight gain. Our previous study revealed that the reduced body weight gain could be achieved in Crj:CD(SD)IGS rats by supplying low protein diet (CR-LPF) instead of a diet with relatively high protein content (CRF-1) [3]. In this paper, different survival rates in Crj:CD(SD)IGS rats are described in relation to the different body weight gain caused by feeding two types of diets with different protein content. In addition, the survival rate was compared to that in Crj:CD(SD) rats fed ordinary commercial diet CRF-1.

MATERIALS AND METHODS

Animals and Husbandry: In experiment I, 100 male and 100 female Crj:CD(SD)IGS rats, at 4 weeks of age, were obtained (October 2 and 9, 1996) from Charles River Japan Inc. (Hino Breeding Center, Japan). The animals were acclimatized for 2 weeks and healthy animals were used at 6 weeks of age. The animals were housed individually in hanging stainless-steel wire mesh cages in an animal room under the following conditions: temperature at 23 ± 3℃, relative humidity at 50 ± 20%, air ventilation at 10 to 15 times per hour and 12-hour illumination (07:00 to 19:00). The animals were supplied commercial low protein feed (approximately 18% crude protein), CR-LPF (Oriental Yeast Co., Ltd., Japan), and tap water *ad libitum* for 104 weeks. In experiment II, 75 male and 75 female Crj:CD(SD)IGS rats (IGS rats) and 75 male and 75 female Crj:CD(SD) rats (CD rats), at 4 weeks of age, were obtained (July 27, 1998) from Charles River Japan Inc. (Hino Breeding Center, Japan). The animals were housed in the same manner in experiment II, excepting that both IGS and CD rats were supplied ordinary commercial diet CRF-1 (approximately 23% crude protein) *ad libitum* for 104 weeks.

Observations and examinations:

General condition: The general condition of the animals was checked daily. The animals found dead or moribund were necropsied soon after discovery.

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.

RESULTS AND DISCUSSION

Survival rate: The survival rates in IGS rats fed CRF-1 or CR-LPF diet and those in CD rats fed CRF-1 are shown in Figures 1 (males) and 2 (females). The survival rates in male rats are summarized in the following Table 1-1.

Table 1-1. Summary of the survival rates in male Crj:CD(SD)IGS and Crj:CD(SD) rats.

Animal	Crj:CD(SD)IGS		Crj:CD(SD)
Diet	CR-LPF	CRF-1	CRF-1
Experimental No.[a)]	I	II	II
No. of animals used	100	75	75
Week of experiment			
0 (initial)	100[b)]	100	100
26	100	98.7	97.3
52	99.0	97.3	90.7
78	87.0	72.0	69.3
91	81.0	53.3	41.3
104	54.0	29.3	22.7

a): Experiment I was carried out from October 1996 to October 1998 and Experiment II was carried out from August 1998 to August 2000.
b): Survival rate (%)

The terminal survival rate in male IGS rats fed CR-LPF was 54.0% and was the highest among the three groups. The terminal survival rate in male IGS rats decreased to 29.3% when the ordinary commercial diet CRF-1 was supplied and was approximately half of that for CR-LPF. The terminal survival rate in male CD rats fed CRF-1 was 22.7% and slightly lower than that in IGS rats fed CRF-1.

The survival rates in female rats are summarized in the following Table 1-2.

Table 1-2. Summary of the survival rates in female Crj:CD(SD)IGS and Crj:CD(SD) rats.

Animal	Crj:CD(SD)IGS		Crj:CD(SD)
Diet	CR-LPF	CRF-1	CRF-1
Experimental No.[a)]	I	II	II
No. of animals used	100	75	75
Week of experiment			
0 (initial)	100[b)]	100	100
26	100	98.7	100
52	98.0	96.0	98.7
78	85.0	81.3	62.7
91	69.0	60.0	36.0
104	49.0	38.7	25.3

a): Experiment I was carried out from October 1996 to October 1998 and Experiment II was carried out from August 1998 to August 2000.
b): Survival rate (%)

The terminal survival rates in female rats were similar to those in males. That is, the terminal survival rate in female IGS rats fed CR-LPF was 49.0% and was the highest among the three groups. The terminal survival rate in female IGS rats decreased to 38.7% when the ordinary commercial diet CRF-1 was supplied and lower than that for CR-LPF. The terminal survival rate in female CD rats fed CRF-1 was 25.3% and lower than that in IGS rats fed CRF-1 and approximately half of that for IGS rats fed CR-LPF.

Body weights: The growth curves in IGS rats fed CRF-1 or CR-LPF diet and those in CD rats fed CRF-1 are shown in Figures 3 (males) and 4 (females). The changes in body weight in male rats are summarized in the following Table 2-1.

Table 2-1. Summary of body weight changes in male Crj:CD(SD)IGS and Crj:CD(SD) rats.

Animal	Crj:CD(SD)IGS		Crj:CD(SD)
Diet	CR-LPF	CRF-1	CRF-1
Experimental No.[a)]	I	II	II
No. of animals used	100	75	75
Week of experiment			
0 (initial)	183±7[b)]	214±11	213±14
26	585±57	675±75	724±86
52	669±79	805±117	875±132
78	730±99	835±150	888±168
91	737±103	825±152	856±141
104	716±110	814±157	804±132
Gain (104 weeks)	533±109	603±150	598±127

a): Experiment I was carried out from October 1996 to October 1998 and Experiment II was carried out from August 1998 to August 2000.
b): Mean±S.D. (unit: g)

The mean terminal body weight in males in IGS rats fed CR-LPF, IGS rats fed CRF-1 and CD rats fed CRF-1 was 716, 814 and 804g, respectively, and the body weight in IGS rats fed CR-LPF was the lowest among the three groups. The body weight gain during a 104-week observation period was the lowest in IGS rats fed CR-LPF, followed by IGS and CD rats fed CRF-1.

The changes in body weight in female rats are summarized in the following Table 2-2.

The mean terminal body weight in females in IGS rats fed CR-LPF, IGS rats fed CRF-1 and CD rats fed CRF-1 was 446, 529 and 667g, respectively, and the body weight of IGS rats fed CR-LPF was the lowest among the three groups. The body weight gain during a 104-week observation period was lowest in IGS rats fed CR-LPF, followed by IGS rats fed CRF-1 and then by CD rats fed CRF-1.

Food consumption: The food consumption in IGS rats fed CR-LPF or CRF-1 and that in CD rats fed CRF-1 is shown in Figures 5 (males) and 6 (females). In males, the food consumption in IGS rats fed CR-LPF was the lowest of the three groups. The food consumption in IGS rats fed CRF-1 was lower than in CD rats fed

Table 2-2. Summary of body weight changes in female Crj:CD(SD)IGS and Crj:CD(SD) rats.

Animal	Crj:CD(SD)IGS		Crj:CD(SD)
Diet	CR-LPF	CRF-1	CRF-1
Experimental No.[a)]	I	II	II
No. of animals used	100	75	75
Week of experiment			
0 (initial)	141±7[b)]	156±10	168±11
26	306±33	351±43	424±80
52	361±47	442±74	552±141
78	419±65	502±106	604±203
91	444±80	532±139	693±173
104	446±88	529±126	667±182
Gain (104 weeks)	304±86	374±123	501±180

a): Experiment I was carried out from October 1996 to October 1998 and Experiment II was carried out from August 1998 to August 2000.
b): Mean±S.D. (unit: g)

CRF-1. In females, the food consumption in IGS rats fed CR-LPF was the lowest of the three groups. The food consumption in IGS rats fed CRF-1 was comparable to that in CD rats fed CRF-1 for the first half, but became lower for the latter half.

As stated above, a high survival rate in Crj:CD(SD)IGS rats fed CR-LPF was coincident with small body weight gain accompanied by low food consumption for both sexes. This was also true for female IGS and CD rats fed CRF-1; that is, the survival rate was higher in IGS rats, and the body weight gain and food consumption were smaller in IGS rats than in CD rats. Therefore, it was suggested that the most important factor affecting the survival rate was body weight gain and low body weight gain might be attributable to a relatively poor appetite for CR-LPF rather than low protein content.

