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Induction of Tryptophan Pyrrolase Activity in Starving Rabbits of Different Ages.* (31149)

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Previous studies(1-3) have shown that liver tryptophan pyrrolase activity (LTP) is increased in female rats and rabbits subjected to total starvation. In adult female rats(2) a bimodal response occurs with peak activities at 4 hours and between 2-7 days after the animals were placed on a starvation regimen. In young adult female rabbits(1) the bimodal response occurred within 18-36 hours and after 7-11 days from the beginning of starvation. In contrast to these findings, LTP activity in adult male rats remains relatively constant during 8-13 days of starvation(2,4). More recent experiments have demonstrated that induction of LTP activity by starvation or by injection of L-tryptophan in male and female rabbits is a function of age of the animals.

Experimental methods. Young 30-day-old male and female New Zealand rabbits weighing about 600 g were obtained commercially. The animals were maintained in the laboratory for all experiments up to 90 days of age. Older animals ranging in age between 4-5 months (80%) and 10 months (20%) were obtained commercially and distributed throughout the experiments. The data for 4-10-month-old animals were pooled because no significant differences were apparent between these ages. All animals were maintained in individual cages on commercial rabbit chow at 74°F.

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Rabbits were starved by complete removal of food but water was available *ad libitum*. For substrate induction experiments, the animals were sacrificed 6 hours after intraperitoneal injection with 0.5 g L-tryptophan per kg body weight suspended in distilled water. Control animals were injected with distilled water. In all animals, the injection volume was limited to 10 ml per kg body weight.

After sacrifice by cervical fracture, the livers were removed, chilled in ice and LTP activity determined as previously described in detail(1) by measuring the rate of kynurenine formation during a 4-hour incubation period with the diazotization procedure of Knox and Mehler(5). Exogenous hematin was not added to any of the assays(6). Liver protein was determined in the homogenates by micro-Kjeldahl procedures using 6.25 as the conversion factor. Liver protein determinations were omitted for animals injected with L-tryptophan. As previously shown(1-2), expression of enzyme activity based on wet liver weight or protein yields essentially comparable results.

The data for "2 kg" female rabbits are presented for comparative purposes and are taken from Rosenthal, Barack and Haessler (1). These animals are estimated to be approximately 60-75 days of age but the exact birth dates are unknown. Due to an error in arithmetic, the values presented in(1) are too high by a factor of 0.65 and the data shown in the present report have been cor-

rected. This error does not alter the relationship of the data or the interpretation.

Results. As shown in Fig. 1, the induction of LTP activity is a function of age of the animal and length of time of starvation. Young 30-day-old female rabbits respond maximally to starvation in a regular fashion with significant increases being apparent within 24 hours from the beginning of starvation. In older animals, ranging between 4-10 months of age, starvation for as long as 11 days is associated with minimal LTP induction. The bimodal response of "2 kg" female rabbits is not apparent in either 30-day-old or 4-10-month-old animals. During starvation, young 30-day-old animals lose weight more rapidly than do older animals (Fig. 2).

The LTP activity in rabbits starved for 24 hours (Fig. 3, 4) is maximal in young animals between 30 and 75 days of age. Although male rabbits appear to respond somewhat sooner than female rabbits, this difference is of doubtful significance. In control, fed animals, LTP activity for both sexes is

