Recovery from Stress in Thyroidectomized Rats.* (22564)

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It has been reported by Hess *et al.* (1) that the increased adrenal response of thyroidectomized rats to stress is not due to a difference in pituitary ACTH release. The following experiments were designed to determine whether the lower adrenal ascorbic acid values after stress in operated animals are due to a failure of the hypothyroid rat to metabolize the vitamin. Is the adrenal ascorbic acid in stressed-thyroidectomized rats lower than controls because they cannot rebuild their adrenal content? If such is the case, then one would expect to find lower adrenal ascorbic acid values in operated animals at various periods of time of recovery from a stress.

Methods. Male rats of the Holtzman strain were thyroid-parathyroidectomized at an age of 6 weeks, and together with intact animals, maintained on Purina Laboratory Chow pellets and tap water *ad libitum* for an additional 6 weeks. Some of the animals from each group then were injected intravenously with ACTH (Armour Corticotropin) at dosages of 5 milliunits and 15 milliunits/100 g of body weight, under Nembutal anesthesia. Saline-injected animals (0.1 cc/100 g of body weight) from both groups served as controls. Groups of operated and intact rats were sacrificed at 1, 6, 12 and 24 hours after treatment, left adrenal and thymus glands weighed on a torsion balance, and adrenal glands assayed for ascorbic acid content according to the method of Roe and Kuether(2).

Results. One hour after injection of both doses of ACTH, significant depletions of adrenal ascorbic acid are observed in both operated and intact rats compared to saline-injected controls (Table I). Only in the adrenal of the thyroidectomized rat, at the higher dose level, is the depletion of ascorbic acid more striking. The depletion is significantly lower than in similarly treated intact rats.

Adrenal ascorbic acid analyses at periods of 6, 12 and 24 hours after treatment (Table I) reveal no significant differences between ACTH-injected thyroidectomized and intact animals nor between the ACTH-treated rats and their saline-injected controls (with the exception of one group of intact rats).

In the group of animals sacrificed one hour after treatment, relative weights of adrenal and thymus glands show no significant differences. Six hours after treatment, the intact rats receiving the higher dose of ACTH reveal larger adrenal glands than other intact and operated animals; thymus gland weights do not differ between groups of operated and intact rats. Relative organ weights of animals autopsied 12 hours after treatment show no significant differences in adrenal size between operated and intact rats, but the thyroidectomized animals have significantly smaller thymus glands. The adrenal glands of operated and intact rats 24 hours after treatment show no differences, whereas some decrease in size of the thymus glands of some thyroidectomized rats is evident.

Discussion. That thyroidectomized rats elicit a greater response to a given injection of ACTH, compared to intact animals, is evident from the adrenal ascorbic acid figures reported for operated animals receiving the higher dose of the trophic hormone one hour before autopsy. If these lower values in ACTH-injected thyroidectomized rats are due to a failure of the hypothyroid animal to rebuild the adrenal ascorbic acid after depletion or to destroy the circulating ACTH at a normal rate, then analyses in operated rats at later periods of time should also be lower. Such is not the case. As soon as 6 hours after ad-

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TABLE I. Adrenal Ascorbic Acid (mg/100 g Tissue) after Graded Doses of ACTH.

Group	Hr after treatment	Saline	ACTH 5 mu dose	ACTH 15 mu dose
Intact	1	333 ± 10† (15)	273‡ ± 11† (21)	270‡ ± 11† (18)
Thyroidectomized	1	376 ± 21 (23)	239‡ ± 13 (26)	201‡§ ± 17 (25)
Intact	6	394 ± 9 (11)	383 ± 10 (13)	386 ± 8 (14)
Thyroidectomized	6	375 ± 9 (14)	406 ± 16 (14)	401 ± 19 (14)
Intact	12	425 ± 7 (16)	397 ± 8 (17)	392‡ ± 7 (17)
Thyroidectomized	12	399 ± 16 (14)	390 ± 15 (14)	417 ± 18 (14)
Intact	24	463 ± 6 (10)	486 ± 7 (12)	465 ± 11 (10)
Thyroidectomized	24	465 ± 17 (11)	498 ± 18 (15)	457 ± 21 (15)

† Stand. error.
‡ Difference from saline-inj. controls statistically significant.
§ " " similarly treated intact rats statistically significant.
Numbers in parentheses are No. of animals in each group.

ministration of ACTH, thyroidectomized rats show the same degree of recovery of adrenal ascorbic acid as intact animals. Likewise, no differences exist at 12 and 24 hours after treatment either. On a formal basis, one can interpret the data to indicate that 6 hours may be a long enough period for the slower process of recovery in the thyroidectomized rats to "catch-up" with that of intact rats. However, this possibility would seem to be less likely.

