Experimental and Clinical Studies of Radiosensitizers in Brain Tumors, with Special Reference to BUdR-Antimetabolite Continuous Regional Infusion-Radiation Therapy (BAR therapy)*

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Introduction:

It was found out in the middle of this century that pyrimidine analogues, especially halogenated deoxyuridine, were incorporated into deoxyribonucleic acid of dividing cells. At the beginning of 1960, Djordjevic & Szybalski1 and Kaplan et al2 showed in vitro experiment that the cells having incorporated such analogues were radiosensitized, that is, sensitivity of the cell to irradiation became much higher, without any other physiological and biological effects to the cell if not irradiated. Following their studies, radiosensitizing ability of halogenated deoxyuridine, especially 5-bromo 2' deoxyuridine (BUdR) and 5-iodo 2' deoxyuridine (IUdR) were confirmed by many investigators in strained human cell lines such as human cell D 93', D 98Az', H Ep I (human epidermoid carcinoma of the cervix)3, ascitic P-388 (lymphocytic leukemia)4, L cell5 and Hepatoma 1926.

These pyrimidine analogues are incorporated into deoxyribonucleic acid of the cells as thymidine substitute and not into ribonucleic acid. Differences between the cells having incorporated BUdR (or IUdR) and the cells of normal nucleic acid are almost none, namely, there is neither metabolic change nor mitotic disability, but when irradiated, the former are much more radiosensitive than the latter. This fact arouses a keen interest from a view-point of radiotherapy. Malignant tumor cells have higher mitotic rate and accordingly, are thought to be much more vigorous in synthesizing DNA than normal cells. Therefore, the rate of uptake of halogenated pyrimidine instead of thymidine into DNA should be much higher in tumor cells than in normal cells, and the destructive effect of irradiation to the...

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cells should be enhanced especially in the tumor cells. The only disadvantage of BUdR or I UdR in clinical administration is that they are extensively dehalogenated by the liver. This is resolved by using local arterial infusion in administration of the drug. This method is also worth-while in a viewpoint of selective administration. Most of the brain tumors are malignant but, generally speaking, they are not very sensitive to irradiation with some exceptions such as medulloblastoma, pinealoma and pituitary adenoma. The authors thought it very promising if the brain tumors were radiosensitized by these halogenated pyrimidines and concomitantly treated with irradiation.

In this paper, the authors have tried to confirm the uptake of the pyrimidine analogues into the brain tumor cells and to see their radiosensitizing effect in tissue culture and finally to show some cases of its clinical application.

Monolayer culture of human brain tumors:

Since several years ago, the authors have been studying tissue culture of brain tumors with roller tube technique obtaining a fairly good results. However, this method was found to have some disadvantage for quantitative experiment such as screening test of anticancerous drugs, and the monolayer culture was thought to be preferable. Therefore, the authors improved the trypsinization-monolayer method, which had been thought to be inadequate to the primary culture of brain tumors, so as to fit with brain tumors. With this improved method, we have obtained so good results that almost all of various brain tumors showed flourishing growth in culture. The method is as follows.

A piece of a brain tumor, which was taken at operation, was minced with knives. At least 1-2 grams of the tumor was necessary as the material. The minced tumor was washed with Gey's solution (1000 rpm., 10 min.) and put in Gey-trypine solution for 10 min. at 37°C, followed by mixing with a magnetic stirrer for another 10 min. Trypsin used was Difco 1:250 and its concentration was about 0.002%, although it should be somewhat variable according to the sort and the consistency of the tumor. The cell suspension thus prepared was filtered with platinum mesh (280 and 2150) and washed with Gey's solution again (1000 rpm., 5 min.). Adequate amount of culture medium was added onto the sediment (cells) and one ml. of this suspension was poured into each small glass cubic culture bottles. The optimal concentration of the suspension was about 3 million cells per ml. Usually about 20 bottles were prepared from 1 gram of tumor piece. The culture medium was NCTC 109 or Eagle's basal medium added with 20% of calf serum. Exchange of the medium in every 9-14 days was usually sufficient.
Up-take of BUdR into the brain tumor cell in vitro:

On the third to the 14th day of the culture, in which a good proliferation was achieved, $^3$H-BUdR (5-bromo 2'deoxyuridine-6-tritium) was added into the medium and after incubation of some period, the uptake of BUdR was examined by radioautography. The incubation time of BUdR varied from 30 min. to 5 days according to the aim of the experiment and the generation time of the cultured cell. Followings are some examples.

