TABLE II

	C.p.m./cm² at infinite thickness	
	Ba tetrahydroPGA	HCHO dimedone
Before treatment with 0.1 *M* HCHO	7,168	—
After 1st treatment	6,556	561
After 2nd treatment	6,194	220

As a final proof of dissociation, saturated dimedone solution was added to a solution of Ba tetrahydroPGA (6,194 c.p.m./cm² at infinite thickness) in O_2-free water. White crystals immediately commenced to separate and after standing were dissolved by the addition of ethanol. The Ba tetrahydroPGA was precipitated, washed with ethanol and prepared for radioactivity determination. It had 2,060 c.p.m./cm² at infinite thickness. The ethanolic supernatants from the precipitation of the barium tetrahydroPGA were treated with carrier formaldehyde and the dimedone compound crystallized. It had 1,750 c.p.m./cm² at infinite thickness. The expected radioactivity of the formaldehyde calculated from the loss of activity of the Ba tetrahydroPGA was 1,420 c.p.m./cm².

These results indicate that formaldehyde combines non-enzymically with tetrahydroPGA to form N^5-hydroxymethyltetrahydroPGA, a compound which dissociates appreciably in solution. On incubating enzyme, glycine and N^5-hydroxymethyltetrahydroPGA labelled in the hydroxymethyl group with ^{14}C, approximately 69 % of the ^{14}C appeared in the serine synthesised. A similar result was obtained with N^5-hydroxymethyl-N^{10}-formyltetrahydroPGA. Present evidence therefore suggests that N^5-hydroxymethyltetrahydroPGA reacts on the enzyme surface with the Schiff's base of glycine and pyridoxal phosphate to form the Schiff's base of serine and pyridoxal phosphate with regeneration of tetrahydroPGA.

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[1] R. L. BLAKLEY, *Biochem. J.*, (1957) (in the press).
[2] R. L. KISLIUK, *Federation Proc.*, 15 (1956) 289.

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Preliminary Notes

Studies on the nature of steroid 11-β hydroxylation

Previous work in several laboratories has shown that adrenal preparations are able to hydroxylate steroids at the 11-position in the presence of TPNH and molecular oxygen[1,2]. Studies with ^{18}O have indicated that the oxygen atom which is introduced is derived exclusively from the gaseous atmosphere and not from the solvent[3,4,5]. These findings suggest a similarity between the mechanisms for the hydroxylation of steroids and of aromatic compounds, as well as a number of apparently unrelated oxidations[6,7].

The present communication deals with further studies on steroid 11-β hydroxylation carried out with extracts of calf adrenal acetone powder which were prepared by homogenization in 0.1 *M* tris(hydroxymethyl)aminomethane (TRIS) buffer, pH 7.4, containing 0.5 % digitonin and in which enzyme activity was not sedimentable on centrifugation at 100,000 × *g* in 1 h. The reaction was detected by a modification of the method described by SWEAT[8] based on the fact that in acid-ethanol mixtures, 11-hydroxysteroids display an intense fluorescence not shown by their 11-deoxy analogues. The substrates were 4-pregnen-21-ol-3,20-dione (DOC) and 4-pregnene-17,21-diol-3,20-dione (compound *S*), which on 11-hydroxylation yielded 4-pregnene-11,21-diol-3,20-dione (compound *B*) and 4-pregnene-11,17,21-triol-3,20-dione (compound *F*), respectively. These reaction products were further identified by paper chromatography[9].

The data presented in Table I indicate that in the presence of TPNH and O_2, at least two distinct enzymes were required to catalyze the overall reaction. Furthermore, it can be seen that one of these enzymes was present not only in the adrenal, but also in extracts of rabbit liver.

Rat spleen and lung were also found to reactivate the aged adrenal extract. Liver catalase, horseradish peroxidase, hydrogen peroxide, or a hydrogen peroxide generating system (glucose and glucose oxidase) were unable to replace this second, widely disseminated, enzyme fraction.

TABLE I

ENZYME REQUIREMENTS FOR 11-β HYDROXYLATION

The reaction mixture contained TPNH, 0.2 μmoles; DOC, 0.058 μmoles; TRIS buffer, pH 7.4, 8 μmoles; and enzyme in a volume of 1.0 ml. 10 min incubation at 37° C in air.

Enzyme	*μmoles product formed*
Initial adrenal mitochondrial extract	0.030
Frozen and thawed mitochondrial extract (Extract II)	0
Extract II + liver extract	0.041
Extract II + boiled liver extract	0.001
Liver extract	0
Liver extract + boiled extract II	0

Additional observations, some of which are shown in Table II, suggest that at least one of the components involved in the hydroxylation of DOC differs from that required for compound *S* hydroxylation. This conclusion is based on the wide variation in the ratio of activities towards *S* and DOC from preparation to preparation and in the same preparation after ageing or freezing and thawing.

TABLE II

RELATIVE HYDROXYLATING ACTIVITIES OF SEVERAL ADRENAL PREPARATIONS

Reaction conditions as in Table I with substrate as indicated below.

Preparation	*Relative activity*		*DOC/S*
	S	*DOC*	
1	26	71	2.8
2	7	60	8.6
3	0	0	—
3 + liver extract	5	74	14.8
4	0	39	—

It would appear, then, that 11-β hydroxylation is a more complex process than imagined heretofore, and that the concept of an "11-β hydroxylase" which functions for all substrates regardless of their structure, cannot adequately explain the present results.

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[1] M. HAYANO AND R. I. DORFMAN, *J. Biol. Chem.*, 211 (1954) 227.
[2] A. C. BROWNIE AND J. K. GRANT, *Biochem. J.*, 62 (1955) 29.
[3] M. HAYANO, M. C. LINDBERG, R. I. DORFMAN, J. E. H. HANCOCK AND W. VON E. DOERING, *Arch. Biochem. Biophys.*, 59 (1955) 529.
[4] B. M. BLOOM, M. HAYANO, A. SAITO, D. STONE AND R. I. DORFMAN, *Federation Proc.*, 15 (1956) 222.
[5] M. L. SWEAT, R. A. ALDRICH, C. H. DE BRUIN, W. L. FOWLKS, L. R. HEISELT AND H. S. MASON, *Federation Proc.*, 15 (1956) 367.
[6] B. B. BRODIE, J. AXELROD, J. R. COOPER, L. GUADETTE, B. N. LADU, C. NUTOMA AND S. UDENFRIEND, *Science*, 121 (1955) 603.
[7] C. MITOMA, *Arch. Biochem. Biophys.*, 60 (1956) 467.
[8] M. L. SWEAT, *Anal. Chem.*, 26 (1954) 773.
[9] I. E. BUSH, *Biochem. J.*, 50 (1952) 370.

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