

Adrenalectomy and exposure to stress led to increased ACTH production in rats, while injection of cortisone inhibited its production.

Changes in the relative percentages of basophil cells were observed in the zone bordering the pars intermedia of the pituitary. It is concluded that the source of ACTH in rats is the basophil cells located mainly in the zone bordering on the pars intermedia of the pituitary.

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It is still debated which cells of the anterior lobe of the pituitary are responsible for production of adrenocorticotrophic hormone (ACTH). Two different views are held at present. The adherents of one cite convincing evidence that basophil cells participate in ACTH production [1, 11, 12, 14, 16, 21]. This has been demonstrated under the optical microscope by study of the adenohypophysis stained by various methods [1, 14, 16], and also by immunohistological investigations [10, 12]. Electron-microscopic studies have revealed some morphological similarity between corticotrophic and thyrotrophic cells [8, 10, 17], but according to some reports, ACTH is produced by cells of the gonadotrophic series [18, 21].

Supporters of the other view insist that chromophobe or agranular cells are the source of ACTH [3, 4, 19, 20]. Siperstein [19], for instance, using an autoradiographic method, distinguished cells in the adenohypophysis of adrenalectomized rats with the highest level of protein renewal, which she designated as chromophobes. She concluded that this chromophobe type of cell is responsible for ACTH production. Subsequently Siperstein confirmed this conclusion by electron-microscopic studies [20].

Unfortunately there is no method of specific staining of corticotrophic cells at present available which could help to solve the important problem of the source of ACTH. Likewise there is no data in the literature to indicate whether corticotrophic cells are uniformly distributed throughout the adenohypophysis or whether they are mainly grouped in one particular zone. The determination of their selective localization could help with the further study of cells of this type both with the optical and electron microscopes.

Adrenalectomy and exposure to stress are known to stimulate ACTH production [2, 5]. Conversely, an excess of cortisone in the body inhibits ACTH production [6, 7, 15].

The object of the present investigation was to study the cell composition of the anterior lobe of the pituitary at times of increased and diminished ACTH production and thereby to attempt to determine what type of cell is responsible for ACTH production and the principal localization of these cells.

#### EXPERIMENTAL METHOD

Experiments were carried out on female albino rats weighing 200-280 g and males weighing 160-180 g. In experiment I the female rats were divided into three groups. Both adrenals were removed from the rats of group 1 (8). The animals (9) of group 2 received an intraperitoneal injection of 6 mg cortisone daily; group 3 (7 rats) acted as control. All the animals were sacrificed 7 days after the beginning of the experiment. In experiment II two groups of female rats were used. The animals of one group (8) were exposed to stress, while the other group (6 rats) acted as control. Stress stimulation consisted of placing the experimental animals in a bath of cold water (5°) in which they were compelled to swim. The rats were subjected to this treatment daily for 6 days. The first session lasted 5 min, and each successive session was lengthened

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TABLE 1. Relative Percentages of Different Types of Cells in the Adenohypophysis after Adrenalectomy, in Controls, and after Administration of Cortisone to Female Rats

Experimental conditions	Zone of adeno-hypophysis	Acidophil cells (in %)	Basophil cells (in %)	Chromophobe cells (in %)
Adrenalectomy (7 days)	Peripheral	34.93	6.18	58.89
	Central	38.21	7.02	54.77
	Bordering on pars intermedia	35.34	6.04	58.26
Control	Peripheral	34.77	7.48	57.75
	Central	37.86	7.84	54.30
	Bordering on pars intermedia	34.66	8.87	56.47
Administration of cortisone (7 days)	Peripheral	31.91	7.08	61.01
	Central	37.80	6.51	55.69
	Bordering on pars intermedia	35.41	6.99	57.60

TABLE 2. Relative Percentages of Different Types of Cells in the Adenohypophysis of Female Rats Exposed to Stress and Intact Female Rats

Experimental conditions	Zone of adeno-hypophysis	Acidophil cells (in %)	Basophil cells (in %)	Chromophobe cells (in %)
Stress stimulation (6 days)	Peripheral	35.68	8.16	56.18
	Central	38.27	7.01	54.17
	Bordering on pars intermedia	34.69	9.81	55.50
Control	Peripheral	34.44	7.32	58.24
	Central	38.76	7.53	53.71
	Bordering on pars intermedia	35.80	7.02	57.18

by 30 sec, so that the last exposure to stress continued for 7 min 30 sec. The animals were sacrificed immediately after the last exposure. Control animals were sacrificed at the same time. In experiment III, carried out on males, the animals were divided into three groups. Both adrenals were removed from the rats of group 1 (8 animals). The rats of group 2 (7) received a subcutaneous injection of 0.5 ml 2% formalin solution daily into the thigh, thus causing stress stimulation. The animals (6) of group 3 acted as control. Adrenalectomized animals were sacrificed 14 days, and those exposed to stress 7 days after the beginning of the experiment. In all the experiments the pituitary glands of the rats were fixed in mercuric chloride-formol mixture, embedded in paraffin wax, and sections were cut to a thickness of 5  $\mu$ . In sections stained by the method of McManus and Hotchkiss [9, 13] all three types of cells in the anterior lobe of the pituitary (acidophil, basophil, chromophobe) were counted in three zones: peripheral, central, and bordering on the pars intermedia. More than 2000 cells in each zone were counted in each animal. The relative percentages of all three types of cells were calculated and the results were subjected to statistical analysis by the Fisher-Student method.

#### EXPERIMENTAL RESULTS

Statistical analysis of the results revealed a significant decrease in the percentage of basophils in the zone bordering on the pars intermedia of the pituitary in adrenalectomized female rats and those receiving cortisone compared with the controls ( $P=0.001$ ,  $P=0.006$ ).

TABLE 3. Relative Percentages of Different Types of Cells in the Adenohypophysis of Control and Adrenalectomized Male Rats and Male Rats Exposed to Stress

Experimental condition	Zone of adeno- hypophysis	Acidophil cells (in %)	Basophil cells (in %)	Chromophobe cells (in %)
Control	Peripheral	35.16	15.13	49.71
	Central	40.77	13.93	45.30
	Bordering on pars inter- media	37.28	15.65	47.07
Adrenalectomy (14 days)	Peripheral	32.41	15.58	52.01
	Central	39.03	14.11	46.86
	Bordering on pars inter- media	30.58	22.52	46.90
Stress stimulation (7 days)	Peripheral	31.11	16.06	52.83
	Central	39.39	14.33	46.28
	Bordering on pars inter- media	31.44	22.42	46.14

The decrease in the percentage of basophils after adrenalectomy was unexpected. However, this result can be explained by the early time of death of the animals, when the basophils were producing secretion intensively and had become indistinguishable from the chromophobes. In animals sacrificed 14 days after the beginning of the experiment a statistically significant increase in the number of basophils was found after adrenalectomy (experiment III).

In experiment II a statistically significant increase in the relative percentage of basophil cells occurred in the same zone in females exposed to stress compared with the controls ( $P = 0.002$ ).

In experiment III, in response to adrenalectomy and stress, a statistically significant increase in the percentage of basophils was also found in the zone bordering on the pars intermedia (in both cases  $P < 0.0001$ ). All other fluctuations in the relative percentage of cells of the adenohypophysis were not statistically significant.

Hence, in response to all procedures associated with changes in the level of ACTH secretion, whether in female or male rats, significant changes took place in the number of basophil cells only in the zone bordering on the pars intermedia (Table 1-3).

It can thus be concluded from the results of this investigation that the source of ACTH in rats is the basophil cells located mainly in the zone bordering on the pars intermedia of the pituitary.

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