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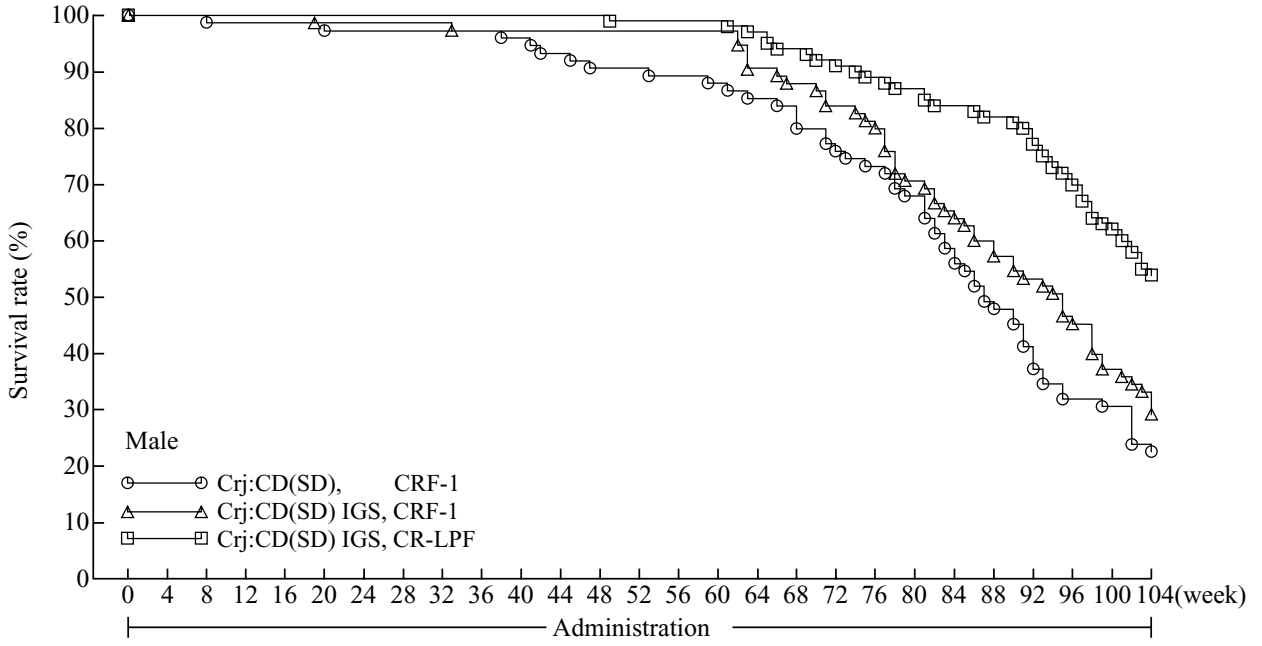

