Mechanisms of Ageing and Development, 5 (1976) 109–124

PATHO-PHYSIOLOGIC FINDINGS AFTER CHRONIC TRYPTOPHAN DEFICIENCY IN RATS: A MODEL FOR DELAYED GROWTH AND AGING

PAUL E. SEGALL* and PAOLA S. TIMIRAS**

Department of Physiology-Anatomy, University of California at Berkeley, Berkeley, Calif. 94720 (U.S.A.)

(Received December 4, 1975)

SUMMARY

Long-Evans female rats three weeks, three months and 13–14 months of age were placed on tryptophan-deficient diets for periods ranging from a few months to nearly two years. Growth was interrupted during the period of tryptophan-deficiency, but when the animals were returned to a complete diet, they gained weight and grew to normal size. Ability to reproduce, as indicated by litter production, was present at 17–28 months of age in rats which had been deprived of tryptophan, whereas no controls over 17 months of age produced any offspring. Other signs of delayed aging in the experimental group included, at advanced ages, greater longevity, as well as later onset in the appearance of obvious tumors, and better coat condition and hair regrowth. Many of these effects were also seen in pair-fed controls (fed a diet equal in amount to that eaten by the tryptophan-deprived rats, but with l-tryptophan added).

It is hypothesized that tryptophan deficiency delays growth, development and maturation of the central nervous system (CNS), in particular, by decreasing the levels of the neurotransmitter serotonin, for which tryptophan is the necessary precursor. In a parallel experiment, chronic treatment with *d*, *l*-parachlorophenylalanine, an inhibitor of brain serotonin synthesis, from weaning until adulthood, also inhibited growth (body weight) and delayed sexual maturation (age of vaginal opening). These observations suggest that diets deficient in tryptophan or restricted in calories can affect maturation and aging by interfering with CNS protein synthesis, or neurotransmitter metabolism, or both.

---

*Graduate student and teaching associate.

**Professor of Physiology.

INTRODUCTION

Of the many ways in which investigators have sought to alter the rate of mammalian aging, dietary manipulation is perhaps historically the only method producing convincing results. Several workers in the area have carefully investigated the life extending effect of caloric restriction, and have shown that the rate of aging can, indeed, be retarded under certain control conditions [1–4]. The present investigation is part of a larger project attempting to examine the effect of long term dietary tryptophan restriction on the process of aging in the rat; with this procedure, it is hoped to understand more fully the basic nature of aging itself, as well as to begin evolving more precise techniques by which aging in mammals can be delayed in the laboratory, and eventually, the clinic.

Diets low in tryptophan have been successfully employed to delay growth and maturation in the chick [5, 6]. It has further been shown that rats fed on tryptophan-deficient diets have lowered brain serotonin levels [7, 8] and can be kept in a state of maturational and growth arrest for long periods of time [9]. When fed a complete, standard diet (Purina Rat Chow), they will then proceed to grow rapidly and eventually reach normal adult body weight [9]. The ability of these rats to maintain homeostasis when stressed has been studied in order to develop an assessment of their rate of physiological aging as compared with control animals. Newly weaned rats fed low tryptophan diets for 8–13 months and then allowed to grow to adult size on Purina Rat Chow regain their normal body temperature after it is depressed by whole body ice water immersion at rates characteristic of much younger animals; such better thermoregulatory adjustments suggest that aging effects are delayed while the rat is maintained on the tryptophan deficient diet [9].

Tryptophan-deficient diets, as they are low in only one specific and well-defined component, have the distinct advantage of narrowing down the mechanism(s) of action of nutritional deficiencies on aging. For example, as tryptophan is the precursor of the neurotransmitter serotonin, as well as an essential amino acid in protein synthesis, we can explore the age retarding effectiveness of blocking each of these separately, *i.e.*, to deplete brain serotonin by administering *d, l*-parachlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, *i.e.*, of serotonin synthesis, as used in these experiments, or to block protein synthesis, eventually by the use of drugs such as puromycin.

Studies on monoamine levels in the brain with aging [10] suggest that changes in serotonin may play a role in the development of senescence [11]. As brain serotonin levels have been shown to be decreased in response to dietary tryptophan deficiency [7, 8], techniques which alter drastically and chronically levels of CNS monoamines [12] may produce useful modifications in the aging process itself.