During the periods of recovery from ACTH injections, all of the operated and most of the intact rats had adrenal ascorbic acid values which did not differ significantly from saline-injected controls. Only one group of intact rats, 12 hours after treatment with the higher dose of ACTH, revealed adrenal ascorbic acid values significantly different from its saline control group. The adrenal ascorbic acid value of this group did not differ from its control by a large margin; the difference, though small, was just statistically significant. However, in no instance during the recovery periods did the adrenal ascorbic acid values of operated and intact rats differ.

The relative weights of the adrenal glands of thyroidectomized and intact rats reveal no significant differences, with the exception of one group of intact animals. The intact rats receiving 15 milliunits of ACTH and autopsied 6 hours later displayed larger adrenal glands than those of thyroidectomized animals sacrificed at the same time. Since the same situation does not persist in the groups of animals autopsied at later periods of time, one can assume that the higher dose of ACTH was no more effective in producing adrenal enlargement in intact than in operated rats at the dose levels and periods of time employed. No explanation on a physiological basis can be offered for the apparent adrenal enlargement in that one group of intact rats.

For the most part, the relative thymus weights of all groups of thyroidectomized animals are less than those of intact rats. In several groups, the differences are statistically significant. Referring to the thymolytic action of adrenal cortical hormones(3,4), the data suggest more active secretion on the part of the adrenals of operated than of those of intact rats.

The lower ascorbic acid values in stressed-thyroidectomized rats are not due to smaller nor inactive adrenal glands, are not due to a difference in amounts of ACTH released from the pituitary gland, and are not due to any failure on the part of the adrenal of the operated rat to rebuild the vitamin. One only can conclude that there is some inherent difference in the response of the adrenal of the thyroidectomized rat to a given stress, as compared to the intact animal, but the basis for this difference has not been determined.

Summary. 1) To determine whether the lower adrenal ascorbic acid value after stress in the thyroidectomized rat is due to failure of the hypothyroid animal to rebuild the vitamin, rats were thyroidectomized, injected with ACTH, and sacrificed at various periods of time following treatment. The adrenal ascorbic acid level was followed during the recov-

ery periods. 2) One hour after injection, operated rats showed a greater depletion of adrenal vitamin than intact animals. If the lower values in operated-stressed rats are due to failure to rebuild ascorbic acid, then analyses of adrenals of operated animals at later periods of time should also be lower. Such is not the case. Six hours after treatment, thyroidectomized rats show the same degree of recovery of adrenal ascorbic acid as intact animals. The basis for the difference in response of the thyroidectomized rat to a given stress has not been determined.

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Use of Copper-Amino Acid Complexing for Determining Amino Acid Esterase Activity.* (22565)

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Spies and Chambers(1) described an adaptation of a method for the quantitative determination of amino acids which is based on the formation of a colored copper-amino acid complex. The method is limited to the determination of N-unsubstituted amino acids which form soluble complexes. Since copper does not form a complex with amino acid esters, the method lends itself to the determination of an amino acid liberated as a result of ester hydrolysis. The present paper describes an application of the copper method to the determination of lysine resulting from the enzymatic hydrolysis of lysine ethyl ester. In our laboratory, this procedure was found to be simple and easily adaptable to routine enzymatic analysis.

Materials. Copper phosphate suspension is prepared by the addition of 0.21M cupric chloride to 1600 ml of 0.18M trisodium phosphate with stirring. After centrifugation for 5 minutes at 2000-3000 rpm, the precipitate is washed by resuspension in 2000 ml of 0.1M borate buffer, pH 7.2. The precipitate is collected, again washed and then suspended in 2000 ml of the buffer. Two hundred and forty grams of reagent grade sodium chloride are added and the suspension is adjusted to pH 7.2 with 5N sodium hydroxide. The preparation is similar to that used by Schroeder *et al.*(2). L-Lysine ethyl ester dihydrochloride (LEE) is prepared as described by Werbin and Palm(3) and L-lysine monohydrochloride was purchased from Nutritional Biochemicals. Both were stored in a desiccator over phosphorous pentoxide. Twice recrystallized trypsin (Worthington) was used as the LEE esterase.

Method. Two ml of copper phosphate suspension are added to 2 ml of the test solution contained in a 15 ml conical centrifuge tube. The tube is shaken intermittently by hand for 5 minutes and excess copper phosphate removed by centrifugation for 2 minutes at 2000 rpm. The blue colored supernatant fluid is decanted into a Klett microtube and read in the Klett Summerson Photoelectric Colorimeter using a #66 (Red) filter.

Lysine standard curve. Since many of our hydrolysis experiments were carried out between pH 6.2 and 7.3, we investigated the effect of pH on the color intensity of the copper-lysine complex. Lysine standard curves

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