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The Absorption and Metabolism in Rats of Small Oral Doses of Dimethylnitrosamine

IMPLICATION FOR THE POSSIBLE HAZARD OF DIMETHYLNITROSAMINE IN HUMAN FOOD

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1. Groups of rats were given one dose of the carcinogen dimethylnitrosamine by gastric intubation. The dose was varied between 10mg/kg body wt. and 1 μg/kg body wt. 2. The dose was rapidly absorbed. 3. The methylation of liver DNA resulting from the administration of this carcinogen was proportional to dose. This suggests that small doses are absorbed from the gut with no more loss than large doses. 4. As the dose was decreased there was a disproportionately greater decrease in the alkylation of kidney DNA, and when the dose was less than 40 μg/kg body wt. the methylation of kidney DNA was no longer detectable. This possibly explains why small amounts of dimethylnitrosamine in the diet do not induce kidney tumours. 5. Comparison of the relative alkylation of liver DNA and kidney DNA resulting from an oral and from an intravenous dose of dimethylnitrosamine suggest that small amounts of dimethylnitrosamine absorbed into the portal blood from the gut are completely metabolized by the liver and do not enter the general circulation. 6. The implications of these results for the possible hazard of dimethylnitrosamine in human food is discussed.

Small amounts of carcinogenic *N*-nitroso compounds are found in human food (Egan & Hubbard, 1975; Scanlan, 1975) and possibly may be formed in the acid contents of the stomach by the reaction between nitrite and amines in the diet (Sander, 1971*a*,*b*,*c*). One carcinogenic nitrosamine found in human food is dimethylnitrosamine. Prolonged feeding of this compound to the rat produces liver cancer (Magee & Barnes, 1956), and although the minimum dietary amount needed to induce in rats a detectable increase in cancer incidence (2mg/kg of diet) (Terracini *et al.*, 1967) is far greater than the amount of this carcinogen in the diet of man (Egan & Hubbard, 1975), there have been worries that this contamination is a hazard to health. It is obvious that large doses of the nitrosamine cross the gut wall because they reach the liver in amounts sufficient to induce cancer. However, dimethylnitrosamine can be destroyed by the bacteria of the gut (Rowland & Grasso, 1975), and as it is also possible that the intestine itself can metabolize small amounts of nitrosamine, it may be that the very small quantities found in human food are rendered innocuous before absorption. If they were, then the minor contamination of food by this compound would be less alarming.

Dimethylnitrosamine is known to be converted by metabolism in the liver, kidney and lung of the rat, and in the liver of man, into an active alkylating agent (Magee & Farber, 1962; Montesano & Magee, 1970), and it has been suggested that the reaction between this alkylating agent and the DNA of the tissue is the cause of this form of cancer. We have used the measurement of the alkylation of DNA in liver and kidney to assess whether small oral doses of dimethylnitrosamine (down to 1 μg/kg body wt.) are absorbed from the gut of the rat without degradation and reach the liver and kidney. The results show that the smallest dose given (1 μg/kg body wt.) reaches the liver with proportionally no more loss than occurs with larger doses (up to 10mg/kg body wt.).

A single large dose, or a few slightly smaller doses, of dimethylnitrosamine produce kidney tumours in the rat (Magee & Barnes, 1962; Riopelle & Jasmin, 1963, 1969). However, if a greater total dose of

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dimethylnitrosamine is given by prolonged feeding of a daily dose, which, because of the toxicity of the material, is necessarily small, liver tumours but not kidney tumours are induced (Magee & Barnes, 1967). The experiment reported here suggests a possible explanation for the failure of these repeated small oral doses to induce kidney cancer. Whereas large doses of dimethylnitrosamine produced alkylation of DNA in both rat liver and kidney, after a small oral dose of the compound alkylation could be detected in liver DNA but not in the DNA of the kidney.

Materials and Methods

Chemicals

Dimethylnitrosamine (BDH, Poole, Dorset, U.K.) was purified by distillation (b.p. 151°C). [^{14}C]-Dimethylnitrosamine was prepared from [^{14}C]-dimethylamine (The Radiochemical Centre, Amersham, Bucks., U.K.) by the method of Dutton & Heath (1956). The radioactive material (specific radioactivity either 28 or 57mCi/mmol) was diluted with unlabelled dimethylnitrosamine unless otherwise stated.

Animals

Wistar-derived female rats (150–200g) from the Courtauld Institute colony were used.

