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THE EFFECTS OF HYPOPHYSECTOMY AND CONTINUOUS FOOD RESTRICTION, BEGUN AT AGES 70 AND 400 DAYS, ON COLLAGEN AGING, PROTEINURIA, INCIDENCE OF PATHOLOGY AND LONGEVITY IN THE MALE RAT

A. V. EVERITT, N. J. SEEDSMAN and F. JONES

Department of Physiology, University of Sydney, N.S.W. 2006 (Australia)

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SUMMARY

Hypophysectomy in young male Wistar rats aged 70 days, followed by cortisone acetate replacement therapy throughout life, retarded the rate of aging of tail tendon collagen fibres, inhibited the development of certain diseases of old age (renal disease, cardiac enlargement, hind limb paralysis, and various endocrine and non-endocrine tumors) and significantly prolonged the duration of life. Almost identical anti-aging effects were obtained by lowering the food intake of intact rats to that of hypophysectomized rats, from age 70 days until death. Hypophysectomy in middle age, at 400 days, even with cortisone acetate replacement therapy, produced a sharp increase in the mortality rate; the surviving rats exhibited significantly reduced aging of collagen fibres and of the kidney as measured by protein excretion. Food restriction begun at 400 days also inhibited renal aging, but had no demonstrable effect on collagen aging during the first 100 days. These studies suggest that procedures such as hypophysectomy and food restriction do not switch off an aging mechanism in youth but probably exert a continuing inhibitory influence on certain aging processes throughout life.

INTRODUCTION

A reduction in the food intake of the rat from puberty onwards prolongs life [1–7], delays the onset of diseases of old age such as chronic nephrosis [8–10], tumors [9, 11, 12], periarteritis [9], myocardial degeneration [9] and skeletal muscle degeneration [9], and retards the aging of collagen fibres in tail tendon [13–15]. Since chronic food restriction has been found to inhibit the secretion of most pituitary hormones [16–18], the anti-aging effects of food restriction may be due to the reduced secretion of a pituitary aging factor [19]. The first tests of this hypothesis showed that hypophysectomy in the young rat retarded the aging of tail tendon collagen [20, 21] and prevented the develop-

ment of proteinuria [22]. Despite the reduced rate of aging, hypophysectomy markedly shortened the duration of life [21, 23]. This suggested that the pituitary gland also secreted a life-maintaining factor. Later work showed that replacement therapy with only one hormone, cortisone acetate, restored life duration to normal [24, 25], thereby suggesting that pituitary adrenocorticotropic hormone (ACTH, which controls corticosteroid secretion) is a life-maintaining factor. The present paper compares the long-term effects of hypophysectomy and food restriction begun in youth with the effects of the same treatments begun in middle age. This is to determine whether these treatments act by switching off an aging mechanism in youth or act by continuous inhibition of aging processes throughout life.

MATERIALS AND METHODS

Animal groups and treatment

At age 70 days (2 months) 35 male Wistar rats (conventional rats outbred at Sydney University) were hypophysectomized, using the intra-aural technique of Koyama [26] and 25 were sham operated. One hundred days later, at age 170 days (6 months), 27 hypophysectomized rats were selected at random from the 29 survivors that were satisfactorily hypophysectomized on the basis of growth failure and testicular atrophy. Completeness of hypophysectomy was assessed at autopsy and two rats with pituitary fragments were rejected from the study.

At age 400 days (13 months) a further 30 male Wistar rats were hypophysectomized and another 20 were sham operated. By age 500 days (17 months) only 18 of the 21 survivors were considered to be satisfactorily hypophysectomized and suitable for the long-term study on the basis of weight loss, testicular atrophy and general health.

All hypophysectomized rats received 0.5 mg of cortisone acetate (Roussel Pharmaceuticals, Sydney) per 100 g of body weight by subcutaneous injection once per week throughout life.

