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Effect of Terephthalic Acid on the Activities of *p*-Dimethylaminoazobenzene Metabolizing Enzymes in Rat Liver

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The reductase[2] and demethylase[3] activities of rat liver fed the *p*-dimethylaminoazobenzene (DAB) diet with or without 2.5% disodium terephthalate (Na_2TPA) were measured to clarify the participation of terephthalic acid (TPA) in DAB metabolism. Male rats of Wistar strain were fed the three different experimental diets for 2 or 4 weeks, which were supplemented with 0.06% DAB, with 2.5% Na_2TPA and 0.06% DAB, or with 2.5% Na_2TPA (DAB, TPA plus DAB or TPA diets, respectively). Although neither reductase nor demethylase activities were increased by the TPA diet feeding, they were maintained by TPA plus DAB diet when compared with the notable decrease by DAB feeding. From these findings, it was suggested that Na_2TPA did not activate these enzymes, but prevented the decline of their activities induced by DBA.

The effects of two TPA derivatives, dimethyl terephthalate (DMT) and β-hydroxyethyl terephthalate (BHET), on these enzyme systems were also studied. DMT elevated the reductase activity only when it supplemented the basal diet. BHET fairly increased the reductase activity when it was given with DAB. Both derivatives were not superior to Na_2TPA in their efficacies.

Furthermore, the content of protein bound aminoazo dyes in the liver of rat fed DAB plus TPA or BHET (but not DMT) were much lower than the content on the DAB diet feeding. The potentiating effect of TPA on DAB metabolism may be supported by this result.

There have been several studies concerning the role of terephthalic acid (TPA) in elevating the concentrations of some antibiotics in blood plasma and their efficacies against certain diseases.[4-9] The potentiating and maintaining effect of TPA on the plasma thiamine level was recently shown.[10,11] It was also reported that TPA encouraged the growth of the fowl fed the undernutritional food, suggesting that the fowl fed the TPA diet could utilize the nutrients more fully than otherwise.[12] These extensive works may imply that TPA plays some role in homeostasis of the body, yet this has not been adequately demonstrated. No toxicity of TPA was ascertained in mouse[13] and rat.[14]

It is well known that *p*-dimethylaminoazobenzene (DAB) is metabolized through three possible ways in the rat liver; demethylation, hydroxylation principally at the 4'-position,

1) Location: *Tsukiji 5-chome, Chuo-ku, Tokyo.*
2) Reductase is used here to refer to the enzyme system which reduces *p*-dimethylaminoazobenzene and related dyes to the corresponding diamine and aniline derivatives.
3) Demethylase is used here to refer to the enzyme system which N-demethylates *p*-dimethylaminoazobenzene and related dyes.
4) G.R. Eggert and R.F. Elliot, The 10th Animal Feed Symposium of American Cyamid Company, Agricultural Division, 65 (1959).
5) E.H. Peterson, W.L. Hendrix, and L.D. Braddy, *Poult. Sci.*, **38**, 235 (1959).
6) K.E. Price and Z. Zolli, Jr., *Avian Diseases*, **3**, 135 (1959).
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9) D.C. Shelton and N.O. Olson, *Avian Diseases*, **2**, 450 (1958).
10) A. Hoshi, R. Yanai, and K. Kuretani., *Jap. J. Zootech. Sci.*, **38**, 1 (1967).
11) K. Kuretani, A. Hoshi, and H. Hirayama, *Jap. J. Zootech. Sci.*, **36**, 511 (1965).
12) I. Tasaki and S. Myoga, *Jap. Poult. Sci.*, **3**, 115 (1966).
13) H. Nagasawa and K. Kuretani, *Jap. J. Zootech. Sci.*, **36**, 392 (1965).
14) H. Nagasawa and K. Kuretani, unpublished data.

and reductive cleavage of the azo linkage. It was reported that the activities of demethylase and reductase in the rat liver were increased by the administration of 3-methylcholanthrene which inhibited the DAB carcinogenesis.[15]

The present experiment deals with the activities of reductase and demethylase in the liver of rat given DAB with or whithout disodium terephthalate (Na_2TPA), as one step of investigating the participation of TPA in DAB metabolism.

Experimental

Animals——A large number of male rats of the Wistar strain were kept 2 or 3 each in a wire cage (25×30×15 cm) and given the commercial diet (CA-1; the product of Central Laboratory of Experimental Animal, Tokyo) until the start of the experiment. When the animals attained the body weight of 200 to 250 g they were offered the basal diet for about 10 days.

