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Response of Two Strains of Rats to a High-Protein Diet Containing Sucrose or Cornstarch (41154)

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Abstract. Male BHE (carbohydrate-sensitive strain) and Wistar rats were fed from weaning to 400 days of age 45% casein diets containing sucrose or cornstarch. At this high protein intake type of carbohydrate had no effect on any of the parameters measured. Several significant differences, however, were observed between the two strains. Food utilization for weight gain was less efficient for young Wistar than for young BHE rats. BHE rats had a higher incidence and level of fat in the liver, and degree of nephrosis in the kidneys. Kidney calcium deposits were negligible in either strain. Urinary protein excretion was very low in young rats but increased with age. Urinary protein electrophoretic patterns changed with age; faster moving proteins were affected by age × strain interactions. At 400 days of age patterns were similar for both strains and probably related to the development of nephrosis. Total serum cholesterol and percentage prealbumin were higher, whereas serum albumin and very-low-density lipoproteins were lower in BHE than in Wistar rats. These differences probably were associated with kidney pathology as related to strain susceptibility.

Studies have demonstrated that the BHE[1] rat is very susceptible to nephrosis and that sucrose in combination with a high cholesterol diet accelerates the kidney damage (1–3). Renal disease (3) is the most frequent cause of death for male BHE rats. Genetic studies have demonstrated that heredity is a predominant factor in the development of nephrosis (4) and that lipid metabolism is abnormal in BHE rats (5–12). When fed selected dietary components, the BHE and Wistar strains differed in the following responses: serum protein distribution (13, 14); concentrations of serum and liver lipid fractions (3, 15, 16); serum, liver, and kidney enzyme activities (17); intestinal transport of sugars (18); serum insulin levels (18); and concentrations of hepatic metabolites (19). Several of these studies examined the effects of source or level of either protein (4, 5) or carbohydrate (3, 14, 16, 18–20) on the pathology and/or biochemistry of the BHE strain.

Little information is available on the extent to which the dietary carbohydrate may influence the response to high-protein diets and aggravate existing kidney conditions. The objective of this study was to determine the effect of the carbohydrate source, sucrose vs cornstarch, on tissue pathology and selected biochemical parameters, when two strains of rats differing in susceptibility to kidney disease and liver fat infiltration were fed a high level of dietary protein. The carbohydrate-sensitive BHE rat (20) provided a model for individuals that are prone to renal dysfunction. The Wistar rat served as the control.

Materials and Methods. Male weanling BHE or Wistar rats were fed *ad libitum* diets containing, g/100 g: casein, 45.0; either sucrose or cornstarch, 32.7; fat blend, 16.0; salt mix,[2] 4.0; nonnutritive

[1] A strain developed by crossing albino (Yale strain origin) and black and white hooded rats (Pennsylvania State College origin).

[2] The salt mix supplied in mg/4 g mix: potassium phosphate monobasic, 1560; calcium carbonate, 1530; sodium chloride, 560; magnesium sulfate, 230; ferric citrate, 110; manganese sulfate·H_2O, 9.5; potassium iodate, 3.2; cupric sulfate·$5H_2O$, 1.9; zinc chloride, 9.4; cobalt chloride·$6H_2O$, 0.091.

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fibre (cellulose), 2.0; vitamin mix,[3] 0.3. The composition of the fat blend approximated that normally consumed in the U.S. based on household food consumption information reported in 1955 and was comprised of 10 fats, g/16 g fat blend: butterfat, 3.95; lard, 3.85; hydrogenated vegetable oil, 2.86; beef tallow, 2.53; cottonseed oil, 1.21; margarine, 1.05; soybean oil, 0.33; corn oil, 0.11; olive oil, 0.055; peanut oil, 0.055. The "typical American diet" provides 44% calories from fat. The two carbohydrate diets containing 45% casein provide 32% calories from fat, which, although considerably more than suggested for the rat, does approach that eaten in the U.S.

The rats, 15 per dietary group for each strain, were housed individually in a temperature- and humidity-controlled room with a normal light cycle. Distilled water was available at all times. Food consumption and body weights were recorded weekly. At 75, 175, and 400 days of age the rats were placed in metabolism cages and urine was collected quantitatively for 16 hr overnight in a tube positioned in a minifreezer set at 5°. During the collection period all rats were fasted. After the final urine collection at 400 days of age, the rats were anesthetized by ip injection of sodium amytal (20 mg/100 g body wt). The body cavity was opened, blood was collected by cardiac puncture, and necropsy was performed immediately. Liver and kidneys were excised and weighed. Tissue sections were prepared for routine histological examination. Methods for histology (2) and grading systems of structural changes for the liver (3) and kidney (2) have been described previously.

