as presumptive evidence that inadequate piloerection is one factor in the production of restraint hypothermia, other mechanisms cannot be excluded.

Summary. Clipping of restrained rats resulted in a much lesser additional body temperature fall than a similar treatment of nonrestrained animals. This is interpreted as supporting the hypothesis that the less erected hair of the restrained animal results in a greater rate of heat loss than the fully erected hair of the nonrestrained animal.

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Cytology and Hormone Content of Rat Pituitary Glands Following Adrenalectomy. (23037)

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The problem of the cellular source of corticotropin has been investigated most frequently in experimental animals by studying pituitary cytology after alteration of normal pituitary-adrenal gland relationship, accomplished by adrenalectomy, administration of pituitary extracts, corticotropin or adrenal cortical hormone. Because these experimental procedures influence also the secretion of hormones other than corticotropin(1-3), changes which occur in pituitary cytology should be interpreted not only in terms of the alteration produced in the secretion of corticotropin, but with respect to other pituitary hormones as well. The present investigation is an attempt to localize the site of corticotropin production by correlating cytological changes in the rat's pituitary gland following adrenalectomy with alterations in content of several of the hormones elaborated by this gland.

Materials and methods. Sprague-Dawley male rats weighing 200-250 g were bilaterally adrenalectomized and maintained on saline drinking water for 28, 56 and 100 days. Pituitary glands were investigated histologically with Masson trichrome stain, the PAS, acid hematin and Bodian protargol methods as described previously(4,5). An average of 1585 cells were counted in each gland in making differential cell counts from both trichrome- and protargol-stained sections. Pituitaries of control rats and animals adrenalectomized for 100 days were assayed for their relative hormone content. Glands used for these analyses were either homogenized in saline and assayed the following day, or they were frozen in dry ice-acetone or liquid nitrogen, lyophilized and stored in a deep freezer. In separate assays, using 22- to 26-day-old hypophysectomized female rats as recipient test animals, the relative content of gonadotropins, thyrotropin and growth hormone was estimated by comparing ovarian and uterine weight and histology, thyroid weight and I^{131} uptake, and epiphyseal plate response(6) respectively. Recipient animals received a total of 5 or 10 mg of pituitary tissue (wet weight) injected in divided doses over a 4-day period. Pituitary thyrotropin content of 6 control and 6 adrenalectomized rats was assayed also according to Levey *et al.*(7). One dosage level was employed, an amount of donor pituitary tissue equivalent to 1/50th of the gland weight, was administered intravenously to 6 test animals for each pituitary gland assayed. Corticotropin content of the pituitary was measured by the ascorbic acid depletion test(8) with 3 dosage levels, according to Halmi and Bogdanove(9), being employed in each assay.

Results. Weight and cytology of pituitary glands. Pituitary glands of 193 adrenalectomized and 184 control animals were weighed, the mean weights 28, 56 and 100 days after adrenalectomy being 8.7, 9.3 and 11.1 mg respectively. Corresponding weights of glands

TABLE I. Relative Percent of Cell Types in Pituitary Glands of Adrenalectomized Male Rats.

Group	Acidophils	β-Basophils	δ-Basophils	Chromophobes	Argyrophils*
Control	39.0 ± 1.5†	2.6 ± .3	4.2 ± .3	54.2 ± 2.6	5.3 ± .4
Adrx- 28	32.1 ± 2.1	2.6 ± .1	3.6 ± .3	61.7 ± 3.4	4.2 ± .4
- 56	38.6 ± 1.7	1.8 ± .2	3.8 ± .1	56.4 ± 2.1	3.3 ± .3
-100	40.8 ± 1.3	1.1 ± .1	4.0 ± .3	55.1 ± 4.0	2.9 ± .2

* Determined from FAA-Protargol preparations; others from Bouin-Masson preparations.
† Determinations made from 8 pituitary glands in each group. Mean ± stand. dev.

TABLE II. Relative Gonadotropic, Thyrotropic and Growth Hormone Activity of Pituitary Glands of Control and Adrenalectomized Rats.