Food intake control groups were commenced at 70 and 400 days. These animals were supplied with the same amount of food as that consumed by hypophysectomized rats, which is about 40% of the *ad libitum* intake of intact rats. In order to control food intake it was necessary to house these animals one per cage. Twenty-five rats were food restricted from age 70 days, but only five rats from age 400 days because it was suspected that large middle-aged rats would not adapt to the severe reduction in food intake. Rats were intermittently fasted, being fed three times per week.

All rats were housed in an air-conditioned room at 28 °C exposed to artificial light for 12 hours per day and fed a commercial cubed rat food (Doust and Rabbidge, Sydney). The composition of the diet was protein 20.2%, fat 3.2%, fibre 3.5%, calcium 1.37%, phosphorus 0.98%, and it had a metabolizable energy value of 2.95 kilocalories per gram. The food did not contain fish meal or cod liver oil. No additional vitamins were given to either food-restricted or hypophysectomized rats.

Measurements on living animals

Body weights of all animals were measured on a Mettler P1000 balance at weekly intervals for the first 4 weeks and thence at 100-day intervals until natural death.

Food intakes were determined at ages 70, 100, 200, 400, 450 and 600 days. For measurement of food intake each cage of rats was supplied with 500 g of food and 24 hours later the food that remained, including that spilled, was estimated.

Tail tendon collagen fibre breaking times in 7 M urea at 50 °C were measured at ages 400, 450, 600, 700, 800 and 950 days using the method of Boros-Farkas and Everitt [27].

The 24-hour protein excretion was estimated in urine collected from rats placed in metabolism cages without previous adaptation. Data were collected at ages 70, 150, 400, 500, 600, 700, 900 and 1150 days. Protein was measured chemically using the trichloroacetic acid Ponceau S dye method of Pesce and Strande [28].

The presence of clinically observable disease such as respiratory disease, hind limb paralysis and skin or testicular tumors was observed at 100-day intervals.

Autopsy data

At death, the duration of life was recorded and an autopsy was performed to determine the presence of pathological lesions. Wet organ weights were determined for testes, adrenals, thyroids, pituitary, kidney, heart ventricles and thoracic aorta. Autopsy data for studies begun at 70 days were obtained from 164 rats aged 800 days or more, consisting of 88 controls, 40 hypophysectomized and 36 food-restricted rats. For studies begun at 400 days data were obtained from only 5 hypophysectomized rats aged 800 days or more.

Thickness of the thoracic aorta was calculated from the wet weight (moistened in 0.9% NaCl and the excess fluid blotted on filter paper) and the area of the wet aorta laid out on graph paper for measurement.

Results

Body weight

In the normal intact rat, body weight (Fig. 1) increased rapidly during growth reaching a peak at 500 days, plateaued for a variable period according to life duration, and then declined by 10–30% during the last 200 days.

Hypophysectomy at 70 days led to an immediate loss of weight, followed by stabilization at a level of about 80% of the initial weight. In completely hypophysectomized rats this weight remained relatively constant until the terminal phase when body weight declined by 10–20% during the last 200 days.

Food restriction begun at 70 days, resulted in a fall in body weight which after adaptation rose to a stable level, averaging 80% of the initial weight.

Hypophysectomy at 400 days led to a marked fall in body weight (Fig. 1). Animals that were able to adapt stabilized their body weight at about 60% of the weight at opera-

