Effects of Thyroxine and Cortisol on Liver Threonine Dehydratase and Tryptophan Pyrrolase in Rats Fed a High Protein Diet* (33605)

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Many enzymes of amino acid catabolism increase in activity in rats fed a high protein diet (1–3) or injected with glucocorticoids (4). Among enzymes that have been examined in some detail are tryptophan pyrrolase, the activity of which is elevated in rats fed a high protein diet (5) or injected with glucocorticoids (6), with its substrate (7), or with various other compounds (8). Threonine dehydratase is also elevated in rats fed a high protein diet or injected daily with cortisol (9–12) or made diabetic (13) but the activity of this enzyme does not increase in rats injected with its substrate (9).

There have been few studies of the influence of hormonal treatments other than with glucocorticoids (12) on the responses of enzymes of amino acid catabolism in rats fed a high protein diet. Thyroxine administration has been shown to depress the activities of several pyridoxal phosphate requiring enzymes (3, 14–16) but the influence of this hormone on enzyme activities *in vivo* has received much less attention than have the adrenal glucocorticoids. Some observations on the effects of cortisol and thyroxine on the activities of tryptophan pyrrolase and threonine dehydratase in rats fed a high protein diet are reported in this paper.

Experimental procedure. Animal care. The experimental animals were male, Holtzman or Sprague-Dawley rats weighing 100–150 or 200–300 g. Hypophysectomized and thyroidectomized rats were obtained from the Chicago Hormone Assay Laboratory. One group of thyroidectomized animals was obtained from the Charles River Breeding Laboratory, Inc. Animals were housed individually in suspended cages with wire mesh bottoms. Upon receipt, they were fed *ad libitum* an adequate purified diet for at least 3 days to allow them to adjust to the new environment. They were then fed the experimental diets for 4 days. Thyroidectomized rats were given 1% calcium gluconate in drinking water throughout the experiments. Hypophysectomized and thyroidectomized animals were used 10 to 20 days after the operation, but in each experiment the time after the operation was the same for all rats.

Diets. The control diet (17) contained 25% casein, 5% salt mixture, 5% corn oil, 0.2% choline chloride, and 0.5% vitamin mix and sucrose to make 100%. Increases in the casein content were at the expense of sucrose. Normal rats were fed the diets in dry form; hypophysectomized and thyroidectomized rats were fed diets in gel form. The gel diets were prepared as follows: all of the dry ingredients except for the vitamins and 4% of agar were mixed together; the agar and an amount of water equal to the weight of the dry components (twice the weight for the 80% casein diet) were brought to a boil; when the agar was dissolved completely, the solution was mixed vigorously with the dry components. After the suspension had cooled a little, the vitamins and choline chloride were added with continuous mixing until the diet became homogeneous. It was then poured into a polyethylene container, allowed to cool, covered with an air-tight lid and stored at 4°.

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Treatment of animals. Hydrocortisone acetate (Abco Dealer Inc., New York, N. Y.) was injected in a suspension as obtained (each ml contained: hydrocortisone acetate 50 mg; polysorbate 80, 0.4%; sodium carboxymethylcellulose 0.5%; benzyl alcohol 0.9%; sodium chloride 0.9% and water). L-Thyroxine hydrochloride pentahydrate (Nutritional Biochemicals Corporation) was dissolved in 0.005 *N* sodium hydroxide at a concentration of 100 μg per 0.1 ml and injected as such. All injections were intraperitoneal and were given daily for 4 days. Although the intraperitoneal route is not necessarily the most effective it is commonly used in enzyme induction studies. Animals were killed 24 hr after the last injection and food was not withheld from them unless otherwise noted. The doses of cortisol and thyroxine were as shown in the results.

Rats were killed with a decapitator and the blood was allowed to drain. The liver was removed promptly, wrapped in plastic wrapping material, frozen immediately in dry ice and kept frozen until used for the enzyme assays.