The present study was designed to determine whether long-term tryptophan deficiency could retard or arrest other aspects of physiological

TABLE I

TRYPTOPHAN-DEFICIENT DIET (GENERAL BIOCHEMICALS, INCORPORATED TD-74312)*

Ingredients	*g/kg*
Corn, ground yellow	600
Casein hydrolysate, acid	200
Sucrose	4
Gelatin	50
Jones–Foster salt mix	50
Vitamin mix (GBI)	30
Corn oil	50
Torula yeast	8
Desiccated liver	8

*In some rats, especially in those placed on the experimental diet in adulthood, body weight was reduced to life-threatening levels while on the T- diet; in these cases, the animals were fed Purina Rat Chow for 1–4 days until their body weight was increased by 5–20%, and then returned to the T- diet.

aging, such as the decline in reproductive ability, the increase in tumor incidence, the degeneration of the coat and the increase in mortality, that are observed in the typical senescent population, as well as to initiate a rational pharmacological approach to this problem.

MATERIALS AND METHODS

Animals

All rats were females of the Long-Evans strain from our colony started in 1911, and maintained under optimal husbandry conditions. For these animals, considerable information on growth, development, reproduction and other aspects of their physiology is available.

All animals were maintained 2 or 3 per cage on a 12-hour day-night cycle at 22–27 °C and supplied with fresh water *ad libitum*. Efforts were made to equalize the amount of handling of the animals, by having the same investigator performing animal care services (feeding, changing of cages, weighing, etc.).

Diets

Three different types of diets were used: Purina Rat Chow, tryptophan deficient diets (T-) and tryptophan deficient diets to which adequate amounts of tryptophan (2.0 g/kg) were added (T+). Inasmuch as we were interested in producing the most marked growth arrest still compatible with survival, several degrees of tryptophan deficiency were tested; the diet shown in Table I was found the most satisfactory in preventing growth and permitting survival.

A first series of experiments was designed to establish whether the synthetic diet, even though lacking tryptophan, was otherwise capable of

TABLE II

SOME PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF TRYPTOPHAN-DEFICIENT DIETS

Treatment and experimental procedures				*Behavioral pathology*	*Reproductive activity*			*Overall appearance*	*Obvious tumors (incidence)*		*Mortality*	
	Initial number of rats	*Age at onset of experiment*	*Duration of diets*	*Proportion of rats convulsing**	*Proportion of rats bearing litters from 17-28 months*	*Ages of oldest mothers at last litters***	*Number of offspring per litter*	*Proportion of animals with good coat at 30 months*	*Age at tumor onset****	*Proportion of animals with obvious tumors by 30 months*	*Proportion of survivors at 23 months*	*at 30 months†*
Control												
Purina Rat Chow *ad libitum*	14	3 weeks	To death	0/14	0/11	a	a	1/6	18–? months	5/14	12/14	6/12
Pair-feeding (T+)	3	3 weeks	Pair-fed to 23 months, *ad lib*, to death	0/3	1/1	26 months	10	1/1	36 months	0/1	1/3	1/1
Weight restriction (Purina Rat Chow)	3	3 weeks	To death	0/3	a	a	a	a	a	a	0/3	0/3
Experimental												
Tryptophan deficient diet (T- 1)	24	3 weeks	T- to 2.5–23 months, Purina to death	5/24	4/6	28 months	3–12	5/7	29–? months	1/7	8/24	7/7
T- 2		3 months	T- to 17 months, Purina to death	1/3	2/2	20.5 months	10	1/2	31–? months	0/2	2/3	2/2
T- 3	3	13–14 months	T- to 26–27 months Purina	0/3	b	b	b	2/2††	28–40 months	1/2	2/3	2/2

T-4	3	24–26 months	To death	0/3	b	b	b	a	b	b	a	0/3
T-5	2	36 months	To death	0/2	b	b	b	0/2	b	b	a	a

*Rats were observed for convulsions only during the period when feeding T- diet.
**Only those mothers over 17 months of age included.
***Experiment not completed at time of Table preparation.
†Only those animals surviving at 23 months are included here.
††Coat condition good at 27 months, but degenerated in both animals by 30 months for unknown reasons (in 1 rat, perhaps due to tumor occurrence).
a Information does not apply.
b Information was not collected.

providing for all requirements for growth and maturation; some animals were fed a diet similar to that deficient in tryptophan, but to which the amino acid had been added again. These animals showed similar weight and general developmental progress as those fed the commercial Purina Rat Chow and therefore, in subsequent experiments, this latter diet was fed to all controls unless otherwise specified. The specific dietary regimens are listed in the following section.

Experimental protocol

A total of 55 rats were divided into four major groups:

Group I, control animals fed *ad libitum* Purina Rat Chow.

Group II, pair-fed controls, were fed the same amount of T+ diet as consumed daily by the rats on the T- diet.

Group III, weight restricted controls were fed on Purina Rat Chow, but in amounts small enough to match body weight of the rats on the T- diet.

