Effect of ACTH on Blood Glutathione and Sulfhydryl Content of Liver and Kidney in Rats.* (18664)

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(Introduced by Maxwell Finland)

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The effects of the 11, 17 oxysteroids of the adrenal cortex on carbohydrate metabolism have long been recognized(1). ACTH may induce glycosuria and hyperglycemia in some human patients and such disturbances in the metabolism of carbohydrate have been accompanied by a lowered blood glutathione (GSH)(2,3).

It has been suggested that some of the effects of the adrenal cortical hormones may be explained on the basis of interference with transphosphorylation enzyme systems(4,5). Sulfhydryl (SH) groups have been shown to be essential for a variety of enzymic activities, including many enzyme systems concerned with anaerobic glycolysis and phosphorylation(6,7). Therefore, the effect of ACTH on the SH contents of rat blood, liver and kidneys was investigated and the effect of the hormone on the ascorbic acid contents of the organs was also determined.

Methods. Twenty-four white male rats of the Sprague-Dawley strain, each weighing between 160 and 190 g, were allowed to feed *ad lib.* on a standard pellet diet. Six animals were given 3 mg of ACTH‡ (Armour standard LA-1A) subcutaneously every 8 hours for 3 days, and 6 animals received 3 mg of ACTH every 8 hours for 10 days. Prior experiments had shown that this schedule of dosage produced sustained eosinopenia in the rats. The remaining 12 animals served as controls, but in order to avoid nonspecific adrenal activation associated with injection alone they were not given control injections(8). Following a fast of 4-10 hours, each animal was anesthetized with ether. Three ml of blood were collected in potassium and ammonium oxalate following section of the tail. Portions of the right lobe of the liver and decapsulated kidney, each about 500 mg in size, were quickly removed from the anesthetized animal and weighed to within .05 mg on a torsion balance. The tissues were then placed in Potter-Elvehjem homogenizing tubes containing ice-cold 0.85% saline which had previously been boiled. The final volume was brought to 5.0 ml, and the tissues were then homogenized. The ascorbic acid contents of the homogenates were determined by the macromethod of Mindlin and Butler(9), substituting 1.0 ml of homogenate for 1.0 ml of plasma. The nonprotein SH was measured by the amperometric method described by Kolthoff and Harris for mercaptans(10), adapted for use with amino acids and proteins by Benesch and Benesch (11), and further modified by Weissman, Schoenbach, and Armistead(12). Two ml of

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1. Ingle, D. J., Sheppard, R., Oberle, E. A., and Kuizenga, M. H., *Endocrinol.*, 1946, v39, 52.

2. Conn, J. W., Louis, J. H. and Johnston, M. W., *J. Lab. and Clin. Med.*, 1949, v34, 255.

3. Kass, E. H., Ingbar, S. H., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 669.

4. Colowick, S. P., Cori, G. T., and Slein, M. W., *J. Biol. Chem.*, 1947, v168, 583.

5. Verzar, F. and Montigel, C., *Helv. Chim. Acta*, 1942, v25, 9.

6. Barron, E. S. G. and Singer, T. P., *J. Biol. Chem.*, 1945, v157, 221.

7. Griffiths, M., *Arch. Biochem.*, 1949, v20, 451.

8. Kass, E. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, 1951, v37, 458.

9. Mindlin, R. L. and Butler, A. M., *J. Biol. Chem*, 1937-38, v122, 673.

10. Kolthoff, I. M. and Harris, W. E., *Ind. and Eng. Chem.*, Anal. Ed., 1946, v18, 161.

11. Benesch, R. and Benesch, R. E., *Arch. Biochem.*, 1948, v19, 35 and 1950, v28, 43.

12. Weissman, N., Schoenbach, E. B., and Armistead, E. B., *J. Biol. Chem.*, 1950, v187, 153.

the homogenate were diluted with 16 ml of boiled distilled water and 2.0 ml of 22% sulfosalicylic acid were then added. Five ml aliquots of the protein-free filtrate were brought to a volume of 50 ml with 90% methanol, and the SH contents were titrated in duplicate with .001 N silver nitrate. The results were expressed as mg of glutathione. The addition of several drops of a solution of sodium ethylene diamine tetraacetate (EDTA)§ stabilized both the SH and ascorbic acid contents of the filtrates by chelating any heavy metal ions present. Several more drops of EDTA were added to the filtrate-methanol mixture prior to titration. The total SH was determined by the method of Hughes(13). Methylmercury iodide in toluene was equilibrated with aliquots of the homogenate at pH 8.0, and the remaining methylmercury iodide titrated in duplicate with diphenylthiocarbazone. "Protein SH" was determined by subtracting the SH equivalent of the nonprotein SH from the total SH. Nitrogen concentrations of the homogenates were determined by the method of Johnson(14). The blood GSH was measured by the method of Woodward and Fry(15), and the blood sugar determined by the method of Reinecke(16).