		Recipients (22-26-day-old hypophysectomized female rats)								
		Gonadotropins			Thyrotropin			Growth hormone	
Donor	Dose,* mg donor pituitary	No.	Ovary, mg	Uterus, mg	No.	Thyroid, mg	I^{131}, % A.D.†	No.	Epiphyseal plate, μ
		22	13.3 ± 2.6‖	33.8 ± 8.1	8	3.8 ± .20	.05 ± .002	13	203 ± 11
Control	5				6	5.25 ± .22	1.72 ± .25	6	324 ± 13
	10	10	28.2 ± 4.2	126 ± 12	8	6.61 ± .61	2.83 ± .45	6	373 ± 10
Adrx-100‡	5				7	4.10 ± .37	1.21 ± .35	5	327 ± 9
	10	14	19.8 ± 2.8	96.5 ± 9.2	8	4.84 ± .40	1.73 ± .10	7	352 ± 13
	100 μg GH§							6	302 ± 9
	200 " "							8	348 ± 11

* Administered in divided portions for 4 days. † A.D. = Administered dose of I^{131}; 3 μc, 4 hr prior to autopsy. ‡ Adrx-100 = Adrenalectomized 100 days. § GH = Beef growth hormone, Armour, Lot No. 285-183. ‖ Mean ± stand. dev.

from control animals were 8.8, 9.1 and 10.1 mg. The relative percentages of cell types in pituitary glands of control and adrenalectomized rats are summarized in Table I. In Masson-stained sections, acidophils did not exhibit notable alteration in relative number or appearance following adrenalectomy. Beta basophils, identified by their staining with aldehyde fuchsin, were reduced at 100 days after adrenalectomy to approximately one-half the number present in control glands. Delta basophils, whose cytoplasmic granules are colored by the light green component of the Masson stain, were not notably altered in number. At 56 days after adrenalectomy, hypertrophy of the delta basophils was noticeable, and there was present at 100 days some degree of vacuolization of these cells. Adrenalectomy produced a slight but consistent decrease in number of PAS-positive basophils, although the staining reaction of individual cells varied considerably. Staining of acidophils by acid hematin for phospholipids was not altered from that observed in control glands. The relative number of argyrophilic cells declined progressively following adrenalectomy and at 100 days after operation was reduced to approximately one-half the number present in control glands (Table I).

Hormone content of pituitary glands. Growth hormone. Estimation of pituitary growth hormone content of control animals and animals adrenalectomized for 100 days revealed no difference between the two groups, injection of 5 or 10 mg of control or experimental pituitary glands producing similar epiphyseal plate responses (Table II). Daily injection of 100 or 200 μg of growth hormone* produced epiphyseal plate responses approximately equal to that produced by administration of these amounts of pituitary tissue respectively.

Thyrotropin. Pituitary thyrotropic hormone content, as measured by thyroid weight and radioiodine uptake in hypophysectomized test animals, was decreased 100 days after adrenalectomy (Table II). I^{131} uptake produced by glands of adrenalectomized rats was significantly less than that produced by

* Beef growth hormone, Armour, Lot No. 285-183, kindly supplied by Dr. Sanford L. Steelman.

TABLE III. Corticotropin Content in Pituitary Glands of Adrenalectomized Male Rats, Assayed by the Adrenal Ascorbic Acid Depletion Test.

Groups	Mean response at indicated dose 1.25*	.62	.31	Slope of log-dose response curve	M	Antilog of M	SM	Limits of error, P = .95
Control (6)†	30.1§ (26.0–36.2)‖	20.2 (7.5–28.2)	15.0 (8.1–24.2)	25.1	.5611	3.640	.098	2.91–4.56
Adrx-28‡ (8)	39.4 (33.8–50.4)	35.0 (22.0–38.8)	28.2 (18.4–35.3)	19.0				
Control (6)	28.7 (18.4–35.0)	18.9 (11.3–30.1)	13.3 (2.5–23.3)	25.6	.5372	3.445	.091	2.79–4.25
Adrx-56 (12)	42.4 (33.0–44.6)	32.6 (15.2–43.5)	27.1 (14.2–33.0)	25.5				
Control (6)	28.2 (20.3–32.5)	19.8 (12.0–33.1)	11.8 (3.0–20.3)	29.2	.3164	2.072	.086	1.70–2.53
Adrx-100 (9)	35.3 (29.4–41.3)	30.6 (18.6–34.4)	20.3 (12.5–30.2)	27.5				

* Dose = % of donor pituitary/100 g recipient. † No. of pooled pituitaries. ‡ Adrx = Adrenalectomized 28, 56 and 100 days. § Response = Mean of difference between ascorbic acid in left and right adrenal of recipients, expressed in % of concentration in left adrenal. ‖ Range, with 6 test animals in each instance.

control pituitaries ($P = < 0.05$ at both 5- and 10-mg dosage levels). The mean thyrotropin content of 6 control pituitary glands assayed by the method of Levey *et al.*(7) was 83 mμ/mg, while that of 6 animals adrenalectomized for 100 days was 39 mμ/mg.