Group IV, experimental animals fed on the T- diet. These rats were subdivided depending on the age at which the diet was started in: T- 1, T- diet started at 3 weeks of age (at weaning); T- 2, T- diet started at 3 months of age (young adult rats); T- 3, T- diet started at 13–14 months of age (middle-aged rats); T- 4, T- diet started at 24–26 months of age (old animals) and T- 5, T- diet started at 36 months of age (very old animals).

Animals in subgroups T- 1, T- 2 and T- 3 were returned to the commercial diet after approximately 1/2 to nearly 2 years on the T- diet; the animals in T- 4 and T- 5 subgroups were maintained on the T- diet until death.

A summary of the animal groups and their treatments is presented in Table II.

Physiologic parameters

Body weight was measured at regular intervals (once a day to once a week and once a month) throughout the experimental period. General appearance, motor activity and behavior of animals was followed throughout the experiment, with special attention to incidence of tremors and of seizures.

Reproductive activity was estimated by counting the number of successful pregnancies and of offspring in each litter after placing the female with a young adult male for 2 weeks.

Coat condition was assessed by gross examination as well as by study of hair growth. In controls and rats of the T- 3 subgroup, two well-circumscribed areas, at the base of the tail and the thigh, were shaved of hair, and hair regrowth was observed after one week.

Pathologic parameters

Animals were carefully checked for the presence of tumors as evidenced by gross inspection during the lifespan (especially in old age) and at autopsy.

Mortality was measured after both controls and experimental animals had reached 23.5 months, *i.e.*, when all animals originally on the T- diet or on pair-feeding had been returned to the Purina diet *ad libitum*.

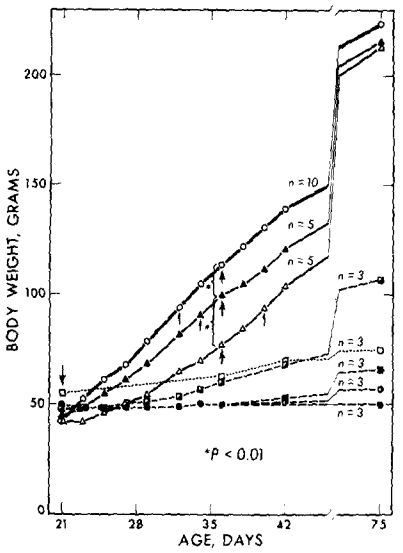

Fig. 1. *Growth-retarding effects of varying levels of tryptophan deficiency and of PCPA administration.* Symbols used as follows: controls fed Purina Rat Chow *ad libitum* (—○—); pair-fed controls (....□....). Of the rats placed on the T- diet, one group (--●--) received the diet indicated in Table I, the others received this same diet to which varying amounts of tryptophan (mg/kg/ of diet) were added as follows: 100 (--◑--), 200 (--■--), 500 (--◪--). PCPA (mg/kg body weight) was injected subcutaneously on alternate days in two doses: 200 (–▲–) and 400 (–Δ–). Full arrows indicate onset (↑) and end (↓) of injection series, half arrows (↿) indicate age of vaginal opening.

Drug administration

In a separate experiment conducted to investigate the effects of subcutaneous injections of *d, l*-parachlorophenylalanine (PCPA), a total of twenty 21-day-old rats was divided into 4 groups of 5 animals each; two experimental groups received every other day 200 mg/kg body weight and 400 mg/kg body weight, respectively; the PCPA was dissolved in 0.9% saline with the addition of a few drops of Tween 80 and each animal received a volume of 1 ml/40 mg of PCPA; the other groups served as controls and were injected with the vehicle only, in amounts comparable with those used in the two experimental groups.

RESULTS

Growth and survival

As shown previously [9], the tryptophan deficient diet was capable of significantly reducing body weight gain in those animals for which the diet had been started at weaning (T- 1), the severity of growth inhibition was parallel to the severity of the deficiency (Fig. 1) and the effects of the dietary restriction lasted for as long as 22 months (Fig. 2a). When the animals on the T- diet were returned to the Purina Rat Chow, they resumed growth and eventually reached control values (Figs. 2b and 3). Whereas in

