

tion even through the enzyme may not be 100% pure. (2) Bovine antifibrinolysin, 5 mg per ml of blood, failed to inhibit the histamine release by antigen as shown by the data of Table VI.

The evidence presented here applies only to the mechanism of histamine release from rabbit blood cells by antigen *in vitro* and may have no bearing upon the mechanism of histamine release by antigen in other species. However, in accord with our results is the recent work by Scroggie and Jaques (14), which indicates that in the dog the activated plasma protease is not necessary for the anaphylactic release of histamine in the liver.

The above evidence indicates that any

activation of profibrinolysin or any increase in plasma protease activity, which may accompany the *in vitro* histamine release by antigen, is only a coincidental phenomenon and is not a prerequisite for the histamine release.

*Summary.* The failure of excessive concentrations of soy bean trypsin inhibitor and bovine antifibrinolysin to inhibit the histamine release by antigen and the poor and inconsistent histamine release by bovine fibrinolysin indicate that the *in vitro* anaphylactic release of histamine from rabbit blood cells does not depend upon fibrinolysin activity.

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### Release of Adrenocorticotrophic Hormone by Direct Application of Epinephrine to Pituitary Grafts.\* (17760)

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It is now known that the injection of epinephrine is followed in normal rats by a fall in the adrenal ascorbic acid(1) and in circulating blood eosinophiles(2). Long(3) has suggested that in many of the conditions associated with an increased degree of activity of the pituitary-adrenal system, a preliminary release of epinephrine is a contributing factor. On the other hand, Sayers and Sayers(4) have found that there is an inverse relationship between the blood levels of adrenocorticotrophic hormone (ACTH) and adrenal corti-

cal hormone (ACH) that determines the relative secretory activity of each gland. They regard the effect of epinephrine as due to its capacity to increase the utilization of cortical hormone and thus reduce its level in the blood traversing the pituitary.

In order to determine whether epinephrine is capable of directly increasing the secretion of ACTH from the anterior pituitary, the following experiment was performed. A group of adult, male, albino rats of the Sprague-Dawley strain, weighing about 250 g each, were hypophysectomized by the parapharyngeal approach, and 3 days later homologous transplants of pituitary tissue from female adult rats of the same strain were inserted into the anterior chamber of the right eyes. Seven rats survived the operations and 6 weeks later all had well vascularized grafts growing in the anterior chamber of the eye. The eosinophile decline in the peripheral blood was selected as an index of adrenal cortical activity

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2. Recant, L., Forsham, P. H., and Thorn, G. W., *Fed. Proc.*, 1948, v7, 99.

3. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.

4. Sayers, G., and Sayers, M., *Recent Progress in Hormone Research*. Vol. II. Academic Press, New York, 1948.

and hence of ACTH release from the grafts. The subcutaneous injection of epinephrine (.02 mg/100 g) brought about a definite eosinopenic response in three hours, the mean fall being  $-46 (\pm 3)\%$ . Previous studies had demonstrated that a series of hypophysectomized rats given a similar dose of epinephrine showed a mean rise of  $+20 (\pm 8)\%$ .

In order to test the effects of the local application of epinephrine directly on pituitary tissue, under light Nembutal anesthesia a small slit was made with a scalpel in the sclero-corneal junction and .05 cc of a 1-250,000 solution of epinephrine was instilled directly into the anterior chambers with a fine needle. All 7 rats showed a definite eosinopenic response in three hours, with a mean fall of  $-44 (\pm 2)\%$ .

An interval of 3-5 days was allowed to elapse between successive experiments on any given rat and then an identical procedure was carried out, using the same dose and concentration of epinephrine in the normal eyes. None of the rats showed any significant change in eosinophile count over a period of 3 hours, the mean variation being  $+6 (\pm 4)\%$ .

Since it was of interest to determine if endogenous epinephrine released from the adrenal medulla would activate the grafted pituitary tissue each rat was given—as a painful stimulus which would not produce any gross physiological stress or extensive tissue damage—a subcutaneous injection of 1.0 cc of hypertonic (10%) saline. All animals showed eosinopenic responses in 3 hours, with a mean fall of  $-40 (\pm 5)\%$ .

As a final experiment on this series of 7 animals, the eyes containing the grafts were enucleated, and 3-5 days following enucleation each rat was given another subcutaneous dose of epinephrine (.02 mg/100 g). Following removal of the grafted pituitary, none of the animals showed an eosinopenic change. In fact, the mean change was a rise of  $+13 (\pm 6)\%$ , comparable to the rise of  $+20 (\pm 8)\%$  noted in a series of eight non-grafted hypophysectomized rats.

One of the grafts was assayed for ACTH by grinding the fresh tissue with 1.0 cc of isotonic saline containing 2 drops of .1 N NaOH.

This extract was then injected intravenously into each of two hypophysectomized rats in the amount of 0.1 cc/100 g. Both rats showed eosinopenic responses (mean  $-54\%$ ) and a fall in total ascorbic acid (mean  $-28\%$ ).

It is of interest to note that the adrenal glands of the hypophysectomized rats with homologous pituitary grafts averaged 10.3 mg/100 g in weight, while the adrenal glands of non-grafted hypophysectomized rats, after a corresponding time interval following operation, averaged 5.7 mg/100 g in weight. The weights of adrenal glands of normal rats in our studies have averaged 14.0 mg/100 g. It would appear then that not only do these grafts have the ability to release ACTH in response to stimulation by systemic or local epinephrine, but also release steadily enough ACTH to support, at least partially, the weight of the adrenal glands. There is no evidence at the moment that these grafts release either growth hormone or gonadotrophic hormone since the animals did not grow and the testes were completely atrophic.

This experiment, taken in conjunction with those recently reported by Cheng *et al.*(5) and Fortier and Selye(6), would indicate that no neural or neurohumoral connections with the central nervous system are necessary for the release of ACTH from the anterior lobe of the pituitary.

**Summary.** 1. Homologous grafts of pituitary tissue to the anterior chamber of the eye of the hypophysectomized rat retain the ability to secrete ACTH, both spontaneously and in response to activation by epinephrine.

2. The effect of epinephrine in releasing ACTH may be brought about by its direct application to anterior pituitary tissue.

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6. Fortier, C., and Selye, H., *Am. J. Physiol.*, 1949, v159, 433.

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