A Study on Phosphorus Uptake of Brain Tumor by Radioactive Isotope in Relation to Histological Findings

by

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1 Introducion

The prognosis of brain tumor has not been fully clarified yet owing to the facts that, it depends greatly on various conditions; e. g. where the tumor originates in the brain and to which direction it is growing. On the other hand, the histological feature of the tumor not always accords to the clinical course.

Intracellular metabolism is generally increased in tumor tissues and phosphorus compounds are known to play important roles in carbohydrates and nucleic acid metabolism, thus it will be easily presumable that phosphorus metabolism will be increased in tumor tissues in which a large growth activity is present. Such a tendency has been noted in human cancer tissue or in tumors of animals. Trials in which radioactive P^{32} has been used to determine the malignancy of tumors or to differentiate a malignant tumor from benign one, have been reported by many scholars: REDDY et al[23]. applied P^{32} to human ovarian tumor, TUDWAY[35] to intraocular tumor, NAKAYAMA[21] to tumors of alimentary tract and again BAUER[3] et al. used it to cutaneous tumors. All the above reporters observed the higher concentration of isotope in malignant tumors than benign tumors or inflammatory lesions. However, the concentration in malignant tumor was not more than 3 times as high as that of normal tissue taken as the control.

SELVERSTONE[30][31][32], on the other hand, reported far higher concentration of isotopes in brain tumors when P^{32} or I^{131} was administered to patients who had the brain tumor. Furthermore, some scholars noted that the rate of this concentration paralleled to the histological malignancy of tumor to a certain degree. However, these studies were not enough to substantiate their conclusion owing to the limited number of cases, unsatisfactory histological determination or the lacking of chemical analysis. The present study was made to investigate the manner of isotope uptake in details and compare this result to the histological picture and the clinical feature. Thus, P^{32} was given to patients of brain tumor with an idea to clarify some nature of these tumors.

2 Materials and methods

Patients who were undergoing the operation for brain tumor were selected. About 20 hours prior to the operation, 1.0 to 2.0mc of P^{32} was injected intravenously or intramuscularly. Isotope used was obtained from Japan Isotope Association and P^{32} was contained in dilute hydrochloric acid solution in the form of ortho-phosphoric acid. The

solution was sterilized and properly diluted with RINGER-LOCKE's solution.

The tumor tissue and normal brain and muscle tissue obtained during the operative procedure, were minced on the ice cold glass plate, washed briefly in cold normal saline then excess fluid was removed. A portion was put into formalin fixative for the histological examination, and the other portion of 300mg to 1000mg wet weight of tissue was weighed and transfered into ice cold ELVEHJEM-POTTER homogenizer. The tissue was homogenized with 6cc of 10% perchloric acid. Before homogenizing, necrotic and hemorrhagic portion or blood coagule were carefully removed and only homogenous and well preserved portion was selected.

The homogenate was fractionated according to SCHNEIDER's[22)28)29)35)] method. Since this method is simple and can be completed relatively in short period of time, and it meets the necessity of radioactivity determination in short time so that only small amount of P^{32} can be administered to patients to give the least harm to them. MOORE et al[17)]. have studied that, when P^{32} was administered to the human body, it is concentrated in brain and brain tumor tissue in large amount within few hours then gradually released, but between 16 and 48 hours after the injection, the relatively constant P^{32} concentration was maintained in these tissues. The present experiment was scheduled to be completed during this stable period to minimize the effect of time factor because the time of obtaining the tissue could not be anticipated exactly since it depended on the progress of operative procedure.

SCHNEIDER[25)26)27)] and DAVIDSON et al[4)5)6)7)]. have made detailed research on the contents of each fraction divided by the method above mentioned: The acid soluble fraction contains phosphorus of phosphate mononucleotides, carbohydrate compounds and low molecule phosphate esters. The phospholipid fraction contains phosphorus in various compounds such as cephaline, acetal phospholipids or inositol lipids. The nucleic acid fraction contains DNA, RNA and inositid phosphorus. The phosphoprotein fraction contains various kinds of phosphoprotein.

The obtained homogenate was centrifuged in refrigerator, and the precipitate was again extracted with 4cc of ice cold 10% perchloric acid solution. Both supernatants were mixed together as the acid soluble fraction. Next the residue was extracted with 5cc of 80% ethanol then with 5cc of pure ethanol, and then 3 : 1 ethanol-ether mixture extraction for 3 minutes was done 3 times in 70°C. All these supernatants were put together as the phospholipid fraction. The precipitate was put into 90°C bathing for 15 minutes with 5cc of 5% perchloric acid. This was centrifuged and the precipitate was washed with 5cc of 5% perchloric acid. These supernatants were mixed together as the nucleic acid fraction. 6cc of 2% NaOH aqueous solution was added to the final residue and this was put into boiling water for 10 minutes to complete the hydrolysis. The obtained solution is the phosphoprotein fraction. Each solution was put into calibrated test tube and the volume was corrected with each extracting solution so that the final volume of each fraction became 10cc acid soluble fraction, 25cc phospholipid fraction, 10cc nucleic acid fraction and 6cc phosphoprotein fraction respectively.