[3] The vitamin mix contained, mg/0.3 g mix: menadione, 0.4; thiamine·HCl, 1.0; riboflavin, 1.0; pyridoxine·HCl, 0.5; niacin, 0.6; D-calcium pantothenate, 4.0; choline chloride, 30.0; vitamin B_{12} with mannitol (0.1%), 4.0; biotin, 0.04; folic acid, 0.4; paraminobenzoic, 20.0; *i*-inositol, 40.0. DL-methionine, 200 mg, made up the remainder of the 0.3 g vitamin mix. In addition the following supplements were fed once a week, percomorph oil, two drops supplying 395 IU of vitamin A and 56 IU of vitamin D, and DL-α-tocopherol acetate, 36 mg/0.1 ml cottonseed oil.

The volume of urine excreted was measured and if necessary the sample was filtered through glass wool to remove coarse particles such as hair. All samples were refrigerated and analyzed within 24 hr of the start of the collection to prevent changes in protein fractions that might occur during freezing and thawing. Protein was estimated by a turbidimetric method (21). Samples with a low-protein concentration were concentrated by dialyzing against a 1:1 mixture of powdered sucrose and cane sugar from 30 to 90 min (22). The urinary proteins were resolved in a discontinuous pH gradient system on polyacrylamide gel (Fig. 1) according to the method of Ornstein and Davis (23) and then evaluated densitometrically.

For separation of serum, blood was allowed to clot for 30 min at room temperature then held 30 min on ice. Total protein content was determined by an ultramicro biuret method (Beckman/Spinco Ultramicro Technical Bulletin No. 6074c). Blood urea nitrogen (BUN) was measured indirectly by the urease method of Kaplan (24). Total serum cholesterol was determined by the method of Bowman and Wolf (25). Prestained serum lipoprotein was resolved by acrylamide gel electrophoresis on a vertical slab by the procedure of Raymond *et al.* (26).

Serum protein was separated as described for urine protein (Fig. 1). The reproducibility of the relative amounts of the rat serum protein fractions separated by disc electrophoresis is satisfactory except for albumin (27). We designated the protein components by the nomenclature used for human serum proteins (23). Rat serum protein was separated into 12–19 components. The most prominent anodal band was albumin and was preceded by one and occasionally by two or three prealbumin bands. The second most-pronounced band was transferrin. Another frequently observed protein migrated slightly faster than the main transferrin component and stained for ferric ion. Percentage distribution of both the separated protein components and the lipoprotein components was measured densitometrically.

The data were statistically tested by

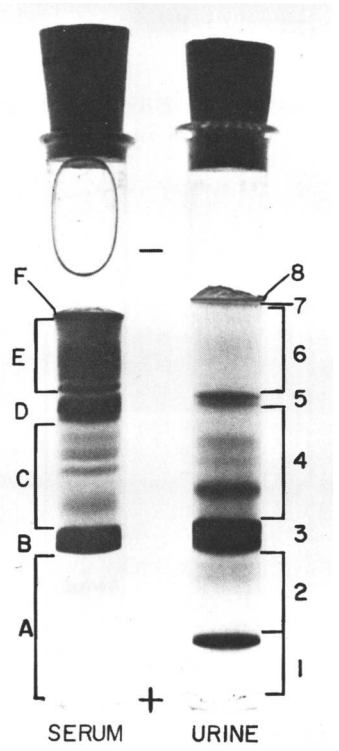

FIG. 1. Polyacrylamide gel (disc) electrophoretic patterns of serum and urinary proteins for comparison. Serum fractions: (A) prealbumin; (B) albumin; (C) postalbumin; (D) transferrin; (E) fast haptoglobin/γ-globulin; (F) slow haptoglobin/slow α-globulin, and β-1 lipoprotein. Urine zones: 1 and 2, fast-moving complex; 3, albumin like; 5, stains for iron, transferrin like.

analysis of variance. No interactions occurred between kind of dietary carbohydrate and strain of rat for any measurement made in this study utilizing a high-protein diet. Therefore, only main effects of these two variables are presented in the tables and discussed in the results. The treatment means for urine were compared by the Duncan multiple range test (28) when the F values were significant at the 5% level. Differences among treatments were considered significant at $P < 0.05$. The significance of the data for incidence of kidney damage and fatty livers was tested by χ^2 analysis (29).

