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LOW TRYPTOPHAN DIETS DELAY REPRODUCTIVE AGING

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SUMMARY

Newly weaned female rats fed diets severely deficient in the essential amino acid tryptophan show marked delays in reproductive aging, with conception and delivery occurring as late as 36 months. The rate of aging in these rats seems inversely related to both their early growth rates and the accessibility of brain tryptophan. The subsequent age retardation may depend on a reduction in both early cell loss and rate of brain maturation.

Key words: Lifespan; Ovaries; Serotonin; Nutrition; Pineal

Previously studied juvenile animals fed diets restricted in the essential amino acid tryptophan [1–4] or severely limited in the amounts of food ingested daily [5–9], experienced a delay in growth and maturation which was sustained into old age, even if later provided a normal diet. Age-related impairment of homeostasic responses to cold [1], reproductive competence, and coat quality is delayed and onset of tumorigenesis and mortality at late age postponed [2]. Rats maintained on the tryptophan-restricted diet for a minimum of 1 year gave birth to litters at ages 20–28 months, when control animals were infertile [2], and the current experiments extend this period to 3 years.

Based upon preliminary studies, we employed two low-level tryptophan diets – a less severely restricted (T-40%) and a more severely restricted (T-30%) – and a standard diet, Purina Rat Chow (Table I). Female Long-Evans rats (40–48 ani-

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TABLE I

FORMULATION OF EXPERIMENTAL TRYPTOPHAN DIETS

In addition to the listed diets, a control diet (100% tryptophan) was formulated by adding 1.5 g of L-tryptophan to the T-30% diet and reducing the amount of corn by an equal amount. The assayed tryptophan level was 2.07 g comparable to that for Purina Rat Chow (5012). It was established in corollary experiments that the rates of growth, development, and aging were similar with both complete diets and, for convenience, Purina Chow was adopted as control. Tryptophan, an essential amino acid, is the precursor of the neurotransmitter, 5-hydroxytryptamine (serotonin). Corn is particularly low in the essential amino acid, tryptophan, and was therefore selected as the primary protein source for these diets. All diets were prepared according to our instructions by Teklad Test Diets, Madison, WI. L-Tryptophan was assayed microbiologically by the method of Wooley and Sebral, *J. Biol. Chem.*, *157* (1945) 141, by Raltech Scientific Services, division Ralston Purina Co.

Ingredients	*30% tryptophan (T-30%) (g/kg)*	*40% tryptophan (T-40%) (g/kg)*	*Purina Rat Chow (g/kg)*
Casein hydrolysate, acid (salt-free)	150.0	150.0	–
Corn, ground yellow	722.0	721.85	–
Torula yeast	8.0	8.0	–
L-Methionine	2.0	2.0	–
L-Isoleucine	4.0	4.0	–
Sucrose	4.0	4.0	–
Corn oil	50.0	50.0	–
Mineral mix, Jones-Foster	50.0	50.0	–
Vitamin mix, Teklad	10.0	10.0	–
L-Tryptophan	–	0.15	–
Assayed tryptophan levels	0.62	0.81	2.10

mals/diet group) maintained under optimal conditions of husbandry, were fed the three diets *ad libitum* commencing at weaning. Weekly body weights were recorded and gross behavior was noted. Although rats on T-30% diets showed higher first year mortality than controls (42% *vs.* 0%), mortality at late ages was reduced (at 3 years 85% *vs.* 93%): two T-30% rats survived at 4 years while all controls were dead at 3.5 years. Surviving experimental animals ranging in age from 23 to 30 months were transferred from the restricted to the control diet and were mated at least 2 months after the transfer. Two of ten experimental animals produced litters, twice; eleven controls mated between 17 and 21 months of age did not produce offspring. Unique to the two rats which successfully reproduced at late ages was a very slow growth rate occurring conjointly with behavioral abnormalities (*e.g.* convulsions), a more youthful coat and no observable tumors.

Female rats, in general, stop reproducing at 15–18 months [10]. In this study, one of the experimental rats gave birth at 33 months (1016 days). Maintained until 30 months (915 days) on the T-30% diet, this rat weighed 77 g at 1 year (one-quarter of the control weight); at 2 years its weight had doubled but

remained significantly lower than that of coetaneous controls (Fig. 1) even after transfer to the Purina diet. After 2 months on Purina, she was mated and produced a litter of five pups, one surviving to adulthood. A second mating resulted in a second litter of five stillborn pups at 36 months (1035 days). A second T-30% rat, also severely restricted in body growth, placed on Purina at 23 months (701 days), produced a litter of seven pups (five surviving to adulthood) at 27 months (814 days), and yet another litter of five pups (four surviving to adulthood) at 30 months (920 days). A third mating at 33 months was unproductive. Surviving offspring of both dams matured normally in terms of whole body growth and reproductive function. Conception and delivery at 32–36 months in these experiments and 20–28 months in our previous studies [2] should be considered of physiological significance and not merely anecdotal.

