Distribution of Radioactive Phosphorus in Normal and Diseased Brain Tissue. Experimental and Clinical Observations. (18959)

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The purpose of this study is to determine the relative amounts of radioactive phosphorus in normal and diseased mammalian brain tissue following parenteral administration of the isotope. The clinical application of this isotope to the practical problems of brain tumor localization and delineation by other workers(1,2) stimulated interest in revealing what pathological change in tissue would alter the normal isotope content.

Methods. A. Experimental. Adult cats were utilized. A series of traumatic, purulent and ischemic lesions were made in the cerebral hemispheres of the animals. In addition, it was possible to make observations on the fetal brain tissue and the adult brain tissue of three pregnant animals. The animals which underwent surgical procedures received the radioactive isotope 5-6 days following operation in the cases of the traumatic and ischemic lesions and at varying intervals in excess of 6 days in the cases of the purulent lesions. The isotope was administered by vein in the form of weak phosphoric acid in doses varying from 9 to 39 microcuries of P-32 per kilo body weight, the average dose being 18 microcuries per kilo. The animals were sacrificed in from 24 to 96 hours after the injection, and the brain tissues were removed and sectioned. The areas of lesions were removed en bloc and were matched by control blocks of tissue from the contralateral hemisphere. Cerebellar and brain stem blocks were sampled in certain instances also. The tissues were chilled, weighed in the wet state and homogenized with distilled water to a consistency which permitted their being drawn into a 1.0 ml serological pipette. 1.0 ml aliquots of the homogenates were placed in dry, weighed metal capsules for drying and assay. Similar aliquots were fractionated into the acid soluble, phospholipid and nucleic acid containing constituents by the method of Schneider(3,4). These chemical fractions were dried in capsules for assay. The dried films were assayed for radioactive content in lead-shielded counting chambers by the use of a Geiger-Muller, bell type counter (2.0 mg/cm^2 window thickness, Background 0.45 counts/second). A minimum of 3500 counts was taken, and in all instances where a sufficient material was available duplicate determinations were made. B. Clinical. Brain tumor cases anticipating surgery received sterilized solutions of the weak phosphoric acid containing the isotope in doses approximating 7 microcuries per kilo body weight. The material was given by vein 12 to 48 hours before operation. Control tissue was biopsied at operation to compare with the abnormal tissue. The tissues were treated in the same way as outlined above.

Results. The results of the tissue assay were expressed (a) in counts per second per gram dry weight of tissue and (b) in a ratio, termed the "differential absorption ratio" (D.A.R.) which is obtained by dividing the actual counts per second of one gram of specimen (calculated as wet weight) by the theoretical count of an equal weight of tissue if the isotope were uniformly distributed throughout the body. Such an expression, while it has no absolute value, is useful in comparing the results from different animals and cases in which varying the dose of isotope per unit body weight could not be avoided. The results of assay of the tissue fractions were expressed in percent of recovery of the total isotope content.

A. *Experimental Material.* 1. *Fetal tissue.* (3 animals). 24 hours after the administra-

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tion of the isotope the fetal brains showed small differences in assay from the maternal tissue of the same animal. Forty-five and 48 hours after injection the dry weight values of the fetal brains rose to 2.5-4.0 times the maternal brain values. The differential absorption ratios (D.A.R.'s) revealed definite but less striking differences in favor of the fetal tissue. Approximately 50% of the isotope was recovered in the acid soluble fractions of the maternal tissues while 60% was present in this fraction in the assay of the fetal tissues.

Ratio of C/S/G dry wt Adult : Fetal	D.A.R. Adult : Fetal
24 hr 1 : 1.9	.12 : .12
45 hr 1 : 2.5	.26 : .35
48 hr 1 : 4	.19 : .45

2. *Traumatic lesions.* (6 animals). Higher isotope assays were observed in the traumatized areas of the hemispheres than in control hemisphere tissue of the same animals. Lesions produced by the electrosurgical unit showed dry weight assays 3.6 and 3.7 times higher than control tissue assays. Lesions produced by blunt instruments showed smaller differences. In all instances more isotope was recovered in the acid soluble tissue fraction than in any other.

