the cellular elements of the blood, and preserves them longer than sodium citrate and the oxalates. For routine blood examination, a 10% aqueous solution (maximum solubility) is preferable; 0.005-0.01 cc will keep 5 cc of blood from coagulating.

If blood is kept at 5°C, the red cell and leucocyte counts do not show any significant changes for from 3-5 days. If blood is collected in paraffinized containers, the platelet count remains unchanged for 24 hours; after this time a slight decrease is noted (6%). After the initial drop, there is no appreciable change in the count for about 4 days, as compared with a decrease of about 50% in sodium citrate. Hemoglobin, bilirubin and sedimentation speed remain unchanged for several days, if the blood is kept at 5°C and not shaken excessively. The stainability for the cellular elements remains unchanged for a week or longer.

The agglutinogens of the red cells and the oxydase reaction of granulocytes are not destroyed by Sequestrene NA 2. The red cells are strongly agglutinated by their specific antisera, and the oxydase reaction remains positive after blood has been preserved for 3 weeks at 4-5°C.

When given *per os,* Sequestrene NA 2 is practically atoxic for mammals. Rats fed maximum doses did not show any toxic symptoms except diarrhea. Ninety-six per cent of the administered Sequestrene was excreted unchanged in the feces of rats fed massive single doses. The coagulation time was not altered significantly, and no pathological changes in the cellular elements of the blood were noted.

Dyckerhoff found that rabbits tolerated parenteral injection of as much as 80 mg of ethylenediamine tetra-acetic acid per kg, without toxic symptoms. When 100 mg per kilo were given, the animals developed tetany, but recovered rapidly upon administration of calcium ion. Rabbits tolerated intravenous injections of 40-80 mg per kg daily for 10 days. When given intravenously, Martin(2) found 50 mg toxic for dogs. The animals recovered when injected with 100 mg of calcium gluconate. Sequestrene was well tolerated when injected simultaneously with 100 mg of calcium gluconate.

Preliminary experiments for substituting di-sodium Sequestrene for sodium citrate in preserving human blood, have shown that it preserves blood longer if kept at 4-5°C. 1.5 g di-sodium Sequestrene dissolved in 100 cc of 5% glucose, will keep 400-500 cc of blood from coagulating. The di-sodium salt does not caramelize when sterilized with glucose; the sterilized solution has a pH around 5. Twenty human transfusions with blood preserved in the above solution, did not show any ill effects. No tetany, or changes in the coagulation time were noted. Further transfusion experiments are in progress and will be reported later.

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Mechanism of Adrenal Involution in the Rat After Treatment with Thiouracil. (18578)

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Previous work has shown that prolonged treatment with thiouracil will produce atrophy and damage of the adrenal cortex(1-3). In the rat, this decrease in the weight of the

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TABLE I. Effect of ACTH (L-41-L) on Weight and Ascorbic Acid of Adrenal Gland.

No. of rats	Body wt, g	ACTH, mg	Adrenal gland: Wt, mg	Adrenal gland: Adrenal wt / Body wt, g × 100	Adrenal gland: mg % ascorbic acid	% increase in: Adrenal wt	% increase in: Adrenal wt / Body wt × 100
				Thiouracil			
5	150	—	25.4 ±1.3*	16.9 ±1.6	427	—	—
5	167	6	36.7 ±1.7	21.9 ±0.8	453	44.0	29.5
				Control			
5	189	—	45.1 ±3.1	23.8 ±2.1	450	—	—
5	200	6	63.0 ±4.8	31.5 ±1.8	496	40.1	33.3

* Standard error.

adrenal gland is obvious by the 6th week of treatment with a 0.1% solution of thiouracil in the drinking water(4). If the treatment is continued for a year or more, hemorrhage and necrosis may be observed in the adrenals of such animals(5-7). It is apparent that these changes could result either from a direct action of thiouracil on the adrenal gland or be mediated via the hypophysis cerebri. It has been suggested also that thiouracil affects the adrenal gland indirectly through suppression of thyroid hormone production. The lack of thyroxine could conceivably lead to a myxedematous adrenal(8). The present investigation was undertaken to determine whether the atrophic changes in the adrenal cortex were due in part to a failure in the secretion of ACTH by the pituitary or to a failure of the adrenal gland to respond to ACTH. This was tested by determining the response of the atrophic, adrenal gland of the thiouracil-treated rat to exogenous ACTH.

Material and methods. Fifty-seven male rats of the Harvard-Wistar strain were separated into 4 experimental groups. Two groups of rats were given 0.1% thiouracil in their drinking water starting at 21 days of age and maintained on this regime throughout the entire experiment. The remaining two groups were kept as controls. At 105 days of age, *i.e.* after 12 weeks of treatment with the antithyroidal drug, one group of thiouracil-treated rats and one group of control rats were injected twice daily for 6 days with ACTH.‡ All animals were killed by exsanguination 24 hours after the last injection of ACTH. The adrenal, thyroid and pituitary glands were dissected out and weighed to the nearest tenth of a milligram on a torsion balance. In some of the animals, both the adrenals and pituitaries were analyzed for ascorbic acid by the method of Roe and Keuther(9).

Results. In the first experiment, the rats received a total dose of 6 mg of ACTH in 6 days and were killed 24 hours after the final injection (Table I). The adrenal glands of the control rats weighed 45.1 mg as compared with 63.0 mg for the rats treated only with ACTH, an increase of 40.1%. The animals receiving only thiouracil had adrenals weighing 25.4 mg while the thiouracil-treated rats that were also injected with ACTH possessed adrenals weighing 36.7 mg, an

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TABLE II. Effect of ACTH [58-0(50)] on Adrenal Gland of Thiouracil Treated Rat.

