Suppression of Adrenal Compensatory Hypertrophy by Hypothalamic Lesions.* (21943)

BROOKE D. FULFORD AND S. M. MCCANN. (Introduced by F. B. Benjamin.)
From the Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia.

There is general agreement that lesions of the median eminence suppress the acute adrenal cortical response to stress(1,2,3). Interruption of the supraopticohypophyseal tract as evidenced by diabetes insipidus appears to be responsible for this failure of the normal stress-induced discharge of adrenocorticotrophin (ACTH)(4). In the adrenalectomized rat maintained on desoxycorticosterone, acute stress results in very high levels of blood ACTH. If rats with hypothalamic lesions are adrenalectomized and subjected to acute stress, no detectable ACTH can be found in blood(5). In addition to acute stress, it is known that adrenalectomy results in an increase in pituitary ACTH output(6,7). This increase can not be demonstrated in adrenalectomized rats with hypothalamic lesions, and this suggests that the increased ACTH secretion following adrenalectomy may also be dependent upon hypothalamic activity(5). However, small increases in blood ACTH concentration above the normal, unstressed level would not be detected by the assay employed. It was of interest, therefore, to determine whether the compensatory hypertrophy which follows unilateral adrenalectomy would occur in rats with hypothalamic lesions. This hypertrophy is presumably due also to increased ACTH discharge consequent to a fall in blood steroids following unilateral adrenalectomy(8,9). The present results indicate that lesions which interrupt the supraopticohypophyseal tract suppress the compensatory hypertrophy following unilateral adrenalectomy, but they also suggest that a small response may be present.

Methods. Bilateral lesions were produced in the hypothalamus of adult male rats of the Wistar strain with the aid of a Krieg stereotaxic instrument. Three weeks to 2 months postoperatively, the rats were subjected to left unilateral adrenalectomy under ether anesthesia. Two weeks later the right adrenal was removed. Control rats without lesions were similarly studied. Adrenals were weighed to the nearest 0.1 mg on a torsion balance. Because of variations in initial adrenal weights, all results are expressed as the percentage change in right adrenal weight after unilateral adrenalectomy as compared to the weight of the previously removed left adrenal.

Results. Twelve normal rats subjected to unilateral left adrenalectomy exhibited an hypertrophy of the right adrenal equal to $51 \pm 8\%$, when it was removed 2 weeks later. Since previous work has shown that the blockade in the acute adrenal stress response in rats with lesions correlates well with the degree of interruption of the supraopticohypophyseal tract as judged by increased water intake(4), the compensatory adrenal hypertrophy of the control rats and of 48 rats with lesions of the hypothalamus was plotted against their daily water intake in Fig. 1. It

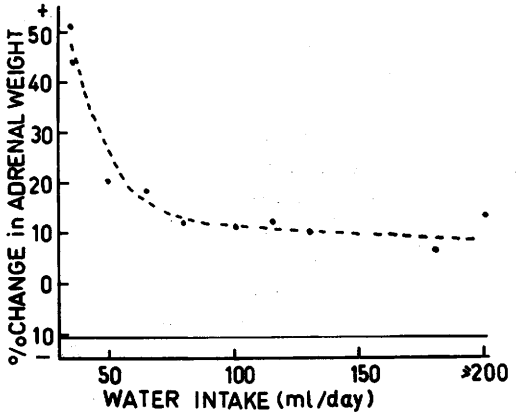

FIG. 1. Suppression of compensatory adrenal hypertrophy in rats with diabetes insipidus. Daily water intake on abscissa is plotted against percentage change in right adrenal wt. Each point represents mean response of 4 or more rats for each increment of 10 ml or a multiple thereof of water consumption per day. Solid line represents result found in rats with lesions where both adrenals were removed simultaneously.

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can be seen that the degree of hypertrophy fell off very rapidly as water intake increased above the normal level of 35 ml/day. At levels of water intake above 100 ml/day, the compensatory hypertrophy fell to a minimal value of approximately 10%, and it remained at this figure with increasing values of water intake. The mean increase in right adrenal weight of all 20 animals with water intakes of 100 ml/day or more was $+ 9 \pm 4\%$. In another series of rats with hypothalamic lesions, when the 2 adrenals were removed simultaneously, the right adrenal weighed on the average $11 \pm 2\%$ less than the left adrenal. Consequently, in the rats with diabetes insipidus the response to unilateral adrenalectomy was $+ 9 \pm 4\%$ plus $11 \pm 2\%$, or a total gain of approximately 20% above the expected weight of glands from this location in rats with lesions. This gain is 30% of the gain exhibited by normal animals. This small residual response was statistically significant if compared to the results obtained when both adrenals were removed simultaneously in the rats with lesions ($P<0.01$).

Nearly all rats gained weight during the experimental period. Five of the 53 rats with lesions, however, lost 10% or more of their body weight during this time, and all of these 5 animals exhibited hypertrophy of the remaining adrenal, the mean response being $+ 56 \pm 14\%$, a value similar to that of the control rats without lesions. This hypertrophy in rats with weight loss was independent of the water intake which varied between 90 and 380 ml/day. The results in these rats were excluded from consideration in Fig. 1.

Testicular atrophy occurred in only 6 of the 52 rats studied, a result in harmony with previous studies indicating that suppression of adrenal function can occur in the absence of testicular atrophy(1,5).

