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EFFECTS OF DIET AND MICROSOMAL ENZYME INDUCTION ON THE TOXICITY OF DIMETHYL NITROSAMINE

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SUMMARY.—Feeding a protein free diet protects rats against the lethal and hepatotoxic effects of dimethyl nitrosamine. The protection cannot be reversed by starvation or by treatments which restore microsomal enzyme activity (phenobarbital or DDT). The cause of the protective effect is unknown.

DIMETHYL NITROSAMINE (DMN) is thought to exert both its acute toxic, and its carcinogenic actions only after conversion to a toxic metabolite. This reaction takes place predominantly in the microsomes of the liver, through an enzyme system similar to that which hydroxylates, and de-alkylates most drugs, and metabolises foreign compounds such as carbon tetrachloride (Magee, 1964).

It was of interest to know whether the toxic effects of dimethyl nitrosamine were affected by low protein diets, which markedly reduce microsomal hydroxylating activity (McLean and McLean, 1966).

We had already found that the activity of these enzymes was the predominant limiting factor in carbon tetrachloride toxicity (McLean and McLean, 1965, 1966). Rats fed protein free diets become resistant to carbon tetrachloride, but when inducers of microsomal hydroxylating activity such as phenobarbitone or DDT are given, the rats become sensitive again. Similar experiments were carried out for dimethyl nitrosamine, but the results were surprisingly different.

MATERIALS AND METHODS

Albino rats of either Porton or Carworth strains weighing 100–150 g. were used. The rats were fed either stock cubes or else purified diets containing no protein (McLean and McLean, 1966). The rats were treated with dicophane (DDT) 100 mg./kg. as a single s.c. injection in oil one week before exposure to DMN, phenobarbitone was given either as 3 daily i.p. injections of 100 mg./kg. or else in the drinking water at a dose of 1 mg./ml. for 5 days or longer, before giving DMN (Marshall and McLean, 1968).

The LD50 was determined and calculated by the method of Weil (1952) using 5 groups of 4 rats after preliminary trials had shown the correct dose range. Deaths were recorded for 10 days after injection. The groups fed protein free diets were returned to stock diet one week after dosing with DMN. The groups given phenobarbital in the drinking water were returned to tap water at the same time as DMN was given.

A redistilled sample of DMN was the gift of Prof. Peter Magee. It was dissolved in 0·15 M saline immediately before use and injected i.p. Plasma isocitric dehydrogenase, and bilirubin were measured as previously described (McLean and McLean, 1966).

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RESULTS

Table I shows that rats fed the protein free diet are substantially protected against the lethal effects of DMN, and that this protection is not affected by injection of DDT.

Phenobarbitone pre-treatment also failed to alter the mortality from DMN in rats fed either stock or protein free diets.

TABLE I.—*Effect of Diet and DDT on the Lethal Effects of Dimethyl Nitrosamine*

Diet and treatment	LD50 (mg./kg.)	95 per cent probability limits
Cube diet	46	40–52
Protein free	79	72–87
Protein free + DDT	81	68–98

Male Porton rats were fed the diets and treated with DDT for 1 week before injection of DMN. The technique and calculations are described in the section on Methods.

The results in Table I were obtained in the summer of 1965 and similar results continued to appear in 1966, and were found again in the summer of 1968. However, late in 1967 and early in 1968 a change in the sensitivity of rats to the lethal effects of DMN was noticed at this and several other laboratories (W. Butler and P. Swann, personal communication). Though protein depletion still protected, the LD50 for both stock and protein depleted animals fell markedly. The cause of this variation has not been found.

TABLE II.—*Factors Altering the Effects of 90 mg./kg. Dimethyl Nitrosamine on Plasma Isocitric Dehydrogenase and Bilirubin in Rats*

Diet	Treatment	n	Log plasma isocitric dehydrogenase activity (mμmoles/ml./min.)	Plasma bilirubin (mg./100 ml.)
Cube diet	—	9	3·00 ± 0·13	1·54 ± 0·47
Cube diet	DDT	5	3·21 ± 0·16	1·58 ± 0·32
Protein free	—	18	1·94 ± 0·36	0·48 ± 0·17
Protein free	DDT	6	2·17 ± 0·26	0·48 ± 0·13
Protein free	Phenobarbitone	5	2·26 ± 0·35	0·36 ± 0·13
Protein free	Cortico steroids	12	1·42 ± 0·55	0·42 ± 0·17
Protein free	Fasted 18 hr.	5	2·08 ± 0·19	0·93 ± 0·07
Control (no DMN)	All groups	8	0·15 ± 0·14	0·19 ± 0·02

Male rats weighing 150 g. were fed on protein free diet or on stock cubes. The DDT groups were given a single dose of DDT 1 week before DMN. The phenobarbitone group, were given 3 injections of phenobarbitone before DMN. Cortisone or hydrocortisone were given as a single dose of 10 mg. 18 or 2 hr. before DMN or else both doses were given. The 3 corticosteroid treated groups did not differ significantly and have been combined. Rats were killed 24 hr. after dosing with DMN. Results are expressed as means ± standard deviation. The difference between the groups fed cube diet, and the group fed protein free diet is significant ($P < 0·001$) for both bilirubin and ICD values.

