TABLE I. Results of Wounds in Groups 1, 2 and 3.

Normal	14
Cortisone inhibited	35
Reversal of cortisone inhibition	32
Failure of complete cortisone inhibition	3
Failure of complete reversal of cortisone inhibition	3
Total	87

induce wound healing(12). Cortisone would then appear to function as an inhibitor of wound healing by virtue of its ability to modify inflammation(1,2).

Summary. 1. Local applications of tissue culture extracts will completely reverse cortisone inhibition of wound healing. 2. The granulation tissues of these wounds are histochemically similar.

1. Howes, E. L., *Surgery,* 1950, v28, 177.
2. Spain, D. M., and Malomut, Norman, *Science,* 1950, v112, 335.
3. Carrel, A., and Burrows, M. T., *J. Exp. Med.,* 1911, v13, 387.
4. Pearse, A. G. E., *Histochemistry, Theoretical and Applied,* Little Brown Co., Boston, 1953, 550.
5. Nachlas, M. M., and Seligman, A. M., *J. Biol. Chem.,* 1949, v181, 353.
6. Asbbell, R., and Seligman, A. M., *Endocrinology,* 1949, v44, 565.
7. Barnett, R. J., and Seligman, A. M., *Science,* 1952, v116, 323.
8. Fischer, A., *Nature,* 1930, v144, 113.
9. ———, *Chem. Products,* 1940, v3, 79.
10. ———, *Acta Physical Scand.,* 1942, v4, 207.
11. Davidson, J. N., and Waynouth, C., *Nutrition Abst. and Rev.,* 1944, v14, 1.
12. Carrel, A., and Ebeling, A. H., *J. Exp. Med.,* 1922, v36, 385.

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Adrenocortical Function in Hypo- and Hyperthyroidism.* (21195)

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Adrenal cortical hypertrophy following thyroid administration has been demonstrated many times since the work of Hoskins on the guinea pig(1). Its dependence on the presence of the pituitary was shown by Smith, Greenwood and Foster(2) in the course of metabolic studies with small doses of thyroxine in rats. Conversely, the cortical atrophy following thyroidectomy has frequently been noted (*e.g.* Evans, Simpson, and Pencharz (3)). The morphological picture of cortical atrophy resulting from thyroidectomy, and hypertrophy from treatment with thyroid hormone in the rat, has recently been illustrated by histological and cytochemical methods by Deane and Greep(4) and by Feldman(5). The question of the functional response of the adrenal cortex in hypothyroidism has been raised by Hill *et al.*(6) on the basis of impaired eosinopenic response to the ACTH test (Thorn *et al.*(7)) in certain hypothyroid patients and its improvement following thyroid hormone therapy in some of these patients. The ACTH test was adapted to use in experimental animals (dogs and rats) by Recant *et al.*(8). It offered a promising approach to the study of adrenocortical function in thyroid deficient and hyperthyroid rats. The work of Gabrilove and Soffer(9) on the ascorbic acid depletion response of propylthiouracil treated rats suggested another approach.

The plan followed in the present work was to study: A) the eosinopenic response to various stimuli such as ACTH or epinephrine, and B) the ascorbic acid depletion response to ACTH, in hypothyroid rats before and after thyroxine therapy, as compared with normal rats, and with normals rendered hyperthyroid by thyroxine administration.

Procedure. Male rats of the Long-Evans strain were surgically thyroidectomized at 21 days of age. After a prolonged interval, those which had shown a marked retardation in growth, and various external signs of thyroid deficiency (such as thinning of hair and accumulation of subcutaneous fat) were further screened for completeness of thyroidectomy