	Ratio of C/S/G dry wt Control : Lesion	D.A.R. Control : Lesion
Blunt instrument	1 : 1.2	.15 : .25
	1 : 1.7	.21 : .38
	1 : 1.9	.24 : .62
	1 : 2.9	.07 : .18
Electro-unit	1 : 3.6	.13 : .50
	1 : 3.7	.20 : .80

% recovery in chemical fractions (avg figures)	Control	Lesion
Acid soluble	57	49
Phospholipid	25	27
Nucleic acid	Not significant	

3. *Purulent lesions.* (7 animals). No walled off abscess was produced. In 2 animals grossly localized collections of liquefied pus were produced. The dry weight assays of the tissue of the lesion gave values from 3-3.5 times the control assays. One animal with an area of localized meningitis and a ventriculitis but without an area of cerebral suppuration showed an assay in the altered area of 1.7 times the control. The other 3 animals, one with inspissated pus and agar in its ventricle and 2 with only minimal cortical contusions revealed only minor deviations from the normal values of the control tissues.

	Ratio of C/S/G dry wt Control : Lesion	D.A.R. Control : Lesion
	1 : .9	.16 : .17
	1 : 1.2	.12 : .14
	1 : 1.4	.19 : .30
	1 : 1.7	.10 : .19
	1 : 2.9	.27 : .66
	1 : 3.6	.14 : .54
Foreign body	1 : 9.1	.09 : .55

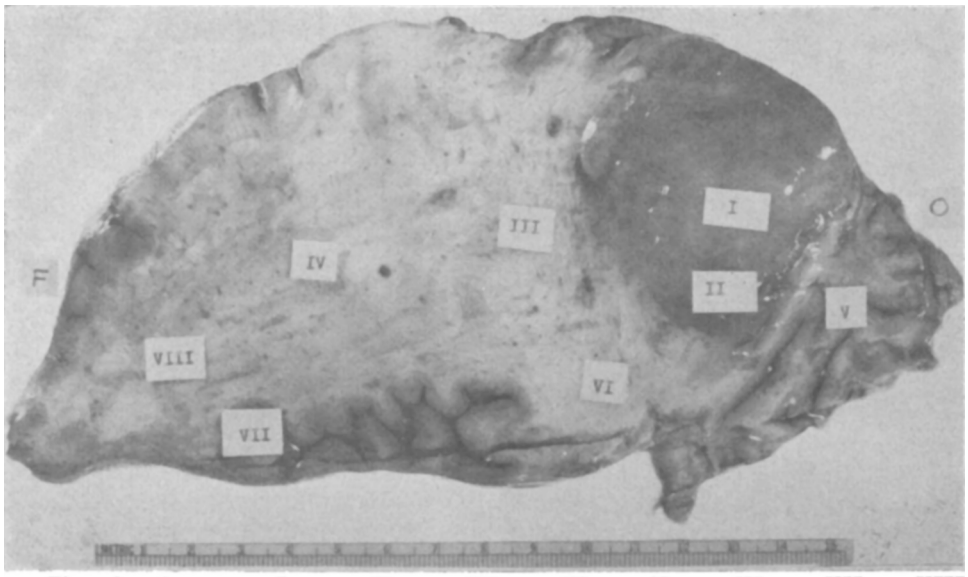
A single experiment in which "Saraka" seeds were implanted into the cerebral hemisphere revealed higher assays in the liquefied mass and surrounding "edematous" brain tissue than in the case of any purulent lesions studied.

4. *Ischemic lesions.* (4 animals). Hemorrhagic softenings were produced by clipping and coagulation of the middle cerebral complex of vessels in the Sylvian fissure. The central portions of the lesions as well as the adjacent cerebral tissue demonstrated dry weight assays of 4.0-4.5 times the values of the opposite hemisphere. The D.A.R.'s showed slightly smaller differences. The relative isotope concentration in the chemical fractions did not appear different between the abnormal and control tissue. Two animals which developed no ischemic lesions showed no important differences between the tissues assayed excepting where a local area of coagulation necrosis raised the isotope content.