Results. The data for food intakes and body weights are summarized in Table I. At this high level of dietary protein, food intakes, and body weights were comparable for BHE and Wistar rats regardless of carbohydrate source. During rapid growth (<100 days of age) Wistar rats consumed more food than the BHE rats, but body weights were similar. The BHE rat, however, was more efficient than the Wistar rat in utilizing either high-protein diet in gaining weight ($P < 0.05$).

Table II presents the data on liver and kidney weights, as well as the histopathology for the rats at 400 days of age. At this level of protein, the kind of dietary carbohydrate did not significantly affect the weight of either organ, the occurrence and extent of structural changes in the kidney or fat deposition in the livers. Nevertheless, BHE rats had a higher incidence ($P < 0.05$) of fatty livers and degree ($P < 0.01$) of fat deposited than the Wistar rats. Kidney damage was prevalent in both strains, but hyalin deposition, an indication of the severity of nephrosis (2), was significantly ($P < 0.01$) greater in BHE than in Wistar rats. In this study, kidney damage is defined as the occurrence of nephrosis (19 rats), hydronephrosis (3 rats), or calcium deposits (3 rats). The degree of calcium deposition was low regardless of strain or kind of carbohydrate.

By the sulfosalicylic acid method, protein was not detected in 80% of the urines of the 75-day-old animals. Urine had to be concentrated before analysis by electrophoresis. By 175 days of age protein was measurable in the urine of 80% of the rats. Nevertheless, the total amount excreted overnight was generally less than 100 mg, even at 400 days of age. The percentage distribution of the protein separated by disc electrophoresis is given in Table III for each strain at 75, 175, and 400 days of age. As many as 20–30 protein components were observed. Because mobilities differed slightly and some components were absent in urines of some younger animals excreting small amounts of protein, individual proteins were grouped into eight zones. The zones were designated numerically. Zone 1 migrated most rapidly toward the anode and zone 8 remained at origin. Zone 3 migrated at a rate similar to that of serum albumin and sometimes appeared as two components. Zones 3 and 4 constituted over

TABLE I. EFFECTS OF DIETARY CARBOHYDRATE AND RAT STRAIN ON FOOD INTAKE AND BODY WEIGHT[a]

	Dietary carbohydrate		Rat strain	
	Sucrose	Cornstarch	BHE	Wistar
		Body weight (g)		
Age (days)				
Weaning, 21	45 (30)[b]	46 (30)	44 (30)	46 (30)
70	258 (30)	266 (30)	260 (30)	264 (30)
98	325 (30)	344 (30)	328 (30)	342 (30)
119	354 (30)	372 (30)	357 (30)	370 (30)
168	397 (28)	403 (29)	408 (27)	393 (30)
400	497 (10)	488 (15)	497 (15)	485 (10)
		Food intake (g)		
70	597 (30)	593 (30)	572 (30)	618 (30)**
98	1014 (30)	1036 (30)	986 (30)	1064 (30)**
119	1325 (30)	1358 (30)	1299 (30)	1385 (30)*
168	2103 (28)	2108 (29)	2061 (27)	2146 (30)
400	5512 (10)	5501 (15)	5467 (15)	5563 (10)
		Diet efficiency[c]		
21–400	12.4 (10)	12.1 (15)	11.8 (15)	12.7 (10)*

[a] Significant differences due to main effects, dietary carbohydrate or rat strain, are denoted as follows: $*P < 0.05$, $**P < 0.01$.

[b] Number of animals denoted in parentheses.

[c] Ratio of total intake to total weight gain.

