potency of 135 mu/gland. This compares with a mean TSH-SR of 48 birds of 2.5 mμ/100 g body weight. By a similar method (6) the TSH in human plasma was reported as 0.003 mμ/ml, a t½ of 30 minutes, and an estimated TSH-SR of 260 mμ/day. No data on body weight were reported.

The short t½ of 13.7 minutes reported for the normal rat(7) would suggest that the t½ of TSH in the fowl would also be relatively short. Although it is believed that determinations of the blood t½ of hormones can result in valuable data, the t½ does not necessarily lead to an accurate measurement of the time duration in which an injected dose of TSH would exert an effect on the thyroid gland resulting in the release of thyroidal-I^{131}.

In fact, in a previous study(10) of fowls whose thyroidal-I^{131} release had been blocked with thyroxine, intravenous injections of TSH resulted in a renewed release of thyroidal-I^{131} and this release continued to occur 10 to 24 hours beyond the time of injection. This indicates that although TSH is cleared from the blood rapidly its effect on the thyroid is prolonged.

Summary. A method of estimating the daily TSH-SR of fowls is described, depending on a comparison of the normal thyroidal-I^{131} release rate where recycling of I^{131} is blocked by a goitrogen, endogenous TSH release is blocked with thyroxine at the TSR level and by producing a comparable thyroidal-I^{131} release rate with the daily subcutaneous injections of TSH assayed against a USP Reference Standard TSH. TSR and TSH-SR of 2-year-old New Hampshire hens were shown to be significantly higher during late spring than during late summer to early fall. No significant difference was observed between TSR and TSH-SR of 1-year-old and 2-year-old hens tested concurrently during late summer. However, there was no significant correlation between TSR and TSH-SR of these hens.

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Liver Tryptophan Pyrrolase Activity in Fed and Starved Female Rabbits.* (29541)

HAROLD L. ROSENTHAL, BRUCE M. BARACK AND ISOLDE HAESSLER
Dept. of Physiological Chemistry, Washington University School of Dentistry, St. Louis, Mo.

Liver tryptophan pyrrolase (LTP) may be induced directly by its substrate l-tryptophan(1,2), or closely related analogues such as d-tryptophan(3,4), α-methyl tryptophan (3,4) and allylisopropylacetamide(5). Glucocorticoids(1,6) will also stimulate LTP activity. These substances are among the effective inducers of LTP in either normal or adrenalectomized rats. Many substances such as sodium chloride(7) and allylisopropyl-

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TABLE I. Liver Tryptophan Pyrrolase Activity of Starved and Refed Rabbits.

	No.	Body wt, kg	Liver wt, g	LTP activity, μM/g/hr ± S.E.
Control	6	2.02	88.1	.59 ± .15
Starved — 36 hr	5	1.85	49.6	3.03 ± .34
" + 36 hr refed	4	2.02	69.0	.93 ± .24
" + 72 " "	4	2.12	86.2	.40 ± .12
Starved — 7 days	6	1.57	39.3	2.69 ± .21
" + 24 hr refed	6	1.78	61.7	.92 ± .15
" + 72 " "	4	2.02	96.8	.22 ± .04
" + 7 days refed	2	2.05	84.8	.32 ± .25
" + 14 " "	4	2.18	99.6	.16 ± .05

acetamide(5) may indirectly induce enzyme formation by mediation of hormonal mechanisms via the adrenal glands presumably due to stimulation of glucocorticoid production. Knox(8) has recently discussed the adaptive control of tryptophan pyrrolase and tyrosine transaminase systems in intact animals and suggested that specific or non-specific stimuli may elicit a common response, the bodily reactions associated with fear, hunger, rage, and pain. Of these bodily reactions, starvation appears to be most amenable for study under controlled conditions. It seemed pertinent therefore, to study the induction of LTP in fed and starved rabbits.

