Primary Neoplasms in Dog Liver Induced by Diethylnitrosamine

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SUMMARY

Clinicopathological, angiographic, and histological studies were made on liver neoplasms in adult male mongrel dogs after administration of diethylnitrosamine. Primary hepatic neoplasms of various types developed in 14 male mongrel dogs upon administration of diethylnitrosamine for over 50 weeks. Grossly, the surface of the liver of these animals appeared finely granular, rubbery, and hard. Single or multiple gray or yellowish tumors with areas of hemorrhage 1 to 9 cm in diameter were found in the livers. Clinicopathological analysis showed that the erythrocyte count, hematocrit, and hemoglobin content of the dogs gradually decreased during administration of diethylnitrosamine while the alkaline phosphatase activity and serum cholesterol level gradually increased. Malignant neoplasms of the liver were detectable by angiography in animals before sacrifice, and hemangiomas and hemangioendothelio- mas were easily diagnosed by selective hepatic angiography. The liver neoplasms were of the following histological types: 3 fibromas, 4 leiomyomas, 1 hemangioma, 10 hemangioendotheliomas, 4 fibrosarcomas, 2 leiomyosarcomas, 1 hepatocellular carcinoma, 1 cholangiocarcinoma, and 1 undifferentiated cell carcinoma. In nonnodular areas of the livers, oval cell infiltration, bile duct proliferation, fibrosis in periportal areas, and development of nodular hyperplasias were usually observed.

INTRODUCTION

There have been many reports on the carcinogenic effects of nitroso compounds on the livers of small experimental animals (12, 17, 18, 24, 27, 31). Primary neoplasms of the liver have mainly been studied on biological, histological, histochemical, and biochemical levels, but in many cases 3-dimensional or clinicopathological studies on primary liver neoplasms in experimental animals were not sufficient. Recently, hepatic neoplasms in rabbits, dogs, and monkeys induced by diethylnitrosamine, 2-acetylaminofluorene, and aflatoxin B1 were reported by several investigators (1, 8–10, 13–16, 20–22).

The present experiments were on the process of induction of primary hepatic neoplasms in dogs treated with diethylnitrosamine. Comparative analyses were made on the clinicopathology, angiographic results, morphology, and histology of liver neoplasms induced by diethylnitrosamine in dogs.

MATERIALS AND METHODS

Diethylnitrosamine was purchased from Tokyo Kasei Co., Tokyo, Japan. Adult male mongrel dogs, weighing 10 to 20 kg, were given drinking water containing 50, 100, or 500 ppm of diethylnitrosamine for 2 to 50 weeks. Solutions of diethylnitrosamine were given to individual dogs ad libitum from Monday to Saturday throughout the experiment. The solutions were refrigerated and protected from the light. All the dogs received commercial stock diet, 35 g/kg body weight (Oriental Co., Tokyo, Japan), each morning. The dogs were housed in individual steel cages in an air-conditioned room at 24°C and weighed weekly before being fed.

Blood Chemistry. Once a month blood samples were taken to examine the erythrocyte and leukocyte counts; hematocrit; and the contents of hemoglobin, SGPT, SGOT, total protein, albumin, albumin/globulin ratio, alkaline phosphatase, total cholesterol, and γ-globulins. Erythrocytes, the hematocrit, hemoglobin, and leukocytes were counted with a microcell counter (TOA Medical Electronics). SGOT, SGPT, alkaline phosphatase, total cholesterol, and total protein were measured with a Hitachi Type 400 automatic analyzer. Electrophoresis of serum protein was performed in a Beckman Model R-101 microzone cell with a cellulose acetate membrane (membrane folin). The albumin/globulin ratio was measured by electrophoresis.