They were divided into eight groups of 6 animals each and offered the respective experimental diet and water *ad libitum*.

The changes of activities of DAB metabolizing enzymes within 4-week DAB feeding were considered to have significant importance, as Miller and Miller have reported that the content of protein bound aminoazo dyes reached the maximum in 4 weeks under the DAB diet feeding.[16] Therefore, the feeding period of either 2 or 4 weeks was selected. Food consumption and body weight were measured every three days.

Experimental Diets——The experimental diet for each group was as follows:

Groups and feeding periods (2 weeks)	(4 weeks)	Experimental diet
I	V	basal diet (BD)
II	VI	BD+2.5% Na_2TPA (TPA diet)
III	VII	BD+0.06% DAB (DAB diet)
IV	VIII	BD+2.5% TPA+0.06% DAB (TPA+DAB diet)

The basal diet was composed of crushed half-polished rice grain (3 kg), olive oil (60 ml) and casein (28 g). TPA (Teijin, Ltd.) was neutralized to Na_2TPA with NaOH.

Assay for Reductase and Demethylase——After the experimental feeding, the rats were fasted for 17—20 hr, before they were sacrificed by decapitation. Fifty mg liver as 10% homogenate in water was used per flask, and two flasks were prepared for each sample; the one was incubated aerobically at 37.5° for 30 minutes and the other stood in ice bath as control. The assay rof reductase and demethylase activities was performed by the original method of Conney, *et al.*[15] except that 200 μg of DAB or 100 μg of 3-methyl-4-monomethylaminoazobenzene (3-CH_3-MAB) per flask were used for the respective enzyme assay. The enzyme activities per 50 mg of liver were expressed as the amounts of destroyed DAB for reductase and of formed 3-methyl-4-aminoazobenzene (3-CH_3-AB) for demethylase.

Results

The Body Weight Change and the Liver Weight

The body weight was little influenced by first 2-week feeding of DAB, but, as may be expected from previous experience, notable decreases were observed after further 2-week feeding of DAB diet (−12.9%) and of DAB+TPA diet (−15.7%). No body weight change was caused by Na_2TPA addition irrespective of feeding period.

The relative liver weight (liver weight per 100 g body weight) in each group is presented in Table I. Little changes in relative liver weights were observed by TPA feeding. On the other hand, DAB and DAB+TPA feeding caused the increase in relative liver weight at 2 week feeding periods. At 4 week feeding period, further increase in relative liver weight by DAB feeding was observed, while DAB+TPA feeding maintained the relative liver weight at 2 week feeding period.

15) A.H. Conney, E.C. Miller, and J.A. Miller, *Cancer Research*, **16**, 450 (1956).
16) E.C. Miller and J.A. Miller, *Cancer Research*, **7**, 468 (1947).

Table I. The Relative Liver Weight in Each Group

Experimental diet[a]	Feeding period of experimental diet: 2 weeks Group	2 weeks R.L.W.[b](g)	4 weeks Group	4 weeks R.L.W.[b](g)
BD	I	2.68±0.08	V	2.48±0.07
BD+Na_2TPA	II	2.68±0.04	VI	2.68±0.08
BD+DAB	III	3.38±0.14	VII	3.83±0.11
BD+DAB+Na_2TAP	IV	3.19±0.12	VIII	3.23±0.12

Mean ± S.E.
a) BD: Basal diet (See the text about the composition)
DAB: *p*-Dimethylaminoazobenzene
Na_2TPA: Disodium terephthalate
b) R.L.W.: Relative Liver Weight
P<0.01: III-I, IV-I, VII-V, VII-VIII
P<0.05: VII-III

The Reductase Activity

The reductase activity of each group is shown in Fig. 1. In the 2-week feeding period, no difference in the reductase activity was found between groups I and II, but group IV was significantly higher than group III in which the activity was fairly reduced by DAB feeding ($P<0.01$). The similar tendencies were observed in the series of 4-week feeding although the difference between groups VII and VIII became smaller.

