The Influence of Starvation Upon Hepatic Drug Metabolism in Rats, Mice, and Guinea Pigs (35674)

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Phenobarbital, 3-methylcholanthrene, DDT and testosterone are examples of more than 200 different compounds which have been classified as enzyme inducing agents. These agents represent the chemical inducers, and they have been fairly well defined as to dosage and site of action. Other inducing agents, or conditions, are thus far classed as nonspecific and include cold exposure, radiation, hind-limb ligation and starvation (1).

In the rat different inducing agents cause different patterns of induction in the oxidative enzymes. For example, phenobarbital stimulates the metabolism of hexobarbital, ethylmorphine, aniline, and *p*-nitroanisole, while 3-methylcholanthrene stimulates the metabolism of aniline and *p*-nitroanisole but depresses the metabolism of hexobarbital and ethylmorphine (2). Starvation stimulates the metabolism of aniline and depresses the metabolism of aminopyrine (3), similar to the pattern of induction produced by 3-methylcholanthrene.

Inducing agents, however, do not necessarily produce the same pattern of change in oxidative drug metabolism in different species. Treatment of rats with phenylbutazone stimulated 6β, 7α, and 16α hydroxylation of testosterone, while treatment of dogs with phenylbutazone stimulated only 6β and 16α hydroxylation of testosterone, having no effect on 7α hydroxylation (1).

It was of interest to determine if the effects produced by starvation were qualitatively the same in different species, like phenobarbital, or dissimilar, like phenylbutazone. If the changes were qualitatively similar in different species, then starvation might cause those changes in oxidative drug metabolism, via similar mechanisms in the different species.

Methods. The final weights of the male rats, male guinea pigs, and male mice used for these studies are indicated in Table I. No significant difference was noted in initial body weights among groups of a given specie. Rats and guinea pigs were kept in wire bottom cages while mice were housed on wood shavings in plastic cages. Food, but not water, was removed from experimental groups 1, 2, or 3 days before sacrifice.

Animals were weighed and then guillotined. The liver was excised, rinsed in 1.15% KCl, blotted dry, weighed, and homogenized in 2 vol of 1.15% KCl. After homogenization (Ultra-Turrax, 30 sec), guinea pig and rat liver homogenates were diluted 1:3 with 1.15% KCl so that each milliliter contained 111 mg of whole mouse liver. Mouse liver homogenate was centrifuged at 9000*g* for 20 min and the supernatant, containing 500 mg whole liver equivalents/ml, was decanted. All metabolism values are expressed on the basis of milligrams of whole liver or milligram whole liver equivalents.

Incubation mixtures were prepared which contained (μmoles): NADP, 4; glucose-6-phosphate, 50; nicotinamide, 20; $MgCl_2$, 25; and phosphate, 500; buffered to pH 7.4. Also included were the appropriate substrates (μmoles): ethylmorphine, 10; *p*-nitroanisole, 6; or aniline, 6. The final volume of the incubation mixture was 5 ml, and for rats and guinea pigs it contained 111 mg of whole liver regardless of substrate. However, for mice, the vessels containing aniline or ethylmorphine received supernatant from 250 mg of whole liver while those containing *p*-nitroanisole received supernatant from 500 mg of whole liver.

In rats and guinea pigs, the metabolism of

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TABLE I. Final Weights of Rat, Guinea pig, and Mouse.[a]