Conduct of animal experiments

(*a*) *Absorption of dimethylnitrosamine from the gastrointestinal tract.* Four female rats were allowed water but no food for 18h before administration of [^{14}C]dimethylnitrosamine (2mg/kg body wt.; 5μCi) by stomach intubation. The rats were killed 15min, 30min, 1h and 2h later. The contents of stomach and intestine were washed out with 20ml of water. Solids were removed from the washings by centrifugation and the radioactivity in 1ml of the washing was measured by scintillation counting after solution in 0.6% diphenyloxazole in toluene/Triton X-100 (4:1, v/v).

(*b*) *Alkylation of liver and kidney DNA of the rat resulting from an oral dose of various quantities of dimethylnitrosamine.* The rats were allowed water but were not fed for 18h before administration of the nitrosamine. [^{14}C]Dimethylnitrosamine was diluted with unlabelled dimethylnitrosamine for the range of doses from 10 to 0.1mg/kg body wt., but no unlabelled dimethylnitrosamine was used to prepare the smaller doses. The dimethylnitrosamine was given by gastric intubation. Four animals were given each dose. The animals were killed 4h after administration of the nitrosamine. The livers (and kidneys) of the four animals were combined and DNA was prepared from them.

Preparation of DNA and determination of the 7-methylguanine produced in it by the carcinogen

DNA was prepared from the liver and kidneys of the rats, and analysed for the content of 7-methylguanine by the methods previously described (Swann & Magee, 1968).

Results and Discussion

The rate of disappearance of dimethylnitrosamine from the gastrointestinal tract after a dose had been given by gastric intubation was measured to discover whether the nitrosamine was absorbed or retained in the gastrointestinal tract. In agreement with Heading *et al.* (1974), it was found that an oral dose of dimethylnitrosamine rapidly disappears from the gastrointestinal tract of the rat. Less than 2% of the radioactivity from an oral dose of [^{14}C]dimethylnitrosamine could be recovered from the contents of the stomach or intestine 15min after administration. These oral doses reach the liver and are there transformed by metabolic processes to a methylating agent which reacts with liver DNA. The amount of methylation of liver DNA was directly proportional to the dose over a range of doses from 10mg/kg body wt. to 1μg/kg body wt. (Fig. 1), suggesting that small doses of the carcinogen are absorbed from the gut with no more loss than large doses.

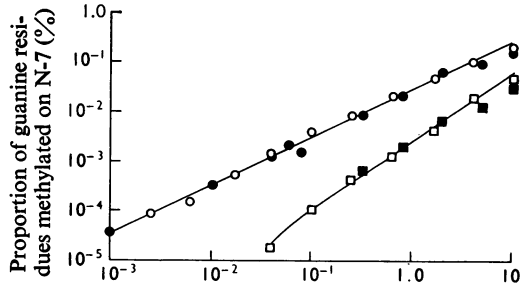

Fig. 1. *Methylation of the N-7 position of guanine in rat liver and kidney DNA by various oral doses of dimethylnitrosamine*

After 16h without food, female rats were given dimethylnitrosamine by stomach tube. The alkylation of the nucleic acids by each dose of dimethylnitrosamine was measured by analysis of the DNA from the combined livers (or kidneys) of four rats. Animals were killed 4h after administration of the nitrosamine and the amount of 7-methylguanine produced in the DNA by the carcinogen was determined as described in the Materials and Methods section. A logarithmic scale is used for both abscissa and ordinate. Liver: ●, first experiment, ○, second experiment; kidney: ■, first experiment, □, second experiment.

After high oral doses the carcinogen also reaches the kidney, and methylation of kidney DNA could also be detected. However, as the dose was decreased there was a disproportionately greater decrease in the amount of methylation of kidney DNA, and when the dose was less than 40μg/kg body wt. no methylation of kidney DNA could be detected (Fig. 2). The finding that the smaller oral doses of dimethylnitrosamine did not give rise to detectable methylation of DNA in the kidney of the rat gives an explanation for the observation that, although large doses of dimethylnitrosamine produce kidney tumours (Magee & Barnes, 1962; Riopelle & Jasmin, 1963, 1969), prolonged treatment in which a larger total dose is added in low concentration to the diet does not (Magee & Barnes, 1956, 1967).

