Biochemical Pharmacology, Vol. 18, pp 2288–2292. Pergamon Press. 1969. Printed in Great Britain

Effects of dietary thiamine intake on hepatic drug metabolism in the male rat*

(*Received* 10 *December* 1968; *accepted* 28 *March* 1969)

MANY FACTORS have been shown to alter the rate at which drugs are metabolized by hepatic microsomal preparations. Certain environmental conditions,[1–3] some drugs,[4–7] stress[8, 9] and starvation[10–11] may increase the rate; whereas other drugs,[5, 6, 12] heavy sucrose feeding,[10] protein and lipid deficiency,[13, 14] and the blockade of protein synthesis[4] may depress the rate of drug metabolism. It is evident that all drug metabolic pathways are not equally affected by experimental intervention. Adrenalectomy,[15] castration,[11, 16] hypoxia, epinephrine or morphine administration[15] and starvation[17] impair hexobarbital metabolism in male rats, but not metabolism of aniline.

With these facts in mind and realizing the importance of thiamine in several pathways involving carbohydrate metabolism, the present study was initiated to investigate the role of thiamine in drug metabolism. Two of the drugs utilized (hexobarbital and aniline) represent substrates whose rates of metabolic degradation are affected differently by other forms of dietary variation (i.e. starvation).

MATERIALS AND METHODS

Animals. Eighty-four white, male, Sprague–Dawley rats (Holtzman Co., Madison, Wis.), weighing approximately 50 g each, were placed on a standard laboratory test diet consisting of casein (16 per cent), sucrose (73 per cent), non-nutrient cellulose (4 per cent), salt mix (4 per cent) and corn oil (3 per cent) with varying amounts of thiamine HCl added to provide the animals with an average daily intake of zero, 0·03, 0·11, 0·20, 0·49, 1·0, 2·9, 7·0, 15·0, 30, 50, 100, 500, and 2000 μg thiamine HCl. Six control rats maintained on Purina lab chow were also included in the experiment.

Enzyme preparation. After 21–23 days on these diets, the rats were decapitated, and their livers were quickly removed and homogenized with 2 vol. of cold 1·15% KCl using a motor-driven Teflon-glass tissue homogenizer. After centrifuging the homogenate at 9000 *g* for 20 min at 0°, the supernatant containing the microsomes was decanted.

Enzyme assays. The 9000 *g* supernatant (1·0 ml) was mixed with a solution (3·0 ml) containing NADP (1·0 μmole), glucose 6-phosphate (25 μmoles), nicotinamide (100 μmoles), magnesium sulfate (25 μmoles) and substrate in appropriate quantities. The final volume was adjusted to 5·0 ml with 0·1 M phosphate buffer (pH 7·4). The substrates used were hexobarbital (3 μmoles), aniline (10·0 μmoles) and heptachlor (0·5 μmoles).

The mixtures were incubated for 30 min at 37° under air in a Dubnoff metabolic shaker. Reactions were stopped by transferring an aliquot of the incubation mixture to the appropriate extraction medium previously chilled in an ice bath. The rate of aliphatic hydroxylation of hexobarbital was determined by measuring the disappearance of substrate by the method of Cooper and Brodie;[18] aromatic hydroxylation of aniline was determined by measuring the formation of *p*-aminophenol by a modification of the indophenol method of Brodie and Axelrod[19] as reported by Kato and Gillette.[15] Heptachlor metabolism was determined by the method of Greene and Wade.[20] The biuret method of Gornall *et al.*[21] was used to quantitate protein content of the 9000 *g* supernatant of the liver homogenates.

