The toxicity of dimethylnitrosamine (DMN) in man was recognized in the past (13, 14) but received relatively little attention. Acute toxicity tests in rats showed that DMN is highly toxic (LD₅₀ about 30 mg/kg of body weight), all the animals dying with severe centrilobular hemorrhagic necrosis of the liver (2). Similar results were obtained in the rabbit (16). The compound was later found to be carcinogenic in rats, inducing tumors of the liver (1, 23, 31), kidney (1, 24, 25, 39), and lung (1, 39). The carcinogenicity of many other nitroso compounds has also been demonstrated in rodents, and systematic investigation of possible correlations between chemical structure and carcinogenic activity are now in progress (7—11).

Following the suggestion that DMN must be metabolized before becoming tumorigenic, it was shown that enzymic demethylation of the compound is followed by methylation of cell constituents, presumably as a result of the intracellular formation of a methylating agent (3, 20, 26, 27).

The present experiment was undertaken primarily to study the possible carcinogenic effect of DMN on another species, the mouse. In view of the particular susceptibility of newborn rodents to the action of carcinogenic chemicals (5, 12, 18, 19, 21, 22, 28—30, 33, 35, 37, 38), the action of DMN in newly born mice was also investigated.

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† On leave of absence from the Toxicology Research Unit, Medical Research Council Laboratories, Carshalton, Surrey, England (present address).

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MATERIALS AND METHODS

BALB/c inbred mice, originally obtained through the courtesy of Dr. Kurt Stern, University of Illinois, Chicago, and since 1961 bred in our laboratory by brother-to-sister mating, were used. Each litter was housed with its mother until weaned, then separated according to sex. All mice were housed in plastic cages with sterilized granular cellulose bedding in groups of ten, and were given Rockland diet in pellets and tap water ad libitum with the exception to be described.

The carcinogen used was dimethylnitrosamine (N-nitrosodimethylamine, Eastman Organic Chemicals, Rochester, New York), purified by distillation (b.p. 150°C.). The experimental and control groups and the treatments are briefly described as follows.

Group 1.—DMN was dissolved in the drinking water as a 0.001 per cent solution and was given continuously for 141 days to adult and by doses of 1.5, 15, 30, and 75 μg in 0.05 ml of physiologic saline in a single subcutaneous injection to newborn BALB/c inbred mice. The treatment resulted in the induction of lung adenomas, hemangiomas, and hemangiosarcomas mainly in the liver, adenomas of kidney, hepatomas, and liver cell carcinomas. Since benign and malignant liver cell tumors were found only in animals treated at birth, the influence of age on oncogenic response is discussed.

Group 2.—One litter consisting of seven siblings was treated by S. C. injection in the dorsal region on the day of birth with a tuberculin syringe with a 30-gauge needle. Each received 1.5 μg of DMN (1 mg/kg of body weight, average body weight being 1.5 gm.) in 0.05 ml of physiologic saline.

Group 3.—Three litters consisting of 22 siblings were treated in the same way as Group 2 with 15 μg of DMN (10 mg/kg of body weight).

Group 4.—Four litters consisting of 28 siblings were treated in the same way as Group 3.

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### TABLE 1
**Survival Rate and Distribution Pattern of Tumors in DMN-treated and Control BALB/c Mice**

