# Effects of Ionizing Radiations on Central Nervous System in Tissue Culture

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# 中枢神経組織培養に及ぼす放射線の影響

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中枢神経組織の放射線による機能的な影響を検討する目的で次の如き実験を行つた.

1) 仔猫の脳室内面の組織を培養し，培養7日目に $^{60}Co$ 照射を行い，照射前後の上衣細胞繊毛運動の状態の変化を検討した．又 Ra 針を用い顕微鏡下に照射し，照射中もその繊毛運動を観察した．これらにより in vitro に於ては上衣細胞は放射線に対し極めて抵抗性が強い事が知られた．一方 in vivo に於ては上衣細胞もそれ程放射線に対し抵抗性が強くはなく，又 in vitro に於ても酸素欠乏等に対しては感受性が高い事は従来より知られており，従つて上衣細胞の放射線による影響に血管障害を含む間接的影響が強く関与している事が推定される.

2) 仔猫脳室内面の組織を切出し，スライドグラスに移植後これに $^{60}Co$ 照射を行い，次で incubation を開始，培養7日目に培養組織の outgrowth の状態を対照群と比較した．10,000R 以下のものでは明瞭な差が認められず，30,000R以上照射のものに著明な細胞の発育及び游走の抑制が認められた.

3) 仔猫に $^{60}Co$ 頭部照射を行い，照射1時間後，脳室内面の組織を培養し，培養7日目にその outgrowth の状態を対照群と比較したが，3,000R以上のものに著明な細胞の発育，游走の抑制が認められた.

4) 幼若 I.C.R. 系マウスに $^{60}Co$ γ線，3,000R頭部照射を行い，照射1時間後にその小脳皮質を培養し，培養5日目の培養組織の面積増大の程度を測定し，対照群のそれとの間に統計的に有意の差がある事が認められた．上記2)，3)，4)より放射線の培養中枢神経組織の発育に及ぼす影響は in vitro 照射と in vivo 照射との間に著明な差がある事が認められ，2) に於ける変化は直接的影響によるものであり，3) 4) に於ける変化は間接的影響も加わったものと考えられる．これ等の間に著明な差がある事は中枢神経組織の機能的影響に循環障害等を含む間接的影響が大きな役割を果している事を示唆していると考えられる.

A great deal of investigation with regard to the cytological and chemical effects of ionizing radiation on the central nervous system has clarified the sensitivity of the interstitial cell system[3)7)-9)11)13)14))22)34)]. Effects of ionizing radiations on the ependyma cells, however, have been reported only by few investigators[8)14)34)]. Brownson has indicated that the choroid plexus, ependymal and subependymal cells of 9 month old rats receiving X-irradiation with doses between 1,000 and 5,000 R to the head underwent variable changes in the structure and greater evidence of swelling and vacuolization was noted at lower levels of

X-irradiation[8]. The sensitivity of ependyma cells in vitro to X-irradiation was not observed in the previous investigations.

The technique of tissue culture offers a means of direct and continual observations of any experimental manipulation on a group of living cells. Effects of ionizing radiations on the central nervous system seems to be induced not only directly but also indirectly through the damage to vascular systems by irradiation. Therefore, investigations with the tissue culture method is considered to be significant in order to observe the direct disturbances of the central nervous system by irradiation excluding the factors of vascular systems.

In almost all of the previous investigations with regard to the effects of irradiation on the central nervous tissues, studies have been restricted only to the organic changes of the tissues. Since, in most cases, the central nervous tissues are remarkable in their ability to withstand a massive dose of radiation before their organic changes are observed, the tissues have been considered to be radio-resistant. However, possibility can not be excluded that the tissues are functionally radiosensitive. Therefore, studies are also important in researching the radiosensitivity of the central nervous system. From these considerations the studies were designed to investigate the radiation effects on the ciliary motility of ependyma cells.

Ependyma cells have been studied both in vivo and in vitro by several investigators[1)2)6)15)17)]. Hild has pointed out that cultured ependyma cells were astonishingly resistant to the action of some unphysiological conditions[15)17)].

This investigation (part 1) was undertaken to determine the sensitivity of ependyma cells in vitro to the γ-irradiation. The ventricular linings obtained from kittens younger than 1 month of age were cultivated in vitro and the tissue fragments containing the ependyma cells were exposed to γ-irradiations after 7 days in culture. Then, observations on the ciliary activity of ependyma cells in culture were made and the comparison with that of controls were performed in this experiment.

Radiation effects on the growth of cultured central nervous tissue have been studied by Levi et al and Goldring[20.13)]. However, no reports with regard to the inhibition of growth activity of the cultures from the central nervous tissue of mammalian exposed to γ-irradiation have presented. Therefore, the following investigations (part 2) were undertaken to determine the effects of γ-irradiation on the growth in tissue culture of kitten cerebral cortices younger than 1 month of age and of cerebellar cortices of 3 day old ICR-strain mice emphasizing the difference of radiosensitivity between in vitro and in vivo exposure. In order to observe the direct as well as indirect effects of γ-irradiation on the growth of cerebral and cerebellar cortices in tissue culture, the experimental groups were classified roughly into two groups: Group 1) Cerebral cortices were excised from normal kittens, irradiated in vitro. and then cultured. Group 2) Kittens or ICR-strain mice were irradiated in vivo (total head irradiation) and cerebral or cerebellar cortices were removed at 1 hour after irradiation and then cultured: Comparison on the growth activity of both groups in culture was made.

