Metabolism of Drugs. III[2]. The Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (1).

In earlier papers in this series,[1,2] it was reported that a metabolite of ethylhexabital (5-cyclohexenyl-5-ethylbarbituric acid) was isolated from the urine of rabbits receiving ethylhexabital and its chemical structure was confirmed as 5-(3′-oxo-1′-cyclohexen-1′-yl)-5-ethylbarbituric acid.

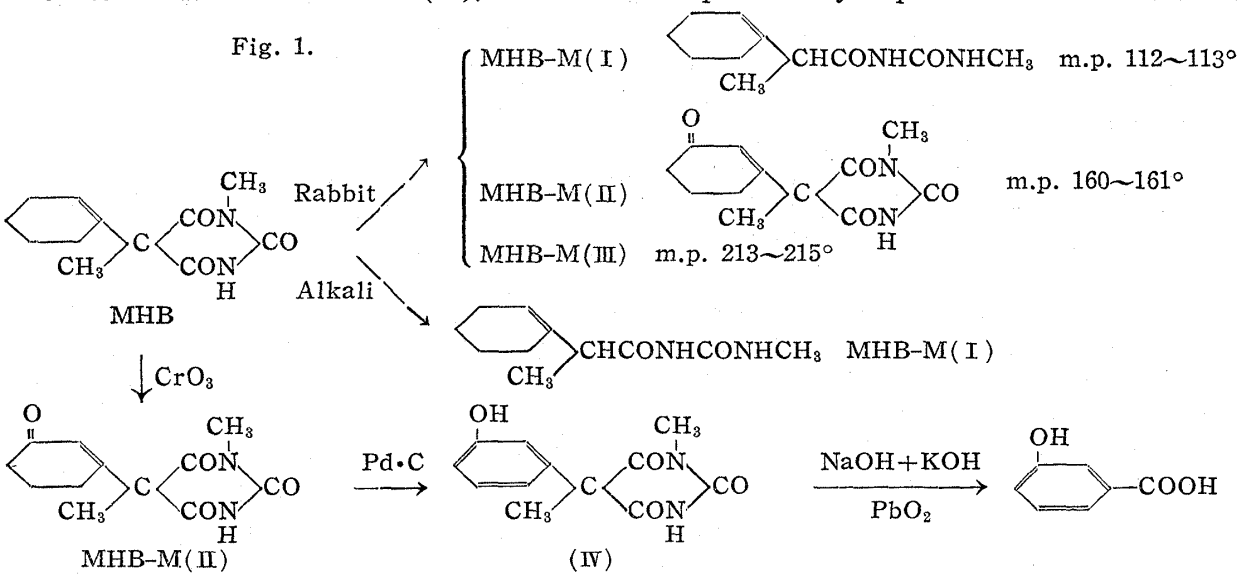
By analogy with this, the present study on the metabolic fate of methylhexabital J. P. (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid, Evipan) was undertaken and the structures of two of the metabolites were established.

The urine of rabbits receiving MHB by stomach tube was continuously extracted with ethyl acetate at pH 4~5 for 15 hours and chromatographed through an alumina column. The first eluate was a mixture of metabolites and the latter principally contained urea. The following metabolites were isolated from this mixture: (I) Colorless needles, m.p. 112~113°; (II) colorless plates, m.p. 160~161°(decomp.); (III) colorless plates, m.p. 213~215°(decomp.).

By admixture, the structure of (I) was confirmed as cyclohexenylmethylaceto-N-methylureide, m.p. 113~114°, which was prepared by the decomposition of MHB-Na according to Sato's method.[3] Hitherto, several studies *in vivo*[4~9] on the destruction of the barbituric acid ring have been described, but in these cases, either only a negligible amount of the destroyed metabolites or the presumptive results had been obtained.

(II) was found by elementary analyses and admixture to be identical with an oxidation product, m.p. 160~161°, of MHB with chromic acid. Further, the formation of a 2,4-dinitrophenylhydrazone, m.p. 228~230°(decomp.), established this substance to be a ketone. With reference to (II), Bush *et al.*[10] previously reported that a metabolite

Fig. 1.



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of m.p. 162~164° was isolated from the urine of dogs receiving MHB and they assumed its structure to be 5-cyclohexenonyl-3,5-dimethylbarbituric acid by the elementary analysis, the formation of an oxime, and the ultraviolet absorption spectrum. It is assumed that (II) is identical with the Bush's product, but they have not chemically clarified the position of the ketone in the cyclohexenyl ring yet.

In our present experiments, (II) was converted to 5-hydroxyphenyl-3,5-dimethylbarbituric acid (IV) by aromatization reaction with 5% palladium-charcoal and (IV) was hydrolyzed and oxidized to *m*-hydroxybenzoic acid by alkali fusion according to the procedures described in the previous paper.[2] Thus, the structure of (II) was confirmed as 5-(3'-oxo-1'-cyclohexen-1'-yl)-3,5-dimethylbarbituric acid. The structure of (III) is being studied at present. The above reactions may be represented as shown in Fig. 1.

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August 1, 1955.

The *in vitro* Metabolism of Ethylhexabital by Rat Liver Slices.

From the fact that the treatment with some liver poisons, partial hepatectomy, or complete nephrectomy prolonged the duration of anesthesia produced by various barbiturates in animals, it was suggested[1] that the liver and kidneys played important roles in the detoxication of barbiturates *in vivo*. By the use of the *in vitro* technique, it was shown that slices or brei of the liver or kidneys were capable of metabolizing the barbiturates[2] and that some metabolites isolated by the *in vivo* experiments were also identified, e.g. a ketoevipal from Evipal[3] and pentobarbital from Thiopental.[4] Utilizing the liver homogenate fortified with some cofactors and substrates, studies on enzymatic systems involved in the biotransformation of barbiturates were carried out.[3,5]

Previously, it had been reported[6,7] that 5-(3'-oxo-1'-cyclohexen-1'-yl)-5-ethylbarbituric acid (3-keto-EHB)† was isolated from the urine of rabbits receiving ethylhexabital (EHB, Phanodorm). In this communication, it is shown that 3-keto-EHB is produced by the *in vitro* metabolism of EHB.

Stoppered Erlenmeyer flasks (50 cc.) containing 500 mg. of liver slices of male rat and various amounts of EHB in a total volume of 10 cc. of Krebs-Ringer phosphate buffer (pH=7.4), saturated with oxygen and containing 0.2% glucose, were shaken in a Wahrburg bath at 38° for 0.75 to 6 hrs., the shaking rate being approximately 120 oscillations per minute. Oxygen was passed through the mixture for one minute

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† 3-Keto-EHB is the same as EHB-M in the preceding papers.