Figure 1. Survival rate of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

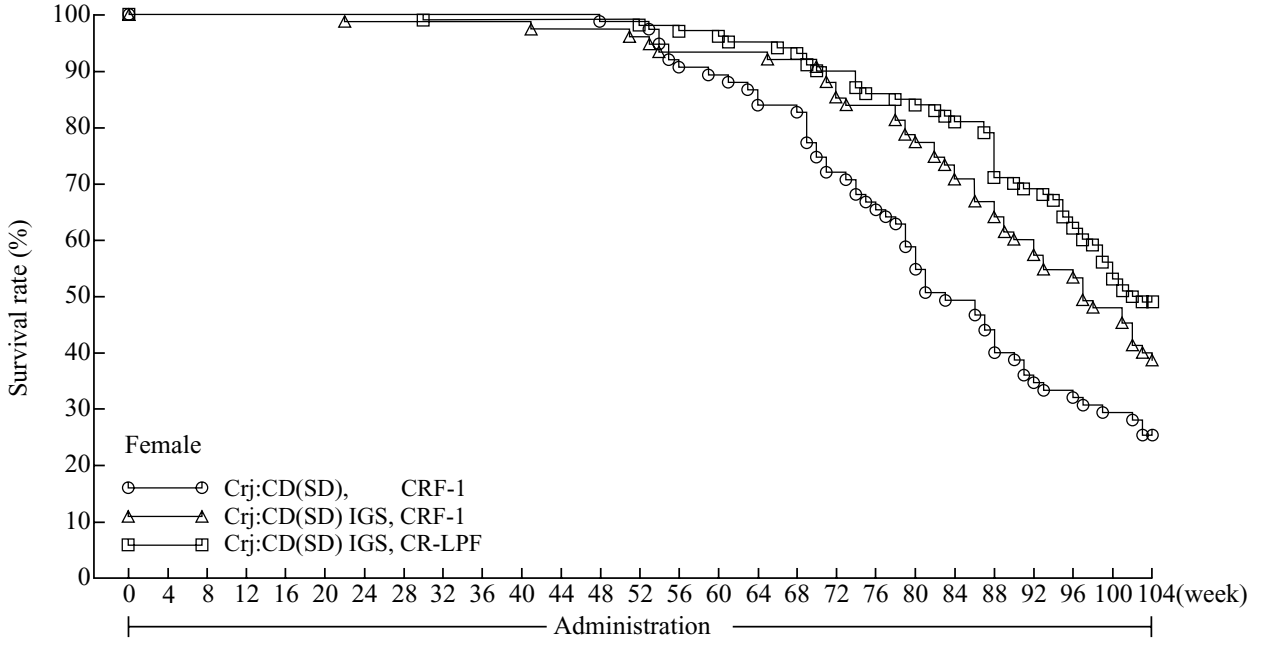

Figure 2. Survival rate of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

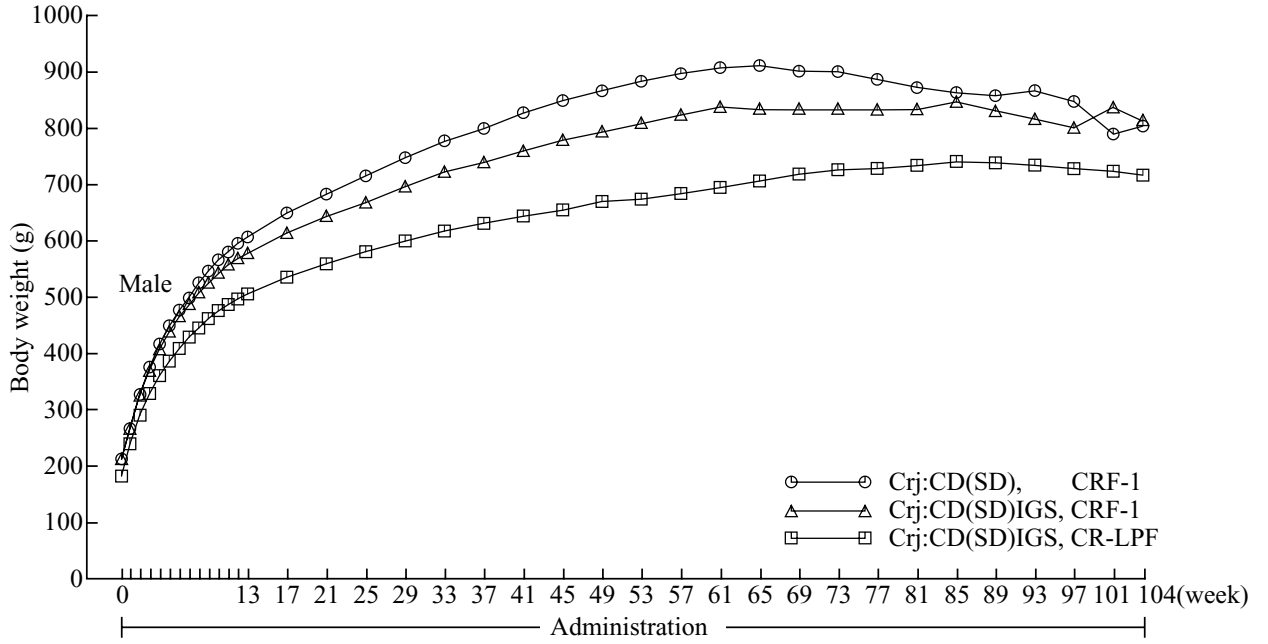

Figure 3. Body weight of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

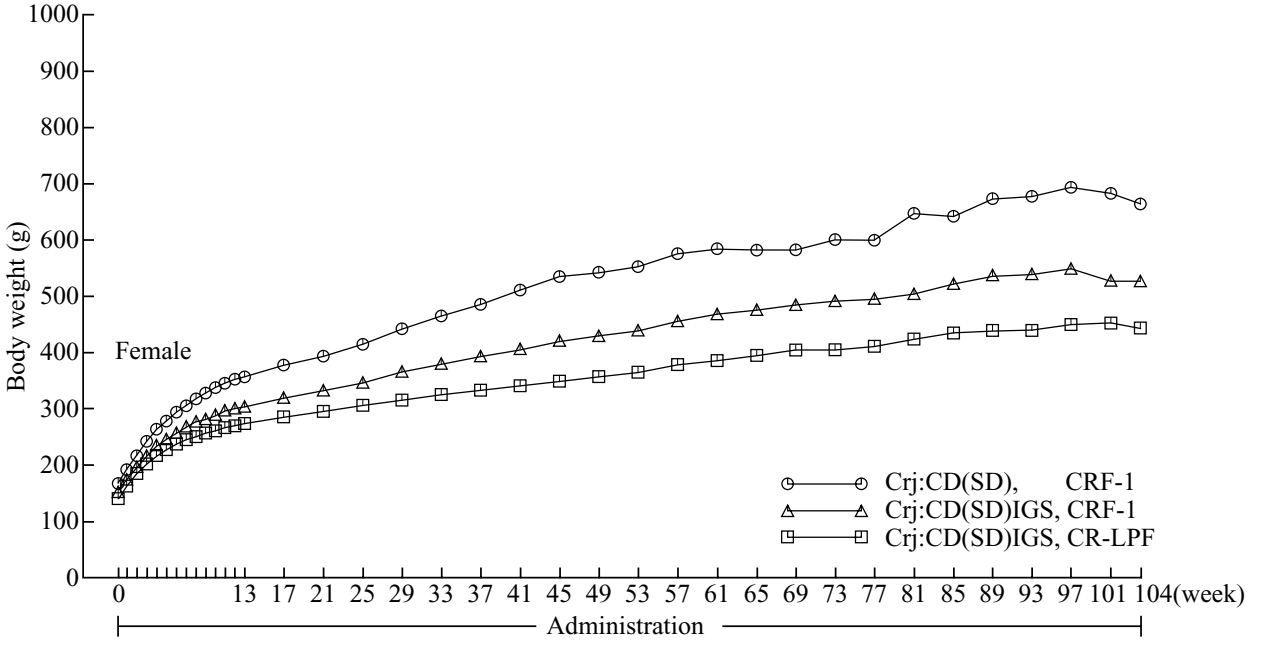

Figure 4. Body weight of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

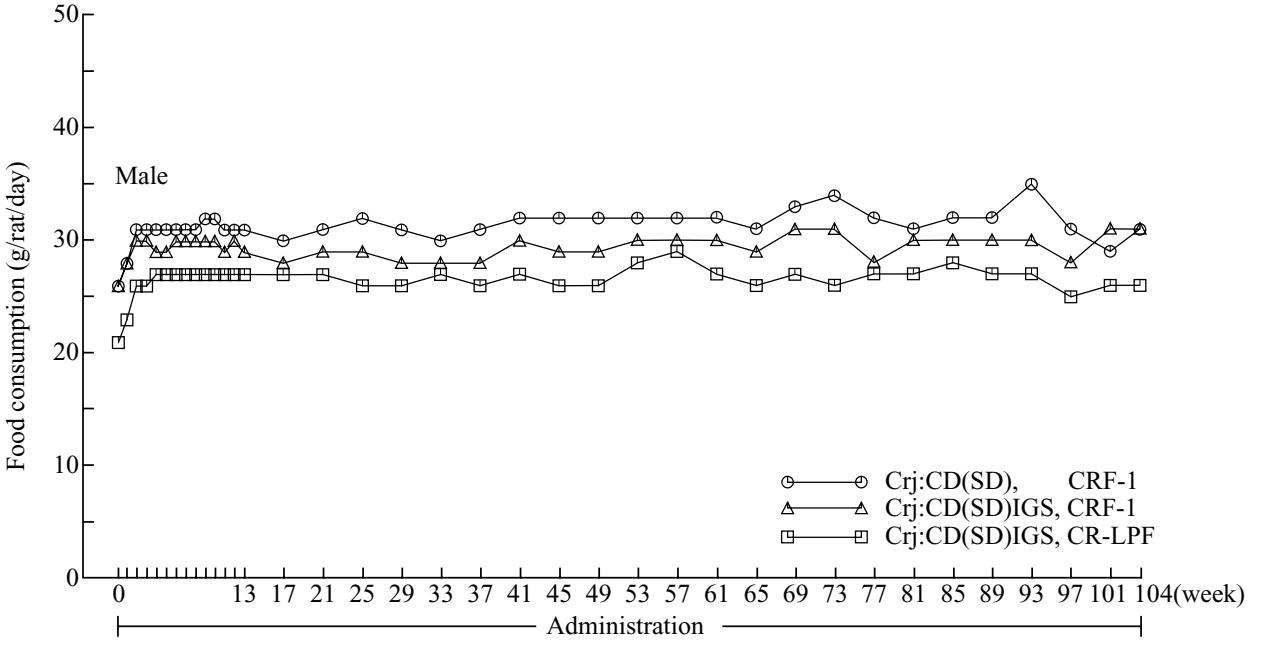

Figure 5. Food consumption of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

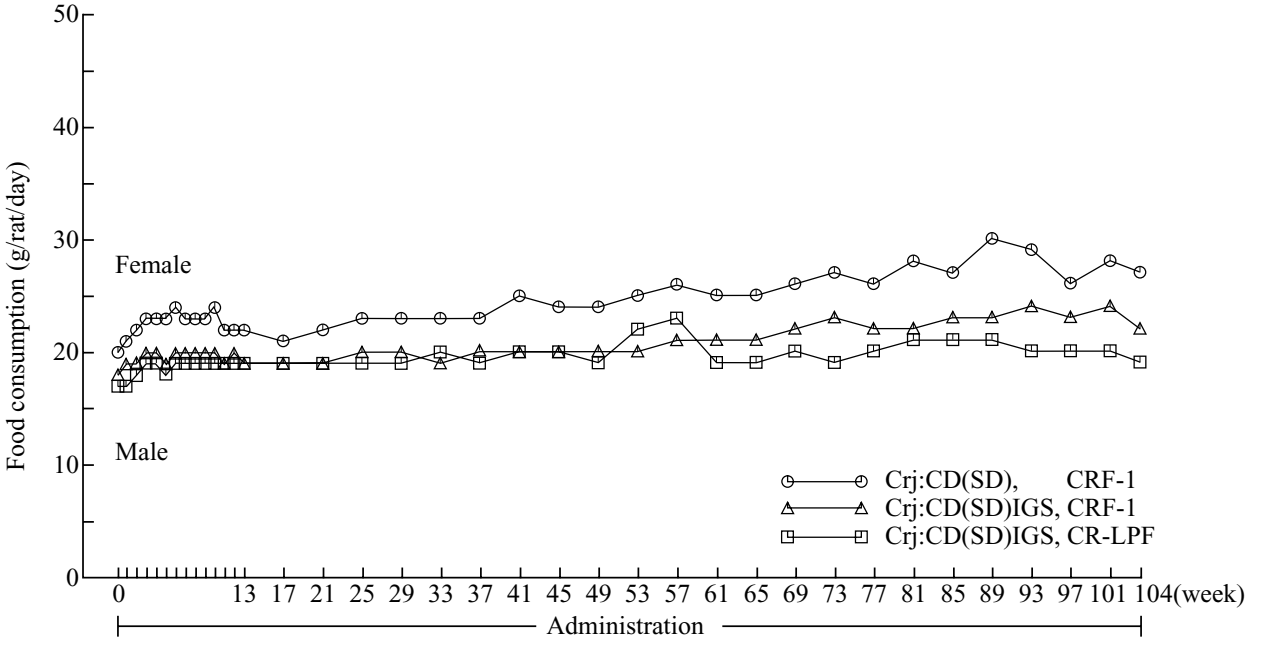

Figure 6. Food consumption of Crj:CD (SD) rats and Crj:CD (SD) IGS rats fed commercial diet CRF-1 or CR-LPF

Compilation of Spontaneous Neoplastic Lesions and Clinical Chemistry Data in Crl:CD® (SD)IGS Rats From Control Groups Maintained on Dietary Restriction

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ABSTRACT. We present control group data compiled from control groups of Crl:CD®(SD)IGS rats from six separate two-year studies in North America utilizing dietary restriction, also known as measured feeding or dietary optimization. These data include survival, incidence of neoplastic lesions, hematology and clinical chemistry. Two-year survival in males ranged from 44 to 77%, and from 40 to 72% in females. Tumors of the endocrine system (pituitary, thyroid, adrenal medulla, pancreatic islets) were the most frequent neoplasms in males and were the only tumors with more than a 10% incidence. Females had an incidence of greater than 10% for pituitary, thyroid, and mammary tumors, as well as for endometrial stromal polyps.
— Key words: dietary restriction, neoplasms, clinical pathology, Crl:CD®(SD)IGS rats, survival

CD(SD)IGS-2000: 263-277

INTRODUCTION

It is accepted within the scientific community that the conditions under which laboratory animals are maintained can influence the outcome of studies conducted as part of the preclinical safety assessment process. Rodents are the most commonly utilized model for the study of potential toxic and carcinogenic effects of food additives, drugs and chemicals. Safety assessment studies are designed to control for as many confounding variables as possible in order to elucidate dose and treatment-related changes, while keeping animal numbers as small as possible for meaningful statistics. The development of both the Food and Drug Administration (FDA), Good Laboratory Practice (GLP) guidelines and more recently the International Conference on Harmonization (ICH) guidelines have been aimed at reducing variability within and among laboratories as well as defining reproducible standardized testing procedures for preclinical safety assessment studies (2,6).

One variable that is not always well controlled in safety assessment studies, particularly those conducted in rodents, is caloric intake. Many rodent studies use *ad libitum* feeding, which can lead to uncontrolled growth. Some investigators believe that such caloric excess leads to "nutritional overdosing" and may affect the interpretation of results from toxicity and carcinogeniciity studies (6).

Since caloric excess has been shown to be a major nongenetic risk factor associated with morbidity and mortality in man, it is reasonable to assume that the same adverse association would hold true for other species as well. In recent years, several industrial and pharmaceutical laboratories have initiated a regimen of dietary restriction through measured feeding. This procedure is also referred to as dietary optimization. The results of these controlled feeding studies suggest that moderate caloric restriction leads to a lower incidence of degenerative diseases, decreases the incidence of spontaneous neoplasms and increases in longevity when compared to the traditional *ad libitum* feeding regimen (1, 3, 4, 5, 6, 7, 8, 9, 10).