Hepatic Angiography. After administration of diethylnitrosamine for 50 weeks, angiograms of selected dogs were taken under general anesthesia. A single preshaped radiopaque polyethylene catheter (length, 60 cm; external diameter, 2.1 mm; internal diameter, 1.3 mm) was introduced into the dogs via the femoral artery. The tip of the catheter was placed in the celiac artery and then in the common hepatic artery, using an image intensifier and X-ray television equipment. Then, Isopaque 350, 1 ml/kg body weight, was injected as fast as possible by hand. The average speed of injection was 3.3 ml/sec, which was comparable to the speed obtained using an automatic injector. Angiograms were taken with an Elma-Schoe

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nander cut film changer programmed at 2 films/sec for 13 sec. Angiograms were taken in the anteroposterior position in all dogs and in the lateral projection as well in 10 dogs. The exposure values were 0.08 to 0.10 sec, 400 ma, and 80 to 85 kV. A grid with 100 lines/inch, Simens Diamant intensifying screens, and Kodak R.P. 54 films were used. The film focus distance was 100 cm, and the focal spot of the tube was 1.2 x 1.2 mm.

**Histopathological Study.** Postmortems were carried out on all dogs that died or were killed when they became moribund. The liver and metastatic lesions in other organs were fixed in 10% buffered formalin solution, embedded in paraffin, and sectioned at a width of 5 µm.

Sections were routinely stained with hematoxylin and eosin, and selected tissues were stained with Mallory’s or Van Gieson’s stain or by silver impregnation or the periodic acid-Schiff reaction.

**RESULTS**

**Clinical Findings**

All dogs lost their appetites after the administration of water containing diethylnitrosamine, but when diethylnitrosamine treatment was stopped they regained their normal appetites. Experimental dogs showed a slight weight gain. Several dogs died of pneumonia or hemoptysis, while others died during anesthesia. The changes in body weight of all tumor-bearing dogs are shown in Tables 1 and 2.

**Hematology and Serum Chemistry**

Changes in the erythrocyte and leukocyte counts, the hematocrit, and the hemoglobin content are summarized in Table 2. Anemia in dogs shortly before death was characterized by a decreased number of erythrocytes, with a decreased hematocrit and hemoglobin content. SGOT, SGPT, alkaline phosphatase, and total cholesterol increased markedly before death or at the end of the experimental period. The total protein values generally remained normal but decreased before death or at the end of the experiment with a decrease in the percentage of albumin and an increase in that of α₁, α₂, β₂, and γ-globulins.

**Angiography**

From the hepatic angiographs obtained, it was possible to distinguish gross benign and malignant tumors before operation, but it was difficult to differentiate malignant tumors histologically because correct diagnoses of malignant neoplasms from angiograms were impossible until the tumors reached a certain size. Hemangioma and hemangiendotheliomas showing vascular proliferation through the tumor were more easily diagnosed than other benign tumors, such as leiomyomas and fibromas, in which blood vessels did not develop. Angiographic findings in experimental malignant hepatic tumors such as the cholangiocarcinoma, the hepatocellular carcinomas, and the leiomyosarcomas (but not the fibrosarcomas) were dilation, abruption, and narrowing of the affluent arteries and fine vascular formations within the tumors and faint, prolonged capillary perfusion. Hemangioma and hemangiendotheliomas characterized showed dilation of the affluent arteries and a dense, hypervascular, mottled appearance within the tumors.

**Histopathology of Livers**

Various histological types of primary hepatic neoplasms developed in 14 dogs treated with diethylnitrosamine.

**Fibromas.** Three of the 14 dogs developed fibromas. Tumor tissues were composed of fibrous elements. There were few cells, and mitotic figures were rare. Fibrous elements were stained red with Van Gieson’s stain.

**Leiomyomas.** Four of the 14 dogs developed leiomyomas. Tumor tissues consisted of bundles of plain muscle fibers. No mitosis was visible, and the cells were stained yellow with Van Gieson’s stain.