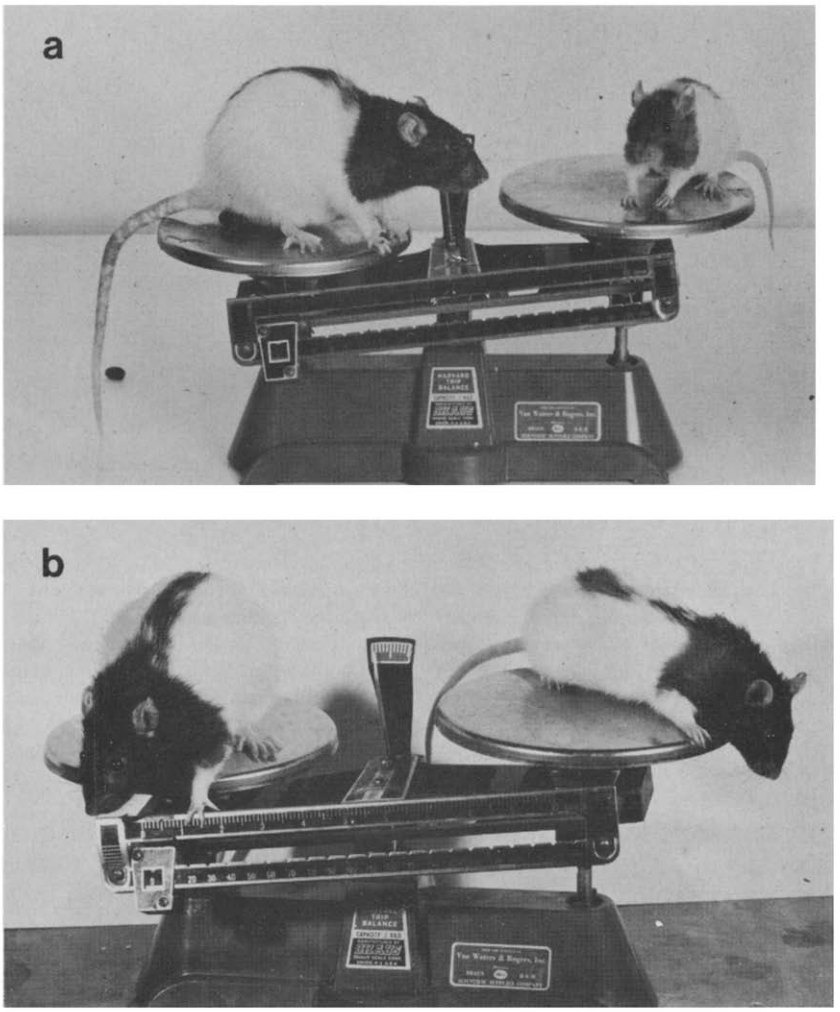

Fig. 2. *Effects of tryptophan deficiency on body growth. a.* Right, 23 month old rat placed at 21 days of age on a tryptophan deficient diet and maintained on this diet for 22 months. Left, control rat of corresponding age on Purina Rat Chow *ad libitum. b.* Right, the same experimental rat as in *a*, after 46 days on the Purina Rat Chow; left, same control as in *a*.

the T- 1 animals body weight remained essentially unchanged or somewhat increased (although always significantly lower than corresponding controls) during the time of dietary restriction (Figs. 1 and 3), when the T- diet was initiated at a later age (groups T- 2, T- 3) body weight was markedly decreased, but reverted to normal after returning to the Purina (Table III).

As shown in Fig. 3, the pair-fed controls receiving the T- diet supplemented with tryptophan, in daily amounts comparable with the food consumed by the T- rats, gained weight less than controls fed *ad libitum*, but significantly more than the corresponding T- rats. On the other hand, the

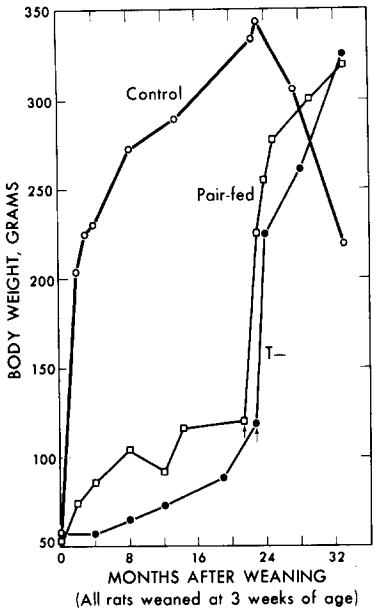

Fig. 3. *Weight changes of three representative rats,* one fed the T-diet for 22 months (—●—) one pair-fed for 22 months a T + diet (—□—) and one control fed Purina Rat Chow *ad libitum* (—○—). Diets were started at 21 days of age for the Pair-fed and T- rats. Arrows represent change to Purina Rat Chow diet *ad libitum*. Note that in the control rat shown here, body weight started to decline at approximately 24 months of age while in the pair-fed and T- rats, body weight continued to rise at this age.