2cc each of these samples were put into a small metal can and desiccated completely, then radioactivity of each sample was determined by GEIGER-MÜLLER tube. (Mica window

type, B-1B, SHIMAZU Co.) In this procedure, samples of the acid soluble fraction and the nucleic acid fraction were neutralized with a measured amount of ice cold 10% KCl solution. The supernatant was used for radioactivity determination. By this procedure, no radioactivity was found in the precipitate and self absorption of each sample was less than 5% so that this error could be disregarded. Each determination was carried out for the enough period of time to bring down the error at most less than 10%. Usual correction of background and decay was done so that obtained value of each fraction will represent the count per minute, per gram of tissue at the time of operative removal of each tissue.

The background count was fairly stable throughout this experiment and it ranged between 30 to 36 cpm.

Next, a certain amount of each sample was ashed in micro-KJELDAHL frask with 0.8cc of 60% perchloric acid solution with heating at 180°C. Thus phosphorus in sample was turned into the inorganic form, then amount of phosphorus was determined by ALLEN's[1] colorimetric method: The blue coloration was measured with electrophotometer by the absorption rate at 660mμ. 0.5cc each sample of acid soluble and phospholipid fraction were used for this determination and 1.0cc in the case of other fractions, because the formers contain a large amount of phosphorus. The obtained value was calculated into phosphorus amount per gram of fresh sample. Each determination was done in duplicated or triplicated samples and the mean value was taken.

Tissues fixed in formalin were used for the histological preparation. Hemotoxylin-eosin stain was done. Since the entire tissue utilized in this experiment was so small that weighed only 1.5 to 2.0g, it may be justifiable to presume that the portion used for chemical analysis and the one for histological examination may not differ greatly. However, in histological examination, when the tissue which was thought to be tumor tissue, did not contain tumor at all or contained it only partially or when the tissue taken as the normal tissue was invaded by tumor cells, such cases were excluded from the study.

Samples used in this experiment were as follows:

Normal cerebral tissue	28
Normal cerebellar tissue	10
Normal muscle tissue	10
Miningioma	6
Hemangioblastoma	2
Malignant lymphoma	1
Glioblastoma	4
Ependymoblastoma	2
Malignant astrocytoma	1
Astrocytoma	4
Oligodendroglioma	4
Medulloblastoma	1
Carcinoma (metastatic)	4
Pituitary adenoma	2
Schwannoma	4

Craniopharyngioma	1
Ependymoma	1
Total number of tumors	37

3 Results

1) Phosphorus contents in tumor, brain and muscle tissue

Obtained tissues were divided into groups of cerebrum, cerebellum, muscle and various kinds of tumor. Amount of phosphorus was expressed as mg per gram of each tissue. Table 1 illustrated the mean value and the standard deviation of each groups. Amount of phosphorus in tumor was lower than that of normal brain and higher than that of muscle. Fractionated results showed that the brain tissue contained a large amount of phospholipid and this was the reason for a large content of phosphorus in brain tissue. As for the phosphorus in nucleic acid fraction, the amount was more in tumor than in cerebrum and equal to that of cerebellum. This may be due to the difference in cellularity between cortex of cerebrum and cerebellum or tumor tissue, and also due to that the tumor tissue contains more nucleic acid.

No remarkable difference was present between histologically malignant tumors and benign ones or glioma group and meningioma group. However, within the limit of glioma group, there was a tendency that the more malignant tumor contained the more phosphorus in every fraction.

2) Uptake of isotope

When the radioactivities of these samples are to be compared, it is necessary to study on the value expressed as the ratio of radioactivity of tissue to that of control, or the ratio of radioactivity against the amount of phosphorus of the sample. Thus following three indices were calculated and shown in tables.

(i) c. p. m. ratio

This indicates the ratio of count per minute of each sample of tumor tissue against that of control tissue.

Table 1 Amount of phosphorus

tissue	acid soluble	phospholipid	nucleic acid	phosphoprotein	total phosph.	no. of cases
cerebrum	0.542±0.193	1.716±0.515	0.127±0.042	0.052±0.023	2.437±0.597	28
cerebellum	0.670±0.146	1.623±0.182	0.413±0.153	0.057±0.020	2.764±0.252	10
muscle	0.821±0.277	0.213±0.063	0.091±0.043	0.017±0.010	1.143±0.344	10
malignant tumors	0.662±0.213	0.774±0.627	0.331±0.141	0.046±0.029	1.812±0.682	15
metastatic carcinoma	0.578±0.079	0.475±0.052	0.332±0.163	0.059±0.033	1.443±0.224	4
benign tumors	0.669±0.377	0.558±0.195	0.308±0.202	0.039±0.032	1.573±0.666	27
meningioma	0.736±0.256	0.652±0.196	0.394±0.205	0.052±0.050	1.832±0.585	8
hemangioblastoma	0.531±0.116	0.499±0.131	0.224±0.041	0.035±0.012	1.290±0.241	4
schwannoma	0.677±0.059	0.690±0.190	0.296±0.105	0.027±0.012	1.690±0.352	3
glioma	0.610±0.272	0.653±0.553	0.285±0.169	0.037±0.024	1.585±0.720	22
malignant glioma	0.740±0.287	0.922±0.700	0.318±0.114	0.037±0.028	2.017±0.747	11
oligodendroglioma	0.590±0.044	0.519±0.118	0.280±0.082	0.040±0.018	1.428±0.161	4
astrocytoma	0.347±0.070	0.379±0.076	0.147±0.028	0.022±0.010	0.895±0.171	5

(ii) specific activity

It indicates the c. p. m. per 1γ of phosphorus in each sample.