**Hemangioma.** One dog developed hemangioma. The tumor tissue was composed of newly formed blood vessels,

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### Table 1

<table>
<thead>
<tr>
<th>Observation period (wk)</th>
<th>Body wt (kg)</th>
<th>Liver</th>
<th>Ascites (ml)</th>
</tr>
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<td>Total</td>
<td>Total intake of diethyl nitrosamine (mg)</td>
<td>Initial</td>
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<td>73</td>
<td>52</td>
<td>52</td>
<td>3120</td>
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<tr>
<td>129</td>
<td>58</td>
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<td>67</td>
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<td>68</td>
<td>6720</td>
</tr>
<tr>
<td>130</td>
<td>64</td>
<td>70</td>
<td>3840</td>
</tr>
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</tr>
<tr>
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<td>70</td>
<td>80</td>
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</tr>
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</tr>
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<td>110</td>
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</tr>
<tr>
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<td>70</td>
<td>113</td>
<td>4200</td>
</tr>
<tr>
<td>87</td>
<td>58</td>
<td>124</td>
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</tr>
<tr>
<td>105</td>
<td>57</td>
<td>175</td>
<td>5580</td>
</tr>
</tbody>
</table>
but proliferation of sinusoids lined with endothelial cells was rare.

Fibrosarcomas. Four of the 14 dogs developed fibrosarcomas. The spindle-shaped cells were irregularly arranged and showed numerous mitotic figures. Stromal areas occasionally appeared necrotic or hemorrhagic.

Leiomyosarcomas. Two of the 14 dogs developed leiomyosarcomas. The tissue was composed of polygonal cells and many mitotic figures were visible. Hemorrhagic areas and hyalinization of the stromal tissues were frequently observed. Tumor cells were stained pale yellow with Van Gieson's stain.

Hemangioendotheliomas. Ten of the 14 dogs developed hemangioendotheliomas. These tumor tissues consisted of networks of newly formed capillaries and irregularly shaped endothelial cells which frequently showed mitotic figures.

Hepatocellular Carcinoma. One dog developed a hepatocellular carcinoma. The tumor tissue consisted of liver cells that were very irregular in size and arrangement. Mitotic figures and giant cells were frequent. Vascular spaces and proliferation of sinusoidal cells were indistinct. Hemorrhagic and necrotic areas were also observed.

Cholangiocarcinoma. One dog developed a cholangiocarcinoma. The tumor cells had an adenomatous pattern. The lining cells resembled those of the bile ducts. Giant cells were rare.

Undifferentiated Cell Carcinoma. One dog developed an undifferentiated cell carcinoma. The tumor cells were polyhedral with chromatin-rich nuclei. Mitotic figures were not frequently observed. Liver cell cords in tumor areas showed atrophic changes.

Nonneoplastic Areas of Liver. Proliferation of connective tissue in portal areas was usually visible, and oval cells or bile duct proliferation was found in all the dogs. Areas of nodular hyperplasias were also demonstrated in all the dogs.

Other Organs. Six of the 14 dogs developed squamous cell carcinomas of the nasal cavity, and 1 dog had hemangioendothelioma of the spleen.

The total experimental period, total intake of diethylnitrosamine, body weight, liver weight, and histological findings in the livers of these dogs are summarized in Table 3.

Autopsy findings and the clinical details of the dogs treated with diethylnitrosamine are as follows.

Case 1, Dog 73, received water containing 50 ppm of diethylnitrosamine for 52 weeks; it died of pneumonia. Angiography was not done. At the time of autopsy, the presence of ascites was measured at 5000 ml, and the liver weighed 344 g. There were marked cirrhotic changes in the right and left lobes of the liver. A neoplastic area, 7 mm in diameter, was found in the middle lobe. This was identified histologically as a hemangioma. No other organs showed neoplastic or metastatic changes.