Experimental procedure or materials and methods. Female white New Zealand rabbits obtained commercially and weighing about 2 kg were maintained in individual cages at 74°F on commercial rabbit chow fed *ad libitum.* Starved animals were obtained by complete removal of all food, but water was available to all animals *ad libitum.* Appropriate animals were injected intraperitoneally with distilled water, 0.154 M sodium chloride or various concentrations of l-tryptophan dissolved or slurried in distilled water at a constant volume dose of 15 ml/kg body weight. The animals were restrained on an animal board for not more than 3 minutes during the injection procedure. Animals were killed by cervical fracture; the livers were immediately placed in ice and a 20-30 g portion was homogenized in a Waring Blendor with 7 volumes of cold 0.14 M KCl adjusted to pH 7.5 with M/3 disodium phosphate. Body weight and liver weights were recorded. Liver protein was determined in the homogenates by micro-Kjeldahl using 6.25 as the conversion factor. The tryptophan pyrrolase activity of the homogenates was immediately determined by measuring the rate of kynurenine formation during a 4 hour incubation period by the diazotization procedure of Knox and Mehler(9). Exogenous hematin was not added to any of the assays(10).

Results. The lag phase observed in 95% of 162 analyses of individual rabbit liver homogenates varies from 30 minutes for homogenates of high LTP activity to 120 minutes for homogenates with low activity (Fig. 1A). In 5% of the homogenates, the lag phase was absent and aberrant assays occurred in 2% of the determinations (Fig. 1B).

Effect of starvation on LTP activity. In control rabbits fed *ad libitum,* LTP activities are low and average 0.59 μmoles kynurenine formed/gram wet tissue/hour (Table I). However, when the animals are deprived of food for from 18 hours to 11 days, LTP activities were elevated (Fig. 2), and a bimodal response was apparent. Maximal LTP activities were observed 28 hours after initiation of starvation, decreasing somewhat at 72 hours and again increasing to maximal LTP activity between 7 and 11 days. The bimodal response is apparent when LTP activity is expressed on either a liver wet weight or liver protein basis. During the 11 day starvation regimen, the animals lost 30% of their initial body weight. When animals previously starved for 36 hours or 7 days were refed, LTP activities returned to normal in approximately 50 hours and enzyme activity remained consistently lower than control values during a 14 day experimental period (Table I).