The histopathology data presented in this publication were gathered from six toxicology studies of approximately 104 weeks duration, while the clinical chemistry data was gathered from eight toxicology studies of approximately 3 months duration. In all of the studies moderate dietary restriction (70-80% of maximum *ad libitum* food consumption) was employed. All studies were conducted in accordance with Good Laboratory Practice regulations of the US Food and Drug Administration or the Environmental Protection Agency and/or the Standard Operating Procedures of the participating laboratory. All studies were performed in the United States or Canada, by contract laboratories, academic institutions or industrial toxicology facilities. All studies were conducted in support of in-house research or marketing permits. The data presented were provided to us by the individual laboratories or gathered from the published literature.

PURPOSE:

The purpose of this compilation is to offer the study director, reviewing toxicologist and/or study pathologist some reported incidences of neoplasms and clinical chemistry data in Crl:CD® (SD)IGS rats maintained on a regimen of dietary restriction. It is not our purpose to endorse one type of feeding regimen over another. This document was prepared for informational purposes only. Diagnoses of the various neoplasms in the compilations are intentionally grouped in a manner to provide the user with a range of reported incidences of similar types of lesions. This compilation is not intended in any way to propose a system of standardized nomenclature nor does it separately include each and every reported variant of each lesion. For the clinical laboratory data presented, it is recognized that different analytical methods, as well as environmental and technique-related variables, can influence the values obtained for a particular parameter. For these reasons, care should be taken in using these data which are not intended as a substitute for adequate study controls or historical data collected within an institution.

COMMON STUDY PARAMETERS:

The 14 studies included in this publication were initiated between 1995 and 1998. All studies used Crl:CD® (SD)IGS rats from Charles River Laboratories. The rats in these studies were from control groups of dietary or gavage studies and were approximately 4-8 weeks of age at study initiation. Some groups were untreated while others received 1.0% polyethelene glycol;

0.5% aqueous methylcellulose; 1.0% aqueous carboxymethylcellulose; or deionized water as the vehicle control.

Rats included in this publication were singly housed in stainless steel wire mesh cages with free access to water. The animal rooms were generally maintained at average temperatures of 72 ± 5 degrees Fahrenheit with an average relative humidity of 30-70%. A 12hr/12hr light/dark cycle was employed in all studies. Since these studies were conducted in different facilities, there was some variation in environmental conditions. However, the overall environmental conditions were not considered by those performing the studies to have had any effect on the quality or integrity of the studies. Rats were fed measured amounts of Purina PMI Certified Rodent Chow 5002 with a physiological fuel value of 3.4 kcal/g. The amount of feed consumed ranged from 14.5 to 21 grams for females and 20.5 to 25.2 grams for males.

Clinical laboratory evaluations are reported for the 12-week interval. The rats were fasted overnight and blood was collected from the retro-orbital sinus in all studies. Serum chemistry determinations were performed using a Hitachi-Model 717 analyzer and hematology determinations were made using the Technicon H*1E analyzer by Bayer.

DATA SETS PRESENTED:

Survival data are from the 104 week studies only and are presented by study as the actual number surviving to terminal sacrifice and as percent survival at terminal sacrifice (Tables 1 and 2). The survival data is also presented in graphic form (Graphs 1 and 2)

The overall incidences of all neoplastic lesions observed in any organ are reported and are summarized in Tables 3 and 4. These data also include neoplastic lesions from rats that died or were found moribund and killed prior to terminal sacrifice. It does not include information from rats that were killed for an interim sacrifice.

The serum chemistry and hematology data are separated by sex and presented by individual study group in Tables 5, 6, 7 and 8. Whenever necessary, results were converted to match the units more commonly used and presented here. Due to variations in methodology used to obtain these values and the intrinsic variations among studies and laboratories, it was not considered appropriate to combine study group means into overall means.

Table 1. Summary of Individual Study Information and Survival/Males-104Weeks

Study Identification	1	2	3	4	5	6
Study Initiation Date (year)	1995	1995	1995	1995	1995	1995
Total Number Necropsied	58	60	50	50	50	50
Number Surviving to Termination	38	46	32	37	22	34
% Survival	65.52	76.67	64.00	74.00	44.00	68.00

Table 2. Summary of Individual Study Information and Survival/Females-104 Weeks

Study Identification	1	2	3	4	5	6
Study Initiation Date (year)	1995	1995	1995	1995	1995	1995
Total Number Necropsied	60	60	50	50	52	52
Number Surviving to Termination	35	43	32	33	21	29
% Survival	58.33	71.67	64.00	66.00	40.38	55.77

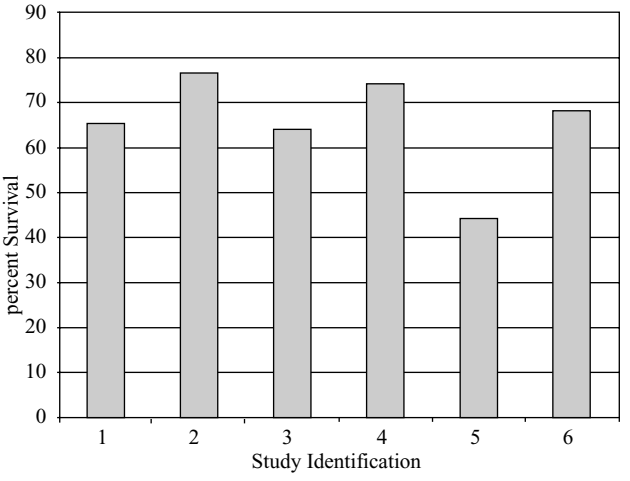

Figure 1. Male Survival-104 Weeks

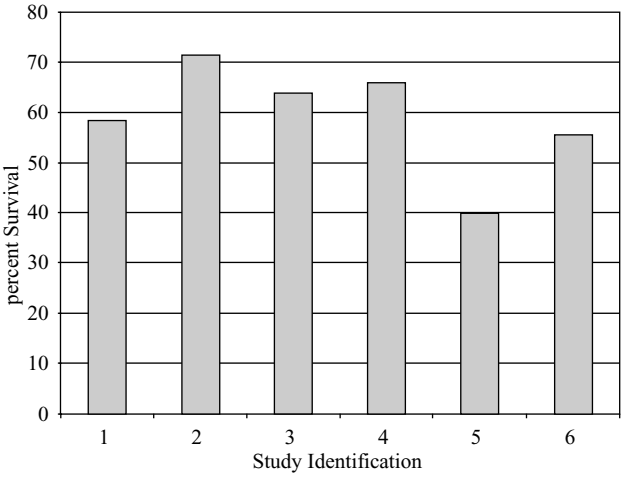

Figure 2. Female Survival-104 Weeks

Table 3-1. Neoplasms/Males-104 Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	**# STUDIES**	**# LESIONS**	**OF TOTAL**	**DIAGNOSIS**	**% FOUND**	**% FOUND**
ORAL CAVITY	6	318				
Hard Palate, Squamous Cell Carcinoma		1	0.31	1	1.67	1.67
SALIVARY GLAND	6	318				
Adenoma		1	0.31	1	2.00	2.00
Adenocarcinoma		1	0.31	1	2.00	2.00
STOMACH	6	318				
Non-glandular Mucosa/Squamous Cell Papilloma		1	0.31	1	1.72	1.72
Leiomyosarcoma		1	0.31	1	2.00	2.00
SMALL INTESTINE	6	318				
Adenocarcinoma		1	0.31	1	2.00	2.00
Leiomyoma		1	0.31	1	2.00	2.00
Leiomyosarcoma		1	0.31	1	2.00	2.00
LARGE INTESTINE	6	318				
Cecum, Leiomyoma		1	0.31	1	2.00	2.00
LIVER	6	318				
Hepatocellular Adenoma		7	2.20	5	2.00	4.00
Hepatocellular Carcinoma		10	3.14	5	2.00	6.00
PERITONEUM	6	318				
Osteogenic Sarcoma		1	0.31	1	2.00	2.00
Paraganglioma		1	0.06	1	2.00	2.00
NASAL CAVITY	6	318				
LUNG	6	318				
KIDNEY	6	318				
Adenoma/Tubular Adenoma		1	0.31	1	2.00	2.00
Liposarcoma		1	0.31	1	2.00	2.00
URINARY BLADDER	6	318				

