SPECIES DIFFERENCE IN DRUG METABOLISM
BY LIVER MICROSONES IN ALLOXAN
DIABETIC OR FASTED ANIMALS

(I) THE ACTIVITY OF DRUG-METABOLIZING ENZYMES
AND ELECTRON TRANSPORT SYSTEM

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In previous papers, it was reported that the activity of drug-metabolizing enzymes of liver microsomes was decreased in alloxan diabetic or fasted male rats (1, 2). The hexobarbital hydroxylation and aminopyrine N-demethylation by liver microsomes were decreased in these animals, but the aniline and xoxazolamine hydroxylations were not decreased. In contrast to the results obtained with male rats, the hexobarbital hydroxylation and aminopyrine N-demethylation were not decreased in the microsomes from female rats. These results thus appeared to be related to the fact that the hexobarbital hydroxylation and aminopyrine N-demethylation are markedly stimulated by androgen, whereas the aniline and xoxazolamine hydroxylations are not stimulated (1, 2). From these results and others, it has been supposed that the decrease in the hexobarbital hydroxylation and aminopyrine N-demethylation by liver microsomes from alloxan diabetic or fasted male rats is due to an impairment of the mechanism of androgen-dependent stimulation for these oxidative activities (1, 2).

On the other hand, the hexobarbital hydroxylation and aminopyrine N-demethylation by liver microsomes of mice and rabbits were not stimulated by androgen, and the lack of the androgen-dependent stimulating mechanism for the drug-metabolizing enzymes has been suggested (3, 4). Therefore, it is reasonable to speculate that, if the decrease in the hexobarbital hydroxylation and aminopyrine N-demethylation by the microsomes from alloxan diabetic or fasted male rats is due to an impairment of the androgen-dependent stimulating mechanism, the decrease in the oxidative activity does not occur in male mice and rabbits and that the sex difference in the alteration of drug-metabolizing enzymes due to alloxan diabetes or starvation is not observed in the liver microsomes from mice and rabbits.

In the present studies, these possibilities were investigated through comparative studies on the effect of alloxan diabetes and starvation on the liver microsomes of both sexes of rats, mice and rabbits.
MATERIALS AND METHODS

Male and female rats of the Wistar strain, weighing about 180 and 160 g, respectively, male and female mice of the dd strain, weighing about 26 and 22 g, respectively, and male and female rabbits, weighing about 2.3 and 2.0 kg, respectively, were used. The rats and mice were treated subcutaneously with alloxan, 170 and 410 mg/kg, respectively, 5 days before killing and the rats, mice and rabbits were fasted for 48, 36 and 72 hours, respectively, before experiment. The animals excreting more than 0.5% glucose into urine were used as the diabetic animals. The liver 10,000 × g supernatant and microsomal fractions were prepared as described in a previous paper (5).

The hexobarbital hydroxylation was determined by the disappearance of the substrate according to the method of Cooper and Brodie (6), the aminopyrine N-demethylation was determined by measuring the formation of 4-aminantipyrine according to the method of La Du et al. (7), and the aniline hydroxylation was determined by the formation of p-aminophenol according to the method described by Kato and Gillette (1).

The incubation mixture consisted of 1 ml of 10,000 × g supernatant fraction equivalent to 250 mg liver, 20 μmoles of glucose-6-phosphate, 0.8 μmole of NADP, 25 μmoles of nicotinamide, 25 μmoles of MgCl₂, 0.7 ml of 0.2 M sodium phosphate (pH 7.4), hexobarbital (2 μmoles), aminopyrine (5 μmoles) or aniline (5 μmoles), and water to a final volume of 2.5 ml. The mixture was incubated at 37°C for 30 minutes under air.

Microsomal NADPH-cytochrome c reductase was determined according to the method of Williams and Kamin (8). The microsomal protein content was measured by the method of Lowry et al. (9). The cytochrome P-450 content was determined by the difference spectrum of the carbon monoxide complex as described in the previous paper (5) and the results are expressed as μmole per mg microsomal protein according to Omura and Sato (10).

RESULTS

1. Effect of alloxan diabetes or starvation on the aminopyrine N-demethylation and hexobarbital hydroxylation by liver microsomes

As reported in a previous paper (4), clear sex difference in the aminopyrine N-demethylation and hexobarbital hydroxylation was observed only in rats, but not in mice and rabbits (Tables 1 and 2). The aminopyrine N-demethylation and hexobarbital hydroxylation by liver microsomes from the alloxan diabetic animals were decreased in male rats and increased in female rats and male and female mice as shown in Table 1. Similarly, the starvation caused marked decrease in the aminopyrine N-demethylation by liver microsomes in male rats, but it caused significant increase in female rats and in both sexes of mice and rabbits (Table 2). The hexobarbital hydroxylation by liver microsomes from fasted animals was decreased in male rats and increased in female rats, but the activity was not significantly affected in both sexes of mice and rabbits.

