Long-Term Feeding Effects of Browned Egg Albumin to Rats

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The long-term effects of feeding Maillard browned egg albumin to rats were investigated. Rats were fed from 1 to 12 months. The weight gain and assays of serum components and tissue enzyme activities failed to show any significant differences after 1 month. In the second month, the rats fed browned diet exhibited a lag in weight gain relative to the control group. Longer feeding periods of browned protein resulted in higher serum glutamate oxalate transaminase (SGOT), serum alkaline phosphatase (SAP), blood urea nitrogen (BUM), urine specific gravity, and lower hemoglobin and hematocrit levels as well as enlargement of some organs compared to control pair-fed groups. In short, the effects of the browned diet seemed to intensify as the length of the feeding period increased. This study further substantiates our previous observations that the poor nutritional quality of browned protein is not restricted to the loss of amino acids and that inhibitory substances might have been formed during the browning process.

The Maillard, or nonenzymatic browning reaction between reducing sugars and proteins is known to cause serious deterioration of food quality during processing and storage (Mauron et al., 1955; Nesheim and Carpenter, 1967; Sgarbieri et al., 1971; Tanaka et al., 1975; Amaya et al., 1976; Lee et al., 1977; Tanaka et al., 1977; Kimiagar et al., 1978). The reduced value of the brown products does not seem to be limited to the loss of amino acids since supplementation of the diet with those amino acids could not completely restore its biological value (Rao et al., 1963; Sgarbieri et al., 1973). This suggests the possible formation of some inhibitory or antinutritional compounds during Maillard reaction, the presence of which cannot be detected with short-term nutritional assays. Moreover, the short-term feeding effects reported in the literature (Fink et al., 1958; Adrian, 1974) seem to be due in part to nutritional deficiency and not specifically the browning compounds. Furthermore, there are no reports concerning the effects of long-term feeding of brown compounds available in the literature.

The present study was conducted to investigate the long-term feeding effects of browned products with the objective of eliminating food inadequacy as a variable factor. This was accomplished by selecting a control ratio resembling the nutritional quality of browned diet and by pair-feeding the rats. The observed effects therefore could be attributed only to the brown compounds.

MATERIALS AND METHODS

Preparation of Browned Egg Albumin. Egg albumin (EA) powder (Nutritional Biochemical Company, Cleveland, OH) and glucose (D-glucose, anhydrous, Fisher Scientific Company, Fairlawn, NJ) were combined in a diet mixer (Hobart Co. Model A-120, Troy, OH) in the ratio of 3:2, respectively. Distilled water was added to give a moisture content of approximately 15%. The mixture was stored at 37 °C in a sealed glass chamber for 10 days. Relative humidity inside of the chamber was kept at 68% using a 40% sulfuric acid solution. The brown product first was freeze-dried and then ground in a ball mill for 1 h to obtain a fine powder.

Preparation of the Brown and Control Diets. In selecting a control diet for this experiment, the following factors were considered. (1) The diet should be isocaloric and isonitrogenous, and its nutritional quality must be as close to the brown diet as possible. (2) Since brown diet is known to depress appetite and pair-feeding should be attained naturally, the control diet should not be overly palatable to rats.

The PER assay was used to formulate an appropriate control diet with a protein value similar to browned protein. In a preliminary study, different ratios of egg albumin (EA) and a mixture of nonessential amino acids (NEAA) were mixed in a proportion recommended by Sauberlich (1961) and fed to male rats. A diet containing 5% egg albumin plus 5% NEAA was found to have virtually the same PER value as the browned protein. Accordingly, it was decided, in the absence of complete information concerning the exact nature of compounds formed during the browning process, to proceed with the diet containing 5% egg albumin plus 5% NEAA, as the most suitable control diet.

As a further measure to insure the propriety of the control diet, an additional diet (diet D) with 5% egg albumin which had been shown in our laboratories to yield the same feed efficiency as diets B and C was included in this study. Diet D served as a control for diet C to determine whether the addition of nonessential amino acids would induce any changes in the data obtained. Twenty-four rats (four males and four females in each feeding period) were pair-fed diet D, along with groups B and C.