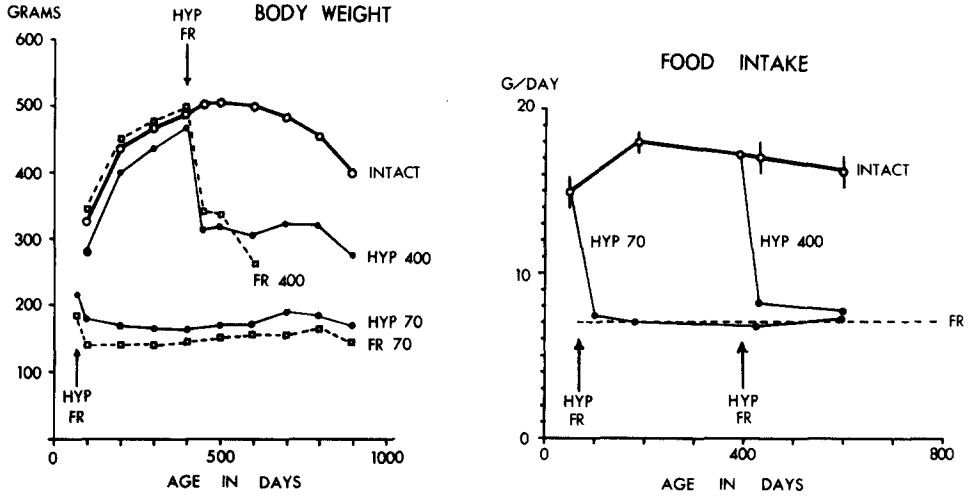

Fig. 1. The effect of hypophysectomy (HYP) and food restriction (FR) commenced at 70 and 400 days on body weight at different ages in male Wistar rats. Both hypophysectomy and food restriction abolished growth in young rats and produced severe weight loss when started in middle age. The middle age (400 days) FR curve shows the body weight at different ages of the only rat to live to 600 days. Other curves show the mean weights of rats surviving beyond 900 days, and are based on data from 4 INTACT controls, 6 HYP 70 days, 4 FR 70 days and 3 HYP 400 days.

Fig. 2. The effect of hypophysectomy (HYP) at 70 and 400 days on the food intake (mean ± S.E.) of male Wistar rats at different ages. Hypophysectomy sharply reduced food intake. Food-restricted (FR) rats were fed an average of 7 g of commercial rat cubes per day, which is the daily food consumption of the hypophysectomized rat.

tion and remained at this level until the terminal phase. Food restriction begun at 400 days, produced a sharp fall in body weight and a high mortality. In all 5 rats studied body weight continued to decline until death.

Food intake

In animals fed *ad libitum,* food intake (Fig. 2) increased during early growth and then remained relatively constant until the terminal phase, when it declined. In rats hypophysectomized at 70 days, food intake dropped acutely from the *ad libitum* level of 15 g/day to 7 g/day; this level was maintained throughout life until the terminal phase when food intake declined. In rats hypophysectomized at 400 days, food intake also dropped acutely from the *ad libitum* level of 18 g/day to 7 g/day and was maintained until the terminal phase.

Collagen fibre aging

In the intact controls the breaking time of tail tendon collagen fibres, in 7 M urea at 50 °C, increased progressively with age (Fig. 3). Both hypophysectomy at 70 days and food restriction begun at 70 days markedly retarded the aging of collagen. Fibres from a 900-day-old rat hypophysectomized at 70 days or food restricted from 70 days have