(iii) specific activity ratio

This is the ratio of specific activity of tumor samples to that of control.

The c. p. m. ratio represents the ratio of radioactivity of each fraction of the entire tissue, the specific activity represents the turn over rate of phosphorus of the tissue, and the specific activity ratio shows the comparison of this turn over rate of tumor tissue with that of control. Moreover, an attempt was made to deduce the grade of malignancy of brain tumors: At first, the comparison of above mentioned indices was made between well known malignant tumors such as metastatic carcinoma or glioblastoma and histologically and clinically verified benign tumors such as astrocytoma or meningioma. Then, on the ground of the data thus obtained, other tumors of which malignancy was not clearly known were studied in the same way to deduce the possible grade of malignancy.

Tumors were divided into following groups according to the histological similarity:

1 Meningioma group (meningioma, hemangioblastoma etc.)
2 Glioma group
3 Carcinoma
4 Pituitary adenoma
5 Schwannoma
6 Craniopharyngioma

They were arranged in the order of higher c. p. m. ratio in each group and shown in tables. Mark ○ indicates known malignant tumors, ● indicates known benign tumors and △ were put on those tumors of which malignancy is in question histologically and clinically.

1) First, the result in which brain tissue was taken as the control will be discussed.

(i) Acid soluble fraction

Even in the cases of astrocytoma or oligodendroglioma which had the lowest c. p. m. ratio they showed twice as many count as control brain. In some tumors, this ratio was as high as 20 to 30 times. This generally accord with the tissue malignancy with exceptions of benign schwannoma or pituitary adenoma. Specific activity showed generally similar tendency with more exceptions. In specific activity ratio, this relationship with malignancy became more indistinct.

(ii) Phospholipid fraction

Table 3 showed the values of phospholipid fraction. There was parallelism of isotope uptake and histological malignancy in glioma group. Specific activity ratio showed generally markedly high values but this was considered to be due to unusually low specific activity of this fraction in normal brain. In brain tissue, in spite of the presence of a large amount of phospholipid, P^{32} uptake was quite low.

(iii) Nucleic acid fraction

Table 4 showed the same values of this fraction. There was a wide range of variation of c. p. m. ratio from 0.7 to 1.5 of astrocytoma to 40 of glioblastoma. This c. p. m. ratio was well paralleled to the histological malignancy with only few exceptions. Similar tendency was noted on specific activity and it's ratio.

Table 2 Acid soluble fraction

case	c.p.m. ratio	s. a.	s.a. ratio	histological findings
meningioma group				
S.A	15.95	40.07	19.2	●fibrocytic meningioma
T.N.	11.38	7.16	5.3	●meningocytic meningioma
T.T.	8.38			○malignant lymphoma
S.I.	6.84	8.32	3.4	●meningocytic meningioma
Y.K.	6.48	73.78	3.1	△hemangioblastic meningioma (juvenile type)
E.W.	6.15	9.23	6.6	●fibrocytic meningioma
M.M.	4.62	5.19	6.4	●hemangioblastoma
S.A	3.79	24.44	3.6	●hemangioblastic meningioma (adult type)
Y.K.	2.90	17.50	3.8	●hemangioblastoma
glioma group				
M.S.	20.81	7.71	32.1	○ependymoblastoma
Y.M.	14.60	67.38	28.6	○glioblastoma
S.Y.	13.00	35.84	15.2	△malignant astrocytoma
K.N.	12.20	26.23	11.1	△oligodendroglioma (ependymoma-like pattern)
M.Y.	11.89	41.85	6.7	○giant celled glioblastoma
E.K.	10.76	54.45	11.0	○ependymoblastoma
M.K.	8.28	8.72	5.1	○medulloblastoma
M.H.	8.14	8.79	3.7	○glioblastoma
M.S.	7.06	27.73	8.0	○glioblastoma
T.S.	6.67	12.86	5.2	△ependymoma
I.H.	6.42	16.07	7.7	△oligodendroglioma (ependymoma-like pattern)
H.S.	4.26	17.31	10.7	●piloid astrocytoma
Z.O.	4.09	23.35	3.5	△oligodendroglioma
N.M.	3.47	18.42	7.7	△astrocytoma (cerebral)
N.T.	2.81	8.04	3.0	●piloid astrocytoma
T.H.	1.90	8.69	5.4	●piloid astrocytoma
K.Y.	1.73	1.49	2.2	△oligodendroglioma
carcinomas				
Y.F.	15.10	10.51	6.7	○metastatic carcinoma
T.M.	11.67	13.70	1.4	○embryonal cell carcinoma
T.K.	8.41	27.06	12.5	○metastatic carcinoma
H.K.	7.76	21.16	9.4	○metastatic carcinoma
pituitary adenomas				
E.T.	24.28	16.16	13.9	●chromophobe adenoma
K.O.	21.93	19.40	10.1	●eosinophilic adenoma
schwannomas				
T.T.	21.10	37.83	22.1	●schwannoma
M.Y.	15.96			●schwannoma
K.M.	12.62	29.83	13.1	●schwannoma
T.S.	12.07	30.01	8.1	●schwannoma
craniopharyngioma				
M.T.	4.95	8.17	1.8	●craniopharyngioma