Gonadotropin. Pituitary glands of adrenalectomized rats produced significantly less ovarian weight ($P = < 0.01$) and less uterine weight ($P = < 0.05$) than did those of corresponding control animals (Table II). Ovarian histology of recipient test animals revealed no qualitative difference in action of gonadotropins from control and experimental glands, both stimulating follicular growth without corpora lutea formation and repairing the interstitial tissue ("cartwheel" chromatin of the nuclei) to approximately the same degree.

Corticotropin. Adrenalectomy led to a 2- to 3½-fold increase in pituitary corticotropin content (Table III). After removal of adrenal glands, there occurs an initial drop in pituitary corticotropin content(10), followed by a rise to normal content in 7 days(11), and continued increase thereafter to 2-3 times the normal content 21 days after adrenalectomy(12). Results indicate that maximum corticotropin content is reached at 28 or 56 days after adrenalectomy and may decline somewhat at 100 days.

Discussion. Results of our study offer several correlations between changes in cytology and hormone content following adrenalectomy. The observations of an apparent normal growth hormone content of the pituitary gland of adrenalectomized rats correlated with a normal relative number of acidophils may add additional evidence in favor of this cell as the source of growth hormone. Because other pituitary hormones (corticotropin, thyrotropin) may exert antagonistic or synergistic effects upon growth hormone in this method of assay, some caution must be exercised in interpreting the results produced by administration of whole pituitary extracts.

The observation of a decrease in thyrotropin store of the pituitaries of adrenalectomized animals together with a fall in number of aldehyde-fuchsin staining beta basophils, adds to the increasing evidence that this cell is the secretor of thyrotropin.

The PAS-demonstrable glycoprotein granules of delta basophils are considerd to represent the gonadotropic hormones(13). Knigge(5) has presented evidence indicating that fluctuations in gonadotropic hormone content of the pituitary are: (a) reflected also by changes in certain silver-staining (argyrophilic) cells in the pars distalis; and (b) that these "argyrophilic cells" are probably identical with delta basophils, stained by a procedure in which certain cytoplasmic granules are blackened with silver. With the protargol silver method, the reduction in silver-staining granules of delta basophils after adrenalectomy correlated with the observed fall in gonadotropin content of the pituitary.

In considering the cellular source of corticotropin, the following observations are pertinent: (1) corticotropin was the only hormone whose content was increased in the pituitary after adrenalectomy; and (2) delta basophils, as revealed by the Masson method, were the only cells which showed cytological signs of increased activity. Increased titers of corticotropin are present in blood following adrenalectomy(11), suggesting that the pituitary is secreting as well as storing more of this hormone. These facts, together with the other data presented in this study, contribute to the conclusion that the delta basophil is the site of corticotropin production. It is felt, however, that the cytoplasmic granules of delta basophils, as revealed in Masson preparations, do not *per se* reflect corticotropin content of the cell. In acute or short-term experiments, no satisfactory single staining procedure is currently available for detecting changes in activity of delta basophils as related to corticotropin secretion.

Summary. 1. Adrenalectomy produced no significant change in weight of rat's pituitary gland. Cytologically, beta basophils decreased in number progressively while acidophils were not altered in number or appearance. The relative per cent of delta basophils was unaffected, but these cells were hypertrophied and exhibited some vacuolation 56 and 100 days after adrenalectomy. The silver-staining capacity of delta basophils, as revealed with protargol, was markedly reduced. 2.Gonadotropic and thyrotropic hormone contents of pituitary were decreased after adrenalectomy, while the corticotropin content was elevated 2- to 3-fold. No difference in growth hormone content was found when whole pituitaries were analyzed by the epiphyseal plate test. 3. Correlations between pituitary cytology and hormone content of the gland suggest that the delta basophil is the source of corticotropin.

Appreciation is expressed to Dr. Harold Levey for the thyrotropin assay according to Levey, Cheever and Roberts (1956).

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