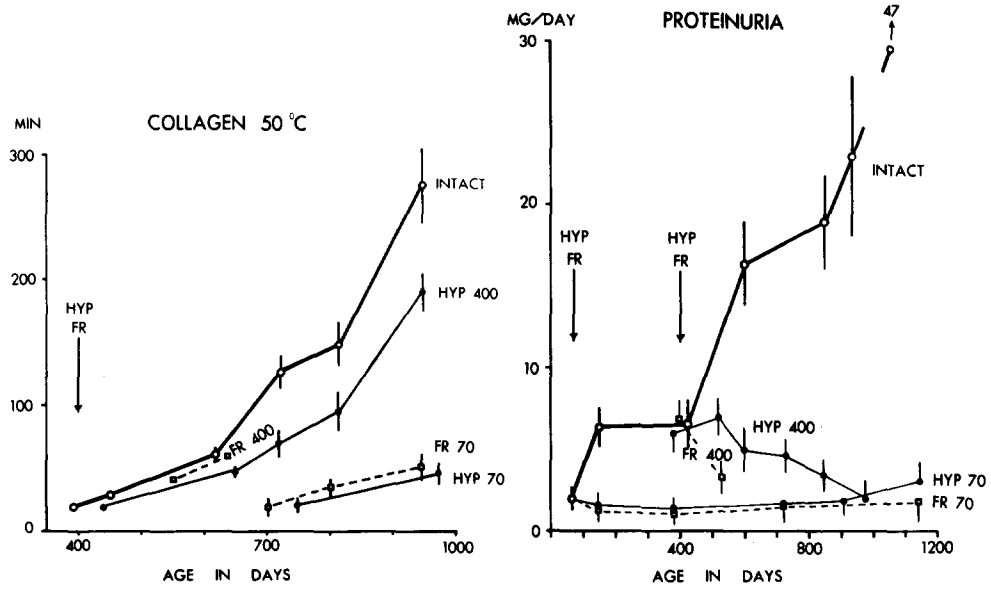

Fig. 3. The effect of hypophysectomy (HYP) and food restriction (FR) commended at 70 and 400 days on the biological age of tail tendon collagen fibres as measured by the breaking time in minutes (mean ± S.E.) of an isolated fibre under a load of 2 g when immersed in 7 M urea at 50 °C. There were 4–6 rats for each point. Both hypophysectomy and food restriction retard the aging of collagen fibres in tail tendon.

Fig. 4. The effect of hypophysectomy (HYP) and food restriction (FR) commenced at 70 and 400 days on the urinary excretion of protein in mg/day (mean ± S.E.) at different ages. There were 5–8 rats for each point. Both hypophysectomy and food restriction suppressed the development of proteinuria. Neither hypophysectomy at 70 days nor food restriction from 70 days affects plasma protein levels [34].

breaking times corresponding to those of a 500-day-old control. Hypophysectomy at 400 days produced a small but significant retardation of collagen aging. In the food restriction study commenced at 400 days there was no significant depression in collagen aging during the first 100 days; no further data were obtained due to the high mortality rate of these animals.

Protein excretion

In intact control rats protein excretion increased progressively throughout life (Fig. 4). Hypophysectomy at 70 days and food restriction from 70 days abolished the rise in protein excretion with age. Hypophysectomy at 400 days produced a progressive decline in protein excretion, which by 1000 days had reached the low level found in animals hypophysectomized at 70 days. Food restriction commenced at 400 days also produced a significant fall in protein excretion.

Life duration

The mean life duration of intact rats fed *ad libitum* was 785 ± 34 (S.E.M.) days with a maximum of 1120 days. The survival curve is seen in Fig. 5.

TABLE I

TUMOR FREQUENCY AT AUTOPSY IN MALE WISTAR RATS OF THE UNIVERSITY OF SYDNEY STRAIN, AGED 800 DAYS OR MORE

	Controls	*Food restricted* (from 70 days)*	*Hypophysectomized*	
			at 70 days	*at 400 days*
Number of rats	88	36	40	5
Endocrine** (thyroid, adrenal and testis)	48%	0%	3%	0/5
Non-endocrine	37%	15%	3%	0/5
Total	64%	15%	5%	0/5

*No rat food restricted from age 400 days survived to 800 days.
**Pituitary tumors have been excluded. In this series 26% of controls had pituitary tumors and 9% of rats had pituitary tumors without other endocrine tumors. The latter 9% of control rats were excluded from this study to permit comparison with hypophysectomized rats.