Fig. 1 shows a culture of a glioblastoma. 5 $\mu$c/ml. (0.78 $\mu$g/ml.) of $^3$H-BUdR was added to the medium on the 7th day of the culture and it was incubated for 5 days. In this case, 5 $\mu$g/ml. of thymidine had been contained in the basal medium.

Fig. 2 is a cerebellar astrocytoma. The cells were incubated in the medium containing 10 $\mu$c/ml. (0.156 $\mu$g/ml.) of $^3$H-BUdR for 2 days. In this case, 2 $\mu$g of thymidine had been contained in the basal medium.

Fig. 3 is a case of ependymoglioblastoma. On the 5th day of the culture, 1 $\mu$c/ml. (0.156 $\mu$g/ml.) of $^3$H-BUdR was administered and it was incubated for 2 days.

As these figures show, black grains of $^3$H-BUdR were found only on the nuclei. On the contrary, $^3$H-uridine was once taken up into the nuclei within 1-2 hours of incubation, but after 12-24 hours, it came into the cytoplasm. BUdR stays permanently in the nucleus.

Fig. 4a shows a culture of an oligodendroglioma (8 day) after the incubation in $^3$H-uridine for one hour. The grains still remain in the nuclei.

Fig. 4b shows the same culture after 24 hours. Most of the gains have moved into the cytoplasm.

Table 1. Labelling index (L.I.) of thymidine and generation time (G.T.) in various brain tumor cells in vitro

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>L.I.</th>
<th>G.T.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastomas</td>
<td>4%</td>
<td>5-8 days</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>1%</td>
<td>17-26 days</td>
</tr>
<tr>
<td>Oligodendrogliomas</td>
<td>1%</td>
<td>17-26 days</td>
</tr>
<tr>
<td>Meningiomas</td>
<td>8%</td>
<td>2-4 days</td>
</tr>
</tbody>
</table>

As BUdR is incorporated into DNA as a substitute of thymidine, the rate of incorporation of the two is thought to be the same. Table 1 shows the labelling index of thymidine in various cells. The labelling index of BUdR was found, in our hands, to be about the same as that of thymidine. This fact seemed to indicate that both thymidine and BUdR did not alter generation time of the cell and BUdR was taken up in dividing cells in
the same rate as that of thymidine.

As the radiosensitizing effect of BUdR is dependent on the amount of BUdR replacing thymidine\(^2\), it is important to find a way to facilitate the incorporation of BUdR into the cell. The concentrations of BUdR and thymidine in the culture medium were thought to be one of the most important factors. Though accurate quantitative experiment was difficult by our present method, the relationship between the amount of uptake and the concentration in the medium could be estimated by calculating the number of the grains in the nuclei (grain index).

Fig. 2 and 5 show cultures of same astrocytoma. Fig. 2 is a culture after the incubation in the medium with 0.156 \(\mu\)g/ml. (1 \(\mu\)g/ml.) of BUdR and 2 \(\mu\)g/ml. of thymidine for 2 days. Fig. 5 is 1.56 \(\mu\)g/ml. (10 \(\mu\)c/ml.) of BUdR and 2 \(\mu\)g/ml. of thymidine respectively. Comparing both figures, it is apparent that if the concentration of thymidine in the medium is constant the amount of BUdR in the cell increases in proportion to the concentration of BUdR in the medium. Fig. 6a and 6b show the same result in FL cells.