Table 3 Phospholipid fractiion

case	c. p. m. ratio	s. a.	s. a. ratio	histological findings
meningioma group				
S.A	41.18	12.34	102.8	●fibrocytic meningioma
T.T.	9.32			malignant lymphoma
S.I.	6.06	5.87	9.9	●meningocytic meningioma
S.A.	6.02	8.85	25.3	●hemangioblastic meningioma (adult type)
Y.K.	5.95	8.39	9.4	●hemangioblastoma
T.N.	5.42	2.31	21.0	●meningocytic meningioma
E.W.	5.25	3.51	11.0	●fibrocytic meningioma
M.M.	4.58	2.96	13.5	●hemangioblastoma
Y.K.	2.43	21.78	5.2	hemangioblastic meningioma (juvenile type)
glioma group				
M.Y.	46.14	12.38	82.5	giant celled glioblastoma
S.Y.	16.91	13.72	40.4	malignant astrocytoma
E.K.	16.38	14.57	48.6	ependymoblastoma
Y.M.	12.14	11.94	30.6	glioblastoma
M.K.	10.82	4.03	26.9	medulloblastoma
M.S	9.28	12.12	11.8	glioblastoma
Z.O.	7.91	6.83	22.0	△oligodendroglioma
K.N.	7.72	5.45	16.0	△oligodendroglioma (ependymoma-like pattern)
I.H.	6.65	4.18	27.9	△oligodendroglioma (ependymoma-like pattern)
N.M.	5.04	11.93	23.9	astrocytoma (cerebral)
T.S	4.75	4.99	16.6	ependymoma
M.S.	1.18	1.55	31.0	ependymoblastoma
M.H.	3.28	2.86	8.4	glioblastoma
T.H.	2.66	2.44	12.2	●piloid astrocytoma
K.Y.	1.45	1.63	1.7	oligodendroglioma
N.T.	0.95	1.18	3.7	●piloid astrocytoma
H.S.	0.79	1.23	3.7	●piloid astrocytoma
carcinomas				
Y.F.	53.60	6.90	345.0	metastatic carcinoma
H.K.	12.01	8.86	46.6	metastatic carcinoma
T.M.	1.58	11.10	16.3	embryonal cell carcinoma
T.K.	3.67	9.56	12.9	metastatic carcinoma
pituitary adenomas				
E.T.	32.50	6.37	91.0	●chromophobe adenoma
K.O	21.93	11.34	87.2	●eosinophilic adenoma
schwannomas				
T.T.	77.71	19.63	178.5	●schwannoma
T.S.	12.82	20.90	46.5	●schwannoma
K.M.	8.28	10.18	17.3	●schwannoma
M.Y.	5.40			●schwannoma
craniopharyngioma				
M.T.	6.01	4.56	16.3	●craniopharyngioma

Table 4 Nucleic acid fraction

case	c. p. m. ratio	s. a.	s. a. ratio	histological findings
meningioma group				
T.T.	47.04			○malignant lymphoma
Y.K.	11.99	14.12	2.0	○hemangioblastic meningioma (juvenile type)
S.A.	7.24	5.22	2.6	●fibrocytic meningioma
S.I.	5.24	1.80	1.3	●meningocytic meningioma
S.A.	4.15	8.23	7.6	●hemangioblastic meningioma (adult type)
T.N.	2.88	0.81	0.7	●meningocytic meningioma
Y.K.	2.80	2.06	2.0	●hemangioblastoma
M.M.	1.18	0.77	2.2	●hemangioblastoma
E.W.	1.01	0.69	0.6	●fibrocytic meningioma
glioma group				
M.Y.	47.69	10.05	19.7	○giant celled glioblastoma
M.S.	13.13	2.24	11.9	○ependymoblastoma
S.Y.	9.40	5.28	4.7	○malignant astrocytoma
E.K.	9.32	11.91	5.9	○ependymoblastoma
Z.O.	8.89	3.00	1.4	△oligodendroglioma
K.N.	7.75	4.43	1.0	○oligodendroglioma (ependymoma-like pattern)
Y.M.	7.58	1.87	1.3	○glioblastoma
M.H.	7.33	2.65	2.4	○glioblastoma
M.S.	6.87	4.41	2.4	○glioblastoma
M.K.	4.64	1.18	6.6	○medulloblastoma
I.H.	3.03	1.47	1.4	△oligodendroglioma (ependymoma-like pattern)
K.Y.	2.33	1.36	1.6	△oligodendroglioma
T.H.	1.62	1.12	7.0	●piloid astrocytoma
N.T.	1.28	1.21	3.1	●piloid astrocytoma
N.M.	0.80	2.76	0.7	○astrocytoma (cerebral)
H.S.	0.70	3.07	2.6	●piloid astrocytoma
carcinomas				
Y.F.	228.00	3.99	3.3	○metastatic carcinoma
T.K.	19.42	12.12	11.4	○metastatic carcinoma
H.K.	19.33	6.71	9.2	○metastatic carcinoma
T.M.	2.34	7.36	1.4	○embryonal cell carcinoma
pituitary adenomas				
E.T.	12.12	1.39	2.3	●chromophobe adenoma
K.O.	7.55	6.94	2.9	●eosinophilic adenoma
schwannomas				
T.T.	41.31	5.40	90.0	●schwannoma
M.Y.	18.14			●schwannoma
T.S.	12.79	4.34	17.4	●schwannoma
K.M.	4.38	4.08	1.2	●schwannoma
craniopharyngioma				
M.T.	1.02	1.63	0.7	●craniopharyngioma

(iv) Phosphoprotein

This fraction according to SCHNEIDER's method is known to be easily contaminated by phosphorus other than phosphoprotein. The amount of phosphorus in this fraction was quite little. For these reasons, further improved and detailed technique seems to be necessary for proper evaluation. Probably due to such an error in procedure, the obtained data showed no apparent relationship with tissue malignancy, although true evaluation of this fraction is left in future.

