Uptake of Radioactive Phosphorus in Experimental Tumors

I. Comparison of Radioactivity in Neoplastic and Normal Ocular Tissue*

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For several years we have been pursuing clinical investigations relating to uptake of radioactive phosphorus (P³²) by neoplastic and inflammatory lesions within the eye, and to the development of criteria for diagnostic interpretation and evaluation of differential radioactive counts in normal and diseased tissues. This method of diagnostic study of eye lesions was suggested by previous experimental and clinical studies (2, 9, 12, 13-16) on uptake of radioactive material by malignant tissue, particularly the work by Selverstone and his associates (16), who, in 1949, demonstrated the usefulness of P³² in the localization of brain tumors. The close relationship, both embryologically and histologically, between the central nervous system and the structures of the eye made ophthalmologic investigations in this area logical and inevitable. In our first studies (18), our immediate aim was to develop a reliable diagnostic test for malignancy in questionable cases presenting retinal detachment. During the past 3 years, data have been accumulated in approximately 100 cases of intraocular lesions of various types (10, 11, 19). The technic has been modified and improved, particularly by the development of special ocular Geiger counter probes, which made possible more accurate radioactive counts over posterior ocular lesions (17).

As this work continued, it became increasingly desirable to build a firmer foundation for these empirical clinical observations by means of controlled experiments in animals. In planning such investigations, it was possible to take advantage of what had already been learned in two areas of experimentation in which a great volume of work had previously been done.

1. We could avail ourselves of a tested method of producing tumors in the eye, i.e., the technic of transplantation of malignant tumors into the anterior chamber, which Greene and his collaborators (4-6) at Yale had been perfecting for several years. Data from their experiments were helpful in selecting tumors for transplantation most suitable for our purpose.

2. We could also take advantage of knowledge gained by many investigations carried out during the past 15 years on the uptake of radioactive material by various types of malignant tissue (2, 9, 12-16), and particularly on the behavior in various tissues of P³², as sodium hydrogen phosphate (NaH₂PO₄). Since phosphorus plays a central role in the metabolism of all types of cells and because this element has a beta-emitting isotope, P³², it has been effectively used in a great variety of situations to measure differences in cellular activity (1, 3, 7, 8).

Our experimental investigation was planned along the following lines: (a) to grow neoplastic tissue in animals' eyes to provide experimental material that could be compared with human eye tumors and to test the uptake of P³² (as NaH₂PO₄) in these experimental lesions; (b) to measure the uptake of P³² by malignant neoplastic tissue, in comparison with that by benign tumors and simple inflammatory lesions; (c) to correlate uptake of P³² with cellular structure and to study the metabolism of phosphorus and its intracellular distribution.

The present report deals with the first phase of this investigation in which a series of experiments was carried out to determine (a) the levels of radioactivity in malignant and normal tissue as a function of time, or the rate of uptake of radioactive phosphorus by these various tissues, (b) the distribution of radioactivity in the neoplastic growth compared with normal tissue at a given time after

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injection of $^{32}$P, and (c) the relationship between radioactive uptake and neoplastic growth, by comparison of in vivo radioactive counts with histologic studies.

**MATERIALS AND METHODS**

**Transplantations**

Among animal tumors, the Murphy rat lymphosarcoma, the Brown-Pearce rabbit carcinoma (generously supplied by Dr. Harry S. N. Greene), a rat glioblastoma, and the S-91 mouse melanoma were selected as suitable for our experiments. Eighty transplants of the lymphosarcoma to the eyes of guinea pigs and rabbits (Fig. 1) resulted in ten (12 per cent) “takes.” The Brown-Pearce carcinoma grows rapidly and easily in the rabbit eye (Fig. 2), and retransplants from this tumor furnished a steady supply of material for transplantations of neoplastic tissue and for uptake measurements. All of 60 transplantations in rabbit eyes grew successfully and occupied a good portion of the anterior chamber (Fig. 3). The strain of glioblastoma was induced by implantation of small pellets of 3-methylcholanthrene into the cerebrum of white rats (Fig. 4). This tumor was transferred to the flank and also to the anterior chamber, where 75 per cent of the transplant grew satisfactorily (Fig. 5). The S-91 melanoma grows slowly but, like the Brown-Pearce carcinoma, provides practically 100 per cent yield of tumor tissue for investigation.

Heterotransplantation of tissue from fifteen different human cancers was carried out in 88 guinea pigs, in eleven of which satisfactory tumor growth occurred in the anterior chambers (12 per cent) (Table 1). In addition, twelve of these transplanted tumors are in a dormant stage, i.e., they are expected to show accelerated neoplastic growth later.