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by determination of metabolic rate. (This was done by means of a simple apparatus for measuring oxygen consumption in terms of the volume of water replacing it dropwise in a closed system. Basal conditions were approximated as closely as possible by training the animals in the apparatus, and by the use of a 20 to 24-hour fasting period before the determination.) Control standards for metabolic rate were developed on groups of intact males of the same age. These had an average fasting body weight of 450 g and a metabolic rate of 41.4 ± 1.57 Cal/m²/hr. The thyroidectomized rats selected for use in the experiments to follow averaged 196 g in body weight and had metabolic rates averaging 27.5 ± 0.69 Cal/m²/hr or 36% below normal. The average rate was raised approximately to the normal value (41.1 ± 1.34 Cal/m²/hr) in 14 days, by a daily dose of 0.005 mg thyroxine (Squibb). A group of normal rats was given a daily dose of 0.100 mg thyroxine for 10 days, resulting in an average metabolic rate of 51.3 ± 1.58 Cal/m²/hr, or 19% above control values, with definite signs of hyperthyroidism. *Eosinophils* were counted directly by the method of Dunger(10), as modified by Recant *et al.*(8). Materials used to evoke the eosinopenic response were ACTH and epinephrine. The ACTH preparation used (Armour, Lot 71-2 (50) c) was potent in the adrenal weight maintenance test at a daily dose of 1.0 mg for 15 days, in hypophysectomized 40-day-old male rats. The minimum dose for adrenal ascorbic acid depletion was 0.5 μg intravenously. The epinephrine used was Adrenaline chloride (Parke, Davis and Co.) 1:1000 in aqueous solution; a fresh ampoule was opened for each experiment. The *adrenal ascorbic acid* depletion tests were carried out after the method of Sayers *et al.*(11). The hypothyroid rats used were some of those previously used in eosinophil tests, at which time they had received thyroxine therapy. A sufficient interval had elapsed before the ascorbic acid test, so that all the effects of therapy had been lost, and the rats had regressed to the hypothyroid state. As a terminal check on completeness of thyroidectomy, the radioactive iodine uptake of the neck tissues was measured in a scintillation counter. Most of the thyroidectomized rats had iodine uptakes less than 1% of the normal uptake. Six of the 19 reported had uptakes 5 to 9% of normal, but responded in no way differently from the remainder of the group, justifying their inclusion as thyroid deficient.

Results. A. The Eosinophil test. Before the effects of ACTH or of epinephrine could be measured, it was found necessary to minimize the effect of handling and withdrawal of blood from the tail tip (an effect amounting to a 30 to 50% decrease in circulating eosinophils). This was accomplished by use of a mild sedation with nembutal (Abbot, veterinary) given initially at a dose of 3 mg/100 g body weight, intraperitoneally, 2 hours before the taking of the first blood sample. Stabilization was dependent upon the *renewal* of sedation by small supplementary doses at hourly or half-hourly intervals, as required (after the method of Recant *et al.*(8), as described by P. H. Forsham in a personal communication). Otherwise, the administration of a single dose of nembutal lowered the eosinophil count, as reported by a number of workers (Sawyer and Parkerson(12); Dumm and Ralli(13)). The eosinophil count was stabilized in this way, so that not more than 5 to 15% of the decrease was attributable to the sampling technic, in all rats being tested with ACTH or epinephrine.

The results of intraperitoneal injection of 3 mg of ACTH are shown in Fig. 1. The

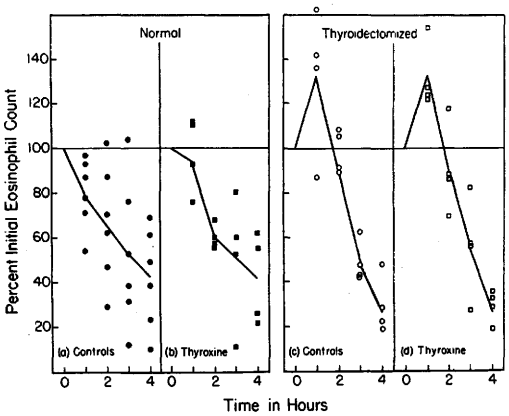

FIG. 1. Effect of ACTH on circulating eosinophils of normal and thyroidectomized male rats, with and without thyroxine treatment. (Nembutal sedation.)

