The Effects of Dietary Alterations on 3-Methyl-4-methylaminoazobenzene *N*-Demethylase Activity[1] (36254)

RUTH E. BILLINGS[2] AND LEE W. WATTENBERG
Department of Pathology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Microsomal enzyme systems are capable of metabolizing a wide variety of compounds not normally present in the animal body. An important property of these enzymes, which is pertinent to the present study, is that an increase in their activity can be induced by a number of aromatic compounds (1–3). In addition, studies of the effects of diet on one of these systems, benzo[*a*]pyrene (BP) hydroxylase, have shown that, in the small intestine, the level of activity is dependent upon the intake of an exogenous inducer or inducers present in the diet (4, 5). In several species of rodents, experiments in which animals were fed a purified diet for a period of about 7 days or starved for 24 hr or longer showed either a total loss of BP hydroxylase activity in the small intestine or, at most, the retention of a weak activity. In contrast, animals fed a variety of crude diets have substantial levels of BP hydroxylase activity in the small intestine. With dietary alterations such as those described above, profound effects on BP hydroxylase activity do not occur in all tissues. Thus the BP hydroxylase activity of the liver is considerably more refractory to change as a result of dietary alteration than is that of the small intestine.

Experiments on the effects of diet on microsomal enzyme activity of the small intestine have been carried out thus far only with the BP hydroxylase system. The question arises as to whether this sytem is unique or if other microsomal enzymes metabolizing foreign compounds will behave similarly. In the present study, the effect of dietary manipulation on the activity of the 3-methyl-4-methylaminoazobenzene (3-MMAB) *N*-demethylase system in the small intestine and liver was determined. It was shown that the response of this microsomal system to dietary alterations is similar to that of BP hydroxylase.

Materials and Methods. Female Sprague Dawley rats, 7 to 8 weeks of age, obtained from Simonsen Laboratories, Minneapolis, MN, were used in all experiments. The rats were fed Purina Rat Chow, Ralston Purina Co., unless otherwise specified. In an initial study, the effect of oral administration of 3-methylcholanthrene (MC) on 3-MMAB demethylase activity was determined (Table I). For this purpose, 4 rats were each given 4 ml of sesame oil containing 20 mg of MC by oral intubation once daily for 4 days. Control groups of rats received either sesame oil (vehicle control) or nothing. Animals were sacrificed on day 5. In a second study, the effects of dietary alteration were determined. Rats were separated into three groups of 6 animals. One group was placed on "normal protein test diet," Nutritional Biochemicals Corp., Cleveland, OH, for 7 days. This is a purified diet consisting of vitamin-free casein, 27%; starch, 59%; corn oil, 10%; salt mixture, 4%, and a complete vitamin supplement. A second group was fasted for 48 hr prior to sacrifice. A third group was continued on the Purina Rat Chow diet throughout the experiment. This experiment was carried out twice and recorded in Table II, Expts. 1 and 2.

In all experiments, the animals were sacrificed by cervical fracture; and the liver and small intestine were removed. The mucosa, obtained from the proximal 20 cm of the small intestine, was used for enzymatic assay.

[1] Supported by U.S. Public Health Service Grant 09599 from the National Cancer Institute.

[2] Present address: Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, Indiana 46206.

TABLE I. Effect of 3-Methylcholanthrene on 3-Methyl-4-methylaminoazobenzene *N*-Demethylase Activity of Liver and Intestinal Mucosa.

Material administered	3-MMAB *N*-demethylase activity[a] (nmoles of HCHO/g of tissue/30 min)	
	Liver	Intestinal mucosa
None	665	65
Sesame oil (vehicle control)	775	90
3-Methylcholanthrene (20 mg/day × 4)	1110	340

[a] Each value is the average of duplicate determinations on pooled tissue from four rats.

The 3-MMAB *N*-demethylase activity was determined by the method of Takemori and Mannering (6), except that formaldehyde was measured by a modification of the method of Belman (7). For this procedure tissues were placed in an ice bath and homogenized in 4 vol of 1.15% KCl solution with a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The homogenates were centrifuged at 9000*g* for 20 min at 4° in a Servall angle centrifuge. All tissues were assayed the same day they were prepared. One milliliter (equiv to 200 mg of wet wt of liver or intestinal mucosa) of 9000*g* supernatant was pipetted into a 25 ml Erlenmeyer flask containing 0.6 μmole of 3-MMAB, 250 μmoles of sodium/potassium phosphate (pH 7.4), 45 μmoles of semicarbazide, 50 μmoles of magnesium chloride, 20 μmoles of nicotinamide, 110 μmoles of glucose-6-phosphate, 1 mg of TPNH, and sufficient water to bring the total reaction volume to 3 ml. "Blank" reaction mixtures contained all components except substrate. Known amounts of formaldehyde were added to the incubation mixtures instead of 3-MMAB and were used to standardize the procedure.

The flasks were incubated with shaking at 37° for 30 min, then deproteinized by the addition of 3 ml of 30% trichloroacetic acid solution. After centrifugation to remove the precipitates, the supernatants were distilled; and 4 ml of distillate was collected. Two milliliters of Nash reagent consisting of 4 *M* ammonium acetate, 0.02 *M* acetyl acetone at pH 6, were added to the distillate and heated at 37° for 60 min. The resulting fluorescence was measured with a Farrand Model A fluorometer, equipped with a primary Corning 5-60 filter and a secondary Corning 3-71 filter.