Case 2, Dog 129, received water containing 50 ppm of diethylnitrosamine for 58 weeks and then water without diethylnitrosamine for 5 weeks. No angiogram was taken. The animal died of cachexia 63 weeks after the start of the experiment. The autopsy revealed no ascites, and the liver weighed 810 g. Grossly, the surface of the liver appeared smooth and a large, whitish yellow neoplasm, 8 x 9 cm in
Liver Tumors in Dogs Induced by Diethylnitrosamine

Table 3
Histopathological findings in the liver in male dogs treated with diethylnitrosamine

<table>
<thead>
<tr>
<th>Dog</th>
<th>Bile duct proliferation</th>
<th>Oval cell infiltration</th>
<th>Fibrosis</th>
<th>Nodular hyperplasia</th>
<th>Primary neoplasm of the liver</th>
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</thead>
<tbody>
<tr>
<td>73</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>Hemangioma</td>
</tr>
<tr>
<td>129</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>Leiomyosarcoma, hemangioendothelioma</td>
</tr>
<tr>
<td>79</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>Fibroma</td>
</tr>
<tr>
<td>96</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Undifferentiated cell carcinoma</td>
</tr>
<tr>
<td>130</td>
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<td>+</td>
<td>++</td>
<td>+</td>
<td>Fibrosarcoma, hemangioendothelioma</td>
</tr>
<tr>
<td>98</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>133°</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Hemangioendothelioma</td>
</tr>
<tr>
<td>80°</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>Leiomyosarcoma, hemangioendothelioma</td>
</tr>
<tr>
<td>104°</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>Fibrosarcoma, hemangioendothelioma</td>
</tr>
<tr>
<td>131</td>
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<td>+</td>
<td>+</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>90°</td>
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<td>+</td>
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<td>+</td>
<td>Fibrosarcoma, hemangioendothelioma</td>
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<tr>
<td>109°</td>
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<td>+</td>
<td>+</td>
<td>Fibrosarcoma</td>
</tr>
<tr>
<td>87°</td>
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<td>+</td>
<td>+</td>
<td>Hemangioendothelioma</td>
</tr>
<tr>
<td>105°</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Fibrosarcoma, leiomyosarcoma, hemangioendothelioma, hepatocellular carcinoma</td>
</tr>
</tbody>
</table>

* Squamous cell carcinoma of the nasal cavity.
* Hemangioendothelioma of the spleen.

...diameter, was seen in the right inner lobe. Six small nodules, 1.0 to 1.5 cm in diameter, were seen in other lobes (Fig. 1). Histologically, 5 of the neoplastic lesions were identified as leiomyosarcomas and 1 in the right inner lobule was identified as a hemangioendothelioma. Both the lung and kidney had metastatic areas that looked essentially the same as the primary lesions (Fig. 2). Case 3, Dog 79, received water containing 100 ppm of diethylnitrosamine for 7 weeks and then water without diethylnitrosamine for 17 weeks. This was followed by water with 100 ppm of diethylnitrosamine for 43 weeks. No angiogram was taken. The animal died of bronchopneumonia 67 weeks after the start of experiment. At the time of autopsy, the volume of ascites was 1800 ml, and the liver weighed 265 g. The surface of the liver showed diffuse granular changes. One tumor, 0.7 cm in diameter, was found in the right inner lobe; 1 of 1.0-cm diameter in the middle lobe; and 1 of 1.2-cm diameter in the left lateral lobe. Histologically, the tumor of the right inner lobule was identified as a hemangioendothelioma (Figs. 5 and 6), that of the middle lobe as a fibrosarcoma, and that of the left lateral lobe as a fibroma. No metastatic changes were seen.

Case 4, Dog 96, received water containing 100 ppm of diethylnitrosamine for 56 weeks and then water without diethylnitrosamine for 12 weeks. No angiography was made. The dog died of general fatigue 68 weeks from the start of the experiment. At the time of the autopsy, the volume of ascites was 1800 ml, and the liver weighed 265 g. The surface of the liver showed diffuse granular changes. One tumor, 0.7 cm in diameter, was found in the right inner lobe; 1 of 1.0-cm diameter in the middle lobe; and 1 of 1.2-cm diameter in the left lateral lobe. Histologically, the tumor of the right inner lobule was identified as a hemangioendothelioma (Figs. 5 and 6), that of the middle lobe as a fibrosarcoma, and that of the left lateral lobe as a fibroma. No metastatic changes were seen.