RESULTS

After 3 weeks on the experimental diets, body and liver weights were found to have increased in proportion to thiamine intake ($r = 0{\cdot}86$ and 0·89 respectively). The weight changes were not uniform throughout the entire range of thiamine consumption, however. Neither body nor liver weights

*This investigation was supported in part by grants from the U.S. Public Health Service (GM 14694 and AM 08595) and by the University of Georgia Office of General Research. This is publication No. 722 from the Institute of Comparative Medicine, University of Georgia. Preliminary reports of this work were presented at the Spring meeting of FASEB, 1968.

deviated from those of thiamine-deficient rats until intakes exceeded 1·0 μg day. At intakes exceeding 50 μg day, no further increases were noted, and these weights were not unlike those fed the laboratory chow diet. Liver weight as per cent of body weight increased from 4·31 ± 0·15 in the thiamine deficient rats to 5·20 ± 0·10 in rats receiving in excess of 30 μg thiamine day ($r = 0{\cdot}48$; $P < 0{\cdot}01$). Control rats fed laboratory chow had liver weight to body weight ratios of 5·19 ± 0·20. The protein content of the 9000 *g* supernatant of liver homogenates was not significantly altered by dietary thiamine levels ($r = -0{\cdot}20$) and varied from 95·63 ± 6·80 to 115·96 ± 7·00 mg/g of liver. Lab chow controls contained 91·3 ± 7·4 mg protein g of liver.

The effects of various levels of dietary thiamine for 3 weeks on the metabolic rate for hexobarbital, aniline and heptachlor *in vitro* are shown in Figs. 1, 2 and 3. There was no significant change in hexobarbital metabolism consistent with changes in thiamine intake ($r = -0{\cdot}16$). It appears from these data, however, that a maximum rate of hexobarbital metabolism occurred in rats receiving at least 2·9 but not more than 30 μg thiamine per day (Fig. 1) and that these rats were not significantly different in their ability to hydroxylate hexobarbital from rats fed the laboratory chow.

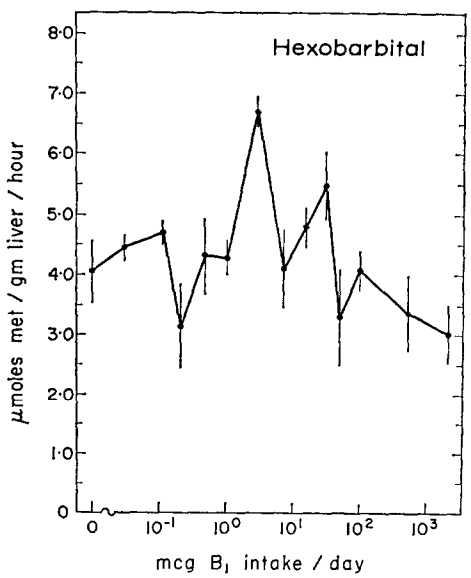

FIG. 1. Metabolism *in vitro* of hexobarbital by liver preparations from rats receiving from 0 – 2000 μg thiamine in their diets per day for 21–23 days. Vertical bars represent S.E. of mean. Group sizes vary from three to twelve animals. Lab chow controls metabolized 5·02 ± 0·41 μmoles/g liver/hr.

Heptachlor metabolism was depressed markedly (Fig. 2) as thiamine intake was increased above 1 μg day ($r = -0{\cdot}74$). The half-maximal change occurred at intakes of about 8–10 μg/day. Thiamine intake in excess of 100 μg per day did not further depress the heptachlor metabolic rate *in vitro*.

Increasing thiamine content in the diet increasingly depressed microsomal metabolism of aniline (Fig. 3), with maximum depression occurring with levels of thiamine of 100 μg or greater/day ($r = -0{\cdot}85$). The half-maximal change occurred at intakes of about 1 μg/day.

The relative rates of drug metabolism, when calculated per unit of body weight or per milligram of microsomal protein, did not vary significantly from those calculated per gram of liver, and the points of maximum rate of deviation in the various groups appear to be consistent regardless of the method of calculation. It should be noted that all three pathways were examined on every liver homogenate and that the divergent rates of metabolism were considerably greater than could be ascribed to individual variation between rats.

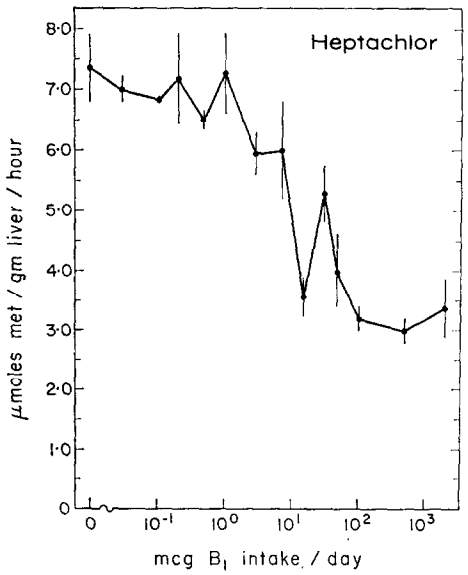

FIG. 2. Inhibition of metabolism *in vitro* of heptachlor in rats fed diets containing varying levels of thiamine for 21–23 days. The values represent the means of three to twelve animals ± S.E. Lab chow controls metabolized 4·96 ± 0·47 μmoles/g liver/hr.

