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THE ANTI-AGING ACTION OF HYPOPHYSECTOMY IN HYPOTHALAMIC OBESE RATS: EFFECTS ON COLLAGEN AGING, AGE-ASSOCIATED PROTEINURIA DEVELOPMENT AND RENAL HISTOPATHOLOGY

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SUMMARY

Hypophysectomy in young male Wistar rats aged 70 days, like food restriction begun at the same age, retarded the life-long rate of collagen aging in tail tendon fibres and inhibited the development of age-associated proteinuria and renal histopathology. Hypothalamic lesions which increased the food intake of hypophysectomized rats from 7 g to 15 g/day and produced obesity did not alter the rate of either collagen aging or proteinuria development, nor reduce life expectancy, but increased the incidence of abnormal glomeruli. In the intact rats elevation of food intake from 7 g to 15 g/day increased the rate of proteinuria development, but did not affect the rate of collagen aging. Hypophysectomy was found to have a greater anti-collagen aging effect than food restriction, when food intakes were the same in both groups. These studies suggest a pituitary-hormonal effect on collagen aging and a food–pituitary-hormone-mediated effect on the development of age-associated proteinuria.

Key words. Collagen fibres, Proteinuria, Renal histopathology, Hypophysectomy, Hypothalamic obesity

INTRODUCTION

It is well known that long-term caloric restriction begun in the young rat prolongs life [1–6], reduces the incidence of a number of diseases in old age including age-associated renal disease [1–6] and retards the physiological aging of collagen [6–8]. Less well known is the observation that hypophysectomy in the young rat has similar anti-aging effects in later life [6, 9–12].

Soon after hypophysectomy in the young male Wistar rat aged 50–70 days there is a sharp reduction in food intake from about 15 g/day in intact rats to 7 g/day in hypophysectomized rats [6, 9]. This raises the question of whether the anti-aging effects of

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hypophysectomy are due to the reduced food intake or to the loss of pituitary hormones which increase the rate of aging. An earlier study [11] had indicated that the anti-aging action of hypophysectomy on tail tendon collagen may be greater than that of food restriction. This suggested a residual collagen aging effect due to pituitary hormones. The present study examines the effect of raised food intake (from hypothalamic lesions) on the anti-aging action of hypophysectomy on the aging of tail tendon collagen and on the development of age-associated renal disease.

MATERIALS AND METHODS

Animals and treatments

A total of 142 conventional male Wistar rats of the University of Sydney strain were investigated.

In the main study data were collected between age 70 days and natural death from 91 rats arranged in five treatment groups consisting of 20 intact rats eating *ad libitum* an average of 19 g of food per day (INTACT 19), 15 intact on 15 g of food per day (INTACT 15), 20 intact on 7 g of food per day (INTACT 7), 22 lean hypophysectomized rats eating 7 g of food per day (LEAN HYP 7) and 14 obese hypophysectomized rats on 15 g of food per day (FAT HYP 15). Obesity was due to accidental hypothalamic damage during the hypophysectomy operation. Rats were hypophysectomized at age 70 days using the intra-aural technique of Koyama [13]. Completeness of hypophysectomy was assessed at autopsy and 3 lean and 2 fat rats with pituitary fragments in the sella turcica were rejected. None of the hypophysectomized rats (either fat or lean) had high water intakes (in excess of 45 ml/day) or abnormal rectal temperatures (outside the range of 35.5–38.0°C). All hypophysectomized rats received weekly subcutaneous injections of cortisone acetate (Roussel Pharmaceuticals, Sydney) at a dosage of 0.5 mg per 100 g of body weight during the course of the study.

A further 19 rats (6 *ad libitum* fed intact, 8 lean hypophysectomized, and 5 obese hypophysectomized) were sacrificed by guillotining in the mid afternoon at ages between 510 and 640 days for estimation of plasma corticosteroids by the competitive binding assay of Murphy [14] and measurement of the wet weights of perirenal and epididymal fat pads. Before sacrifice deep-body temperatures were measured during the afternoon on four successive days, using a No. 402 Yellow Springs thermistor probe inserted 5 cm into the rectum. Temperatures were read on a CIG Medishield digital thermometer.

Another 10 food-restricted intact rats (eating 7 g of food per day since age 50 days) and 12 hypophysectomized rats (operated at age 50 days) were used to provide additional tail collagen fibre data. Glomerular histopathology was studied in a further 8 *ad libitum* fed intact rats aged 100 and 500 days.

All rats were housed in an air-conditioned room at 28°C and exposed to artificial light for 12 h per day. They were fed a commercial cubed rat food (Doust and Rabbidge, Sydney) of composition 20.2% protein, 3.2% fat, 3.5% fibre, 1.37% calcium and 0.98%

phosphorus. No additional vitamins or minerals were given to either food-restricted or hypophysectomized rats.

Serial measurements on living animals

Food intakes were determined at ages 70, 200 and 500 days. For measurement of food intake each cage of rats was supplied with 500 g of food and 24 h later the food remaining including that spilled was estimated. Estimations were made on 3 consecutive days. In the following week food intake was measured over a 3-day period. Water intakes were measured concurrently with food intakes by supplying daily 800 ml of water to each cage of rats and measuring the volume remaining at the end of each day; this was performed only at age 500 days.

Body weights of all animals were measured on a Mettler P1000 balance at 2-weekly intervals until age 200 days and then at 100-day intervals until natural death.

Tail tendon collagen fibre breaking times in 7 M urea at 40°C were measured on the same animals at ages 170, 320, 620 and 740 days using the method of Boros-Farkas and Everitt [15]. Collagen fibres were removed from living rats under ether anaesthesia.

The 24-h protein excretion was estimated in urine collected from rats placed in metabolism cages without previous adaptation. Protein excretions were measured on the same animals at 70, 360, 620 and 700 days using the trichloroacetic acid Ponceau S dye method of Pesce and Strande [16]. Creatinine measurements were made at 620 days using the Folin alkaline picrate method [17].

