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EFFECTS OF STARVATION AND REFEEDING ON THE OXIDATION OF DRUGS BY LIVER MICROSOMES*

R. KATO

Department of Pharmacology and Department of Toxicology, National Institute of Hygienic Sciences, Tamagawayoga-machi, Setagaya-ku, Tokyo-to, Japan

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Abstract—The metabolism of hexobarbital, aminopyrine and aniline by liver microsomes were increased in rats fasted for 72 hr and they were drastically decreased by refeeding on a standard diet as well as on sucrose. The activities of drug metabolism in the rats refed on the standard diet for 24 hr and 48 hr were about 35 and 52 per cent of control values, respectively.

Moreover, since the activities per total liver also markedly decreased by refeeding of the fasted rats, the decrease in the activities could not be ascribed to increase of liver weight.

On the other hand, sucrose feeding decreased the metabolism of drugs by liver microsomes and they were increased by starvation, but they were not altered by refeeding on the standard diet. The activities of NADPH dehydrogenase and NADPA-cytochrome *c* reductase and the content of P-450 in liver microsomes markedly decreased by refeeding of the fasted rats on the standard diet. The relationship between the activities of drug metabolisms and NADPH dehydrogenase and content of P-450 was discussed.

MANY drugs and foreign compounds have been oxidized by enzymes localized in liver microsomes in presence of NADPH and molecular oxygen. The activities of these enzymes are markedly altered by administration of some drugs and anabolic hormones and in abnormal physiological states.[1–6] The activities and toxicities of drugs, therefore, are markedly influenced by altered metabolism of drugs in the above-mentioned conditions.[1–3,7,8] In a previous paper it was demonstrated that the metabolism of drugs by liver microsomes was markedly decreased in fasted male rats, in contrast, it was increased in fasted female rats.[5] In the present investigation, the effects of starvation and refeeding on the activities of aniline hydroxylase, hexobarbital, oxidase and aminopyrine N-demethylase in the female rat are investigated. Since the refeeding after the starvation markedly depressed the metabolism of drugs in liver microsomes to below the control level, the activities of NADPH dehydrogenase (E.C.1.6.99.1) and NADP-cytocerome *c* reductase (E.C. 1.6.2.3.) and the contents of P-450 and cytochrome b_5 in liver microsomes were determined to investigate the mechanism of the depression in the metabolism of drugs.

MATERIALS AND METHODS

Female rats of the Sprague–Dawley strain, weighing about 160–180 g were used. The rats were fed on a standard diet, on sucrose or fasted as stated in the text.

* Preliminary report of this work has been given at the Annual Meeting of the Biochemical Society of Japan (Seikagaku, **37**, 545 1965).

Enzyme preparation. Rats were killed by decapitation and the liver was removed, chopped into small pieces, washed well and homogenized in a Teflon-glass homogenizer with 4 vol. of ice-cold 1·15% KCl solution. The homogenate was centrifuged at 9000 *g* for 15 min and the supernatant fraction was then centrifuged at 105,000 *g* for 1 hr to sediment the microsomes.

The microsomal pellet was suspended in ice-cold 1·15% KCl at a concentration such that 1·0 ml of suspension was equivalent to 400 mg of liver.

Enzyme assays. The 9000 *g* supernatant (3·0 ml) were mixed with 2·0 ml of a solution containing NADP (1·0 μmoles), glucose-6-phosphate (40 μmoles), magnesium chloride (25 μmoles), nicotinamide (50 μmoles), sodium phosphate buffer (140 μmoles, pH 7·4) and various substrates (3 μmole of hexobarbital, 5 μmole of aminopyrine or aniline).

The mixtures were incubated in a Dubnoff metabolic shaker for 30 min at 37° under air.

