Radioautography of Cerebral Tumors Employing P^{32}.* (17885)

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(Introduced by A. B. Hastings)

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Radioactive phosphorus, given intravenously to patients with brain tumors, reaches concentrations in the tumor tissue which is ordinarily from 5 to 100 times as high as that in the normal cerebral tissue(1). A remarkable variation in P^{32} activity in adjacent regions of the same tumor was early recognized. For example, Geiger-Mueller counting of biopsy specimens provided crude but dramatic evidence that necrotic areas in a malignant glioma might take up only 0.1% as much P^{32} as regions of rapid growth(1). More precise correlation of these variations in the rate of uptake of P^{32} with microscopic appearance has been difficult. The heterogeneous character of certain gliomas makes it impossible in many cases to obtain specimens of uniform histologic pattern sufficiently large for assay by Geiger-Mueller counting. Since the grains of a photographic emulsion provide, in effect, a mosaic of minute "counters", a radioautographic technic permits the assay of P^{32} in each area of a tissue section, at the microscopic level. This communication describes the use of such a technic in order to secure a map of the varying uptake of radioactive phosphorus in brain and brain tumor as it is related to histologic pattern.

Method. Brain and brain tumor tissue have been obtained from patients in whom radioactive phosphorus has been used for the localization of cerebral tumors by means of a probe Geiger-Mueller counter(2,3). A single intravenous dose of from 0.5 to 4.0 millicuries of carrier free radioactive phosphate ion is given to presumptive brain tumor patients preferably from 12 to 72 hours before craniotomy. At operation an attempt is made to secure specimens of tumor for microscopic autography with a minimum of trauma. Control samples of normal gray and white matter are obtained when removal of overlying or adjacent cerebral tissue is necessary in order to expose the tumor. Samples from regions of different gross appearance are taken and immediately frozen at -15°C. In patients who come to postmortem examination, gross radioautographs may also be made, in which case the brain is removed, frozen rapidly, and sectioned either coronally or horizontally.

Autography of microscopic sections. Since conventional histologic fixatives were found to leach an appreciable fraction of the P^{32} activity from the tissue and to produce major shifts of the isotope within the tissue, all autographs have been made using unfixed quick-frozen material. Tissue sections are cut in a cold room at -15°C using a modification of the Linderstrøm-Lang technic (4,5). The sections for autography are cut at 20μ since, with the dosage levels permissible in humans, thinner sections often contain insufficient P^{32} to give an adequate image. These sections are allowed to melt on gelatin coated slides where they dry with little or no distortion. In the dark room a

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5. Coons, A. H., personal communication.

strip of Eastman No-screen x-ray film 2.5 x 7.5 cm is pressed firmly against the tissue section with a second glass slide and the entire 'sandwich' is sealed tightly with cellulose tape. The completed preparation is wrapped in opaque paper and stored in the cold room for the duration of the exposure, which is determined by the method of Steinberg and Solomon(6). Sections immediately preceding and following each 20μ section are cut at 8μ, fixed in formalin, and stained with hematoxylin-eosin for histologic correlation. When available, a sample of normal brain from each operation is mounted on the same slide with the tumor, providing a control which will necessarily receive exposure and development identical to those of the tumor section. Activity of tumor tissue can then be expressed relative to that of normal brain (1) without the necessity for further correction factors. At the conclusion of the calculated exposure, usually 7 to 20 days, the films are removed in the dark room and developed under controlled conditions. Each film is processed for 5 minutes in 50 cc of fresh Kodak D19 developer in a water bath at 20° $\pm$ 0.3°C. It is then stopped with 1% acetic acid and fixed and hardened in Kodak F5. Rigorous standardization of development is essential if it is desired to attempt quantitative comparison of autographs from different cases(6).