<table>
<thead>
<tr>
<th>Group no. and Treatment</th>
<th>No. of Mice Injected at Birth</th>
<th>Effective No. of Animals</th>
<th>Survivors at Weeks of Age</th>
<th>Animals with:</th>
<th><strong>Lung adenomas</strong></th>
<th><strong>Hemangiomas</strong></th>
<th><strong>Hemangiosarcomas</strong></th>
<th><strong>Hepatomas</strong></th>
<th><strong>Liver cell carcinomas</strong></th>
<th>Other neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>Latent period*</td>
<td>No.</td>
<td>%</td>
<td>Latent period*</td>
</tr>
<tr>
<td>Group 1. 0.001% DMN in drinking water for 141 days</td>
<td>—</td>
<td>46♂</td>
<td>44 40 15 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>41</td>
<td>89.1</td>
<td>26(15-40)</td>
<td>15†</td>
</tr>
<tr>
<td>Group 2. 1.5 μg. DMN by S.C. injection at birth</td>
<td>7</td>
<td>3♂</td>
<td>3 3 3 3 2 2 2 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group 3. 15 μg. DMN by S.C. injection at birth</td>
<td>22</td>
<td>10♂</td>
<td>10 10 10 8 7 6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>56</td>
<td>5**</td>
</tr>
<tr>
<td>Group 4. 30 μg. DMN by S.C. injection at birth</td>
<td>28</td>
<td>5♂</td>
<td>5 5 3 2 2 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>2(24-30)</td>
<td>2**</td>
</tr>
<tr>
<td>Group 5. 75 μg. DMN by S.C. injection at birth</td>
<td>27</td>
<td>1♂</td>
<td>1 1 1 1 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>57</td>
<td>1**</td>
</tr>
<tr>
<td>Group 6. Untreated controls</td>
<td>—</td>
<td>51♂</td>
<td>49 48 48 48 45 29 11 6 1</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>64</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* * In weeks.
† Nine in livers; one in liver, ovary, and spleen; one in liver and pararenal tissue; one in liver and ovary; one in liver and lymph node; one in pararenal tissue; one in ovary and peripheral nerve.
‡ Five in livers; one in liver and spleen; one in liver, spleen, ovary, and lung; one in ovary; one in uterus; one in salivary gland; one in pancreas; one in pararenal tissue.
§ One adenoma of kidney (40).
# Eight in livers; one in pararenal tissue.
$ Nine in livers; two in liver and lymph node; one in liver and pancreas.
|| One squamous-cell carcinoma of skin (30); one papillomatosis of gall bladder (38); one adenoma of kidney (34).
** In livers.
†† One malignant lymphoma, stem cell type (30).
‡‡ One skin papilloma (79).
§§ One malignant lymphoma, lymphoerytic type (48).
necropsy was performed on all animals except three males are summarized in Table 1. In Group 1, in which the treatment markedly reduced the survival in the various incidences, a significant number of benign and malignant liver cell carcinomas were seen. One was composed of a densely populated mass of stypical cells without any trabecular structure (Figs. 9 and 10); the other type exhibited neoplastic cells in a cordlike arrangement, separated by large, dilated vascular spaces (Figs. 11 and 12). These malignant tumors were morphologically similar to those previously reported induced in the Syrian golden hamster with carbon tetrachloride (4).

In addition, a small number of other types of tumor appeared in the treated and control animals and, therefore, with the possible exception of the two adenomas of kidney, cannot be attributed to the treatment.

RESULTS

The survival rates at weaning in animals given injections at birth is recorded in Table 1. A considerable number of siblings died in the group treated by injection with 30 μg.; the solitary survivor in the 75-μg. group indicates a close lethal dose effect. The survival rates after weaning are also recorded in Table 1. It can be seen that with the exception of the group receiving 1.5 μg., the treatment markedly reduced the survival in the various groups, including the animals treated at adult age.

The number, incidence, and latent period of the tumors are summarized in Table 1. In Group 1, in which the mice were treated with DMN in the drinking water, a high incidence of lung adenomas and a considerable number of hemangiommas and hemangiosarcomas, mainly in the liver, were found. Summarizing the results of Groups 2, 3, 4, and 5, in which the mice were treated at birth with different doses of DMN, it appears that in addition to the induction of lung adenomas, hemangiommas, and hemangiosarcomas (often in the liver), which exhibited various incidences, a significant number of benign and malignant liver cell tumors were obtained.

The microscopic examination of lung tumors revealed the typical appearance of benign adenomas (Fig. 1), and in a few instances their morphology suggested malignancy since they invaded blood vessels and bronchii or showed many mitoses. It is notable also that of the 224 mice observed, 64 (35 in the control group) developed nodular, usually multiple, lung necroses.

At necropsy several animals had internal hemorrhage and the pleural and peritoneal cavities were filled with blood. The macroscopic appearance of the lesions in the liver was as follows: all lobes showed irregularity in size and shape and were often distorted and shrunken (Fig. 2). The livers were often nodular, and the majority contained varying numbers of hemorrhagic cysts. Microscopically, in the early stages there were hyperemia and dilation of the liver sinusoids, often accompanied by severe centrlobular necroses (Fig. 3). Later on, blood lakes were also seen in several animals. Our criterion for the diagnosis of hemangiomma was the characterization of many layers of proliferating endothelial cells (Fig. 6). To be considered a hemangiosarcoma, a lesion had to be composed of highly anaplastic cells forming vascular clefts and invading the surrounding tissue endovascularly (Fig. 7), occasionally giving metastases to various organs (Fig. 8). In animals treated at birth, we frequently saw hyperplastic nodules in the liver (Fig. 4). The task of distinguishing between hyperplastic nodules and benign hepatomas was difficult, and our diagnosis was based primarily on size, ability to compress surrounding parenchyma, and presence or absence of portal tracts and central veins in the nodule (Fig. 5).

Two main types of liver cell carcinomas were seen. One was composed of a densely populated mass of stypical cells without any trabecular structure (Figs. 9 and 10); the other type exhibited neoplastic cells in a cordlike arrangement, separated by large, dilated vascular spaces (Figs. 11 and 12). These malignant tumors were morphologically similar to those previously reported induced in the Syrian golden hamster with carbon tetrachloride (4).

In addition, a small number of other types of tumor appeared in the treated and control animals and, therefore, with the possible exception of the two adenomas of kidney, cannot be attributed to the treatment.