Ovaries from aged rats were compared between control and experimental animals and with the ovaries of young mature females. By 24 months, most ovaries from control rats contained large cysts and extensive corpora lutea with little evidence of primary or maturing follicles; in T-30% animals, corpora lutea were also prominent, but ovaries contained few cysts and retained a considerable

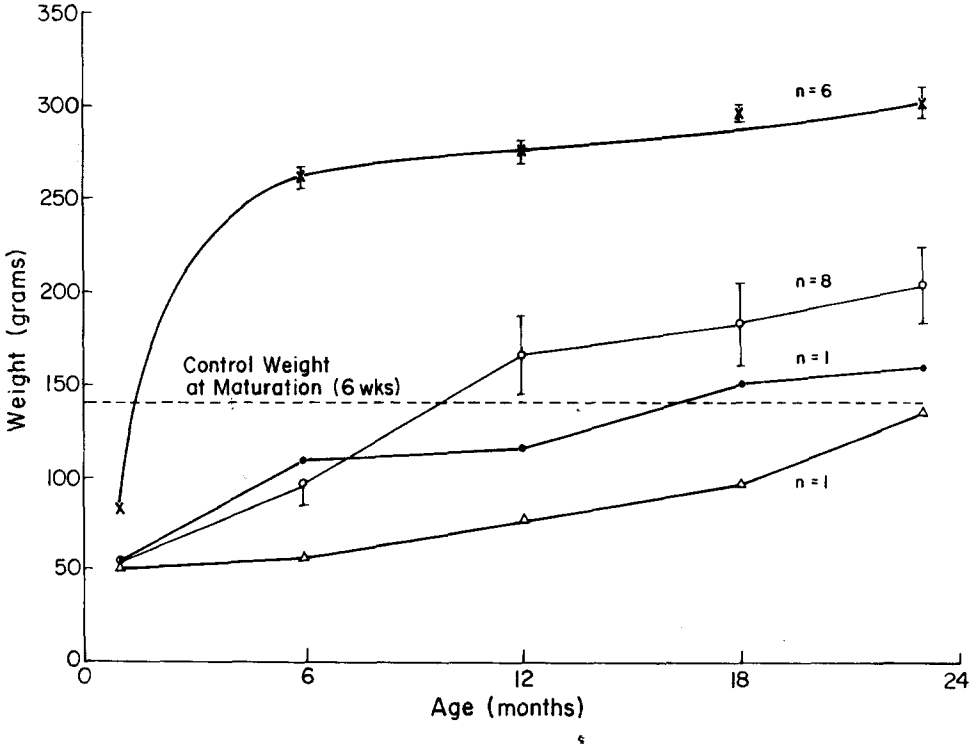

Fig. 1. Relative growth rates of (×) control rats, (○) rats which were infertile at late ages despite their previous low tryptophan diets, and the two experimental animals which produced young after (●) 30 months and (△) 33 months of age. Note that the animals which remained for longer periods below the weight normally attained at female reproductive maturity showed a more delayed reproductive senescence.

number of follicles with developing oocytes. Comparison of the number of ova per ovary between control and T-30% animals shows twice as many ova in the tryptophan-deficient rats (control = 2.31 ± 0.75; T-30% = 5.25 ± 0.92; $p < 0.025$). Electron-microscopically, follicles from control animals 18-months or older were generally empty or contained degenerating oocytes (Fig. 2A), whereas in T-30% at 30 months intact follicles persisted (Fig. 2B). Although the cells appeared to have been viable before fixation, their appearance differed from ovarian follicle cells from young mature animals (Fig. 2C). Characteristic of cells that have aged [11,12], the most striking ultrastructural changes in the older oocyte were the loss of spherical symmetry of the nucleus, sparse uneven distribution of cytoplasmic protein, irregular mitochondrial forms, a paucity of endoplasmic reticulum and the presence of debris-containing (presumably lysosomal) organelles. Some follicular cells in the 30-month-old T-30% ovary appear extremely dense; however,

