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Influence of dietary stress on microsomal enzyme activity in immature mice*

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THE MICROSOMAL enzymes concerned with drug metabolism are inactive in the newborn and increase in activity until the onset of maturity.[1] In the mature animal these enzymes are apparently quite labile in that their level can be decreased by starvation,[2] or by feeding for 7 days a diet low in proteins.[3] The stability of these enzymes has not been studied in weanling animals who are actively synthesizing them. However, when weanling mice were maintained on a diet low in protein from weanling to early maturity, they exhibited a prolonged hexobarbital sleeping time.[4] It was also noted in this study that liver supernatants derived from such animals metabolized less hexobarbital per gram of liver than controls. However, it was not determined whether this was due to a decrease in the level of the microsomal enzymes or of the cofactors. It was of interest, therefore, to determine whether a low protein dietary stress would decrease microsomal enzyme levels in these weanlings as readily as it does in the adult animal.

CF-1 male mice, 21 days old, were randomly divided upon arrival and offered one of two diets and water *ad libitum*. The two isocaloric diets used in this study differed only in protein and carbohydrate content and have been described previously.[4] The mice were decapitated, bled, and the livers excised and homogenized (33% in 0·1 M phosphate buffer, pH 7·4) in an all glass homogenizer. The homogenates were centrifuged at 9000 *g* for 60 min. The supernatant, containing microsomes plus soluble fraction, was centrifuged at 105,000 *g* for 60 min. The microsomal pellet was washed with buffer, recentrifuged at 105,000 *g*, and resuspended in phosphate buffer. The microsomal suspension was used for determination of the rate of hexobarbital metabolism and for estimation of nitrogen concentration. Microsomal nitrogen concentration was determined by the micro-Kjeldahl method.[5]

The incubation mixture contained 2 ml of the microsomal fraction, 0·676 μmole NADP, 0·96 μmole isocitric acid, 0·06 units of isocitric dehydrogenase, 25 μmole $MgCl_2$, 50 μmole nicotinamide and 1 μmole hexobarbital in a final volume of 4 ml of 0·1 M phosphate buffer. The mixtures were incubated in a Dubnoff metabolic shaker for 1 hr at 37° with oxygen as the gas phase. The rate of hexobarbital metabolism was determined by estimating the disappearance of substrate.[6]

It can be seen in Table 1 that the microsomal nitrogen per g liver and the total microsomal nitrogen are lower after both 7 and 28 days in the animals maintained on the low protein diet. However, the

TABLE 1. HEPATIC MICROSOMAL NITROGEN*

	7 Days on diet		28 Days on diet	
	(mg N/g liver)†	(mg total microsomal N)‡	(mg N/g liver)†	(mg total microsomal N)‡
Control (27% Casein)	88·6 ± 5[a]§	79·68[c]	93·6 ± 3[b]	154·28[d]
Experimental (8% Casein)	64·5 ± 3[a]	38·68[c]	75·8 ± 3[b]	72·77[d]

* Microsomal suspension prepared from pooled livers of 20–30 mice.
† Microsomal nitrogen, mg/g liver; mean of 6 determinations ± S.D.
‡ Calculated from mean liver weight.
§ Statistical comparison of numbers with the same lettered superscript: *a,b,c,d* = $P(t) < 0{\cdot}001$.

total microsomal nitrogen content of the liver increases approximately 100 per cent in both groups during the first 3 weeks of experimental feedings.

As can be seen in Table 2, the microsomal drug-metabolizing activity per g liver did not differ at 7 days between the experimental and control groups. However, when calculated per mg microsomal

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nitrogen, the metabolic activity is 37 per cent higher in the low protein experimental group than in the control

After 4 weeks of experimental feeding, the microsomes derived from liver of mice maintained on the low protein diet metabolized only 58 per cent as much hexobarbital as the control when calculated per g liver. When the comparison is made per mg nitrogen, the low protein livers metabolized 73 per cent as much hexobarbital as the control microsomes. It should also be noted that the microsomal activity increased in both the control and experimental groups from the 7- to 28-day period, when calculated on a total liver weight basis, but decreased in the experimental group when calculated per mg nitrogen or per g of liver.

TABLE 2. HEXOBARBITAL METABOLISM BY LIVER MICROSOMES FROM MICE MAINTAINED ON AN EXPERIMENTAL PROTEIN DIET FOR 7 AND 28 DAYS

μM Metabolized/hr	Control (27% casein)		Experimental (8% casein)	
	7 days	28 days	7 days	28 days
Per g liver*	0·551 ± 0·033[a]†	0·794 ± 0·008[d]	0·512 ± 0·019[a]	0·462 ± 0·007[d]
Per mg microsomal nitrogen*	0·0638 ± 0·008[b]	0·0934 ± 0·003[e]	0·0867 ± 0·003[b]	0·0670 ± 0·003[e]
Per total liver‡	0·460[c]	1·310[f]	0·307[c]	0·444[f]

* Mean of 4 determinations ± S.D.

† Statistical comparison of numbers with the same lettered superscript: *a,c* = P(t) N.S.; *b* = P(t) < 0·05; *d,e,f* = P(t) <0·001.

‡ Calculated from mean liver weight of 20–30 mice.

Since the drug-metabolizing activity per g of liver was the same at 7 days in both the control and experimental groups while the total microsomal nitrogen levels differed by as much as a factor of two, it is evident that the decreased nitrogen level in the low protein group is primarily due to a loss of protein other than that concerned with drug metabolism. Thus, in contrast to what has been reported to occur in the adult animal exposed to protein dietary stress for 1 week,[3] the drug-metabolizing enzymes in the weanling mouse are relatively resistant to the effects of this stress. However, after 4 weeks of feeding the low protein diet, it is evident that a marked decrease in the rate of microsomal hexobarbital metabolism is now present in these mice. Since this is true both on a per g liver and per mg nitrogen basis, it is possible that there has been an actual loss of drug-metabolizing enzymes from the livers of mice maintained on the low protein diet. Thus the study indicates that during the period of early maturation, when there is an active synthesis of liver enzymes, the hepatic drug-metabolizing enzymes are more resistant to the effects of dietary stress than they are in the adult mouse when enzyme synthesis has become more stabilized.

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