50% of the protein material excreted. Zone 5, usually two sharply defined bands, stained for iron and migrated at the same rate as serum transferrin. Rat urine proteins separated by disc electrophoresis (30) and by starch gel electrophoresis (31) have been characterized as only partially originating from serum. Some are related to the kidney

TABLE II. EFFECTS OF DIETARY CARBOHYDRATE AND RAT STRAIN ON WEIGHTS AND HISTOLOGICAL EVALUATION OF LIVER AND KIDNEY AT 400 DAYS OF AGE[a]

	Dietary carbohydrate		Rat strain	
	Sucrose	Cornstarch	BHE	Wistar
Number of rats	10	15	15	10
Liver weight (g)	11.01	10.35	11.11	9.86
Incidence[b] of fat (%)	60	47	73	20*
Fat[c]	0.9	0.5	1.0	0.2**
Kidney weight (g)	1.69	1.85	1.91	1.60
Incidence[b] of kidney damage (%)	70	80	93	70
Hyalin[c]	1.5	1.4	2.0	0.6**
Calcium[c]	0.1	0.2 (13)[d]	0.1	0.2 (8)[d]

[a] Significant differences due to main effects, dietary carbohydrate or rat strain, are denoted as follows: $*P < 0.05$, $**P < 0.01$.

[b] Incidence values were tested by χ^2 analysis for independence in a 2×2 table and a single degree of freedom.

[c] Histological scores of fat, hyalin and calcium based on a scale of 0 for no damage to 4+ for the most extensive deposits.

[d] Values in parentheses denote change in the number of rats.

TABLE III. EFFECTS OF RAT STRAIN AND AGE ON DISTRIBUTION OF URINARY PROTEINS (% OF TOTAL PROTEIN) SEPARATED BY DISC ELECTROPHORESIS[a]

	Strain						Probability		
	BHE			Wistar			Strain	Age	Interaction
Age (days)	75	175	400	75	175	400			
Number of rats	15	15	15	10	10	10			
Protein zone									
1	20.9a	8.3c	2.9d	13.3b	10.8bc	2.8d	—	0.01	0.01
2	12.8a	8.4bc	7.7c	13.4a	5.0d	10.5ab	—	0.01	0.01
3[b] (Albumin)	25.9b	35.3a	38.9a	29.3b	26.2b	40.0a	—	0.01	0.01
4	30.5b	23.8c	18.2d	33.2b	40.5a	19.8cd	0.01	0.01	0.01
5[b] (Transferrin)	3.2c	10.2b	13.9a	3.8c	8.6b	12.4a	—	0.01	—
6	2.2c	3.4ab	4.0a	2.6bc	2.6bc	4.4a	—	0.01	—
7	2.9c	8.5b	11.9a	2.8c	5.0c	9.0b	0.01	0.01	—
8	1.6b	2.0ab	2.4a	1.6b	1.4b	1.2b	0.05	—	—

[a] Means on a given line not followed by the same letter are significantly different ($P < 0.05$) as determined by Duncan's multiple range test (28).
[b] Tentative identity indicated in parentheses.

(30) and others designated as unique to urine (31).

Dietary carbohydrate did not influence urinary protein excretion, either totally or on a percentage distribution basis, at any age (data not shown). Age, however, produced a shifting in the percentage distribution of all protein components except for the slowest moving protein in zone 8. The significant interactions ($P < 0.01$) show that at each age level, the two strains responded differently to the high-protein diet with respect to the fast moving protein components of zones 1 through 4. Of the fastest moving proteins (zone 1) the percentage was higher in the young BHE rat (75 days of age) than in the Wistar rat. In Wistar rats zone 1 proteins did not differ between ages 75 and 175 days. However, the values for zone 1 were low for both strains at 400 days of age. The percentage of albumin-like material, zone 3, increased at an earlier age in BHE than in Wistar rats. The iron-containing proteins of the zone 5 increased with age for both strains.

Data on selected blood serum constituents of rats, 400 days of age, are shown in Table IV. Neither urea nitrogen nor total protein differed significantly with diet or strain. The slightly higher total protein

TABLE IV. EFFECTS OF DIETARY CARBOHYDRATE AND RAT STRAIN ON SERUM CONSTITUENTS AT 400 DAYS OF AGE

	Dietary Carbohydrate		Rat Strain	
	Sucrose	Cornstarch	BHE	Wistar
Number of rats	10	15	15	10
Urea nitrogen (mg/dl)	16.2	16.5	17.0	15.5
Total protein (TP) (g/dl)	6.62	6.14	6.54	6.02
Prealbumin (% of TP)	0.81	0.77	1.01	0.45**
Albumin (% of TP)	25.8	26.3	25.2	27.4*
Postalbumin 2 (% of TP)	2.44	2.67	1.91	3.58**
Fast haptoglobin/γ-globulin-1 (% of TP)	9.00	7.38	8.84	6.70**
Total cholesterol (mg/dl)	186	159	206	116*
Very-low-density lipoprotein (% of total lipoprotein)	16.8	18.6	12.2	26.2**