Case 6, Dog 98, received successively water containing 500 ppm of diethylnitrosamine for 2 weeks, water without diethylnitrosamine for 10 weeks, water with 100 ppm of diethylnitrosamine for 18 weeks, water without diethylnitrosamine for 17 weeks, and water with 50 ppm of diethylnitrosamine for 32 weeks. A hepatic angiogram taken after 70 weeks showed 2 tumors in the liver. The larger tumor showed abnormal vascularity. The dog died of general fatigue 80 weeks after the start of the experiment. No ascites were seen during the autopsy, and the liver weighed 490 g. Its surface showed multiple, irregular, cirrhotic nodules. One tumor, 2.5 x 2.1 cm in diameter, was found in the right middle lobe, and one, 0.8 x 0.4 cm in diameter, was found in the right middle lobe. Both were whitish yellow and were identified histologically as fibrosarcomas (Figs. 7 and 8). No metastatic changes were seen.

Case 7, Dog 133, received water containing 50 ppm of diethylnitrosamine for 70 weeks and then water without diethylnitrosamine for 10 weeks. An angiogram taken after 80 weeks showed a tumor with abnormal vascularity. The dog died of bronchopneumonia 80 weeks after the experiment began. The autopsy revealed no ascites, and the liver weighed 480 g. The surface was irregular, and a hemorrhagic liver tumor, 2.9 x 2.4 cm in diameter, was found. The tumor was identified histologically as a malignant hemangioendothelioma. No metastatic change was seen.
The dog also had a squamous cell carcinoma of the nasal cavity.

Case 8, Dog 80, successively received water containing 100 ppm of diethylnitrosamine for 24 weeks, water without diethylnitrosamine for 12 weeks, water containing 100 ppm of diethylnitrosamine for 28 weeks, and then water without diethylnitrosamine for 17 weeks. Two angiograms of the liver were made after 78 and 81 weeks. A hemangiomatous lesion of the left lobe with markedly increased vascularity and density was detected from the angiogram after 78 weeks. The animal became moribund and was killed after 81 weeks had elapsed from the start of the experiment. The autopsy revealed no ascites. The liver weighed 540 g, its surface appearing irregular or slightly cirrhotic, and 2 hemorrhagic tumors measuring 1.4 x 0.9 and 0.6 x 0.4 cm in diameter were found in the left lateral and median lobes, respectively. They were diagnosed histologically as a hemangioendothelioma and a leiomyoma. No metastatic change was seen. The animal had a squamous cell carcinoma of the nasal cavity.

Case 9, Dog 104, received water containing 100 ppm of diethylnitrosamine for 24 weeks, water without diethylnitrosamine for 12 weeks, water with 100 ppm of diethylnitrosamine for 20 weeks, water without diethylnitrosamine for 6 weeks, water with 100 ppm of diethylnitrosamine for 14 weeks, and then water without diethylnitrosamine for 28 weeks. From angiograms of the liver taken after 77, 92, and 102 weeks, a hemangiomatic lesion of the right inner lobe was identified 92 weeks later. In the angiogram, the tumor tissue of the hemangioendothelioma showed markedly increased vascularity and uneven density, but areas of fibrosarcoma did not appear abnormal. The dog became moribund and was killed after 102 weeks. No ascites were seen at the time of the autopsy. The liver weighed 345 g and showed diffuse cirrhotic changes. Two tumors were found in the right inner lobe (1.7 x 0.5 and 0.4 x 0.7 cm), 1 in the right middle lobe (3.0 x 4.7 cm), and 3 in the right lateral lobe (1.0 x 0.5, 0.6 x 0.6, and 0.7 x 0.7 cm). The tumors of the right inner and lateral lobes were identified histologically as hemangioendotheliomas and that of the right middle lobe as a fibrosarcoma. No metastatic changes were seen, but squamous cell carcinoma was found in the nasal cavity.