Table 3-2. Neoplasms/Males-104 Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
TESTIS	6	318				
Interstitial Cell Tumor, Benign		9	2.83	5	2.00	6.00
Mesothelioma, Benign		1	0.31	1	2.00	2.00
SEMINAL VESICLE	6	318				
PROSTATE	6	318				
SKIN	6	318				
Basal Cell Tumor, Benign		4	1.26	2	1.72	5.01
Keratoacanthoma, Benign		9	2.83	4	1.72	8.00
Papilloma/Squamous Cell Papilloma		9	2.83	6	1.67	6.90
Tricoepithelioma, Benign		1	0.31	1	1.72	1.72
Fibroma		12	3.77	6	1.67	5.16
Fibrosarcoma		3	0.94	2	2.00	4.00
Hemangiosarcoma		1	0.31	1	1.72	1.72
Histiocytic Sarcoma		1	0.31	1	2.00	2.00
MAMMARY GLAND	6	318				
Fibroadenoma		3	0.94	3	2.00	2.00
ADRENAL	6	318				
Cortex, Adenoma		3	0.94	3	1.67	2.00
Cortex, Carcinoma		2	0.63	2	2.00	2.00
Pheochromocytoma, Benign		15	4.72	5	1.67	14.00
PANCREAS	6	318				
Acinar Cell, Adenoma		2	0.63	2	2.00	2.00
Islet Cell, Adenoma		48	15.09	6	4.00	32.00
Islet Cell, Carcinoma		13	4.09	4	5.01	10.00
PITUITARY	6	318				
Adenoma		101	31.76	6	14.00	54.00
Carcinoma		4	1.26	2	3.34	3.44
Pars Intermedia, Adenoma		2	0.63	2	4.00	4.00
Craniopharyngioma		1	0.31	1	2.00	2.00

Table 3-3. Neoplasms/Males-104 Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
THYROID	6	318				
C-Cell, Adenoma		28	8.81	5	6.00	18.00
C-Cell, Carcinoma		11	3.46	4	1.67	14.00
Follicular Cell, Adenoma		4	1.26	4	1.72	2.00
Follicular Cell, Carcinoma		4	1.26	3	1.67	4.00
PARATHYROID	6	318				
Adenoma		2	0.63	2	1.72	2.00
BRAIN	6	318				
Astrocytoma, Benign		4	1.26	3	1.67	4.00
Astrocytoma, Malignant		2	0.63	2	1.67	2.00
Granular Cell Tumor, Benign		5	1.57	4	1.67	4.00
Meningioma, Malignant		1	0.31	1	1.72	1.72
Meningeal Sarcoma		1	0.31	1	1.67	1.67
SPINAL CORD	6	318				
Glioma, Malignant		1	0.31	1	2.00	2.00
PERIPHERAL NERVE	6	318				
SKELETAL MUSCLE	6	318				
Fibrosarcoma		2	0.63	1	4.00	4.00
Hemangioma		1	0.31	1	2.00	2.00
Rhabdomyosarcoma		1	0.31	1	2.00	2.00
BONE	6	318				
Osteosarcoma		1	0.31	1	2.00	2.00
HEART	6	318				
Mesothelioma, Benign		1	0.31	1	1.72	1.72
Schwannoma, Malignant		2	0.63	2	2.00	2.00
BLOOD VESSEL	6	318				
Aorta, Paraganglioma, Benign		2	0.63	2	1.67	1.72
BONE MARROW	6	318				

Table 3-4. Neoplasms/Males-104 Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
SPLEEN	6	318				
Hemangiosarcoma		2	0.63	2	2.00	2.00
THYMUS	6	318				
Thymoma, Malignant		1	0.31	1	1.67	1.67
LYMPHORETICULAR SYSTEM	6	318				
Lymph Node, Hemangioma		2	0.63	2	2.00	2.00
WHOLE BODY/MULTIPLE ORGAN	6	318				
Lymphoma		4	1.26	4	1.67	2.00
Histiocytic Sarcoma		14	4.40	6	2.00	8.00
EYE	6	318				
EAR	6	318				
Zymbal's Gland, Carcinoma		2	0.63	2	2.00	2.00
Pinna, Schwannoma, Benign		2	0.63	2	1.67	1.72

Table 4-1. Neoplasms/Females-104Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
ORAL CAVITY	6	324				
Squamous Cell Carcinoma		2	0.62	2	1.67	1.67
STOMACH	6	324				
Non-glandular Mucosa, Carcinoma		1	0.31	1	1.92	1.92
SMALL INTESTINE	6	324				
Adenocarcinoma		1	0.31	1	1.67	1.67
Leiomyoma		2	0.62	2	1.92	1.92

Table 4-2. Neoplasms/Females-104Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
LARGE INTESTINE	6	324				
Leiomyosarcoma		1	0.31	1	1.92	2.00
LIVER	6	324				
Hepatocellular Adenoma		5	1.54	3	2.00	4.00
Hepatocellular Carcinoma		2	0.62	2	1.92	1.92
PERITONEUM	6	324				
NASAL CAVITY	6	324				
KIDNEY	6	324				
Adenoma/Tubular Adenoma		1	0.31	1	1.92	1.92
Lipoma		1	0.31	1	1.67	1.67
Mesenchymal Tumor		1	0.31	1	1.92	1.92
URINARY BLADDER	6	324				
Papilloma		1	0.31	1	1.67	1.67
Transitional Cell Carcinoma		1	0.31	1	1.92	1.92
OVARY	6	324				
Granulosa Cell Tumor, Benign		2	0.62	1	3.34	3.34
Theca Cell Tumor, Benign		1	0.31	1	1.92	1.92
Luteoma		1	0.31	1	1.92	1.92
UTERUS	6	324				
Endometrium, Adenocarcinoma		7	2.16	4	2.00	3.84
Endometrial Stromal Polyp		25	7.72	6	2.00	13.44
Endometrial Stromal Sarcoma		4	1.23	3	1.67	3.84
Fibroma		1	0.31	1	1.67	1.67
Leiomyosarcoma		1	0.31	1	2.00	2.00
Schwannoma, Malignant		1	0.31	1	1.92	1.92
CERVIX	6	324				
Squamous Cell Carcinoma		1	0.31	1	1.92	1.92
Fibroma		1	0.31	1	1.92	1.92

Table 4-3. Neoplasms/Females-104Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	# STUDIES	# LESIONS	OF TOTAL	DIAGNOSIS	% FOUND	% FOUND
VAGINA	6	324				
Polyp		1	0.31	1	1.67	1.67
Squamous Cell Carcinoma		1	0.31	1	1.92	1.92
CLITORAL GLAND	6	324				
SKIN	6	324				
Basal Cell Carcinoma		1	0.31	1	1.92	1.92
Squamous Cell Carcinoma		2	0.62	2	1.67	1.67
Tricoepithelioma, Benign		1	0.31	1	1.92	1.92
Fibroma		1	0.31	1	1.67	1.67
Fibrosarcoma		1	0.31	1	1.92	1.92
Sarcoma		1	0.31	1	1.92	1.92
Histiocytic Sarcoma		1	0.31	1	1.92	1.92
Schwannoma, Malignant		1	0.31	1	1.92	1.92
MAMMARY GLAND	6	324				
Adenoma		36	11.11	6	5.01	16.00
Adenocarcinoma		51	15.74	6	6.68	26.88
Fibroma		2	0.62	2	1.67	1.67
Fibroadenoma		79	24.38	6	19.20	38.00
Carcinosarcoma		1	0.31	1	2.00	2.00
ADRENAL	6	324				
Cortex, Adenoma		4	1.23	2	1.92	5.01
Cortex, Carcinoma		1	0.31	1	1.67	1.67
Pheochromocytoma, Benign		1	0.31	1	1.677	1.67
PANCREAS	6	324				
Islet Cell, Adenoma		14	4.32	5	2.00	7.68
Islet Cell, Carcinoma		2	0.62	2	2.00	2.00
PITUITARY	6	324				
Adenoma		156	48.15	6	22.00	69.12
Carcinoma		7	2.16	6	3.34	7.25

Table 4-4. Neoplasms/Females-104Weeks

		TOTAL		# STUDIES		
		# ORGANS	PERCENT	USING THIS	MINIMUM	MAXIMUM
LOCATION AND TUMOR	**# STUDIES**	**# LESIONS**	**OF TOTAL**	**DIAGNOSIS**	**% FOUND**	**% FOUND**
THYROID	6	324				
C-Cell, Adenoma		20	6.17	4	3.84	17.28
C-Cell, Carcinoma		9	2.78	3	1.67	12.00
Follicular Cell, Adenoma		1	0.31	1	2.00	2.00
Follicular Cell, Carcinoma		2	0.62	1	3.34	3.34
PARATHYROID	6	324				
Adenoma		3	0.93	2	1.92	3.34
BRAIN	6	324				
Astrocytoma, Benign		2	0.62	1	3.34	3.34
Astrocytoma, Malignant		2	0.62	1	3.84	3.84
Granular Cell Tumor, Benign		3	0.93	3	1.92	2.00
NERVE	6	324				
SKELETAL MUSCLE	6	324				
BONE	6	324				
HEART	6	324				
SPLEEN	6	324				
Mesothelioma, Benign		1	0.31	1	1.67	1.67
THYMUS	6	324				
Thymoma, Malignant		1	0.31	1	2.00	2.00
WHOLE BODY/MULTIPLE ORGAN	6	324				
Lymphoma. Malignant		1	0.31	1	1.92	1.92
Leukemia		1	0.31	1	1.67	1.67
Histiocytic Sarcoma		6	1.85	3	3.34	3.84
EYE	6	324				
EAR	6	324				
Zymbal's Gland, Carcinoma		1	0.31		1.92	1.92