Results. The findings in the blood are shown in Table I and those in liver and kidney are given in Table II. The blood sugar values in the rats receiving ACTH, under the conditions of fasting and anesthesia, were moderately elevated as compared with the controls. None, however, were in the diabetic range. Random urine samples showed only an occasional trace of sugar, and none of the animals was glycosuric following the short fast on the day it was sacrificed. No change in the blood GSH occurred under the influence of ACTH, nor were the erythrocyte counts significantly altered. The GSH content per million erythrocytes was in the normal range for rats(17).

TABLE I. Effect of ACTH on Blood Glutathione (GSH), Sugar and Erythrocyte Count of Rats.

ACTH given*	Blood GSH per million RBC, $\mu g \times 10^2$	RBC, $\times 10^6$/cu. mm	Blood sugar, mg/100 ml
Control	7.9 ± 1.2†	5.8 ± 0.8	109 ± 12
3 days	7.4 ± 1.5	6.7 ± 1.3	142 ± 18
10 days	7.6 ± 1.0	6.1 ± 0.9	138 ± 8

* 3 mg every 8 hr, subcutaneously.

† Values are expressed as the means ± standard deviation.

The mean nonprotein SH concentration in the kidney decreased within 3 days, but was not reduced further at the end of 10 days. These changes were significant statistically. There was no change in the mean nonprotein SH concentration in the liver after 3 days, but it was significantly increased after 10 days of administration of ACTH.

The mean concentration of ascorbic acid in the kidney was reduced in those rats given ACTH for 3 days, but showed no further change at the end of 10 days of therapy. No significant changes occurred in the hepatic concentration of ascorbic acid in either group receiving ACTH.

The "protein-SH" concentration per mg of nitrogen in either kidney or liver did not change significantly during the administration of ACTH.

ACTH apparently did not induce alterations in the hydration of the tissues as measured either by total nitrogen determinations or by erythrocyte counts.

Discussion. The blood GSH of human patients receiving ACTH has been reported to decrease in those individuals manifesting hyperglycemia or glycosuria(2,3). In these experiments, the blood GSH of the rats receiving ACTH was unchanged. The alterations in tissue nonprotein SH suggest that the measurement of GSH in the blood need not reflect the status of nonprotein SH compounds in the tissues. A dissociation between the blood and

§ "Sequestrene," kindly supplied by Alrose Chemical Co., Providence, R. I. (See Ref. 11).

13. Hughes, W. L., Jr., *Cold Spring Harbor Symposia,* 1949, v14, 79.

14. Johnson, M. J., *J. Biol. Chem.,* 1941, v137, 575.

15. Woodward, G. E., and Fry, G. E., *J. Biol. Chem.,* 1932, v97, 465.

16. Reinecke, R. M., *J. Biol. Chem.,* 1942, v143, 351.

17. Schultze, M. O., and Elvehjem, C. A., *J. Biol. Chem.,* 1936, v116, 711.

TABLE II. Effect of ACTH on Nitrogen, Sulfhydryl, and Ascorbic Acid Contents of Livers and Kidneys of Rats.

ACTH given*	Nonprotein SH, mg GSH/100 g	Total N, mg/100 mg	Protein SH, mg/100 mg N	Ascorbic acid, mg/100 g
		Liver		
Control	195 ± 14.6†	2.70 ± .36	1.43 ± .35	24.4 ± 1.7
3 days	196 ± 21.6	—	—	21.8 ± 3.9
10 days	228 ± 11.6‡	2.60 ± .12	1.36 ± .33	24.1 ± 3.0
		Kidney		
Control	166 ± 7.6	2.77 ± .42	1.05 ± .32	22.1 ± 2.1
3 days	129 ± 12.0‡	—	—	13.0 ± 2.4‡
10 days	119 ± 5.1‡	2.80 ± .29	1.29 ± .44	15.0 ± 2.1‡

* 3 mg every 8 hr, subcutaneously.
† Values are expressed as the means ± stand. dev.
‡ Differences from controls are statistically significant ($P < 0.01$ by "t" test of Fisher) (30).

tissue concentrations of GSH has also been demonstrated in scurvy(18). The values obtained for the hepatic nonprotein SH by the amperometric method agree quite well with hepatic GSH values obtained by the specific manometric method(19), indicating the GSH is the principal acid-soluble nonprotein SH compound in the liver. This correlates well with observations that the livers of rats contain enzymes capable of synthesizing but not hydrolyzing GSH(20). The increase in the hepatic nonprotein SH may therefore be explained by specific activation of enzymic synthesis of GSH in the liver, or by an increase in the availability to the liver of the constituent amino acids of glutathione, resulting from either increased hydrolysis of GSH in the kidney or from a catabolic phase of protein metabolism.