Autopsy data

In the 91 rats of the main study the duration of life was recorded and an autopsy performed. The sella turcica of all hypophysectomized rats was examined for pituitary fragments. The lungs were grossly examined for respiratory disease. The kidneys and hearts were weighed for the assessment of hypertrophy using the criteria of Everitt *et al.* [6].

Four obese and 3 lean hypophysectomized rats, when moribund, were perfused with 10% formol saline and serial coronal sections of the hypothalamus cut at 50 μm and stained with cresyl violet to locate hypothalamic lesions.

Kidneys were collected from 20 old moribund rats (12 intact and 8 hypophysectomized) in the main study for histological assessment of age-associated renal disease using techniques described earlier [18]. A further 8 intact rats (4 young, 110–135 days, and 4 middle-aged, 370–540 days) provided additional data on age changes. Kidneys were fixed in formol saline, embedded in paraffin, 7-μm sections were cut and stained with periodic Schiff's reagent. A sample of 100–200 glomeruli in 16 random fields of kidney cortex of area 13 mm^2 were examined for proteinaceous casts and abnormal glomeruli.

Statistical analysis

Data are presented as mean ± standard error of the mean. The effects of treatments in the main collagen and proteinuria study were assessed by two-way analysis of variance

[19]. The rates of age-associated change were measured as the slopes of the collagen-age and proteinuria-age lines calculated by the method of least-squares [20]. Student's *t*-test was used for statistical comparisons between slopes and between means [20]. Data on glomerular abnormalities and cast counts were subjected to one-way analysis of variance and the studentized range test for differences between individual means [20].

RESULTS

Age-related changes in body weight, collagen and protein excretion were computed from serial data on the 23 rats (5 INTACT 19, 4 INTACT 15, 5 INTACT 7, 5 LEAN HYP 7, and 4 FAT HYP 15) which lived more than 850 days. Other parameters were measured only in young and/or middle-aged rats.

Food intake

Ad libitum fed intact rats increased their food intake (Fig. 1) from 15.0 ± 1.2 g/day ($n = 8$) at 70 days to 19.1 ± 1.4 g/day ($n = 10$) at 200 days and 18.8 ± 1.0 g/day ($n = 10$) at 500 days. In rats hypophysectomized at 70 days food intake dropped to. 6.9 ± 0.7 g/day at 200 days ($n = 10$), and 6.8 ± 0.6 g/day (range 5.3–8.5) at 500 days ($n = 10$). However, in the fat hypophysectomized rats food intake was 14.8 ± 1.0 g/day at 200 days ($n = 6$) and 15.1 ± 0.8 g/day (range 12.5–16.4) at 500 days ($n = 6$), and was significantly greater ($p < 0.01$) than that of lean hypophysectomized rats. Two fat rats,

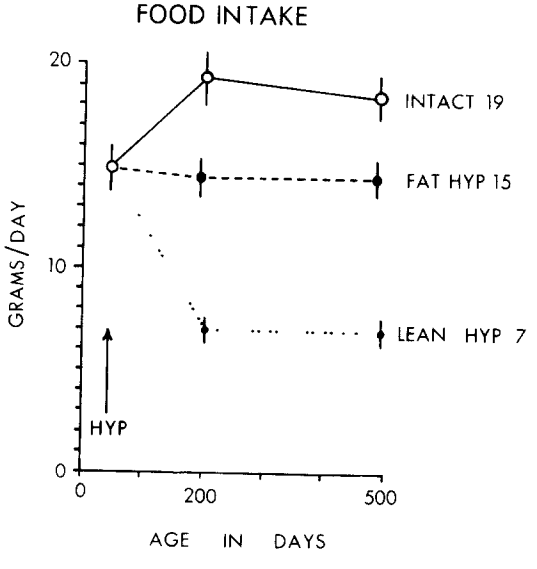

Fig. 1. The effect of hypophysectomy (LEAN HYP 7) and hypophysectomy with hypothalamic lesions (FAT HYP 15) at age 70 days on food intake at different ages in conventional male Wistar rats. The intact controls (INTACT 19) ate 15 g of food per day at 70 days rising to 19 g in fully grown rats. Data are plotted as mean ± S.E.

not in the main study, ate 18.0 and 18.8 g/day at age 200 days. No food-restricted rat (INTACT 7 and INTACT 15) spilled food in this study.

Water intake

At age 500 days daily water intakes were similar in *ad libitum* fed INTACT 19 rats (32 ± 3.1 ml/day, $n = 10$) INTACT 15 rats (29.0 ± 2.5 ml/day, $n = 5$), FAT HYP 15 rats (30.6 ± 3.6 ml/day, $n = 6$) and LEAN HYP 7 rats (28.1 ± 4.4 ml/day, $n = 8$), but lower ($p < 0.01$) in INTACT 7 rats (17.4 ± 1.2 ml/day, $n = 8$), compared with INTACT 19, INTACT 15 and FAT HYP 15 rats.

Body weight

In the sham-operated rats fed *ad libitum* (INTACT 19), body weight (Fig. 2) increased during growth reaching a maximum of 513 ± 15 g at 500 days and then slowly declined in old age.

Hypophysectomy at 70 days (LEAN HYP 7) produced a fall in body weight which stabilized at about 180 g or 80% of the initial weight. Intact rats (INTACT 7) fed the same amount of food (7 g/day) stabilized at about 165 g or 75% of their initial weight.

Fat hypophysectomized rats (FAT HYP 15) continued to gain weight reaching a maximum of 417 ± 18 g at 400 days. Intact rats (INTACT 15) fed the same quantity of food (15 g/day) also continued to grow reaching a maximum of 364 ± 16 g at 400 days. These maxima were not significantly different.