Table 5. Summary of Hematological Parameters from Individual Studies - Males - 12 Weeks

Study Identification	Study A		Study B		Study C		Study D		Study E	
Study Start Date	Oct-96		May-97		Apr-97		Nov-96		Feb-97	
Number of Animals	15		15		15		15		15	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Erythrocytes, million/mm^3	8.24	0.34	8.32	0.28	7.89	0.59	8.33	0.37	8.39	0.33
Hematocrit, %	43.9	1.5	43.6	1.3	41.7	3	46	1.6	44.1	1.5
Hemoglobin, gm/100 ml	14.6	0.4	15.1	0.5	14.7	1.1	15.2	0.4	15.3	0.5
Mean Corp Hgb Conc, gm/dl	33.2	0.6	34.7	0.5	35.3	0.6	33	0.5	34.8	0.5
Mean Corp Hgb, picograms	17.7	0.5	18.2	0.6	18.7	0.4	18.2	0.5	18.3	0.4
Mean Corp Vol, cubic microns	53.2	0.9	52.5	1.5	52.9	1.4	55.3	1.5	52.5	1.4
Leukocytes, 1000/mm^3	11.48	1.43	11.88	2.26	11.21	2.79	10.46	1.42	9.87	2.38
Neutrophils, %	12.4	5.7	13.6	7.7	12.6	10	10.5	3.9	9.6	2.2
Neutrophils, cells/mm^3	1398	590	1572	818	1646	2244	1070	299	945	309
Lymphocytes, %	82.8	6.1	80.1	7.7	80.4	10.1	83	4.6	84.3	2.9
Lymphocytes, cells/mm^3	9531	1512	9562	2280	8785	955	8720	1460	8327	2076
Monocytes, %	1.8	0.6	2.7	0.6	3.4	0.6	3.1	0.7	2.8	0.9
Monocytes, cells/mm^3	212	78	315	99	379	114	317	68	279	120
Eosinophils, %	1.3	0.5	1.4	0.6	1.2	0.5	1.6	0.6	1.5	0.7
Eosinophils, cells/mm^3	156	67	162	74	137	56	163	66	141	55
Basophils, %	0.5	0.1	0.5	0.1	0.3	0.1	0.4	0.1	0.3	0.1
Basophils, cells/mm^3	58	17	61	27	34	12	44	14	34	16
Large Unstained Cells, %	1.1	0.2	1.8	0.5	2.1	0.4	1.4	0.4	1.4	0.3
Large Unstained Cells, cells/mm^3	127	29	212	88	234	89	144	43	143	50
Platelets, 1000/mm^3	800	110	835	101	852	124	847	123	855	81
Study Identification	Study F		Study G		Study H					
Study Start Date	Jun-97		Oct-97		Jul-98					
Number of Animals	15		15		15					
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.				
Erythrocytes, million/mm^3	8.05	0.38	8.24	0.3	8.19	0.26				
Hematocrit, %	42.7	1.5	43.2	1.1	42.3	1.4				
Hemoglobin, gm/100 ml	14.9	0.6	15	0.3	14.8	0.4				
Mean Corp Hgb Conc, gm/dl	35	0.3	34.8	0.4	35.1	0.5				
Mean Corp Hgb, picograms	18.6	0.6	18.3	0.6	18.1	0.4				
Mean Corp Vol, cubic microns	53.1	1.7	52.5	1.6	51.7	1.2				
Leukocytes, 1000/mm^3	10.9	2.79	9.78	2.64	12.9	4.38				
Neutrophils, %	10.8	3.6	9.5	3.1	10.8	4				
Neutrophils, cells/mm^3	1150	390	908	303	1349	647				
Lymphocytes, %	82.9	4.2	84.1	3.3	80.7	5.9				
Lymphocytes, cells/mm^3	9071	2539	8255	2406	10453	3739				
Monocytes, %	2.8	0.8	2.9	0.6	4.1	1.6				
Monocytes, cells/mm^3	299	105	286	113	524	273				
Eosinophils, %	1.3	0.6	2	1.2	1.4	0.7				
Eosinophils, cells/mm^3	137	53	176	83	178	88				
Basophils, %	0.3	0.1	0.3	0.1	0.4	0.1				
Basophils, cells/mm^3	37	19	27	16	60	34				
Large Unstained Cells, %	1.8	0.4	1.3	0.3	2.6	1				
Large Unstained Cells, cells/mm^3	201	71	126	52	332	180				
Platelets, 1000/mm^3	821	87	765	107	865	86				

Table 6. Summary of Hematological Parameters from Individual Studies - Females - 12 Weeks

Study Identification	Study A		Study B		Study C		Study D		Study E	
Study Start Date	Oct-96		May-97		Apr-97		Nov-96		Feb-97	
Number of Animals	15		15		15		15		15	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Erythrocytes, million/mm^3	7.23	0.3	7.47	0.24	7.43	0.3	7.74	0.37	7.54	0.27
Hematocrit, %	39.7	1.8	40.6	0.9	40.6	1.2	44	1.9	40.5	1.3
Hemoglobin, gm/100 ml	13.5	0.5	14.3	0.5	14.5	0.6	14.3	0.6	14.6	0.4
Mean Corp Hgb Conc, gm/dl	34.1	0.5	35.1	0.5	35.7	0.5	32.6	0.4	36	0.5
Mean Corp Hgb, picograms	18.7	0.4	19.1	0.7	19.5	0.5	18.5	0.5	19.3	0.6
Mean Corp Vol, cubic microns	54.9	1.2	54.4	1.4	54.7	1.5	56.9	1.7	53.7	1.6
Leukocytes, 1000/mm^3	7.91	2.35	10.69	3.33	8.17	1.44	7.58	2.35	8.22	1.65
Neutrophils, %	10.3	5.6	14.2	7.9	9	3.6	10.7	8.5	7.9	2.6
Neutrophils, cells/mm^3	869	681	1535	1029	711	231	905	1140	635	193
Lymphocytes, %	85.4	6.2	80.6	8.6	85.3	4.2	84	9.4	87	3
Lymphocytes, cells/mm^3	6697	1837	8601	2895	6991	1416	6259	1711	7161	1533
Monocytes, %	1.9	0.6	2.2	1	2.5	0.9	2.4	1.1	2.2	0.6
Monocytes, cells/mm^3	155	72	235	121	201	67	195	140	174	40
Eosinophils, %	1.2	0.5	1.2	0.5	1.4	0.5	1.4	0.5	1.4	0.6
Eosinophils, cells/mm^3	93	35	127	52	114	55	104	47	112	57
Basophils cells/mm^3	34	21	51	29	21	12	24	9	24	9
Basophils, %	0.4	0.1	0.4	0.1	0.2	0.1	0.3	0.1	0.3	0.1
Large Unstained Cells, %	0.8	0.2	1.3	0.2	1.5	0.3	1.2	0.4	1.4	0.3
Large Unstained Cells, cells/mm^3	67	29	145	56	125	32	92	48	112	29
Platelets, 1000/mm^3	817	132	787	102	815	93	818	152	877	132
Study Identification	Study F		Study G		Study H					
Study Start Date	Jun-97		Oct-97		Jul-98					
Number of Animals	15		15		15					
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.				
Erythrocytes, million/mm^3	7.5	0.26	7.62	0.39	7.41	0.43				
Hematocrit, %	40.8	1.3	40.8	1.6	39.6	1.6				
Hemoglobin, gm/100 ml	14.5	0.5	14.3	0.5	14	0.5				
Mean Corp Hgb Conc, gm/dl	35.5	0.3	35	0.3	35.4	0.5				
Mean Corp Hgb, picograms	19.3	0.6	18.8	0.6	18.9	0.7				
Mean Corp Vol, cubic microns	54.4	1.5	53.6	1.7	53.5	1.9				
Leukocytes, 1000/mm^3	9.37	2.17	7.5	2.64	7.57	1.55				
Neutrophils, %	9.1	5.2	9.5	3.8	7.7	2				
Neutrophils, cells/mm^3	832	524	743	534	577	172				
Lymphocytes, %	84.9	6.3	84.7	4.5	85.1	2.3				
Lymphocytes, cells/mm^3	7982	2063	6324	2078	6443	1358				
Monocytes, %	2.7	1.1	2.8	0.9	3.3	1.1				
Monocytes, cells/mm^3	254	126	205	73	250	98				
Eosinophils, %	1.3	0.6	1.5	0.7	1.6	0.5				
Eosinophils, cells/mm^3	112	46	110	54	120	42				
Basophils cells/mm^3	27	11	21	14	24	9				
Basophils, %	0.3	0.1	0.3	0.1	0.3	0.1				
Large Unstained Cells, %	1.7	0.5	1.2	0.3	2	0.5				
Large Unstained Cells, cells/mm^3	162	61	94	66	156	54				
Platelets, 1000/mm^3	832	110	790	110	880	57				