Discussion. The present results indicate that compensatory adrenal hypertrophy is suppressed in rats with hypothalamic lesions. This is interpreted to mean that such lesions at least partially block the augmented secretion of pituitary ACTH which normally ensues when the blood level of corticoids is lowered as a result of unilateral adrenalectomy. A small response still occurs in such rats. Furthermore, if the animals lose significant weight during the course of the study, they still manifest compensatory hypertrophy. This hypertrophy is presumed to be due to some additional stress which appears to be capable of evoking a discharge of ACTH when acting over this 2-week time interval even in the rat with hypothalamic lesions. It has been previously demonstrated that rats with extremely severe diabetes insipidus show adrenal hypertrophy although the acute adrenal stress response is blocked(4,5). Sufficient rats with severe diabetes insipidus have not been subjected to unilateral adrenalectomy to allow conclusions as to whether they would still respond with compensatory hypertrophy.

The lesions in the present study were designed to interrupt the supraopticohypophyseal tract in the rostral portion of the median eminence. Damage to other structures undoubtedly occurred, but the correlation between diabetes insipidus and reduction in the response to unilateral adrenalectomy agrees with the results of previous work in which it was demonstrated that blockade of the acute adrenal stress response in rats could be correlated only with injury to the supraopticohypophyseal tract(4).

Our results are in good agreement with those recently reported by Ganong and Hume in the dog(10), who correlated a complete absence of compensatory hypertrophy with lesions which destroyed an estimated 50% or more of the median eminence. In our experience a small response is still present in rats with lesions.

The secretion of ACTH which normally follows adrenalectomy is believed to be caused by the fall in blood level of corticoids following removal of the adrenals(5,7). In animals with appropriate hypothalamic lesions there is a suppression of this ACTH secretion. It appears that this suppression may have two possible explanations. Either the steroids normally act directly upon the hypothalamic or other nervous structures to inhibit neural activity when the blood level is raised and to stimulate it when the level falls. Or if the steroids act directly upon the cells of the anterior pituitary gland, then the sensitivity of

these cells to the level of cortical steroids present in the blood must be affected by the neurohumor presumed to be secreted by the hypothalamus. Our data do not permit us to decide which of these possibilities is correct.

Conclusions. The compensatory adrenal hypertrophy which normally follows unilateral adrenalectomy is suppressed in rats with diabetes insipidus produced by hypothalamic lesions.

1. McCann, S. M., *Am. J. Physiol.*, 1952, v171, 746.
2. Hume, D. M., *Ann. Surg.*, 1953, v138, 548.
3. Anand, B. K., Raghunath, P., Dua, S., and Mohindra, S., *Indian J. Med. Research*, 1954, v42, 2.
4. McCann, S. M., and Brobeck, J. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 318.
5. McCann, S. M., and Sydnor, K. L., *ibid.*, 1954, v87, 369.
6. Gemzell, C. A., Van Dyke, D. C., Tobias, C. A., and Evans, H. M., *Endocrinol.*, 1951, v49, 325.
7. Sydnor, K. L., and Sayers, G., *ibid.*, 1954, v55, 621.
8. Tepperman, J., Engel, F. L., and Long, C. N. H., *ibid.*, 1943, v32, 373.
9. Ganong, W. F., *ibid.*, 1954, v55, 117.
10. Ganong, W. F., and Hume, D. M., *ibid.*, 1954, v55, 474.

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Plaque Formation of Poliomyelitis Viruses on Human Amnion Cell Cultures.* (21944)

JØRGEN FOGH AND ROSEMARY O. LUND. (Introduced by W. M. Stanley.)
From the Virus Laboratory, University of California, Berkeley.

In a previous publication(1) human amnion cells were reported to support propagation of poliomyelitis viruses, and it was indicated that tissue cultures obtained from this readily available tissue could provide a source for the large scale production of poliomyelitis virus.

In the present work plaque formation in monolayer tissue cultures of amnion cells by the 3 types of this virus was obtained, similar to the plaque formation by this virus in monkey kidney and testis cultures(2) and human cancer (HeLa) cell cultures(3). It is, therefore, possible to employ this tissue also for the quantitative assay of poliomyelitis virus.

Material and methods. Virus. Poliomyelitis viruses, type 1, strain Mahoney; type 2, strain MEF-1; type 3, strain Saukett, obtained from Dr. Jonas Salk in 1952, were passed several times in monkey kidney cells, 3-4 times in HeLa cells and finally 1 or 2 times in monkey kidney cells. The final virus preparations were stored at −60°C. *Cells.* Monolayer cultures of human amnion cells were prepared in 50 mm petri dishes as previously described(1). After centrifugation the cell pack was diluted 1:120 and the cultures were grown at 37°C in a 3% CO_2-atmosphere in a medium consisting of 20% ox serum and Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate (Nutritional Biochemicals). The culture fluid was replaced by fresh medium after incubation for 4-5 days. The cell sheet was usually confluent after 10-14 days. *Infection and overlay.* The cultures were washed 3 times with buffer solution at 37°C after removal of the nutrient fluid and inoculated with 0.3 ml of various dilutions of the virus preparation. After permitting adsorption of the virus for at least 30 minutes at 37°C, the plates were overlayed with 3-4 ml of melted agar containing nutrient fluid. 100 ml nutrient fluid consisted of 55 ml 1% Difco yeast extract in distilled water, 6 ml 5% bovine albumin powder V (Armour) in Hanks' solution, 9 ml 5% $NaHCO_3$ in distilled water and 30 ml 10 times concentrated Hanks' solution without phenol red(4). One part of 3% agar was melted and kept in a water bath at 44°C. One part of the nutrient fluid was mixed with an equal volume of dis-

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