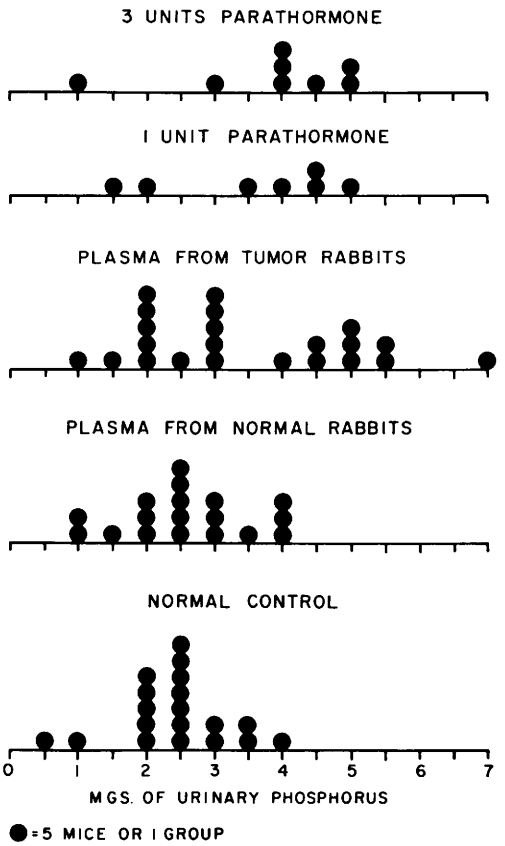

FIG. 1

nous parathormone. Lemon(2) used a change in the urinary phosphorus-to-calcium ratio as method for bio-assay of parathormone-like activity. In the present experiment the phosphaturic effect is used as index of this activity. It is demonstrated (Fig. 1) that commercial parathyroid extract, under the conditions of this experiment, does cause increase in urinary phosphorus. A similar effect was observed in 8 of 22 groups of mice receiving hypercalcemic rabbit plasma, and statistical analysis indicated this to be highly significant difference from groups receiving normal rabbit plasma.

Histopathologic study of hypercalcemic VX-2 carcinoma rabbits shows absence of parathyroid hyperplasia. Also excision of tumor reverses hypercalcemia, indicating likelihood that circulating parathormone-like substance is produced by tumor.

Summary and conclusions. Serum from hypercalcemic VX-2 carcinoma rabbits has a phosphaturic effect similar to exogenous parathormone when injected into mice. The most likely explanation is production of parathormone-like substance by tumor tissue.

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Food Restriction and Lipogenesis in the Rat. * (29195)

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Restriction of feeding time of rats to a period of 1-2 hours per day has been reported to increase activity of lipogenic mechanisms over that observed in animals permitted to eat spontaneously throughout the day(1-4). Hollifield and Parson(5) have recently confirmed that the lipogenic mechanisms are markedly increased in rats allowed access to food for only 2 hours per day and added the interesting and practical observation that, within a few days after this time-restricted feeding program was initiated, the rats trained to eat in a 2-hour period con-

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sumed greater quantities of food than did their controls on spontaneous, *ad libitum* feeding. Hollifield and Parson noted that after 10 weeks of experimental feeding, time-restricted fed animals were 30% heavier than were the controls. This observation led the authors to interesting speculations regarding the causes and treatment of obesity.

It has been our observation that restriction of feeding time of rats usually results in a body weight gain less than that of *ad libitum*-fed controls. We therefore, attempted to reproduce the experiment of Hollifield and Parson(5) and the results are reported here.

Methods. Male rats of the Sprague-Dawley strain were housed in individual, screen-bottomed cages at an environmental temperature of 23 ± 1°C and with 12 hours of light each day. In the 2 experiments to be reported, the animals were fed either Purina Chow laboratory pellets (3.6 cal/g; 69, 23, 5% from carbohydrate, protein, fat) or a high carbohydrate diet (HCD) (3.9 cal/g; 71, 21, 5% from carbohydrate, protein, fat). Rats on 2-hour feeding regimen were fed *ad libitum* from 9:00 a.m. to 11:00 a.m. daily. Food intake was measured 5 days each week and body weight once weekly. At termination of experimental feeding (10 weeks or 4 weeks), rats were fasted for 24 hours, then allowed food for 2 hours and sacrificed under sodium pentobarbital anesthesia (6 mg/100 g intraperitoneally). Incorporation of acetate-1-C^{14} into lipids by adipose tissue (epididymal fat pad) *in vitro* was determined by a modification of the procedure of Baruch and Chaikoff (6). Free fatty acid content of adipose tissue was measured by the procedure of Dole(7). Livers were quickly removed, frozen and glycogen determined by the procedure of Russell and Bloom(8). Carcass water was estimated by drying for 72 hours (to constant weight) at 80°C under vacuum. On aliquots of ground, dried tissue, total fat was measured by a Soxhlet procedure using acetone and ethanol as solvents; nitrogen was measured by a micro-Kjeldahl procedure. Protein was calculated by multiplying the nitrogen value by the factor 6.25. Ash was determined by the method of Brown(9).

