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.

*Pharmaceutical Institute*
*Medical Faculty*
*University of Kyushu*
*Katakasu, Fukuoka*

Hisao Tsukamoto (塚元久雄)
Hidetoshi Yoshimura (吉村英敏)

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## 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.

at the beginning of the incubation period. After incubation, the flasks were immersed in a boiling water bath for five minutes and inactivated. The flask content was made acidic with one drop of concentrated hydrochloric acid and extracted with ether. Ether layer was separated, treated with anhydrous sodium sulfate and activated charcoal, filtered, and evaporated. The residue was dissolved in methanol, and chromatographed on a paper with butanol saturated with 5 $N$ ammonia as the developer. After drying the paper, two parts of the paper strip, Rf 0.20~0.45 for 3-keto-EHB and Rf 0.45~0.70 for EHB, were cut out, eluted with pH-11 borate buffer, and their absorption was determined at 230, 239, and 250 m$\mu$ by a spectrophotometer. From the optical density at 239 m$\mu$, the amounts of 3-keto-EHB and EHB were calculated. Analytical recovery was approximately 90%. The accompanying table shows the result of the *in vitro* metabolism of EHB. All data shown are average of three or four determinations. Further details of these experiments will be reported in a near future.

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| Incubation period (hrs.) | EHB added, $\gamma$ | EHB remained, $\gamma$ | 3-Keto-EHB formed, $\gamma$ |
|---|---|---|---|
| 3 | 256 | 104 | 51 |
| 3 | 320 | 158 | 69 |
| 3 | 400 | 201 | 71 |
| 3 | 500 | 264 | 116 |
| 3 | 625 | 399 | 123 |
| 0.75 | 500 | 416 | 56 |
| 1.5 | 500 | 367 | 83 |
| 3 | 500 | 271 | 104 |
| 6 | 500 | 246 | 137 |
| 3* | 500 | 439 | 0 |

* The flask was immersed in a boiling water bath before the incubation.

*Pharmaceutical Institute*
*Medical Faculty*
*University of Kyushu*
*Katakasu, Fukuoka*

Eigo Takabatake (高畠英伍)

August 1, 1955.