Preparation of Tissue Homogenates. Liver and whole small intestine were cut into small pieces with dissecting scissors and mixed with approximately two parts ice-cold saline as rapidly as possible and were homogenized by hand in a glass tissue homogenizer with a Teflon pestle (Arthur Thomas Co. Philadelphia, PA). A mechanical homogenizer was not used so as to avoid heat generation which could possibly destroy the enzyme activity. The homogenate was then centrifuged at 12000 rpm for 20 min in an automatic refrigerated centrifuge (Sorval Inc.). The supernatant was collected and kept frozen at −20 °C for further analysis.

Relative Organ Weight Determinations. Liver, kidney, testes, spleen, heart, and lung were kept in 0.9% saline for approximately 1 h before they could be weighed. The tissues were blotted on paper towels and trimmed of excess fat. The kidneys were decapsulated before weighing. The stomach was cut open, emptied, and weighed. All rats were sacrificed between 10 a.m. and noontime.

Analytical Methods. Blood urea nitrogen (BUN) was

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Table I. Ingredients Common to All Diets

	% total diet
dextrine	53.5
corn oil	8
salt mixture[a]	5
vitamin mixture[b]	1
sucrose	16
dextrose[c]	6.7
total	90

[a] Jone, Foster salt mixture, ICN Pharmaceutical Inc., Cleveland, OH. [b] Vitamin fortification mixture, Nutritional Biochemical Co., Cleveland, OH. [c] In group B, a part of this dextrose was present in the bound form to egg albumin in the brown compound. The amount of browned egg albumin was added to the diet adjusted in accordance with its egg albumin and dextrose composition.

Table II. PER Values for Control and Brown Diets

diet	PER (mean ± SD)
brown	1.1 ± 0.2
control (5% egg albumin + 5% nonessential aa)	1.1 ± 0.1
untreated egg albumin	3.0 ± 0.15

determined using a Sigma Chemical Co. kit (Sigma Chemical Co., 1975a). Serum glucose was assayed with glucostat reagent set (Worthington Biochemical Co., Freehold, NJ). Serum protein was determined by the Biuret method. Serum glutamate–oxalate transaminase (SGOT) and serum glutamate pyruvate (SGPT) were determined colorimetrically according to Richterich (1969). Serum alkaline phosphatase (SAP) was measured according to the method of Neuman and Van Vreedendaal (1967). Blood hemoglobin was determined using a kit purchased from Sigma Chemical Co. (Sigma Chemical Co., 1975b). Liver GOT and GPT were determined according to Richterich (1969). The sucrase activity in the small intestine was measured by the method of Dahlquist (1964). Small intestinal dipeptidase (glycylvaline) activity was determined by the method of Josefsson and Lindberg (1965). The protein content in the supernatants of the liver and tissue homogenates were measured by the Lowry method (1951). Urine specific gravity was measured using a pycnometer.

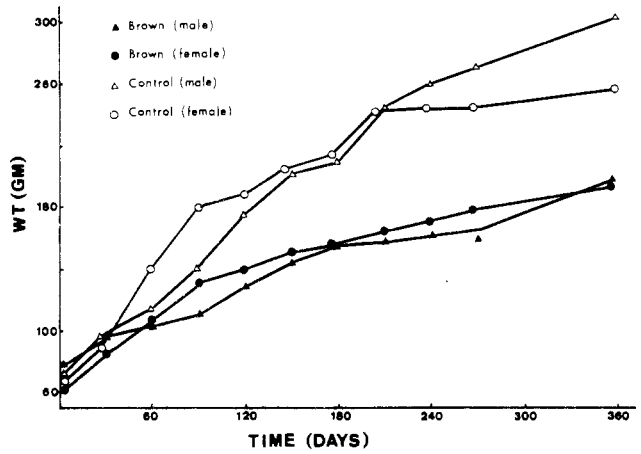

Figure 1. Growth curve for rats on brown and control diets. (Average of five or six rats in each group; the weight gains graphed here are for the rats which were fed for 12 months.)