Aliphatic hydroxylation of hexobarbital was determined by measuring the disappearance of substrate by the method of Cooper and Brodie.[9] Aromatic hydroxylation of aniline was determined by measuring the formation of *p*-aminophenol by the phenolindophenol method of Brodie and Axelrod[10] as modified by Sasame and Gillette.[5] N-Demethylation of aminopyrine was determined by measuring the formation of 4-aminoantipyrine as described by La Du *et al.*[11] Microsomal NADPH-dehydrogenase was assayed by the disappearance of NADPH as described by Gillette *et al.*[12] Microsomal NADPH-cytochrome *c* reductase was determined by the method of Williams and Kamin.[13]

Estimation of P-450 and cytochrome b_5. The amount of P-450 was determined by measuring the difference spectrum of a microsomal preparation in a Beckman DK_2 spectrophotometer with cuvettes of 1 cm optical path. One ml of microsomal suspension, equivalent to 250 mg of liver, and 1·8 ml of 0·1 M phosphate buffer (pH 7·4) were placed in Thunberg-type cuvettes and chilled in an ice-cold water bath. Then 0·2 ml of NADPH (2·0 μmoles) was added and the gas-phase was immediately replaced with oxygen-free nitrogen (reference cell) or oxygen-free carbon monoxide (sample cell). The difference spectrum was measured at the time of maximal absorbance. The amount of cytochrome b_5 was similarly determined by measuring the difference spectrum. In this case the nitrogen-phase cuvette was used as sample cell and air-phase cuvette without addition of NADPH was used as reference cell. The content of P-450 was expressed as mμmole/mg microsomal protein by the difference of the optical densities between 450 mμ and 490 mμ according to Omura and Sato.[14] The content of cytochrome b_5 was expressed as mμmole/mg microsomal protein by the difference of the optical densities between 423 mμ and 408 mμ according to Omura and Sato.[14]

RESULTS

1. *Effect of refeeding after starvation on the metabolism of hexobarbital, aminopyrine and aniline by liver microsomes*

Starvation of female rats increased the hydroxylation of hexobarbital by liver microsomes equivalent to 1 g wet weight of liver. However, the increased hydroxylation of hexobarbital in the fasted female rats was markedly decreased by refeeding on the standard chow diet or sucrose. As shown in Fig. 1, the hydroxylation of hexo-

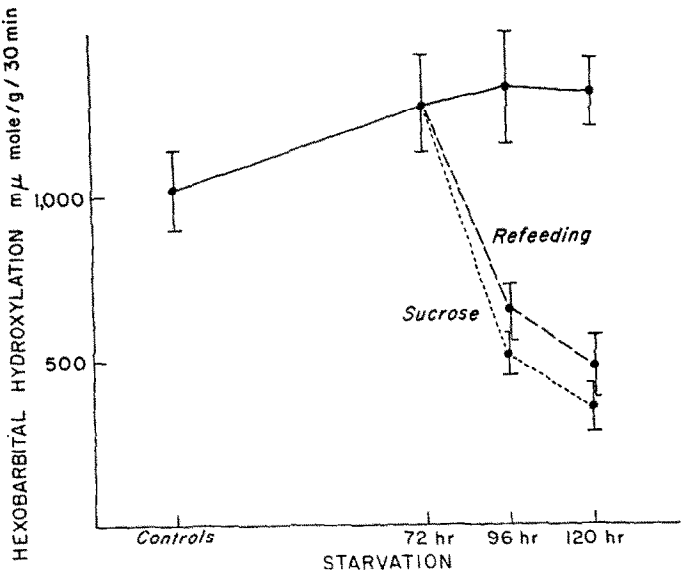

FIG. 1. Effect of refeeding after starvation on the metabolism of hexobarbital by liver microsomes of female rats. The rats were fasted for 72 hr and refed on a standard chow diet or on surcose for 24 hr and 48 hr. The results were expressed by amount of hexobarbital metabolized for 30 min by microsomes equivalent to g wet weight of liver and expressed as averages ± S.D. from eight rats.