	Ratio of C/S/G dry wt Control : Lesion	D.A.R. Control : Lesion
No infarct	1 : .9	.07 : .06
	1 : 1.5	.07 : .12
Gross infarcts	1 : 4.3	.12 : .41
	1 : 4.8	.12 : .50

Assay of the chemical fractions revealed no consistent alteration from normal values in the lesions produced.

% recovery in chemical fractions (avg figures)	Control	Lesion
Acid soluble	52	49
Phospholipid	26	27
Nucleic acid	Not significant	



Tissue location	I	II	III	IV	V	VI	VII	VIII
Counts/sec/gram dry wt	96.61	828.15	72.30	2.87	9.77	22.37	12.25	6.07
	103.06	811.40	79.47	3.38	10.18	23.61	13.04	—
Differential absorption ratio	0.052	0.488	0.050	0.003	0.009	0.016	0.009	0.006

Glioblastoma Multiforme 19 hr after 6.6 μc $_{15}P^{32}$/kg

FIG. 1. Analysis of P^{32} content in brain slice. Note correspondence between area I within the tumor's necrotic area and area III in uninvolved white matter.

B. *Clinical material.* Tissues from 8 brain tumor cases were obtained as surgical specimens. In every case the tumor tissue assay exceeded the control tissue assay several-fold. The ratios of the dry weight content of isotope of control vs. tumor tissue were as follows:

Tumor type	Control	Tumor
Glioblastoma multiforma	1 :	7.7
	1 :	12.8
	1 :	18.6
	1 :	64.8
Ependymoblastoma	1 :	22.6
Astrocytoma	1 :	6.2
Chromophobe adenoma of pituitary	1 :	3.6
Unclassified (glioma?)	1 :	7.5

Sufficiently large specimens in 2 of the glioblastoma cases permitted the assay of soft, necrotic tissue within the tumor mass as well as firm, neoplastic tissue. The isotope content of the degenerated tissue was several times below that of the firm tissue. The ratios of the dry weight assays were as follows:

Control	Necrotic area	Firm area
1 :	3.7 :	18.6
1 :	7.9 :	64.8

One of these 2 cases is illustrated (Fig. 1) in a manner to show the areas of specimens which were sampled and assayed. Histological study of the same areas provided evidence that there was no tumor tissue in areas III to VIII. Area I was soft and necrotic whereas area II was firm, cellular tumor. The assay figures are tabulated beneath the brain slice. Assay of the chemical fractions revealed the major portion of the recovered isotope to be in the acid soluble fractions of both tumor and control tissues. There was a tendency for the relative content of isotope to be higher in the phospholipid and lower in the acid soluble fractions for the tumor tissue than for the control tissue. These variations did not exceed 10% of the amount of recovered isotope.

% recovery in chemical fractions (avg figures)	Control	Tumor
Acid soluble	74	69
Phospholipid	9	15
Nucleic acid	Not significant	

Discussion. It appears from these observations that the lesions of the brain which were studied have a higher content of P-32 following its intravenous administration than the corresponding control brain tissue of each preparation. We cannot reason from the results of animal work to those of clinical material, but it is noteworthy that in general the 8 brain tumor cases showed higher ratios between control and abnormal tissue than did the experimental lesions. Areas of the brain tumors which were degenerating or necrotic revealed lower values than firm areas of tumor. It would seem that areas of brain tissue destruction, whether in experimental lesions or in areas of tumor degeneration, exhibit higher isotope content than normal brain but usually lower values than "viable" brain tumor tissue.