(v) Isotope uptake in total phosphate

On comparison of radioactivity in each fraction, it was noted that 60 to 70% of total count resulted from acid soluble fraction and 20 to 25% from phospholipid fraction. Therefore, isotope uptake was greatly dependent on that of acid soluble fraction.

Table 5 showed the values of the total phosphate fraction and it showed the similar tendency to that of acid soluble fraction.

2) Cases when muscle tissue was taken as control

When normal brain tissue was selected as a control, the influence of blood-brain-barrier, or the brain edema was inevitable. Therefore, for the purpose of comparison, the muscle tissue also was selected as a control in following cases: 2 cases each of glioblastoma, oligodendroglioma, pituitary adenoma and schwannoma and 1 case each of medulloblastoma, malignant lymphoma, ependymoma and astrocytoma.

The result was shown in Table 6. Grossly similar tendency was seen as in the case in which brain was taken as the control, but specific activity ratio was generally lower especially in phospholipid fraction. It may be justifiable to presume that this is due to the character of tissue component since phospholipid phosphorus in muscle is very low.

3) Isotope uptake and histological findings

Judging from above results, the malignancy of tumor is the most dependable on c. p. m. ratio especially in those of nucleic acid and acid soluble fraction. However, these fractions are composed of various compounds and it has been reported that the composition of these compounds is different in various tumors. Therefore, c. p. m. ratio of these two fractions only cannot be the solid ground of malignancy of tumor. However, if all the indices of each fraction are taken into consideration, isotope uptake of tumors of which malignancy is known, seems fairly well parallel to the malignancy of tumor judged on the basis of histological findings and clinical picture. Then those tumors of which clinical malignancy is not completely understood will possibly be evaluated from their isotope uptake.

(i) Hemangioblastic meningioma

Among the tumors called hemangioblastic meningioma, there are two types[14]. One is called adult type and the histological picture is nearly the same as that of cerebellar hemangioblastoma. The other is called juvenile type and it has high cellular density and may be called hemangiosarcoma or undifferentiated meningioma. The latter is more frequently encountered. In this series, there were one each of these types. The adult type was of benign form showing the similar isotope uptake in every index with cerebellar hemangioblastoma. Contrary to this, the juvenile type had higher value as compared with other tumors of meningioma group suggesting increased growth velocity.

This finding agrees well with clinical observation. These juvenile type tumors had

Table 5 Total phosphorus

case	c. p. m. ratio	s. a.	s. a. ratio	histological findings
meningioma group				
S.A.	17.64	19.70	27.0	●fibrocytic meningioma
T.T.	9.22			○malignant lymphoma
T.N.	8.81	1.53	12.6	●meningocytic meningioma
S.I.	6.48	5.82	5.3	●meningocytic meningioma
E.W.	5.20	5.66	8.3	●fibrocytic meningioma
Y.K.	5.10	40.56	4.5	△hemangioblastic meningioma (juvenile type)
S.A.	4.17	15.76	10.7	●hemangioblastic meningioma (adult type)
M.M.	4.14	3.44	8.6	●hemangioblastoma
Y.K.	3.48	10.36	4.4	●hemangioblastoma
glioma group				
M.Y.	14.88	24.39	16.0	○giant celled glioblastoma
M.S.	12.81	4.49	49.9	○ependymoblastoma
Y.M.	12.78	25.07	27.5	○glioblastoma
E.K.	11.37	28.95	19.7	○ependymoblastoma
M.K.	8.43	6.14	10.6	○medulloblastoma
S.Y.	8.22	19.44	21.4	△malignant astrocytoma
M.H.	7.71	6.00	6.6	○glioblastoma
M.S.	7.31	16.41	13.5	○glioblastoma
I.H.	6.27	9.77	13.0	△oligodendroglioma (ependymoma-like pattern)
Z.O.	4.76	12.06	6.0	△oligodendroglioma
N.M.	3.08	13.13	9.4	△astrocytoma (cerebral)
K.N.	2.68	13.30	14.6	△oligodendroglioma (ependymoma-like pattern)
H.S.	2.51	8.30	9.2	●piloid astrocytoma
T.H.	1.98	4.27	7.8	●piloid astrocytoma
N.T.	1.74	5.31	4.0	●piloid astrocytoma
K.Y.	1.61	1.46	3.2	△oligodendroglioma
carcinomas				
Y.F.	21.57	7.61	33.1	○metastatic carcinoma
H.K.	9.12	13.11	19.3	○metastatic carcinoma
T.K.	6.99	16.75	15.0	○metastatic carcinoma
T.M.	4.81	11.03	7.5	○embryonal cell carcinoma
pituitary adenomas				
E.T.	23.92	9.19	25.5	●chromophobe adenoma
K.O.	20.55	15.99	18.8	●eosinophilic adenoma
schwannomas				
T.T.	28.53	25.73	49.5	●schwannoma
T.S.	12.32	22.28	22.7	●schwannoma
K.M.	9.56	15.40	12.7	●schwannoma
M.Y.	9.26			●schwannoma
craniopharyngioma				
M.T.	4.20	5.65	6.8	●craniopharyngioma

recurrence within 10 years in our experiences although the recurrence was not as rapid as sarcoma.

