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Adrenal Cortical Hormone and Ant. Pituitary Extract on Carbohydrate Levels in Fasted Hypophysectomized Rats.*

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Hypophysectomized rats are unable to maintain their carbohydrate stores during fasting as do normal animals.[1] Certain anterior pituitary extracts are capable of preventing completely the changes found in short fasts, such as 8 hours. In longer fasts, however, while muscle glycogen stores are held at normal or supernormal levels, the liver glycogen and blood glucose are not maintained by this treatment.[2] This difference in the response of liver and muscle has led to the suggestion that more than one factor may be concerned in the maintenance of carbohydrate levels in the fasted hypophysectomized rat.[3]

Because of the possibility that the adrenal cortex may mediate some of the effects of the anterior pituitary on carbohydrate metabolism, the relationship of the adrenocorticotropic substance of the A.P. and of the adrenal cortical hormones to the factor or factors mentioned above has recently been investigated. That the maintenance of muscle glycogen stores is possible in the absence of the adrenal cortex was early found;[2] and Bennett, working in this laboratory, has recently presented much additional evidence that the factor maintaining muscle glycogen (the "glycostatic" factor) is not the adrenocorticotropic factor.[4] Bennett also showed that adrenocorticotropic preparations on chronic administration (that is, with maintenance or repair of the adrenal cortex) could effect consider-

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1 Russell, J. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 279; Russell, J. A., and Bennett, L. L., *Am. J. Physiol.*, 1937, **118**, 196.

2 Russell, J. A., and Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 406.

3 Russell, J. A., *Endocrinol.*, 1938, **22**, 80.

4 Bennett, L. L., *Endocrinol.*, 1938, **22**, 193.

able increases in the liver glycogen and blood sugar levels of fasted hypophysectomized rats.[5] From a consideration of these facts, it seemed possible that 2 pituitary factors affecting the carbohydrate stores in these animals were (1) a "glycostatic" substance acting directly on the muscle glycogen, and (2) the adrenocorticotropic hormone affecting also the liver glycogen and thence blood sugar levels. Evidence supporting this suggestion is here presented.

In the experiments reported here (Table I), hypophysectomized rats 2-4 weeks post-operative were fasted 24 hours and given during this time either Kendall's crystalline Compound B from the adrenal cortex‡ or a standard alkaline extract of beef anterior pituitary[2] or a combination of the 2 substances. When these experiments were partly completed, the report of Long and Katzin appeared with data on the effects of cortical extract on the carbohydrate levels of fasted hypophysectomized rats.[6] The figures are entirely confirmatory of those we obtained using Kendall's crystalline Compound B alone. Marked increases in liver glycogen content were produced; and blood sugar levels were normal or a little above. However, in neither the series of Long and Katzin nor in our several groups were the muscle glycogen values maintained at fully normal levels. In spite of the use of high doses of the hormone and the production of extremely high liver glycogen levels, normal muscle glycogens were not obtained except in isolated cases. These results are entirely parallel to those which Bennett found after treatment of the rats for some days with adrenocorticotropic extracts.

On the other hand, the effects of acute treatment with anterior pituitary extracts, of which an example is given here, are very different from those of either the adrenocorticotropic hormone or of the adrenal cortex substance. Although approximately 6 times the dosage required to produce a maximal effect was used, the action of the anterior pituitary extract was evident solely on the muscle glycogen stores; in this case the muscle glycogen levels were maintained completely. With the combination of the 2 types of treatment—the crystalline Compound B with injection of the anterior pituitary extract—the stores of muscle glycogen and of liver glycogen were both much higher than with either type of treatment alone (compare Groups 3 and 5, Table I). While some synergism may have occurred in this case, it seems more probable that the effect was additive, the maintenance of glycogen in the muscles by the anterior

[5] Bennett, L. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 50.

‡ We wish to thank Dr. E. C. Kendall for this substance.

[6] Long, C. N. H., and Katzin, B., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 566.

TABLE I.
Carbohydrate Levels of Hypophysectomized Rats Fasted 24 Hours with and without Adrenal Cortical Hormone* and Anterior Pituitary Extract.