No. of rats	Body wt, g	ACTH, mg	Adrenal gland: Wt, mg	Adrenal gland: Adrenal wt / Body wt, g × 100	Adrenal gland: % increase in Adrenal wt	Adrenal gland: % increase in Adrenal wt / Body wt × 100
				Thiouracil		
10	202	—	36.6 ±0.81*	18.5 ±0.67	—	—
5	165	12	46.6 ±3.8	28.2 ±2.3	27.3	48.4
3	182	24	50.3 ±1.6	27.6 ±1.6	30.6	52.4
				Control		
11	223	—	55.4 ±3.9	24.6 ±0.71	—	—
5	194	12	64.6 ±2.6	32.7 ±1.2	17.3	33.7
3	209	24	75.1 ±8.1	35.9 ±4.5	35.0	44.7

* Standard error.

increase of 44%. In spite of the disparity in body weight of the rats due to the depressing effect of thiouracil, comparison of the adrenal weights expressed as mg of adrenal per 100 g of rat gave results comparable to those noted above. The injection of ACTH increased the adrenal weights of the control rats from 23.8 for the uninjected to 31.5 mg % body weight for the injected rats, an increase of 33.3%. The adrenal glands of the thiouracil-treated rats weighed 16.9 mg % body weight while the adrenals of the thiouracil-treated rats injected with ACTH weighed 21.9 mg % body weight, an increase of 29.5%. The ascorbic acid content of the adrenals was 450 mg % for the control rats and 496 mg % for the control rats injected with ACTH. An ascorbic acid content of 427 mg % was found in the adrenal glands of the thiouracil-treated rats and 453 mg % in the adrenal glands of the thiouracil-treated rats injected with ACTH. As could be expected, hyperplasia of the thyroid was observed in the rats receiving thiouracil or thiouracil and ACTH. The thyroids of the normal rats weighed 17.3 mg and after thiouracil treatment 74.6 mg. After injection of ACTH, the normal rats showed an average thyroid weight of 16.7 mg and the thiouracil-treated rats an average thyroid weight of 51.9 mg.

The above experiment was repeated with a second batch of ACTH (Lot No. 58-9[50]) and comparable results were obtained (Table II). The normal, control rats had adrenal glands weighing 55.4 mg, while rats injected with 2 and 4 mg of ACTH had adrenal weights of 64.6 and 75.1 mg respectively. The adrenals of the thiouracil-treated rats weighed 36.6 mg and after injection of 2 and 4 mg of ACTH an increase in adrenal weight to 46.6 and 50.3 mg respectively was obtained.

Discussion. Atrophy of the adrenal cortex after thiouracil treatment and the inability to obtain hypertrophy of the gland following exposure to cold led Zarrow and Money(4) to suggest that the failure was due either to a decreased ACTH production by the pituitary gland or decreased sensitivity of the adrenal gland to ACTH. The second hypothesis was examined in the present investigation and found to be untenable. A comparison has been made of the sensitivity of the adrenal gland of the normal and thiouracil-treated rat to ACTH. The per cent increase in the weight of the adrenal gland was approximately the same for both the normal and thiouracil-treated rats after treatment with ACTH. Thus it would appear that the atrophic, adrenal gland of the thiouracil-treated rat is not only capable of responding to ACTH by an increase in size but has approximately the same degree of sensitivity as the adrenal gland of a normal rat. This would indicate

a failure on the part of the pituitary gland to secrete the trophic hormone.

A possible explanation for the failure of the hypophysis to produce ACTH after treatment with thiouracil may reside in the limited numbers and types of cells found in the gland of such animals. It is conceivable that when the pituitary is forced to secrete excessive amounts of TSH, as occurs after thiouracil treatment, it can only do so by a decrease in its production of ACTH. Such an explanation has been offered for the decreased release of TSH and gonadotrophins and increased production of ACTH after stress(10,11). Thus the atrophy of the adrenal gland or the failure to obtain hyperplasia after exposure to cold in thiouracil-treated rats would indicate functional damage to the pituitary part of the pituitary-adrenal axis. It is not to be denied, however, that a decreased amount of ACTH is still being secreted in view of the partial maintenance of the ascorbic acid of the adrenal gland and the depletion of the adrenal-ascorbic acid on exposure to cold(12). This would indicate that greater amounts of ACTH are needed for adrenal hyperplasia than for the adrenal-ascorbic acid depletion. The fact that the pituitary of normal animals can secrete increased amounts of both TSH and ACTH (as seen after exposure to cold) does not invalidate the above hypothesis as the situations are not comparable. In the thiouracil-treated rat the stimulus is such as to bring about an increased secretion of only one of the factors (TSH) resulting in an early appearance of ACTH deficiency. In cold stress the initially increased TSH output eventually decreases as the animal remains under the influence of the stress and as the pituitary secretes greater amounts of ACTH.

Conclusion. 1. Thiouracil produces an involution of the adrenal cortex. 2. The atrophic adrenal gland of the thiouracil-treated rat retains its sensitivity to ACTH. 3. It is suggested that the involution of the adrenal gland of the thiouracil rat is caused by a decreased ACTH secretion by the pituitary gland. This decreased ACTH level may result from the excessive TSH production in the pituitary caused by the thiouracil treatment.

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Response of Plasma to Excess of Thromboplastin as a Measure of Prothrombin Activity.* (18579)

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The one-stage method of prothrombin determination proposed by Howell(1) and the two-stage procedure employed by Bordet(2) were used for much important work on prothrombin, but the results obtained with those methods were not regarded as quantitative, since large amounts of prothrombin remained unconverted to thrombin and therefore escaped measurement. Smith and coworkers (3) introduced the two principles which subsequently proved to overcome some of the shortcomings of these two methods. They

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