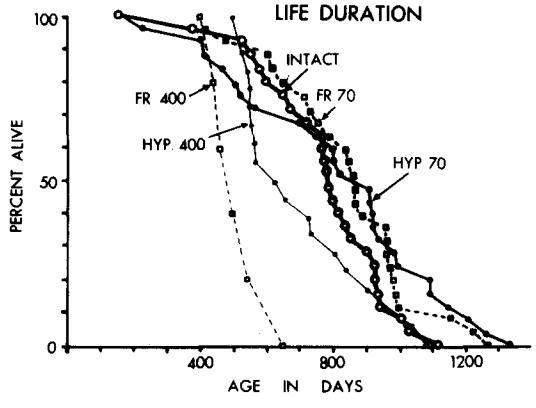

Fig. 5. The survival curves of male Wistar rats hypophysectomized (HYP) at, or food-restricted (FR) from, ages 70 and 400 days compared with INTACT controls fed *ad libitum*. Hypophysectomized rats received 1 mg of cortisone acetate subcutaneously per week throughout life. Hypophysectomy and food restriction commenced in young rats (70 days) prolonged life significantly, but in middle age (400 days) these treatments shortened life. In all groups except the FR400 rats deaths occurring during the first 100 days have been excluded. The high mortality of the FR400 and HYP400 rats is probably related to the sudden drop in food intake from 17 g/day to 7 or 8 g/day.

Hypophysectomy at 50 days without replacement therapy was earlier shown in this laboratory [23] to reduce mean life duration to 500 days. In the present study hypophysectomy at 70 days followed by weekly injections of cortisone acetate increased life duration to 916 ± 46 days, which is significantly greater than that of the intact rats. Food restriction begun at 70 days had a similar effect on life duration, which averaged 858 ± 38 days. In this study the maximum life duration was 1342 days for hypophy-

TABLE II

AUTOPSY DATA ON MALE WISTAR RATS OF THE UNIVERSITY OF SYDNEY STRAIN, AGED 800 DAYS OR MORE

	Controls	*Food restricted* (from 70 days)*	*Hypophysectomized*	
			at 70 days	*at 400 days*
Number of rats	88	36	40	5
Lung congestion	72%	75%	55%	3/5
Thoracic haemorrhage	9%	3%	8%	0/5
Cardiac ventricular enlargement**	21%	0%	0%	0/5
Renal enlargement**	8%	0%	0%	0/5
Hind limb paralysis	71%	0%	0%	0/5

*No rat food restricted from age 400 days survived to 800 days.
**Organ weight more than 50% greater than the mean weight in controls at age 500 days, when body growth had ceased.

sectomized rats and 1282 days for food-restricted rats. The life duration study was commenced at age 170 days, thus deleting deaths caused by the acute effects of surgical injury or failure to adapt to food restriction.

Both hypophysectomy (with cortisone therapy) and food restriction from 400 days produced a high mortality rate. The mean life duration of the hypophysectomized rats (after deleting deaths during the first 100 days) was 708 ± 58 days, with a maximum of 1090 days. For the entire food-restricted group of 5 rats the mean was 518 ± 54 days and maximum 652 days.

Tumors

In Table I the incidence of tumors of endocrine glands (thyroid, adrenal and testis) and non-endocrine tumors is recorded in 88 intact controls, 36 food-restricted (from 70 days), 40 hypophysectomized (at 70 days) and 5 hypophysectomized (at 400 days) rats living 800 days or longer. The total incidence of tumors in the controls was 64%, which was markedly greater than that in hypophysectomized (at 70 days) rats 5% and food-restricted rats 15%. No tumors were present in the 5 rats hypophysectomized at 400 days. Endocrine tumors are the main tumors in these animals, being found in 48% of controls, but are seen only rarely in hypophysectomized or food-restricted rats. Pituitary tumors were found in 26% of intact rats in this age group. The 9% of controls which had pituitary tumors without other endocrine tumors were excluded from this autopsy study, to permit comparison with hypophysectomized rats.

Lung disease

In Table II it is seen that the incidence of congested lungs in controls (72%) was not affected significantly by either hypophysectomy at 70 days (55%) or food restriction from 70 days (75%).

Haemorrhage

A small number of rats died as a result of haemorrhage, which almost always occurred in the thoracic cavity. The frequency of this abnormality was not affected by either hypophysectomy or food restriction (Table II).

Cardiac enlargement

Heart ventricle weights in controls increased from a mean of 0.77 ± 0.02 (S.E.) g at 70 days to 1.20 ± 0.05 g at 500 days and 1.76 ± 0.11 g at 1100 days. In hypophysectomized (at 70 days) rats the mean weights were 0.52 ± 0.05 g at 500 days and 0.76 ± 0.09 at 1100 days; and in food-restricted (from 70 days) rats 0.53 ± 0.06 g at 500 days and 0.74 ± 0.10 g at 1100 days.