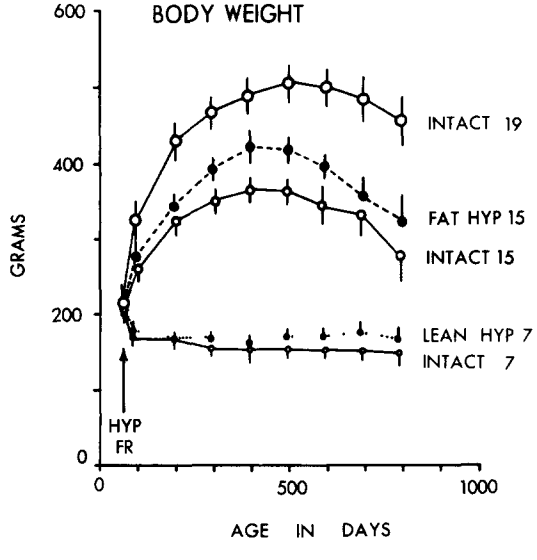

Fig. 2. The effect of hypophysectomy (LEAN HYP 7) and hypophysectomy with hypothalamic lesions (FAT HYP 15) at age 70 days on body weight at different ages in conventional male Wistar rats. Three groups of intact rats were fed *ad libitum* (INTACT 19) or food-restricted (FR) to 15 g of food per day (INTACT 15) or 7 g (INTACT 7). Data are plotted as mean ± S.E.

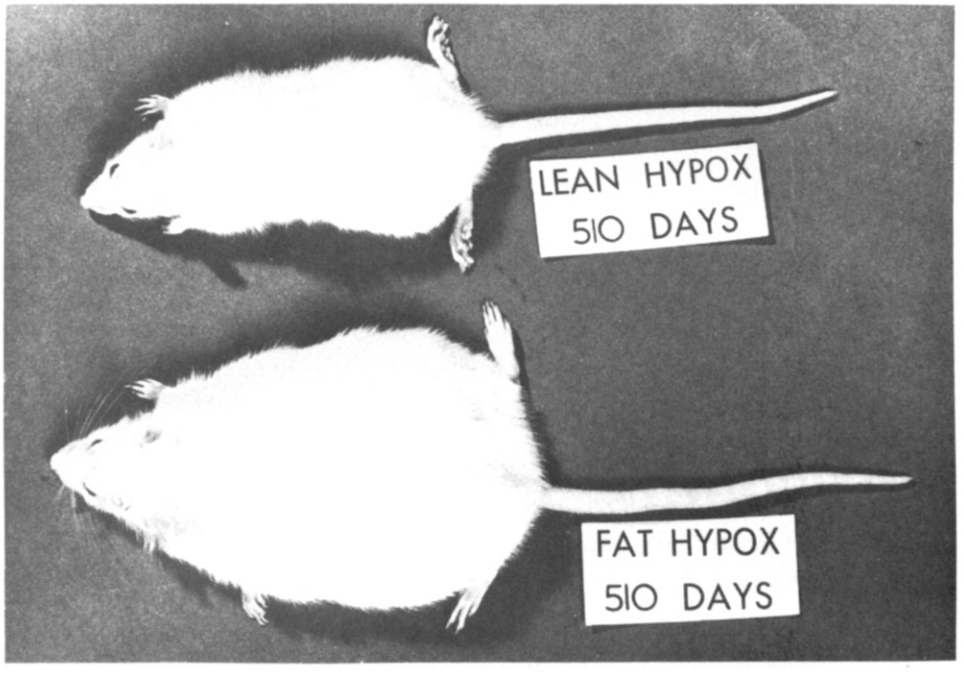

Fig. 3. The gross appearance of a lean hypophysectomized rat (7 g of food per day) and a fat hypophysectomized rat with a hypothalamic lesion (15 g of food per day). Rats were aged 510 days.

TABLE I

WET WEIGHTS OF ABDOMINAL FAT DEPOTS IN LEAN AND OBESE HYPOPHYSECTOMIZED RATS COMPARED WITH *AD LIBITUM* FED INTACT RATS AGED 510–640 DAYS

	Lean hypophysectomized rats	*Obese hypophysectomized rats*	*Control rats fed ad libitum*
Number of rats	5	5	6
Body weight (g ± S.E.)	208 ± 9	379 ± 19**	486 ± 16**
Perirenal fat pad (g ± S.E.)	3.83 ± 0.53	12.17 ± 1.82**	7.49 ± 1.12*
Epididymal fat pad (g ± S.E.)	4.16 ± 0.67	14.06 ± 1.67**	8.96 ± 0.63**
Perirenal + epididymal fat pads (g ± S.E.)	7.99 ± 0.89	26.22 ± 3.26**	16.45 ± 1.66**
Perirenal + epididymal fat pads (g per 100 g ± S.E.)	3.81 ± 0.36	7.33 ± 0.58**	3.35 ± 0.27

*$p < 0.05$ compared with lean hypophysectomized rats.
**$p < 0.01$ compared with lean hypophysectomized rats.

Weight of fat depots

In external appearance fat hypophysectomized rats were obviously obese compared with lean hypophysectomized rats (Fig. 3). The wet weights of perirenal and epididymal fat depots (Table I) in 5 obese hypophysectomized rats were approximately three times those of 5 lean hypophysectomized rats and twice those of 6 intact *ad libitum* fed rats of similar age. When allowance was made for difference in body size the combined weight of perirenal and epididymal fat depots per 100 g of body weight was approximately the same in lean hypophysectomized rats (3.81%) as in *ad libitum* fed intact rats (3.35%), but was significantly greater ($p < 0.01$) in fat hypophysectomized rats (7.33%) compared with values in lean hypophysectomized and *ad libitum* fed intact rats.