**Radiosensitizing ability of BUdR in vitro:**

The radiosensitizing ability of BUdR has been already studied in various strained cells and microorganisms and reported by many investigators\(^3\). The authors tried to elucidate the radiosensitizing effect of BUdR in human brain tumor cells in vitro. At present, we have no established strained brain tumor cell lines in our laboratory and it was thought to be rather difficult to do quantitative study using primary culture of brain tumors.\footnote{Fig. 6a and 6b show the same result in FL cells.}

However, since constant good growth has become to be obtained by our trypsinization-monolayer technique in primary culture of brain tumors as stated before, we applied it in investigation of the effect of BUdR in vitro. This method is simple in technique and of constant result and is thought to be also applicable to a routine screening test on clinical use of BUdR.

The method is as follows. After obtaining a good proliferation of brain tumor cells in culture, 10–40 \(\mu\)g. of BUdR was added into the medium and the bottles were incubated at 37°C for the period corresponding to the generation time of the tumor cell obtained from labelling index of \(^3\)H-thymidine. Then the bottles were irradiated with X-ray of various amount of dose (500–8,000 r) in serial divided bands. Control bottles without BUdR were also irradiated at the same time. Besides the brain tumors, the authors also examined the effect of BUdR on FL cells. In this case, we passaged 30\(\times\)10\(^4\) cells/ml. of FL in cubic bottles and after the incubation of 24 hours in the medium containing 20 \(\mu\)g/ml. of BUdR, the bottles were irradiated in the same manner as in brain tumors. In both cases, after the irradiation, the bottles were incubated at 37°C further for 7–20 days. Then the materials
were fixed and stained and the effect of the irradiation on the cells was judged based mainly on the population of the cells and their morphological changes.

Fig. 7 shows the effect of BUdR and irradiation on FL cells. Comparing with the control group, the BUdR plus irradiation group shows marked decrease in number of the cells and exhibits morphological changes. Stainability is also reduced in this group.

Fig. 8 is a case of oligodendroglioma. As seen in the case of FL strain, the cells affected by BUdR are more sensitive to irradiation. In the BUdR plus irradiation group, comparing with the control, we can see pyknosis of the nuclei, decrease of stainability and fibrous change of the cells. Cell population is markedly reduced in this group. As shown in these experiments, it is apparent that the cells having incorporated BUdR in vitro are much more sensitive to irradiation. Furthermore, the authors would like to emphasize that there is no inhibitory effect on the cells in vitro of BUdR itself even in a long term culture such as more than one hundred days.

Enhancement of BuDR incorporation into the nucleus by antimetabolites:

It is said that more the amount of BUdR taken up into the tumor cell, more radio-sensitive is the cell. Therefore, in clinical administration of BUdR, it is important to find out any means to facilitate the incorporation of BUdR into the cells. Although it is still not known of the mechanism of replacement of thymidine by BUdR, the amount of BUdR in the nucleus increase, as stated before, in proportion to the amount of BUdR in the medium if the concentration of thymidine is unchanged. However, in clinical use, we cannot give so much amount of BUdR to the patient, one reason of which is that BUdR is extremely expensive now. Therefore, the authors pursued the way to obtain maximal incorporation of BUdR into tumor cells in minimal dose of its administration. Various chemicals were examined and the authors found out that very small amount of methotrexate (amethopterin), 5-fluoro 2'deoxyuridine and 5-fluorouracil enhanced the uptake of BUdR into the cell nucleus in vitro.