TABLE III

EFFECTS ON BODY WEIGHT OF A TRYPTOPHAN DEFICIENT DIET INITIATED AT ADULTHOOD AND ADMINISTERED FOR 13–14 MONTHS

Body weight (g)

Young adults

Rat number	*3 months of age (start of T- diet)*	*17 months of age (return to Purina Rat Chow)*	*19 months of age*
W-1	221	164	315
G-2	257	204	343

Middle-aged adults

Rat number	*13–14 months of age (start of T- diet)*	*26–27 months of age (return to Purina Rat Chow)*	*31–32 months of age*
G-1	354	213	301
BH_2	313	207	322

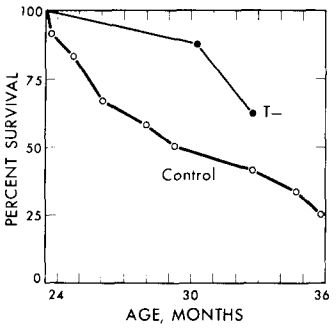

Fig. 4. *Survival curves of control and experimental rats after 23.5 months of age.* The curve for experimental animals (—●—) represents combined values for rats placed on T- diet at 21 days of age (n = 6) and at 3 months of age (n = 2); only those fed the experimental diet for more than 6 months included. Because of a difference in the timetable of the experiments, studies on control (—○—) rats (n = 12) were started at an earlier date than that of T- rats, hence, their longer time of observation. Experiments are still in progress, and therefore, neither control nor experimental curves are completed.

weight restriction controls, *i.e.*, maintained at a constant reduced weight similar to that of the T- rats, died within one month of the onset of the experiment (Table II).

Data on survival of control and experimental animals are summarized in Table II and Fig. 4. As shown in Table II, survival from weaning to 23 months of age was greater in controls fed *ad libitum* (86%) than in T- rats (33%). When T- diet was initiated at a later age, survival at 23 months was 66%. After 23 months of age, when all experimental animals had been returned to the Purina diet *ad libitum*, mortality rate was lower in the animals which had been on the T- diet than in controls (Fig. 4). When one considers survival at advanced ages, that is, after approximately two years of age, one observes that at 30 months, survival in controls was 50%, whereas in the T- group the survival was 100%, except in those animals in which the T- diet had been started at two years of age or more. Also the pair-fed showed a survival of 100% although only one animal remained at this time.

In the PCPA experiment, growth was significantly inhibited by the drug and this inhibition was dose-related (Fig. 1); when the inhibition in body weight induced by PCPA is compared with that induced by the T- diet, a marked difference is observed, with the highest dose of PCPA producing a less marked inhibition than the least severe degree of T- (Fig. 1). Survival of the animals was followed until 75 days of age and no deaths were recorded during this period.

Behavioral pathology

All T- rats showed tremors when handled and five out of 27 were observed to convulse either spontaneously or during handling at one or several times during the period of tryptophan deprivation. The convulsions, tonic-clonic in nature, appeared as early as 50 days after the rats had been placed on the diet and were followed by prolonged postictal depression.

Seizures were not lethal except in one case and most animals recovered without any obvious after-effect. No seizures occurred in the controls. In the pair-fed, neither tremors nor seizures were observed, and these animals were more active than the T- and control rats.

No overt behavioral abnormalities were observed in the PCPA-injected rats.

Reproductive activity

The number of animals capable of producing litters decreased with age. Of the 11 controls that were caged with a young adult male for two weeks between the ages of 17 and 21 months, none had offspring. On the other hand, the one pair-fed rat that survived after 23 months, had one 10-pup litter at 26 months of age.

Of the T- rats, four out of six animals, placed on the T- diet at 3 weeks of age (T- 1) and returned to the Purina diet 8–22 months later, gave birth to offspring at ages ranging from 17 to 28 months. The same animals were repeatedly mated and repeatedly produced offspring; for example, one T- 1 animal, returned to Purina diet at 13 months of age, produced litters at 20, 23.5, 26 and 28 months of age, the litters containing 3 to 7 surviving offspring each. However, some abnormalities in delivery and offspring survival were noted in a few animals. In one case, the pregnant rat which had previously delivered viable offspring died at delivery at 24 months of age (even though the 12 fetuses were fully developed) and in another case, the 26 month old mother ate some of the pups. Two out of two rats placed on the T- diet starting at 3 months of age (T- 2) and maintained for 14 months on the T- diet, produced litters of 10 pups each at 20.5 months of age; however, in one case, cannibalism of the pups occurred.

In the PCPA injected rats, the age when more than 50% of the test rats showed vaginal opening was significantly delayed (4 days in the low dose experiment, 8 days in the group receiving high doses) as compared with controls (Fig. 1).

Tumors

In the controls, gross examination revealed the presence of tumors (presumably of mammary and uterine origin) in the abdominal region in 5 out of 14 animals. The onset of the tumors was at 18 months in one animal and at 24, 25, 28 and 30 months in the others.