Case 10, Dog 131, received water containing 50 ppm of diethylnitrosamine for 70 weeks and then water without diethylnitrosamine for 35 weeks. Angiographs of the liver were taken after 72 and 92 weeks. From these, 2 hemangiomatic lesions were detected after 72 weeks; this was exemplified by their increased density and vascularity, but an area of fibroma appeared normal. The dog died after left hepatic lobectomy at 105 weeks and was autopsied. Ascites content was measured at 1500 ml, and the smooth-surfaced liver weighed 450 g. Two liver tumors 1.7 and 0.5 cm in diameter were found in the right inner and middle lobes, respectively. Histologically, 2 larger tumors were identified as hemangioendotheliomas and another smaller one as a fibroma. No metastatic lesion was seen.

Case 11, Dog 90, received water containing 500 ppm of diethylnitrosamine for 2 weeks, water without diethylnitrosamine for 10 weeks, water containing 100 ppm of diethylnitrosamine for 18 weeks, water without diethylnitrosamine for 18 weeks, water with 50 ppm of diethylnitrosamine for 32 weeks, water without diethylnitrosamine for 10 weeks, water with 50 ppm of diethylnitrosamine for 3 weeks, and then water without diethylnitrosamine for 16 weeks. A hemangioendothelioma was diagnosed by the increased vascularity and density in angiograms of the liver made after 92 and 110 weeks. The dog became moribund and was killed after 110 weeks and autopsied. The liver weighed 290 g and there were no ascites. One tumor, 0.3 x 0.3 cm in diameter, was found in the left lateral lobe; 1, 1.0 x 0.8 cm in diameter, in the inner lobe; and 1, 0.8 x 0.8 cm in diameter, in the right inner lobe. Nontumorous areas were essentially smooth.

Histologically, the tumors were identified as a hemangioendothelioma (in the left lateral lobe), cholangiocarcinoma (in the left inner lobe), and leiomyoma (in the right inner lobe). Metastatic changes of the cholangiocarcinoma were not seen, but squamous cell carcinoma was found in the nasal cavity and metastatic foci of it were observed in the lung.

Case 12, Dog 109, received water containing 50 ppm of diethylnitrosamine for 70 weeks and water without diethylnitrosamine for 43 weeks. Angiograms of the liver were made after 91 and 113 weeks, and hemangiomatic lesions were recognized from the angiograph after 91 weeks. In the angiograms hemangioendotheliomas showed abnormal vascularity, but the leiomyoma appeared normal. The dog became moribund and was killed and autopsied after 113 weeks. No ascites were seen. Diffuse cirrhotic changes were seen in the liver surface with 1 hemorrhagic nodule, 0.5 x 0.4 cm in diameter, in the right inner lobe, and 2 tumors, 3.3 x 1.5 and 0.8 x 0.7 cm in diameter, in the left inner lobe. The tumor of the right lobe and larger tumor of the left lobe were identified histologically as hemangioendotheliomas and the smaller tumor of the left lobe was identified as a leiomyoma. No metastatic change was seen. A squamous cell carcinoma was found in the nasal cavity with metastatic lesions in both lungs.

Case 13, Dog 87, successively received water containing 500 ppm of diethylnitrosamine for 2 weeks, water without diethylnitrosamine for 10 weeks, water with 100 ppm of diethylnitrosamine for 18 weeks, water without diethylnitrosamine for 18 weeks, water with 50 ppm of diethylnitrosamine for 32 weeks, water without diethylnitrosamine for 10 weeks, water with 50 ppm of diethylnitrosamine for 5 weeks, and then water without diethylnitrosamine for 24 weeks.