ANIMALS AND FEEDING EXPERIMENT

Male and female Sprauge-Dawley CD strain rats (Charles River Breeding laboratories, Wilmington, MA) were caged individually in an automatically light- and temperature-controlled room. They were fed Rat Chow (Purina Lab. Chow, Ralston, Purina Co.) for 48 h and then divided into four groups. Group A (three males and three females in each feeding period) was fed diet A ad libitum. This group was included to provide a general base line for the experiment. Group B (five or six rats of each sex per treatment, i.e., a total of 44 rats for four feeding periods) was fed brown diet. Group C (equal number of rats to group B) was fed diet C. The rats in this group were pair-fed with group B, i.e., the average food intake of the rats on brown diet in each sex was fed to the control group of the same sex the next day. Group D was fed in the same manner as group C.

All diets contained ingredients as listed in Table I. Additional ingredients of the formula were as follows: diet A (ad libitum), 10% untreated, pure egg albumin; diet B (brown), 10% browned egg albumin (excluding the dextrose component); diet C (control), 5% untreated egg albumin plus 5% nonessential amino acids; diet D, 5% un-

Table III. Body Weight, Relative Organ Weight, and Biochemical Values in Rats Fed for 1 Month

	brown, mean ± SD, $n = 6$	control, mean ± SD, $n = 5$	signif. of differ.
body wt	85 ± 8	87 ± 5	NS[a]
relative organ wt			
liver	4.82 ± 0.39	4.23 ± 0.74	NS
kidneys	1.19 ± 0.11	1.24 ± 0.08	NS
testes	1.78 ± 0.27	1.81 ± 0.13	NS
spleen	0.201 ± 0.02	0.215 ± 0.01	NS
heart	0.426 ± 0.03	0.463 ± 0.02	NS
lungs	0.842 ± 0.10	0.917 ± 0.04	NS
cecum	2.53 ± 0.58	1.99 ± 0.01	S
small intestinal dipeptidase (glycylvaline), μm of substrate (60 min)$^{-1}$ (mg of protein)$^{-1}$	7.5 ± 2.0	6.5 ± 2.2	NS
small intestinal sucrase	3.62 ± 1.4	2.83 ± 1.4	NS
liver GOT,[b] μm of substrate (min)$^{-1}$ (mg of protein)$^{-1}$	580 ± 110	492 ± 148	NS
liver GPT,[c] μm of substrate (min)$^{-1}$ (mg of protein)$^{-1}$	240 ± 180	289 ± 72	NS
BUN,[d] mg %	18.4 ± 11	8.7 ± 1.8	NS
serum glucose, mg %	106 ± 8	72 ± 31	S
serum protein, g %	4.72 ± 0.66	5.32 ± 0.59	NS
SAP,[e] IU	205 ± 64	172 ± 38	NS
SGOT,[f] IU	23 ± 5	23 ± 4.5	NS
SGPT,[g] IU	26 ± 3	27 ± 3	NS
hematocrit	38.3 ± 5.3	38.2 ± 4.3	NS

[a] NS = not significant, S = significant. [b] Glutamate oxalate transaminase. [c] Glutamate pyruvate transaminase. [d] Blood urea nitrogen. [e] Serum alkaline phosphatase. [f] Serum glutamate oxalate transaminase. [g] Serum glutamate pyruvate transaminase.

Table IV. Organ Weight to Body Weight Ratios of Rats Fed ad lib (A), Brown (B), and Control (C) Diets (Mean ± SD)