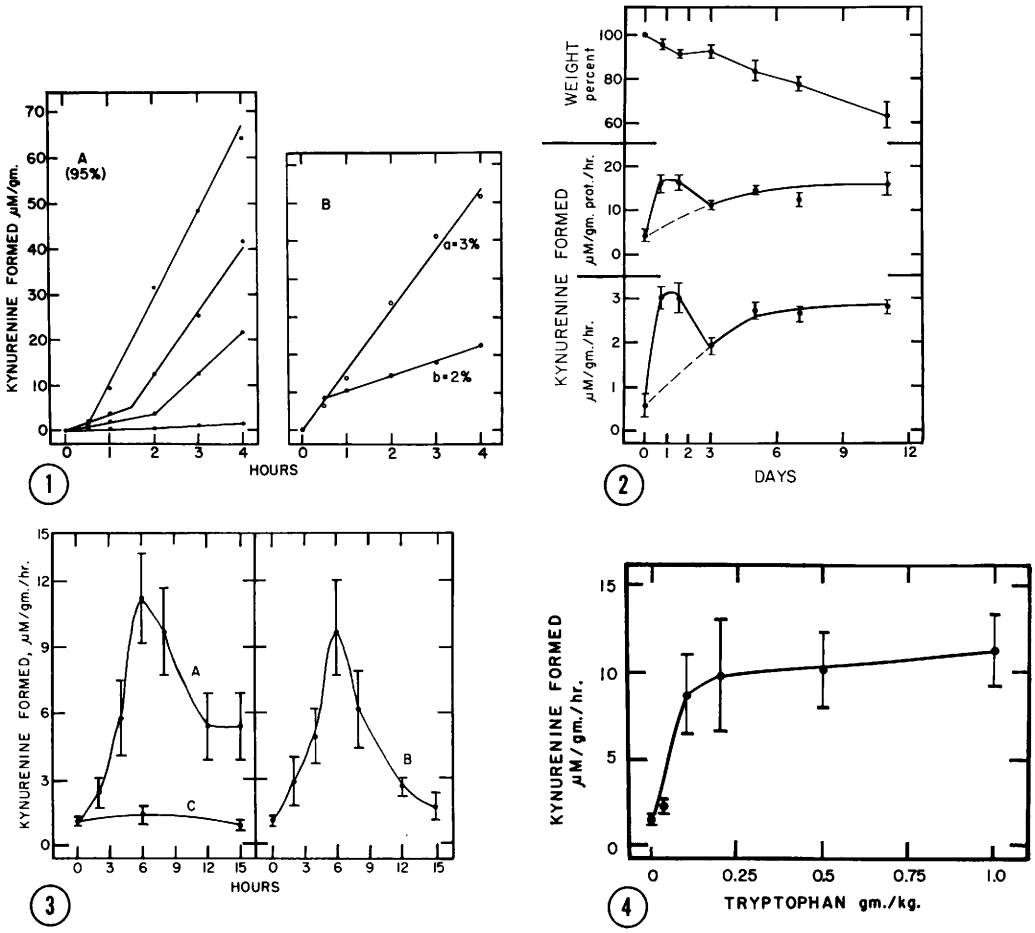

FIG. 1. Kynurenine formation in liver homogenates vs time. A. Typical response of liver with low and high activities occurring in 95% of assays. B. Aberrant responses occurring in 5% of assays. System: Twenty-one ml liver homogenate (1:8 in 0.14 M KCl), 60 mM phosphate, 3 mM L-tryptophan, pH 7.5. Total volume of 70 ml, 37.5°C.

FIG. 2. Relationship of liver kynurenine formation and body weight loss of female rabbits vs days of starvation. Each point represents average values ±1 S.E. obtained with 5-7 animals.

FIG. 3. Induction of LTP in rabbit liver from fed animals by intraperitoneal injection of L-tryptophan or distilled water. Curve A = 1.0 g L-tryptophan/kg body wt. Curve B = 0.2 g L-tryptophan/kg body wt. Curve C—distilled water control. Each point represents average values ±1 S.E. obtained with 3-6 rabbits.

FIG. 4. Induction of LTP in rabbit liver from fed animals vs injected dose of L-tryptophan. Each point represents average values ±1 S.E. obtained with 5-7 animals.

Induction of LTP activity in fed and starved rabbits. Maximal LTP activities were induced 6 hours after intraperitoneal injection of 0, 0.2 or 1.0 g l-tryptophan in distilled water/kilogram body weight (Fig. 3). Fifteen hours after injection, the LTP activity of animals injected with 1.0 g/kg body weight was considerably elevated over control values, while the 0.2 g/kg body weight dose approached initial control values. Rabbit LTP induction is very sensitive to injections of l-tryptophan and a measurable response may be obtained by an injection of as little as 25 mg l-tryptophan/kg body weight (Fig. 4), and a half maximal inductive response was obtained with approximately 75 mg l-tryptophan. Furthermore, restraint of the animals for 3 minutes, during the time for intraperitoneal injection of distilled water or saline followed by immediate sacrifice,

TABLE II. Effect of Injection Procedure on LTP in Female Rabbits.

Conditions*	No.	LTP activity, μM/g/hr ± S.E.
Control—No injection	6	.59 ± .25
Exp—Distilled H_2O, 0 hr	6	1.12 ± .24
" 6 "	5	1.41 ± .43
Saline, 0 hr	7	.70 ± .25
" 6 "	6	3.54 ±1.45

* Animals injected I.P. with 15 ml distilled water or saline per kg body wt.

results in normal LTP values in saline injected animals and a 2-fold increase of LTP for water injected animals as compared with the values obtained in unrestrained uninjected animals. In animals injected with 0.154 M sodium chloride and sacrificed 6 hours post injection, LTP activities were 2-3 fold higher than in similar animals injected with distilled water (Table II). For these reasons, tryptophan injections in water solution were used to avoid, as much as possible, the nonspecific saline stimulating effects.

That starved animals are more sensitive to LTP induction following injection of distilled water or l-tryptophan is shown in Table III. In animals starved for 36 hours or 7 days, 1 gram l-tryptophan/kg body weight induces twice as much enzyme as may be induced in fed animals.