For example, Fig. 9 shows the result in FL cells thus treated. 0.156 \( \mu g/ml \) (specific activity 1 \( /\mu c/ml \)) of \( H^3\)-BuDR was given 3 days after a passage of FL cells and incubated for 48 hours in the medium with 1 \( \mu g/ml \) and 100 \( \mu g/ml \) of methotrexate respectively. The controls contained 0.156 \( \mu g/ml \) and 1.56 \( \mu g/ml \) of \( H^3\)-BuDR, both without methotrexate. The variance in grain index between Fig. 9c and Fig. 9d is due to the quantitative difference of BUdR administered. The cells incubated with methotrexate (Fig. 9a, b) show remarkable increase of grain index compared with the cells in Fig. 9c and are almost of the same grain index of the cells in Fig. 9d, which are
administered 10 times as much as amount of BUDR. Consequently, it is apparent that a very small amount of methotrexate (even in the order of \(\gamma/ml\)) accelerates the uptake of BUDR into the cell nuclei. The result of the same experiment on an oligodendroglioma is shown in Fig. 10. The cells in Fig. 10a were administered 0.156 \(\mu g/ml\) (1 \(\mu c/ml\)) of \(^{3}H\)-BUDR alone and cells in Fig. 10c were administered the same amount of BUDR with 100 \(\gamma/ml\) of methotrexate. In Fig. 10b the concentration of \(^{3}H\)-BUDR was 1.56 \(\mu g/ml\) (10 \(\mu g/ml\)) and free of methotrexate. One can easily notice that the grain index in the cells in Fig. 10b and 10c is almost the same.

From these results it may be assumed that a small amount of methotrexate would accelerate the uptake of BUDR into the nuclei of the brain tumor cells in clinical administration too, though there may be some differences between the cells in vitro and in vivo.

As for other chemicals which we examined, the results are summarized in Table 2.

**Table 2.** Change of the amount of \(^{3}H\)-BUDR incorporated into brain tumor and FL cells by the co-administration of various antimetabolites, and folic acid.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mtx.</th>
<th>Antimetabolites 5-FU</th>
<th>FUDR</th>
<th>Folic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg/dl</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>0.001 mg/dl</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>-</td>
</tr>
</tbody>
</table>

Mtx.: methotrexate, 5-FU: 5-fluoro-uracil, FUDR: 5-fluoro 2'deoxyuridine, +++: marked increase, ++++: very marked increase, +++++: most marked increase, -: moderate decrease, --: marked decrease

**Clinical administration of BUDR:**

Clinical application of BUDR as a radiosensitizer has been already carried out in several hospitals. However, the method of administration and the dosage of BUDR and irradiation are still not established.

The principle of our method consisted of the administration of BUDR with a small amount of methotrexate (or other antimetabolite) by means of continuous arterial (carotid) infusion for more than one generation time of the tumor cell and simultaneous irradiation.

The authors usually performed decompressive craniotomy and took out some specimen of the tumor. Then, after the pathological diagnosis has been confirmed, we put a catheter into the regional internal carotid artery and infused continuously, by a small pump, 500–1000 mg. of BUDR and 1–5 mg. of methotrexate a day, which were dissolved in about 750 ml. of saline. At the same time, the tumor was put in tissue culture and the basic
investigations such as those of the generation time and radiosensitivity of the tumor were carried out.

Usually, the authors continued the infusion for 3 to 6 weeks. For example, in cases of astrocytoma and oligodendrogloma, the infusion was continued for 4 to 6 weeks and of glioblastoma for 3 to 4 weeks. Irradiation started on the 4th or 5th day of the infusion and was repeated every day. Daily dose of the irradiation was about 200 r (tumor dose) and the total dose is 5,000–6,000 r (tumor dose) or more. During the irradiation, though it was a very short time less than one hour, the infusion was temporarily interrupted and the infusion tube was filled with heparin solution. The authors are now testing a very small portable infusion pump, and when it is completed, the infusion can be continued even during the irradiation.

As no sufficiently long time has passed to judge the long-term follow up result of this therapy, the authors checked the effect of this therapy by measuring decrease of the tension in the craniotomized area and the pressure of cerebrospinal-fluid on lumber puncture and by observing improvement of the neurological symptoms and signs and the rentogenological (angiographic, pneumoencephalogrphic, etc.) evidence. Routine investigations such as blood count, urinalysis, serum electrolytes, liver function were done periodically during the course of the therapy.

The authors have carried out this therapy on 5 cases of brain tumors. Followings are two illustrative cases.

Case 1. I.O., 37 year old male

Clinical history: The patient started to have convulsive seizures about two years prior to admission and since about the same time he noticed to have headache and memory disturbance. He visited the Neurological Department of our Hospital about 6 months prior to admission and was referred to us. At that time, however, there was no neurological abnormality except slight tremor on both hands and we decided to leave him on observation. Then he again became to have headache and nausea and the tremor on both hands began to increase. His vision fell down quickly to almost blindness He was reexamined at our out-patients clinic and admitted.