feeding period	diet	wt, g	organ weight to body weight ratio, % body weight							
			liver	kidneys	testes	spleen	heart	lung	cecum	stomach
3 months	A	321 ± 37[b,c]	2.86 ± 0.42	0.740 ± 0.13	0.950 ± 0.01[b]	0.157 ± 0.02	ND[d]	ND	ND	ND
	B	121 ± 17[a,c]	3.78 ± 0.93	0.836 ± 0.15	1.83 ± 0.34[a]	0.171 ± 0.05	ND	ND	ND	ND
							ND	ND	ND	ND
							ND	ND	ND	ND
	C	161 ± 24[a,b]	3.52 ± 0.63	0.754 ± 0.06	1.50 ± 0.29[a]	0.172 ± 0.02	ND	ND	ND	ND
							ND	ND	ND	ND
6 months	A	411 ± 126[b,c]	2.48 ± 0.27[b]	0 702 ± 0.09[c]	0.77 ± 0.04[b,c]	0.183 ± 0.05	0.290 ± 0.01[b,c]	0.421 ± 0.06[b,c]	0.959 ± 0.25[b,c]	ND
										ND
	B	155 ± 70[a,c]	4.03 ± 1.36[a]	1.10 ± 0.36[a,c]	1.78 ± 0.03[a]	0.268 ± 0.09[c]	0.493 ± 0.15[a,c]	0.743 ± 0.19[a]	2.10 ± 0.97[a]	ND
										ND
	C	207 ± 12[c,b]	3.28 ± 0.50	0.820 ± 0.08[a,b]	1.58 ± 0.15[a]	0.188 ± 0.04[b]	0.405 ± 0.04[a,b]	0.694 ± 0.10[a]	1.55 ± 0.23[a]	ND
										ND
12 months	A	429 ± 111[b,c]	2.64 ± 0.28[b]	0.685 ± 0.06[b]	0.712 ± 0.06[c]	0.126 ± 0.01[b]	0.267 ± 0.02[b,c]	0.430 ± 0.08[b,c]	1.74 ± 0.31[b]	0.533 ± 0.05[b,c]
	B	196 ± 80[a,c]	3.38 ± 0.70[a,c]	0.857 ± 0.13[a,c]	1.12 ± 0.18	0.154 ± 0.02[a]	0.398 ± 0.07[a]	0.631 ± 0.12[a,c]	2.93 ± 1.1[a,c]	1.03 ± 0.27[a,c]
	C	277 ± 36[a,b]	2.80 ± 0.33[b]	0.701 ± 0.07[b]	0.958 ± 0.04[a]	0.148 ± 0.03	0.367 ± 0.04[a]	0.543 ± 0.06[a,b]	1.61 ± 0.40[b]	0.710 ± 15[a,b]

[a] Significantly different from group A: $P < 0.05$ or less. [b] Significantly different from group B: $P < 0.05$ or less. [c] Significantly different from group C: $P < 0.05$ or less. [d] Not determined.

treated egg albumin plus 5% dextrine.

Rats were fasted for 18 h before being sacrificed by decapitation. Blood was collected in test tubes and heparinized capillary tubes. Hemacrit and hemoglobin values were determined on the spot. A kit (Sigma Chemical Co. Technical Bulletin No. 525, 1975b) in which Drabkin's reagent was employed was used to determine hemoglobin values. The rest of the blood was immediately placed in an automatic refrigerated centrifuge (Sorvall Superspeed RC 2-B, Servall Inc., Norwalk, CT) and centrifuged at 5000 rpm for 15 min at 4 °C. Serum was collected and frozen at −20 °C.

RESULTS AND DISCUSSION

Table II shows the PER values for control and browned diets. This table indicates that the protein value of the control and brown diets are virtually identical. The weight gain, relative organ weight, and biochemical determinations in rats fed brown and control diets for 1 month are reported in Table III. There were no significant differences in the values for serum protein and hematocrit between the brown and control groups. This suggests that the two diets are nutritionally similar as was also evident from the PER assay. The results of relative organ weights indicated that, except for cecum, no differences existed between the control and experimental groups after 1 month of feeding. Similarly, tests of liver function (liver GOT, liver GPT and SAP, SGOT and SGPT) did not reveal any difference between the two groups (Table III). The activities of small intestinal digestive enzymes also failed to show any significant differences after 1 month of feeding (Table III). These results further indicate that the assumption of nutritional similarity of the two diets is correct and diet C (5% egg albumin plus 5% nonessential amino acids) can be used as a control for the brown diet.