Angiographs of the liver were taken after 96 and 124 weeks, showing hemangiomatic lesions of left lateral lobe and mesenchymal tumor lesions of the left inner lobe after 96 weeks. In the angiograms, areas of hemangioendotheliomas and fibrosarcomas showed irregular vascularity and density (Figs. 13 and 14). The dog became moribund due to hemorrhage from a duodenal ulcer after 124 weeks. Ascites was not found. The liver surface appeared finely granular and 3 tumors in the left lateral lobe (1.7 x 0.8, 0.4 x 0.2, and 1.1 x 1.1 cm) and 1 white tumor, 2.0 x 1.2 cm in
diethylnitrosamine for 2 weeks, water without diethylnitrosamine for 10 weeks, water with 100 ppm of diethylnitrosamine for 18 weeks, water without diethylnitrosamine for 18 weeks, water with 50 ppm of diethylnitrosamine for 33 weeks, water without diethylnitrosamine for 11 weeks, water with 50 ppm of diethylnitrosamine for 4 weeks, and water without diethylnitrosamine for 79 weeks. Angiography of the liver was taken after 85, 105, 124, 150, and 174 weeks, and in these angiographs areas of the leiomyosarcoma, hemangioendotheliomas, and hepatocellular carcinoma showed irregular vascularity and density (Figs. 13 and 14). After 124 weeks, hemangiomatous tumors of the left inner lobe and right lateral lobe and mesenchymal tumors of the right inner lobe were recognized by angiography. After 175 weeks the dog died after receiving anesthesia during angiography. Upon autopsy, no ascites were seen, and the liver weighed 990 g and showed a total of 26 diffuse cirrhotic tumors of various sizes. Larger tumors of the right lateral lobe were up to 3.3 cm in diameter. Some tumors appeared yellowish or hemorrhagic.

Histologically, the larger tumors were identified as leiomyosarcomas (Figs. 9 and 10). The tumors showing central necrosis were identified as hepatocellular carcinomas (Figs. 11 and 12), and many small nodules were identified as fibromas or hemangioendotheliomas.

**DISCUSSION**

In the present experiments, primary neoplasms of the liver were induced in dogs by p.o. administration of diethylnitrosamine for over 50 weeks. In previous studies, primary liver neoplasms induced in rats and mice by diethylnitrosamine were mainly epithelial tumors with a relatively lower incidence of mesenchymal tumors (17, 18, 31). However, most of the primary liver neoplasms induced in dogs treated with diethylnitrosamine were mesenchymal tumors, with only a few epithelial tumors (8-10, 27, 28). The histology and histogenesis of hemangioendotheliomas induced in mice by diethylnitrosamine have been reported by several investigators (18, 24, 31). A high incidence of hemangioendotheliomas was induced in rats and mice by administration of diethylnitrosamine. In the present work hemangioendotheliomas of the liver were also induced with very high incidence in dogs by using diethylnitrosamine, and no difference was found between the histological pattern in dogs and those in rats and mice.

In this work the incidence of epithelial tumors in the liver, including hepatocellular carcinomas in dogs treated with diethylnitrosamine, was lower than that of nonepithelial tumors of the liver. Schmähl et al. (27) reported that the diethylnitrosamine-induced hepatomas in dogs were all leiomyosarcomas. However, in the present study, many mesenchymal tumors were found in the liver, such as fibromas, fibrosarcomas, leiomyomas, and leiomyosarcomas. These findings suggest that there is a difference in tissue or cell affinity for diethylnitrosamine in dogs, rats, and mice. These differences may depend on immunological or histological differences. Further analyses are required on the different responses of the liver of dogs and rats or mice to diethylnitrosamine.

In man the incidence of primary, nonepithelial liver tumors is very low (2, 3). However, the hepatocellular, cholangiocellular, or undifferentiated cell carcinomas in dog liver induced by diethylnitrosamine is very similar to that seen in human cases. Moreover, these diethylnitrosamine-induced tumors in dogs were essentially the same as those induced in rats or mice by other chemical carcinogens (6, 7, 11, 12, 29). The primary, nonepithelial tumors (fibromas, leiomyomas, fibrosarcomas, and leiomyosarcomas) induced in dogs by diethylnitrosamine in the present study seemed to arise from the periportal tissue of the liver. Hirao et al. (10) reported that hemangioendotheliomas of the liver have the same ultrastructural patterns in dogs and man. Previously, Takayama et al. and Otsuka et al. suggested that hemangiomatous tumors induced by diethylnitrosamine in mouse liver are formed by proliferation or hyperplastic changes of sinusoidal endothelial cells. Thus, the histogenesis of hemangioendotheliomas in the liver induced by diethylnitrosamine might have the same histogenesis in dogs and mice.