On examination, he had marked papilloedema and loss of visual acuity (blind on the left and 10 cm n. d. on the right). Three was right homonymous hemianopia. Memorizing ability has been markedly disturbed. He might have some dysphasia and dysacalcuria, which have been masked by marked memorizing disturbance, but no motor and sensory signs. Left carotid angiography was performed and revealed a large space occupying mass in the left temporal region, which was thought to be a glioma.

Clinical course: Left lateral craniotomy was performed under general
anesthesia. The convolutions of the exposed cortex were flat, pale and somewhat hard on palpation. The tumor invaded diffusely in almost whole of the left temporal lobe and extended into the lower part of the parietal lobe. Specimens were taken from several parts of the tumor for histological examination and tissue culture. For a good external decompression, the dura was closed with a supplemental patch of the facia lata, and the bone flap was removed. Post operative course was uneventful and the left carotid artery was catheterized 12 days after the operation. The infusion of BUdR (600 mg/day) and methotrexate (6 mg/day) was started from the day of the catheterization and continued for 34 days. Total dose of the irradiation was 5,283 r tumor dose.

The bulging in the craniotomized area started to reduce on the 7th day of radiation therapy and the area showed marked depression after 2 weeks. Papilloedema was also markedly reduced, but visual field was not widened. Memorizing disturbance was improved gradually and almost fully recovered at the time of the end of the therapy. Clinical course and the results of the various investigations were shown in Table 1.

The authors opened the flap again for exploration one week after finishing the radiation therapy and biopsy was done. Fig. 11a shows the tumor at the time of the first operation and Fig. 11b was at the second exploration. We can see a marked necrosis over the area where the tumor infiltration was noticed on the first operation. Histological findings are in Fig. 12. Fig. 12a shows a specimen which was taken from the central part of the tumor on the first operation and Fig. 12b shows that of peripheral region of the tumor. Fig. 13a shows a specimen which has taken from the central part of the tumor on the second operation and Fig. 13b illustrates a specimen of the peripheral part of the tumor on the second operation respectively.

It is well known that pathological characteristics often vary considerably place by place in the same tumor. Therefore the authors tried to take specimens as large as possible and from as many places as possible on explorations. Anyhow, difference between the characteristics of the specimens of the first and the second exploration is marked. In the specimen after the therapy, we can hardly see the tumor cells.

Case 2. S.S. 30 year old male

Clinical history: This patient had a clinical history of a sudden onset of severe headache, nausea and vomiting about one month prior to admission. Since then he was in a drowsy state for about fortnight. He recovered from the drowsy state gradually but continued to have headache and vomited several times a day. About one week before the admission, he was noticed to have right sided abduccens paresis.
On admission, he still had severe headache and nausea and both fundi showed marked papilloedema. He had right sided sixth nerve palsy but otherwise there was no neurological abnormality. Plain craniograms were normal except some widening of the sella but the left carotid angiograms showed a marked shift of the anterior cerebral artery to the right and there was abnormal vascularization in the left frontal region, suggesting a large space-occupying lesion probably glioblastoma. Left frontal craniotomy was carried out and anterior two thirds of the left frontal lobe was removed. Though the tumor seemed to invade far in the anterior parietal and temporal lobes, we gave up further manipulation because internal decompression was fully achieved and the pathological evidence was glioblastoma. Two weeks after this primary operation, the left carotid catheterization was carried out and the infusion of BUdR (600 mg/day) and methotrexate (6 mg/day) was started. The infusion was continued for 3 weeks and the irradiation by Co⁶⁰ was started on the 4th day of the infusion. The irradiation was continued for 40 days and the total dose reached to 5,000 r tumor dose. During the therapy, the symptoms and signs of the increased intracranial pressure disappeared gradually. Fig. 14a, b and c show the left carotid angiograms pre-operative, post-operative and post-irradiative respectively. In this case, re-exploration after the BUdR-irradiation therapy is not yet performed. Clinical course and the results of examinations are grossly illustrated in table 4.