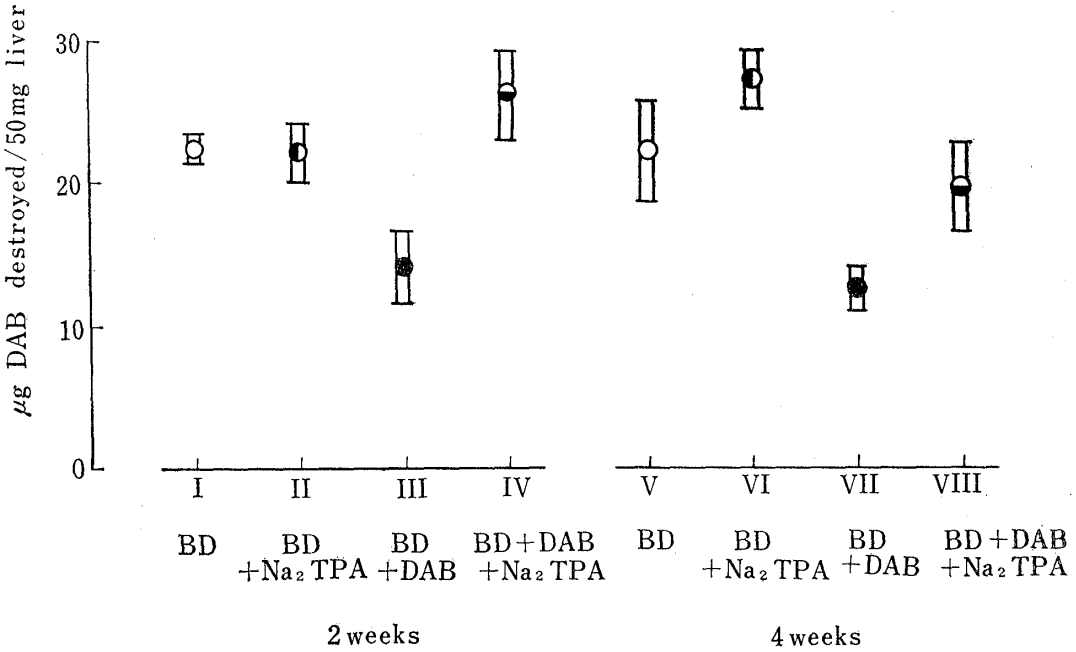

Fig. 1. Reductase Activities in the Liver of Rats Fed Na_2TPA with or without DAB for 2 or 4 Weeks

Mean±S.E.
P<0.01: I-III P<0.05: V-VII, III-IV

The Demethylase Activity

The demethylase activity of each group is shown in Fig. 2.

The demethylase activity was decreased by 2-week feeding of DAB, but little difference was observed between groups III and IV. On the other hand, this activity in group VIII was significantly higher than that in group VII ($P<0.05$).

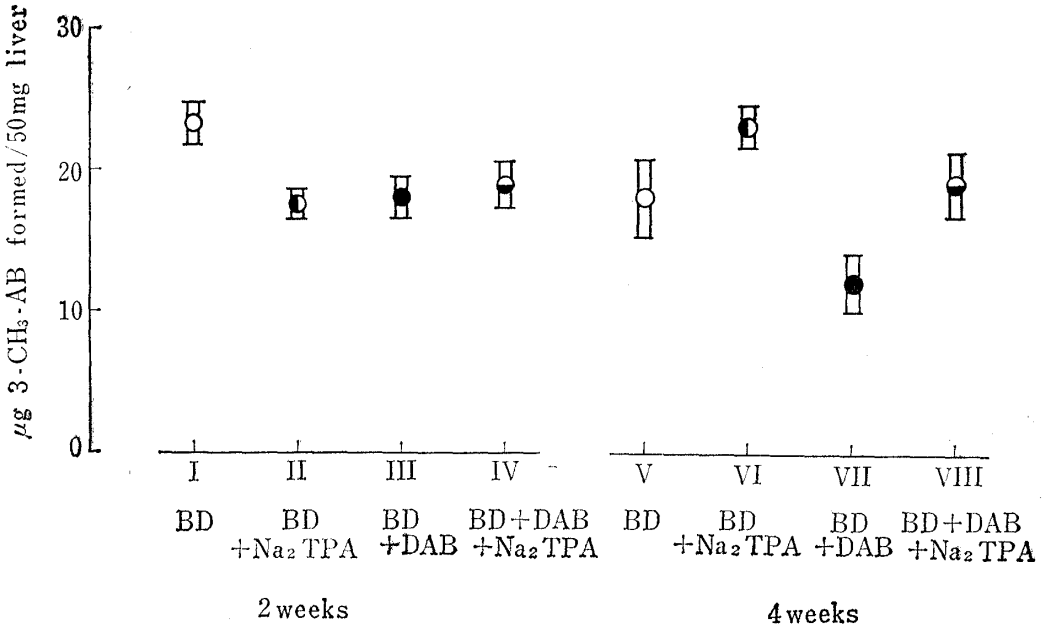

Fig. 2. Demethylase Activities in the Livers of Rats Fed Na_2TPA with or without DAB for 2 or 4 Weeks