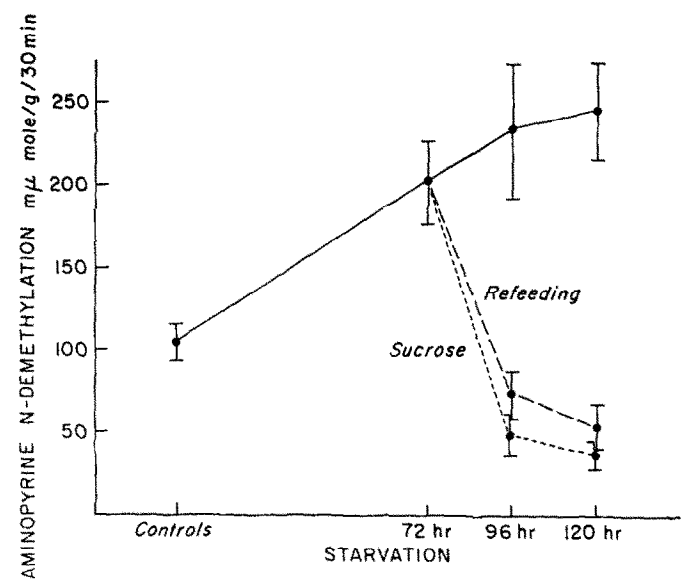

FIG. 2. Effect of refeeding after starvation on the metabolism of aminopyrine by liver microsomes of female rats. The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr. The results were expressed by amount of aminopyrine metabolized for 30 min by microsomes equivalent to g wet weight of liver and expressed as averages ± S.D. from eight rats.

TABLE 1. EFFECT OF REFEEDING AFTER STARVATION ON LIVER PROTEIN AND LIVER MICROSOMAL PROTEIN

	Body wt (g)	Liver wt (g)	Liver protein (mg/g)	Microsomal protein (mg/g)	Total microsomal protein (mg)
1. Control	183 ± 7 (8)	7·11 ± 0·80 (8)	165 ± 4 (8)	24·2 ± 0·4 (8)	172 ± 9 (8)
2. Starvation	142 ± 3 (8)	4·36 ± 0·35 (8)	176 ± 6 (8)	25·7 ± 0·6 (8)	112 ± 6 (7)
3. Starvation + refeed. 24 hr	157 ± 4 (8)	7·45 ± 0·55 (8)	158 ± 7 (8)	22·0 ± 0·4 (8)	164 ± 8 (8)
4. Starvation + refeed. 48 hr	165 ± 7 (8)	8·00 ± 0·79 (8)	163 ± 4 (8)	23·7 ± 0·3 (8)	190 ± 11 (8)
5. Starvation + sucrose 24 hr	145 ± 4 (8)	6·66 ± 0·47 (8)	156 ± 5 (8)	20·5 ± 0·6 (8)	137 ± 7 (8)
6. Starvation + sucrose 48 hr	149 ± 5 (8)	6·86 ± 0·54 (8)	159 ± 6 (8)	20·2 ± 0·4 (8)	139 ± 8 (8)

The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr.
The results were expressed by amount of drugs metabolized for 30 min by 1 g of wet wt liver and expressed as averages ± S.D. from eight rats.
The numerals in brackets indicate numbers of determinations.

barbital was increased by 25 per cent after 72 hr starvation, but it was drastically decreased by 49 per cent and 60 per cent, respectively, following the refeeding and sucrose feeding for 24 hr and it was still decreased by the refeeding for 48 hr.

Starvation of female rats markedly increased the N-demethylation of aminopyrine and the hydroxylation of aniline by liver microsomes (Figs. 2 and 3).

However, the refeeding of fasted female rats on the standard chow diet or sucrose markedly decreased the N-demethylation of aminopyrine and the hydroxylation of aniline. It was of interest that the increased metabolism of drugs by liver microsomes of fasted female rats was markedly decreased by refeeding on the standard diet as well as feeding on sucrose. Further experiments showed that the metebolism of drugs turned to increase following initial decrease by the refeeding for 72 hr and it recovered to normal level after 5 days of the refeeding.

These results were expressed by the metabolism of drugs per g wet weight of liver, but as shown in Table 1, the liver weight was markedly altered by fasting and refeeding.

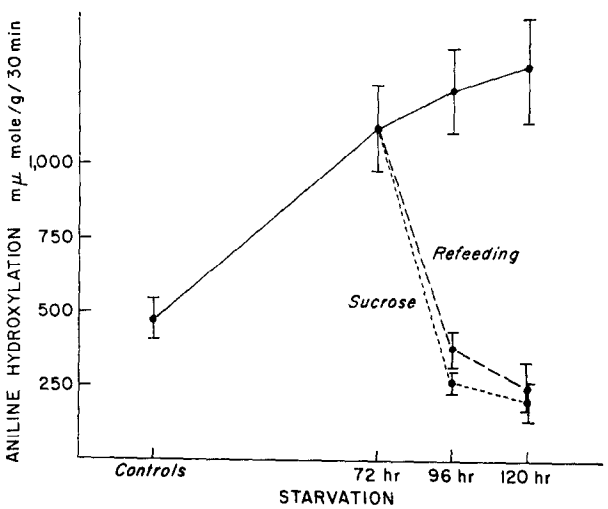

FIG. 3. Effect of refeeding after starvation on the metabolism of aniline by liver microsomes of female rats. The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr. The results were expressed by amount of aniline metabolized for 30 min by microsomes equivalent to g wet weight of liver and expressed as averages ± S.D. from eight rats.