Table 3.

<table>
<thead>
<tr>
<th>Serum GOT</th>
<th>42</th>
<th>44</th>
<th>35</th>
<th>800</th>
<th>156</th>
<th>55</th>
<th>26</th>
<th>27</th>
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<tbody>
<tr>
<td>Glu</td>
<td>137</td>
<td>39</td>
<td>43</td>
<td>650</td>
<td>175</td>
<td>60</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

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Discussions:

Based on the clinical and pathological findings, in case 1 and case 2, this combined therapy of BUDR, methotrexate and irradiation is thought to be very promising for brain tumors which can not be extirpated, though reliable evaluation should be made after a sufficient time has passed to see the follow up result.

Anyway the advantage of this therapy for malignant brain tumors is thought to be as follows.

1. Most of the brain tumors are solitary and rarely metastasize.
2. The surrounding tissues (normal brain cells) have almost no mitotic ability, namely, they hardly incorporate radiosensitizers into their DNA.
3. The normal brain cells are protected by the blood-brain barrier from these drugs, whereas the tumor cells are exposed to these drugs became of break down of the barrier.
4. Regional infusion of the drug is easy.

Though it has been confirmed in vitro that methotrexate accelerates BUDR incorporation, its mechanism is still unknown. Recently, Young & Hodas\(^{16}\) reported that methotrexate enhance the uptake of thymidine into the cell in vitro and the authors also confirmed that in FL cells. To explain these facts the authors have a hypothesis that methotrexate, or other antimetabolites of the same kind such as 5-fluorouracil and 5-fluoro 2'deoxuryridine does not block the process through which thymidine is incorporated into DNA, but does inhibit the process of the production of thymidine in the cell. Therefore, if thymidine (or BUDR) is given together with methotrexate or the other antimetabolite to the cell, this newly given thymidine (or BUDR) is mainly
utilized in composing DNA, since the cell itself can not produce thymidine any more because of the inhibition. We are preparing another paper on this point.

Though further study is necessary to find out the best drug for this purpose, the complementary administration of these antimetabolites is very promising for enhancement of the effects of the BUdR-radiation therapy. In our impression, the complementary use of methotrexate for this therapy is very useful, but leucopenia and other side effects of methotrexate appear rather quickly compared with methotrexate infusion therapy only, although these side effects rapidly disappear by a momentary interruption of the administration.

Summary

1. Method of trypsinization-monolayer culture of brain tumors was briefly stated.
2. Uptake of BUdR into brain tumor cell nuclei was confirmed by means of tissue culture.
3. Radiosensitizing effect of BUdR was confirmed using monolayer-culture of brain tumor cells and FL cells. This effect was seemed to increase in proportion to the amount of BUdR incorporated into the cell nuclei.
4. The uptake of BUdR into the cell nuclei was enhanced by a small amount of antimetabolites such as methotrexate, 5-FU and FUdR in vitro.
5. Clinically, continuous intracarotid infusion of BUdR with small amount of methotrexated was performed in several brain tumor patients and simultaneous irradiation was done. We convinced that this BUdR-Antimetabolite-continuous regional infusion-radiation therapy — BAR therapy — is very promising in treating malignant brain tumors.

Acknowledgment

We wish to express our thanks to Prof. T. Miyagawa and the staffs of the Dept. of Radiologry, University of Tokyo, for much assistance and to Prof. J. Nakai and T. Yoro of the Dept. of Anatomy, University of Tokyo, for helpful advice and instruction.

References:

Fig. 1. Globlastoma multiforme incubated for 2 days with 0.75 µg/ml of H\textsuperscript{3}-BUdR (specific activity 5 µc/ml) and 5 µg/ml of thymidine.

Fig. 2. Astrocytoma incubated for 2 days with 0.156 µg/ml of H\textsuperscript{3}-BUdR (specific activity 1 µc/ml) and 2 µg/ml of thymidine.