Assay procedures. The activity of tryptophan pyrrolase was measured by the method of Knox and Auerbach (6). The incubation mixture consisted of 0.3 *M* phosphate buffer, pH 7.0, 1 ml; 10% liver homogenate, 1 ml; 0.02 *M* L-tryptophan, 0.3 ml; and water, 1.7 ml. The activity of threonine dehydratase was measured by the method of Goldstein *et al.* (9). The incubation mixture consisted of 0.1 *M* pyrophosphate buffer, pH 8.3, 1.4 ml; 0.02 *M* pyridoxal phosphate, 0.1 ml; 0.5 *M* L-threonine, 0.5 ml; and 100,000*g* supernate of 10% liver homogenate, 0.5 ml. The units of threonine dehydratase or trytophan pyrrolase are defined, respectively, as 1 μmole of α-ketobutyrate or kynurenine formed per hour under the assay conditions. The protein content was determined by the method of Lowry *et al.* (18).

The activity of threonine dehydratase as reported by different authors for rats fed a control diet varies greatly. Goldstein *et al.* (9) reported that under their assay conditions the normal value for threonine dehydratase in 250–300 g rats was 8–11 units/mg of protein, which is the highest among several reported values. Our results for 200–300 g rats are in agreement with Goldstein's and Knox's values, ranging from 9.8 to 11.5 units/mg of protein.

Since activity per 100 g of body weight [(units/g of liver × liver wt. × 100)/body wt.] takes into account changes in liver size and should be indicative of the total enzymatic capacity of the animal, results calculated on this basis and expressed as percentage of the control value are presented in the figures. Actual values for the control groups are given with each figure.

Results. Intact rats. In rats (initial av body wt. 256 g) fed a control diet (25% casein) or a high protein diet (80% casein) *ad libitum,* daily injections of L-thyroxine (i.p., 400 μg/rat per day for 4 days) (Table I) depressed the activity of threonine dehydratase by 40% (control diet) and 55% (high protein diet). Thyroxine treatment did not significantly alter the activity of tryptophan pyrrolase of rats fed either diet.

The enzyme changes throughout the study followed the same pattern whether the results were expressed per milligram of protein, per gram of fresh liver, or per 100 gram of body weight as shown in Tables I and II.

Thyroxine treatment (50 μg/day) depressed by about 50% induction of threonine dehydratase activity by cortisol, but did not affect induction of tryptophan pyrrolase activity of rats fed the 25% casein control diet (Table II and Fig. 1).

The combination of a high protein diet and cortisol injections increased the activity of threonine dehydratase 23-fold over that of rats fed the 25% casein control diet and not injected with hormones (Fig. 2). However, when rats fed the high protein diet were injected with both cortisol and thyroxine the activity of threonine dehydratase was about 35% below that of the cortisol-treated group. The activity of tryptophan pyrrolase was not affected by thyroxine treatment.

Thyroxine administration did not depress threonine dehydratase activity of rats fed an 80% casein diet in the experiment reported in

TABLE I. Effect of Thyroxine on Activities of Threonine Dehydratase and Tryptophan Pyrrolase of Rats Fed 25 or 80% Casein Diet.

Casein content of diet (%)	Treatment	Body wt. (g)	Δ[a] (g)	Liver wt. (g)	Protein content of liver (mg/g)	Threonine dehydratase (units/hr)			Tryptophan pyrrolase (units/hr)		
						Per mg of protein	Per g of fresh liver	(unit/100 g of body wt.)	Per mg of protein	Per g of fresh liver	(unit/100 g of body wt.)
25	—	269	+14	13.3	148	9.86	967	4750 ± 85[b]	0.010	1.45	7.14 ± 0.42
80	—	268	+12	14.3	156	47.20	5020	26,700 ± 390	0.031	4.82	25.80 ± 1.89
25	Thy[c]	235	−21	8.5	175	6.59	804	2900 ± 336	0.010	1.63	5.87 ± 0.56
80	Thy	243	−13	9.8	179	24.60	2900	12,500 ±2100	0.029	5.20	20.90 ± 1.75

[a] Change in body wt.
[b] Mean ± SE for 4 rats/group.
[c] Four hundred μg of thyroxine/rat per day.