Autography of gross specimens. The beta particles of P^{32} (E_{max} 1.7 Mev) penetrate soft tissues (assuming density 1.0) to a maximum depth of 7 mm(7). This degree of penetration makes it possible to employ thick "slabs" of tissue (up to 7 mm) in order to obtain a survey autograph in minimum time. A large specimen may thus be studied within a period of 72 hours, for gross regions of high and low P^{32} uptake. Such autographs provide resolution which, although coarse, suffices to indicate areas of special interest for further study. The more laborious technic of microscopic radioautography may then be concentrated upon such areas. Since only 15% of the initial activity of the tissue decays in the first 72 hours, exposure time for the microscopic radioautographs is not unduly prolonged by this preliminary survey. The thick frozen section of brain obtained at operation or autopsy, including tumor, is kept constantly in a cold room at -10 to -15°C. Surfaces are trimmed so that irregularities will not impair the resolution of the autograph. Mean surface radioactivity is then estimated by means of a portable Geiger-Mueller counter and exposure time estimated (6). The specimen is covered with a single layer of aluminum foil (2.3 mg/cm^2) in order to protect the photographic emulsion; Eastman No-screen x-ray film is then applied under Wratten 6B light to each flat surface.§ The tissue and films are placed between cardboards, wrapped in opaque paper and stored in the cold room for the duration of the exposure. Films are routinely processed as described for microscopic radioautography. The frozen tissue specimen is photographed to provide a permanent record of its gross morphology.

Densitometry. Comparison of P^{32} uptake in various areas of a microscopic tissue section requires precise matching of corresponding areas in the radioautograph. In heterogeneous tumors it is desirable to measure densities in areas as small as 100μ in diameter. At the present time matching is accomplished by simple superposition, taking advantage of the configuration of the outlines of the tissue and the autograph. While this method is reliable in regions which can be readily identified, especially at the periphery of the section, it is difficult to be certain of the matching in central regions. A system employing simultaneous macroprojection of tissue and autograph for precise point-to-point correlation is now being developed. The radioautograph to be studied is projected on a ground glass screen through a microscope at suitable magnification. The machined aperture of the search unit of a Photovolt 500A

6. Steinberg, D., and Solomon, A. K., *Rev. Scient. Instr.*, 1949, v20, 655.

7. Glendenin, L. E., *Nucleonics*, 1948, v2, 12.

§ Three sheets of film may be applied to each surface. The additional autographs so obtained, although inferior in resolution, assist in the recognition of any artefacts in the primary autographs.

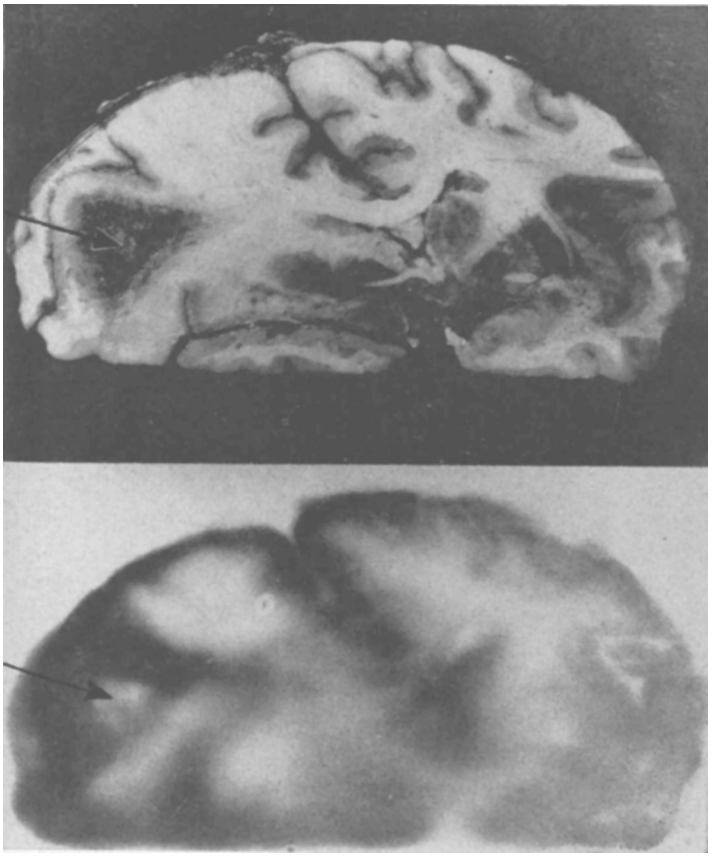
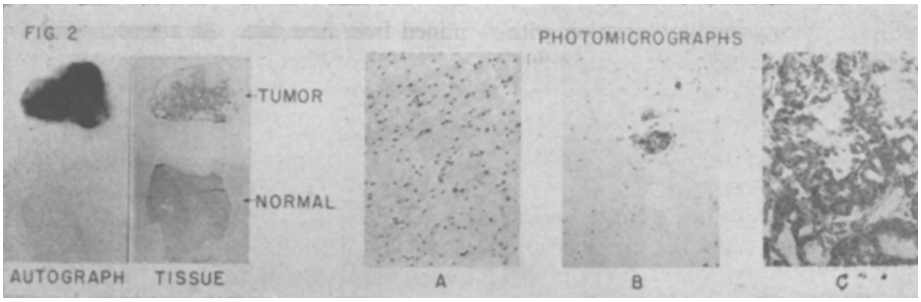