Days of fasting	Rat	Guinea pig	Mouse
		Body wt	
0	214.8 ± 3.9 (4)	425.2 ± 14.6 (5)	39.3 ± 0.67 (19)
1	179.5 ± 2.1 (4)[b]	411.2 ± 19.7 (5)	35.1 ± 1.08 (20)[b]
2	157.0 ± 1.9 (4)[b]	377.2 ± 11.6 (5)[b]	31.4 ± 0.78 (16)[b]
3	140.8 ± 4.6 (4)[b]	352.6 ± 5.5 (5)[b]	30.3 ± 0.98 (17)[b]
		Liver wt	
0	10.86 ± 0.18 (4)	18.00 ± 1.15 (5)	2.34 ± 0.04 (19)
1	6.00 ± 0.09 (4)[b]	13.85 ± 0.44 (5)[b]	1.62 ± 0.06 (19)[b]
2	5.37 ± 0.37 (4)[b]	12.86 ± 0.50 (5)[b]	1.57 ± 0.06 (16)[b]
3	4.64 ± 0.21 (4)[b]	13.85 ± 1.55 (5)	1.60 ± 0.10 (17)[b]
		Liver wt/100 g of body wt	
0	4.93 ± 0.21 (4)	4.22 ± 0.15 (5)	5.99 ± 0.12 (19)
1	3.34 ± 0.08 (4)[b]	3.41 ± 0.24 (5)[b]	4.72 ± 0.25 (19)[b]
2	3.41 ± 0.39 (4)[b]	3.40 ± 0.06 (5)[b]	5.06 ± 0.24 (16)[b]
3	3.29 ± 0.16 (4)[b]	3.93 ± 0.46 (5)	5.33 ± 0.34 (17)

[a] Values are means ± SE. Numbers in parentheses represent number of animals making up mean.

[b] Group is different from 0 days fasting (control) animals at level of $p < 0.05$.

ethylmorphine (4), *p*-nitroanisole (5), and aniline (3) was measured by standard techniques (with the exception that 5% phenol was used in the color formation for *p*-aminophenol). In mice the metabolism of ethylmorphine, *p*-nitroanisole, and aniline was determined by continuous flow technique (6).

Comparison of the group means was carried out using a multiple *t* comparison programmed for use on the IBM 2741.

Results. Body weight. After 3 days starvation, rats had lost 35%, mice 25%, and guinea pigs 18% of their original body weights (Table I). Although water was provided *ad libitum* to all animals, only starved guinea pigs exhibited fluid-filled digestive tracts. Since guinea pigs were watered from a common reservoir, this observation was not quantitated; and, therefore, it is not certain if the fluid-filled digestive tract was the result of excessive water consumption or pathological water retention. Whatever the source, the fluid may have been the reason for the smaller weight loss by guinea pigs since all species, including guinea pigs, had less abdominal fat after starvation.

Liver weight. All three species groups, which had been starved 1 day had about 40% less total liver than controls fed *ad libitum* (Table I). No further significant decrease occurred in guinea pigs or in mice. Total liver weight in rats declined significantly between 1 and 2 days and again between 2 and 3 days starvation, although the magnitude of decrease was somewhat less than during the first day of starvation. No significant difference in liver weight per 100 g of body weight was noted among animals starved for 1 day or more.

Ethylmorphine metabolism. The metabolism of ethylmorphine (Table II) was significantly depressed in guinea pigs after 1 day of starvation. In the rats the decline was noticeable after 1 day, but was not significant until the third day of starvation. Although ethylmorphine metabolism in the guinea pig was less than control values for all time periods of starvation, the reduced levels were significant only at the 1 and 3 day periods. In mice the metabolism of ethylmorphine was stimulated by starvation, and was increasing even after 3 days of starvation.

p-Nitroanisole metabolism. Starvation elevated the metabolism of *p*-nitroanisole significantly in rats and mice, but not in guinea pigs after 2 days (Table II). In mice the metabolism of *p*-nitroanisole paralleled the metabolism of ethylmorphine and was still

TABLE II. Metabolism.