Liver weight in the fasted rats decreased by 39 per cent, but it increased by 71 per cent and 83 per cent, respectively, in the rats refed for 24 hr and 48 hr. However, liver protein per g wet weight of liver did not significantly alter and liver microsomal protein per g wet weight of liver slightly increased in the fasted rats and it slightly decreased in the refed rats and sucrose-fed rats (Table 1). Thus, as shown in Table 2, the hydroxylation of hexobarbital by total liver decreased by 23 per cent in the fasted rats and the refeeding for 24 hr and 48 hr resulted in further decreases of 11 per cent and 33 per cent, respectively, and sucrose feeding for 24 hr and 48 hr resulted in further decreases of 40 per cent and 58 per cent, respectively. On the other hand, hydroxylation of aniline by total liver was increased 46 per cent in the fasted rats and the refeeding for 24 hr and 48 hr resulted in decreases of 58 per cent and 61 per cent,

respectively, and sucrose feeding for 24 hr and 48 hr resulted in decreases of 66 per cent and 72 per cent, respectively.

TABLE 2. EFFECT OF REFEEDING AFTER STARVATION ON TOTAL LIVER ACTIVITIES OF THE METABOLISM OF HEXOBARBITAL, AMINOPYRINE AND ANILINE

	Hexobarbital hydroxylation (mμmole/30 min)	Aminopyrene N-demethylation (mμmole/30 min)	Aniline hydroxylation (mμmole/30 min)
1. Control	7217 ± 984 (8)	739 ± 58 (8)	3335 ± 587 (8)
2. Starvation	5520 ± 581 (8)	881 ± 71 (8)	4874 ± 658 (7)
3. Starvation + refeed. 24 hr	4895 ± 721 (7)	543 ± 53 (8)	2041 ± 351 (8)
4. Starvation + refeed. 48 hr	3832 ± 754 (8)	449 ± 67 (8)	1922 ± 423 (8)
4. Starvation + sucrose 24 hr	3417 ± 403 (8)	320 ± 42 (8)	1745 ± 259 (8)
6. Starvation + sucrose 48 hr	2415 ± 438 (8)	247 ± 55 (8)	1372 ± 419 (7)

The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr. The results were expressed by amount of drugs metabolized for 30 min by total liver and expressed as averages ± S.D. from eight rats.
The numerals in brackets indicate numbers of determinations.

TABLE 3. EFFECT OF REFEEDING AFTER STARVATION ON THE ACTIVITY OF NADPH DEHYDROGENASE OF LIVER MICROSOMES

	Microsomal NADPH dehydrogenase		
	Activity per mg microsomal protein (mμmole/mg/5 min)	Activity per g wet liver (mμmole/g/5 min)	Activity per total liver (mμmole/5 min)
1. Control	52·9 ± 2·4 (8)	1280 ± 84 (8)	9103 ± 803 (8)
2. Starvation	73·4 ± 5·2 (7)	1885 ± 156 (8)	8219 ± 913 (8)
3. Starvation + refeed. 24 hr	35·7 ± 3·1 (8)	788 ± 98 (8)	5870 ± 610 (8)
4. Starvation + refeed. 48 hr	43·0 ± 3·3 (8)	1021 ± 151 (8)	8166 ± 1075 (8)
5. Starvation + sucrose 24 hr	32·2 ± 3·9 (8)	661 ± 69 (8)	4400 ± 513 (8)
6. Starvation + sucrose 48 hr	34·9 ± 3·5 (8)	686 ± 85 (8)	4706 ± 656 (8)

The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr. The results were expressed as averages ± S.D. from eight rats.
The numerals in brackets indicate numbers of determinations.