These results showed that the sex difference in the effect of alloxan diabetes or star-
TABLE 1. Effect of alloxan diabetes on the aminopyrine N-demethylation and hexobarbital hydroxylation by liver microsomes.

| Species | Sex | Control (m\(\mu\)mole/g/30 min) | Alloxan diabetes (m\(\mu\)mole/g/30 min) | Difference (%) |
|---------|-----|-------------------------------|---------------------------------|---------------|
| Rats    | M   | 593±25                        | 294±17                         | -50*          |
|         | F   | 138±7                         | 202±14                         | +46*          |
| Mice    | M   | 323±14                        | 374±18                         | +16*          |
|         | F   | 279±12                        | 324±17                         | +16*          |

Hexobarbital hydroxylation (m\(\mu\)mole/g/30 min)

| Species | Sex | Control | Starvation | Difference (%) |
|---------|-----|---------|------------|---------------|
| Rats    | M   | 3120±148| 1709±153   | -46*          |
|         | F   | 1187±81 | 1585±142   | +34*          |
| Mice    | M   | 1205±57 | 1480±89    | +23*          |
|         | F   | 1256±48 | 1544±75    | +27*          |

The rats and mice were treated subcutaneously with alloxan, 170 and 410 mg/kg, respectively, 5 days before the experiment. The results are given as means±S.E. from 8-12 determinations. Pooled livers from 3 mice were used for one determination. The asterisks indicate significant differences (p<0.05) from control values.

TABLE 2. Effect of starvation on the aminopyrine N-demethylation and hexobarbital hydroxylation by liver microsomes.

| Species | Sex | Control (m\(\mu\)mole/g/30 min) | Starvation (m\(\mu\)mole/g/30 min) | Difference (%) |
|---------|-----|-------------------------------|---------------------------------|---------------|
| Rats    | M   | 585±24                        | 302±21                         | -48*          |
|         | F   | 132±8                         | 195±13                         | +47*          |
| Mice    | M   | 301±13                        | 410±17                         | +36*          |
|         | F   | 278±12                        | 405±18                         | +46*          |
| Rabbits | M   | 512±34                        | 622±49                         | +21*          |
|         | F   | 498±38                        | 587±52                         | +18*          |

Hexobarbital hydroxylation (m\(\mu\)mole/g/30 min)

| Species | Sex | Control | Starvation | Difference (%) |
|---------|-----|---------|------------|---------------|
| Rats    | M   | 3085±158| 2053±143   | -32*          |
|         | F   | 1171±69 | 1565±121   | +34*          |
| Mice    | M   | 1187±63 | 1338±92    | +13           |
|         | F   | 1224±52 | 1389±68    | +13           |
| Rabbits | M   | 2250±171| 2458±221   | +9            |
|         | F   | 2187±188| 2461±208   | +13           |

The rats, mice and rabbits were fasted for 48, 36 and 72 hours, respectively before the experiment. The results are given as means±S.E. from 8-12 determinations. Pooled livers from 3 mice were used for one determination. The asterisks indicate significant differences (p<0.05) from control values.

2. Effect of alloxan diabetes or starvation on the aniline hydroxylation by liver microsomes

In contrast to the results obtained with the hexobarbital hydroxylation, there was no clear sex difference for the aniline hydroxylation in liver microsomes of rats as well as in mice and rabbits. As shown in Table 3, the aniline hydroxylation by liver micro-
TABLE 3. Effect of alloxan diabetes or starvation on the aniline hydroxylation by liver microsomes.

| Species | Sex | Aniline hydroxylation (µmole/g/30 min) |
|---------|-----|---------------------------------|
|         |     | Control | Alloxan diabetes | Difference (%) |
| Rats    | M   | 577±33  | 864±59  | +50* |
|         | F   | 432±24  | 762±55  | +76* |
| Mice    | M   | 1131±56 | 1439±89 | +27* |
|         | F   | 1069±59 | 1404±101| +29* |
|         |     | Control | Starvation | Difference (%) |
| Rats    | M   | 589±57  | 662±68  | +46* |
|         | F   | 448±31  | 812±56  | +81* |
| Mice    | M   | 1150±53 | 1621±89 | +41* |
|         | F   | 1082±50 | 1530±73 | +41* |
| Rabbits | M   | 682±49  | 939±80  | +38* |
|         | F   | 661±53  | 841±67  | +27* |

See the legends for Tables 1 and 2.

The activity of NADPH-cytochrome c reductase in liver microsomes represents the rate of NADPH reduction at the first step of the NADPH-linked electron transport system.