The renal nonprotein SH concentration as determined amperometrically was higher than that reported with the manometric method of GSH determination(19). The presence of a GSH-hydrolyzing enzyme in the rat kidney(20) may explain the occurrence in the kidney of large amounts of acid-soluble SH compounds other than GSH, and the changes induced by ACTH may represent loss of GSH or other SH compounds, or both. The decrease in the nonprotein SH of the kidneys of rats receiving ACTH is of interest in view of the evidence that altered renal tubular function occurs in human beings given large doses of ACTH or cortisone(21). The marked effects of various agents which inactivate sulfhydryl groups on the function and structure of the renal tubules are well known(22,23). It is of interest that the content of protein SH in neither kidney nor liver was altered by ACTH, despite the occurrence of changes in the nonprotein SH. Whether, as has been suggested(24), a diminution in GSH alone can alter in the kidney or elsewhere the activity of enzymes which depend on intact sulfhydryl groups remains to be determined.

Although the blood sugar values of the treated animals were higher than those of the controls, the possible hyperglycemic effect of the ether anesthesia(25) prevents precise interpretation. The hyperglycemia of the rats receiving ACTH may represent an increased responsiveness of these rats to anesthesia as a result of the increased glycogen content of the livers(26).

No explanation is apparent for the striking

18. Bersin, T., Koster, H., and Jusatz, H. J., *Z. physiol. Chem.*, 1935, v235, 12.

19. Woodward, G. E., *J. Biol. Chem.*, 1935, v109, 1.

20. Binkley, F. and Nakamura, K., *J. Biol. Chem.*, 1948, v173, 411.

21. Ingbar, S. H., Kass, E. H., Burnett, C. H., Relman, A. S., Burrows, B. A., and Sisson, H. J., *Proc. Second Clinical ACTH Conference*, Blakiston Co., 1951, v1, 130.

22. Gilman, A., Philips, F. S., Koelle, E. S., Allen, R. P., and St. John, E., *Am. J. Physiol.*, 1946, v147, 115.

23. Farah, A. and Maresh, G., *J. Pharm. and Exp. Therap.*, 1948, v92, 73.

24. Barron, E. S. G. and Singer, T. P., *Science*, 1943, v97, 356.

25. Beecher, H. K., *The Physiology of Anesthesia*, p236, Oxford Univ. Press, 1938.

decrease in the renal concentration of ascorbic acid under the influence of ACTH. Increased urinary excretion of ascorbic acid, possibly as a result of a change in renal function, has been reported in human beings receiving ACTH(27). The administration of doses of cortisone adequate to suppress the growth of granulation tissue does not significantly alter the concentration of ascorbic acid in the plasma or muscles of rabbits(28). There is evidence suggesting that GSH serves to maintain ascorbic acid in the reduced state *in vitro*, and the amounts of ascorbic acid and GSH in the tissues may vary together under certain circumstances(18,29). Whether the reduction in renal nonprotein SH observed in this experiment had any causal relationship to the concomitant decrease in the concentration of ascorbic acid is not clear at this time.

Summary. Rats given ACTH for 3 to 10 days in doses sufficient to produce sustained eosinopenia when compared with controls, showed: (1) no change in the blood glutathione; (2) decreased nonprotein sulfhydryl contents of the kidneys; (3) increased nonprotein SH contents of the livers; (4) markedly decreased renal ascorbic acid; (5) no change in the ascorbic acid contents of the livers; and (6) no change in the protein sulfhydryl contents of the livers or kidneys.

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26. Hartman, F. A. and Brownell, K. A., *The Adrenal Gland,* p237, Lea and Febiger, 1949.

27. Beck, J. C., English, M. M., Hackney, J. W., and Mackenzie, K. R., *J. Clin. Invest.* (Abst.), 1950, v29, 798.

28. Howes, E. L., Plotz, C. M., Blunt, J. W., and Ragan, C., *Surgery,* 1950, v28, 177.

29. Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C., *J. Biol. Chem.,* 1937, v117, 237.

30. Fisher, R. A., *Statistical Methods for Research Workers,* 10th Ed., 1946, Oliver and Boyd, London.

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Propagation of Poliomyelitis Virus in Cultures of Monkey and Human Testicular Tissues.*† (18665)

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It was recently established that propagation in tissue culture of members of the poliomyelitis group of viruses can be affected in extraneural tissues(2-5) as well as in neural tissues(1-4). Human embryonic brain and cord were employed for the cultivation of the MV(1), Lansing(2-4), and Brunhilde(3) strains; a mixture of human embryonic skin, muscle and connective tissue for the Lansing(2-4) and Brunhilde(3) strains; human foreskin and subcutaneous tissues from patients ranging in age from 4 to 11 years for the Lansing strain(3); human embryonic intestine for the Lansing strain(2,4); human adult testicular tissue for the Lansing and Hof.

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‡ National Research Council Fellow in Poliomyelitis.

1. Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 357.

2. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science,* 1949, v109, 85-87.

3. Weller, T. H., Robbins, F. C., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v73, 153.

4. Milzer, A., Levinson, S. O., Vanderboom, K., and Adelman, P., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 136.

5. Smith, W. M., Chambers, V. C., and Evans, C. A., *Northwest Med.,* 1950, v49, 368.