[a] Significant differences due to main effects, dietary carbohydrate or rat strain, are denoted as follows: *$P < 0.05$, **$P < 0.01$.

means for sucrose-fed rats and for BHE rats may have resulted from the milkiness or opalescence present in a few sera. Since there were no significant differences in serum total protein, the individual proteins are presented as percentages of the total protein. The pattern of distribution differed between strains but not between carbohydrates. By 400 days of age BHE rats had relatively more prealbumin and fraction 1 of haptoglobin/γ-globulin, and less albumin and fraction 2 of postalbumin than Wistar rats.

Total serum cholesterol was almost twice as high in BHE as in Wistar rats ($P < 0.05$). No carbohydrate difference was observed. Serum cholesterol was high for the starch-fed as well as sucrose-fed rats. The distribution of the lipoproteins on acrylamide gel was compared with lipoprotein fractions separated by preparative ultracentrifugation (32). The four major fractions separated on the gels were designated according to their flotation by ultracentrifugation. The two fastest migrating components were high density fractions and accounted for 60 to 75% of the lipid-staining material on the gel. The third fraction was low-density lipoprotein. The slowest moving fraction, was a very-low-density lipoprotein. In rats at 400 days of age no differences in lipoprotein distribution could be attributed to dietary carbohydrate; however, the percentage of very-low-density lipoprotein migrating in the serum was only about half as high for BHE as for Wistar rats ($P < 0.01$).

Discussion. Food consumption surveys have repeatedly shown that protein intakes in the U.S. (33) and other affluent nations are, on the average, more than twice that recommended. A continual high-protein diet may have deleterious effects on renal function as recently suggested by Robertson *et al.* (34–36). Carbohydrate energy in the human diet is chiefly provided by sucrose and starch. They are not metabolically equivalent. In any population some individuals apparently are sensitive to the sucrose component in the diet. The BHE strain of rats used in the present study is heterogeneous, differs from other strains (such as Wistar) with respect to energy metabolism, and provides individuals that are responsive to dietary carbohydrate.

In many studies, dietary carbohydrate sources have been compared using rats and other animal species as models, but the protein level of these diets was either adequate or below recommended levels. Carcass composition was studied (37) in BHE rats that were fed either 46% lactalbumin or 50% wheat gluten with sucrose or cornstarch. Food consumption was greater with sucrose than with cornstarch for both high levels of protein. Consequently, the sucrose-fed rats were heavier and deposited more carcass fat than the cornstarch-fed rats. Under our experimental conditions cornstarch-fed rats ate as much as the sucrose-fed rats and their body weights were comparable at each period of time over the 380 days. Since our experimental design differs considerably from the earlier study (37), further comparisons are not warranted.

In another study, BHE and Wistar rats fed a mixed source of protein at the 30% level for 330 days again exhibited different responses with sucrose and cornstarch. Livers (16) and kidneys (3) were larger when the diet contained sucrose rather than cornstarch. Sucrose caused a higher deposition of protein, cholesterol, and noncholesterol lipid in the liver than cornstarch (16). However, serum cholesterol concentration was not affected by the kind of dietary carbohydrate (16). Our present study, confirmed that serum cholesterol levels were not significantly affected by the kind of dietary carbohydrate.

Durand *et al.* (3) found that nephrosis occurred more frequently and the extent of kidney damage (hyalin casts) was greater when the carbohydrate was sucrose rather than cornstarch. In our study kidney damage, incidence, and severity, was similar in cornstarch-fed and sucrose-fed rats. Comparison of the hyalin ratings in both studies indicated that hyalin cast development was equivalent when sucrose was fed. In our study, twice as much hyalin deposition occurred when cornstarch was fed at the higher level of protein intake. Durand *et al.* (3) also reported that daily protein excre-

tion was higher for the sucrose-fed rats (356 mg) than for the cornstarch-fed rats (213 mg). These values are higher than those in our present study. The sulfosalicylic acid method that we used, however, provides only an estimate and is less sensitive than other methods, particularly at low concentrations (38).