TABLE 4. Effect of alloxan diabetes or starvation on the activity of NADPH-cytochrome c reductase in liver microsomes.

| Species | Sex | NADPH-cytochrome c reductase (µmole/mg protein/3 min) |
|---------|-----|--------------------------------------------------|
|         |     | Control | Alloxan diabetes | Difference (%) |
| Rats    | M   | 498±32  | 469±40  | −6 |
|         | F   | 367±24  | 421±34  | +15 |
| Mice    | M   | 421±19  | 498±28  | +18* |
|         | F   | 443±24  | 521±33  | +17* |
|         |     | Control | Starvation | Difference (%) |
| Rats    | M   | 485±34  | 467±45  | −4 |
|         | F   | 367±28  | 418±33  | +14 |
| Mice    | M   | 429±21  | 564±29  | +31* |
|         | F   | 418±23  | 559±33  | +34* |
| Rabbits | M   | 425±31  | 487±32  | +15 |
|         | F   | 401±34  | 451±41  | +13 |

See the legends for Tables 1 and 2. The activity is expressed as µmole cytochrome c reduced per mg microsomal protein per 3 minutes.
The activity of NADPH-cytochrome c reductase in liver microsomes from the diabetic rats was not significantly affected, but it was slightly increased in the diabetic mice (Table 4). The NADPH-cytochrome c reductase activity in the fasted rats and rabbits was not significantly affected, but it was increased in the fasted mice. In addition, the content of microsomal protein in all animals was not significantly affected by the alloxan diabetes or starvation.

4. Effect of alloxan diabetes or starvation on the cytochrome P-450 content in liver microsomes

The content of cytochrome P-450 in liver microsomes from the diabetic animals was slightly increased in female rats, but it was not affected in male rats, and male and female mice (Table 5). Moreover, the cytochrome P-450 content was increased in the fasted rats and mice, but it was not significantly affected in rabbits.

5. Effect of alloxan diabetes or starvation on the hexobarbital hydroxylation or aniline hydroxylation per unit of cytochrome P-450 by liver microsomes

Since cytochrome P-450 is known as the oxygen-activating component as well as the substrate-binding site in drug oxidation by liver microsomes (10-12), the hexobarbital or aniline hydroxylation per unit of cytochrome P-450 was calculated for the results obtained from normal and the diabetic or fasted animals.

The hexobarbital hydroxylation per unit of cytochrome P-450 was markedly decreased in the diabetic or fasted male rats, but it was not significantly affected in the diabetic or fasted female rats and both sexes of mice and rabbits (Table 6). These results suggested that the alteration in the hexobarbital hydroxylation in the microsomes from the diabetic or fasted female rats and both sexes of mice and rabbits is related to

| Species | Sex | Control | Alloxan diabetes | Difference (%) |
|---------|-----|---------|------------------|----------------|
| Rats    | M   | 0.95±0.06 | 1.08±0.09       | +14            |
|         | F   | 0.70±0.04 | 0.85±0.06       | +21*           |
| Mice    | M   | 1.09±0.06 | 1.18±0.08       | +8             |
|         | F   | 1.03±0.04 | 1.12±0.06       | +9             |
| Rats    | M   | 0.99±0.06 | 1.18±0.05       | +19*           |
|         | F   | 0.73±0.05 | 1.04±0.06       | +42*           |
| Mice    | M   | 1.11±0.05 | 1.36±0.08       | +23*           |
|         | F   | 1.04±0.04 | 1.29±0.07       | +24*           |
| Rabbits | M   | 1.81±0.13 | 1.78±0.15       | -2             |
|         | F   | 1.74±0.10 | 1.76±0.12       | +1             |

See the legends for Tables 1 and 2.
the alteration in the cytochrome P-450 content, but other factor may be involved in the decrease in the hexobarbital hydroxylation by liver microsomes from the diabetic or fasted male rats.

On the other hand, the aniline hydroxylation per unit of cytochrome P-450 was increased in liver microsomes from the diabetic or fasted rats and rabbits, but it was not significantly affected in the mice (Table 7).

**DISCUSSION**

In the present investigations, it has been demonstrated that the decrease in the hexobarbital hydroxylation and aminopyrine N-demethylation by the alloxan diabetes
or starvation was observed only in liver microsomes from male rats, but not in the microsomes from the diabetic or fasted male mice and rabbits. The hexobarbital hydroxylation or aminopyrine N-demethylation by liver microsomes was rather increased in the diabetic or fasted female rats and both sexes of mice and rabbits (Tables 1–4). On the other hand, the aniline hydroxylation was not decreased by the alloxan diabetes or starvation in the microsomes from all animals tested, but it was increased significantly (Tables 5 and 6).