Growth. The growth curve for the rats on brown and control diets is shown in Figure 1. Rats in both groups gained weight equally for the first month. After this period, those on diet B started to lag behind the diet C group. As the rats were strictly pair-fed, the growth retardation in the brown group indicated the gradual precipitation of an effect which was not present and/or evident at the start of the feeding. Since the difference in absorption between the brown and control diets has been offset by feeding diets with the same PER values, it is safe to assume that the decline in the growth rate is not due to the original amino acid pattern or to the absorption rate of amino acids. Thus, the effect on growth appears to be beyond the absorption level being achieved through metabolic processes in the body.

Table IV contains data for body weight and relative organ weight of rats fed browned and control diets. The data for group D rats are not reported in this article. This is because after all the assays were conducted on this group of rats, the statistical analyses revealed that none of the values obtained in this group was significantly different from that of group C ($P < 0.10$) and concluded that the addition of 5% nonessential amino acids to diet C will not significantly modify the data in comparison with diet D. Thus, for the sake of brevity, we refrained from reporting group D values as they are similar to the values in group C.

From Table IV it is noted that the values for rats on browned diet generally possess a larger standard deviation of the mean, relative to the control group. This indicates a differential response by individual rats to the browned diet.

Table V. Urine Specific Gravity of Rats Fed Browned and Control Diets for 6 Months

diet	specific gravity, mean ± SD
brown	1.0549 ± 0.008[a]
control	1.0310 ± 0.010

[a] Significantly different from control group ($P < 0.01$).

On the average, the rats on brown diets weighed, compared to rats fed diet C, 30% less after 3 months and 25% less after both 6 and 12 months of feeding. The liver in rats fed browned diet was on the average 23% larger than group C and 62% larger than group A after 6 months. The kidneys also were enlarged after both 6 and 12 months of feeding.

The cecum and stomach of rats on the brown diet were significantly heavier than those in the control group. The occurrence of heavier stomach in group B is believed to be due in part to the change in the rate of stomach emptying observed in this study. The stomach of rats in the brown group were full after 18 h of fasting. The slower rate of stomach emptying was also observed previously (Tanaka et al., 1974). The size of the cecum in group B increased as the feeding period continued.

The spleen and heart in rats fed brown diet for 6 months were enlarged compared to the control group. The lung was also heavier after 1 year. These effects could be secondary to the effects on kidneys and liver since the latter organs are more susceptible to damage.

It should be pointed out that, although it is not apparent from Table IV, some effects of browned products were manifested in male rats after 3 months of feeding. This will be discussed later in this paper.

The specific gravity of urine in rats fed browned diet for 6 months was significantly higher than for the control group (Table V). This increase, along with the kidney enlargement suggest some abnormalities in kidney function.

The activity of serum alkaline phosphatase (SAP) in group B was increased, relative to group A, for all feeding periods, and the change was not significantly different from group A. After 1 year of feeding, SAP of group B was 130% higher than the control group (Table VI).

The increase in the activities of SAP and SGOT on one hand and the enlargement of liver on the other may imply physiological damage to the liver of rats fed browned diet. Furthermore, histopathological examination of the liver revealed the accumulation of a black-brown pigment of an unknown nature, the results of which will be reported separately.

It is our belief that the origin of the SAP is the liver since SGOT, another liver-specific enzyme, was also higher. The increased activities of SAP and SGOT merit more thorough investigation as possible indicators of the site and mode of action of brown compounds. By measuring the activities of alkaline phosphatase and GOT in the small intestine, liver, and kidneys it will be determined whether the stress is at the absorption level in the small intestine or in the metabolic process, i.e., liver or possibly kidneys.

Serum glucose was higher in rats fed brown diet after 6 months despite the fact that part of the glucose in the brown diet was bound to amino acids in the form of brown compounds. This elevated activity is another indication of a physiological stress, the exact nature of which cannot be ascertained with the present experiment, and should be explored further.

BUN of group B was higher compared to group C after 6 months of feeding but not significantly so after 1 year of feeding. BUN values were actually lower as compared to group A after 1 year. This would probably be due to the higher protein intake in group A.