In the T- rats, (T- 1 and T- 2), one out of 9 showed a tumor, the occurrence of the tumor becoming first evident at 29 months of age (Table II).

Coat condition

With aging, the coat of the rat undergoes progressive changes characterized by increased yellow color (in the albino rats), coarseness and sparsity of the hair, alopecia patches, etc. These aging changes were well evident in the control rats especially after 30 months of age; they were less apparent in the T- rats of corresponding age in which the hair preserved a more youthful

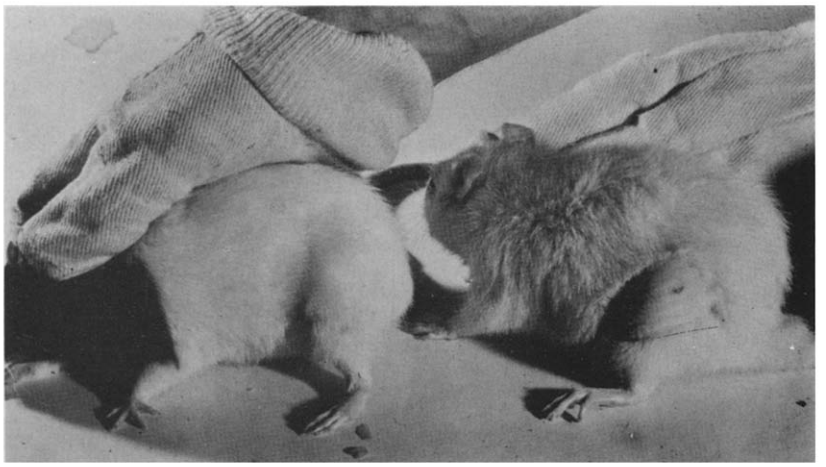

Fig. 5. *Aging of the coat in experimental and control rats.* Left, 27 month old experimental animal fed Purina Rat Chow until 13 months of age and then fed a tryptophan-deficient diet. This animal was returned to a Purina diet at 26 months of age. Right, control fed Purina Rat Chow. Both animals were shaved on upper hind leg 5 days prior to the date when photograph was taken. Note better regrowth of shaved area in experimental animal, as well as its better (and youthful looking) overall coat condition.

appearance in color, texture and quantity. When a specific body area was shaved and hair regrowth followed, hair grew faster in the T- rats (placed on the T- diet in adulthood) than in controls. An example of changes with age in hair condition in one control and one experimental animal is shown in Fig. 5 and the observations for all animals are summarized in Table II.

Cataracts

Cataracts can be easily detected by observation in albino rats. Among the T- rats, there were three albinos, two of which showed cataracts as early as 3 months of age. None of the albino controls showed cataracts.

DISCUSSION

Our previous experiments have shown that rats maintained on long-term tryptophan deficient (T-) diets and then fed Purina Rat Chow retain the capability of undergoing homeostatic (temperature) adjustments at later ages better than do controls continuously fed the Purina diet [9]. The present observations suggest that feeding rats a T- diet for varying periods of time and starting at selected ages from weaning to old age, will affect also other physiologic and pathologic parameters. For example, rats fed a T- diet from weaning to 23 months of age and severely arrested in their growth during this period, demonstrate the capability of growing to normal size once they are placed on the Purina diet, or, in the case of animals given the T- diet at an older age, to regain the weight loss during the period of

tryptophan deficiency (Table III, Figs. 2a, 2b and 3). In addition, the rats maintained on the T- diet show, when placed on the Purina diet, not only a period of fertility lasting until 28 months of age – an age well past reproductive activity in control rats [2, 13, 14] – and a more youthful appearance of their coat associated with a faster hair regrowth than controls of the same age, but also an apparent prolongation of the lifespan (Table II, Fig. 4). In parallel with this greater physiologic competence, the age for the onset of spontaneous tumors in the rats kept on the T- diet seems to be delayed well beyond that of control animals fed *ad libitum* (Table II).

The pair-fed rats in our experiments, and the caloric restricted rats in the experiments of others [1–4], also show a prolonged lifespan, an ability to reproduce at advanced ages, a better overall coat condition at late ages and a delayed onset of tumors. These animals, however, seem to differ from those on the T- diets in their early growth patterns (lesser degree of growth inhibition, Figs. 1 and 3), as well as in the type of their behavioral responses: hyperactivity in the pair-fed [15] as opposed to tremors and increased convulsibility in the T- rats [8]. Indeed, it is possible to postulate that chronic caloric restriction and long-term tryptophan deficiency may produce their effects through related mechanisms involving primarily maturation and aging of the brain.