**Preparation of transplant.**—Immediately after the tumor specimen was obtained, it was refrigerated in a sterile Petri dish. Portions for transplantation were selected somewhat away from the surface, well into the cellular portion of the neoplasm. A portion of the tumor was subjected to microscopic histologic study before transplantation, to be compared later with anterior chamber growths.

**Technic of transplantation.**—Introduction of tumor tissue into the anterior chamber of animals’ eyes was carried out essentially according to the technic developed by Greene (6). Animals were anesthetized with nembutal administered intraperitoneally in guinea pigs and rats and intravenously (into the ear) in rabbits. The eye was fixed and a small keratome introduced into the anterior chamber at the 12 o’clock position. If the tumor tissue was of firm consistency, it was introduced into the anterior chamber with a small lens forceps, and if it was spongy a trocar was inserted through the wound and the injection made with a syringe. Sterile conditions were maintained—an important feature in success of the procedure. After its insertion, the tissue fragment was massaged into the anterior chamber angle at the 6 o’clock position by pressure on the corneal surface with an iris spatula.

**Observation.**—Animals were observed regularly, to follow the progress of neoplastic growth in the anterior chamber. Anterior chamber tumors evolve through three stages (93): suspension, while tumor fragments lie loosely in the anterior chamber; nidation, when fragment organizes and becomes attached to the iris, with vascularization; and growth, when the tumor increases in size by multiplication of cells and increased vascularity. When satisfactory growth of tumor had occurred in the anterior chamber, in vivo determination of radioactive phosphorus by the tumor and normal eye tissue was carried out.

**TABLE 1**

<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>Animals Transplanted</th>
<th>Takes</th>
<th>Latent Period</th>
<th>Maximum Size</th>
<th>Time of Maximum Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scirrhus carcinoma, breast</td>
<td>24</td>
<td>2</td>
<td>1 month</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Glioma</td>
<td>10</td>
<td>1</td>
<td>6 weeks</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cylindroma of lung</td>
<td>6</td>
<td>5</td>
<td>5 weeks</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Supraclavicular node</td>
<td>5</td>
<td>5</td>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic breast carcinoma</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, bladder</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, stomach</td>
<td>5</td>
<td>2</td>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, prostate</td>
<td>6</td>
<td>2</td>
<td>3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, breast</td>
<td>8</td>
<td>5</td>
<td>4 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, renal pelvis</td>
<td>4</td>
<td>1</td>
<td>2 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, colon</td>
<td>4</td>
<td>1 growing</td>
<td>5 weeks</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Carcinoma, corneal limbus</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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eyes the tumor fragments became lodged behind the iris and grew in the region of the ciliary body and posterior segment of the eye.

**In Vivo COUNTING PROCEDURE**

**Special eye counter probes.**—In our first clinical experiments involving radioactivity counts over the eye, it became evident that special probe counters were desirable, or necessary, especially in taking readings over posterior portions of the eye. Through experience, our preference is for Geiger counters rather than scintillation counters. Hence clinical end-window and angle-window counter probes were developed and have been used successfully on the eyes of patients (10). These clinical counter probes were used in our first experiments on animals’ eyes, but we then developed a much smaller mica-window Geiger counter tube (17) which has greatly facilitated the in vivo counting procedure in these animal experiments.

In vito counting procedure.—The eye was enucleated and immediately sectioned through the tumor. One half of the specimen was used for preparation of histologic sections. From the other half of the eye, samples of tumor, sclera, or uveal tract were obtained for assay of radioactivity. Such counts must be made on fresh specimens, for if tissues are allowed to remain in fixative for any length of time there is loss of radioactivity by leeching.

The aliquots were weighed wet (wet weights of tumors ranged from 0.3 to 0.8 gm.) and placed in porcelain counting dishes, dissolved in concentrated nitric acid, and then dried, thus yielding thin flat specimens. After drying, samples were counted under identical conditions, with the use of a conventional laboratory Geiger counter. Results were recorded, after correction for background radiation, as counts/min/gm of sample. Ratio of these values for tumor to normal tissue was then determined.