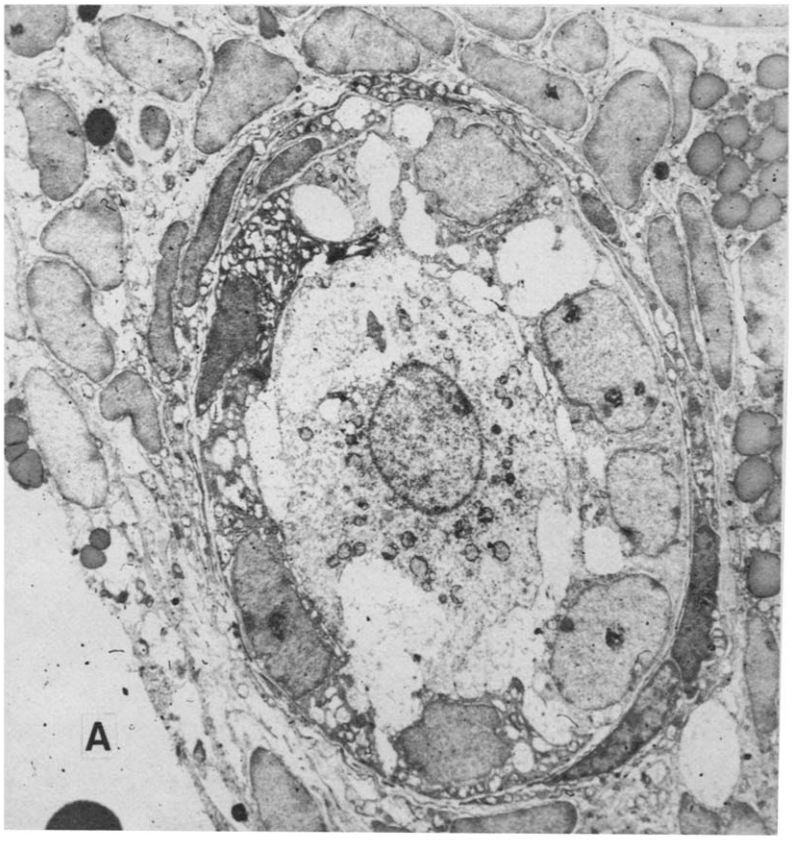

Fig. 2. Transmission electron micrograph of ovarian follicles from (A) an 18-month control rat, (B) a 30-month tryptophan-deficient rat, and (C) a 50-day control rat. Although the oocyte (large central cell) from the 30-month tryptophan-deficient animal shows age change, it appears more intact than the vacuolated oocyte from the 18-month-old control. Ovaries were perfused with glutaraldehyde, post-fixed with OsO_4, stained *en bloc* with lead aspartate, dehydrated in alcohol, embedded in plastic and then in Sparrs epoxy resin, thin-sectioned and photographed with a Philips EM201. ×2040.

similar dense cells were also noted in some 50-day-old follicles. Fertility depends on an available supply of oocytes established well before birth (and, therefore, before the animals are placed on the tryptophan-restricted diet), these oocytes being gradually lost throughout the lifespan. Our data show that age-related oocyte loss occurs at a slower rate in tryptophan-deficient animals than in controls.

Food consumption is spontaneously reduced upon feeding the tryptophan-restricted diet; the rats are thus also calorie restricted and this may be, at least partially, responsible for some of the age-delaying effects [5–9]. Tryptophan restriction, as an experimental tool, has the advantage over daily food restriction in that it eliminates the need to measure the amount of food administered each day [13].

The delay in aging is less effective if the low-tryptophan diet is started at 3 months, after brain maturation and puberty have occurred, rather than at 3