TABLE II. Effect of Thyroxine on Activities of Threonine Dehydratase and Tryptophan Pyrrolase of Cortisol-Treated Rats Fed a Control Diet.

Treatment		Body wt. (g)	Δ[a] (g)	Liver wt. (g)	Protein content of liver (mg/g)	Threonine dehydratase (units/hr)			Tryptophan pyrrolase (units/hr)		
Cortisol (mg)	Thyroxine (μg)					Per mg of protein	Per g of fresh liver	(unit/100 g of body wt.)	Per mg of protein	Per g of fresh liver	(unit/100 g of body wt.)
—	—	134	+32	7.0	197	4.1	421	2250 ± 325[b]	0.006	1.14	6.0 ± 0.37
10	—	89	−13	7.8	178	44.8	5060	44,400 ±6650	0.032	5.67	50.2 ± 3.93
10	200	82	−20	6.4	196	16.9	1950	15,100 ±1700	0.023	4.56	36.0 ± 2.96
10	100	82	−20	6.7	185	22.1	2470	21,300 ±1230	0.029	5.56	45.5 ± 5.85
10	50	86	−16	6.5	186	26.3	2970	22,400 ±2550	0.029	5.28	40.1 ± 4.35
—	200	115	+13	5.0	224	1.9	235	1030 ± 160	0.006	1.25	5.4 ± 0.32

[a] Change in body wt.
[b] Mean ± SE for 4 rats/group.

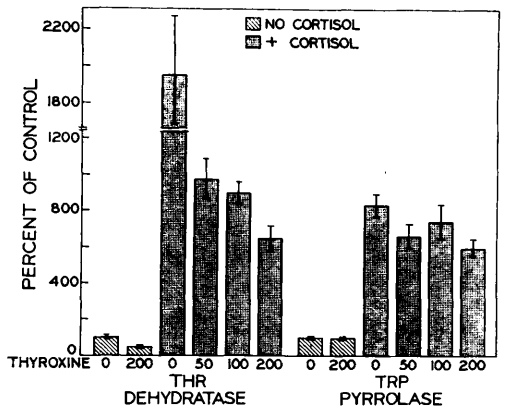

FIG. 1. Effects of thyroxine and cortisol on activities of liver tryptophan pyrrolase and threonine dehydratase of rats fed control diet (25% casein). Male rats (100–110 g) were given intraperitoneal injections of 10 mg of cortisol and various amounts (as indicated under the figure) of thyroxine (μg per rat) daily for 4 days; four rats per group; units/100 g of body weight/hr for control groups receiving no hormonal treatment: threonine dehydratase, 2250; tryptophan pyrrolase, 6.0.

Fig. 2 whereas in the experiment reported in Table I it depressed the activity of this enzyme in rats fed the high protein diet by nearly 50%. The rats used in the first experiment (Table I) weighed 256 g; those used in the second weighed only 110 g. The doses of thyroxine used were 400 μg for 256 g rats and 200 μg for 110 g rats. When the two trials were repeated, nearly identical results were obtained but in another study in which food had been withheld from the animals the night after the last injection, threonine dehydratase activity of young rats fed a high protein diet and injected with thyroxine was depressed. A further experiment revealed that thyroxine treatment depressed threonine dehydratase activity of young rats fed a high protein diet only if they were kept without food overnight before they were taken for the enzyme determinations.[3] Values for rats allowed access to food until they were killed for the enzyme determinations were essentially the same as those reported in Fig. 2. However, values for rats (110–130 g) fed the 80% casein diet but kept without food overnight were: for those not injected with thyroxine, 12,700 units/100 g of body wt./hr; for those injected with 200 mg of thyroxine/day, 8900 units/100 g of body wt./hr.

