[Chem. Pharm. Bull. 19(4) 827—830 (1971)]

UDC 615.31.076.9 : 577.15.084

Studies on Drug Metabolism. XII.[1] Activity of Liver Microsomal Drug-Metabolizing Enzymes in Human Liver

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(Received December 24, 1970)

An attempt was made to estimate the activity of microsomal drug-metabolizing enzymes of the human liver at the time of death using post-mortem human livers. The human livers were placed under various conditions, and from the rate of loss of the activities, the activities at the time of death were estimated. The increase in the formation of formaldehyde-like substance in proportion to the period after death was also observed.

Introduction

Although difference between species, especially that between man and laboratory animals, is one of the important problems for applying results obtained with laboratory animals to man, only a few studies on the enzyme activities using human liver have been reported.

Kuntzman, *et al.*[3] compared enzymatic activities of human and rat's liver as regards the ring hydroxylation of 3,4-benzopyrene, the side-chain oxidation of pentobarbital, the O-dealkylation of acetophenetidine, and the N-dealkylation of 3-methyl-4-monomethylaminoazobenzene.

Creaven, *et al.*[4,5] studied the activities in the course of time of liver enzymes in hydroxylation of biphenyl and coumarine, using rat and rabbit liver kept at room temperature or 37° or in deep freeze. They found that the activities disappeared in the liver kept without freezing, while only a small loss was seen in the frozen liver. Moreover, their preliminary studies on five samples of human liver obtained in routine *post-mortems* (4—28 hr after death) showed that four of these livers were able to hydroxylate biphenyl, the fifth one giving only traces of hydroxylation, and only one hydroxylated coumarine to an appreciable degree.

In the present work, we investigate whether the disappearance of hepatic enzyme activities is close to linear after death in the livers of rats, monkeys, and humans. Then an attempt is made to estimate the enzyme activities at zero time after death.

Moreover, as in a preliminary experiment a formaldehyde-like substance was found to increase as the time increases after death, this phenomenon was examined in all the subjects used.

Experimental

Liver——1. Monkey: Four male adult monkeys (*Macaca irus* F. Cuvier) weighing 1.1—4.0 kg were used. Each of the three livers was soaked in 1.15% KCl solution and kept at 36°, 7°, and 24°. The 4th monkey died of acute illness, its liver was isolated from the body 7 hr after death, kept at 24°. The livers were used the time course study on enzyme activities.

1) Part XI: H. Kitagawa, T. Kamataki and Y. Tanabe, *Chem. Pharm. Bull.*(Tokyo), **19**, 221 (1971).
2) Location: *1-33 Yayoi-cho, Chiba-shi.*
3) R. Kuntzman, L. C. Mark, L. Brand, M. Jacobson, W. Levin and A. H. Conney, *J. Pharmacol. Exptl. Therap.*, **152**, 151 (1966).
4) P. J. Creaven, D. V. Parke and R. T. Williams, *Biochem. J.*, **85**, 5 (1962).
5) P. J. Creaven and R. T. Williams, *Biochem. J.*, **87**, 19 (1963).

2. Rat: Five adult Wistar rats were killed by a blow on the head, and the carcasses were placed in a temperature controlled room at 24°. The livers were isolated at different intervals after death and the activities were measured.

3. Man: Adult human livers which were isolated in judicial or administrative dissections with known time of death were soaked in 1.15% KCl solution and kept at 24° in a temperature controlled room. By cutting off about 10 g of the liver at intervals of 9—12 hr, the activities of drug-metabolizing enzymes of liver microsomes were determined. The livers used in this experiment were obtained from individuals without any history of drug poisoning or liver illness.

Measurement of Drug-Metabolizing Enzyme Activities——1. Preparation of Liver Microsomal Fraction: Ten grams of liver were added with 4 volumes of 1.15% KCl solution, and homogenized in a Potter type Teflon homogenizer in ice. The homogenate was centrifuged at −2.0—2.0°,9000×*g* for 20 min. The supernatant was used as the liver microsomal fraction.

2. Preparation of Incubation Mixture: Aniline (0.5 mg) and aminopyrine (1 mg) were used as substrates for estimating the hydroxylase and N-demethylase activities of each substrate. The contents of co-factors have been described previously.[6]

3. Determination of *p*-Aminophenol (Estimation of Aniline Hydroxylase Activity): We used the same method as that described previously,[6] which is a modification of the method of Lester[7] and Brodie.[8]

4. Determination of 4-Aminoantipyrine (Estimation of Aminopyrine N-Demethylase Activity): The method of determination has also been described previously,[6] which is a slight modification of the method of Brodie.[9]

5. Determination of Formaldehyde-Like Substance: Formation of a formaldehyde-like substance, which was found to increase in the human liver kept at 24° in proportion to the period after death, was determined by the method of Nash.[10] The calibration curve was made from pure formaldehyde.