Table 7. Summary of Serum Chemistry Parameters from Individual Studies - Males - 12 Weeks

Study Identification	Study A		Study B		Study C		Study D		Study E	
Study Start Date	Oct-96		May-97		Apr-97		Nov-96		Feb-97	
Number of Animals	15		15		15		15		15	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
A/G Ratio	1.1	0.1	1.1	0.1	1.1	0.1	1.3	0.1	1.2	0.1
Albumin, g/dl	3.4	0.1	3.4	0.1	3.3	0.2	3.5	0.1	3.4	0.1
Alkaline Phosphatase, u/l	146	46	125	32	118	25	104	22	121	31
ALT, u/l	31	4	32	5	31	5	27	5	35	5
AST, u/l	77	9	90	14	91	15	110	32	103	17
Calcium, mg/dl	10	0.3	9.9	0.3	10.1	0.2	9.6	0.3	9.7	0.2
Chloride, mEq/l	103	2	105	1	105	1	105	1	104	2
Cholesterol Total, mg/dl	57	11	59	11	61	12	57	8	59	9
Creatinine, mg/dl	0.6	0	0.6	0	0.6	0	0.6	0	0.6	0.1
Glucose, mg/dl	197	22	156	19	167	26	154	17	173	20
Phosphorus, mg/dl	7.2	0.5	7	0.5	7.2	0.4	7.3	0.4	7	0.6
Potassium, mEq/l	4.6	0.3	4.7	0.3	5	0.2	5.1	0.4	5	0.3
Sodium, mEq/l	141	1	143	1	142	1	143	1	142	2
Total Protein, g/dl	6.5	0.2	6.4	0.2	6.1	0.3	6.3	0.2	6.4	0.3
Triglycerides, mg/dl	62	21	75	32	90	33	64	13	75	22
Urea Nitrogen, mg/dl	12	2	13	2	13	2	14	3	15	2
Study Identification	Study F		Study G		Study H					
Study Start Date	Jun-97		Oct-97		Jul-98					
Number of Animals	15		15		15					
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.				
A/G Ratio	1.2	0.1	1.2	0.1	1.3	0.1				
Albumin, g/dl	3.3	0.2	3.4	0.1	3.4	0.1				
Alkaline Phosphatase, u/l	117	22	115	20	121	22				
ALT, u/l	31	3	31	4	35	6				
AST, u/l	92	20	88	17	92	18				
Calcium, mg/dl	9.9	0.2	9.8	0.2	9.9	0.2				
Chloride, mEq/l	107	2	104	1	102	1				
Cholesterol Total, mg/dl	55	11	62	12	61	6				
Creatinine, mg/dl	0.6	0	0.6	0	0.6	0				
Glucose, mg/dl	175	23	159	29	174	18				
Phosphorus, mg/dl	7.2	0.6	7.2	0.6	7.1	0.5				
Potassium, mEq/l	5	0.2	4.8	0.3	4.8	0.2				
Sodium, mEq/l	143	1	141	1	142	1				
Total Protein, g/dl	6.1	0.3	6.2	0.2	6.0	0.2				
Triglycerides, mg/dl	72	26	79	46	66	20				
Urea Nitrogen, mg/dl	15	1	14	3	13	2				

Table 8. Summary of Serum Chemistry Parameters from Individual Studies - Females - 12 Weeks

Study Identification	Study A		Study B		Study C		Study D		Study E	
Study Start Date	Oct-96		May-97		Apr-97		Nov-96		Feb-97	
Number of Animals	15		15		15		15		15	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
A/G Ratio	1.3	0.1	1.1	0.1	1.3	0.1	1.3	0.1	1.3	0.1
Albumin, g/dl	3.8	0.2	3.5	0.1	3.7	0.2	3.5	0.2	3.7	0.3
Alkaline Phosphatase, u/l	97	21	117	44	72	17	97	28	68	25
ALT, u/l	34	25	27	5	26	4	30	6	28	4
AST, u/l	83	26	102	17	95	13	116	33	97	14
Calcium, mg/dl	10.3	0.3	10	0.2	9.8	0.3	9.6	0.4	9.8	0.2
Chloride, mEq/l	107	2	107	2	105	2	105	2	105	1
Cholesterol Total, mg/dl	71	14	72	8	79	13	66	16	72	11
Creatinine, mg/dl	0.6	0.1	0.6	0	0.6	0	0.6	0	0.6	0.1
Glucose, mg/dl	186	18	177	16	171	19	183	27	154	23
Phosphorus, mg/dl	6.3	0.5	5.9	0.6	6	0.6	5.8	0.6	5.6	0.6
Potassium, mEq/l	4.3	0.3	4.5	0.3	4.4	0.3	4.5	0.3	4.3	0.1
Sodium, mEq/l	142	2	141	1	142	1	141	2	143	1
Total Protein, g/dl	6.6	0.3	6.5	0.3	6.5	0.4	6.3	0.4	6.4	0.4
Triglycerides, mg/dl	51	13	75	27	59	16	64	32	62	24
Urea Nitrogen, mg/dl	12	2	11	2	13	2	12	2	13	2
Study Identification	Study F		Study G		Study H					
Study Start Date	Jun-97		Oct-97		Jul-98					
Number of Animals	15		15		15					
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.				
A/G Ratio	1.3	0.1	1.3	0.1	1.5	0.1				
Albumin, g/dl	3.7	0.3	3.7	0.2	3.9	0.2				
Alkaline Phosphatase, u/l	65	17	79	38	76	26				
ALT, u/l	33	6	25	7	26	3				
AST, u/l	106	18	94	24	84	14				
Calcium, mg/dl	10.1	0.4	9.8	0.3	9.9	0.2				
Chloride, mEq/l	107	1	108	2	103	1				
Cholesterol Total, mg/dl	73	20	74	14	72	9				
Creatinine, mg/dl	0.6	0.1	0.6	0.1	0.6	0.1				
Glucose, mg/dl	170	11	181	35	169	13				
Phosphorus, mg/dl	6.1	0.7	6	0.5	5.6	0.6				
Potassium, mEq/l	4.4	0.3	4.4	0.3	4.4	0.2				
Sodium, mEq/l	142	2	141	2	141	1				
Total Protein, g/dl	6.5	0.5	6.5	0.4	6.5	0.3				
Triglycerides, mg/dl	61	28	64	29	53	28				
Urea Nitrogen, mg/dl	13	2	14	2	13	2				

SUMMARY TABLE CALCULATIONS FOR NEOPLASTIC LESIONS:

The following is a description of how each of the parameters in the tables was calculated.

Number of Studies (# Studies)

This is the number of studies in which a particular tissue/organ was examined. In this presentation, the number of studies is always 6.

Total Number of Organs (Total # Organs)

This number represents the sum of the total number of tissues or organs examined in all control groups from all studies combined. Widespread tumors which showed involvement of multiple organs were listed on the basis of total number of animals examined. Occasionally a tumor would be noticed in a tissue not designated for histological examination by the study protocol. In these instances, the tumor incidence was based on the total number of animals examined as any such tumor or lesion would have been noticed on gross examination of the animal. Autolysis of tissues did not routinely exclude tissues from diagnosis. Tissue numbers were adjusted only if the individual study table indicated that some tissues were missing or inadequate for examination. Some laboratories presented data separately for different regions within an organ (i.e., duodenum, jejunum and ileum) while most presented data by the organ (i.e., small intestine). When data were presented separately by organ region, it was grouped under the organ and calculations were based on the number of organs examined.

Total Number of Lesions (# Lesions)

This represents the total number of occurrences of this lesion in the specified organ in all studies examined.

Percent of Total

These values represent the percent incidence of a particular lesion/diagnosis in the total number (all studies combined) of a particular organ examined. These values were calculated by dividing the total number of lesions by the total number of organs/animals examined and multiplying by 100 to express the values as a percent. Values are expressed to the second decimal place. Some caution is indicated in using this number, since not all pathologists or institutions will include all diagnoses in their lexicon.

Number of Studies Using This Diagnosis

This is the number of studies in which a particular diagnosis was reported. This number may be useful in interpreting the overall incidence (percent of total) of a particular diagnosis, see above.

Minimum and Maximum Percent Found (Minimum and Maximum % Found)

The range reported is the lowest and highest percent incidence for each lesion from the studies where the diagnosis was made. Therefore, if a study did not include a particular diagnosis, it was excluded from these calculations. The minimum and maximum percent found values should be considered in conjunction with the Number of Studies Using the Diagnosis.

The individual study percentages, Minimum % Found and Maximum % Found, were calculated by dividing the number of times each diagnosis was made by the total number of organs examined in each study and then multiplying the resultant value by 100 to express it as a percent. Values are expressed to the second decimal place.

SYNONYMS FOR NEOPLASTIC LESIONS:

Synonymous terms or diagnoses were frequently encountered in different studies, and were combined under a single, often broad diagnosis, which was considered to be the primary diagnosis. Although some effort was made to use currently acceptable terms, it is beyond the scope of this publication to propose a system of preferred diagnoses. A current trend in toxicologic pathology is to simplify tumor classification (i.e., "lumping" as opposed to "splitting") and the categories of neoplasms used in this publication are considered to be consistent with that trend. The synonyms which were included in the various diagnoses are presented in the synonym list which follows. Where possible, terminology is consistent with the classification system proposed by the Society of Toxicologic Pathologists.

