found in either the sera or the saliva of the human and guinea pig; on the other hand, saliva of the rabbit contained about ¼-½ the bactericidin activity found in the sera. In the case of the rat, however, an animal which consistently demonstrated a high serum titer, none was detected in the saliva. The basis for this unexpected dichotomy was sought.

The results with the saliva of the rat indicate that the cofactor is present, whereas the non-dialyzable heat labile portion is lacking. This is confirmed by the inability of heat-inactivated rat serum to be reactivated by untreated rat saliva. Also, addition of cofactor did not potentiate the sysem, whereas addition of rat saliva to the dialyzed rat serum, from which the cofactor is missing, reestablished the bactericidal system.

The components of the saliva are suspected to be derived mainly from the plasma. As in the case of the kidney, the process is selective and, of course, variation between species is expected. For example, lysozyme, an antimicrobial enzyme found in high concentration in the polymorphonuclear leucocyte, was found to be present in both saliva and serum of the rat(11). Intravenously administered penicillin passed into the salivary secretions of the rat in high concentration(11). The present findings serve to emphasize the selective capacity of the salivary glands and the variability in this selectivity among the mammals studied.

*Summary.* 1. Sera and saliva from humans and guinea pigs possessed very low or undetectable amounts of the bactericidin for *B. subtilis*. 2. Rabbit saliva possessed ¼-½ the rabbit serum level. 3. The serum of the rat contained high levels of this bactericidin, whereas rat saliva demonstrated no activity. The non-dialyzable portion of the bactericidal system was lacking in the saliva; the cofactor was present.

1. Jacox, R. F., *J. Exp. Med.*, 1950, v92, 101.
2. Myrvik, Q. N., Weiser, R. S., *J. Immunol.*, 1954, v74, 9.
3. Myrvik, Q. N., Soto-Figueroa, E., Naff, G., Henelt, E., *ibid.*, 1958, v81, 118.
4. Myrvik, Q. N., Leake, E. S., *ibid.*, 1959, v84, 247.
5. Florey, N., *Brit. J. Exp. Path.*, 1930, v11, 251.
6. Nemes, J. L., Wheatcroft, M. G., *Oral Surg., Oral Med., Oral Path.*, 1952, v5, 653.
7. Zeldow, B. J., *J. Dent. Res.*, 1959, v38, 798.
8. ———, *ibid.*, 1960, v40, 446.
9. Kahn, R. H., *Pfluger Arch. Physiol.*, 1922, v196, 400.
10. Bernarbe, M. A., Fabian, F. W., Rosen, S., Hoppert, C. A., Hunt, H. R., *J. Dent. Res.*, 1956, v32, 326.
11. Zeldow, B. J., unpublished observations.

Received October 23, 1961. P.S.E.B.M., 1962, v109.

## Glycogen in Adrenal Cortex of Sodium Depleted Rat.* (27155)

RICHARD B. COHEN AND JOHN D. CRAWFORD (Introduced by E. B. Taft)
*Departments of Pathology and Pediatrics, Harvard Medical School, James Homer Wright Pathology Laboratories and Children's Service, Massachusetts General Hospital Boston, Mass.*

The glycogen content and distribution in the adrenal cortex of the white rat has been studied by a number of investigators(1,2). As demonstrated histochemcially by the periodic acid Schiff (PAS) reaction, glycogen in this species concentrates in granular form in the cytoplasm of the cells of the fascicular and reticular zones, and is either absent or present in very small quantities in the glomerulosa. Haynes *et al.*(3,4) suggest that adrenocorticotropin (ACTH) acts to stimulate adrenal phosphorylase activity *via* an effect on adenosine-3′-5′-monophosphate. In the light of this theory the presence of considerable stored glycogen in the inner zones would seem reasonable since this is the area which responds most acutely to stress or ACTH and, therefore, should require a read-

* This research was supported by U. S. Public Health Service Grants.

ily available carbohydrate substrate. The participation of glycogen in this response is suggested by its rapid loss from the cortex of rats given ACTH or submitted to acute stress(1,2). Since these stimuli affect mainly the fascicular and reticular zones it was of some interest to observe glycogen in the adrenal cortex of sodium depleted rats. Sodium depletion provides what appears to be a selective stimulus to the glomerulosa, with the added technical advantage of affording a wide, easily observed zone of cells considered to be of the glomerulosa type.

*Material and method.* Twenty 80 g male white rats were acclimated to the animal room on standard diets (Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.) and tap water *ad lib.* for a period of 2 weeks. Ten of these animals were then placed on a low sodium diet (Nutritional Biochemicals Corp., Cleveland, Ohio, Na content 4.33 meq/kg) and distilled water while the remaining animals were maintained as controls on regular diet and tap water. Half the animals were sacrificed at 2 weeks and the remainder after 3 weeks. All animals were killed by a blow on the head and adrenals promptly removed. One adrenal was fixed immediately in 10% formalin and the other frozen on dry ice. Multiple 30 $\mu$ cross sections were cut from each frozen adrenal in a cryostat (−18°C). These sections were placed on coverslips and fixed for 20 minutes in absolute alcohol, then submitted to the PAS reaction. The alcohol serves both to fix the tissue and also extracts considerable lipid. Alternate sections were predigested with saliva prior to application of the PAS procedure. The susceptibility of the PAS positive material to salivary diastase digestion was taken as confirmation of its identity as a glycogen. Blocks were made of the formalin fixed sections, embedded in paraffin and 10 $\mu$ sections cut on a rotary microtome and stained with hematoxylin and eosin.