(ii) Subtype of meningioma and hemangioblastoma[14]

There was no appreciable difference in isotope uptake between fibrocytic meningioma and meningocytic meningioma or hemangioblastoma.

(iii) Peculiar type ependymoma[2]

This tumor may also be called oligodendrogliomatous ependymoma. It has the pattern of ependymoma but major part of tumor cells is composed of oligodendrocytes. Two cases were encountered in this series. Their values of indices were situated between those of other oligodendroglioma and ependymoma. This tumor often grows to considerable size but usually well demarcated from surrounding brain tissue and when totally removed, recurrence is rare and good prognosis can be expected.

(iv) Glioblastoma

In the tumors of this group, in spite of frequent presence of small necrotic foci, the uptake is usually much higher than astrocytoma although there was considerable variation in values. This may be understandable because the histological picture of this tumor is

Table 6

case	c. p. m. ratio	specific activity ratio	histology
Acid soluble fraction			
T.T.	7.04		◯ malignant lymphoma
M.K.	5.11	1.9	◯medulloblastoma
F.M.	4.39	5.5	◯glioblastoma
Z.O.	1.05	5.8	◯oligodendroglioma
T.S.	3.60	3.1	△ependymoma
H.N.	3.22	2.9	●piloid astrocytoma
M.S.	2.36	3.6	◯ glioblastoma
K.Y.	0.43	0.7	oligodendroglioma
E.T.	6.69	5.7	●pituitary adenoma
M.K.	4.32		●pituitary adenoma
K.M	3.10	5.7	●schwannoma
M.Y.	2.57		●schwannoma
Phospholipid fraction			
T.T.	16.17		◯ malignant lymphoma
F.M.	335.06	116.6	◯glioblastoma
M.K.	50.89	10.6	◯ medulloblastoma
Z.O.	38.60	11.5	◯ oligodendroglioma
T.S.	18.46	13.1	△ ependymoma
M.S.	11.08	5.8	◯ glioblastoma
H.N.	8.31	2.4	●piloid astrocytoma
K.Y.	3.18	1.2	◯ oligodendroglioma
E.T.	25.71	11.6	●pituitary adenoma
M.K.	14.57		●pituitary adenoma
M.Y.	379.18		●schwannoma
K.M.	9.68	1.9	●schwannoma

case	c. p. m. ratio	sppecific activity ratio	histology
Nucleic acid fraction			
T.T.	35.10		○malignant lymphoma
T.S.	37.62	7.2	ependymoma
F.M.	16.57	3.6	○glioblastoma
M.S.	11.71	3.9	○glioblastoma
Z.O.	11.10	1.3	△oligodendroglioma
H.N.	7.48	2.4	●piloid astrocytoma
M.K.	1.69	1.0	○medulloblastoma
K.Y.	2.96	0.8	△oligodendroglioma
M.K.	24.29		●pituitary adenoma
E.T.	8.08	1.1	●pituitary adenoma
K.M.	7.14	2.5	●schwannoma
M.Y.	2.49		●schwannoma
Total phosphorus			
T.T.	8.55		○malignant lymphoma
M.K.	6.10	1.9	○medulloblastoma
F.M.	5.94	4.4	○glioblastoma
Z.O.	5.17	3.6	△oligodendroglioma
T.S.	4.28	2.4	○ependymoma
H.N.	3.85	2.1	●piloid astrocytoma
M.S.	3.09	2.7	○glioblastoma
K.Y.	0.86	0.7	△oligodendroglioma
E.T.	7.86	1.2	●pituitary adenoma
M.K.	5.56		●pituitary adenoma
K.M.	4.05	3.1	●schwannoma
M.Y.	3.77		●schwannoma

quite variable and there are many necrotic foci which have no cellular activity. One case was the so-called giant celled glioblastoma, which has been said to be quite malignant. The uptake also supported this showing remarkably high uptake.

(v) Cerebral astrocytoma[14]

One case was histologically diagnosed as a malignant astrocytoma. This tumor was composed mostly of astrocytes but had higher cellular density and occasionally bizarre cells were mixed. The isotope uptake of this tumor was essentially the same as that of glioblastoma. Contrary to this, the other cerebral astrocytoma had similar histological appearance to that of cerebellar piloid astrocytoma. Also the uptake of this tumor was similar to that of cerebellar piloid astrocytoma.

(vi) Schwannoma and pituitary adenoma

These were known as benign tumors but showed high isotope uptake. The proper explanation to this was difficult within the limit of present experiment but there would be a particular phosphorus metabolism which was independent of cell division of these tumors, or differing from glioma, the brain tissue as a control may not be suitable to accurate evaluation of cell activity in these tumors.

Considering these facts, it may be difficult to compare glioma to these tumors on the same standard such as isotope uptake.