Since maximum body weight is reached at 500 days in intact rats, increases in heart weight beyond this age are unrelated to body weight, and hence must be either a normal age-related increase or a pathological process. We have arbitrarily defined abnormal cardiac enlargement as heart ventricles in excess of 1.8 g, which is 50% greater than the mean weight in intact controls at 500 days. In experimental cardiac hypertrophy in young animals the weight increase is usually 50–70% [29]. Applying this criterion to the autopsy data we found that 19 out of 88 controls, or 21%, had abnormal cardiac enlargement (Table II). No rat hypophysectomized at 70 days or 400 days, or food-restricted from 70 days, had heart ventricles larger than 1.8 g, the maxima being 1.35, 1.51 and 1.50 g, respectively.

Renal enlargement

The mean weight of two kidneys in controls increased from 2.08 ± 0.09 (S.E.) g at 70 days to 3.01 ± 0.15 g at 500 days and 3.79 ± 0.26 g at 1100 days. The corresponding values in rats hypophysectomized at 70 days were 1.05 ± 0.03 g at 500 days and 1.15 ± 0.05 g at 100 days; and in rats food restricted from 70 days, 1.52 ± 0.04 g at 500 days and 1.75 ± 0.18 g at 1100 days.

Applying similar criteria of renal enlargement as for cardiac enlargement, the upper limit of normal was arbitrarily set at 50% greater than the mean intact control value at 500 days; that is, 4.5 g. Using this value we found that 7 out of 88 controls, or 8%, had abnormal renal enlargement (Table II), whereas none of the hypophysectomized or food-restricted rats had kidneys of that size. The heaviest kidneys in rats hypophysectomized at 70 days were 1.57 g, in those hypophysectomized at 400 days 1.99 g, and 3.22 g in those food restricted from 70 days.

Hind limb paralysis

In control rats aged 800 days or more 71% were found to develop hind limb paralysis. However, no hypophysectomized or food-restricted rat in this laboratory, even up to the age of 1515 days, has ever been found to have this disease. Rats with this disease have normally functioning fore limbs, and in propelling themselves forward drag their paralysed hind limbs. There is gross atrophy and degeneration of the gastrocnemius muscle in the hind legs. The principal histological changes are progressive loss of the

pattern of striations, fragmentation of muscle fibres, fatty infiltration and collagenous fibrosis. These changes are not seen in rats hypophysectomized early in life [30].

Aortic wall thickening

With increasing age, the thoracic aortic wall thickened from 0.18 ± 0.01 (S.E.) mm at 70 days to 0.24 ± 0.01 mm at 500 days and 0.28 ± 0.02 mm at 1000 days. There were 10 rats in each group. Wet thoracic aortic weight increased accordingly from 27 ± 1 mg at 70 days to 44 ± 2 mg at 500 days and 67 ± 4 mg at 1000 days. Following hypophysectomy and commencement of food restriction at 70 days, aortic wall thickness was reduced to 0.13 ± 0.01 mm at 200 days and then increased progressively with age to 0.18 ± 0.01 mm at 800 days in both groups. Compared with controls aortic wall thickness was significantly less in both food-restricted and hypophysectomized rats at the ages studied.

DISCUSSION

This study clearly demonstrates that both hypophysectomy in young rats aged 70 days and food restriction begun at 70 days retard the aging of collagen, inhibit the development of various diseases of old age and prolong life. It is, however, necessary for hypophysectomized rats to receive corticosteroid replacement therapy throughout life for the life span to be prolonged. Cortisone therapy is not necessary for retardation of collagen aging, nor for the delayed onset of proteinuria, which both occur in hypophysectomized rats not receiving replacement therapy [23].