Creatinine excretion

Urinary creatinine is an index of muscle mass [21]. At age 620 days mean creatinine excretion per 100 g of body weight was significantly lower ($p < 0.01$) in fat hypophysectomized rats (2.02 ± 0.086 mg/100 g, $n = 7$) than in lean hypophysectomized rats (2.81 ± 0.127, $n = 10$), intact *ad libitum* fed rats (2.98 ± 0.132, $n = 10$) or INTACT 15 rats (3.25 ± 0.18, $n = 6$). Thus obese hypophysectomized rats appeared to have less muscle tissue per 100 g of body weight than rats in these other groups.

Plasma corticosteroids

Five days after injection, corticosteroid levels in cortisone-treated lean hypophysectomized rats (3.8 ± 0.4 μg/dl, $n = 4$) were higher ($p < 0.05$) than those in untreated hypophysectomized rats (all <2 μg/dl, $n = 3$), but less ($p < 0.05$) than the basal levels (13.7 ± 3.5, $n = 4$) in intact *ad libitum* fed rats of the same age. The limit of sensitivity of the assay was 2 μg/dl.

Rectal temperature

Deep body temperatures were not affected by the level of food intake, hypophysectomy or hypothalamic lesions. Mean temperature remained between 36.3 and 37.2°C for all treatments, with a range from 35.6 to 37.8°C, for rats aged 510–640 days.

Collagen fibre aging

In the intact *ad libitum* fed controls (INTACT 19) the breaking time of isolated tail tendon collagen fibres in 7 M urea at 40°C increased progressively with age (Fig. 4) from 28.0 ± 2.1 min at 172 days to 56.1 ± 3.1 min at 325 days and 166.2 ± 8.7 min at 744 days. Food-restricted intact rats (INTACT 15 and INTACT 7) and hypophysectomized rats (FAT HYP 15 and LEAN HYP 7) had shorter breaking times than *ad libitum* fed controls (INTACT 19) at ages 300 and 700 days.

Analysis of variance (Table II) revealed significant effects ($p < 0.01$) of both age and treatments. In addition, there was a significant interaction ($p < 0.01$) between treatment and age (treatments × ages) which represents the difference in slopes of regression lines.

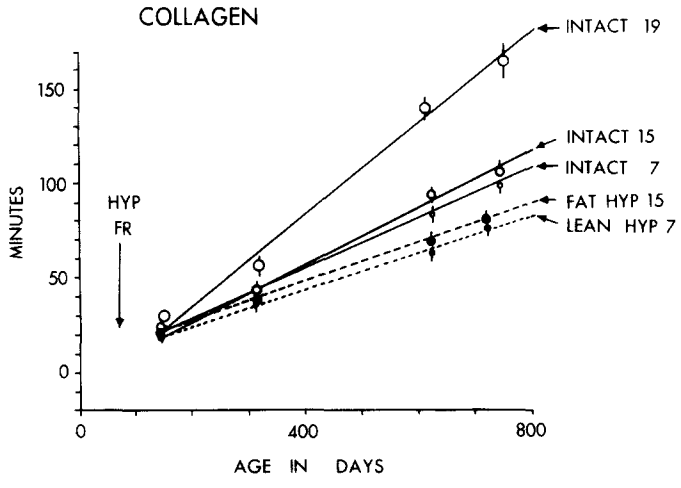

Fig. 4. The effect of hypophysectomy (LEAN HYP 7) and hypophysectomy with hypothalamic lesions (FAT HYP 15) at age 70 days on the aging of tail tendon collagen fibres as measured by the breaking time in minutes (mean ± S.E.) of an isolated fibre under a load of 2 g when immersed in 7 M urea at 40°C. Each point is the mean of fibre breaking times from 4 or 5 rats. Serial data at four ages were obtained from 23 rats (5 INTACT 19, 4 INTACT 15, 5 INTACT 7, 5 LEAN HYP 7 and 4 FAT HYP 15) which lived more than 850 days. INTACT 19 rats refers to *ad libitum* fed animals eating 19 g of food per day, INTACT 15 to rats fed 15 g of food per day, *etc.* The collagen aging rate (slope of the regression line) in hypophysectomized rats is slower than that in intact rats eating the same amount of food. Statistics are in Tables II and III.

The slope of the regression line is a measure of the rate of aging. In intact rats regression line slopes (Table III) were less ($p < 0.01$) in food-restricted rats (INTACT 15 and INTACT 7) than in *ad libitum* fed rats (INTACT 19). There was no difference in collagen aging rate (slope) between rats eating 15 g or 7 g of food per day, whether hypophysectomized (FAT HYP 15 *vs.* LEAN HYP 7) or intact (INTACT 15 *vs.* INTACT 7).

TABLE II

ANALYSIS OF VARIANCE OF COLLAGEN FIBRE BREAKING TIMES

Source of variation	*d f.*	*Sum of squares*	*Mean square*	*F between*	*F within*
Treatments	4	28 964.40	7 241 10	67.08**	–
Rats in treatments	18	1 942.84	107.94	–	–
Total between rats	22	30 907.24			
Ages	3	103 346.34	34 448 78	–	577 13**
Treatments X ages	12	17 176.75	1 431.40	–	23 98**
Ages X rats in treatments	54	3 223.41	59.69	–	–
Total	91	154 653.74			

**$p < 0.01$.

TABLE III

REGRESSION LINE SLOPES ± S.E. FOR COLLAGEN FIBRE BREAKING TIME–AGE DATA IN FIG. 4

Slope is reported as min/day

Food intake (g/day)	*Intact rats*	*Hypophysectomized rats*
19	0.242 ± 0.011**[a]	–
15	0.149 ± 0.0058	0.098 ± 0.012**
7	0.132 ± 0.060	0.092 ± 0.006**

**$p < 0.01$ compared with corresponding intact group.
**[a]$p < 0.01$ compared with INTACT 15.

Two fat hypophysectomized rats eating 18–19 g of food per day, aged 325 days, had breaking times of 32 and 39 min, which were similar to those of HYP 7 (37.6 ± 2.0) and HYP 15 (42.5 ± 2.7) rats. These rats died at 368 and 604 days.

