Radioactive phosphorus uptake test
An in vitro analysis of choroidal melanoma and ocular tissues

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The concentration of radioactive phosphorus in uveal melanoma and normal parts of the eye was determined in vitro in 14 eyes. The eyes were enucleated after a positive $^{32}$P uptake test. Portions of the melanoma as well as normal choroid, retina, sclera, lens, and vitreous were analyzed. The $^{32}$P uptake test had been performed at various intervals after intravenous administration of $^{32}$P from 24 to 556 hr. The in vitro uptake of $^{32}$P was compared to cell type, tumor volume, time of testing, percent uptake measured clinically, and specific activity. The only positive correlation was between percent uptake measured clinically and $^{32}$P concentration (dpm/gm). A higher concentration of phosphorus in melanoma resulted when carrier-free $^{32}$P was used. A negative correlation existed between number of hours from injection to clinical measurement of percent uptake, although melanoma to normal choroid ratios did not change from 24 to 72 hr. No correlation was found between uptake and tumor volume. The sample was small; however, we saw no correlation between $^{32}$P uptake and degree of malignancy.

Key words: radioactive phosphorus, choroidal, melanoma, uveal, malignancy

Radioactive phosphorus ($^{32}$P) concentrates in malignant tissue to a greater degree than in normal tissues. This fact has been known since 1941 and is the basis for its use in ophthalmology as an aid in the diagnosis of malignant melanoma of the choroid. Some authors believe that a high $^{32}$P uptake correlates well with the presence of an intraocular malignancy. However, $^{32}$P uptake has not correlated with histologic features, or prognosis and more recently has given false results. The results with choroidal hemangioma have also proven to be variable. The uptake of $^{32}$P by the suspected tumor is measured against a normal area of the same eye and expressed as a percentage. How positive the $^{32}$P uptake should be to rely on it for enucleation remains unanswered, although most would agree to greater than 100%. The range between 50% and 100% remains a gray zone with different authors using different percent uptake for positives, 50%, 60%, 60-14 and 85%. The purpose of this study was to measure phosphorus concentration by in vitro analysis of $^{32}$P. We then sought to correlate this with $^{32}$P uptake in vivo as well as a number of
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prognostic factors. This was done in an attempt to explain previous failure to correlate $^{32}$P uptake in vivo and degree of malignancy, and to determine the reason for the wide range of uptake seen with similar tumors. We examined eyes at different intervals after injection of $^{32}$P to find an optimal time for performing the test. We believed that by examining the melanoma and the normal tissues we might find a time of optimum tumor to background ratio.

Materials and methods

Fourteen patients were given radioactive phosphorus (10 $\mu$Ci/kg) intravenously. Measurement of the radioactivity from the suspected melanoma and a control area was performed with the standard surgical techniques at varying intervals after injection (24 to 556 hr). Most testing was at a 24, 48, or 72 hr interval. Eight patients were women (age range, 52 to 86) and six were men (age range, 51 to 67). All testing was done in the operating room, with the control site being in the same eye in a quadrant opposite the area of tumor. The lesion was localized with indirect ophthalmoscopy and transillumination. Radioactivity was measured with either a silicone solid state probe or a Geiger-Mueller probe. A minimum of three series of 60 to 100 sec accumulated counts were taken over the tumor and over the control area. Percent uptake was determined as follows:

\[
\% \text{ uptake} = \frac{\text{average counts over tumor} - \text{average counts over control}}{\text{average counts over control}} \times 100
\]
Table I. Phosphorus-32 uptake in human choroidal melanoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Time (hr)</th>
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<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>F</td>
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<td>7</td>
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<td>13</td>
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<td>M</td>
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<tr>
<td>14</td>
<td>76</td>
<td>F</td>
<td>48</td>
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</table>