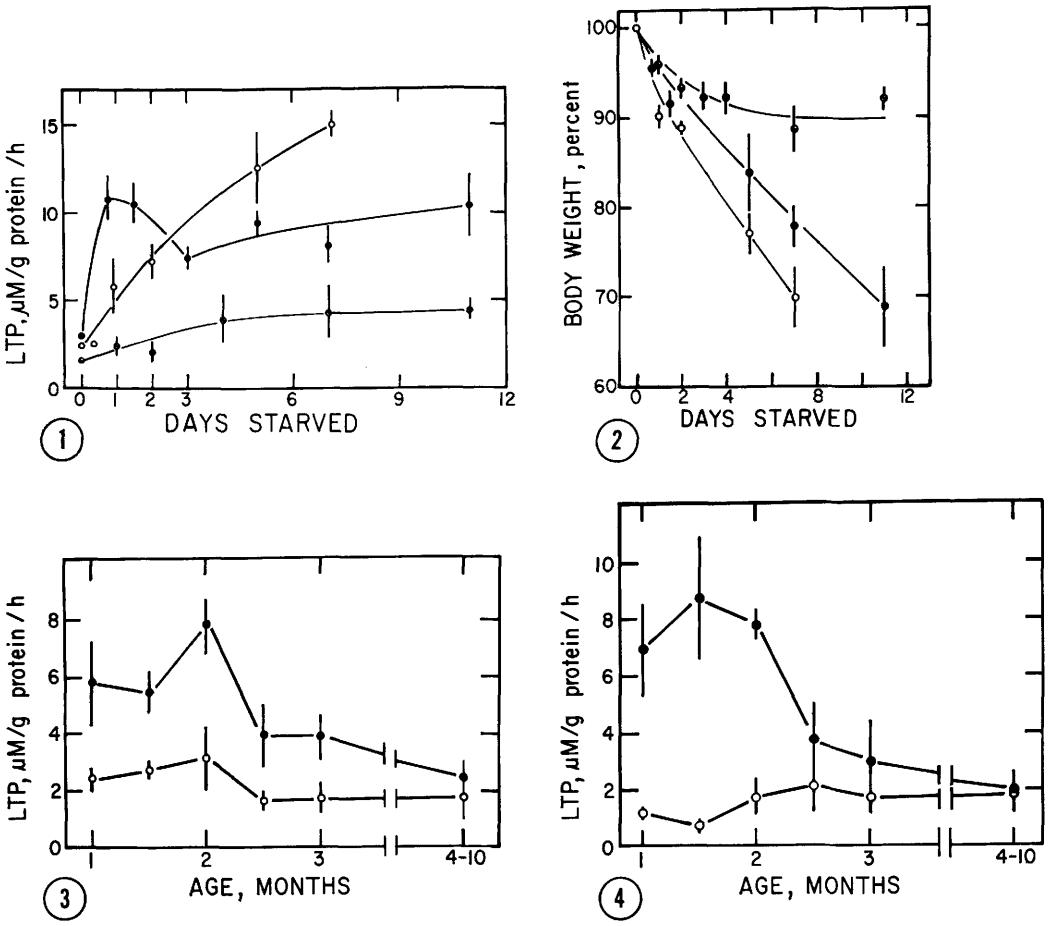

FIG. 1. Liver tryptophan pyrrolase activity *vs* days of starvation for female rabbits. 30-day-old —○—, "2 kg" —●—, 4-10 months —Θ—. Each point represents average values ± 1 S.E. (vertical bars) obtained with 4 to 10 animals.

FIG. 2. Body weight *vs* days of starvation for female rabbits. See legend for Fig. 1.

FIG. 3. Liver tryptophan pyrrolase activity *vs* age of female rabbits fed *ad libitum* (—○—) or starved for 24 hours (—●—). Each point represents average values ± 1 S.E. (vertical bars) obtained with 6-10 animals.

FIG. 4. Liver tryptophan pyrrolase activity *vs* age of male rabbits fed *ad libitum* (—○—) or starved for 24 hours (—●—). Each point represents average values ± 1 S.E. (vertical bars) obtained with 5-6 animals.

TABLE I. L-Tryptophan Induction of LTP Activity in Female Rabbits of Varying Age.

Group*	Age of animals		
	30 days	"2 kg"	4-10 mo
Control (uninjected)	.33 ± .06 (10)	.43 ± .17 (6)	.28 ± .13 (8)
Water injected	.15 ± .03 (4)	.91 ± .28 (5)	.68 ± .35 (5)
L-tryptophan injected	2.26 ± .47 (4)	6.48 ±1.33 (5)	1.51 ± .45 (4)
L-tryptophan/H_2O	15×	7×	2×

* Average values expressed as μM kynurenine formed/g liver/hr ± S.E., for number of animals given in parentheses. Animals injected I.P. with 0.5 g L-tryptophan in 10 ml water per kg body weight or water alone. All animals sacrificed 6 hr post injection.

similar and is essentially constant for young and old animals.

Induction of LTP by L-tryptophan injection is also greater in young 30-day-old female rabbits than in older 4-10-month-old animals (Table I). Although LTP activity in uninjected control animals is constant, the effect of intraperitoneal injection of water alone is somewhat variable(1) resulting in a depression of LTP activity in 30-day-old rabbits and slight elevations in older animals.