Stomach:
NONGLANDULAR MUCOSA/SQUAMOUS CELL PAPILLOMA
= papilloma; non-glandular mucosa papilloma; squamous cell papilloma
NONGLANDULAR MUCOSA, CARCINOMA
= squamous cell carcinoma
Liver:
BILE DUCT ADENOMA = cholangioma
Prostate:
CARCINOMA/CARCINOMA NOS = adenocarcinoma
Uterus:
ENDOMETRIAL STROMAL POLYP = polyp
ENDOMETRIUM, ADENOCARCINOMA
= adenocarcinoma; endometrium, carcinoma
ENDOMETRIAL STROMAL SARCOMA = sarcoma
Skin:
BASAL CELL CARCINOMA = malignant basal cell tumor
Mammary Gland:
ADENOMA = cystadenoma
Adrenal:
CORTEX, CARCINOMA= cortex, adenocarcinoma
Pancreas:
ISLET CELL, ADENOMA= islet, adenoma; adenoma NOS
ISLET CELL, CARCINOMA
= islet cell, adenocarcinoma; islet, carcinoma
Pituitary:
ADENOMA = adenoma anterior lobe; adenoma pars distalis
CARCINOMA = carcinoma pars distalis; adenocarcinoma; adenocarcinoma pars distalis
Thyroid:
C-CELL = parafollicular cell

FOLLICULAR CELL CARCINOMA
= follicular cell adenocarcinoma
Body:
WHOLE BODY/MULTIPLE ORGAN
= primary site undetermined

ABBREVIATIONS:

A/G Ratio = Albumin/globulin ratio
ALT = Alanine Aminotransferase
AST = Aspartate Aminotransferase
Mean Corp Hgb Conc = Mean Corpuscular Hemoglobin
Mean Corp Hgb = Mean Corpuscular Hemoglobin
Mean Corp Vol = Mean Corpuscular Volume
NA = Not Available
NOS = Not otherwise specified.

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Appendix

ABSTRACTS OF ORIGINALS

Toxicological Studies on the Preparation of Lysed *Enterococcus faecalis*

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OyoYakuri (Pharmacometrics) 54, 257-265 (1997)

Abstract: The preparation of lysed *Enterococcus faecalis* (LFK) was studied toxicologically in rats and mice. By single oral administration to mice or long term administration (CO days and 188 days) to mice or rats, fatal evidence did not occur even at the largest dose of technical limitations. Furthermore, disturbance of the growth was not found in the whole experimental period and dose related changes or disorders did not appe4r in the organ weight, and in the hematological, biochemical and pathological examination.

4-Week Repeated Dose Oral Toxicity Study of *Enterococcus faecalis* FK-23 Preparation (FK-23) in Rats

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OyoYakuri (Pharmacometrics) 55, 53-60 (1998)

Abstract: Heat treated *Enterococcus faecalis* preparation (FK-23) was studied toxicologically in CDO IGS rats. FK-23 was administered orally to male and female rats at dosage levels of 1% (low dose) and 5% (high dose) in feed for 4 weeks. Furthermore, disturbance of the growth was not found in the whole experimental period and dose related changes or disorders did not appear in thc organ weight, and in the hematological, biochemical and pathological examination.

Effects of Sotalol Hydrochloride, an Antiarrhythmic Drug, Administered to Pregnant and Lactating Rats on Behavioral Observations in their Offspring

Shuichi Kai et al.
Kanagawa Laboratories, Bristol-Myers Squibb K.
Jpn Pharmacol Ther 26, 315-324, 1998

Abstract: Sotalol hydrochloride (sotalol), an antiarrhythmic drug, was administered orally to femael Crj:CD(SD)IGS rats at daily dose levels of 0 (distilled water for injection), 60, 240, and 960mg/kg from day 6 of gestation to postpartum day 20.

Results obtained were as follows:

1) Body weigh gains were slightly suppressed in F_0 dams from days 10 to 20 of gestation at dose level of 960mg/kg. Further, food consumption were significantly decreased in F_0 dams from days 6 to 9 of gestation as well as from postpartum days 14 to 20.

2) Delivery status in F_0 dams as well as number of stillborn pups, number of live pups, birth index and sex ratio of live pups in F_1 rats were not affected by sotalol.

3) Sotalol failed to affect the viability index on postnatal day 4 and weaning index in F_1 rats.

4) Body weight gains were significantly suppressed in both male and female F_1 rats during the postnatal period at dose level of 960mg/kg.

5) Sotalol did not alter learning ability and memory, spontaneous motor activity as well as emotionality in both male and female F_1 rats.

Based on the above results, it was considered that learning ability and memory, spontaneous motor activity as well as emotionality were not affected by sotalol even at higher dose level of 960mg/kg which was a toxic dose level for dams (F_0) and their offspring (F_1).

Oral 3-month Repeated-dose Toxicity Study of Treated Water by Electro Clean in Rats

Tsuneo Kosazuma et al.
Institute of Applied Medicine, Inc., etc.
Jpn Pharmacol T'her 27, 783- , 1999

Effect of Sepimostat mesilate (FUT-187) on Reflux Esophagitis after Gastrectomy in Rats

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Oyo Yakuri (Pharmacometrics) 58 17-22, 1999

Abstract: The reflux esophagitis after gastric surgery is attributed to excessive exposure of the esophageal mucosa to refluxed bile and pancreatic juice from duodenum. There are clinical observations and experimental evidence to suggest that the reflux of duodenal contents into the esophagus develop Barrett columnar metaplasia and/or esophageal cancer. Sepimostat mesilate* (FUT-187) has the inhibitory effects on various proteases, especially trypsin. This study was designed to determine the effect of oral administration of FUT-187 on the reflux esophagitis after gastrectomy in two different experimental models. In the first experiment, three operative procedures were performed in rats, 1) total gastrectomy and Billroth-Ⅱ reconstruction, 2) total gastrectomy and Roux-Y reconstruction, 3) partial gastrectomy and Billroth-Ⅱ reconstruction. In this study, the most severe mucosal ulceration occurred to all of rats underwent total gastrectomy with Billroth-Ⅱ reconstruction at the 14th day after surgery. None of the others models showed esophageal ulcer. Therefore, total gastrectomy with Billroth-Ⅱreconstruction was chosen as the experimental model for reflux esophagitis in the present study. In this model, the area of esophageal ulcer at 14th day after oral administration of FUT-187 for 12 days and trypsin activity of juice in duodenum at 10th day after single administration of FUT-187 were determined. FUT-187 exhibited inhibitory effect on the development of esophagitis and the duodenal trypsin activity in a dose dependent manner. In the second experiment, acute reflux of duodenal contents into the esophagus was induced by keeping polyethylene tube in stomach of rats to cause acute esophageal mucosal injuries. This acute model had a mild mucosal alterations in comparison with the gastrectomized model and similarity clinical cases. Oral administration of FUT-187 had remarkable effect on histopathological legions of esophageal mucosa that were assessed by microscopic findings in this acute models. In conclusion, these studies show that FUT-187 significantly inhibits the duodenal trypsin activity resulting in symptom imrpovements in both severe and mild models. These results suggest that FUT-187 is an effective agent for the treatment of reflux esophagitis after gastrectomy.

Study for Effects of Kyukichoketsuin Extract on Pre and Postnatal Development Including Maternal Function by Oral Administration in Rats -Effects until the Weaning of Pups (F_1)-

Toshio Ihara, Satoru Oneda, Kazuyo Aikou nad Koichi Magata : Shin Nippon Biomedical Laboratories, Ltd. ; Toshio Shirata : Taikoseide Pharmaceuticals Co., Ltd. ; Shigeru Tajima : Kanebo, Ltd.
Jpn Pharmacol Ther 28 399-406, 2000

Abstract: Kyukichoketsuin Extract was administered orally to Crj:CD(SD)IGS rats from Day 6 of gestation to Day 21 of lactation at dose levels of 1.0 and 2.0g/kg/day, in order to assess the effects on pre and postnatal development of pups until weaning including maternal function.

Regarding dams, there were no deaths and no treatment-related effects on clinical signs, delivery and nursing conditions, body weight, food consumption, gestation period, delivery index or gross pathology findings in any group throughout the gestation and lactation periods.

Regarding pups, there were no treatmen-related effects on clinical signs, birth index, external findings, viability indices on Days 4 and 7 after birth, weaning index, sex ratio, body weight until weaning, stomach contents weight, morphological development (pinna detachment, incisor eruption, eyelids opening), functional development (righting reflex, negative geotaxis, pupil reflex, Preyer reflex, pain response) or gross pathology findings (including skeletal findings) in any group.

From the above-mentioned results, it was concluded that the non-toxic dose level of Kyukichoketsuin Extract for dams and pups, under the conditions of thes study, was 2.0g/kg/day and above.