Result

Drug-Metabolizing Enzyme Activities

The aniline hydroxylase activity in the following figures is expressed as μg of *p*-aminophenol formed by 1 g of liver during 1 hr incubation. The results on laboratory animals are shown in Fig. 1.

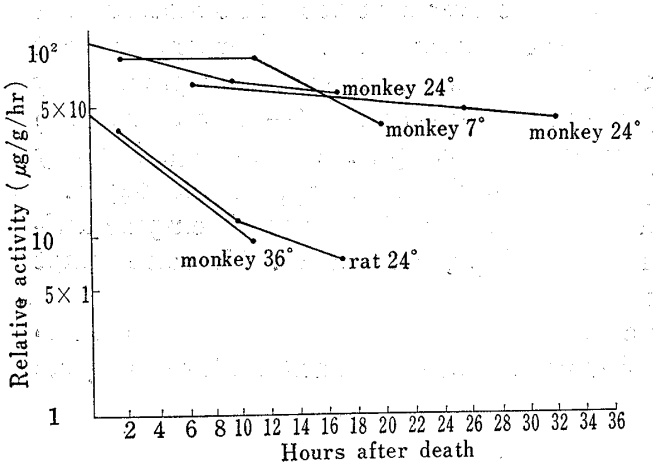

Fig. 1. Periodical Decrease of Aniline Hydroxylase Activity in Monkeys and Rats

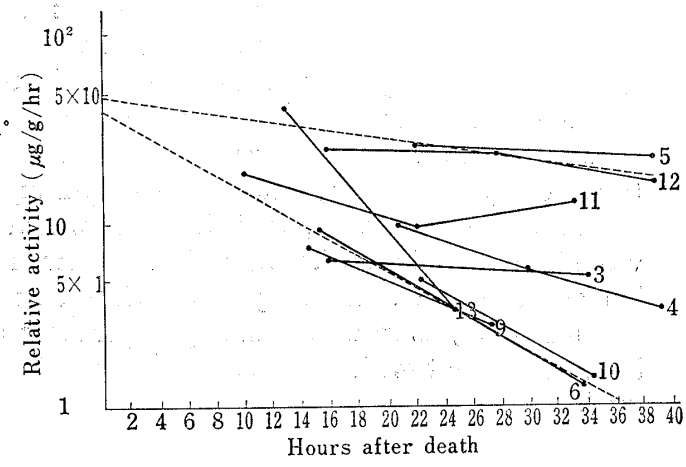

Fig. 2. Periodical Decrease in Aniline Hydroxylase Activity in Human Liver

A monkey liver kept at 7° did not show any significant decrease of activity during first 11 hr but during the following 9 hr the decrease appeared. On the other hand, in the liver kept at 36°, most of the activity was lost during the first 11 hr. At 24°, the loss of activity

6) H. Kitagawa, S. Yoshida and T. Kamataki, *Yakugaku Zasshi*, **88**, 954 (1968).
7) B. B. Lester and L. A. Greenberg, *J. Pharmacol. Exptl. Therap.*, **90**, 68 (1947).
8) B. B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **94**, 22 (1948).
9) B. B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **99**, 171 (1959).
10) T. Nash, *Biochem. J.*, **55**, 416 (1953).

was less than at 36°, and apparently it was linear all along the experiment. At 24°, the rat liver lost its activity more rapidly than the monkey one. About 200 g of human liver was soaked in 1.15% KCl solution and kept at 24°, and 10 g of this liver was used for measurement of the activity, at each time. The time course of the enzyme activity of human liver is shown in Fig. 2.

The numbers in the figure represent the number of subjects used in the experiment. Description of each subject is given in Table I.

The time course of decrease in the activities after death was found in all subjects to a different extent.

Table I. Description of Subject

Liver No.	Age	Sex
1	27	♀
2	25	♂
3	40	♂
4	46	♀
5	28	♀
6	32	♀
7	20	♀
9	57	♂
10	66	♂
11	73	♂
12	39	♀
13	32	♂

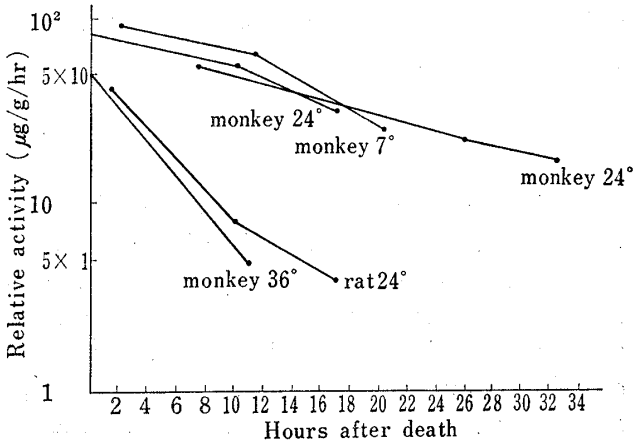

Fig. 3. Periodical Decrease of Aminopyrine N-Demethylase Activity in Monkeys and Rats