Days of fasting	(mμmoles of substrate metabolized/mg of whole liver/min × 10^{-3}) Rat	Guinea pig	Mouse
	Ethylmorphine metabolism		
0	328 ± 18 (4)	157 ± 5 (5)	195 ± 12 (6)
1	303 ± 28 (4)	135 ± 4 (5)[b]	211 ± 10 (6)
2	289 ± 16 (4)	143 ± 5 (5)	266 ± 14 (5)[b]
3	258 ± 24 (4)[b]	133 ± 3 (5)	306 ± 19 (5)[b]
	p-Nitroanisole metabolism		
0	17.3 ± 0.7 (4)	31.3 ± 2.0 (5)	91.0 ± 4.0 (6)
1	24.7 ± 1.7 (3)[b]	28.2 ± 3.0 (5)	113.0 ± 6.0 (6)
2	28.0 ± 1.4 (4)[b]	36.7 ± 2.0 (5)	129.0 ± 7.0 (5)[b]
3	21.2 ± 1.3 (4)[b]	33.8 ± 4.0 (5)	166.0 ± 4.0 (5)[b]
	Aniline metabolism		
0	27.1 ± 2.0 (4)	33.4 ± 1.7 (5)	67.0 ± 3.0 (6)
1	48.4 ± 2.0 (4)[b]	42.2 ± 4.1 (5)	85.0 ± 2.0 (6)[b]
2	50.1 ± 3.0 (4)[b]	47.7 ± 3.1 (5)[b]	95.0 ± 5.0 (5)[b]
3	31.2 ± 6.0 (4)	47.3 ± 1.4 (5)[b]	97.0 ± 6.0 (5)[b]

[a] Values are all means ± SE. Numbers in parenthesis represent number of animals. For mice the numbers represent the number of separate determinations carried out using a pool of 3 or 4 mouse livers/determination.

[b] $p < 0.05$, when compared with fed animals of species group.

increasing after 3 days starvation. In rats the metabolism of *p*-nitroanisole reached a maximum after 2 days starvation and declined after 3 days starvation.

Aniline metabolism. Aniline metabolism was elevated maximally after 2 days starvation in all three species, but after 3 days, while aniline metabolism remained high in the mouse and guinea pig, in the rat it had declined such that it was no longer different from fed controls (Table II). The metabolism of aniline in the rat roughly paralleled the metabolism of *p*-nitroanisole in the rat in that both are higher at 2 days than at 3 days starvation.

Discussion. Starvation is one of many agents or conditions which can influence oxidative drug metabolism in the rat (1). However, little information is available concerning the effects of starvation in other species. It was decided, therefore, to examine the effects of starvation upon different animal species, specifically as related to the metabolism of ethylmorphine, *p*-nitroanisole, and aniline as classical examples of drugs, which undergo oxidative enzymatic degradation by the hepatic microsomes. The changes in metabolism of these three drugs are excellent models for determining the influence of various treatments and species dependent effects on hepatic oxidative enzyme systems.

The data suggest that the enzymes responsible for oxidative metabolism (i) are not interdependent since aniline metabolism in rats increases and then decreases, while ethylmorphine metabolism only decreases; and (ii) are species dependent since starvation decreases ethylmorphine metabolism in the male rat, but increases ethylmorphine metabolism in the male mouse. Furthermore, *p*-nitroanisole metabolism in the guinea pig is not changed significantly by starvation, although significant increases are noted in the mouse and rat.

A recent report by Gram *et al.* (7) indicates that, in the rat, ethylmorphine metabolism is stimulated by starvation and not diminished as reported here. Gram and his coworkers noted that the apparent K_m for ethylmorphine changes with starvation, and that if the substrate concentration used were near the K_m, then an increase in the apparent K_m might be revealed as a decrease in total enzyme activity. However, since the substrate

concentration used in these experiments was far in excess of the apparent K_m reported, it is believed that changes in K_m cannot account for the reported differences.

Summary. Male rats, mice, and guinea pigs were starved for 1, 2, or 3 days; and the metabolism of ethylmorphine, *p*-nitroanisole, and aniline was studied. The metabolism of aniline was stimulated by starvation in mice, rats, and guinea pigs; the metabolism of *p*-nitroanisole was stimulated only in mice and rats; and the metabolism of ethylmorphine was stimulated by starvation only in mice. The results suggest that the oxidative enzyme systems studied are not interdependent, and the pathways studied appear to be species dependent.

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