FIG. 1.
Gross radioautograph and photograph of brain slice containing glioblastoma multiforme. Note that the gray matter shows greater activity than the white but that the areas containing tumor are much denser than the gray. Note also that the necrotic region near the center of the tumor (see arrows) is much less dense than the surrounding tumor.

photometer is applied to the ground glass over the area whose density is to be measured. A standard Ansco optical wedge, projected in the same manner, is used for calibration between each set of readings. The light source, microscope and projection system are maintained in rigid alignment throughout the measurements. From the densities of the radioautographs relative P^{32} uptake can be calculated within $\pm 10\%$ for individual cases(6). Comparison from case to case requires standardization of dose and of time interval between P^{32} injection and operation, which is often made impossible by the clinical needs of the patient. Expressing uptake relative to that of the normal tissue makes the data more nearly comparable with respect to dose.

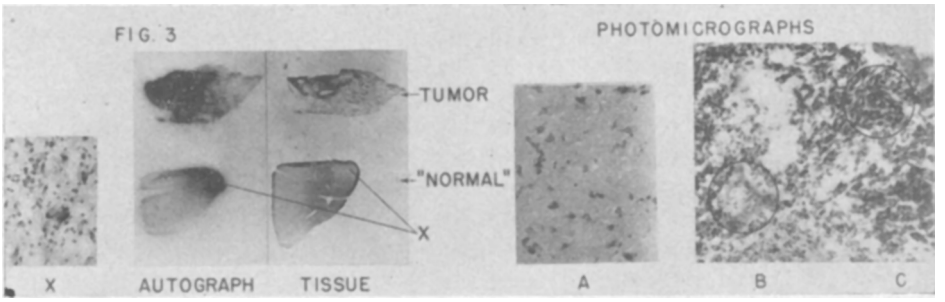
Discussion. Radioautographs of 18 cerebral tumors of various histologic types have been made employing these technics. Fig. 1 illustrates a gross radioautograph together with a photograph of the specimen from which it was made. Areas of interest in the gross autograph are removed from the frozen tissue slab in the cold room for microscopic autography. Fig. 2 illustrates the correlation of histologic appearance with microdensitometric determinations of autographic blackening in a bronchogenic carcinoma metastatic to the temporal lobe. Fig. 3 illustrates similar findings in a glioblastoma multiforme.



	Absolute density	Relative P^{32} content
A. Cerebral white matter near tumor	.07	1.0
B. Cerebral tissue at edge of tumor, containing a few tumor cells	.31	3.5
C. Actively growing region of tumor	.79	8.3

FIG. 2.