Mean± S.E.
P<0.01 : I-II, I-III P<0.05 : VII-VIII

Discussion

The reductase activities in the basal (groups I and V), TPA (groups II and VI) and TPA plus DAB (groups IV and VIII) diet groups did not change distinguishably throughout the experimental periods. On the other hand, these activities in DAB diet groups (groups III and VII) were much lower than those in the other groups at 2 as well as 4 weeks of feeding periods. The similar tendency in the demethylase activity was also recognized at 4-week feeding. These findings show that both enzyme activities are apparently decreased by DAB feeding and addition of Na_2TPA to this diet largely prevented these decreases, though it did not produce activation of the enzymes.

The relative liver weight on the DAB diet at 4-week period was heavier than at 2-week period, whereas on the TPA plus DAB diet there was no increase of relative liver weight at 4-week period. As the rates of the body weight decreases were little different between the TPA and the control groups, Na_2TPA is considered to prevent, to some extent, the liver weight increase owing to regeneration and/or edema caused by DAB. This result may also support the effect of TPA on the maintenance of the liver function.

Table II. The Level of Protein Bound Aminoazo Dyes in the Livers of Rats in Each Group (diets were fed for 4 weeks)

Group		E/100 mg dry weight of liver
VII	BD+DAB	0.465±0.026
VIII	BD+Na_2TPA+DAB	0.325±0.020
XI	BD+DMT+DAB	0.434±0.016
XII	BD+BHET+DAB	0.370±0.014

Mean ± S.E.

The levels of protein bound aminoazo dyes in the liver were also measured by the method of Miller and Miller,[16] using rats of 4-week feedings of DAB and TPA plus DAB under the

same condition as groups VII and VIII. As given in Table II, the level of bound dyes in TPA plus DAB feeding was lowered to 70% of the level in DAB feeding (0.325 *vs.* 0.465 E/100 mg dry weight of liver). It is well known that the addition of riboflavin in the DAB diet lowers the bound dye level of liver and depresses the DAB carcinogenesis.[16] The reductase is also

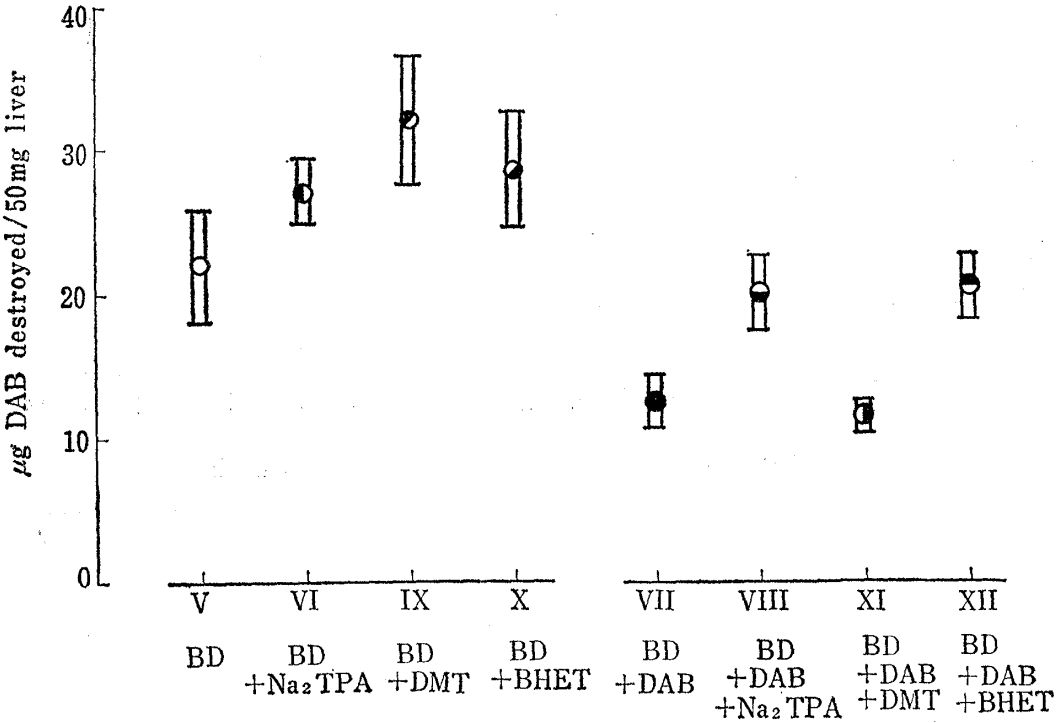

Fig. 3. Reductase Activities in the Livers of Rats Fed TPA Derivatives (DMT, BHET) with or without DAB for 4 Weeks