Table II shows that the rise in plasma bilirubin and isocitric dehydrogenase found after dosing with DMN is much reduced in rats fed protein free diet. The protection afforded by protein free diet is not reversed by DDT, phenobarbitone, cortisone injection, or starvation.

TABLE III.—*The Effect of Fasting and Phenobarbitone on Plasma Isocitric Dehydrogenase and Bilirubin after 60 mg./kg. Dimethyl Nitrosamine in Rats Fed Stock Diet*

Treatment	n	Log plasma isocitric dehydrogenase activity (mμmoles/ml./min. ± S.D.)	Plasma bilirubin mg./100 ml. (mg./100 ml. ± S.D.)
Fed	9	2·44 ± 0·28	1·18 ± 0·74
Fasted	5	2·59 ± 0·05	0·86 ± 0·51
Fed + phenobarbitone	5	2·57 ± 0·51	0·55 ± 0·40
Fasted + phenobarbitone	5	2·33 ± 0·47	0·40 ± 0·21

Female rats were fed stock cubes, with or without 1 mg./ml. sodium phenobarbitone in the drinking water for 10 days. The fasted group had food removed on the evening before dosing with DMN. All rats were given DMN 60 mg./kg. i.p., and killed 24 hr. later.

Table III shows that in rats fed stock diets there is a slight protection against DMN liver damage on fasting and on feeding phenobarbitone.

TABLE IV.—*Effect of Starvation and Adrenalin on Plasma Isocitric Dehydrogenase 48 hr. after 30 mg./kg. DMN in Rats Fed Protein-Free Diet*

Treatment	Log plasma isocitric dehydrogenase activity (mμmoles/ml./min. ± S.D.)	Liver water kg./kg. fat free dry wt. ± S.D.
—	1·32 ± 0·42	3·40 ± 0·093
Starved 18 hr.	1·26 ± 0·11	3·09 ± 0·20
Adrenalin	1·36 ± 0·51	3·46 ± 0·28
Phenobarbitone	1·43 ± 0·30	3·38 ± 0·18
Controls (no DMN)	0·39 ± 0·14	2·54 ± 0·22

Female rats were fed protein free diet for one week, phenobarbital was given in the drinking water for 5 days before DMN. Adrenalin 0·5 mg./kg. was given i.p. 4 hr. before DMN.

Table IV shows that starvation and adrenalin do not significantly alter the response to a dose of 30 mg./kg. DMN, 48 hr. after dosing, in rats fed the protein free diet.

DISCUSSION

A protein free diet clearly protects rats against acute dimethyl nitrosamine poisoning. The LD50 is almost doubled, and indices of liver damage show the same trend. In CCl_4 poisoning the protective effect of protein free diet can be reversed by induction of microsomal enzymes with DDT, or phenobarbitone, and also in part, by starvation. Dimethyl nitrosamine toxicity is evidently not enhanced by starvation, there is even some slight reduction in the toxic effects in starved animals. Glycogen depletion using adrenalin injection also does not alter the liver damage produced by DMN.

Protein free diet reduces the rate of DMN metabolism both *in vivo* in the whole animal and *in vitro* in liver slices (Swann and McLean, 1968). The reduction of DMN toxicity after feeding protein free diet might be attributed to this reduced rate of metabolism in the liver. However, the failure of DDT or phenobarbitone to reverse the " no protein " effects suggests either that the rate of DMN metabolism is not affected by these inducers of microsomal hydroxylating enzyme activity or

else that liver damage does not depend on the rate of DMN metabolism. The first seems possible though unlikely in view of the finding of Orrenius, Ericsson and Ernster (1965) that phenobarbitone injections increase microsomal oxidations using DMN as substrate. The second and more likely explanation is that neither the rate (see Heath, 1962) nor the amount of DMN metabolised in the liver is the predominant factor in DMN liver damage. DMN after conversion to a toxic metabolite such as a carbonium ion must attack cell sites which become accessible, or are protected, depending on the previous diet. The nature of the cell site is not clear, because we cannot tell which of the many alterations in the cell produced by feeding protein free diet, is the one that has produced a protection against DMN (Lijinsky, Loo and Ross, 1968).

The protective effect of diet has been used in that it allows large doses of DMN to be given without killing rats. In this way 100 per cent incidence of kidney tumours has been produced after a single injection of DMN (Swann and McLean, 1968; Magee and McLean, unpublished).

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