*Results.* As observed by others(5,6) sodium depletion caused marked widening of the zona glomerulosa, the result of increased number and size of cells. In 2 weeks the width was doubled to tripled and by 3 weeks of sodium depletion the zone was about 4 times the size of the control glomerulosa. The fascicular and reticular area appeared to be compressed by the expanded glomerulosa. No glycogen was observed in the cells of either control or experimental glomerulosa, thus sharply distinguishing this zone metabolically as well as anatomically from the remaining cortex. The cells of the fascicular and reticular zones of both control and experimental groups contained abundant glycogen (Fig. 1, 2). Glycogen storage appeared to be increased in the outer fascicular

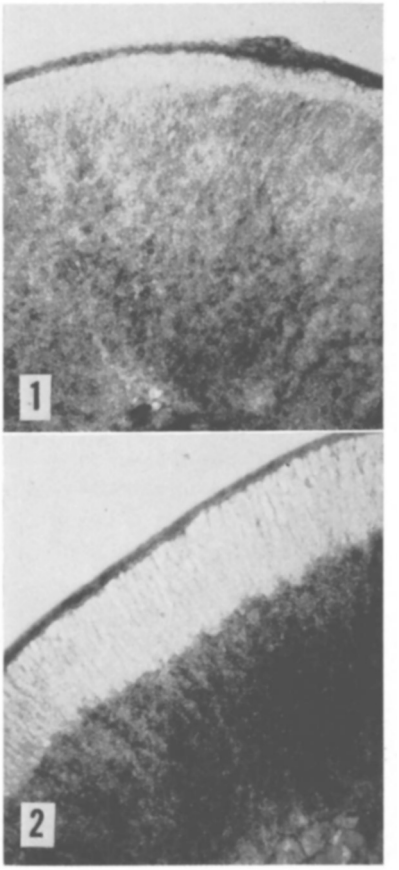


FIG. 1. Glycogen in adrenal cortex of control rat. The staining in the capsule is largely diastase resistant. Immediately beneath the capsule is the glomerulosa which is poor in glycogen. The fascicular zone and particularly the reticularis are rich in glycogen (PAS ×80).

FIG. 2. Glycogen in adrenal cortex of sodium depleted rat. The very broad band of glomerulosa remains glycogen poor. The fasciculata and reticularis retain glycogen. The apparent increase in glycogen in the outer fascicularis may depend on concentration due to compression of inner zones by expanded glomerulosa. (PAS ×80).

zone of the salt depleted animal. This may represent the concentration of glycogen in a slightly compressed area rather than an absolute increase.

*Discussion.* From observations of the control and experimental rat adrenals it is clear that large amounts of stainable glycogen accumulate in the fascicular and reticular zones. Neither the normal glomerulosa nor the glomerulosa stimulated by sodium deprivation appear to accumulate glycogen appreciably. If an important mechanism of ACTH action involves the stimulation of phosphorylase activity it would seem that for this action to be promptly effective stores of glycogen must be present as substrate. The facts that glycogen is accumulated in the inner cortical zones and is not stored in the glomerulosa may provide at least part of the basis for the differing metabolic responses of the zones to stimulation. Without data on glycogen turnover rates no comment can be made on its utilization in a given zone. The data referred to here do not exclude the possibility that glycogen may be actively metabolized in the glomerulosa cells but they do indicate that it is not stored.

*Summary.* Sodium depletion resulted in widening of the glomerulosa zone of the rat adrenal cortex. The enlarged zone resembled the glomerulosa of the control animal in that no glycogen was stored. This contrasted with the fascicular and reticular zones where abundant glycogen was found. It is suggested that differences in glycogen storage may at least in part explain differences in the immediate metabolic response to stimulation of the zones.

1. Planel, H., Guilhem, A., *C. R. Soc. Biol.,* 1958, v152, 165.
2. Cohen, R. B., *Endocrinology,* 1961, v68, 710.
3. Haynes, R. C., Berthet, L., *J. Biol. Chem.,* 1957, v225, 115.
4. Haynes, R. G., *ibid.,* 1958, v233.2, 1220.
5. Deane, H. W., Shaw, J. W., Greep, R. O., *Endocrinology,* 1948, v43, 133.
6. Hartroft, P. M., Eisenstein, A. B., *ibid.,* 1957, v60, 641.

Received October 25, 1961. P.S.E.B.M., 1962, v109.

## Nutritional Effects of β-Methyl-Aspartate and Methyl-Malonate on *Euglena gracilis* and *Ochromonas malhamensis.** (27156)

HELENE A. NATHAN AND HELEN B. FUNK
*Department of Biological Sciences, Goucher College, Towson, Md., and The Haskins Laboratories, New York City*

It was recently reported that the nutritional requirement of *Poteriochromonas stipitata* for Vit. $B_{12}$ could also be satisfied by β-methyl-aspartate(1). The specificity of this organism towards members of the $B_{12}$ group of vitamins is the same as that of *Ochromonas malhamensis* and of mammals (2). Since both *O. malhamensis* and *E. gracilis,* whose specificity is less strict, are widely used to assay Vit. $B_{12}$, we studied the effect of β-methyl-aspartate and of methyl-malonate, both products of $B_{12}$-catalyzed reactions (3,4) on the growth response of *O. malhamensis* and *E. gracilis* to $B_{12}$; full replacement of $B_{12}$ by β-methyl-aspartate or methyl-malonate would mean that the indispensable functions of $B_{12}$ for both protozoa had been identified, and would favor the likelihood that these findings also apply to man.

*Results. Euglena gracilis* Z strain, ATCC 12716 and *Ochromonas malhamensis,* ATCC 11532 were used. Maintenance methods and media, general experimental procedures, media for *E. gracilis* when grown at pH 3.6, and for *O. malhamensis* when grown at pH 5.0 have been described(5). Euglena was grown in *O. malhamensis* medium for experiments at pH 5.0. Ochromonas was grown in the medium described in Table XI by Nathan

* Supported by grant from U. S. Public Health Service.