Hypophysectomized rats (FAT HYP 15 and LEAN HYP 7) had smaller slopes ($p < 0.01$) and hence slower rates of collagen aging than intact rats eating the same quantity of food (INTACT 15 and INTACT 7). Due to the lack of agreement on the relative anti-collagen aging effects of hypophysectomy and food restriction [6,11] an additional study was made. The mean breaking time at 40°C of 10 food-restricted rats was 80.0 ± 3.2 min, which was significantly greater ($p < 0.01$) than 65.8 ± 2.8 min found in 12 hypophysectomized rats of similar age. The mean age of these food-restricted rats was 612 ± 3.6 days (range 585–625) and of hypophysectomized rats 615 ± 3.5 days (range 595–630). Food intakes were approximately 7 g/day in intact rats and 7.1 ± 0.5 g/day in hypophysectomized rats.

Protein excretion

In the intact *ad libitum* fed rats protein excretion (Fig. 5) increased progressively with age from 0.3 ± 0.08 mg/day at 70 days to 5.3 ± 0.44 mg/day at 360 days and 17.8 ± 4.1 mg/day at 800 days. Mean protein excretions at 770–825 days were proportional to food intake; there were significant differences ($p < 0.01$) between food intake groups of 19 and 15 g/day and 15 and 7 g/day. In this age range hypophysectomized rats had a lower protein excretion ($p < 0.01$) than intact rats eating 15 g/day, but there was no difference in rats eating 7 g/day.

Analysis of variance (Table IV) showed significant effects ($p < 0.01$) of age and treatment, as well as a significant interaction ($p < 0.01$) between age and treatment. Regression line slopes (Table V) in intact rats decreased markedly ($p < 0.01$) between food intakes of 19 g (INTACT 19) and 15 g (INTACT 15) and moderately ($p < 0.05$) between 15 g and 7 g (INTACT 7). Thus doubling food intake from 7 g to 15 g per day had no effect on the development of proteinuria in hypophysectomized rats, but it accelerated proteinuria development in intact rats. Protein excretions (1.4 and

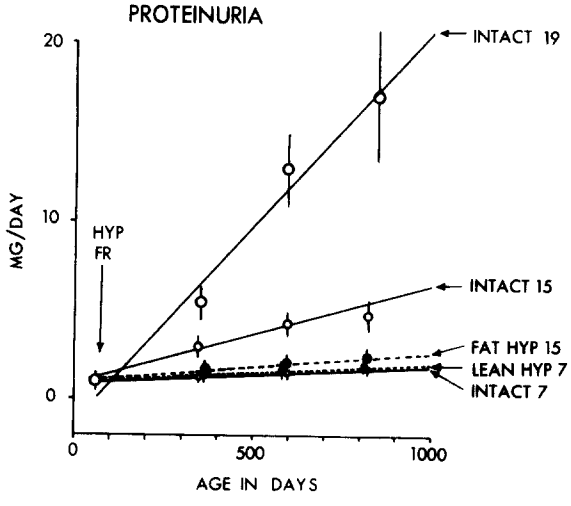

Fig. 5. The effect of hypophysectomy (LEAN HYP 7) and hypophysectomy with hypothalamic lesions (FAT HYP 15) at age 70 days on the urinary excretion of protein in mg/day (mean ± S.E.) at different ages. Each point is the mean protein excretion of 4 or 5 rats. Serial data at four ages were obtained from 23 rats (5 INTACT 19, 4 INTACT 15, 5 INTACT 7, 5 LEAN HYP 7 and 4 FAT HYP 15) which lived more than 850 days. INTACT 19 rats are *ad libitum* fed animals eating 19 g of food per day, INTACT 15 are fed 15 g of food per day, *etc.* Raising the food intake from 7 to 15 g/day increased the development of age-associated proteinuria in intact rats (INTACT 7 *vs.* INTACT 15) but not in hypophysectomized rats (LEAN HYP 7 *vs.* FAT HYP 15). Statistics are in Tables IV and V.

1.6 mg/day) of two HYP 19 rats aged 330 days were similar to those of HYP 7 and HYP 15 rats.

Renal histopathology

The rise in protein excretion with age is associated with the development of renal disease [18]. The incidence of proteinaceous casts (Table VI) per mm^2 paralleled the

TABLE IV

ANALYSIS OF VARIANCE OF PROTEIN EXCRETIONS

Source of variation	*d.f.*	*Sum of squares*	*Mean square*	*F between*	*F within*
Treatments	4	833 74	208.44	15.84**	–
Rats in treatments	18	236.85	13.16	–	–
Total between rats	22	1070.59			
Ages	3	263 30	87.77		16.43**
Treatments X ages	12	553.58	46.13		8.64**
Ages X rats in treatments	54	288 43	5 34		
Total	91	2175 90			

**$p < 0.01$.

TABLE V

REGRESSION LINE SLOPES ± S.E. FOR PROTEIN EXCRETION– AGE DATA IN FIG. 5

Slope is reported as 10^{-3} mg day^{-2}.

Food intake (g/day)	*Intact rats*	*Hypophysectomized rats*
19	22.32 ± 4 33**	–
15	5.49 ± 1.03	1.49 ± 0.63**
7	0.68 ± 0.56**	1.34 ± 0.52

**$p < 0.01$ compared with INTACT 15.

level of proteinuria. In *ad libitum* fed intact rats the count of casts increased with age from 0.3/mm^2 at 120 days to 2.18/mm^2 at 950 days ($p < 0.01$). In old age the count was significantly lower ($p < 0.01$) in hypophysectomized rats (HYP 7 and HYP 15) and in food-restricted rats (INTACT 7).