That tryptophan is an amino acid essential for protein synthesis is well known, and its role as the precursor of the neurotransmitter serotonin has been satisfactorily proven [16]. Neuronal growth and development depend on both protein and neurotransmitter synthesis; thus, a deficiency in either synthesis at an early developmental stage (*e.g.*, in the weaning rat), would arrest or retard some aspects of neuronal maturation. Once brain maturation has occurred (*e.g.*, in the adult or old rat) a decline in protein and neurotransmitter synthesis could produce a variety of atrophic and degenerative changes in neurons. Inasmuch as considerable energy is required for maturation and maintainance of neural activity, a diet severely restricted in calories may have CNS consequences similar to those induced by tryptophan deficiency. Such alterations in CNS maturation and function would then be responsible for the impairment in growth and sexual maturation observed while the animals are subjected to the dietary manipulations described. We know, however, that the brain is endowed with great plasticity, particularly at early ages [17], but also in adult and older animals [18, 19]; thus, once protein and energy requirements are met through a complete diet, normal maturation and function are resumed and possibly also "rebound" phenomena would occur and be manifested by the catch-up growth, the late reproductive activity and the youthful appearance and growth of the coat reported here as well as the increased homeostatic capability reported previously [9], all physiologic adaptations compatible with a longer lifespan. The above interpretation is consistent with the hypothesis that a "program for aging", genetically encoded in the brain [10, 20], may be expressed by a precisely defined timetable of growth, maturation and aging of specific CNS centers. When animals are placed on the T- or calorie-restricted diet the

neuronal activities responsible for the expression of this "program" would be prevented from developing (in the case of young animals) or would regress (in the case of older animals). This phenomenon, however, would be reversible and would result in the prolonged activity of those functions which were "suspended" during the period of dietary restriction.

The synapse has been identified as a target particularly important for CNS growth and aging processes [21] and neurotransmitter changes have been described at this level in several animal species including man [10, 11, 22, 23]. In this respect, the observations presented here on the effects of prolonged tryptophan-hydroxylase inhibition during PCPA treatment support the view that brain depletion of serotonin may be related to alterations in the course of body growth and sexual development [24]. By analogy with the effects of tryptophan deficiency which also depletes brain of serotonin, the data on the effects of PCPA in reducing body weight and delaying onset of vaginal opening can be extrapolated to suggest that aging also may be influenced by this drug and experiments are now in progress in our laboratory to explore this possibility. Thus, these experiments with PCPA may create, in addition to the classical nutritional studies, a potentially more flexible pharmacologic approach to aging retardation. Indeed, recent experiments have shown that the daily administration of large doses of l-Dopa, known to lower brain serotonin levels [25], will increase longevity in mice [12]; it may be noted that striking (*e.g.*, yellowing of the coat, increased incidence of cataracts) similarities exist between the side effects of the drug and the tryptophan deficiency.

Finally, the relationship between neurotransmission and neuroendocrine function may also be considered in the interpretation of some of our observations, particularly body growth, reproductive activity and coat appearance, all hormonally dependent functions. During undernutrition, the pituitary gland produces many of its hormones only at a reduced level [5, 26] probably because of low input from the hypothalamus and higher CNS centers. Long-term tryptophan deficiency has been referred to as producing an effect resembling hypophysectomy [6]. The arrest or inhibition of body growth and the delay in sexual maturation described in our rats during tryptophan deficiency and after PCPA administration may also depend on a transitory period of pituitary insufficiency; after returning to a normal diet, catch-up growth, longer reproductive capability and better coat condition suggest a recovery in pituitary function. Long-term undernutrition in humans can produce marked oscillations in steroid hormone levels during the recovery phase when re-feeding is taking place [26]. Such a recovery pattern resembles the hormonal picture at adolescence before the complete maturation of the higher CNS centers imposes its regulatory control on the hypothalamo-hypophyseal axis. A condition of "delayed adolescence" occurring in the T- and calorie-restricted animals when returned to the normal diet could perhaps account for the "delayed aging" suggested in these animals by the longer duration of reproductive function, the retardation of coat deterioration and the longer survival, as well as the reduced incidence of tumors.

ACKNOWLEDGEMENTS

We are grateful to Jerry Renkin and Susan Ramani for their skillful technical assistance, and especially to Harold D. Waitz for his dedicated help in the maintenance of our animal colony, as well as his aid and advice in several of the experimental procedures. We also wish to thank Drs. A. Vaccari, T. Valcana and W. Vaughan for their helpful criticism of this manuscript.

This research was supported by Public Health Service Training Program NIH Grant #AG-00009.