TABLE II. Effect of Dietary Factors on 3-Methyl-4-methylaminoazobenzene *N*-Demethylase Activity of Liver and Intestinal Mucosa.

Diet	Expt. no.	3-MMAB *N*-demethylase activity[a] (nmoles of HCHO/g of tissue/30 min)	
		Liver	Intestinal mucosa
Purina Rat Chow	1	625	60
	2	665	65
Purified diet[b]	1	655	10
	2	525	5
Fasted, 48 hr	1	660	5
	2	580	0

[a] Each value is the average of duplicate determinations on pooled tissue from 6 rats.

[b] Vitamin-free casein, 27%; starch, 59%; corn oil, 10%; salt mixture, 4%; and a complete vitamin supplement.

Results. In Table I, the effect of MC administration on the 3-MMAB *N*-demethylase activity of small bowel mucosa and liver is shown. MC is a widely used inducer of increased microsomal enzyme activity; and its inducing effect on liver 3-MMAB *N*-demethlyase is well documented (8); but comparable studies on the small intestine had not been performed. In the present work, induction of increased enzyme activity in the liver again was found. In addition, note that an increased 3-MMAB *N*-demethylase activity is also induced in the mucosa of the small intestine. With the procedure employed for inducer administration, *i.e.*, repeated oral doses, the relative increase in activity of the small intestine is greater than that in the liver.

The effects of dietary alteration on 3-MMAB *N*-demethylase activity are presented in Table II. The feeding of a purified diet or starvation both produced a

marked decrease in the activity of this enzyme system in the mucosa of the small intestine, but neither had any significant effect on the activity in the liver.

Discussion. The results obtained in the present study indicate that an inducer (or inducers) in the Purina Rat Chow diet is responsible for most of the 3-MMAB *N*-demethylase activity of the small bowel. In the absence of exogenous dietary inducer, *i.e.*, feeding a purified diet or starvation, the activity falls to only a small fraction of that of rats fed this conventional crude diet. These findings are similar to those obtained with the BP hydroxylase system (4, 5).

Under the conditions employed in the present study, relatively small and inconsistant effects were obtained on liver 3-MMAB *N*-demethylase activity. However, in early studies by Brown *et al.* (9), a decrease in activity of this enzyme in the liver could be demonstrated in rats fed a purified diet. In their work, weanling rats were employed; and experimental diets were fed for 2 to 3 weeks. Under these conditions, which were more drastic than those employed in the present investigation, the liver 3-MMAB *N*-demethylase in female rats fed a purified diet was approximately 60% that of groups of rats fed various crude diets. A comparable situation exists for the polycyclic hydrocarbon hydroxylase system of the rat liver. The dietary alterations used in the present study have no significant effect on the polycyclic hydrocarbon hydroxylase activity of the liver, even though they cause a profound decrease in the activity of this system in the small bowel. However, in studies employing weanling rats, Sims and Grover (10) demonstrated that rats which were fed a condensed milk diet (or continued to nurse on their mothers) had considerably lower levels of activity than animals fed a crude diet.

Data from the current and previous studies now indicate that at least two microsomal enzyme systems in the small bowel and liver can be affected in a similar way by dietary alteration. Microsomal enzyme activity in the small bowel mucosa appears to be much more readily altered by diet than that of the liver. One implication of these findings is that dietary effects on microsomal systems could alter the results of experimental studies in which the compounds metabolized by these systems are employed. In addition, if the microsomal systems of man respond to diet in a similar manner to those of the rat, then the nature of the diet might be of significance in determining the response to a variety of foreign compounds including environmental pollutants.

Summary. The effects of starvation for 48 hr or feeding a purified diet have been determined on the 3-MMAB *N*-demethylase activity of the mucosa of the small intestine and the liver. Both of these dietary alterations produce a marked decrease in activity of this enzyme system in the small intestine but do not have a significant effect on liver. These data indicate that most of the 3-MMAB *N*-demethylase activity in the small bowel is dependent upon the intake of an exogenous inducer or inducers present in the diet.

1. Conney, A. H., and Burns, J. J., *in* "Advances in Pharmacology" (S. Garattini and P. A. Shore, eds.), Vol. 1, p. 31. Academic Press, New York (1962).

2. Wattenberg, L. W., and Leong, J. L., Cancer Res. **25**, 365 (1965).

3. Wattenberg, L. W., Page, M. A., and Leong, J. L., Cancer Res. **28**, 934 (1968).

4. Wattenberg, L. W., Leong, J. L., and Strand, P. J., Cancer Res. **22**, 1120 (1962).

5. Wattenberg, L. W., Cancer **27**, 99 (1971).

6. Takemori, A. E., and Mannering, G. J., J. Pharmacol. Exp. Ther. **123**, 171 (1958).

7. Belman, S., Anal. Chim. Acta. **29**, 120 (1963).

8. Conney, A. H., Miller, E. C., and Miller, J. A., Cancer Res. **16**, 450 (1956).

9. Brown, R. R., Miller, J. A., and Miller, E. C., J. Biol. Chem. **209**, 211 (1954).

10. Sims, P., and Grover, P. L., Nature (London) **216**, 77 (1967).

Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.