nen-3β-ol-20-one,[18] which represents one of the intermediates[3] in our synthesis of cortisone.

(18) H. B. MacPhillamy and C. R. Scholz, *J. Biol. Chem.*, **178**, 37 (1949).

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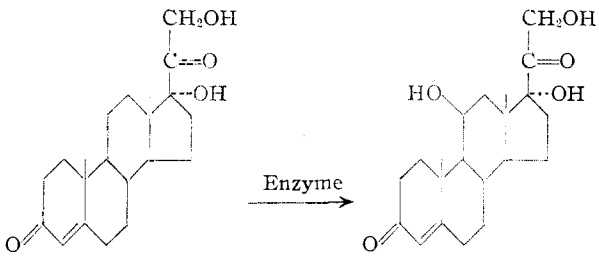
ENZYMATIC SYNTHESIS OF 17-HYDROXYCORTICOSTERONE

Sir:

The biosynthesis of 17-hydroxycorticosterone has been demonstrated in intact adrenal glands,[1] extirpated adrenal glands perfused with a medium containing 17-hydroxy-11-desoxycorticosterone acetate[2] and adrenal gland homogenates to which 17-hydroxy-11-desoxycorticosterone had been added as the substrate.[3] We wish to report the oxidation of 17-hydroxy-11-desoxycorticosterone to 17-hydroxycorticosterone by an enzyme system associated with the insoluble cellular constituents of the adrenal cells.



17-Hydroxy-11-desoxycorticosterone — 17-Hydroxycorticosterone

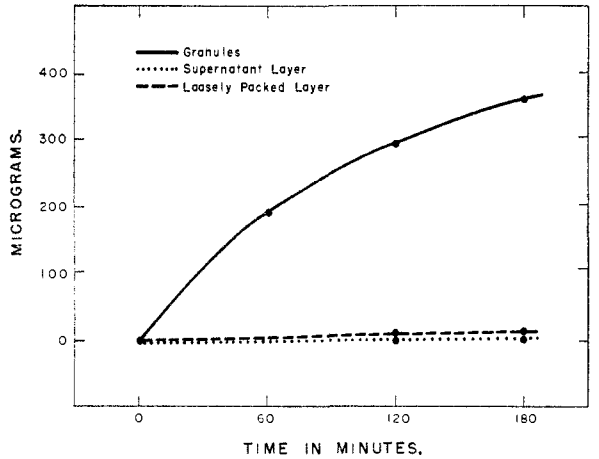


Fig. 1.—17-Hydroxycorticosterone formed in differentially centrifuged fractions of a beef adrenal homogenate incubated with 400 micrograms of 17-hydroxy-11-desoxycorticosterone in a buffer composed of 0.01 *M* glucose, 0.062 *M* NaCl, 0.04 *M* ($NaHPO_4$–NaH_2PO_4, *p*H 7.0), 0.025 *M* KCl, 0.01 *M* sodium fumarate and 0.004 *M* $MgSO_4$.

(1) D. H. Nelson, H. Reich and L. T. Samuels, *Science*, **111**, 578 (1950).

(2) O. Hechter, R. P. Jacobsen, R. Jeanloz, H. Levy, C. W. Marshall, G. Pincus and V. Schenker, *Arch. Biochem.*, **25**, 457 (1950).

(3) D. A. McGinty, G. N. Smith, M. L. Wilson and C. S. Worrel, *Science*, **112**, 506 (1950).

Frozen beef adrenals were thawed at 2°, minced, suspended in buffer solution or 0.88 *M* sucrose solution and homogenized with a Potter–Elvehjem type homogenizer. The resulting homogenates were differentially centrifuged according to the methods of Schneider and Hogeboom[4] and the various supernatant and sedimented layers were tested for enzyme activity.

To test for enzyme activity, portions of the sediments or supernatants in amounts equivalent to 1 g. of original tissue were incubated from one to five hours in 5 ml. of buffer containing 400 micrograms of 17-hydroxy-11-desoxycorticosterone. After incubation, the mixture was extracted with chloroform, the chloroform evaporated to dryness and the residue partitioned between petroleum ether and 70% ethanol. The 70% ethanol phase was evaporated to dryness and the 17-hydroxycorticosterone formed was determined by the fluorescence and by chromogens produced with sulfuric acid. For the identification of the products, the residues of several incubations were combined, dissolved in chloroform and chromatographed on silica gel with 7–10% ethanol in chloroform.

The figure shows the amount of 17-hydroxycorticosterone formed by fractions separated by differential centrifugation between 2000 *g* for ten minutes and 19000 *g* for 30 minutes. It will be seen that essentially all of the activity is in the granules while only traces are present in the supernatant layer or the loosely packed layer above the granules. The reaction proceeds best at a *p*H near the neutral point and at temperatures between 35 and 40°. Above 40° there is a rapid decline in activity. It is inhibited by 0.01 *M* diethyl dithiocarbamate, partially inhibited with 0.01 *M* HCN and disodium versonate and slightly inhibited with 0.01 *M* sodium azide. With the buffer used, fumarate ion is necessary for the reaction. Adenosinetriphosphate does not enhance the rate of conversion.

The product of the reaction has been isolated in crystalline form. The crystals separated in small striated cylinders and cruciform aggregates and melted at 199–205°. A second crystallization yielded crystals which melted at 204–208° and gave no depression of melting point with an authentic sample of 17-hydroxycorticosterone obtained by saponification of 17-hydroxycorticosterone acetate. The product gave a distinct green fluorescence when treated with sulfuric acid and when chromatographed according to the methods of Zaffaroni[5] positioned itself in the regions characteristic of 17-hydroxycorticosterone. Both the specific rotation and infrared spectrum were shown to conform to those of 17-hydroxycorticosterone.

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(4) W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, **183**, 123 (1950).

(5) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *ibid.*, **188**, 763 (1951).