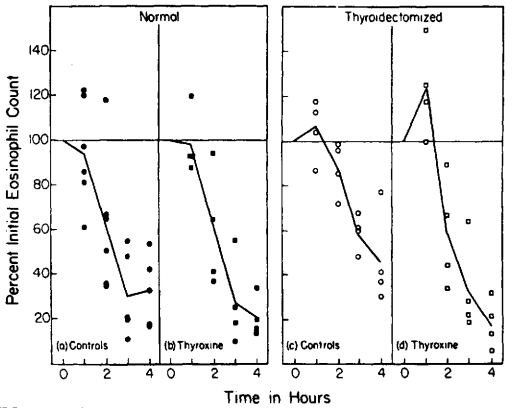

FIG. 2. Effect of epinephrine on circulating eosinophils of normal and thyroidectomized male rats, with and without thyroxine treatment. (Nembutal sedation.)

eosinophil counts of the normal rats showed an average fall of 58% (±8.02) in 4 hours, and a comparable fall, 62% (±8.9) was obtained in a normal group receiving thyroxine. The thyroidectomized rats responded with a decrease of 73% (±4.18) before thyroxine therapy and 72% (±3.48) afterwards. There was, then, no impairment of the ability of the adrenal cortex of the thyroidectomized rats to respond to exogenous ACTH, as measured by this test.

When 0.1 mg epinephrine was used as the stimulus, normal rats responded with a decrease of 73% (±6.22) in eosinophil count (Fig. 2). This response was unaffected by thyroxine treatment (82% ±3.18). The thyroidectomized rats showed some impairment in this response, the average decrease being 56% (±8.58). Thyroxine therapy had a definitely beneficial effect on the response to epinephrine, resulting in an average decrease of 85% (±3.34). This improvement was even more clearly seen when the responses of individual rats, before and after thyroxine treatment, were compared as in Fig. 3. The difference between the responses of thyroidectomized and normal rats to epinephrine was not statistically significant, due to the great individual variation. However, the difference between the thyroxine-treated and untreated thyroidectomized rats was highly significant, and the response of the thyroxine-treated group was equivalent to normal.

B. *Ascorbic acid depletion test.* For the adrenal ascorbic acid depletion test, a dose of 5 μg of the adrenocorticotrophic preparation was chosen, as this was in the significant region of the standardization curve. The assay was carried out in the routine manner, 24 hours after hypophysectomy in 7 "standard" animals (40 days old), in 11 of the thyroidectomized rats and 6 normal controls of the same age, and in 3 hyperthyroid rats.

Table I shows that the decrease in ascorbic acid in the thyroidectomized rats was greater and more uniform than in the normal rats of the same age (132 ± 5.41 mg/100 g adrenal as compared to 83 ± 14.1 mg). Values for the 3 hyperthyroid rats which survived the procedure fell within the normal range. It may also be seen in Table I that the response of the thyroidectomized rats was greater, and that of their normal age controls slightly less, than that of the younger "standard animal".

Discussion. So far as the eosinophil test was concerned, the thyroidectomized rats showed no impairment of adrenocortical response to exogenous ACTH. Impairment of the response to epinephrine would seem to place the site of weakness in the pituitary-adrenocortical system at the pituitary rather than the adrenal level. Evidence for lessened pituitary content of ACTH in thyroidectomized rats has been presented by Halmi and Bogdanove(14). Specificity of the eosinopenic response to epinephrine as an indicator of adrenocortical function has been questioned (15), and thus the significance of the impaired

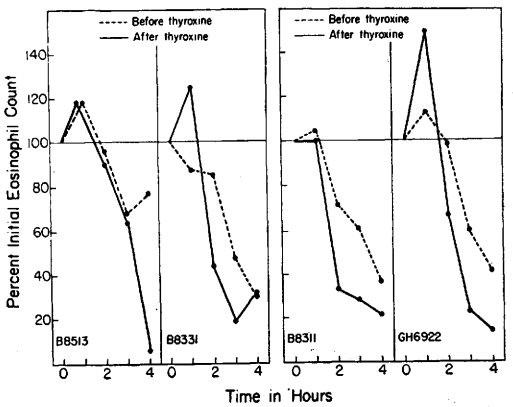

FIG. 3. Individual responses of circulating eosinophils of thyroidectomized male rats to epinephrine, before and after thyroxine treatment. (Nembutal sedation.)