Thyroidectomized rats. The activity of liver threonine dehydratase was two- to threefold higher in thyroidectomized rats than in intact controls (Figs. 3 and 4). Control rats had the same average body weight as the thyroidectomized rats at the time of thyroidectomy. The activity of tryptophan pyrrolase in rats fed a control diet was very little affected by thyroidectomy. Administration of thyroxine to thyroidectomized rats fed a control diet depressed the activity of threonine dehydratase by 45% and that of tryptophan pyrrolase by 30% (Fig. 4). Feeding a high protein diet to thyroidectomized rats resulted in a ten- to thirteenfold increase in threonine dehydratase and about three- to fourfold increase in tryptophan pyrrolase (Figs. 3 and 4). In thyroidectomized rats fed

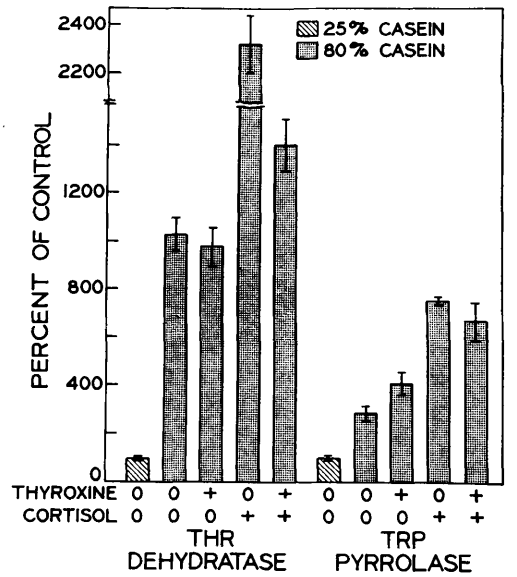

FIG. 2. Effects of cortisol and thyroxine on liver tryptophan pyrrolase and threonine dehydratase of rats (100–110 g) fed an 80% casein diet. Thyroxine (200 μg/rat), cortisol (10 mg/rat) were injected intraperitoneally daily for 4 days; four rats per group; units/100 g of body weight/hr for control groups receiving low protein diet and no hormonal treatment: threonine dehydratase, 2930; tryptophan pyrrolase, 8.3.

[3] This experiment was done in collaboration with John I. Dalezios (19).

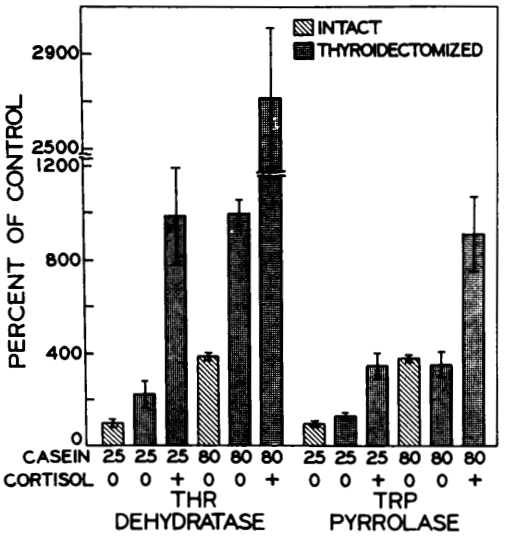

FIG. 3. Effects of cortisol (10 mg/rat) on liver tryptophan pyrrolase and threonine dehydratase of thyroidectomized rats fed a 25% casein (control) or 80% casein diet. Four rats per group; units/100 g of body weight/hr for intact control rats receiving no hormonal treatment; threonine dehydratase 2360; tryptophan pyrrolase, 8.3.

the high protein diet and injected with cortisol the activity of threonine dehydratase increased 22- to 27-fold. Treatment with cortisol had the same effect in thyroidectomized rats fed the control diet as it did in intact rats.

Thyroxine depressed the activity of threonine dehydratase by about 40% in thyroidectomized rats fed a high protein diet, and by about 60% in those fed a high protein diet and also treated with cortisol (Fig. 4). On the other hand, tryptophan pyrrolase activity of thyroidectomized rats fed a high protein diet was not affected by thyroxine and cortisol (Fig. 4).