Hemoglobin and hematocrit values were lower in rats after 6 and 12 months of feeding brown diet. The regression value for hemoglobin and hematocrit after a 1-year feeding period was 0.878 ($P < 0.001$). This observation suggests the merits of future investigation of other blood components, i.e., complete blood cell count and the determination of serum iron saturation, for correlation with results observed in this experiment.

Differences Due to Sex. Tables VII and VIII show the effect of sex on relative organ weights and serum components of rats fed browned and control diets. Male rats showed more susceptibility to brown compounds. After 3 months of feeding, the liver and kidney sizes were greater in male rats, while in females this effect only occurred after a 1-year feeding period. Serum alkaline phosphatase, serum glucose, and BUN increased in male rats after 3 months, while in females they increased only after 1 year of feeding. After 1 year, males had low hemoglobin and hematocrit values, but females were not affected appreciably.

The greater susceptibility of males as opposed to females to physiological stress is common and is reported frequently in the literature (Kuntz et al., 1966; Kinoshita et al., 1966; Irwin and Staton, 1969).

CONCLUSION

The statistical analysis of the data obtained in this study is shown in Table IX. From this table and also from Table VI, it is clear that as the feeding period increases the adverse effects of browned diet become more pronounced. This pattern of effects seems to be cumulative in nature and resembles that of toxic compounds.

Table VI. Hematology Data from Rats Fed ad lib (A), Browned (B), and Control (C) Diets (Mean ± SD)[d]

feeding period	diet	serum alkaline phosphatase, IU	serum GOT, IU	serum glucose, mg/100 mL	BUN, mg/100 mL	serum protein, g/100 mL	Hgb, g/100 mL	hematocrit
3 months	A	96 ± 42[b]	32 ± 4.8	66 ± 9	12.6 ± 3.0	8.2 ± 0.48[b,c]		45.8 ± 1.6
	B	211 ± 92[a]	39 ± 11	87 ± 33	15.4 ± 5.8	6.89 ± 0.84[a]		41.4 ± 4.5
	C	146 ± 28	29 ± 10	79 ± 9	10.1 ± 4.9	6.25 ± 0.58[a]		44.3 ± 3.1
6 months	A	78 ± 21[b]	62 ± 28	85 ± 7	19.4 ± 2.2[c]	6.60 ± 1.4	17.9 ± 0.4	46.7 ± 2.4[b]
	B	136 ± 68[a]	66 ± 18[c]	97 ± 21[c]	19.4 ± 6.4[c]	5.35 ± 1.4	15.1 ± 3.1[c]	38.4 ± 7.7[a,c]
	C	98 ± 30	53 ± 4[b]	71 ± 8[a,b]	12.9 ± 3.0[a,b]	5.84 ± 0.40	18.6 ± 2.2[b]	48.6 ± 4.0[b]
12 months	A	50 ± 23[b]	39 ± 1[b]	76 ± 4[b]	18.4 ± 5.3[b,c]	6.77 ± 0.49	14.9 ± 1.4[b]	46.9 ± 4.1
	B	138 ± 81[a,c]	56 ± 9[a,c]	82 ± 4[a,c]	13.5 ± 3.6[a]	6.38 ± 0.58[c]	12.7 ± 1.0[a,c]	44.4 ± 3.2[c]
	C	60 ± 43[b]	40 ± 8[b]	76 ± 6[b]	12.1 ± 3.4[a]	6.99 ± 0.38[b]	14.8 ± 1.8[b]	50.5 ± 3.8[b]

[a] Significantly different from group A: $P < 0.05$ or less. [b] Significantly different from group B: $P < 0.05$ or less. [c] Significantly different from group C: $P < 0.05$ or less. [d] Five male and five female rats for each treatment.