The incidence of abnormal glomeruli increased with age (Table VI). Stages in the development of glomerular pathology are shown in Fig. 6. Old *ad libitum* fed controls

TABLE VI

INCIDENCE OF ABNORMAL GLOMERULI AND PROTEINACEOUS CASTS IN KIDNEYS IN RELATION TO AGE, FOOD INTAKE AND HYPOPHYSECTOMY

There were 4 rats in each group. Data are reported as mean ± S.E

Pituitary	*Age (days)*	*Food intake (g/day)*	*Abnormal glomeruli (%)*	*Casts per mm²*
INTACT	123 ± 5	*ad lib.*	36.0 ± 3.3[a]	0.03 ± 0.03[a]
	474 ± 44	*ad lib.*	37.0 ± 5.3[a]	0.13 ± 0.03[a]
	957 ± 54	19	81.0 ± 3.6	2.18 ± 0 68
	979 ± 15	15	34 0 ± 1 4[a]	0.98 ± 0.29
	1008 ± 85	7	24.0 ± 2.1[a]	0 08 ± 0 05[a]
HYP (hypophysectomized)				
	851 ± 98	15	44.5 ± 5.2[ab]	0.15 ± 0.06[a]
	1019 ± 17	7	13.8 ± 2.0[a]	0.08 ± 0.05[a]
F			34.66[c]	8 23[c]
Minimum significant range at $p < 0$ 01			20.3	1 58
(studentized range test [19]) at $p < 0$ 05			16 5	1.29

[a]$p < 0$ 01 compared with INTACT 19 (*ad libitum* fed).
[b]$p < 0.01$ compared with HYP 7
[c]$p < 0$ 01.

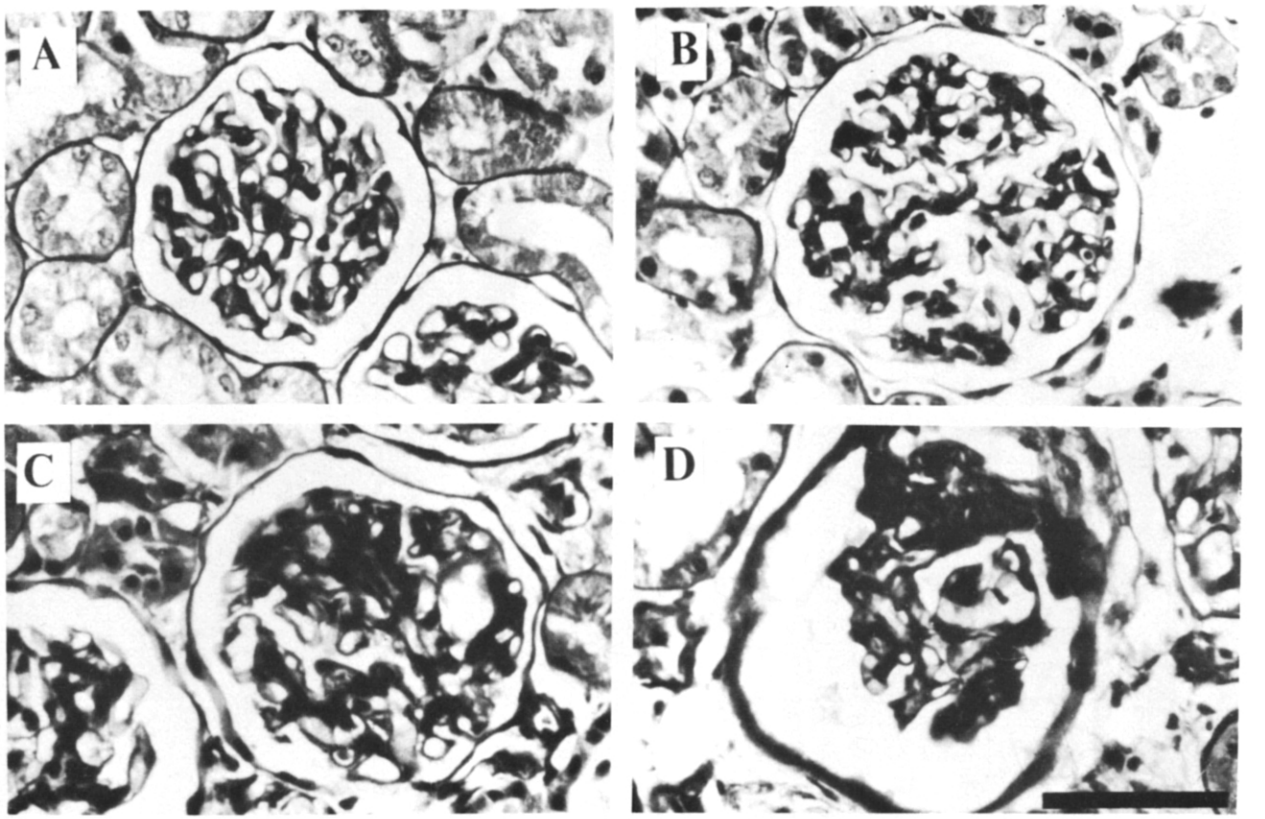

Fig. 6. Light micrographs of glomeruli showing progressive development of abnormalities. All were taken at the same magnification. The scale bar is 50 μm (A) Essentially normal glomerulus from an old LEAN HYP 7 aged 1102 days. It is similar in diameter and appearance to a glomerulus from a young intact *ad libitum* fed rat. (B) Early glomerulopathy in an old FAT HYP 15 rat aged 802 days. It has a larger diameter than A, increased mesangial fibrosis and marked lobulation. (C) Moderate glomerulopathy in an old FAT HYP 15 rat aged 802 days. Basement membrane thickness has increased in both the glomerulus and Bowman's capsule. Mesangial fibrosis is marked and there are fewer patent capillaries than in either A or B. (D) Advanced glomerulopathy in an old INTACT 19 rat aged 795 days. It shows degeneration of the capillary tuft, increased membrane thickness in both the glomerulus and Bowman's capsule, and the presence of a cast in Bowman's space.

