was determined by this technic. (3) The advantages of this technic over the dye method have been discussed.

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Effect of Thyroidectomy on ACTH Content of Rat Adenohypophysis.* (18833)

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The site of formation of ACTH within the adenohypophysis has not yet been conclusively established. Finerty and co-workers (1,2), who observed a transient rise in the number of acidophils after hemiadrenalectomy in the rat, regard this class of cells as the source of ACTH. On the other hand, one of the rat's 2 types of pituitary basophils, the beta cell, has been suggested as the possible producer of the hormone(3). In these studies conclusions were reached by comparing pituitary cell counts with the state of the adrenal gland. It should be recognized, however, that the count of a given pituitary cell type, if due allowance is made for the size and density of granulation of the individual cells, reflects the storage of its secretory material, whereas the condition of the target organ is an index of the level of its governing pituitary principle in the circulation. As storage and discharge of pituitary trophins do not always run parallel, the comparison between the histological structure and the stored amounts of hormones in the pituitary appears to be a more promising approach to the problems of its histophysiology. Since thyroidectomy is known to lead to an extremely conspicuous drop in the number of both the acidophils and the beta cells, while the delta cells become progressively hyperplastic and vacuolated(3), it was of interest to determine how these profound histological changes affect the ACTH stores of the pituitary.

Method. A shipment of 22 young adult male Sprague-Dawley rats (180-200 g) was divided into 2 groups. Nine animals were thyroidectomized, 13 served as controls. The rats received Purina Laboratory Chow and tap water *ad lib.* Thirty days later the members of both groups were sacrificed by decapitation. At this time the thyroidectomized rats weighed 228-256 g (mean 248 g), the intact animals 288-337 g (mean 322 g). The pituitaries of 3 thyroidectomized and 4 control rats were saved for histological examination. The hypophyses of the remaining animals were prepared for a comparative assay of their ACTH content by the ascorbic acid depletion method of Sayers *et al.*(4). In view of the unstableness of ACTH in alkaline solutions(5), lyophilization and subsequent extraction of the glands with saline made alkaline to 0.01 N with sodium hydroxide(6) was abandoned in favor of a technic devised by Sayers(7). The anterior lobes were pooled in a solution of 0.85% sodium chloride made to 10th molar with hydrochloric acid (one pituitary per ml fluid), homogenized and extracted at room temperature. The extracts were transferred to a boiling water bath for 60 minutes,

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TABLE I. Pituitaries of Thyroidectomized Rats Assayed for ACTH Content Against Those of Controls.

Dose†	Thyroidectomized donors (6)* No. recipients	Thyroidectomized donors (6)* Response	Control donors (9)* No. recipients	Control donors (9)* Response
1.25	4	29.2‡ ± 2.7§	5	30.1 ± 1.2
.625	5	17.3 ± 4.1	5	17 ± 1.5
.3125	4	6.4 ± 2.1	4	7.9 ± 1.6
Total	13		14	
Slope of log dose–response curve		39.0		37.1

Significance of diff. between slopes $p = 0.9$

$M = \bar{1}.9727$ $S_M = 0.0548$

ACTH content of thyroidectomy-pituitaries with that of controls at 100% = 94%
Range of potency at 95% level of confidence = 73-118%

* No. of donor rats.
† % donor pituitary per 100 g recipient.
‡ Mean of differences between ascorbic acid concentration in left and right adrenal of individual recipients, expressed as % of concentration in left adrenal.
§ Std error.

centrifuged and the decanted supernatants stored in a deep freeze unit. On the morning of the assay appropriate saline dilutions of the stock solutions were prepared and their pH adjusted to 1.2-2. The extract of the thyroidectomy-pituitaries was assayed against that of the control hypophyses in strict adherence to the design outlined by Burns *et al.*(6). Hypophysectomized male rats (120-160 g) were purchased from the Hormone Assay Laboratories and used 24 ± 3 hours postoperatively. Adrenal ascorbic acid determinations were performed by the method of Roe and Kuether(8). The administration of 0.5 ml per 100 g recipient of a 1:40, 1:80 and 1:60 dilution of the stock solutions was found to be the dose range for which a rectilinear relationship could be established between the log dose and the adrenal ascorbic acid drop. In other words, the hypophysectomized rats received doses equivalent to 1.25, 0.625 and 0.3125% of a donor pituitary, respectively, per 100 g body weight. The analysis of the assay was carried out according to the method of Irwin, as presented by Pugsley(9). Sections from the pituitaries destined for histological examination were stained with aldehyde-fuchsin, hematoxylin, orange and light green. Cell counts were performed in the usual manner.