The amount of alkylation of the kidney DNA is dependent on the amount of carcinogen and on the route of administration. When large doses (10mg or more/kg body wt.) are given, the rat cannot metabolize an appreciable part of the dimethylnitrosamine before it has become evenly distributed in the body water of the rat (Magee, 1956). In this case, liver and kidney are equally exposed to the carcinogen, but because the liver has the greater ability to metabolize the nitrosamine the alkylation of liver nucleic acids is about 4–5 times greater than that of kidney nucleic acids. However, when the dose is given by mouth it passes into the blood that drains the stomach and jejunum. This enters the portal vein and passes through the liver before entering the general circulation. The proportion of the nitrosamine metabolized in the liver increases as the size of the dose is decreased only if the dose is given by mouth. It does not increase if the dose is given by injection into the tail vein. When an oral dose of 40μg/kg body wt. was given, the alkylation of liver DNA was 75 times that of kidney DNA; however, when the same dose was given by injection into the tail vein of the conscious rat, the alkylation of liver DNA was only 4.2 times that of kidney DNA. This ratio is similar to that produced by an oral dose of 10mg/kg (4.18 times). Presumably an increasing proportion of the nitrosamine in the portal blood is metabolized by the liver as the dose given to the animal is lowered, and when a dose less than 40μg/kg body wt. is given by mouth virtually all the nitrosamine in the portal blood is metabolized by the liver before it reaches the hepatic vein and can flow from there to other organs (Fig. 2). Thus the liver prevents nitrosamine reaching other organs. The effectiveness of this barrier is perhaps demonstrated by the failure of prolonged oral administration of dimethylnitrosamine to induce kidney tumours in the rat (Magee & Barnes, 1967).

The lowest dose used in these experiments is comparable with those which man might receive if he ate some of the foods which are known to contain high concentrations of nitrosamines (Egan & Hubbard, 1975; Scanlan, 1975). One must be cautious in extrapolating these results to man because, among other things, it is not known whether the gastrointestinal tract of man can metabolize dimethylnitrosamine; however, if the factors controlling absorption and metabolism are similar in man and rat, these experiments would have some interesting implications. They would imply that small amounts of dimethylnitrosamine in food would be absorbed by man and transformed into the methylating agent, which, through its reaction with tissue, is believed to initiate the transformation of the tissue into a tumour. Although it appears that under particular circumstances recovery from the carcinogenic effect of a chemical may occur (Swann *et al.*, 1976), many extensive studies, in particular by Druckrey (1967), have shown that, in general, doses of carcinogens are cumulative and irreversible in their effect. Thus the alkylation of DNA by even the smallest dose of dimethylnitrosamine is potentially of some consequence.

However, if man and the rat are comparable, these experiments would imply that in a healthy man the metabolism and activation of dimethylnitrosamine in the diet would take place in the liver, and that the liver would remove the nitrosamine from the portal blood and prevent it reaching other organs. The liver of the rat appears to be less sensitive to the carcinogenic action of simple nitrosamines than are many other organs of this animal (Goth & Rajewsky, 1974; Kleihues & Margison, 1974; Margison & Kleihues, 1975; Nicoll *et al.*, 1975); thus to some extent the

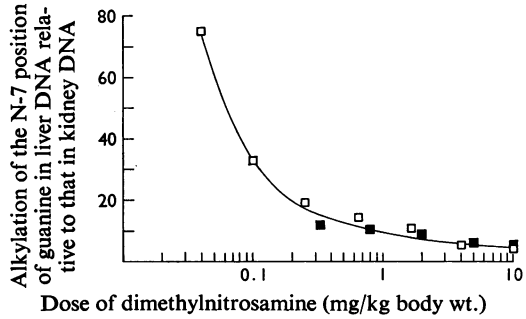

Fig. 2. *Methylation of liver DNA relative to that of kidney DNA*

■, First experiment; □, second experiment. The graph shows, at each dose, the amount of alkylation of liver DNA divided by that of kidney DNA. When 10mg/kg body wt. was given, the alkylation of liver DNA was 4.2 times that of kidney DNA. When 40μg/kg body wt. was given the alkylation of liver DNA was 75 times that of kidney DNA. These ratios were calculated from the experimental results shown in Fig. 1.

preferential metabolism in the liver protects the rat, and might protect man, against the carcinogen. It follows that the hazard of dimethylnitrosamine in the diet might be increased by any damage to the liver which lowered its ability to metabolize the nitrosamine, or which interrupted the normal flow of portal blood through the liver, so that more sensitive organs were exposed to the carcinogen. Several human liver diseases might have this undesirable effect. For example, alcoholic cirrhosis might be expected to produce both kinds of damage. The cirrhotic liver is less able to metabolize drugs than the normal liver (Levi *et al.*, 1968; Marshall & McLean, 1969) and in this disease part of the portal blood by-passes the liver both by collateral circulation and by intrahepatic anastomoses between the portal and hepatic veins (McIndoe, 1928; Hales *et al.*, 1959; Sherlock, 1975).

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