When hypophysectomy is performed in middle age at 400 days the aging of collagen is retarded and renal aging (as measured by proteinuria) is markedly inhibited. Thus the anti-aging effect of hypophysectomy is not due to the suppression of a switching mechanism early in life. It is probably due to the removal of pituitary hormones which have a continuing influence on aging processes at all ages. For example, long-term pituitary growth hormone therapy has been found to accelerate renal aging in hypophysectomized rats, resulting in increased thickening of both Bowman's capsule and tubular basement membranes, as well as increased protein excretion [31]. Most of the effects of pituitary hormones are mediated by target-gland hormones such as corticosteroids, thyroid hormones and sex steroids. Thus cortisone replacement therapy increases the aging of collagen fibres in hypophysectomized rats [19]. Furthermore, thyroxine increases the aging of tail tendon collagen fibres both in young [14] and in old rats [32]. Similarly thyroxine has been shown to accelerate renal aging in young rats [33, 34] and also in old rats [17].

Both hypophysectomy and food restriction have similar effects in delaying the onset of quite a number of different diseases in old age, such as endocrine and non-endocrine tumors, renal disease, cardiac enlargement and hind limb paralysis. The delayed onset of these diseases apparently occurs by mediation of some common mechanism such as the preservation of normal immunity to disease. For example, Gerbase-DeLima *et al.*

[35] have shown that underfeeding in the mouse retards the senescent decline in immunological vigor. In a similar manner, Bilder and Denckla [36] found that hypophysectomy in middle-aged rats restored their youthful immunological responses. Of course, hormonal and nutritional factors may affect aging phenomena by actions at other sites both centrally [37–39] and peripherally [40, 41].

There are certain pathological conditions such as respiratory disease and haemorrhage whose incidence is not affected by either hypophysectomy or food restriction. This indicates that nutritional and hormonal factors present in this study do not affect the development of these diseases.

The relationship between the anti-aging actions of food restriction and hypophysectomy is not clear. There is no doubt that food restriction reduces the secretion of many anterior pituitary hormones [17], because it decreases the production of hypothalamic releasing hormones [18] which control the liberation of anterior pituitary hormones. However, whether there are separate hormonal, metabolic and nutritional actions on cellular aging is not clear. In the case of collagen aging, hypophysectomized rats have significantly lower collagen breaking times than food restricted rats [24, 42], thus suggesting that a pituitary hormone such as ACTH is increasing the aging of collagen fibres in food-restricted rats [19]. In the case of the thyroid hormone there is good evidence that its action is mediated by nutritional or metabolic factors [14].

The life duration of rats is determined largely by the development of pathology, as shown by the prolongation of life in rats hypophysectomized at 70 days or food restricted from 70 days, associated with a delayed onset of pathology. However, in the case of rats hypophysectomized in middle age at 400 days or food restricted from the same age, the life duration is significantly reduced. It is believed that this is because the intake of calories or some essential dietary factors such as vitamins is too low for the normal survival of these animals. We have found [43] that untreated hypophysectomized rats (operated at age 70 days) that over-eat (due to hypothalamic lesions unintentionally produced at the time of operation) live significantly longer (mean life duration 761 ± 54 days) than those that do not over-eat (515 ± 41 days). The poor survival of rats food restricted from age 400 days may well be due to the rapidity of weight loss, whereas a more gradual weight loss similar to that achieved by Weindruch *et al.* [44] might well have prolonged survival. However, it was necessary in this study to restrict the food intake as closely as possible to that consumed by rats after hypophysectomy at 400 days. Hypophysectomized rats survive better than the intact rats on this severe food restriction, possibly because of a lower metabolic rate due to the lack of pituitary hormones. There are now several studies [45–47] which show that less-severe and more gradual food restriction of middle-aged rats will actually prolong life.

Thus there are both nutritional and hormonal factors determining the duration of life as well as the rate of aging. The present study indicates that hormonal (and probably nutritional) factors exert a continuing influence on aging processes throughout life and do not switch on or off an aging mechanism only in young animals.

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