In estimating the enzyme activity at zero time after death as shown in this figure, the human liver activity was assumed to be about 40—50 μg/g/hr, and this activity seems to be not as low as that of animals. The rate of decrease in aminopyrine-N-demethylase activity was similar at 7° and 24° but more rapid at 36°. The activity of the rat disappeared at 24° more rapidly than that of the monkey. The enzyme activity of the rat at zero time after death was estimated to be 53 μg/g/hr, and that of the monkey seemed to be about 60—100 μg/g/hr (Fig. 3).

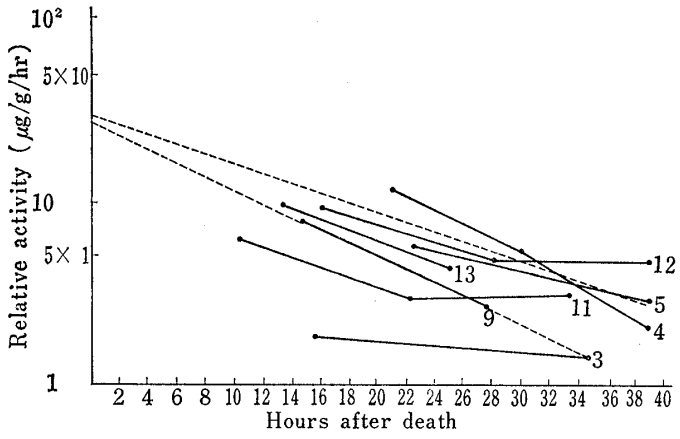

Fig. 4. Periodical Decrease of Aminopyine N-Demethylase Activity in Human Liver

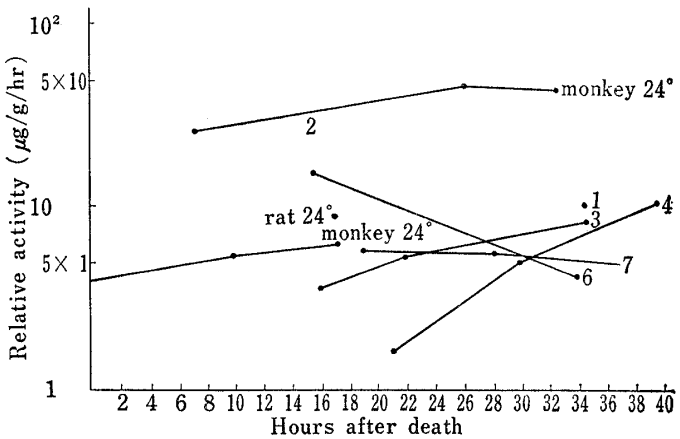

Fig. 5. Increase of the Formation of Formaldehyde-like Substance

The results of the experiment using human liver are shown in Fig. 4. The decrease in the activities are approximately linear after death. The human activity was estimated to be about 30 μg/g/hr.

Formation of Formaldehyde-Like Substance

It was found that, in the incubation mixture incubated at 37° for 30 min after the addition of co-factors to the microsomal fraction, the formation of formaldehyde–like substance detectable with the Nash method increased. This formation was in proportion to the period after death, but was not correlated with the activities of drug–metabolizing enzymes (Fig. 5).

The liver of No. 7 was kept relatively cold from 4 hr after death, and it was kept at 7° in our experiment. The body containing the liver of No. 6 was placed in warmer conditions, when we could get the liver (13 hr after death), the body temperature was 33°. The tendency of increase in formation of the substance was found in all subjects except No. 6 and No. 7. In the experiments with the monkey's and the rat's liver, the increase in the formation of formaldehyde–like substance was also found. Especially in the case of the rat, the formation was slight till 9 hr after death but important after next 8 hr.

Discussion

Although considerable activity of drug–metabolizing enzyme was found in normal human liver even at the time of 10 hr after death, nothing of that kind was found in two livers with cirrhosis. The estimated enzyme activities in the human liver at zero time after death was made on the assuamption that the corpses had been placed at the temperature of the room (24°) after death till our obtaining them. The temperature of the body might be very slowly decreasing before being handed over to us and, therefore, the loss of the activities prior to our experiments might have been as much the same as kept at 36°. Thus the true activities of human livers at the time of death might be higher than those estimated from our experiments.

Acknowledgement We should like to express our appreciation to Professor Dr. Yoshiro Fukuda, School of Medicine, Juntendo University, Professor Dr. Yoshinosuke Miyauchi, School of Medicine, University of Chiba, and Dr. Michio Inui, Medical Examiner office of Tokyo for kind offer of the human livers.