	No. of rats	Blood Glucose mg %	Liver Glycogen mg %	Muscle Glycogen mg %
1. Untreated Rats	10	42 (30-54)	13 (8-20)	350 (250-400)
2. A.C.H., 1 mg total dose; injection every hr during last 8 hr of fast	5	76 (53-106)	137 (46-236)	382 (290-437)
3. A.C.H., 3 mg total dose; fed during 24 hr fasting period	5	72 (57-94)	97 (41-156)	453 (417-475)
4. A.C.H., 3 mg total dose; injected every hr during 24 hr or fed during first 16 hr, injected every hr during last 8 hr	5	90 (73-106)	917 (37-1950)	480 (448-512)
5. A.C.H., 3 mg total dose, fed or injected during 24 hr fasting period, + 1.5 cc A.P.E.† during fast	7	78 (64-103)	440 (179-1370)	629 (530-722)
6. A.P.E., alone (1.5 cc during fast)	6	46 (36-64)	11 (7-13)	565 (550-612)
7. Normal Rats, untreated	15	72 (65-85)	25 (15-60)	525 (490-550)

* Compound B, crystalline, from Kendall.
† Standard alkaline extract of anterior lobes of beef pituitary.

pituitary extract allowing greater accumulation of glycogen in the liver under the influence of the cortical hormone.

The cortical hormone seems able to cause an increase in the glycogen content of fasted animals in which the carbohydrate levels are low before the injections are started, as found by Long and Katzin in a small series and in our experiments (Group 2). It is noticeable in the latter series that whereas the liver glycogen was supernormal and the blood sugar level also up, the muscle glycogen content was scarcely affected. Apparently, then, this substance acts directly on the liver, affecting the muscle glycogen level only more slowly and probably indirectly.

From the results presented here it appears that at least 2 factors with different functions act together to prevent the fall in carbohydrate levels which ordinarily takes place in fasted hypophysectomized rats The factor affecting the muscle glycogen content directly, the "glycostatic" factor, appears to act independently of the adrenal cortex and to be associated with none of the other known anterior pituitary hormones. It does not appear to affect liver glycogen or blood sugar values under the usual conditions, and probably acts by suppressing carbohydrate oxidation.[7] Kendall's Compound

[7] Fisher, R. E., Russell, J. A., and Cori, C. F., *J. Biol. Chem.*, 1936, **115**, 627.

B, or a physiologically similar substance produced when the adrenal cortex is stimulated by adrenocorticotropic extracts of the anterior lobe, causes increases in liver glycogen and blood sugar levels in these animals, only more slowly and incompletely restoring the muscle glycogen levels. When the cortical hormone and a glycostatic extract of the anterior lobe are administered simultaneously, the separate effects of each on liver and muscle are apparent.

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Sensitized Pupillary Dilator and Facial Muscles as Indicators of Sympathetic and Parasympathetic Substances in Blood.*

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It is well known that section of the cervical sympathetic renders the pupillary dilator fibers sensitive to circulating adrenaline. Therefore, any discharge of epinephrine into the blood stream would be indicated by greater dilatation in the denervated than in the normal pupil. The degree of dilatation varies with the concentration of adrenaline or sympathin in the blood and with the sensitivity of the radial fibers of the iris, which in turn depends on the completeness of denervation. This pupillary reaction may be used as an indicator for sympathetic hormonal activity.

An indicator for circulating parasympathetic substance (acetylcholine) may be prepared by sectioning the facial nerve (Bender[1]). This renders the denervated facial muscles sensitive to acetylcholine. In the presence of small amounts of acetylcholine, slow contraction in the paralyzed muscles may be seen. By repeated denervations the sensitivity of the reaction may be maintained for long periods.

On sectioning the superior cervical ganglion on one side and the facial nerve on the opposite, indicators for both sympathetic and parasympathetic substances were obtained. Monkeys, cats, dogs and rabbits were used. The operative procedures were simple, and the two sections were carried out in one period. Usually the left facial nerve and the right cervical sympathetic were cut.

The sensitization phenomenon appeared on the sympathectomized

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1 Bender, M. B., *Am. J. Physiol.*, 1938, **121**, 609.