In our present long-term study of rats fed diets with 45% protein, the values of all parameters tested were similar when either cornstarch or sucrose was the carbohydrate. Apparently heredity was the important factor in the response of the animals in metabolizing the excess protein. In our laboratory, short-term experiments, in which 45% casein was fed to Charles River rats, indicated that excess protein was not used for muscle protein synthesis (39) or for collagen formation in the muscle or skin (39, 40). Most, if not all, of the excess protein is thereby catabolized (41–43) or shunted into energy metabolism (44, 45). Protein catabolism apparently does not differ significantly between the two strains since fasting blood urea nitrogen levels in serum were similar (Table IV). Energy metabolism, however, differs markedly in the BHE and Wistar strains (5–12). Pyruvate kinase (PK) is a key enzyme in the regulation between glycolysis and gluconeogenesis. Adaptation of this enzyme to very high protein diets (>50%) differs between the liver and the kidney; levels of PK are depressed in the liver (44, 45) and elevated in the kidney (46). Therefore, gluconeogenesis is enhanced in the liver and glycolysis is enhanced in the kidney. Chang *et al.* (7) showed that in the liver fat was synthesized more rapidly and a greater portion of radioactivity from glucose appeared in the glycerol of neutral lipids in BHE than in Wistar rats. In our study, the BHE rats fed high protein possibly developed fatty livers because the high influx of amino acids into the liver was diverted for glucose formation and, in turn, into neutral lipid.

Serum cholesterol levels of the BHE rats were approximately double those of the Wistar rat. Marshall *et al.* (5) showed in their genetic studies on inbred BHE rats that serum cholesterol levels were elevated in a certain line and that the level was significantly affected by the amount of dietary protein. It was suggested that the high serum cholesterol levels in that line were related to the higher severity of nephrosis in rats fed high protein (4) and, that a defect in the regulatory mechanism influences the production and channeling of cholesterol between liver and serum. Berdanier *et al.* (6) in their experiments on inbred BHE, BHE, and Wistar rats also fed a high-protein diet (20% casein plus 20% lactalbumin), but for only 80 days after weaning. Serum cholesterol levels were highest in the inbred BHE rats (216 mg/dl) and did not differ significantly between BHE and Wistar rats (154 and 166 mg/dl, respectively).

Elevated serum cholesterol and increased urinary protein excretion are two indexes of nephrosis. Syntheses of plasma lipoprotein and protein are increased in the livers of nephrotics to compensate for the loss of protein, particularly albumin, in the urine that results in hyperlipoproteinemia and hypoalbuminemia. The differences in serum cholesterol concentrations between the two strains of rats fed the high-protein diet in our study probably are also related to the differences in the development of nephrosis in the two strains. In the rat, two-thirds of the total serum cholesterol is transported by the high-density lipoprotein fraction (HDL) (47, 48). Although the percentage distribution of the two HDL fractions did not differ significantly, the percentage of the combined HDL fractions was higher in BHE (75%) than in Wistar rats (62%). Possibly the higher percentage of HDL in BHE rats was related to the elevated cholesterol in their sera. Triglyceride mobilization, however, seems to be impaired in that strain since the percentage of very-low-density lipoprotein (VLDL) in serum was lower and the incidence and degree of fatty livers was higher in BHE than in Wistar rats. VLDL is the primary vehicle for circulating endogenous triglyceride. Fasting and nonfasting serum triglyceride levels also were lower in BHE than in Wistar rats (6).

Total plasma protein did not differ between the two strains and apparently was not altered by the nephrotic condition. However, the hyperprealbuminemia of the

BHE rats is indicative of the development of nephrosis (49). The low albumin percentage in BHE rats is associated with the nephrotic condition. At an early age (75 days) the urinary protein patterns, except for the most rapidly migrating components, were essentially similar for the two strains, thereby indicating that during the period of rapid growth the two strains were equally able to handle the high-protein diet without adverse effects on the kidney. The altered distribution of the protein fractions in the BHE strain at 175 days, especially the increased albumin excretion (fraction 3) is an expression of renal impairment. By 400 days of age the patterns again became similar, corresponding to the onset of nephrosis in the Wistar rat. The results of our study show that consumption of excessive amounts of protein could have deleterious effects on the kidney and that a genetic predisposition to renal disease accelerates the onset.

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