Short Communications and Preliminary Notes

The metabolism of pregnenolone by rat liver homogenate

Pregnenolone (pregn-5-en-3β-ol-20-one) has been assigned an important role in the biogenesis of the adrenocortical steroids since in experiments with isolated bovine adrenal glands it was converted by a one-cycle perfusion to progesterone and, by an eight-cycle perfusion to corticosterone and 17-hydroxycorticosterone[1]. The conversion of pregnenolone to progesterone has also been effected by incubation with preparations of adrenal glands and other endocrine tissues; diphosphopyridine nucleotide (DPN) appears to act as hydrogen acceptor in this system. Although no conversion to progesterone was observed when pregnenolone was incubated with liver and kidney preparations, no attempt was made to ascertain whether the pregnenolone had undergone any other changes during the incubations[2].

The metabolism of pregnenolone by rat liver homogenate has now been investigated more fully. The steroid (200 μg) dissolved in 0.04 ml propylene glycol was incubated in a phosphate-saline medium (pH 7.4) with 150 mg (wet-weight) rat liver as homogenate prepared in 0.15 *M* KCl. Pregnenolone was determined by a modification of a method involving formation of its dinitrophenylhydrazone, separation from other material by chromatography on alumina and estimation of the pregnenolone dinitrophenylhydrazone by spectrophotometry[3]. The results of incubating pregnenolone under a variety of conditions are shown in Table I.

TABLE I

EFFECT OF VARIOUS COMPOUNDS ON THE METABOLISM OF PREGNENOLONE BY RAT-LIVER HOMOGENATE

Compound added	*Concentration mM*	*Gas phase*	*Pregnenolone metabolized* in 2 h, µg*
Nil	—	Air	107 (103–112)
Nicotinamide	20	Air	109 (104–116)
ATP	1.0	Air	110 (104–113)
DPN**	1.0	Air	138 (137–141)
DPN	2.0	Air	148 (148–150)
TPN	1.0	Air	120 (118–123)
Nicotinamide	20	N_2	60 (50– 70)
Controls			6 (3– 12)

* Values are the means of at least two experiments carried out in duplicate; figures in parentheses give the range.

** In experiments involving addition of pyridine nucleotides the homogenate was prepared in KCl-nicotinamide to give a nicotinamide concentration in the final reaction mixture of 20 m*M*.

These results indicate that pregnenolone is metabolized by rat liver *in vitro*. Metabolism is increased by addition of pyridine nucleotides and DPN appears to be a more effective co-factor than TPN under the conditions employed. From the lack of effect of ATP it would appear that high-energy phosphate bonds are not involved. Since metabolism is depressed under anaerobic conditions, an oxidative process may be involved.

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Received July 9th, 1955