The minimum percent uptake was 88% in this series. Enucleation was promptly performed following the positive 32P test. Immediately after enucleation, the eye was dissected, and a small portion of the tumor and normal eye parts (10 to 100 mg samples) (cornea, lens, vitreous, retina, and choroid) were placed into sealed vials. These were autoclaved and then analyzed in the Chemistry Department at Brookhaven National Laboratory. All specimens were digested, and beta counting was done in a liquid scintillation detector. At no time were these specimens exposed to formalin, and all specimens were analyzed in their original vial. Laboratory data concerning activity were expressed as disintegration of 32P per minute per gram of tissue, specific activity (μCi 32P/μmol P), amount of phosphorus (μg), and percent dose per gm. Percent dose per gram was obtained by the following formula:

\[
\text{Percent dose per gram} = \frac{\text{tissue activity (μCi)}}{\text{tissue weight (gm)}} \times \frac{100}{\text{total activity injected (μCi)}}
\]

Clinical data included age, sex, percent uptake 32P, number of hours between 32P injection and measurement of 32P uptake, cell type, pigmentation, mitotic activity, and size (maximum diameter and volume). No correlations were made with fluorescein angiography, scleral invasion, optic nerve invasion, integrity of Bruch's membrane, or length of time of symptoms before enucleation.

Histology was performed in the routine man-
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...that is, the eyes were placed into formalin after the dissection described above had taken place.

The eye, except for the small sample taken for in vitro radioactivity analyses, was placed into formalin and processed for histopathologic diagnosis. The specimens for radioactivity analysis were taken from the apex of the tumor whereas histologic parameters were from the base of the tumor. Degree of pigmentation was expressed as average number of pigment cells per 10 high-power fields (hpf) and mitotic activity as average number of mitotic figures per 10 hpf.

Results

The clinical and laboratory data are given in Tables I and II. The patients were selected at random from those scheduled for the $^{32}$P uptake test. The $^{32}$P uptake test was performed at the following intervals after injection: 24 hr (three patients), 48 hr (seven patients), 72 hr (three patients), and 556 hr (one patient). Fig. 1 is a scattergram of percent $^{32}$P uptake vs. interval after injection.

Scattergrams were also made for specific activity, micrograms of phosphorus, and tumor base diameter vs. percent $^{32}$P uptake. No correlation seemed to exist between these factors. Both percent dose per gram vs. percent $^{32}$P uptake (Fig. 2) and dpm per gram vs. percent $^{32}$P (not shown) had a positive correlation. Cell types were divided into spindle A and B and epithelioid. Fig. 3 relates the cell type and percent $^{32}$P uptake. Cell type was taken from those cells near the base of the tumor. Tumor volume was determined by two methods: (1) length times width times height and (2) a geometric analysis when the shape of the tumor made this reasonable. Fig. 4 shows the lack of correlation and large variation between tumor volume and percent $^{32}$P uptake.

Tumor to background ratios are critical for detection. Fig. 5 shows that there is no variation in the ratio of uptake by tumor to choroid (dpm/gm) vs. time after injection.

Table I shows an obvious increase in disintegrations per minute per gram in patients who received carrier-free $^{32}$P (Patients 11 to 14). This did not vary with cell type but did result in the two patients with epithelioid melanomas having a higher percent uptake. This differentiation (between epithelioid and spindle cell) was not apparent when non-carrier-free $^{32}$P was used (Patients 3 to 7, 9, and 10). We assured ourselves that the former preparation was near carrier-free by analyzing for phosphorus. By our testing there was no more than 0.2 μg/ml phosphorus which is substantially less than the range of the non-carrier-free $^{32}$P (250 to 680 μg); although to be carrier-free there would have to be less than 0.003 μg/mCi.

Fig. 6 shows the lack of correlation between percent $^{32}$P uptake and the number of pigmented cells per 10 hpf. These cells were at the base of the tumor within 1 mm of the choroid. Fig. 7 correlates percent $^{32}$P uptake and numbers of mitoses per 10 hpf at the base of the melanoma. There is wide variation in each group.

Table II lists the uptake of the tumor and various ocular tissues that would contribute to background. Lens, cornea, and vitreous had low uptake compared to the melanoma. Similarly $^{32}$P uptake by choroid and retina were consistently lower than the melanoma. Similarly $^{32}$P uptake by choroid and retina were significantly lower than the choroid. The uptake by muscle may be significant and one should therefore not allow muscle between probe and tumor when testing. Table II and Fig. 5 are evidence that the $^{32}$P uptake test would not be expected to vary significantly
at any time between 24 and 556 hr from injection.