Mean± S.E.
$P<0.05$, XII–VII

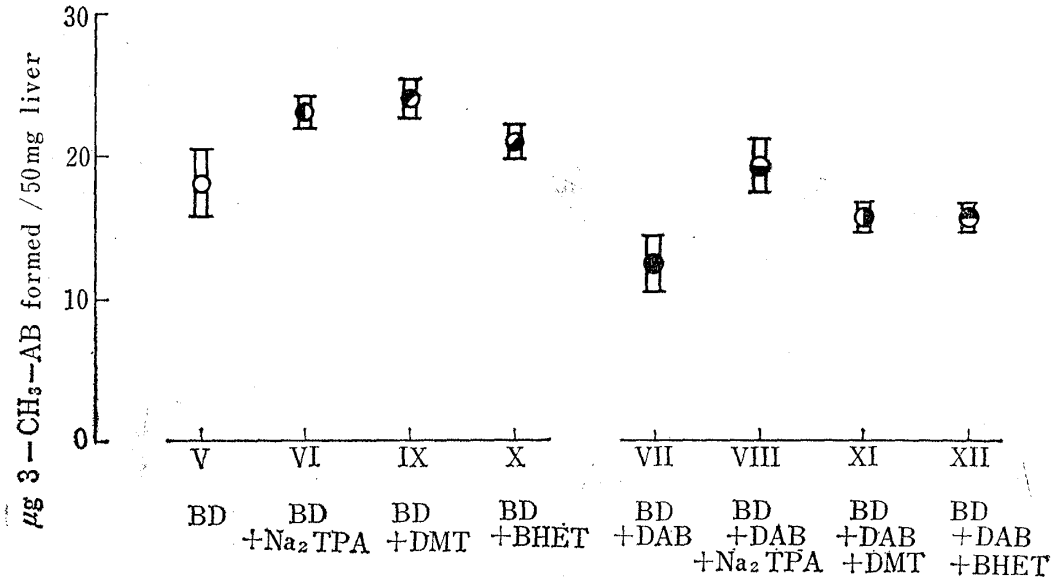

Fig. 4. Demethylase Activities in the Livers of Rats Fed TPA Derivatives (DMT, BHET) with or without DAB for 4 Weeks

Mean± S.E.

reported to be a kind of flavin enzymes.[17] There seems to be some relation between the above facts and the present results that TPA lowered the bound dye levels and restored the reductase activity which was lowered by DAB feeding.

The effects of two derivatives of TPA, dimethyl terephthalate (DMT) and β-hydroxyethyl terephthalate (BHET), on the reductase and demethylase activities were additionally studied. These compounds were supplemented to the diet with or without DAB (groups XI, IX for DMT and XII, X for BHET, respectively) at molar ratio equivalent to 2.5% Na_2TPA and rats were fed these diets for 4 weeks. The enzyme activities of these groups are presented in Fig. 3 and 4. Both derivatives had no effect on the demethylase activity but some effect on the reductase activity. DMT increased the latter activity when it was supplemented to the basal diet (group IX) but not when supplemented to the DAB diet (group XI). BHET distinctly prevented the decrease of reductase activity, which was caused by DAB feeding (group XII). However, the body weight decrease was exceptionally high in BHET feeding regardless of DAB addition (−19.8 and −17.8% in groups X and XII).

The contents of protein bound aminoazo dyes in these groups were illustrated in Table II. The bound dye level in BHET feeding was lowered to 80% of the level in DAB feeding (0.370 *vs.* 0.465 E/100 mg dry weight of liver), but not in DMT feeding (0.434 E/100 mg dry weight of liver).

These results showed that both derivatives were not superior to Na_2TPA in their effects.

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17) G.C. Mueller and J.A. Miller, *J. Biol. Chem.*, **185**, 145 (1950).