Clinicopathological studies on tumor-bearing experimental animals have been reported previously (28). Blood analysis has revealed several changes after treatment with diethylnitrosamine. Diethylnitrosamine is well known as a potent hepatotoxin and hepatocarcinogen; thus, it was suggested that the disturbances in the blood chemistry of dogs treated with diethylnitrosamine might be due to diethylnitrosamine intoxication. It has generally been concluded that the only change in blood chemistry related to development of primary neoplasms during liver tumorigenesis in dogs is a change in α-fetoprotein (28). Clues to the relationship between the amount of α-fetoprotein and the size or histology of tumors may be obtained by further experiments on tumors in dog livers.

In previous works, angiographic or microangiographic observations were made on animals with experimental cirrhosis, hepatomas, and regenerating tumors (4, 5, 19, 23, 25, 26, 30). The present investigation has shown that hemangioendotheliomas and malignant tumors in the liver are more easily diagnosed by angiography than are benign tumors in dog liver. Malignant tumors in the liver with hypervascularity could be diagnosed even when they were small foci. Thus improved techniques may show angiographic analysis to be a good method for early diagnosis of primary liver cancer in man.

The present studies on liver neoplasms in dogs suggest that this model may be useful in studying the early diagnosis, chemotherapy, and surgical treatment of primary liver cancers. For biochemical and metabolic studies on primary liver neoplasms, including hyperplastic nodules induced by diethylnitrosamine, dogs seem to be more suitable as experimental animals than rats or mice.
REFERENCES


Fig. 1. The liver of Dog 129 treated with diethylnitrosamine. One large tumor and multiple nodules are seen in the liver.

Fig. 2. The lung of Dog 129 with many metastatic tumors.

Fig. 3. An area of undifferentiated cell carcinoma of the liver of Dog 96. H & E, × 100.

Fig. 4. Higher magnification of Fig. 3 showing irregular hyperchromatic cancer cells in sinusoidal spaces and atrophic liver cell cords. H & E, × 300.

Fig. 5. An area of hemangioendothelioma in the liver of Dog 130 treated with diethylnitrosamine. Vascular spaces are seen with enlarged endothelial cells. H & E, × 100.

Fig. 6. Higher magnification of Fig. 5 showing irregular endothelial cells and proliferating irregular vascular spaces. H & E, × 400.

Fig. 7. An area of fibrosarcoma in the liver of Dog 98 treated with diethylnitrosamine. Spindle-shaped cells are irregularly arranged. H & E, × 100.

Fig. 8. Higher magnification of Fig. 7 showing many mitotic figures and a loose, edematous stromal area. Tumor cells are irregularly shaped. H & E, × 300.

Fig. 9. An area of leiomyosarcoma in the liver of Dog 105 treated with diethylnitrosamine showing polygonal cells. H & E, × 100.

Fig. 10. Higher magnification of Fig. 9 showing many mitotic figures and irregularly shaped tumor cells. H & E, × 300.
Fig. 11. An area of hepatocellular carcinoma in Dog 105 treated with diethylnitrosamine showing irregular tumor cells. H & E, x 100.

Fig. 12. Higher magnification of Fig. 11. Tumor cells are liver cells that are very irregular in size and arrangement. Mitotic figures and large cells are frequent. H & E, x 300.

Fig. 13. Hepatic arteriogram of Dog 105 treated with diethylnitrosamine for 174 weeks. In a leiomyosarcoma abnormal tortuous vessels are seen with luminal irregularity arising from arteries of the right lateral lobe (black arrows). A hepatocellular carcinoma in the right inner lobe near the hilus shows abnormal tumor vascularity and staining (white arrows). Hemangioendotheliomas in the periphery of the right inner lobe have a characteristic vascularity with multiple dilated vascular channels throughout the tumor (double arrows).

Fig. 14. Hepatic arteriogram of a Dog 105 treated with diethylnitrosamine for 174 weeks. A hemangioendothelioma in the left lateral lobe shows abnormal vascular spaces with numerous dilated, tortuous vessels (double arrows).
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