(INTACT 19) showed focal glomerulosclerosis, proliferation of mesangial cells, thickened basement membranes in Bowman's capsule and the glomerulus itself, and the presence of hyaline casts in glomeruli and convoluted tubules. The glomeruli of the old food-restricted rats (INTACT 7) and lean hypophysectomized rats (LEAN HYP 7) were virtually free of renal pathology and resembled those of the young *ad libitum* fed controls. However, the old fat hypophysectomized rats (FAT HYP 15) showed a greater incidence of abnormal glomeruli than either the INTACT 7 ($p < 0.01$) or LEAN HYP 7 ($p < 0.01$). Initial electron microscopy of FAT HYP 15 glomeruli shows changes often seen in old INTACT 19 rats such as collapsed glomerular capillaries, increased basement membrane thickness, and fused and misshapen foot processes.

Life duration

The mean life duration of 20 intact rats fed *ad libitum* was 756 ± 42 (S.E.M.) days with a maximum of 1087 days. Compared with the *ad libitum* fed intact rats, life duration was significantly greater ($p < 0.05$) in the 19 lean hypophysectomized rats (891 ± 48 days), but not in the 12 fat hypophysectomized rats (872 ± 56 days). The longest-lived lean hypophysectomized rat died at age 1124 days and the longest-lived fat hypophysectomized at 1164 days. The mean life duration of fat rats was underestimated due to the sacrifice of 4 moribund animals for histological studies; the underestimate is probably about 5 days. For other groups the underestimate is less than 5 days.

Compared with the *ad libitum* fed intact group, food restriction significantly ($p < 0.05$) increased life duration to 912 ± 54 days in 15 rats eating 15 g of food per day, but not in 20 rats eating only 7 g of food per day (870 ± 41 days). The maximum life durations were 1188 and 1128 days, respectively.

Incidence of gross pathology

In rats older than 700 days hind-leg paralysis (Table VII) was observed in 5 of the 15 *ad libitum* fed controls during the last 100 days of life. This disease was not seen in any food-restricted or hypophysectomized rat. Rats with this disease drag their paralysed

TABLE VII

INCIDENCE OF DISEASE IN CONVENTIONAL MALE WISTAR RATS AGED 700 DAYS OR MORE

	Controls	*Food restricted*		*Hypophysectomized*	
Food intake (g/day):	*19*	*7*	*15*	*7*	*15*
Number of rats	15	16	13	17	10
Tumours	5/15	1/16	1/13	0/17	0/10
Respiratory disease	12/15	9/16	10/13	10/17	8/10
Cardiac enlargement	3/15	0/16	0/13	0/17	0/10
Renal enlargement	1/15	0/16	0/13	0/17	0/10
Hind-limb paralysis	5/15	0/16	0/15	0/17	0/10

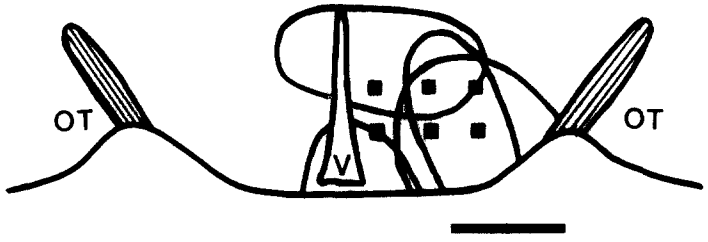

Fig. 7. A diagram of the coronal section of the medial hypothalamus outlining the position of gross lesions in 4 fat hypophysectomized rats from which serial data were obtained on collagen aging and proteinuria development. These lesions overlap the area shown with black squares where lesions were found by Anand and Brobeck [22] to induce hyperphagia. OT = optic tract, V = third ventricle. The interaural coordinate is approximately 6.7 mm in the atlas of Paxinos and Watson [46]. The scale bar is 1 mm. The lesions average 1.5 mm across. In each case the lesions destroyed part or all of the ventromedial nucleus. Other nuclei affected partly are the anterior hypothalamus and possibly the dorsomedial nucleus.

hind legs, while propelling themselves forward with their normally functioning forelimbs.

Chronic respiratory disease was seen grossly at autopsy in 60–80% of all rats. The incidence was not affected by either food restriction or hypophysectomy. No rat had severe respiratory disease in this study.

Gross tumours in rats older than 700 days were found in 5 *ad libitum* fed controls and in 2 restricted rats, but no hypophysectomized rat had a tumour.

Abnormal cardiac enlargement (heart ventricle weight greater than 1.8 g [6]) was seen in 3 *ad libitum* fed controls but not in any food-restricted or hypophysectomized rat.

Hypothalamic lesions

Coronal sections of the basomedial hypothalamus (Fig. 7) of 4 obese hypophysectomized rats in the main study revealed large lesions which overlapped the area where lesions are known to induce hyperphagia [22]. In lean hypophysectomized rats no evidence of damage was seen in 2 animals, but in 1 case there was minimal damage to the infundibular region. These hypothalamic lesions did not change behaviour other than to increase food intake and reduce body movement (qualitative observations only). Deep-body temperature and water intake were not affected by these hypothalamic lesions. There was no evidence of motor or visual disturbances or of aggressive behaviour in these obese hypophysectomized rats.