Furthermore, since the alternations of microsomal proteins per g wet weight of liver were only slight, the activities of microsomal drug-metabolizing enzymes per mg microsomal protein were almost same as the activities per g wet weight of liver. Thus, the increases in N-demethylation of aminopyrine and hydroxylation of aniline in the fasted rats were not due to only the decrease in liver size and more the decreases in hydroxylation of hexobarbital and aniline and N-demethylation of aminopyrine in the refed rats were not due to only the increase in liver size. In these experiments the 9000 *g* supernatants were used as enzyme source and in some experiments the microsomal fractions were used, but the results were both quite similar, therefore the alternation in the metabolism of drugs was not related to some factors in the 105,000 *g* supernatant. Moreover, since increase or decrease of the amount of added NADP and glucose-6-phosphate did not alter the results, the alternations of drug metabolisms is not dependant on changes in cofactor requirements.

2. *Effects of starvation and refeeding on a standard diet after sucrose feeding on the metabolism of aniline, hexobarbital and aminopyrine*

The feeding on sucrose markedly decrease activities of drug-metabolizing enzymes of liver microsomes in female rats as well as male rats.[15,19]

Thus, the effects of starvation and refeeding on a standard diet after sucrose feeding on the activities of drug-metabolizing enzymes of liver microsomes in female rats was investigated. As shown in Fig. 4, sucrose feeding markedly decrease the activity of aniline hydroxylation, but refeeding on the standard diet for 24 hr and 48 hr did not significantly alter the activity and the activity was slightly increased by the refeeding for 72 hr.

On the other hand, the activity of aniline hydroxylation was markedly increased by starvation of sucrose-fed rats. For example, the activity of aniline hydroxylation was increased by 152 and 243 per cent, respectively, following starvation for 24 hr and 72 hr. Almost similar results obtained on the activities of hexobarbital hydroxylation and aminopyrine N-demethylation. Liver weight was decreased 18 per cent in the

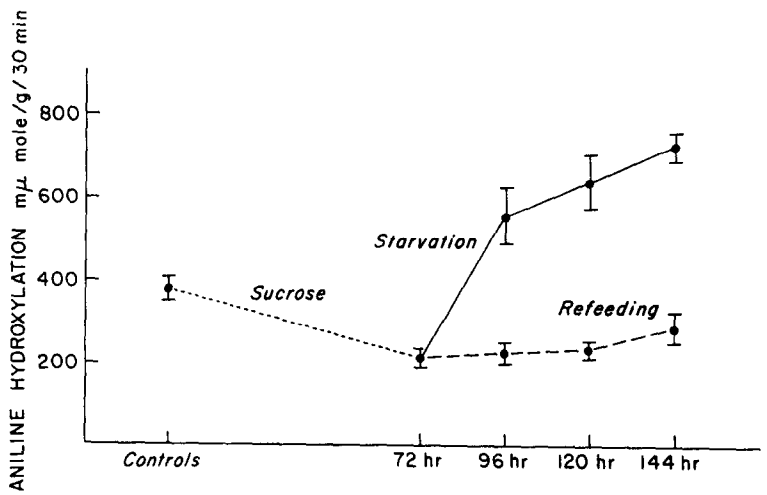

FIG. 4. Effects of starvation and refeeding after sucrose feeding on the metabolism of aniline by liver microsomes of female rats. The rats were fed on sucrose for 72 hr and starved or refed on a standard chow diet for 24 hr, 48 hr and 72 hr. The results were expressed by amount of aniline metabolized for 30 min by microsomes equivalent to g wet weight of liver and expressed as averages ± S.D. from eight rats.

sucrose fed rats and it was further decreased by 22 and 35 per cent, respectively, in the fasted rats for 24 hr and 72 hr. On the other hand, the refeeding for 24 hr, 48 hr and 72 hr resulted in increase in the liver weight by 15, 21 and 24 per cent, respectively.

The total activity of aniline hydroxylation therefore was increased by the refeeding for 24 hr and 48 hr and moreover, it was also increased by the starvation for 24 hr and 72 hr.

3. *Effect of refeeding after starvation on the activities of* NADPH *dehydrogenase and* NADPH-*cytochrome c reductase of liver microsomes*

NADPH dehydrogenase is likely a component of the drug-metabolizing enzyme

systems of liver microsomes and the alternation of NADPH dehydrogenase and NADPH-cytochrome *c* reductase in different experimental conditions was similar to that of the metabolism of drugs.[5,15–17,19]

Thus, it is of interest to investigate whether the alternations of NADPH dehydrogenase and NADPH-cytochrome *c* reductase in the present experiment were similar to that of the metabolism of drugs.

