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# DIMETHYLNITROSAMINE DEMETHYLASE ACTIVITY OF RAT LIVER MICROSOMES AFTER PARTIAL HEPATECTOMY

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**Abstract**—It is known that partial hepatectomy increases the hepatocarcinogenicity of dimethylnitrosamine (DMN). To investigate why this procedure increases the hepatocarcinogenicity of DMN, the activity of liver DMN demethylase was determined on Wistar male rats at different time intervals after partial hepatectomy. The $LD_{50}$ for DMN administered 1 day after partial hepatectomy was also compared to that for the control animals. The enzyme activity reached its lowest point (47 per cent) 1 day after operation, and at 3 days had recovered to about 90 per cent of the control values. The $LD_{50}$ for partially hepatectomized rats 44 hr after i.p. injection of DMN was 114 mg/kg body weight as compared to 82 mg/kg for control animals. The reduction rather than increase of enzyme activity after hepatectomy shows that increased DMN carcinogenicity after hepatectomy is not caused by a compensatory increase of demethylase activity associated with liver regeneration.

Alkylnitrosamines are known to be metabolized to active alkylating agents by the liver microsomal mixed-function oxidase system [1, 2]. However, for induction of liver tumors long exposure to the carcinogen at a low dose level is needed [3]. This is especially true for dimethylnitrosamine (DMN). At high dose levels this compound is extremely toxic to liver cells and produces predominantly kidney tumors [4]. On the other hand, rapidly dividing cells, such as liver cells after partial hepatectomy or the cells of newborn animals, are especially sensitive to cancer production when exposed to different carcinogens [5–11]. However, the endoplasmic reticulum of newborn animals is poorly developed [6] and the levels of the microsomal enzymes involved in drug metabolism are extremely low [12–14]. This agrees with the finding that DMN is less toxic to newborn animals [6], but it does not explain the higher carcinogenicity of the same compound in newborns compared to adults if the activity of the carcinogen activating enzymes is used as a criteria.

The purpose of the present experiment was to study the activity of DMN demethylase at different time intervals after partial hepatectomy and possibly gain some information of the mechanism involved in liver tumor production after partial hepatectomy.

## MATERIALS AND METHODS

For the determination of DMN demethylase activity after partial hepatectomy male Wistar rats from Taconic Park, NY, were used. The average weight of the animals was 159 ± 2.8 g (mean ± S. E.). Median and left lateral lobe partial hepatectomies were performed, according to the method of Higgins and Anderson [15]. The food intake of the animals after hepatectomy was recorded. Three partially hepatectomized and three control rats were killed by exsanguination at 6, 24, 48 and 72 hr, and 1 week after the operation. The livers of the test animals were pooled, as were those of the control animals, and homogenized in 5 vol. (w/v) of 0.25 M sucrose and centrifuged for 20 min at 12,000 *g*. The supernatant solution was centrifuged at 100,000 *g* for 1 hr, producing the microsomal pellet which was suspended in 0.1 M potassium phosphate buffer (pH 7.4). Microsomal demethylase actively was determined in quadruplicate, employing the incubation system of Venkatesan *et al.* [16]. The amount of formaldehyde formed was determined by the method of Nash [17], with the modifications of Cochin and Axelrod [18] and McLean and Day [19]. Microsomal protein was determined by the method of Lowry *et al.* [20]. In the second part of the experiment, eight control and eight partially hepatectomized animals were killed by exsanguination 24 and 48 hr after the operation. Demethylase activity of the individual animals was determined in duplicate. These values were used for statistical evaluation.

For comparison of the toxicity of DMN in partially hepatectomized and control animals, 38 rats (average weight 108 ± 1.62 g) were partially hepatectomized between 7:00 a.m. and noon. On the following day at noon, DMN was injected i.p. into the partially hepatectomized and control rats, at dose levels ranging from 50 to 156 mg/kg. The number of dead animals was recorded 44 hr after the injection of DMN. The $LD_{50}$ for operated and for control rats was calculated from probit regression lines [21].

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Table 1. DMN-demethylase activity and protein concentration of liver microsomes of control rats and partially hepatectomized rats

| Control | | | Time after operation (days) | Partially hepatectomized | | |
|---|---|---|---|---|---|---|
| Demethylase (nmoles/g/hr)* | Demethylase (nmoles/mg/hr)† | Protein (mg/g)* | | Demethylase (nmoles/g/hr)* | Demethylase (nmoles/mg/hr)† | Protein (mg/g)* |
| [667] (650–701)‡ | 24 | 27.7 | 0 | 667 (650–701) | 24 | 27.7 |
| [535] (515–566) | 24 | 22.5 | 0.25 | 402 (386–435) | 17 | 23.8 |
| [413] (390–435) | 19 | 22.0 | 1.00 | 194 (176–215) | 9 | 21.3 |
| [886] (850–936) | 44 | 20.0 | 2.00 | 748 (716–795) | 33 | 22.5 |
| [756] (739–780) | 34 | 22.5 | 3.00 | 702 (686–725) | 31 | 22.5 |
| [758] (720–795) | 35 | 21.9 | 7.00 | 768 (750–780) | 40 | 19.2 |

* Per g of liver.
† Per mg of microsomal protein.
‡ Range.