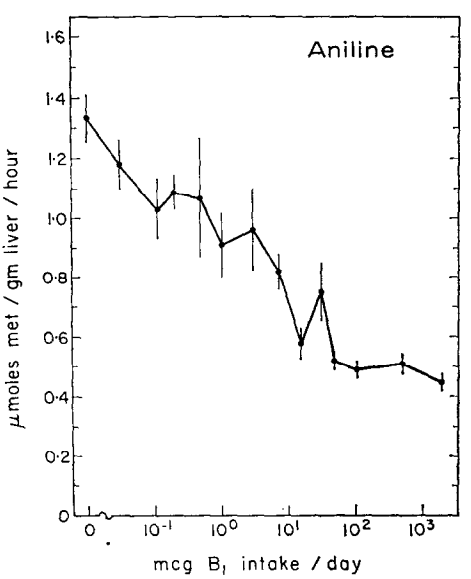

FIG. 3. Effect of thiamine intake on aniline metabolism by rat liver microsomes *in vitro*. The values represent means ± S.E. of three to twelve animals. Lab chow controls metabolized 1·05 ± 0·15 μmoles/g liver/hr.

DISCUSSION

Thiamine is one of the best known and most critically needed of the vitamins. It is needed for maximal growth rate and to prevent the cardiovascular and central nervous system changes characteristic of thiamine deficiency. In the rat the $I_{1/2}$ values (i.e. the dietary intakes corresponding to the half-maximal changes[22]) are reported to be 6·8, 0·95 and 0·25 μg thiamine per day for these three effects respectively.[23, 24] That the $I_{1/2}$ values of heptachlor and aniline are similar to two of these is interesting.

The mechanism by which high dietary thiamine intake depressed two of the three metabolic pathways studied is currently under investigation. Since the food intake of animals on thiamine-poor diets was less than those on thiamine-rich diets, it is possible that some effects produced by thiamine deficiency are related to starvation. Although effects of chronic food restriction on drug metabolism have not been reported, starvation of male and female rats for up to 3 days has been observed to increase aniline metabolism significantly when calculated per gram of liver weight. In the case of hexobarbital, starvation decreased hexobarbital metabolism in the male and increased it in the female.[17]

In this study the mean rate of hexobarbital metabolism by all groups fed the experimental diet was approximately 80 per cent of that by rats fed the lab chow diet. Inhibition in the thiamine-free groups was no greater than that in those receiving high levels. Heptachlor metabolism in lab chow controls was not significantly different from that of rats fed experimental diets containing 7-50 μg thiamine per day and was approximately 67 per cent as great as that of rats fed the thiamine-free diet. Aniline metabolism in rats fed laboratory chow was not significantly different from those on experimental diets containing 0·03 to 7·0 μg thiamine per day. The last observation suggests that factors other than food deprivation were operative, since these low thiamine diets were poorly consumed.

Rats re-fed after experimental starvation are reported to have subnormal rates of metabolism for aniline and hexobarbital.[17] Re-feeding with sucrose or chow diet also resulted in increased liver weights. Although the metabolic rates for aniline and heptachlor in this study varied inversely with thiamine intake, it is yet unknown whether the decreases with diets containing high thiamine were due to excessive intake of sucrose. This explanation seems untenable, however, in view of the differences in the level of thiamine ingestion at which the rates of metabolism of aniline and heptachlor appeared to deviate from those of the thiamine-deficient rats. There is no evidence in these experiments to suggest that the increased intake in high thiamine diets significantly depressed hexobarbital metabolism.

