of brain examined on guinea pig ileum,[1] only those prepared by the method of Adam[4] were devoid of materials interfering with the estimation of histamine.

Acknowledgements—This work was supported by U.S. Public Health Service Research Program Award 2K3-GM-2459-05 and Research Grant GM-10313-01; and by Research Grant 60-G-71 from the American Heart Association. Dr. Carlini was a Postdoctoral Fellow of the Rockefeller Foundation; his present address is Instituto Biologico, Fisiologia Animal, São Paulo, Brazil.

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REFERENCES

1. E. A. CARLINI and J. P. GREEN, *Brit. J. Pharmacol.* **20**, 264 (1963).
2. I. A. MICHAELSON and G. DOWE, *Biochem. Pharmacol.* **12**, 949 (1963).
3. P. A. SHORE, A. BURKHALTER and V. H. COHN, *J. Pharmacol. exp. Ther.* **127**, 182 (1959).
4. H. M. ADAM, *Regional Neurochemistry*, S. S. KETY and J. ELKES, Eds., p. 293. New York, Pergamon Press (1961).

Mechanism of the antinatriuretic action of aldosterone

(*Received* 15 *July* 1963; *accepted* 17 *September* 1963)

ALDOSTERONE characteristically decreases the urinary excretion of sodium and enhances the excretion of potassium. However, little is known about the manner in which this mineralocorticoid exerts its action. Barger *et al.*[1] have infused aldosterone directly into the renal artery of the dog and noted a lag of 30 min or more before the onset of action of this hormone. This was in marked contrast to the almost immediate onset of action of the antidiuretic hormone (ADH). The marked difference in onset of action of these two hormones suggests that ADH acts directly, whereas aldosterone probably acts indirectly. A possible explanation for the lag could be that aldosterone is involved in protein synthesis. Actinomycin D was therefore used to determine whether protein synthesis *de novo* was involved in the delayed onset of action of aldosterone.

The procedure of Kagawa *et al.*[2] was used to assess the effect of the inhibitor on the action of aldosterone. Male Holtzman rats, 190–220 g, were adrenalectomized at zero time and given a 0·9% solution of sodium chloride for drinking water. Food was withdrawn at 6 hr. At 24 hr drinking water was removed and each rat given 2·5 ml of 0·9% sodium choride, subcutaneously. Animals were divided into four groups of four each and treated as follows: (1) no treatment; (2) 1 μg *d*-aldosterone-21-acetate* subcutaneously; (3) 0·3 mg actinomycin D† intraperitoneally; (4) aldosterone and actinomycin D. A 4-hr collection period was employed. Sodium and potassium outputs were determined with a Coleman flame photometer. Data were evaluated by Duncan's new multiple range test.[3] The 0·05 level of probability was the criterion of significance.

The results of this experiment are given in Table 1. Aldosterone produced a significant decrease in the excretion of sodium while significantly increasing the excretion of potassium. Actinomycin alone did not alter the excretion of either ion. However, when actinomycin D was administered to rats also receiving aldosterone, the action of the hormone on sodium excretion was completely blocked. In contrast, potassium excretion was not affected. A separation of this action of aldosterone has been reported previously.[1]

Karlson[4] believes that many hormones exert their action by promoting synthesis of enzymes. He proposes that their site of action is on DNA to somehow cause exposure of DNA receptors. Thus

* The aldosterone was kindly supplied by Dr. Gene Lata, Department of Biochemistry, State University of Iowa.

† The actinomycin D was kindly supplied by Dr. Richard Adamson, National Institutes of Health.

messenger RNA is formed which acts on ribosomes to stimulate specific enzyme production. Ui and Mueller[5] have reported that actinomycin D blocked RNA synthesis in the rat uterus and also blocked the action of the hormone estradiol on the uterus. They concluded that the action of estradiol depended upon the synthesis of new RNA and, consequently, a stimulation of protein synthetic mechanisms.

TABLE 1. EFFECT OF ACTINOMYCIN D ON ALDOSTERONE-INDUCED CHANGES IN THE EXCRETION OF ELECTROLYTES IN ADRENALECTOMIZED RATS

Treatment	Total excretion in 4 hr, mEq Sodium	Potasssium
None	0·41 |	0·08 |
Actinomycin D	0·44	0·13
Actinomycin D and aldosterone	0·36 |	0·22 |
Aldosterone	0·13	0·23
Coefficient of variability	42%	25%

* Values for each treatment represent the means of four animals. In each column, any two means joined by a vertical line are not significantly different. Any two means not so joined are significantly different (5% level). Statistical analysis was performed by analysis of variance using a completely randomized block design and Duncan's new multiple range test.[3]

The blockade by actinomycin D of the action of aldosterone on sodium excretion presented here could be interpreted to occur by the mechanism proposed by Karlson.[4] The delay in onset of action of mineralocorticoids would thus be due to the time necessary to initiate synthesis of a protein involved in the transport of sodium. The lack of any effect of actinomycin D on aldosterone-induced kaluresis would indicate that this action can be separated from the antinatriuretic response and hence is mediated in another manner.

Acknowledgements—This work was supported in part by Grant AM-05298 from the National Institute of Arthritis and Metabolic Diseases.

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REFERENCES

1. A. C. BARGER, R. D. BERLIN and J. F. TULENKO, *Endocrinology* **62**, 804 (1958).
2. C. M. KAGAWA, E. G. SHIPLEY and R. K. MEYER, *Proc. Soc. exp. Biol. (N.Y.)* **80**, 281, (1952).
3. R. G. D. STEEL and J. H. TORRIE, *Principals and Procedures of Statistics*, p. 107. McGraw-Hill, New York (1960).
4. P. KARLSON, *Perspect. Biol. Med.* **6**, 202 (1963).
5. H. UI and G. C. MUELLER, *Fed. Proc.* **22**, 409 (1963).

Localization of acetylcholine, 5-hydroxytryptamine and noradrenaline within subcellular particles derived from guinea pig subcortical brain tissue

(*Received* 27 *September* 1963; *accepted* 1 *October* 1963)

WHEN brain tissue is homogenized in 0·32 M sucrose, nerve endings escape disruption and are snapped off to form nerve-ending particles (NEPs) which can be isolated in a discrete fraction by differential