(vii) Vascularity and cellularity of tumors

It is quite difficult to evaluate exactly the vascularity and the cellularity of certain tissue. HELLER's[11] method was applied in several cases but only too erroneous results were obtained. Therefore, several spots of relatively homogeneous portion were selected on

Table 7 Vascularity and cellularity of tumors

total c. p. m. ratio	vascularity	cellularity	histology
28.53	×	× ×	●schwannoma
21.57	× ×	× × ×	○metastatic carcinoma
20.55	× × ×	× × ×	●pituitary adenoma
17.64		× ×	●fibrocytic meningioma
14.88	× ×	× ×	○giant celled glioblastoma
12.81	×	×	○ependymoblastoma
12.78	×	×	○glioblastoma
12.32	×	× ×	●schwannoma
11.37	×	× ×	○ependymoblastoma
9.24	× × ×	× ×	●hemangioblastoma
9.12	× ×	× ×	○metastatic carcinoma
8.22	× × ×	×	○malignant astrocytoma
7.71	×	× ×	○glioblastoma
6.48	×	× ×	●meningocytic meningioma
6.27	×	× ×	△oligodendroglioma (ependymoma-like pattern)
5.20	×	× ×	●fibrocytic meningioma
5.10	× ×	× × ×	○hemangioblastic meningioma (juvenile type)
4.81	× ×	×	○embryonal cell carcinoma
4.53	×	× ×	●meningocytic meningioma
4.17	× × ×	×	●hemangioblastic meningioma (adult type)
3.08	× ×	×	●astrocytoma
2.68	×	× ×	○oligodendroglioma (ependymoma-like pattern)
2.51	×	×	●piloid astrocytoma
1.98	×	× ×	●piloid astrocytoma
1.74	×	×	●piloid astrocytoma

○ malignant tumor
△ tumor with somewhat malignant tendency
● benign tumor

vascularity: poorly vascular ×; moderately vascular ××; highly vascular ×××

cellularity: (0–40) / (41–80) / (81 and over) cells in one field (×900) of well preserved tumor ..... ×; ..... ××; ..... ×××

histological preparation and counting of cells and vessels were done in certain microscopic fields. The mean value was calculated and shown in Table 7.

No apparent tendency was found on relation of the malignancy of tumor, isotope uptake and cellularity or vascularity. THOMAS[36] made an experimental study on intraocular tumor and reported that, increased vascularity by artificial inflammation gives effect on isotope uptake only in the first 10 minutes after isotope injection. He also found the influence of vascularity on isotope uptake in variously vascularized tumors was noted only in the initial stage of isotope administration. Although this result is not enough to substantiate the solid decision, it can be considered that vascularity and cellularity will not have a large effect.

4 Discussion

In 1948, SELVERSTONE[30][31] administered P^{32} intravenously to those patients of brain tumor and observed the isotope concentration in tumors was 5 to 100 times as high as that of normal brain tissues within short period of time after administation. This concentration ratio was maintained for about two weeks. He applied this fact on the detection and determination of tumor localization. In 1956, he again reported[32] that glioma contains less phospholipid than normal brain tissue and therefore, total phosphorus in glioma was also smaller in amount and that P^{32} concentration in tumor was higher in each phosphorus fraction especially in phospholipid fraction than that of brain tissue. Thus he concluded that it is due to increased cellular activity.

The same finding was obtained in the present experiment, but c. p. m. ratio of phospholipid was not quite accordant to the tumor malignancy. Therefore, high specific activity ratio in phospholipid fraction may be due to low specific activity of brain tissue taken as the control.

There are various components in phospholipid fraction of brain tissue and fairly active P^{32} uptake into inositol lipid or phosphatid acid has been observed in-vivo experiment but the total amount of phospholipid is so large that as a whole, the specific activity may appear low.

In 1952, STAPLETON[34] compared P^{32} uptake of brain tumor to its histological findings and observed gross parallelism between uptake and histological malignancy of tumor. In this experiment, the relationship was observed more clearly in nucleic acid and acid soluble fraction.

In tumor tissues, there are increased anaerobic and aerobic glucolysis and produced energy was used for various reactions which are essential to protein synthesis and cell division. Phosphorus compounds which participate in this carbohydrate metabolism are mostly contained in acid soluble fraction. Therefore, it is natural that activity of acid soluble fraction of highly malignant tumor is elevated. However, the component of this fraction is not completely clarified yet. Thus uptake of acid soluble fraction may be considered to be one of useful indices which may show the malignancy of tumor, although not all the components of this fraction may be directly related to the malignancy of tumors.

The role of phospholipid and phosphoprotein in tumor tissue is also not entirely clear but some of these substances are known to have remarkably high metabolic activity[9][20][24][37].

In this experiment, isotope uptake of these fractions was higher than that of normal tissue. REDDY et al[23]. made in-vivo experiment on ovarian tumors but he could not find any relationship between turnover rate of phospholipid and malignancy of tumor. On the other hand, NAKAYAMA[21] observed considerable increase of P^{32} uptake in phospholipid of cancer of stomach, esophagus and rectum. MIROFF et al[20]. also made in-vivo experiment on C3H ascites tumor and observed some relationship between relative specific activity of tumor and velocity of tumor growth. However, the true role of phospholipid and phosphoprotein metabolism still remained unknown.