Results. The assay data are summarized in Table I. It is evident that there was no significant difference between the amounts of ACTH stored in the pituitaries of intact male rats and of animals which had been thyroidectomized for 30 days. As Table II shows, the thyroidectomy-pituitaries were around 25% heavier than those of the controls, which makes the concentration of ACTH in the former about 70% of that found in the control pituitaries.

The regression of the acidophils and the beta cells in the histologically examined hypophyses of the thyroidectomized rats (Table II) is of the same order of magnitude as that previously observed to occur after complete removal of the thyroid, the acidophils being practically absent and the beta cell count around 8% of the control value. The question arises whether or not there is any cogent evidence that the acidophils and the beta cells behaved in a similar fashion in the assayed pituitaries. It has been pointed out by Griesbach and Purves(10) that the degranulation of the acidophils after complete thyroidectomy can be assessed by gross inspection of the pituitary, which becomes distinctly transparent in this condition. All the hypophyses of thyroidectomized rats used in the present

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10. Griesbach, W. E., and Purves, H. D., *Brit. J. Exp. Path.*, 1943, v24, 174.

TABLE II. Pituitary Weights and Cell Counts.

	No. rats	Wt, mg	Acidophil	Beta	Delta	Vacuolated delta
			%			
Controls	4	7.5	44.48	2.16	8.65	.25
Thyroidectomized	3	9.3	.00	.17	37.90	7.43

assay presented this macroscopic alteration to a very pronounced degree and it can be safely concluded that they must have been largely devoid of acidophilic granulation. As to the beta cell count, it has been previously reported(3), and amply confirmed on additional material, that the regression of this cell type is a more sensitive sign of thyroid hormone deficiency than the degranulation of the acidophils. Thus we have no reason to doubt that the ratio between the beta cell counts of the control and the thyroidectomy-pituitaries in the assay material was similar to that seen in the glands subjected to microscopic examination. The same holds true for the hyperplasia of the delta cells.

Discussion. Since the pituitaries of thyroidectomized rats, which contain negligible amounts of beta- and acidophilic granular material, were found to have an ACTH content almost as high as that of normal glands, it has to be concluded that neither the beta nor the acidophilic granule can be the cytological sign of ACTH storage in the rat hypophysis. The failure of the ACTH levels in the pituitary to rise concomitantly with the thyroidectomy-induced increase in the amount of delta-granular material makes a connection between this hormone and the delta granules appear unlikely, without, however, disproving it unequivocally. The fact that ACTH can not be identified with the histologically demonstrable secretory products of acidophils or beta cells does not preclude the possibility that the principle is produced by these cells and stored in the form of submicroscopic particles, but the burden of proof is on those who claim that any single one of the two cell types is the sole source of ACTH, and the evidence so far marshalled in favor of such views can not be regarded as conclusive. The role of the delta cells in the elaboration of ACTH will be the subject of further studies in this laboratory.

The results presented in this paper also indicate that the reduction in the output of ACTH in hypothyroid rats(11,12) is not primarily due to an inability of the pituitary to elaborate adequate amounts of ACTH on account of a "shift" to thyrotrophin production, as suggested by Zarrow and Zarrow(12), but rather to a disturbance in the release of ACTH. The failure of larger than normal quantities of ACTH to accumulate in the thyroidectomy-pituitary, however, indicates that the pace of ACTH production also must be slowed down to such an extent that the physiological balance of elaboration and discharge is maintained. The presence of not significantly diminished pituitary ACTH stores in hypothyroidism explains the findings that thiouracil-treated rats react to epinephrine with a drop in adrenal ascorbic acid(13) and that the stressor effect of starvation leads to marked adrenal enlargement in thyroidectomized rats(14).

Summary. 1. A comparative assay of the pituitary ACTH content of normal and thyroidectomized rats was carried out by means of the adrenal ascorbic acid depletion method in hypophysectomized rats. 2. No significant difference was found between the ACTH stores of the normal and the thyroidectomy-pituitaries. 3. Since the hypophyses of thyroidectomized rats are extremely poor in acidophilic and beta-granular material, it is concluded that neither the granulation of the acidophils nor that of the beta cells can be the morphological equivalent of ACTH storage. 4. The nature of the defective secretion of ACTH in hypothyroidism is briefly discussed.

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