Restricted feeding time in young male rats. Thirty-two young rats weighing approximately 125 g were divided into 4 groups of 8 each. Group I was fed chow *ad libitum,* Group II chow for 2 hours daily, Group III HCD *ad libitum,* Group IV HCD for 2 hours daily. After 10 weeks of experimental feeding, all animals were killed and analyses were performed.

Restricted feeding time in adult male rats. Twelve adult rats averaging 347 g in weight were divided into 2 groups of 6 each. Group I were fed *ad libitum* and Group II HCD for 2 hours daily. After 4 weeks of experimental feeding, all animals were killed and analyses were performed. One rat of the *ad libitum* control group was lost so that analyses for this group were done on 5 animals.

Results. Food and water intakes of young male rats are shown in Table I. Rats on 2-hour feeding ate significantly less than did *ad libitum* controls regardless of diet, and water intake was proportional to food intake. Rats on 2-hour feeding lost weight during the first week of feeding, then showed a gain in weight less than that of *ad libitum* controls and, at the end of 10 weeks, had average weight 40-45% less than that of the controls (Fig. 1). On both diets, incorporation of acetate-1-C^{14} into lipids (Table II) was significantly increased in animals fed for 2 hours compared with *ad libitum* controls. Although free fatty acid content of adipose tissue was lower in "restricted" groups than in controls, the differences were not statistically significant. Liver glycogen was increased in 2-hour fed animals on both diets but this increase attained significance only with HCD. Results of carcass analyses (Fig. 2) indicate that, if anything, 2-hour fed animals had less fat and a slightly higher proportion of water and ash than did *ad libitum* controls.

Food intakes, and liver glycogen concentrations in adult animals are shown in Table III. As with young rats, adult rats on the 2-hour feeding regimen ate significantly less food than did the *ad libitum* controls. The liver glycogen concentration of the 2 hour-fed animals was significantly higher than that of the *ad libitum* controls. These animals lost weight in the first week and failed to gain throughout the experiment, their final aver-

TABLE I. Effect of Restricted Feeding Time (2 hr/day) on Average Food Intake and Water Intake in Young Male Rats.

Group time (wk)	I (Chow ad lib) (8) Food int (g)	I (Chow ad lib) (8) Water int (g)	II (Chow 2 hr/day) (8) Food int (g)	II (Chow 2 hr/day) (8) Water int (g)	III (HCD ad lib) (8) Food int (g)	III (HCD ad lib) (8) Water int (g)	IV (HCD 2 hr/day) (8) Food int (g)	IV (HCD 2 hr/day) (8) Water int (g)
1	*19.1 ± .66	28.2 ± .94	4.7 ±.31†	11.0 ± .86†	16.3 ±.85	18.3 ±2.41	5.2 ±.32†	9.7 ±1.52‡
2	22.6 ± .56	33.2 ±1.59	8.1 ±.28†	15.9 ± .62†	18.1 ±.13	24.9 ±4.12	10.4 ±.11†	14.7 ±1.39§
3	23.7 ± .37	33.7 ±1.16	9.4 ±.42†	19.0 ± .95†	18.4 ±.56	31.3 ±4.41	10.9 ±.63†	17.8 ±2.50§
4	23.8 ± .43	35.0 ±1.31	10.4 ±.39†	22.0 ±1.02†	19.1 ±.89	27.3 ±2.95	11.2 ±.52†	20.5 ±2.53
5	24.1 ± .53	36.1 ±1.45	11.6 ±.61†	23.4 ±1.58†	18.9 ±.74	28.1 ±2.03	12.7 ±.67†	22.1 ±2.57
6	22.4 ± .37	38.8 ±2.04	11.1 ±.61†	23.4 ±1.99†	17.7 ±.68	29.3 ±3.69	11.8 ±.73†	20.2 ±2.20
7	22.7 ± .75	40.8 ±2.33	10.2 ±.53†	21.5 ±1.64†	18.5 ±.68	31.8 ±4.11	10.1 ±.63†	24.7 ±3.30
8	21.6 ± .43	40.3 ±2.76	10.4 ±.66†	20.9 ±1.90†	18.9 ±.51	32.4 ±4.68	9.8 ±.50†	20.9 ±2.35§
9	22.8 ± .54	41.4 ±2.81	10.5 ±.67†	21.5 ±1.58†	19.1 ±.55	32.8 ±3.63	11.5 ±.66†	20.4 ±2.22§
10	22.1 ±1.58	36.3 ±3.52	11.0 ±.82†	22.5 ±1.85‡	19.1 ±.55	31.7 ±3.63	11.4 ±.66†	19.1 ±2.03‡