Discussion. Previous studies by Nemeth (7) and Nemeth and De La Haba(8) have shown that liver tryptophan pyrrolase activity is essentially absent in livers of fetal rabbits but begins to appear at term and rises to adult values within 24 hours after birth. Similar results have been shown for glucose-6-phosphatase activity(7) and for tyrosine aminotransferase(9). More recently, electrical stimulation of the splanchnic nerve of rabbits has been shown to result in increased activity of liver glycogen phosphorylase and glucose-6-phosphatase activities(10). The present experiments demonstrate that LTP activity may be induced in young rabbits subjected to starvation for as little as 1 day but the LTP activity of adult rabbits is relatively insensitive to 1 or 2 days of starvation. That starvation-induced LTP activity may vary with species is shown by our previous data(2) in which adult female rats demonstrated bimodal increases of LTP activity during 8 days of starvation, similar to those of "2 kg" rabbits, while adult 4-10-month-old female rabbits respond only slightly to starvation stress. While Rivlin and Knox(11) reported that the specific activity of LTP increases with age in fed rats, no significant changes between 30-day-old and 4-10-month-old fed rabbits are apparent in the present experiments. Although it is conceivable that starvation may alter the availability of a ferroporphyrin prosthetic group of the enzyme(6), preliminary experiments showed that addition of hematin to liver homogenates of starved or fed rabbits failed to alter LTP activity.

A more probable explanation of the effect of age on starvation induction of LTP activity may be related to larger fat and protein stores in adult, slowly growing animals as compared with young, actively growing rabbits. The older rabbits contained large amounts of abdominal fat before starvation. These fat deposits decreased during starvation and we observed some edema in these animals(12) which may account, in part, for the relatively minor weight loss. In younger animals, the marked weight loss is presumably due to rapid catabolism of tissue and muscle protein to supply the energy needs of the body. We interpret these observations to mean that rapid catabolism of protein, with the concomitant release of tryptophan, may induce enzyme formation in younger animals. In older animals, the catabolism of fat conserves body protein thus leading to minimal tryptophan release and LTP induction. However, the induction of LTP activity may also be modified by glucocorticoid(13) or estrogenic(14) activity which may be related to age of animals and the ability to respond to conditions of stress. These and other relationships concerning the complex phenomena of starvation induction of LTP activity await further study.

Summary. Induction of liver tryptophan pyrrolase activity occurs readily in young rapidly growing male and female rabbits between 30-75 days of age when subjected to starvation for 24 hours or more. LTP activ-

ity in older rabbits between 4-10 months of age increases only slightly during 11 days of starvation. In the younger 30-75-day-old rabbits, intraperitoneal injection of 0.5 g L-tryptophan per kg body weight induces the enzyme between 7- to 15-fold over control values as compared to about 2-fold for 4-10-month-old animals.

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Inhibition of TRIC Agents by Virus-Induced Interferon.* (31150)

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The causative agents of trachoma and inclusion conjunctivitis (TRIC) have been called viruses in the past. However, TRIC agents possess several characteristics which clearly separate them from typical viruses. They possess a bacteria-like cell wall containing muramic acid; they contain both DNA and RNA; they elaborate enzymes involved in decarboxylation of carbohydrates and in folic acid synthesis; they are inhibited by antibacterial drugs; and at least some stages in the development of TRIC agents involve binary fission(1). Thus it appears that TRIC agents are more closely related to obligate intracellular bacteria or rickettsiae than to true viruses.

Up to the present time, interferons have been known to act only on true viruses. It was, therefore, of interest to determine whether virus-induced interferons would inhibit the replication of the more complex TRIC agents. Early in this work we were made aware of a publication by Mordhorst and Reinecke(2) in which it was claimed that interferon had no effect on TRIC agents. We wish to report here experiments which establish the inhibition of a TRIC agent by virus-induced mouse interferon in L cells.

Materials and methods. Interferon. Mouse interferon for these experiments was prepared by infecting strain L 929 cells, obtained from Dr. J. Youngner, with Newcastle disease virus (NDV) (E4 Herts strain) at a multiplicity of 1.5 pfu/cell (plaque forming units) as measured on chick embryo fibroblasts. The L cells were grown in one liter Blake bottles in a 5% CO_2 incubator at 37°C, using a growth medium of 10% calf serum in Eagle's Minimum Essential Medium (MEM) containing penicillin, streptomycin and mycostatin. Following infection, the cells were maintained on serum free MEM + antibiotics. Supernatants were collected 24 hours after infection. They were adjusted to pH 2 with 0.1 N HCl and held for 5 days at 4°C before neutralization with 0.1 N NaOH.

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