Fig. 3. Ependymo-glioblastoma in 5 day culture with 0.156 µg/ml of H\textsuperscript{3}-BUdR (specific activity 1 µc/ml) and 2 µg/ml of thymidine.
Fig. 4. (a) Oligodendroglioma, 8 day culture, incubated for 1 hour with H²-uridine (1 μc/ml). Note, the grains are found mainly on the cell nuclei.

(b) Oligodendroglioma, 8 day culture, incubated for 24 hours with H²-uridine (1 μc/ml). The grains have moved into the cytoplasm.

Fig. 5. The same astrocytoma with Fig. 2, incubated for 2 days with 1.56 μg/ml of H²-BUdR (specific activity 10 μc/ml) and 2 μg of thymidine.
Fig. 6. (a) FL cells incubated for 2 days with 0.156 μg/ml of H^3-BUdR (specific activity 1 μc/ml).
(b) FL cells incubated for 2 days with 1.56 μg/ml of H^3-BUdR (specific activity 10 μc/ml). Note the increase of grain index of the nuclei.
Fig. 7. EL cells. Left sided photographs show the effect of irradiation only. The upper picture shows the FL cells not irradiated. The middle illustrates the cells irradiated with 1,500 r of X-ray and the lower picture with 2,000 r. Right sided pictures show the BUDR plus irradiation group. No irradiation, 1,500 r and 2,000 r respectively. Note the marked decrease in cell population and morphological changes in BUDR plus irradiation group.
Fig. 8. (a) Oligodendroglioma, incubated for 48 days with 20 μg/ml of BUdR. The characteristics of the cells are almost the same as the cells cultured free of BUdR.

(b) Same Oligodendroglioma, given 8,000 r of X-r cultured for 48 days without BUdR.

(c) Same Oligodendroglioma, given 8,000 r of X-r cultured with 20 μg/ml of BUdR for 48 days.
Fig. 9. (a) FL cells incubated with 0.156 μg/ml of H3-BUdR (1 μc/ml) and 17/ml of methotrexate for 2 days.
(b) FL cells incubated with 0.156 μg/ml of H3-BUdR (1 μc/ml) and 1007/ml of methotrexate for 2 days.
(c) FL cells incubated with 0.156 μg/ml of H3-BUdR (1 μc/ml) only for 2 days, without methotrexate.
(d) FL cells incubated with 1.56 μg/ml of H3-BUdR (10 μc/ml) for 2 days, without methotrexate.
Fig. 10. (a) Oligodendroglioma, 8 day culture, incubated with 0.156 μg/ml of H-3-BUdR (1 μc/ml) for 2 days.

(b) Oligodendroglioma, 8 day culture, incubated with 1.56 μg/ml of H-3-BUdR (10 μc/ml) for 2 days.

(c) Oligodendroglioma, 8 day culture, incubated with 0.156 μg/ml of H-3-BUdR (1 μc) and 100 μg/ml of methotrexate for 2 days. Note the marked increase of grain index, of the nucleus.
Fig. 11. (a) Case I. The first operation. The tumor is invading in almost whole of the left temporal lobe (lower in this photograph) and extending into the lower part of the parietal lobe.

Fig. 11. (b) Case I. The second exploration. Marked necrosis is seen over the area where the tumor was noticed at the first operation.
Fig. 12. (a) Case 1. Specimen taken from the central part of the tumor at the first operation.
(b) Case 1. Specimen taken from a peripheral part of the tumor at the first operation, showing a nest of the tumor cells and calcifications.

Fig. 13. Case 1. Specimen taken from a central part (a) and a peripheral part (b) of the tumor at the second exploration. Gliosis and calcification are marked but the tumor cell nests has disappeared.
Fig. 14. Case 2. (a) Preoperative findings of the left carotid angiography. Marked shift of A. cerebri anterior is observed.

(b) 2 weeks after the operation (before BUdR-irradiation therapy). The shift of A. cerebri anterior is reduced but still exists.

(c) Post-irradiative finding. The shift of A. cerebri anterior has been disappeared.