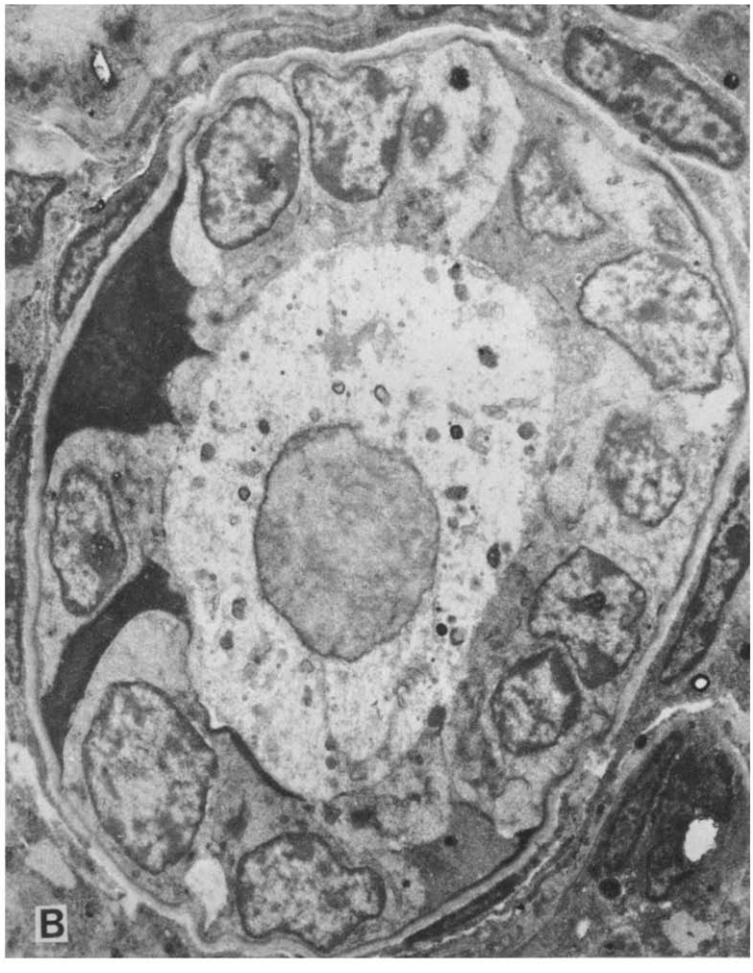

Fig. 2B.

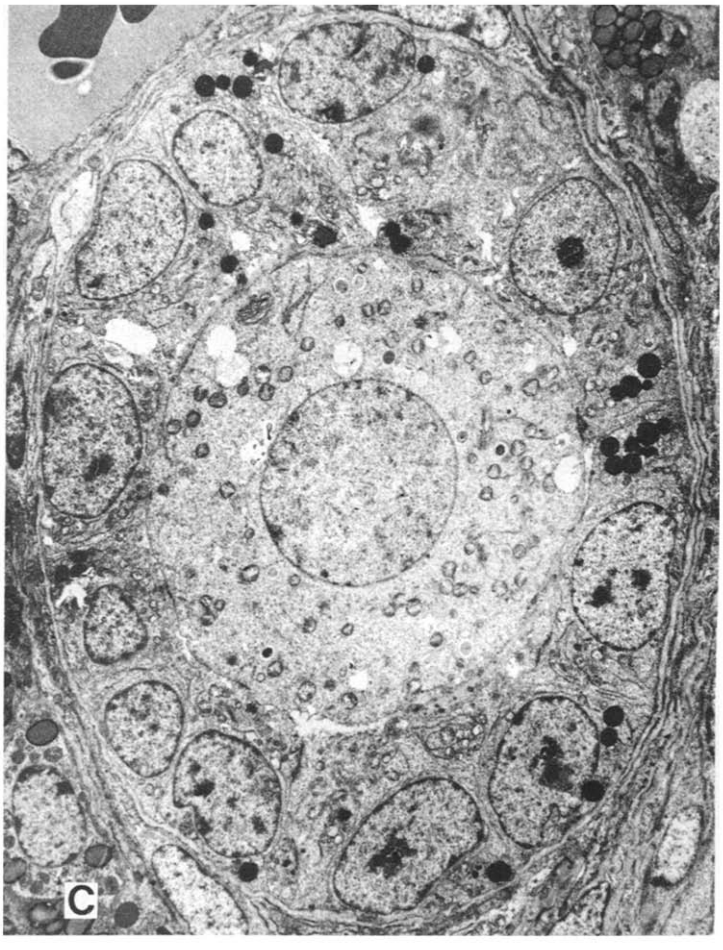

Fig. 2C.

weeks [2]. In adolescent and adult rodents, comprehensive reports have shown that nutritional restriction prolongs (10–40%) the maximal lifespan [9,14,15]. However, severe nutritional restriction imposed on the juvenile rat can extend maximal lifespan over 70% [9]. Our findings that only the most severely affected nutritionally restricted rats were fertile (a manifestation of physiological competence) at late ages suggest that severe nutritional restriction, initiated early, is necessary to produce a maximum delay of the aging process.

With aging, substantial numbers of cerebral cortical cells [16], cerebellar neurons [17], and neurally derived pinealocytes [18] are lost. The loss of pineal cells can be prevented by severe nutritional restriction in the juvenile rat [18]. Thus, low tryptophan feeding may slow down or arrest maturational-related depletion of selected neural populations involved in the control of ovarian function. It is well known that tryptophan is the precursor of the neurotransmitter 5-hydroxytryptamine (serotonin), and that low dietary tryptophan results in low

brain serotonin levels [19]. Therefore low tryptophan feeding may act by reducing serotonin levels in critical tissues, such as the pineal, where serotonin is the essential precursor for melatonin and other potential antigonadotropic factors [20]. In our experiments, brain serotonin, including pineal, is markedly reduced [13,21–22]; this reduction may depress the activity of the pineal gland (*i.e.* reduced antigonadotropic potential) which would, in turn, postpone reproductive aging and/or extend reproductive activity.

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