**RESULTS**

Comparative in vivo and in vitro measurements of radioactivity were made in the eye tumor and in normal ocular tissues in 25 rabbits and 25 guinea pigs, with transplanted tumors of various types, and in vitro counts were made on many additional animals. Table 2 contains the data on ten experiments, which are typical of the entire series, and the curves in Charts 1–6 illustrate the various patterns of uptake response. In every tumor studied, the in vivo measurements indicated a more rapid uptake (1.5–6 times greater) of radioactive phosphorus by the tumor than by normal uninvaded eye tissue. These results were confirmed by in vitro measurements of both the wet and ashed or dry fragments, again with values 1.5–6 or 7 times the average uptake/gm of normal tissue. In general, curves of counts per minute (corrected for
physical decay of P32) plotted against time of observation showed an initial rapid rise reaching a maximum within 10–12 minutes after injection, and then a decline, perhaps rapid at first, followed by a slow decline extending over several days. During the initial rise in radioactivity immediately after injection, the blood levels of P32 also were high. It appears likely that during these first 10–12 minutes, the high counts are dependent largely upon vascularity of the tissues concerned. However, after the injected radioactive phosphorus becomes mixed with the phosphorus of the body and the metabolic processes begin to establish equilibrium in phosphorus exchange, the curves of uptake decline and follow a gradual pattern of decay. In one experiment there was a rise in radioactivity

![Chart 1](chart1.png)

**Chart 1.**—Pattern of radiophosphorus uptake in lymphosarcoma in rabbit eye

![Chart 2](chart2.png)

**Chart 2.**—Pattern of radiophosphorus uptake in lymphosarcoma in rabbit eye

![Chart 3](chart3.png)

**Chart 3.**—Pattern of radiophosphorus uptake in Brown-Pearce carcinoma in rabbit eye
**Chart 4.**—Pattern of radiophosphorus uptake in glioblastoma in rat eye

**Chart 5.**—Pattern of radiophosphorus uptake in S-91 mouse melanoma
count in the normal eye which was explained by accidental contamination of the cornea with radioactive phosphorus. The curve fell to a more nearly average ratio after the eye was irrigated.

In Rabbit 23 (Chart 1), with transplanted lymphosarcoma in one eye, the curve in both eyes displayed almost identical patterns, with initial rapid uptake and gradual decay, but radioactivity counts throughout were significantly higher in the eye containing the tumor. In Rabbit 93 (Chart 2), the curve is somewhat different, but the general ratio between normal and tumor eye is similar. The more gradual rise in counts of the normal eye than of the eye with lymphosarcoma may possibly be explained by the fact that excessive vascularity accompanying the neoplastic growth may have increased the initial uptake of radiophosphorus in the cancerous eye. This tumor was situated well into the base of the iris and ciliary body.

In rabbits with anterior chamber growths of the Brown-Pearce carcinoma (Chart 3), the pattern of radioactive phosphorus uptake was similar. This was also the case with the rat glioblastoma (Chart 4) and the S-91 melanoma (Chart 5). There was a rapid rise in both tumor and normal curves, with the tumor reaching a higher level and maintaining this differential throughout the period of decay.

In Guinea Pig 2 (Chart 6), the slow increase in \( \text{P}^{32} \) uptake by the tumors and in the normal eye tissue is explained by the fact that these animals received the radioactive phosphorus intraperitoneally; this resulted in slower absorption by the tissues. The high count in Guinea Pig 2 (Chart 6) at the end of 24 hours was attributable to an iritis initiated by trauma, and the fact that equilibrium in phosphorus exchange had not yet been attained. Later, the curve assumed the normal pattern. On histologic section, this tumor was found to be situated well into the base of the iris and posterior chamber (Fig. 7, A and B).

Although the material available from heterotransplants of human malignant tumors is somewhat limited, Table 2 demonstrates that uptake of radioactive phosphorus in these growths follows the pattern established by neoplasms of animal origin.

In the in vitro measurements, counts on ashed specimens were a little higher than the wet measurements, but in the majority of experiments both showed significantly higher radioactivity in the
tumorous portion of the eye. In two tumorous eyes examined (Rabbits 94 and 9) the radioactive count of tumor tissue was less than in the iris. The presumed explanation is that the phosphorus turnover of this uveal tissue is high and simulates that of rapidly growing or rapidly dividing cells. In vitro counts in Guinea Pig 2 (Table 2) showed a high count for the cornea, with consequent reduction in ratio of tumor count to cornea count. This tumor had completely filled the anterior chamber, and neoplastic cells had already infiltrated the cornea.

COMMENT

These experimental results appear to follow the same pattern established by differential counts.