Discussion. It is well known that liver tryptophan pyrrolase activity is induced by the substrate and is also mediated by hormonal control under the influence of adrenal stimulation or injections of glucocorticoid. The present experiments indicate the marked ability of rabbits to respond to a changing physiological state under the influence of continuous starvation. Chiancone(11), however, found no alteration of liver LTP activity in rats subjected to fasting for 8-13 days. This difference in response between rabbits and rats may be due to the severe degree of starvation of the rats in Chiancone's experiments. In our experience 50% of totally starved rats will die in 8 days, while rabbits may easily withstand total starvation for 15 days or more. The bimodal response of LTP to starvation in rabbits may be resolved into 2 separate but distinct phases. The first phase, which results in maximal LTP during the first 30 hours of starvation is interpreted to represent a stress response mediated via the adrenal gland. The first response also appears to be superimposed upon a more gradual response, indicated by the dotted line of Fig. 2, which is interpreted to indicate LTP induction due to tryptophan liberation from degradation of muscle and other more labile protein stores. Both responses are completely reversible when the animals are refed.

TABLE III. Tryptophan Induction of LTP in Rabbits.

Group*	No.	LTP activity μM/g/hr ± S.E.	Induced
Fed *ad libitum*			
Control	5	1.41 ± .43	9.8
Exp	5	11.2 ± 2.0	
Starved—36 hr			
Control	5	4.86 ± .60	18.7
Exp	4	23.6 ± 2.1	
Starved—7 days			
Control	6	4.62 ± .87	19.4
Exp	4	24.0 ± 3.2	

* Control animals injected I.P. with 15 ml distilled water and experimental animals injected with 1 g L-tryptophan in 15 ml water per kg initial body wt. All animals sacrificed 6 hr post-injection.

The greater LTP activity of starved rabbits suggests an increased catabolism of tryptophan for energy purposes but it is difficult to reconcile the observed LTP changes with the pathway for tryptophan metabolism. This phenomenon is in contrast to the activity of other enzyme systems, notably cytochrome C reductase(12) and succinic dehydrogenase (13,14) that are reduced during starvation thus reflecting an overall depression of catabolism with a concomitant conservation of energy. The dynamic aspects of adaptive control of metabolic systems by intact animals has recently been presented in detail by Knox(8) who has indicated the complexity of control mechanisms.

Summary. Liver tryptophan pyrrolase activity is elevated from normal values of 0.59 μmoles/g/hour to 3 μmoles/g/hour in female rabbits subjected to starvation. An increase of LTP occurs within 18 hours fol-

lowing deprivation of food, decreases at 3 days and increases again between 7 and 11 days of starvation. In fed rabbits, intraperitoneal injections of 1.0 g l-tryptophan/kilogram body weight induces LTP activities of 10 μmoles/g/hour but in rabbits previously starved for 36 hours or 7 days, a similar injection induces twice as much LTP as in fed animals. Liver tryptophan pyrrolase activity of starved rabbits returns to normal values within 2 days following refeeding.

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Effect of Tween 80 on Cholestyramine-Induced Malabsorption.* (29542)

JOHN J. DU BOIS, PETER R. HOLT, GUNTHER W. KURON, SAMI A. HASHIM AND THEODORE B. VAN ITALLIE
Department of Medicine, St. Luke's Hospital and Institute of Nutrition Sciences, Columbia University

Cholestyramine, a quaternary ammonium anion exchange resin with a styrene-divinylbenzene skeleton, has been shown to be an effective bile acid sequestering agent. Administered by mouth, it forms a nonabsorbable complex with bile acids in the intestinal tract (1,2). In human subjects this interruption of the enterohepatic cycle of bile acids results in a lowering of serum total cholesterol(3), and at higher dose levels in significant steatorrhea(4). In view of the intestinal intraluminal detergent role of bile salts in the emulsification of lipids, it seemed of interest to study the effects of ingestion of a surface active agent on the steatorrhea induced in man by large doses of cholestyramine.

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Polyoxyethelyene sorbitan monooleate (Tween 80), a partial ester of a fatty acid, is a nonionic surface active agent. It has been shown to be non-toxic in man(5). It has no effect on the motility of the small intestine(6), or on the serum total cholesterol (7). It does not affect fat absorption in normal adults(8), and in bile fistula dogs(9). Administration of Tween 80 has been shown to increase fat absorption in patients with steatorrhea secondary to sprue, regional ileitis, and gastrojejunostomy(10).

Methods. Three healthy female adults, aged 22-26 were maintained for 3 to 4 weeks on a constant, homogenized liquid formula diet providing 40 calories per kg body weight per day. Forty per cent of the calories was derived from corn oil (90-100 g of fat/day), 43% from dextrose, and 17% from casein. Cholestyramine was given as a suspension in