Dyes have long been used in investigations of cancer with the hope of developing useful diagnostic and therapeutic agents. The aim in such investigations has been to find a dye which, in the intact animal, stains living tumor cells without staining surrounding tissue. A large number of acid dyes, especially in the azo series, has been rather intensively investigated for such a purpose. From these investigations there has resulted general agreement (1, 11) that acid dyes do not localize in viable tumor cells but accumulate in the stroma of the tumor.

The incorporation of a radioactive isotope into a dye molecule has been achieved with several acid dyes (5, 10). Studies of the distribution of radioactive intense staining of viable tumor cells when administered orally or parenterally. The halogen derivatives of Nile blue 2B were prepared (8) and found to give good staining of tumor cells in mice. The radioactive iodine derivative of Nile blue 2B has been prepared (9). In the present work, this radioactive dye has been administered to animals. It is the intent of this paper to describe these experiments and their results.

MATERIALS AND METHODS

Radioactive Dye.—The dye used is a radioactive iodine derivative of Nile blue 2B. The iodine is organically bound and is not in an ionizable state. The structure and nomenclature of the dye are:

\[
\text{\(5-(p\text{-radioiodobenzylamino})-9\text{-diethylaminobenzo-}\)}
\]
\[\text{\(\text{[a]phenoxazine}\)}
\]

The preparation of this radioactive dye has been described in detail in a previous publication (9). The specific activity of the dye at the start of this investigation was 0.55 millicurie per gram. For injection purposes, an 0.1 per cent solution of the dye in 5 per cent glucose was prepared by adding the dye to sterile glucose solution, boiling gently for 20 minutes, and filtering into a sterile flask.

Animals Used and Method of Administration of Dye.—Inbred mice of the C3H strain were used. The mice were implanted subcutaneously in the right axillary region with either of two tumors native to the strain. The tumors used were a fibrosarcoma (methylcholanthrene induced) and a transplantable spontaneous mammary carcinoma. These tumors have been transplanted through

* Aided in part by a grant to Dr. Margaret R. Lewis from the National Cancer Institute.
many generations with no incidence of spontaneous regression.

The dye was administered by either of two methods. It was administered orally by mixing it into their food to the extent of 0.4 per cent of the weight of the food. It was administered parenterally by subcutaneous injection of the 0.1 per cent solution. The average dose was 0.4 ml. The injection was made on the side of the mouse opposite to that bearing the tumor.

In one experiment the radioactive dye solution was administered intravenously to a normal dog in daily doses of 25 ml. for five days. This experiment was performed for two reasons. First, it was of interest to determine the pattern of distribution of radioactivity in an animal more comparable to the human than is the mouse. Secondly, it is readily possible in the dog and not in the mouse to take a sample of thyroid tissue for radioactivity measurement.

**Measurement of Radioactivity in Tissues.**—Immediately after sacrificing the experimental animal, weighed portions of various tissues were taken for radioactivity measurement. These were prepared for measurement by either of two methods. One method was to extract the minced tissue with methanol acidified with acetic acid and evaporate the filtered extract to dryness in a 40 mm. open Petri dish. Extraction by this technique was sometimes incomplete since measurable radioactivity was present in the tissues after extraction. A more reliable method for preparing the tissues for radioactivity measurement involved digestion of the weighed sample with concentrated nitric acid to which a little silver nitrate solution had been added. The digestion mixture was evaporated to a small volume in a 40 mm. open Petri dish prior to measurement.

The radioactivity of these preparations was measured with a thin window Geiger-Müller counter.

### Experimental and Results

**Distribution of Radioactivity in a Normal Dog.**—One normal dog weighing 9.3 kilograms received intravenously 25 ml. of 0.1 per cent radioactive dye solution daily for five days. No untoward effects were noticed during this period and three hours after the last injection the dog was sacrificed. Autopsy showed that all organs were grossly and microscopically normal. The radioactivity found in the extracts of the various tissues is shown in Table 1. The data of Table 1 show very high radioactivity in the bile, indicating that this is the principal avenue of excretion of the dye. The relatively high radioactivity in the thyroid is probably due to release of iodine in the metabolism of the dye since the thyroid gland is not visibly stained by the dye. Other organic iodine compounds such as those used for cholecystography (e.g. Priodax), are known (7) to significantly affect thyroid function possibly through release of iodine in metabolism. The very low radioactivity in the brain and the absence of staining of the intact central nervous system are of significance in connection with the possible use of the radioactive dye for the localization of intracranial neoplasms.

**Distribution of Radioactivity in Tumor-bearing Mice.**—The distribution of radioactivity in tumor-bearing mice which received a single injection of radioactive dye was studied. The level of radioactivity of the various tissues varied from mouse to mouse depending on the quantity of dye administered and the time intervals involved. In the present study the absolute values of the radioactivity were considered to be of relatively little import, the principal objective being to determine the relative radioactivity in the various tissues. In Table 2 are listed typical results obtained with C3H mice bearing transplanted fibrosarcomata which received a single subcutaneous injection of 0.9 ml. of the 0.1 per cent solution of radioactive dye. The mice were sacrificed at 2.5 hours and at 24 hours following injection and the tissues were digested with acid and silver nitrate prior to measurement of radioactivity.
The data of Table 2 show that all of the radioactive dye is excreted within 24 hours; in part via the kidney and probably to a greater extent via the bile into the intestine. The data further show that when the blood level is high, the concentrations in the liver, kidney and spleen are of the same order of magnitude as that in the tumor. The concentration of dye in muscle is considerably lower than in the tumor.