REFERENCES

1 C. M. McCay, Chemical aspects of ageing and the effect of diet upon ageing, in A. I. Lansing (ed.), *Cowdry's Problems of Ageing*, Williams and Wilkins, Baltimore, 3rd edn., 1952, p. 139.
2 B. N. Berg, Nutrition and longevity in the rat. I. Food intake in relation to size, health and fertility, *J. Nutr.*, 71 (1960) 242.
3 B. N. Berg and H. S. Simms, Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake, *J. Nutr.*, 71 (1960) 255.
4 M. H. Ross, Length of life and nutrition in the rat, *J. Nutr.*, 75 (1961) 197.
5 M. R. McRoberts, Growth retardation of day old chickens and physiological effects at maturity, *J. Nutr.*, 87 (1965) 31.
6 R. S. Gordon, Growth arrest through tryptophan deficiency in the very young chicken, *Sixth Intern. Congr. of Nutrition*, Edinburgh, Scotland, 1963, p. 471.
7 J. D. Fernstrom and R. J. Wurtman, Effect of chronic corn consumption on serotonin content of rat brain, *Nature (London), New Biol.*, 234 (1971) 62.
8 L. D. Lytle, R. B. Messing, L. Fisher and L. Phebus, Effects of long-term corn consumption on brain serotonin and the response to electric shock, *Science*, 190 (1975) 692.
9 P. E. Segall and P. S. Timiras, Age-related changes in thermoregulatory capacity of tryptophan deficient rats, *Federation Proc.*, 34 (1975) 83.
10 C. E. Finch, Catecholamine metabolism in the brains of ageing male mice, *Brain Res.*, 52 (1973) 261.
11 P. E. Segall, C. Miller and P. S. Timiras, Aging and CNS monoamines, *Tenth Intern. Congr. of Gerontology*, Vol. II, Jerusalem, Israel, 1975, p. 33 (abstract).
12 G. C. Cotzias, S. Miller, A. R. Nicholson, Jr., W. H. Matson and L. C. Tang, Prolongation of the lifespan in mice adapted to large amounts of l-dopa, *Proc. Natl. Acad. Sci. U.S.*, 71, 6 (1974) 2466.
13 G. B. Talbert, Effect of maternal age on reproductive capacity, *Am. J. Obstet. Gynecol.*, 102 (1968) 451.
14 H. H. Huang and J. Meites, Reproductive capacity of aging female rats, *Neuroendocrinology*, 17 (1975) 289.
15 E. R. Cornish and N. Mrosovsky, Activity during food deprivation and satiation in six species of rodent, *Animal Behav.*, 13 (1965) 242.
16 S. Garattini and L. Valzelli, *Serotonin*, Elsevier, Amsterdam, 1965.

17 P. S. Timiras, A. Vernadakis and N. Sherwood, Development and plasticity of the nervous system, in G. Jasmin (ed.), *Biology of Gestation*, Vol. II, Academic Press, New York, 1968, p. 261.
18 G. Raisman, Anatomical evidence for plasticity in the central nervous system of the adult rat, in G. B. Ansell and P. B. Bradley (eds.), *Macromolecules and Behavior*, Baltimore University Park Press, 1973, p. 183.
19 W. H. Riege, Environmental influences on brain and behavior of year old rats, *Develop. Psychobiol.*, 4, 2 (1971) 157.
20 C. E. Finch, Enzyme activities, gene function and ageing in mammals, *Exptl. Gerontol*, 7 (1972) 53 (review).
21 P. S. Timiras, Neurophysiological factors in aging: Recent advances, *Tenth Intern. Congr. of Gerontology*, Vol. I, Jerusalem, Israel, 1975, p. 50.
22 D. S. Robinson, Changes in monoamine oxidase and monoamines with human development and aging, *Federation Proc.*, 34 (1975) 103.
23 A. Vernadakis, Neuronal-glial interactions during development and aging, *Federation Proc.*, 34 (1975) 89.
24 J. H. Copenhaver, M. J. Carver, E. A. Johnson and M. J. Saxton, Effect of para-chlorophenylalanine on growth and development of the fetal, neonatal and adult rat, *Biochem. Med.*, 4 (1970) 516.
25 S. Algeri and C. Cerletti, Effects of l-dopa administration on the serotonergic system in rat brain: correlation between levels of l-dopa accumulated in the brain and depletion of serotonin and tryptophan, *European J. Pharmacol.*, 27 (1974) 191.
26 F. G. Mont, Undernutrition, in M. G. Wohl and R. S. Goodhart (eds.), *Modern Nutrition in Health and Disease. Diet and Therapy*, Lea and Febiger, Philadelphia, 1968, p. 984.