* Mean ± S.E.M. † P <.001 ‡ P <.01 § P <.05 compared with ad lib group on same diet.
( ) indicates number of animals.

TABLE II. Effect of Restricted Feeding Time (2 hr/day) on Average Liver Glycogen, Free Fatty Acids of Adipose Tissue and Acetate-1-C^{14} Incorporation into Adipose Tissue in Young Rats.

Group	Liver glycogen (%)	Free-fatty acids (μM/100 mg fat)	Radioactive acetate incorporation (count/min/g fat/0 mass)
I (Chow ad lib) (8)	*2.62 ± .35	.95 ± .12	1837 ± 150
II (Chow 2 hr/day) (8)	3.21 ± .58	.58 ± .15	55143 ± 11044†
III (HCD ad lib) (8)	4.17 ± .28	.64 ± .11	4833 ± 1577
IV (HCD 2 hr/day) (8)	6.45 ± .44†	.39 ± .071	141379 ± 19352†

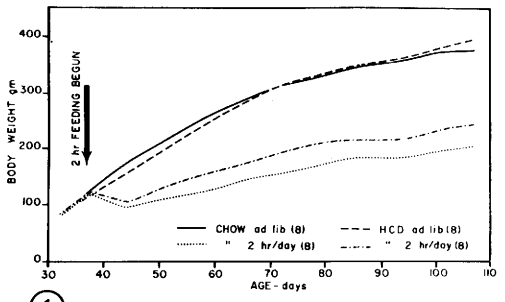
* Mean ± S.E.M.
† P <0.001 compared with ad lib group on same diet.
( ) indicates No. of animals.

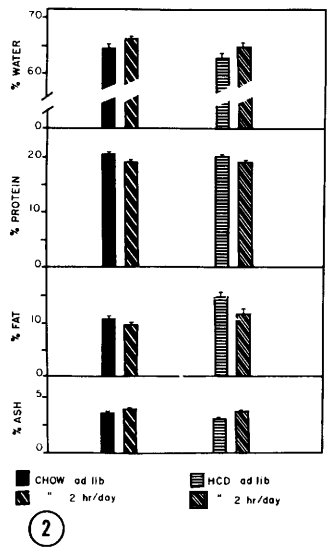
TABLE III. Effect of Restricted Feeding Time (2 hr/day) on Average Food Intake in Older Male Rats.

Food intake (g/day)	Time (weeks) 1	2	3	4
I-HCD ad lib (5)	*14.4 ± 1.10	18.9 ± 1.06	20.1 ± .84	19.5 ± 1.25
II-HCD 2 hr/day (6)	4.0 ± .21†	9.4 ± .70†	11.5 ± .70†	12.3 ± .65†
Liver glycogen (%)				
I-HCD ad lib (5)				3.55 ± .16
II-HCD 2 hr/day (6)				5.27 ± .51‡

* Mean ± S.E.M. † P <0.001 ‡ P <0.02 compared with ad lib group.
( ) indicates No. of animals.

age body weight after 4 weeks being 22% less than that of the controls (Fig. 3).





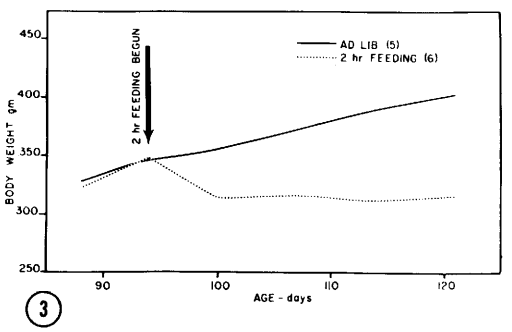


FIG. 1. Body weight changes of young male rats provided with chow or high carbohydrate diet (HCD) for 24 or 2 hr per day.