Since the increase in the hexobarbital hydroxylation and aminopyrine N-demethylation by androgen was observed only in liver microsomes from male rats, but not in male mice and rabbits (12), these results give the evidence to support the view that the alloxan diabetes and starvation impair the androgen-dependent stimulating mechanism for hexobarbital hydroxylation and aminopyrine N-demethylation and cause a decrease in the increased oxidative activity in microsomes of male rats (1-4). Therefore, it is reasonable that the alloxan diabetes or starvation did not decrease the hexobarbital hydroxylation and aminopyrine N-demethylation in male mice and rabbits and did not produce any different effect between the males and females. Similarly, since the aniline hydroxylation is not clearly dependent on the androgen action (12), it is reasonable that the aniline hydroxylation by liver microsomes was not decreased in male animals subjected to the alloxan diabetes or starvation and the hydroxylating activity was rather increased in similar degree in liver microsomes from both sexes of rats, mice and rabbits.

The hexobarbital hydroxylation per unit of cytochrome P-450 was lower in liver microsomes from the diabetic or fasted male rats than in those from the control group. This result suggested that the decreased hydroxylating activity for hexobarbital in the diabetic or fasted male rats might be related to the decreased binding capacity of cytochrome P-450 for hexobarbital. This possibility is discussed in the following paper (13). On the other hand, the increased hydroxylation activity for hexobarbital in liver microsomes from the diabetic female rats, the fasted female rats and male and female mice may be related to the increased amount of cytochrome P-450 content as observed in Tables 5 and 6. Moreover, the aniline hydroxylation per unit of cytochrome P-450 was increased in liver microsomes from the diabetic male and female rats and in the fasted rats and rabbits of both sexes. These results indicated that the binding capacity of cytochrome P-450 for aniline or the reduction rate of cytochrome P-450-aniline complex may be increased in these animals. This possibility is also investigated (13).

SUMMARY

The hexobarbital hydroxylation and aminopyrine N-demethylation by liver microsomes were decreased only when male rats were subjected to alloxan diabetes or starvation, but such decreases were not observed in male mice and rabbits and in the female animals. The aniline hydroxylation by liver microsomes was rather increased in all animals subjected to the alloxan diabetes or starvation. These results suggested that the species difference which was observed between male rats, and male mice and rabbits
concerning the effect of the alloxan diabetes or starvation on the hexobarbital hydroxylation and aminopyrine N-demethylation is related to the sex difference in these activities in liver microsomes of the intact rats. The oxidative activities in liver microsomes of male rats are stimulated by androgen and this stimulating mechanism has been supposed to be blocked by the alloxan diabetes or starvation. Since the androgen-dependent stimulating mechanism is absent in mice and rabbits, it is reasonable that the hexobarbital hydroxylation was not decreased in liver microsomes from the diabetic male mice and rabbits. The aniline hydroxylation is almost independent upon the androgen action, therefore it is reasonable that the aniline hydroxylation was not decreased in the microsomes from all animals studied. The hexobarbital hydroxylation per unit of cytochrome P-450 was decreased only in liver microsomes from the diabetic and fasted male rats and these results suggested a decreased in the binding capacity of cytochrome P-450 for hexobarbital in these rats.

REFERENCES

1) Kato, R. AND Gillette, J.R.: J. Pharmac. exp. Ther. 150, 279 (1965)
2) Kato, R. AND Gillette, J.R.: J. Pharmac. exp. Ther. 150, 285 (1965)
3) Kato, R. AND Takahashi, A.: Mol. Pharmac. 4, 95 (1968)
4) Kato, R., Takanaka, A. AND Takayanaghi, M.: Jap. J. Pharmac. 18, 482 (1968)
5) Kato, R., Takanaka, A. AND Takayanaghi, M.: J. Biochem. 68, 395 (1970)
6) Cooper, J.R. AND Brodie, B.B.: J. Pharmac. exp. Ther. 114, 409 (1955)
7) La Du, B.N., Gaudette, L., Trousof, N. AND Brodie, B.B.: J. biol. Chem. 214, 741 (1955)
8) Williams, C.H., Jr. AND Kamin, H.: J. biol. Chem. 237, 587 (1962)
9) Lowry, O.H., Rosebrough, N.J., Farr, A.L. AND RANDALL, R.J.: J. biol. Chem. 193, 265 (1951)
10) Omura, T. AND Sato, R.: J. biol. Chem. 239, 2370 (1964)
11) Schenckman, J.B., Remmer, H. AND Estabrook, R.W.: Mol. Pharmac. 3, 113 (1967)
12) Kato, R. AND Onoda, K.: Biochem. Pharmac. 19, 1649 (1970)
13) Kato, R., Onoda, K. AND Takanaka, A.: Jap. J. Pharmac. 20, 554 (1970)