TABLE I. Effect of Thyroid Status on Adrenal Ascorbic Acid Depletion Response to a Standard Dose of ACTH (5 μg per 100 g BW, Injected IV).

Type of rat*	No. of rats	BMR, Cal/m²/hr	Body wt, g	Adrenal wt, mg		Adrenal ascorbic acid, mg/100 g adrenal		
				Left	Right	Left	Right	Depletion
Thyroidectomized†	11	26.6	279	13.5	13.5	276	146	132 ± 5.41
Normal controls†	6	37.7	439	22.3	20.3	298	215	83 ± 14.1
Normal + thyroxine†	3	53.7	454	23.4	20.9	309	224	86 ± 15.2
Normal 40-day	7	—	119	10.6	10.3	448	348	100 ± 13.7

* All rats hypophysectomized 24 hr before the test.
† Approximately one year old at time of this test.

response to epinephrine, and its improvement by thyroxine, is not entirely clear.

The adrenal ascorbic acid depletion test indicated an enhanced adrenocortical responsiveness in the hypothyroid state. Finerty and Hess(16) have found an increased ascorbic acid response to the stress of scalding, in thyroidectomized rats. However, caution should be used in the interpretation of all such results, since, as has been pointed out by Freedman and Gordon(17), the same or a slightly greater response *per unit weight of gland* (as the ascorbic acid depletion is reported) may still mean a greatly reduced daily hormonal output from a small as compared with a large adrenal.

Summary. 1. As a means of assessing adrenocortical function, the eosinopenic and adrenal ascorbic acid depletion responses of normal and thyroidectomized male rats, with and without thyroxine treatment, have been examined. 2. With nembutal sedation, the eosinopenic response to ACTH was the same in thyroidectomized as in normal rats and was not influenced by treatment with thyroxine in either group. 3. Under these conditions the eosinopenic response to epinephrine was less in thyroidectomized than in normal rats and was distinctly improved after thyroxine therapy. The response of normal rats to epinephrine was not modified by the administration of thyroxine in doses leading to hyperthyroidism. 4. In the second test used, adrenal ascorbic acid depletion in response to ACTH, the thyroidectomized rats showed a greater and more uniform depletion than did normal rats, with the same dose of ACTH. Hyperthyroid rats responded like the normals.

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1. Hoskins, R. G., *J. Am. Med. Assn.,* 1910, v55, 1724.
2. Smith, P. E., Greenwood, G. F., and Foster, G. L., *Am. J. Path.,* 1927, v3, 669.
3. Evans, H. M., Simpson, M. E., and Pencharz, R. I., *Endocrinology,* 1939, v25, 175.
4. Deane, H. W., and Greep, R. O., *ibid.,* 1947, v41, 243.
5. Feldman, J. D., *Anat. Rec.,* 1951, v109, 41.
6. Hill, S. R., Reiss, R. S., Forsham, P. H., and Thorn, G. W., *J. Clin. Endocrinol.,* 1950, v10, 1375.
7. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., *J. Am. Med. Assn.,* 1948, v137, 1005.
8. Recant, L., Hume, D. M., Forsham, P. H., and Thorn, G. W., *J. Clin. Endocrinol.,* 1950, v10, 187.
9. Gabrilove, J. L., and Soffer, L. J., *Endocrinology,* 1950, v47, 461.
10. Dunger, R., *Muench. med. Wochschr.,* 1910, v57, 1942.
11. Sayers, M. A., Sayers, G., and Woodbury, L. A., *Endocrinology,* 1948, v42, 379.
12. Sawyer, C. H., and Parkerson, G. R., Jr., *ibid.,* 1952, v52, 346.
13. Dumm, M. E., and Ralli, E. P., *ibid.,* 1954, v54, 71.
14. Halmi, N. S., and Bogdanove, E. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 518.
15. Thorn, G. W., *Am. J. Med.,* 1953, v14, 139.
16. Finerty, J. C., and Hess, M., *Am. J. Physiol.,* 1951, v167, 783.
17. Freedman, H. H., and Gordon, A. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 729.

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