Contrary to this, various studies have been made on nucleic acid metabolism and the tumor growth[10)12)18)24)25)26)27)]. It is well known that DNA and RNA in nucleic acid fraction play important roles in protein synthesis. MIROFF et al[20]. reported that the logarithm of tumor growth velocity and that of relative specific activity of nucleic acid showed parallel relationship in C3H ascites tumor. Many scholars such as NAKAYAMA[21], HARRINGTON[12], REDDY et al[23]., MANN et al[19]. and SCHNEIDER et al[25)26)27)] all reported the increased nucleic acid metabolism in tumor tissue. In this experiment, no further attempt to divide the nucleic acid component was made, but major portion of this fraction is composed of DNA and RNA which directly participate in the cell division. Thus it is easily understandable that isotope uptake of nucleic acid fraction fairly well accords with the malignancy of tissue.

The amount of phosphorus was similar to that appeared in SELVERSTONE's[32] report. BODIAN and DZIEWATKOWSKI[16] made a study on monkey brain and the result appeared similar to this experiment. But these values in human brain was considerably lower than that of dog brain reported by LOGAN[16] or that of rat brain by SCHNEIDER[26]. However, the proportion of each fraction was similar to the case of human.

SCHNEIDER[26] reported slight increase of phosphorus contents of acid soluble fraction in experimental tumor but no great difference was present between various tumors.

As for the phosholipid, SCHNEIDER[26] reported no difference in amount of phosphorus in various tumors of rat. The nucleic acid phosphrous was generally increased in tumor tissue. SCHNEIDER's[26] determination on experimental tumor showed considerable similarity of the amount of RNA and DNA in various tumors but there was considerable difference in various normal tissues. All these tendencies were similarly observed in this experiment.

5 Summary

1) Radioactive P^{32} was administered to 37 patients of intracranial tumor and a comparative study was made over isotope uptake and histological and clinical malignancy of tumors from the view-point of growth activity.

2) The parallelism between isotope uptake and malignancy of tissue was most clearly noted in c. p. m. ratio of nucleic acid fraction followed by that of acid soluble fraction. Specific activity also showed similar tendency.

3) The result of this experiment revealed that it is not proper to compare the different natured tumors such as meningioma, glioma, carcinoma, schwannoma or pituitary adenoma on the same standard of isotope uptake. But P^{32} uptake showed fairly good parallel relationship with malignancy of tumor when compared within the groups of glioma

or meningioma.

4) Three indices on P^{32} uptake were proposed. The discussion was made on such tumors as hemangioblastic meningioma, peculiar type ependymoma or cerebral astrocytoma judged according to these indices. The clinical course of these tumors well accorded with the velocity of tumor growth when judged in this manner.

5) Phosphorus content was much greater in normal brain tissue than in tumor tissue and this is mainly due to phospholipid which is contained in brain tissue in large amount. The phosphorus in nucleic acid or in acid soluble fraction was rather more in tumor tissue. Markedly high specific activity ratio of phospholipid of brain tumor may not be due to high metabolic rate of this fraction but due to remarkably low specific activity of this fraction in brain tissue.

6) No relationship was found between P^{32} uptake and vascularity or cellular density of tumors.

7) Results were compared when brain tissue was taken as a control and when muscle was taken each other. As for the relationship between P^{32} uptake and malignancy of tumor, there was no remarkable difference.

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和文抄録

P^{32}による脳腫瘍のアイソトープ摂取量と組織学的所見について

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脳腫瘍におけるアイソトープの摂取様式とその組織像，特に腫瘍の悪性度との関係を見るため脳腫瘍の患者にP^{32}を投与し以下の実験を行なつた．すなわち，得られた組織片につきSchneiderの方法により燐の分画を行ない，その各々について放射能の測定と燐の定量を行なつて得られた結果を組織標本と比較検討した．

このようにして37例の脳腫瘍について検討した結果は次の如くである．

1) 核酸分画のカウント比が組織の悪性度に最もよく一致し，酸可溶性分画のカウント比が之に次ぐが，比活性度も大体組織の悪性度に一致した．

2) 本実験の方法ではMeningiomaやGlioma等とSchwannoma, Pituitary adenoma等を相互に比較するのは適当でないが，同一種類のGliomaとかMeningioma群の中ではアイソトープ摂取量は組織の悪性度所見とよく一致する．

3) P^{32}摂取量の上から，カウント比，比活性度，比活性度率等を基準とし，明らかに悪性であるGlioblastomaや良性であるAstrocytoma等に対し，悪性度が充分明らかでない幾つかの腫瘍を対比させて見ると，その結果は臨床経過にかなりよく適合し，それ等の腫瘍の性格をある程度理解するのに有用であつた．

4) 対照組織には脳及び筋肉組織を用いたが，これ等2つの場合には著明な差は認められなかつた．

5) 燐含有量は腫瘍組織よりも脳組織に多かつたが，これは脳には大量の燐脂質が含まれる故であり，核酸及び酸可溶性分画の燐はむしろ腫瘍に多かつた．脳腫瘍においては脳を対照とするとき，燐脂質分画の比活性度率が非常に高かつたが，これは脳のこの分画の比活性度が非常に低かつたためで，この分画の代謝が特に高かつたためとは考え難い．

6) P^{32}摂取量と組織の細胞密度や血管分布の間には一定の関係が見出せなかつた．