The rates of aniline and heptachlor metabolism were depressed maximally at about 100 μg thiamine intake per day with no further depression occurring with thiamine ingestion up to 2000 μg per day. This is understandable, since it has been shown that thiamine intakes in the diet in excess of 65 μg per day lead to no further increases in thiamine body stores.[25]

It appears that thiamine ingestion is an important determinant in the rate of drug metabolism by the liver of the rat. It remains to be seen whether thiamine deficiency increases the concentration of cofactors required in the metabolic alterations of certain drug molecules or if the administration of thiamine increases synthesis of a repressor.

Present knowledge of the relevance of vitamins, essential fatty acids, and carbohydrate and protein concentrations in the diet is inadequate to explain the complex and varied reactions of individuals to drugs. However, with more knowledge will come a better understanding of the mechanisms involved in drug metabolism and perhaps the means of varying the therapeutic or toxicologic potential of medicinal agents. The present data do clearly demonstrate, however, that currently accepted experimental diets,[26] which provide 20 μg thiamine per day to the rat, may result in marked depression in the metabolism of some drugs and that further additions of thiamine may increase this depression.

Department of Pharmacology,
School of Pharmacy, Home Economics, Nutrition,
Institute of Comparative Medicine,
University of Georgia,
Athens, Ga. 30601, *U.S.A.*

A. E. Wade
F. E. Greene
R. H. Ciordia
J. S. Meadows
W. O. Caster

REFERENCES

1. H. C. FERGUSON, *J. pharm. Sci.* **55**, 1142 (1966).
2. E. S. VESSELL, *Science, N.Y.* **157**, 1057 (1967).
3. A. E. WADE, J. E. HOLL, C. C. HILLIARD, E. MOLTON and F. E. GREENE, *Pharmacology* **1**, 317 (1968).
4. A. H. CONNEY, *Pharmac. Rev.* **19**, 317 (1967).
5. A. H. CONNEY and J. J. BURNS, *Adv. Pharmac.* **1**, 31 (1962).
6. R. KATO, E. CHIESARA and P. VASSANELLI, *Biochem. Pharmac.* **13**, 69 (1964).
7. J. R. GILLETTE, *Advances in Enzyme Regulation* (Ed. G. WEBER), vol. 1, p. 215. Pergamon, Oxford (1963).
8. C. W. DRIEVER, W. F. BOUSQUET and T. S. MIYA, *Int. J. Neuropharmac.* **5**, 199 (1966).
9. R. E. STITZEL and R. L. FURNER, *Biochem. Pharmac.* **16**, 1489 (1967).
10. R. KATO, *Biochem. Pharmac.* **16**, 871 (1967).
11. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
12. J. R. GILLETTE, *Adv. Pharmac.* **4**, 219 (1966).
13. W. M. WEATHERHOLTZ, T. C. CAMPBELL and R. E. WEBB, *Fedn. Proc.* **27**, 551 (1968).
14. W. O. CASTER, A. E. WADE, F. E. GREENE and J. S. MEADOWS, *Fedn. Proc.* **27**,549 (1968).
15. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
16. S. D. MURPHY and K. P. DUBOIS, *J. Pharmac. exp. Ther.* **124**, 194 (1958).
17. R. KATO, *Biochem. Pharmac.* **16**, 871 (1967).
18. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
19. B. B. BRODIE and J. AXELROD, *J. Pharmac. exp. Ther.* **94**, 22 (1948).
20. F. E. GREENE and A. E. WADE, *Pharmacology* **1**, 363 (1968).
21. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177**, 751 (1949).
22. W. O. CASTER, P. AHN, E. G. HILL, H. MOHRHAUER and R. T. HOLMAN, *J. Nutr.* **78**, 147 (1962).
23. J. S. MEADOWS and W. O. CASTER, *Fedn. Proc.* **26**, 525 (1967).
24. J. S. MEADOWS, *The Effect of Thiamine Deficiency on the Metabolism and Physiological Performance of the White Rat*. University of Georgia, thesis (1967).
25. A. S. SCHULTZ, R. F. LIGHT, L. J. CRACAS and L. ATKIN, *J. Nutr.* **17**, 143 (1939).
26. W. F. J. CUTHBERTSON, *Proc. Nutr. Soc.* **16**, 70 (1957).