Discussion

Certain correlations seemed unnecessary because of the physical constraints of the probe and of $^{32}$P. Phosphorus-32 is a pure beta emitter with an average beta energy of 0.7 MeV. The physical half-life is 14.5 days and the average range in tissue is 2 mm. For example the $^{32}$P probe has a 7 mm sensitive area, and therefore a tumor whose base is greater than this would not result in a significant increase in the count rate. Perhaps 2 mm to 7 mm tumors would have a progressive increase in count rate with increased size. Most tumors under 7 mm (~4 disc diameters) should not be subjected to the surgical procedure required for the $^{32}$P test since the probability of malignancy is low.17-18

Recent studies have correlated the uptake of radioactive phosphorus with various clinical parameters.2, 4, 5, 17-31 Some of the variability in uptake may well be due to the vagaries of measuring radioactive phosphorus or to lack of attention to certain parameters of radioactive materials. The specific activity of $^{32}$P varies from batch to batch and as a consequence the absolute amount of phosphorus that each patient receives will vary. The standard dose of 10 $\mu$Ci/kg maintains the amounts of radioactivity per patient, but not the amount of phosphorus. If carrier-free isotope is given, the amount of phosphorus (mg) differs from non-carrier-free $^{32}$P. It appears from our data that where non-carrier-free $^{32}$P was used there is binding of free phosphate in the melanomas and therefore less apparent uptake of the radioactive phosphorus. Our results indicate a difference in uptake when carrier-free material was used; however, more patients need to be studied. In addition, the transfer of materials for analysis as well as storage in formalin may alter results.

The limited tissue penetration of $^{32}$P (2 mm)10 means that only radioactivity from the base of the tumor (1 to 2 mm) is responsible for the counts obtained with an eye probe that is placed against the sclera beneath the tumor. Other tumor-localizing radiopharma-

Fig. 4. Radioactive phosphorus percent uptake and corresponding tumor volume.

Fig. 5. Ratio of melanoma to choroid (dpm/gm) at various times after injection.
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Fig. 6. Percent $^{32}$P uptake correlated to number of pigment cells in 10 hpf.

Fig. 7. Percent $^{32}$P uptake correlated to number of mitoses in 10 hpf.

cousticals may allow for a more accurate assessment of malignancy than $^{32}$P. We found no correlation with many of the prognostic factors of melanoma such as size, pigmentation, and mitoses. It was our intention to correlate $^{32}$P uptake with the various histologic parameters which we examined at the base 1 to 2 mm of the melanomas. This should correspond to the tissue penetration of $^{32}$P. Similarly, samples for in vitro analysis were from the base of the tumors. We realize that melanomas are often not uniform in histologic characteristics and therefore feel that aside from this study it is illogical to make clinical statements relating $^{32}$P uptake to the malignant potential of the entire tumor unless it is less than 2 mm in height. However it was our purpose to establish any correlation given the above limitations. The fact that we were able to positively correlate $^{32}$P uptake with $^{32}$P concentration in vitro adds validity to our other negative correlations.

The appropriate time to measure $^{32}$P uptake clinically has never been determined. Our data support doing the test at any time after 24 hr from injection. This is not surprising since phosphorus stays bound to malignant tissue and the half-life of $^{32}$P is 14.3 days. More patients have to be studied with carrier-free $^{32}$P to confirm these findings. Our findings agree with those of Rao et al., and disagree with those of Char et al., who believed that there was a correlation between degree of malignancy and the $^{32}$P uptake. The main difference is that we did in vitro determination as well as in vivo whereas Char et al. did only the latter. This is difficult to understand, since our studies show a good correlation between $^{32}$P uptake measured clinically and $^{32}$P concentration (Fig. 2). However, both studies lack sufficient numbers of patients to be considered definitive, and there was a large variance in the previous work as there is in ours.

Donald Margouleff, M.D., Division of Nuclear Medicine, North Shore University Hospital, helped with the manuscript. Dennis Greenberg and Joan Briggs provided technical assistance. Dr. Merlyn Rodrigues at Wills Eye Hospital and Dr. Madilyn Kahn at North Shore University Hospital performed the ophthalmic pathology.

REFERENCES