The activities of microsomal NADPH dehydrogenase per mg microsomal protein and per g wet weight of liver was significantly increased in the fasted rats (Table 3), but the activity per total liver was decreased. Refeeding of the fasted rats for 24 hr and 48 hr decreased the activity of NADPH dehydrogenase per g wet weight of liver by 58 and 46 per cent, respectively. Sucrose feeding after the starvation for 24 hr and 48 hr similarly decreased the activity of NADPH dehydrogenase. The activity of NADPH dehydrogenase per total liver was markedly decreased in the rats refed for 24 hr and it recovered to pre-refeeding level in the rats refed for 48 hr, while the activity was markedly decreased in the rats fed on sucrose for 24 hr and 48 hr.

TABLE 4. EFFECT OF REFEEDING AFTER STARVATION ON THE ACTIVITY OF NADPH-CYTOCHROME *c* REDUCTASE OF LIVER MICROSOMES

	Microsomal NADPH-cytochrome *c* reductase		
	Activity per mg microsmal protein (mμmole/mg/5 min)	Activity per g wet liver (μmole/g/5 min)	Activity per total liver (μmole/5 min)
1. Control	374 ± 29 (8)	9·05 ± 0·80 (8)	64·3 ± 6·5 (8)
2. Starvation	418 ± 58 (7)	10·73 ± 1·28 (8)	46·8 ± 4·7 (8)
3. Starvation + Refeed. 24 hr	264 ± 33 (8)	5·81 ± 0·78 (8)	43·3 ± 4·5 (8)
4. Starvation + refeed. 48 hr	289 ± 40 (8)	6·85 ± 1·12 (8)	54·8 ± 5·6 (8)
5. Starvation + sucrose 24 hr	258 ± 43 (8)	5·28 ± 0·78 (8)	35·2 ± 4·3 (8)
6. Starvation + sucrose 48 hr	272 ± 42 (8)	5·51 ± 0·93 (8)	37·8 ± 5·9 (8)

The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr.
The results were expressed as averages ± S.D. from eight rats.
The numerals in brackets indicate numbers of determinations.

Moreover, the activities of microsomal NADPH-cytochrome *c* reductase per mg microsomal protein and per g wet weight of liver were only slightly increased and the activity per total liver significantly decreased in the fasted rats. Refeeding of the fasted rats for 24 hr and 48 hr decreased the activity of NADPH-cytochrome *c* reductase per g wet weight of liver by 46 and 36 per cent, respectively. Sucrose feeding after the starvation for 24 hr and 48 hr similarly decreased the activity of NADPH-cytochrome *c* reductase.

However, the activity of NADPH-cytochrome *c* reductase per total liver was not altered in the rats refed for 24 hr and slightly increased in the rats refed for 48 hr, while the activity was markedly decreased in the rats fed on sucrose for 24 hr and 48 hr.

These results almost agreed with the results in the metabolism of drugs by liver microsomes, although the decrease in the activities of NADPH dehydrogenase and NADPH cytochrome *c* reductase in the rats refed on the standard diet was less marked than that in the metabolism of drugs.

4. *Effect of refeeding after starvation on the contents of* P-450 *and cytochrome* b_5 *in liver microsomes*

Since involvement of microsomal P-450 in the oxidation of drugs by liver microsomes was recently suggested,[17,18] the effect of refeeding after the starvation on the P-450 content was studied. The alternation of cytochrome b_5 content was also investigated in comparison with that of P-450 content. Since cytochrome b_5 is likely not involved in the oxidation of drugs, but it is a kind of haemoprotein as well as P-450. The content of P-450 per mg microsomal protein was only slightly increased in the fasted rats, but the refeeding after the starvation markedly decreased the P-450 content (Table 5). For example, the P-450 content increased only 21 per cent in the fasted rats, but it decreased by 49 per cent and 45 per cent, respectively, in the rats refed on the standard diet for 24 hr and 48 hr. Moreover, the P-450 content decreased by 61 per cent and 72 per cent, respectively, in the rats refed on sucrose for 24 hr and 48 hr.