Hypophysectomized rats. Increasing the protein content of the diet resulted in increases in the activities of both threonine dehydratase and tryptophan pyrrolase in hypophysectomized rats (Fig. 5). Hypophysectomy had little or no effect on the activity of either enzyme in rats fed the 25% casein control diet (Fig. 6). Thyroxine depressed the activities of both enzymes in hypophysectomized rats fed either the control or the high protein diet and injected with cortisol (Fig. 6).

Discussion. Threonine dehydratase activity, but not that of tryptophan pyrrolase was depressed by administration of thyroxine to rats fed a control diet or a high protein diet with or without cortisol injections. In thyroidectomized rats fed either the control or high protein diets, the activity of threonine dehydratase was two- to threefold above values for intact rats but tryptophan pyrrolase activity was not. Evidently thyroxine selectively depressed the activity of threonine dehydratase under these conditions. Although the thyroxine dosage was high in most of these experiments similar effects have been obtained with doses as low as 10μg/100 g of body weight (14, 19).

Rosen *et al.* (20) have reported that thyroxine administration inhibits the response of glutamate-alanine transaminase to cortisol in the rat. Horvath (14), and Litwack *et al.* (15) showed that thyroxine inhibits pyridoxal phosphate-dependent enzymes *in vitro* and suggested that thyroxine inhibits enzyme activity *in vitro* by interacting with pyridoxal. Litwack *et al.* (21) also reported that in rats

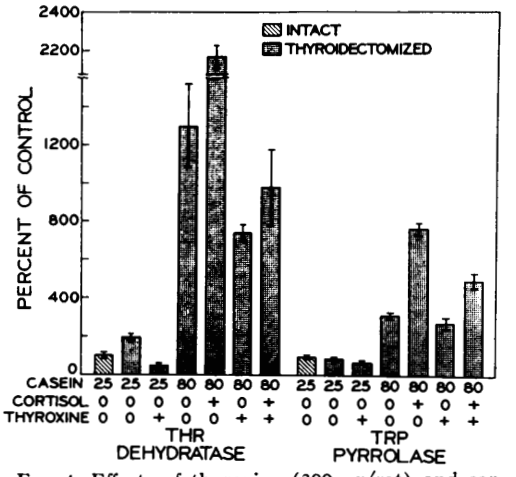

FIG. 4. Effects of thyroxine (200 μg/rat) and cortisol (10 mg/rat) on liver tryptophan pyrrolase and threonine dehydratase of thyroidectomized rats fed a 25% casein (control) or 80% casein diet. Four rats per group; units/100 g of body weight/hr for intact control rats receiving low protein diet and no hormonal treatment: threonine dehydratase, 2480; tryptophan pyrrolase, 7.3.

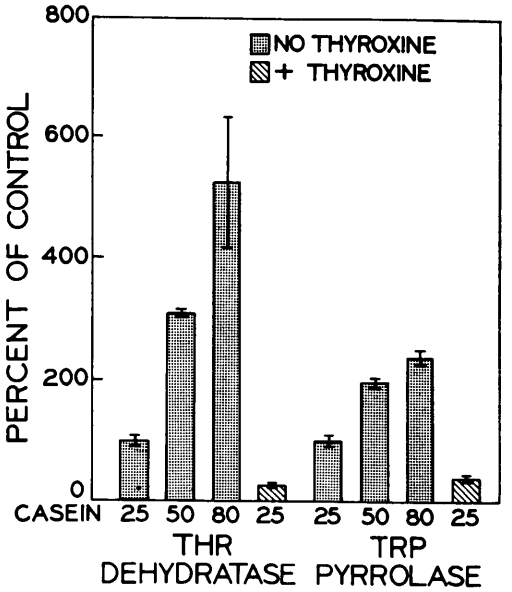

FIG. 5. Effect of casein content of the diet on liver tryptophan pyrrolase and threonine dehydratase of hypophysectomized rats. Five rats per group; units/100 g of body weight/hr for control groups receiving low protein diet and no hormonal treatment: threonine dehydratase, 3740; tryptophan pyrrolase, 11.4.