DISCUSSION

Hypophysectomy produces major changes in the physiology of the animal [10, 23] due to the loss of at least nine hormones causing large decrements in adrenocortical, thyroid, reproductive, metabolic, renal and cardiovascular functions. Despite the severe hormonal deficiencies and depressed body functions of the hypophysectomize rat, there

is evidence that collagen aging is retarded and certain age-associated pathological changes are delayed in onset [6, 9–11]. Cortisone therapy which produces a subphysiological plasma level of corticosteroid enables the hypophysectomized rat to live longer than the intact animal [6].

Collagen aging

This report showed that hypophysectomized rats with large hypothalamic lesions which caused them to double their food intake (from 7 g to 15 g of food per day) and become obese, aged at about the same rate as lean hypophysectomized animals on the basis of the collagen aging test. Such gross and random hypothalamic lesions may have destroyed a number of centres affecting a variety of body functions apart from feeding behaviour and endocrine secretions already depressed by hypophysectomy. Despite this, these lesions produced no significant change in the rate of collagen aging. This suggests either that the hypothalamic centres destroyed have no effect on this collagen aging process, or if they do their effect is mediated by the pituitary.

A further problem with this study was the lack of substantial data from HYP 19 rats. The only two rats available had collagen fibre breaking times at 325 days which were similar to those of HYP 7 and HYP 15 rats. While these data are meagre they do suggest that pituitary hormones, rather than food factors, may be concerned in collagen aging.

A pituitary-hormone effect on collagen aging is also suggested by the observation that hypophysectomized rats have a lower rate of collagen aging than intact animals eating the same amount of food per day (Fig. 4, Table III, and additional data in the text). This confirms an earlier report from this laboratory using the 40°C urea-breaking-time test [11] but is at variance with another study [6] where breaking-time estimations were performed at 50°C rather than 40°C in order to make measurements on very old rats aged 1000 days. It is possible that the 50°C version of this test may have different effects on the various collagen cross-links concerned in the 40°C test.

Proteinuria development and renal histopathology

The earlier studies of Kennedy [24] showed that rats with an intact pituitary but with ventromedial hypothalamic lesions doubled their food intake compared with the normal, became obese, and "developed typical senile kidney lesions about 9 months earlier than the unoperated controls". Genetically obese rats [25, 26] also have high food intakes in association with high urinary protein excretions and renal lesions. In the present study obese hypophysectomized rats (eating 15 g of food per day) when compared with lean hypophysectomized rats (eating 7 g/day) had a significantly higher percentage of abnormal glomeruli in their kidneys (Table VI), although there was no increase in proteinaceous casts or urinary protein excretion. This observation suggests that the incidence of age-associated glomerular pathology may be related to food intake as previously reported [18].

The rate of development of age-associated proteinuria appears to depend on both food intake and pituitary hormones. When food intake was 7 g/day the rate of proteinuria development was the same in hypophysectomized and intact rats (Fig. 5). Raising

food intake to 15 g/day accelerated proteinuria development in intact rats but not in hypophysectomized animals. This suggests that the aging effect of food on proteinuria development is mediated by the pituitary.

Hormonal aging factors

This study suggests that the pituitary secretes one or more aging factors. There is evidence that these factors may be normal pituitary hormones [27] or even hormones not yet isolated [12]. Pituitary growth hormone is reported to increase the incidence of age-associated renal lesions [28] and to accelerate skeletal aging [29]. There is evidence that pituitary adrenocorticotropin and related peptides can influence brain aging [30]. Hormones secreted by pituitary-dependent glands (thyroid, adrenal cortex, ovary, testis) also appear to modulate aging phenomena [27, 30–32]. For example, thyroxine [33] and cortisone [27] have both been found to accelerate collagen aging in rat tail tendon. The low corticosteroid levels observed in hypophysectomized rats in this study would have contributed to their slow rate of collagen aging.

Hyperinsulinemia is a characteristic of hypothalamic-obese rats [34] and is also present when such animals are hypophysectomized [35]. No measurements of plasma insulin were made in the present study. The actions of insulin on aging phenomena are largely unknown. However, in diabetes mellitus (where there is a deficiency of insulin or tissue resistance to insulin) there are reports of accelerated collagen aging both in human tendon [36] and in rat tail tendon [37]. Thus, high insulin levels may have contributed to the lower rate of collagen aging seen in our obese hypophysectomized rats.

Hypothalamic aging areas

The present exploratory study shows that gross hypothalamic lesions in hypophysectomized rats are compatible with a normal life span in the rat. The lesions in these animals appeared to be confined to the basomedial hypothalamus, destroying the ventromedial nucleus and adjacent areas. It is believed that the main effect of these lesions was to raise food intake since water consumption and body temperature were no different from those recorded in intact *ad libitum* fed controls and lean hypophysectomized rats. Raising the food intake was seen to have little or no effect on collagen aging or age-associated proteinuria development in hypophysectomized rats, although it did increase the incidence of abnormal glomeruli in the kidney. It is likely that differences in feeding patterns (food restricted versus *ad libitum*) and changes in metabolism may also have affected the results.

The relationship of specific hypothalamic lesions to whole body aging is relatively unexplored and is urgently in need of investigation. By means of specific hypothalamic lesions it should be possible to localize the putative hypothalamic aging clock or centre [27, 38–40]. A likely candidate for this role is the suprachiasmatic nucleus which appears to be an endogenous clock regulating a number of circadian rhythms such as plasma corticosterone, body temperature, feeding behaviour and the oestrous cycle [41–43]. One study [44] showed that neonatal ablation of the suprachiasmatic nucleus does not

alter the age of puberty onset nor affect the development of pituitary ovarian function in the rat, but the life-long effects of such lesions on aging phenomena are unknown. The work of Clemens and Bennett [45] suggests a role for the preoptic area in ovarian aging, since lesions in this area in young rats induce changes in the oestrous cycle seen only in intact old rats. Once again, the life-long effects of such lesions on aging have not been investigated.

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