Bronchogenic carcinoma, metastatic to right temporal lobe. Patient received 3.9 mc of P^{32} 69.3 hours before operation. Exposure 11 days. Densities were measured in the regions of the radioautograph corresponding to photomicrographic areas A, B, and C.



	Absolute density	Relative P^{32} content
A. Cerebral gray matter with some glial reaction	0.07	1.0
B. Necrotic area in tumor	0.17	1.9
C. Nest of tumor cells	0.65	6.8

X—Tumor infiltration found in presumed normal control.

FIG. 3.

Glioblastoma multiforme, right temporal lobe. Patient received 3.6 mc of P^{32} 24.7 hours before operation. Exposure 26 days. Densities were measured in the regions of the radioautograph corresponding to photomicrographic areas A, B, and C.

The resolution of the autographs is adequate to permit microdensitometric measurements of adjacent areas as shown in the illustration. The radioautographic densities measured in this study reflect the total P^{32} uptake per unit volume of tissue. This uptake is a function both of the total content of phosphorus and of the rate of phosphorus replacement. Chemical analysis of nine brain tumors, including five different histologic types, showed significantly lower total phosphorus content in each case than in its control of normal brain(8). It would therefore appear reasonable to interpret increases in autographic density produced by brain tumor tissue as a reflection of increased metabolic activity.

Summary. Radioautographs of human brain tumors and of normal brain, employing P^{32}, have been made in 18 cases. A frozen section technic, without fixation, has been found necessary in order to prevent redistribution of the isotope within the tissues. Autographic blackening has been consistently much higher in tumor tissue than in brain,

8. Selverstone, B., and Moulton, M., personal communication.

but is subject to considerable variation within certain tumors. These variations, reflecting differences in uptake of radioactive phosphate ion by various portions of the tumor, may be correlated with certain histologic features in the stained sections. Quantitative microdensitometric measurements have been made, and relative P^{32} uptake determined from these data. An attempt is being made to correlate radioautographic features, especially in the gliomas, with histologic appearance and with prognosis.

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Estrogenic and Gonadotrophic Hormone Inhibiting Activity of Some Adrenal Cortical Substances.* (17886)

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The secretion of estrogens by the adrenal cortex has been observed clinically(1-3), and steroids possessing female sex hormone activity have been isolated from adrenal cortical extracts. Englehart(4,5) and Shirrmeister (6) obtained uterine growth with cortical extracts, and Beall(7) isolated estrone from ox adrenals. Desoxycorticosterone acetate has also been found to have estrogenic activity. Hoffman(8) and Gallardo(9) report uterine growth, and van Heuverswyn *et al.* (10) found mammary duct growth with this substance. In view of the administration of commercial adrenal cortical extracts (Lipo Adrenal Cortex, Adrenal Cortex Extract), desoxycorticosterone acetate (DCA), and 11-Dehydro-17-hydroxycorticosterone - 21 - acetate (Merck and Co., Inc.) in large amounts in experimental procedures, it is of practical importance to determine the doses of these substances which cause inhibition of the pituitary gonadotrophic hormone secretion and which result in uterine stimulation.

An ovariectomized immature female rat united in parabiosis with an intact littermate provides an assay technic by which a compound may be tested for both its activity in inhibiting pituitary gonadotrophic hormone secretion and in causing uterine growth. An estrogenic substance administered in an adequate dose to the ovariectomized rat prevents the post-castration hypersecretion of the gonadotrophic hormone and the resultant ovarian hypertrophy in the co-parabiont. The uterine hypertrophy of the castrate rat serves as an index of the estrogenic activity of the injected substance.

Procedures. Thirty-day-old female littermate rats of the Sprague-Dawley strain weighing between 65 and 75 g were joined in parabiosis according to the method of Bunster and Meyer(11) except that metal skin clips were used instead of silk sutures in closing the skin incisions. The right partners were ovariectomized at the time of parabiosis. One group of parabionts was left uninjected

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