Table VII. Effect of Sex on Relative Organ Weights (Gram Percent Body Weight) of Rats Fed Browned (B) and Control[a] (C) Diets

feeding period	diet	body wt, g		liver		kidneys		spleen		heart		lung		cecum		stomach	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 months	B	110 ± 23[c]	132 ± 10[c]	4.16 ± 0.73[c]	3.4 ± 1.0	0.95 ± 0.10[c]	0.725 ± 0.05	0.189 ± 0.06	0.153 ± 0.04	0.46 ± 0.13	0.370 ± 0.54	ND[b] ND	ND ND	ND ND	ND ND	ND ND	ND ND
	C	140 ± 12	182 ± 12	3.08 ± 0.60	3.95 ± 0.26	0.80 ± 0.06	0.71 ± 0.03	0.154 ± 0.02	0.189 ± 0.01	0.430 ± 0.03	0.363 ± 0.02	ND ND	ND ND	ND ND	ND ND	ND ND	ND ND
6 months	B	155 ± 64	155 ± 79	4.6 ± 1.7	3.67 ± 1.1	1.23 ± 0.33[c]	1.02 ± 0.33	0.285 ± 0.15	0.259 ± 0.07	0.482 ± 0.17	0.499 ± 0.16	0.669 ± 0.10	0.779 ± 0.23	2.18 ± 0.61[c]	2.08 ± 0.71	ND ND	ND ND
	C	203 ± 12	212 ± 12	3.30 ± 0.17	3.25 ± 0.74	0.854 ± 0.06	0.785 ± 0.09	0.173 ± 0.02	0.204 ± 0.05	0.430 ± 0.04	0.376 ± 0.03	0.676 ± 0.08	0.676 ± 0.08	1.54 ± 0.07	1.56 ± 0.34	ND ND	ND ND
12 months	B	198 ± 82[c]	194 ± 76	3.28 ± 0.88	3.45 ± 0.63[c]	0.865 ± 0.14[c]	0.849 ± 0.17[c]	0.159 ± 0.02	0.151 ± 0.029	0.408 ± 0.10	0.459 ± 0.17	0.649 ± 0.12[c]	0.620 ± 0.13	2.87 ± 1.2	2.98 ± 0.79[e]	1.06 ± 0.46	1.0 ± 0.16[c]
	C	303 ± 28	256 ± 28	2.77 ± 0.30	2.82 ± 0.38	0.717 ± 0.07	0.687 ± 0.08	0.137 ± 0.02	0.157 ± 0.04	0.354 ± 0.03	0.378 ± 0.05	0.503 ± 0.05	0.577 ± 0.05	1.76 ± 0.42	1.48 ± 0.36	0.639 ± 0.11	0.771 ± 0.15

[a] Control pair-fed. [b] Not determined. [c] Significantly different from control pair-fed group at $P < 0.05$. [d] Significantly different from control pair-fed group at $P < 0.01$. [e] Significantly different from control pair-fed group at $P < 0.001$.

Table VIII. Effect of Sex on Serum Components of Rats Fed Browned (B) and Control (C) Diets (Units Used Are the Same as in Table IV)

feeding period	diet	SAP[1]		SGOT[2]		ser. glucose		BUN[3]		ser. protein		hemoglobin		hemotocrit	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
3 months	B	259 ± 75[e]	179 ± 85	46.5 ± 10	30.7 ± 3.9[e]	110 ± 28[e]	65 ± 23	19.6 ± 5.2[e]	11.1 ± 3.4	7.06 ± 0.77	6.7 ± 1.1	ND ND	ND ND	37.7 ± 3.3[e]	45 ± 1.4
	C	133 ± 30	159 ± 22	37.7 ± 5.8	21 ± 5.2	74 ± 13	84 ± 3.4	12.2 ± 6.3	7.8 ± 2.9	6.57 ± 0.68	5.9 ± 0.44	ND ND	ND ND	44.7 ± 4.5	44.0 ± 2
6 months	B	190 ± 77	104 ± 19	78 ± 22[e]	57 ± 3	105 ± 18[f]	90 ± 22	20.3 ± 9.1	18.1 ± 4	4.83 ± 1.1	5.62 ± 1.6	13.8 ± 4.1	14.7 ± 3.8[e]	37.5 ± 8.2[e]	39.0 ± 8[e]
	C	101 ± 18	96 ± 41	54 ± 1.0	53 ± 6	72.0 ± 8	69 ± 10	12.5 ± 2.9	13.2 ± 3.4	5.64 ± 0.48	6.08 ± 0.32	18.1 ± 2.4	19.0 ± 2.1	47.0 ± 4.9	50.2 ± 2.6
12 months	B	169 ± 86[e]	123 ± 81[e]	56 ± 9.8[f]	56.2 ± 9.5[e]	82.3 ± 6.3	82.5 ± 4.1[f]	13.3 ± 3.4	13.6 ± 3.9	5.95 ± 0.58[e]	6.8 ± 0.39[e]	12.4 ± 0.9[g]	12.9 ± 1.0	42.9 ± 4.1[g]	45.5 ± 2.2
	C	79 ± 48	43 ± 33	38.8 ± 5.8	41.4 ± 9.6	80.3 ± 4.7	73.0 ± 4.0	12.8 ± 4.5	11.5 ± 2.3	6.76 ± 0.30	7.19 ± 0.35	15.6 ± 0.87	14.1 ± 2.1	52.8 ± 1.6	48.6 ± 4.2