FIG. 2. Body composition of young male rats provided with chow or high carbohydrate diet (HCD) for 24 or 2 hr per day. S.E.M. is depicted by vertical bars.

FIG. 3. Body weight changes of adult male rats provided with high carbohydrate diet (HCD) for 24 or 2 hr per day.

Discussion. Hollifield and Parson(5) noted that incorporation of acetate-1-C^{14} into lipids by adipose tissue was increased and free fatty acid content of adipose tissue fell as rate of lipogenesis increased in rats allowed access to unlimited quantities of food for only 2 hours each day. They also noted increased liver glycogen in these animals. Our results with young male rats fed either Purina Chow or a high carbohydrate diet for 2 hours each day are in agreement with the findings of Hollifield and Parson. However, we have been unable to confirm their observation of an increased food intake and weight gain in 2-hour fed animals and, indeed, our results in these respects are opposite to theirs. The reason for these differences in results is not readily apparent.

In our investigation with chow-fed, young, male rats, the strain, sex and starting weight, of the rats, the diet, feeding times and duration were similar to those used by Hollifield and Parson(5). A comparison of body weight changes in our experiment with those observed by Hollifield and Parson reveals that the weight gain of their *ad libitum* controls was considerably less than that of our control animals (140 g compared to 250 g in 10 weeks) which, in our experience, is a normal rate for this strain of rats. Tepperman and Tepperman(2), studying rats trained to eat a day's ration in one hour noted that, after a preliminary period of weight loss, growth rate for "trained" rats nearly equalled that of controls. However, these workers chose only those rats which were gaining weight regularly at the end of the training period; these animals did not gain sufficient weight to attain the body weight of the controls in the experimental period.

Summary. 1. Using young male rats fed chow for only 2 hours daily, the increased lipogenesis *in vitro* and liver glycogen concentration reported by others has been confirmed. The phenomena have been demonstrated also in young male rats fed a purified, high carbohydrate diet for only 2 hours daily. 2. In contrast to the findings of Hollifield and Parson, young rats whose feeding time was restricted to 2 hours daily had a lower food intake and body weight gain than did *ad*

libitum-fed controls whether provided with chow or the high carbohydrate diet. These "restricted" animals had slightly lower proportion of carcass fat and higher proportion of water and ash. 3. Adult male rats when provided with the high carbohydrate diet for only 2 hours daily, ate less than *ad libitum*-fed controls and failed to gain weight.

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A Humoral Antibody Associated with Endotoxin Resistance.* (29196)

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The endotoxins of the gram-negative bacteria are highly toxic, but considerable resistance or tolerance to their pyrogenic and other effects may be induced easily by a series of injections of endotoxic preparations in experimental animals(1) or by infections in man(2). In many of their physiological effects, the endotoxins derived from different bacterial species are quite similar. Animals injected with a particular endotoxin become resistant generally not only to the homologous endotoxin but to serologically unrelated endotoxins derived from other bacterial species. Moreover, the resistance cannot be correlated with specific antibody titer(3) and may be induced in agammaglobulinemic subjects(4). Thus, a classical antibody or antitoxin response has not been invoked to explain endotoxin resistance. The mechanism responsible for endotoxin resistance has been considered generally to be an accelerated nonspecific phagocytosis of endotoxin by the reticuloendothelial system (RES). This concept was based primarily upon the reversal of endotoxin resistance by blockade and other studies associating endotoxin resistance and the functional activity of the RES.

Recent evidence has implicated also a humoral factor. Freedman reported that significant endotoxin resistance could be achieved by passive transfer of serum although he did not invoke a specific immunological mechanism to explain his results(5). Greisman, Carozza and Hills showed further that plasma from resistant animals conferred significant resistance in recipient subjects prepared by RES blockade and postulated that an opsonin may be involved in endotoxin resistance(6). Lastly, Watson and Kim(7) have demonstrated a notable degree of specificity in endotoxin resistance, in contrast to earlier work which indicated a lack of serological specificity. By means of extensive pyrogenic cross resistance tests they obtained results compatible with the presence of non-reciprocal cross-reactive antigens in different endotoxins. These findings and other ancillary evidence led these investigators to propose that endotoxin resistance was related to a classic immune response.

The present study was undertaken to detect the postulated antibody involved in endotoxin resistance by a classical serological

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