appearance of ametachromatic cells in cultured white cells from heterozygous carriers, compared to the more gradual disappearance of metachromasia in cells derived from the homozygous individuals, suggests that this temporal relation may prove to be a convenient way of distinguishing heterozygous from homozygous individuals. Quantitative studies on a larger number of families will be needed to substantiate these preliminary findings.

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Potassium, Corticosterone, and Adrenocorticotropic Hormone Release in vitro

Abstract. *Incubation of rat adenohypophyses in a high concentration of potassium increases adrenocorticotropic hormone release. This increased release is suppressed by the addition of corticosterone to the incubating medium. Our findings are consistent with a process of "stimulus-secretion coupling" proposed for other glands and suggest that corticosterone may operate directly on the adenohypophysial cell membrane to inhibit releasing mechanisms.*

Douglas and co-workers (*1*) have proposed that the release of catecholamines from the adrenal medulla and of vasopressin from the neurohypophysis is initiated by a depolarization of the cell membrane. This depolarization alters the permeability of the cell membrane allowing Ca^{++} to enter the cells. The increased concentration of intracellular Ca^{++} is then thought to activate a process leading to hormone release. The process has been called "stimulus-secretion coupling" (*1*). We have now obtained evidence to suggest that the release of adrenocorticotropic hormone (ACTH) from the adenohypophysis may involve mechanisms similar to those postulated for the adrenal medulla and the neurohypophysis.

Adenohypophyses from rats adrenalectomized 1 month previously were bisected in the midsagittal plane. Pooled batches of half glands were processed immediately for ACTH (*2*). The other halves were incubated at 37°C in (i) Krebs-Ringer bicarbonate with glucose (KRB) (*3*), (ii) KRB containing a fivefold increase in K^+ (28 m*M*), isotonicity being maintained by alteration of the molarity of the NaCl, or (iii) KRB containing corticosterone (1 μg/ml). Solutions were gassed with 95 percent O_2 and 5 percent CO_2. Incubating media were assayed for adrenocorticotropic hormone content; the latter is expressed as a percentage of the content of the nonincubated halves. Parentheses enclose the 95 percent confidence limits (Table 1). All incubations were preceded by a 30-minute incubation in KRB; this medium was discarded. Exposure to a fivefold increase in K^+ significantly increased adrenocorticotropic hormone release.

Table 1. Effect of a high concentration of K^+ on adrenocorticotropic hormone release in vitro. Results are expressed as adrenocorticotropic hormone content of incubating medium (KRB, High K^+-KRB), as a percentage of nonincubated pituitary halves (parentheses enclose 95 percent confidence limits). The medium was drawn off and replaced with fresh medium at 10, 30, and 90 minutes.

Incubation time (min)	ACTH release	
	KRB (%)	High K^+–KRB (%)
1–10	0.45(0.27–0.73)	2.92(2.37–3.58)
10–30	0.41(0.25–0.68)	4.04(3.33–4.91)
30–90	0.51(0.32–0.81)	7.41(5.92–9.27)

We then investigated the effect of corticosterone on this augmented release of ACTH. The addition of corticosterone to the incubating medium significantly reduced the augmented release of ACTH from 13.40 (9.76 to 18.38) percent to 5.23 (4.01 to 6.83) percent, with a 90-minute incubation.

This effect of the high concentration of K^+ is not unique to the release of ACTH, since increased release of thyroid-stimulating hormone and luteinizing hormone have been reported under similar conditions (*4*, *5*). Indeed, Vale and Guillemin (*4*) have stated that increased concentrations of K^+ stimulate the release of ACTH in vitro. However they presented no data to support this statement.

We tentatively assume that this increased release of ACTH is secondary to a decrease in transmembrane potential. Such an effect could, however, have been due simply to a swelling of cells, such as occurs in muscle under similar conditions (*7*). But we found no significant change in weight when adenohypophyses were incubated in KRB containing a fivefold increase in the concentration of K^+.

In this system, high physiological concentrations of corticosterone depress the release of ACTH induced by high concentrations of K^+. In the intact animal, high concentrations of circulating corticosterone suppress ACTH secretion by way of a negative feedback system (*6*). It has not been firmly established where the sensing elements of the feedback system are located. These experiments strongly suggest that corticosterone may operate directly on releasing mechanisms in the adenohypophysial cell membrane.

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