[a] Serum alkaline phosphatase. [b] Serum glutamic oxalic transaminase. [c] Blood urea nitrogen. [d] Not determined. [e] Significantly different from control at $P < 0.05$. [f] Significantly different from control at $P < 0.01$. [g] Significantly different from control at $P < 0.001$.

Table IX. Test of Difference (Student's t Test) between Groups Fed ad lib (A),[a] Browned (B),[b] and Control (C)[c] Diets

feeding period	groups compared	SGOT[d]	ser. glucose	SAP[e]	ser. protein	BUN[f]	hema-tocrit	hemo-globin	body wt	relative organ weight[l]							urine sp grav
										stomach	cecum	liver	kidneys	testes	spleen	heart	lung	
3 months	B vs. C	NS[h]	NS	NS	NS	NS	NS	ND[g]	**	ND	ND	NS	NS	NS	NS	ND	ND	ND
	B vs. A	NS	NS	*	**	NS	NS	ND	***	ND	ND	NS	NS	*	NS	ND	ND	ND
	C vs. A	NS	NS	NS	NS	NS	NS	ND	***	ND	ND	NS	NS	NS	NS	ND	ND	ND
6 months	B vs. C	*[i]	**[j]	NS	NS	**	**	*	*	ND	NS	NS	*	NS	*	*	NS	**
	B vs. A	NS	NS	*	NS	NS	*	NS	***	ND	*	NS	NS	***	NS	*	**	ND
	C vs. A	NS	**	NS	NS	**	NS	NS	***	ND	**	*	*	***	NS	***	**	ND
12 months	B vs. C	***[k]	*	**	**	NS	***	**	*	**	**	*	**	NS	NS	NS	*	ND
	B vs. A	**	**	*	NS	*	NS	*	***	**	*	*	*	*	*	***	**	ND
	C vs. A	NS	NS	NS	NS	**	NS	NS	***	*	NS	NS	NS	***	NS	***	**	ND

[a] Group fed 10% egg albumin diet. [b] Group fed browned diet. [c] Control group. [d] Serum glutamic oxalic transminase. [e] Serum alkaline phosphatase. [f] Blood urea nitrogen. [g] Not determined. [h] Not significantly different ($P > 0.05$). [i] Significantly different at $P < 0.01$. [j] Significantly different at $P < 0.001$. [k] Significantly different at $P < 0.001$. [l] 1, stomach.

Although the question of the toxicity of the browned compounds could not be completely resolved with this experiment, it is our belief that there is sufficient data to substantiate such a possibility. However, since this study was originally designed to investigate the general effects of the long-term feeding of browned food products, no firm conclusion could be made as to the reactive site and the mechanism of the effects of the browned diet as observed in the present study. It can be concluded, however, that the browned protein has certain physiological effects that are not detectable from chemical or short-term nutritional evaluations commonly practiced in the assessment of processed foods.

The possible toxicity of the browned food products, therefore, remains an open issue until the compounds responsible for the aforementioned effects are isolated and identified.

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