### TABLE 2

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>TUMOR</th>
<th>SPECIFIC ACTIVITY</th>
<th>ACTIVITY OF TUMOR RELATIVE TO</th>
<th>HISTOLOGIC FINDINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WET WT. (counts/min/gm)</td>
<td>CORNEA Iris Sclera</td>
<td></td>
</tr>
<tr>
<td>Rabbit 94</td>
<td>Lympho-sarcoma</td>
<td>5,900</td>
<td>1.725</td>
<td>Mass of lymphoid cells in edematous, richly vascularized connective tissue. No necrosis. No radiation effect detected.</td>
</tr>
<tr>
<td>Rabbit 23</td>
<td>Lympho-sarcoma</td>
<td>11,970</td>
<td>2.960</td>
<td>Richly vascularized collagenous connective tissue with collections of immature lymphoid cells.</td>
</tr>
<tr>
<td>Rabbit 9</td>
<td>Lympho-sarcoma</td>
<td>35,600</td>
<td>2.41</td>
<td>Immature lymphoid cells in several masses of discrete lymphocytes. In anterior chamber attachment of neoplasm to ciliary body and iris. Some purulent inflammation.</td>
</tr>
<tr>
<td>Guinea Pig 2</td>
<td>Lympho-sarcoma</td>
<td>59,400</td>
<td>1.155</td>
<td>Tissue mass in anterior chamber partly composed of discretely arranged groups of immature lymphoid cells, with vesiculated nuclei. Occasional mitosis. Viable sarcoma.</td>
</tr>
<tr>
<td>Guinea Pig 5</td>
<td>Lympho-sarcoma</td>
<td>21,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 26</td>
<td>Brown-Pearce carcioma</td>
<td>54,220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 49</td>
<td>Brown-Pearce carcioma</td>
<td>6,728</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea Pig 31</td>
<td>Human cylindroma</td>
<td>25,988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea Pig 42</td>
<td>Human retinoblastoma</td>
<td>1,197</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea Pig 49</td>
<td>Human scirrhous carcinoma</td>
<td>51,288</td>
<td>1.02*</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor eye. † Normal eye.
Fig. 1.—Lymphosarcoma in the anterior chamber of a rabbit eye.

Fig. 2.—Brown-Pearce carcinoma filling one-third of the anterior chamber of a rabbit eye.

Fig. 3.—Histologic section of Brown-Pearce carcinoma from rabbit eye (×490). Tumor occupied anterior segment and consisted of diffuse sheets and masses with some regions of hemorrhage and necrosis. Nuclei in general were large and vesicular. Individual cells show active mitosis.

Fig. 4.—Glioblastoma induced by 3-methylcholanthrene (×400). Actively growing and viable cellular glioma. Cells vary in size, with several giant basal forms. Nuclei are round, oval and elongated, and slightly vesicular. Mitosis very active. Tumor is moderately vascularized. Appearance is that of astrocytoma, glioblastoma group.

Fig. 5.—Glioblastoma well filling anterior chamber of eye of white rat.

Fig. 6.—Method of application of Geiger counter to the eye.

Fig. 7.—A. Lymphosarcoma of guinea pig eye (#2) extending into base of iris and posterior chamber (×95). B. Lymphosarcoma shown in A (×490).
over tumors and other lesions in clinical studies on patients' eyes. Although our investigation of phosphatide metabolism in various kinds of tumor tissue is still incomplete, preliminary results (which will be the subject of a later report) suggest that there is no uniformity of phosphatide activity and that it cannot be correlated with specific types of malignant lesions. Each tumor appears to possess a characteristic phosphatide turnover, independent of pathologic type and independent of phosphatide metabolism in the host. Maximal deposition of phosphatides (P₃₂) in neoplastic tissues may vary from 10 to 50 hours, as revealed in both in vivo and in vitro studies.

**SUMMARY**

Experimental intraocular tumors were produced in rabbits, guinea pigs, rats, and mice by transplantation of neoplastic tissue into the anterior chambers according to the technic developed by Greene. Animal tumors included the Murphy rat lymphosarcoma, the Brown-Pearce rabbit carcinoma, a rat glioblastoma (originally induced by implantation of 3-methylcholanthrene into the rat cerebrum), and the S-91 mouse melanoma. Hetero-transplantation of tissue from fifteen different human cancers was also carried out in guinea pigs. Uptake of P₃₂—as measured with special Geiger counter probes—by the experimental intraocular tumors was compared in vivo and in vitro with that of normal ocular tissues in the same animal. In general, curves of counts per minute plotted against time of observation showed an initial rapid rise reaching a maximum within 10–12 minutes after injection of NaH₂P₀₄, and then a decline, perhaps rapid at first, followed by a slow decline extending over several days. During the initial rise in radioactivity immediately after injection, blood levels of P₃₂ also were high, and initial high counts were probably dependent largely upon tissue vascularity. After establishment of equilibrium in phosphorus exchange, curves of uptake declined and followed a gradual pattern of decay. These experimental results are parallel to those previously established by differential counts over ocular tumors in clinical studies.

**ACKNOWLEDGMENTS**

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