Several factors are thought to influence the uptake of radioactive phosphorus by tissues in general(5-7). (a) The rate of phosphorus turnover or exchange in a given tissue(8,9). (b) The building of new tissue of which phosphorus is an essential constituent(8,9). (c) Mitotic activity(10,11). (d) Temperature(12). (e) Membrane permeability (12,13). (f) The total amount of exchangeable phosphorus in the tissue(9). It is probable that not all of these factors are of importance in this study. Although the total content of phosphorus in normal brain tissue is low in comparison with other soft tissues, the isotope uptake is low and is accomplished at a slow rate(9,14). Equilibrium is reached at a lower level and at a slower rate than in the case of most all other soft tissues of experimental animals studied(15-19). The differences in distribution of the isotope in the chemical fractions between the experimental lesions and control tissues were not of sufficient magnitude to explain the total tissue differences on the basis of any disproportionate increase of the isotope in a given fraction. The destructive processes which broke down capillary and other tissue membranes may have led to changes in permeability to phosphate ions both at the capillary and cell membrane barriers. Such a mechanism is an attractive explanation for the differences observed. However, in the absence of studies comparing the isotope content of the plasma, extracellular and intracellular spaces, we are not justified in passing judgment on the probable importance of such a mechanism.

Observations upon the distribution of phosphorus in brain tumors are few. Cumings(20) reported the vast bulk of the stable element to be in the acid soluble fraction with very little nucleoprotein phosphorus and less phospholipid phosphorus. Erickson and associates (21,22), employing Schneider's methods, found the phosphorus distributed far more evenly between the fractions. However, as has been well shown by Fries and Chaikoff (18) the stable phosphorus distribution may have no relationship to the percentage of total

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radioactive isotope which can be recovered in any given fraction. Our findings agree with those of Erickson and associates(22) who found the P-32 in decreasing amounts in the acid soluble, phospholipid and nucleic acid fractions. Unlike the experience of others with other neoplastic tissue(23,24) we did not find an important alteration in the percentage distribution of the isotope in the chemical compartments compared with control tissue. We recognized that our observations may not have covered a sufficiently long period following the administration of the isotope. From our data, however, we are unable to explain the increase in total tissue content by, for example, a sharp rise in isotope-containing nucleic acid. By studies of the nuclear content of P-32 Marshak(10,11) was able to reach conclusions suggesting the importance of mitotic activity in determining the increases of the isotope above normal in tumor and regenerating tissue of livers of experimental animals. We are unable to provide any data on this point. We would suggest that there are factors at play which influence the brain tumor increases of isotope which are not functioning in the case of the experimental lesions we have studied.

Conclusions. 1. Traumatic, purulent and ischemic lesions of the brains of cats demonstrate increased contents of radioactive phosphorus following intravenous administration of the isotope when compared with control brain tissue. 2. Fetal cat brain tissues demonstrate higher contents of the isotope in 45 and 48 hours after administration than maternal brain tissue of the same animal. 3. Brain tumor tissue from clinical cases reveals greater deviations above the normal of any pathological nervous tissue studied. When the tissue architecture of the tumor is intact the content of the isotope is higher than in degenerating, softened tissue of the same tumor mass. 4. The distribution of the isotope among the three phosphorus containing fractions of the pathological tissues does not show sufficiently marked alterations from the distribution in control tissues to explain the abnormal increases in total tissue content on the basis of a disproportionate increase of any one isotope-bearing fraction. It is suggested that different mechanisms are at play to explain the increase of isotope in destroyed tissue of the brain as compared with the mechanisms at play in neoplastic tissue. 5. Caution must be employed when utilizing radioactive phosphorus in brain tissue to localize or delineate tumor masses by probe methods, for non-tumor tissue may show a differential ratio between normal and abnormal tissue such as to cause errors in interpretation.

The authors are pleased to acknowledge the helpful advice of Dr. A. Earl Walker in whose department this work was carried out. Clinical material was provided through courtesy of Dr. Walker, Dr. Herbert C. Johnson and Dr. Robert G. Fisher. The National Research Council defrayed part of the expense of this study, and the Atomic Energy Commission furnished the P-32. Dr. J. Logan Irvin of the Division of Physiological Chemistry was generous in his suggestions aiding the biochemical procedures.

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Received June 15, 1951. P.S.E.B.M., 1951, v78.