DISCUSSION

The present experiment demonstrates that oral and subcutaneous administration of DMN to adult and newborn mice induces lung adenomas and also hemangiommas and hemangiosarcomas, mainly in the liver. In addition, a considerable number of benign and malignant liver cell tumors were found only in mice treated at birth. Since not a single hepatoma or liver cell carcinoma was seen in the mice treated at an adult age, and because no tumor was found at the site of administration of DMN in either age group, the neoplastic response appears to be qualitatively influenced by the age of the hosts.

This finding is somewhat similar to our previous results, in which the subcutaneous injection of 7,12-dimethylbenz(a)anthracene (DMBA) in adult Swiss mice induced mainly local sarcomas, while the same treatment in newborns gave rise to malignant lymphomas and lung adenomas, but only a few subcutaneous sarcomas (37). The suggested explanation of this result was that either the carcinogen had been retained subcutaneously in the adults and produced local tumors, or the target tissue susceptibility at certain age levels had been responsible for the different response.

The present results, of course, differ from our previous findings with DMBA in Swiss mice. The differences might be due to various reasons. In this instance, DMN was given subcutaneously to newborns and orally to adult mice; in prior studies the carcinogen was administered to both age groups only subcutaneously. Furthermore, DMN is a water-soluble substance, whereas DMBA was injected in an oily solution. In addition, it is notable that no tumor was found at the application site of DMN in either age group.
More recently our attention was directed toward the enzymatic and metabolic studies in this field. The activities of certain liver enzymes in newborn mice were shown to be 5 times smaller than in adults (15). Moreover, it was demonstrated in our laboratory that DMBA persists for a longer period in newborns than in adult mice (6). In accord with this finding, the catabolic rate of urethan was reported considerably higher in adult mice than in younger and newborn animals (17). The results obtained in these different experiments do not explain, of course, the qualitative differences in tumor type observed in adult and newborn mice in the present investigation; however, they do point to the direction in which further studies could be performed.

In relation to incidence and distribution of tumors induced by DMN in different organs of various species, it seems to us of particular importance to correlate these findings with the results obtained in the metabolic studies of radioactive DMN. It was shown that when rats, mice, and hamsters were treated with tritium- and carbon-14-labeled DMN, most of the radioactivity was present as 7-methylguanine in the liver and kidney of the rat, in the liver of the hamster, and in the liver and lung of the mouse (20). Since the induction of tumors by DMN was observed mainly in these particular organs of the various species (23, 24, 34, 36), it is possible that a link exists between the structural changes of RNA and tumor induction.

ACKNOWLEDGMENTS

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Fig. 1.—Lung. Adenoma. 26-week-old female mouse treated orally with DMN; killed 20 weeks after the beginning of the treatment. Hematoxylin and eosin, X 160.

Fig. 2.—Liver. Carcinoma. 56-week-old female mouse treated with 15 ,ug. of DMN at birth, X 12.

Fig. 3.—Liver. Severe hemorrhagic necrosis. 11-week-old male mouse treated orally with DMN; died 7 days after beginning of the treatment. Hematoxylin and eosin, X 95.

Fig. 4.—Liver. Regenerative nodules. 50-week-old female mouse treated with 15 ,ug. of DMN at birth. Hematoxylin and eosin, X 80.
Fig. 5.—Liver. Hepatoma. Note the absence of portal tracts and central veins in the lower part of the picture. 38-week-old male mouse treated with 30 μg. of DMN at birth. Hematoxylin and eosin, × 180.

Fig. 6.—Liver. Hemangioma, showing several layers of proliferating endothelial cells. 28-week-old female mouse treated orally with DMN; killed 20 weeks after the beginning of the treatment. Hematoxylin and eosin, × 95.

Fig. 7.—Spleen. Hemangiosarcoma, exhibiting vascular growth and invasion of the spleen. 30-week-old female mouse treated orally with DMN; killed 22 weeks after the beginning of the treatment. Hematoxylin and eosin, × 110.

Fig. 8.—Lung. Metastasis from the spleen. Same as Figure 7. × 120.
Fig. 9.—Liver. Carcinoma, composed of densely populated atypical cells without any trabecular structure. 58-week-old female mouse treated with 15 μg. of DMN at birth. Hematoxylin and eosin, × 170.

Fig. 10.—Same as Figure 9. × 450.

Fig. 11.—Liver. Carcinoma, composed of neoplastic cells in cordlike arrangement which are separated by dilated vascular spaces. 61-week-old female mouse treated with 30 μg. of DMN at birth. Hematoxylin and eosin, × 130.

Fig. 12.—Same as Figure 11. × 350.
Carcinogenesis Study with DimethylNitrosamine Administered Orally to Adult and Subcutaneously to Newborn BALB/c Mice

Bela Toth, Peter N. Magee and Philippe Shubik

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