**Distribution of Radioactivity in Tumor-bearing Rats.**—The radioactive dye was also administered to a small number of inbred rats bearing transplanted fibrosarcomata. The staining of tumors in the rat is not as good as in the mouse. The limited data obtained with rats showed that the concentration of dye (from radioactivity measurements) was considerably higher in the liver than in the tumor. This species difference may be related to the fact that the rat has no gallbladder whereas the mouse does.

**Effect of Oral Administration of Radioactive Dye on Survival of Tumor-bearing Mice.**—A group of adult C3H mice were implanted subcutaneously with mammary carcinomata from a tumor native to the strain which had been transplanted through several generations. No cases of spontaneous regression had been observed with this tumor. On the thirteenth day after implantation of the tumors, the radioactive dye was administered orally (mixed with the food as 0.4 per cent of the weight of the food). The tumors were definitely palpable and growing at the time the feeding of the dye was started. The administration of the dye was continued for approximately 17 days and then the mice were given ordinary food until they died. Survival time was computed as the number of days from time of implantation of tumor to death.

Another group of adult C3H mice implanted with the same mammary carcinomata served as a control group. They were prepared and fed in the same manner as the previous group except that the dye used was prepared in identical fashion with ordinary iodine instead of radioactive iodine. The data for these two groups is shown in Table 3.

Although the number of animals involved in this experiment is small, a marked difference in the average survival time of the two groups (74 days for the radioactive group, 36 days for the control group) is shown. This indicates that the radioactivity carried to the tumor is a significant factor in prolonging the life of these tumor-bearing mice.

Mice of this same strain implanted with this same tumor which receive no dye survive an average of 25 to 30 days (2). Thus, in the radioactive dye two factors are operative in prolongation of life: the retardation of tumor growth by the dye and the radiation effect of the radioactive isotope.

During the period of treatment with the radioactive dye and until just before death, the mice remained active and in good physical condition. The tumors grew slowly and in two of the mice treated with radioactive dye, there was a temporary but marked diminution in the size of the tumor.

**Effect of Parenteral Administration of Radioactive Dye on Survival of Tumor-bearing Mice.**—Ten adult C3H mice were implanted subcutaneously with fibrosarcomata and the mice were untreated until approximately 2 weeks after implantation at which time the tumors were moderately large. The mice then received subcutaneous injections of 0.4 ml. of the radioactive dye solution on an average of every second day. This treatment was continued for about 16 days following which the mice were untreated. Survival times were recorded. The data are summarized in Table 4.

The survival time of untreated mice bearing the tumor used in this experiment is approximately 20 days (2). The average survival time of the treated mice shown in Table 4 is 51 days, a significant increase over the untreated. In several of the treated mice in this series there was partial sloughing of the tumor and comparatively little viable tumor

### Table 3

<table>
<thead>
<tr>
<th>Radioactive Dye Group</th>
<th>Control Dye Group</th>
</tr>
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<tbody>
<tr>
<td>Days dye fed</td>
<td>Days survived</td>
</tr>
<tr>
<td>17</td>
<td>58</td>
</tr>
<tr>
<td>17</td>
<td>56</td>
</tr>
<tr>
<td>18</td>
<td>62</td>
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<tr>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>14</td>
<td>92</td>
</tr>
<tr>
<td>Ave: 74</td>
<td>Ave: 36</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Period of dye injections (days)</th>
<th>No. of injections</th>
<th>Days survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>12</td>
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</tr>
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<td>10</td>
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</tr>
<tr>
<td>Ave: 9</td>
<td>Ave: 51</td>
<td></td>
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</tbody>
</table>
tissue was present at death. In the case of the mouse which survived 94 days, most of the tumor had sloughed and there remained only a very thin rim of tumor tissue surrounding a small amount of caseous necrotic material.

SUMMARY
1. The radioactive iodine derivative of the oxazine dye Nile blue 2B has been administered to a normal dog and to tumor-bearing mice. The distribution of radioactivity in the tissues of these animals has been determined. In the dog, the radioactivity is very high in the bile and rather high in the thyroid gland. In the tumor-bearing mice, the concentration of dye in the tumor is of the same order of magnitude as in the liver, kidney and spleen.

2. The administration, either orally or parenterally, of this radioactive dye to tumor-bearing mice has been shown to result in marked prolongation of life of the mice. The prolongation of life by the radioactive dye has been shown to be significantly greater than by the same non-radioactive dye.

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The Distribution and Action of a Radioactive Oxazine Dye in Tumor-Bearing Mice

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