fed 2% of thyroid powder in the diet for 3 days the activity of tyrosine α-ketoglutarate transaminase was depressed but after 7 days of feeding this diet, the activity was increased slightly. Knox and Greengard (3) suggested that depressions of pyridoxal phosphate-dependent enzymes in thyroxine-treated animals may be the result of depletion of the coenzyme from the tissues by this treatment with reduction in apoenzyme being a secondary response. Threonine dehydratase is a pyridoxal phosphate-dependent enzyme, whereas tryptophan pyrrolase is not; hence, the differential responses of the two enzymes could be attributed to inactivation or depletion of pyridoxal phosphate. However, the high intake of pyridoxine (4-fold the requirement) by rats used in the present experiments should have prevented pyridoxine depletion during the short time thyroxine was administered. Further, the observation that thyroxine did not depress the activity of threonine dehydratase in young rats fed a high protein diet and allowed to eat freely until the end of the experimental period makes it unlikely that direct interaction of thyroxine with pyridoxal phosphate can account for these observations.

Freedland and Avery (11) reported that including 4% of iodinated casein in the diet of normal rats resulted in a twofold increase in the activity of serine dehydratase. Since serine dehydratase and threonine dehydratase are the same enzyme (9), the observations of Horvath (19) and ourselves that thyroxine injection depresses the activity of threonine dehydratase are in contrast to their findings. However, they (11) fed 4% of iodinated casein and did not inject thyroxine. Further, the size and age of the animals may be important (they did not report the size of their animals). In young animals (100–130 g) fed a high protein diet. thyroxine injection did not depress the activity of threonine dehydratase.

Of particular interest are the elevations of the two enzymes in hypophysectomized rats

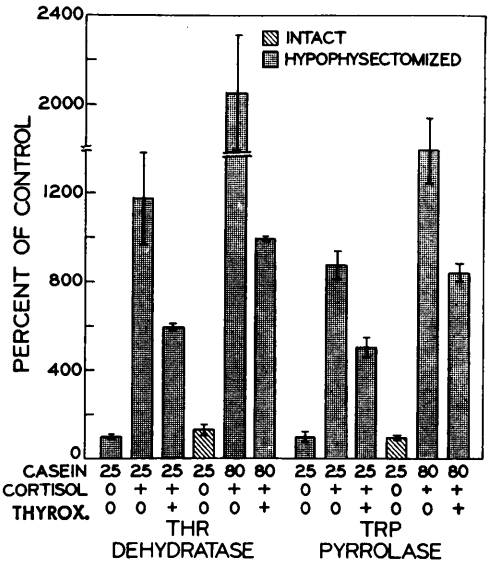

FIG. 6. Effect of thyroxine (200 μg/rat) and cortisol (10 mg/rat) on liver tryptophan pyrrolase and threonine dehydratase of hypophysectomized rats fed a 25% casein (control) or 80% casein diet. Four rats per group; units/100 g of body weight/hr for hypophysectomized control rats receiving low protein diet and no hormonal treatment; threonine dehydratase, 1580; tryptophan pyrrolase, 7.5.

fed a high protein diet. This would indicate that induction of these enzymes by a high protein intake is not mediated by hormones of the pituitary gland. However, responses to a high protein intake are higher in the intact than in the hypophysectomized rat and the response to combined treatment with high protein and cortisol was much greater than to either treatment alone. Rosen *et al.* (22) showed that several enzymes respond to a high protein intake in adrenalectomized rats, and Peraino (12) showed that in intact rats the response to cortisone and a high protein intake are additive. It would appear, therefore, that a high protein diet and cortisol induce this enzyme through different mechanisms. There is considerable evidence (23–25) that cortisol induction is the result of an enhanced rate of enzyme synthesis. There is also evidence that the activities of some enzymes of amino acid catabolism in animals may increase owing to inhibition of enzyme degradation (26). If a high protein intake depresses the rate of enzyme degradation this would account for the greater response of threonine dehydratase to combined treatment with cortisol and a high protein intake than to either treatment alone.