Table 2. DMN-demethylase activity and protein concentration of liver microsomes 24 and 48 hr after partial hepatectomy*

| | | 24 hr | | | 48 hr | | |
|---|---|---|---|---|---|---|---|
| | | Demethylase | | Protein | Demethylase | | Protein |
| Condition | N | (nmoles/g/hr)† | (nmoles/mg/hr)‡ | (mg/g)† | (nmoles/g/hr)† | (nmoles/mg/hr)‡ | (mg/g)† |
| Control | 8 | 1043 ± 20 | 40 ± 2.1 | 27 ± 1.0 | 996 ± 40 | 39 ± 2.2 | 26 ± 0.7 |
| Partially hepatectomized | 8 | 490 ± 40§ | 20 ± 1.9§ | 25 ± 0.9 | 865 ± 36 | 36 ± 1.9 | 25 ± 0.8 |

* Values are expressed as mean ± S. E.
† Per g of liver.
‡ Per mg microsomal protein.
§ Significant at 0.1 per cent level.

## RESULTS

Table 1 shows the effects of partial hepatectomy on demethylase activity. The activity had decreased 6 hr after the operation but reached its lowest point 24 hr after the operation. At 48 hr the enzyme activity had returned almost to the control values. The food intake of the partially hepatectomized rats was 35 per cent of that of controls during the first 24 hr after the operation, 59 and 88 per cent during the following 2 days and 97 per cent during the last 2 days of the experiment.

Table 2 shows the values obtained when demethylase activity was determined for livers from eight control and eight test animals. One day after the operation the enzyme activity was decreased significantly ($P < 0.001$) whereas 48 hr after the operation the activity was about 90 per cent of the values of the control animals.

The $LD_{50}$ for partially hepatectomized animals obtained at 44 hr after the injection of DMN was 114 mg/kg body weight, compared to 82 mg/kg body weight for control animals. The fiducial limits for the former group were 95–137 and for the latter 74–91.

## DISCUSSION

It is generally believed that the toxic and carcinogenic effects of DMN are mediated by the same metabolites [22] and that DMN demethylase is the primary controlling factor in the metabolism of DMN to these toxic and carcinogenic agents [23]. This enzyme is located mainly in the liver, which makes it the chief organ metabolizing DMN. The present experiment shows that the activity of DMN demethylase is lowest 1 day after the partial hepatectomy, a time when the liver is most sensitive to the carcinogenic effects of DMN [24]. The reduced DMN-demethylase activity and DMN toxicity (increased $LD_{50}$), at the time of increased DMN carcinogenesis, suggest that factors which decrease the toxic effect to the cell do not necessarily decrease the carcinogenicity.

Craddock [24, 25] showed that the metabolism of nitrosamines was slowed after partial hepatectomy, which agrees with our finding of decreased DMN-demethylase activity 1 day after the operation. Craddock [5] suggested that replication of damaged DNA after hepatectomy may play an important role in making

the cells more susceptible to the carcinogenic effect of nitrosamines. Besides partial hepatectomy, other insults to the liver, such as administration of carbon tetrachloride, also decreased the activity of DMN demethylase [26] and decreased DMN toxicity but increased its carcinogenicity [26, 27]. Both of these treatments seem to result in slowing of the metabolism of DMN.

The acute toxic effect of DMN is manifested as an extensive centrilobular necrosis which leads, when it reaches significant proportions, to the death of the animal. Because of the decreased enzyme activity after partial hepatectomy this destructive effect of DMN on liver is decreased. In the normal liver with high enzyme activity, however, the administration of DMN results in necrosis of sufficient cells to lead to death from acute toxicity. Chronic DMN treatment, which is necessary for liver tumor production, has been shown to lead to a decreased methylation of liver DNA [28]. In newborn animals with a poorly developed drug-metabolizing enzyme system DMN toxicity is low, but carcinogenicity is high [6]. The present experiment favors the idea that a slowing of DMN metabolism is a predisposing factor for carcinogenicity, while it protects against DMN toxicity. It is possible that cell replication, together with the slow metabolic rate of the carcinogen, form optical conditions for a significant number of liver cells to become transformed to premalignant cells and some of these cells will escape the repair mechanism and become the origin for tumor formation.

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