TABLE 5. EFFECT OF REFEEDING AFTER STARVATION ON THE CONTENT OF P-450 IN LIVER MICROSOMES

	Microsomal P-450		
	Content per mg microsomal protein (mμmole/mg)	Content per g wet liver (mμmole/g)	Content per total liver (mμmole)
1. Control	1·07 ± 0·08 (8)	25·8 ± 2·5 (8)	183 ± 0·15 (8)
2. Starvation	1·29 ± 0·14 (7)	31·3 ± 4·4 (8)	136 ± 0·15 (8)
3. Starvation + refeed. 24 hr	0·72 ± 0·11 (8)	15·9 ± 2·1 (8)	118 ± 0·12 (8)
4. Starvation + refeed. 48 hr	0·72 ± 0·09 (8)	17·1 ± 1·7 (8)	137 ± 0·12 (8)
5. Starvation + sucrose 24 hr	0·60 ± 0·08 (8)	12·2 ± 2·3 (8)	81 ± 0·10 (8)
6. Starvation + sucrose 48 hr	0·49 ± 0·07 (7)	9·8 ± 2·1 (7)	67 ± 0 13 (7)

The rats were fasted for 72 hr and refed on a standard chow diet or on sucrose for 24 hr and 48 hr.
The results were expressed as averages ± S.D. from 8 rats.
The numerals in brackets indicate numbers of determinations.

On the other hand, the content of cytochrome b_5 per mg microsomal protein was altered slightly in the rats refed on the standard diet or sucrose. These results may indicate that the content of P-450 may relate to the activity of drug oxidation, while the content of cytochrome b_5 is not likely to relate to the activity of drug oxidation.

DISCUSSION

It is well known that the activities of many enzymes of the intermediate metabolism are markedly altered by fasting or refeeding.[20,21] Lipid-soluble compounds were metabolized by enzyme systems localized in liver microsomes and these enzyme systems are not considered to play an essential role in intermediate metabolism. However, the metabolism of drugs by liver microsomes of female rats was increased by starvation.[5] In contrast, the metabolism of drugs by liver microsomes of female rats was markedly decreased by feeding on sucrose or protein-free diet.[15,19,23]

These results indicate that the decrease of the metabolism of drugs by liver microsomes of female rats was not simply related to the deficiency of protein. Thus, the mechanism of the increase in the metabolism of drugs is not yet known, but it is

unlikely to assume that the increased activity of drug metabolism by liver microsomes from the fasted rats is due to an artifact in the formation of the microsomes from the endoplasmatic reticulum of the liver of fasted rats. In connexion with this view, Kato *et al* observed that the *in vivo* metabolism of meprobamate and other drugs in female rats increased in fasted rats.[22,23]

It was of interest that the metabolism of drugs by liver microsomes markedly decreased in the refed rats as well as in the sucrose-fed rats. Refeeding of female rats after the starvation for 72 hr increased the weight of liver and decreased the metabolism of drugs by liver microsomes. In fact, the activities of drug metabolisms in the rats refed on the standard diet for 24 hr and 48 hr were about 35 and 52 per cent of control values, respectively.

Similarly, the weight of liver was increased and the metabolism of drugs by liver microsomes was decreased after partial hepatectomy of female rats.[24] Moreover, refeeding of the fasted female rats and partial hepatectomy of female rats markedly decreased the activities of NADPH dehydrogenase and NADPH-cytochrome *c* reductase and the amount of P-450 in liver microsomes.[21]

It was postulated that microsomal NADPH dehydrogenase is a component of drug-metabolizing enzyme systems of liver microsomes and P-450 may act as an oxygen-activating component of liver microsomes.[17,18]

However, it was difficult to conclude from the present works that the decrease in the activity of NADPH dehydrogenase and the amount of P-450 is responsible for the decrease in the metabolism of drugs by microsomes of the refed rats. The decrease in the activity of NADPH dehydrogenase and the amount of P-450 may be related to the decrease in the metabolism of drugs, but it is also probable that these components localize in the same compartment in liver microsomes and the refeeding or heptectomy give a similar influence on the biosynthesis or degradation.

It will need further studies for elucidating factors which may control the activity of drug metabolism and NADPH dehydrogenase and the content of P-450 in different dietary conditions.

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