Changes in the activity of threonine dehydratase in rats injected with thyroxine may also be related to changes in energy metabolism and this might explain the limited responsiveness of young rats that were not starved. Visual inspection of rats receiving thyroxine injections indicated that the larger rats had substantial reserves of fat whereas young rats, which normally have very little body fat, became almost wholly depleted of adipose tissue when they were fed a high protein diet and injected with thyroxine for 4 days. The activities of many enzymes of amino acid catabolism increase when rats are fed a high protein diet, a condition in which energy must be derived from amino acids; and thyroxine treatment increases metabolic rate and, hence, energy consumption. The suppressed response of threonine dehydratase activity to a high protein intake in mature rats treated with thyroxine may therefore be a reflection of preferential utilization of amino acids as a source of energy and diversion of them away from synthesis of labile proteins. However, when body fat is depleted and amino acids must be mobilized even more extensively as a source of energy, as in the young animal treated with thyroxine, enzymes which are required for this, such as threonine dehydratase, may be maintained tenaciously unless a period of starvation is superimposed on the thyroxine treatment.

Summary. The activities of threonine dehydratase (L-threonine hydro-lyase EC4.2.1.16) and tryptophan pyrrolase (L-tryptophan: oxygen oxido-reductase EC1.13.1.12) were measured in tissues from rats subjected to hormonal and dietary treatments. Both treatment with cortisol and a high protein intake resulted in elevations in the activities of these enzymes in intact, hypophysectomized, and thyroidectomized rats. The combined effect of cortisol treatment and a high protein intake on the activities of threonine dehydratase and tryptophan pyrrolase was as great or greater than the sum of the two treatments individually. Thyroidectomy resulted in a two- to threefold increase in the activity of threonine dehydratase, but did not affect the activity of tryptophan pyrrolase. Thyroxine injections depressed threonine dehydratase activity in intact, hypophysectomized, and thyroidectomized rats. Thyroxine also depressed the activity of threonine dehydratase in rats fed a high protein diet or injected with cortisol. Tryptophan pyrrolase activity was not altered in intact rats treated with thyroxine but was depressed in hypophysectomized rats fed control or high protein diets.

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Seasonal Variation of Aortic Ruptures of Turkeys Induced by Diethylstilbestrol* (33606)

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In 1963, it was reported from this laboratory that injections of high levels of diethylstilbestrol (DES) resulted in a high incidence of aortic rupture in turkeys (1). The administration of DES by either the parenteral or oral routes caused mortality from aortic ruptures (2). The mortality resulting from the DES treatments is apparently due to the estrogenic effect since the feeding of dienestrol diacetate also resulted in a high level of mortality (3). In several experiments, various factors have been found to influence the rate of mortality resulting from the injections of DES. It was determined that a higher incidence of mortality occurred on a corn–soybean type diet containing 23% protein and a relatively high level of energy than occurred when the diet contained 20% protein and a high level of oats (4). Increasing the level of dietary sodium chloride from 0.5 to 3.0% resulted in significantly increasing rate of aortic rupture, and reducing the level from 0.05 to 0% resulted in a significantly decreased incidence of aortic rupture (5). Supplementing the diet with thiouracil has been found to increase incidence of aortic rupture (6) as compared to a significant decrease in mortality by the addition of iodinated casein (7).

During the past 7 years, 23 experiments were conducted to study factors which influence aortic ruptures in turkeys induced by diethylstilbestrol. In this series of studies it was observed that the turkeys are more susceptible to aortic rupture in certain periods of the year than in others. The data from these 23 experiments were combined to demonstrate this seasonal effect.

Materials and Methods. Mortality data from 23 experiments involving the use of DES to produce aortic ruptures in turkeys have been summarized. Only the data from those groups receiving DES without addition-

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