## Part 1 Effects of Ionizing Radiations on the Ciliary Activity of Ependyma Cells in Tissue Culture

### Materials and Methods

Culture Techniques: The method employed for culture was the primary culture by means of a roller tube method similar to those in the previous reports[10)16)18)19)23)24)27)28)31)35)38)40)]. Tissue fragments of cerebral cortices were obtained from the third and lateral ventricular linings of kittens in the first month

of life. Three of such fragments, cut approximately 0.5–1 $mm^3$ in Gey's balanced salt solution, were set up on a 12 × 50 mm cover slip of No. 1 thickness in a clot of equal parts of heparinized rooster plasma and tissue extract of 8 day old chick embryos. A cover slip was placed in a roller tube, and 2 ml of fluid nutrient medium (50% human ascitic fluid, 45% Gey's balanced salt solution, containing 300 mg% of glucose, 5% embryonic extract) was added. Penicilline, 1,000 units par ml of nutrient, was also added to insure against chance of contamination. The cultures were maintained at 37°C in a drum designed to complete 8 revolutions par hour. At 7 days in culture a cover slip was removed from roller tube and mounted in closed chambers and cultures were observed under a phase optics. After observation of the ciliary movements the cultures were irradiated.

Irradiation: a) γ-irradiation to the cultures from Co-60 source: The distance from the source to the cultures was 31 cm. The doserate was 240 R/min. Dosimery was carried out with the use of a Siemens Universal Dosimeter. The culculation of the actual radiation exposure of ependyma cells was uncertain because of their intimate contact with glass. The temperature of the cultures during irradiation time of 40 minutes to 14 hours fell to between 21°C and 29°C. The cultures of control were set at the same temperature exactly. b) γ-irradiation to the cultures from Ra-226 source: A radium needle of 30 mg with 1.5 cm active length was placed on the cover slip, so that the distance between the center of the radium needle and the part of the ependyma cells is 3 mm. Dosage culculation was made by Quimby's table. [33] In this condition R/hour was approximately 1,800 R.

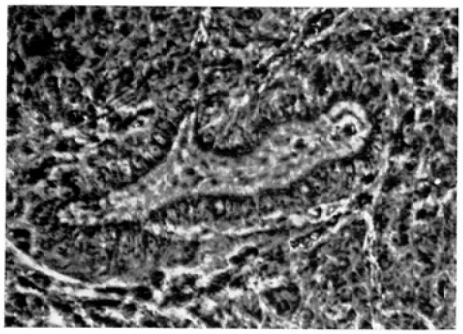
**Observations and Results**

Before and after irradiation the cultures were observed under a phase optics. The general fteatures of the growth pattern were almost the same as those described by Hild.[17] The ependyma cells showed two types of outgrowth patterns, the first being in the form of an epithelial sheet in which the cells became flattened to a considerable degree, attaching to the surface of the cover slip with what seems to be their original basal end. The free surface bearing the cilia was directed toward the culture medium. The second manner in which ependyma cells established themselves in vitro was with the formation of pools or elongated parallel double rows. The apical parts of such elements in one row faced the apical parts of those on the opposite side. The cilia extended into the space between the two rows. The cilia were in constant rapid motion which was apparently very well co-ordinated.

The cilia of ependyma cells in the second manner were mainly observed under a phase optics. The speed of the ciliary motility was observed before and after exposure frequently until the arrest of the motility. The ratio of the speed of ciliary movement before irradiation to that after irradiation was measured. The speed of ciliary movement was culculated by measurement of time required to observe 100 pendel movements of cilia. The continuation time of the motility from the completion of the exposure to the arrest of the motion was also measured and compared with the non-irradiated controls. The temperature of the cultures during observation time fell to between 20°C and 39°C. The ependyma cilia in this condition were shown to be moving at a rate of 3 to 7 times per second before irradiation. The continuation time of the ciliary motility of controls was approximately 70 to 140 hours. In the experiment of b) the movements of cilia irradiated with 13,000, 17,000 and 22,000 R respectively were measured during irradiation from Ra-226 source and the ratio of the speed of these cilia to that of non-irradiated controls was culculated.

The changes in the ciliary motility of the ependyma cells in tissue culture was as follows. There were no significant manifestations of the alteration both in the speed and continuation time at doses below 50,000 R. However, at 60,000 to 100,000 R shortening of continuation time of ciliary movements was found and the rate of ciliary motility became reduced at the end of irradiation. In a few cases in which irradiation was carried out with 60,000 to 90,000 R arrest of the motility was observed when irradiation was completed. In most cases, the ciliary movement was arrested at the end of the exposure to 100,000 R and no observable ciliary motility was found with 200,000 R after completion of exposure. Under microscopical observations during Ra-irradiation no marked alteration of ciliary movement was observed at doses below 22,000 R. Fig. 1. indicates the ependyma cells in the second manner from the third ventricular linings of the kitten younger than 1 month of age. Fig. 2. shows the percentage of speed ratio of ciliary motility before irradiation to that after irradiation. Fig. 3. indicates the percentage of continuation time of ciliary motility of controls with that of experimentals after irradiation.

Fig. 1. Ciliated ependyma cells from third ventricle of kittens younger than 1 month old. 7 days in vitro. The cilia are some' what blurred due to their rapid movement during exposure time. Phase cotrast. × 200 (left) × 400 (rithg)



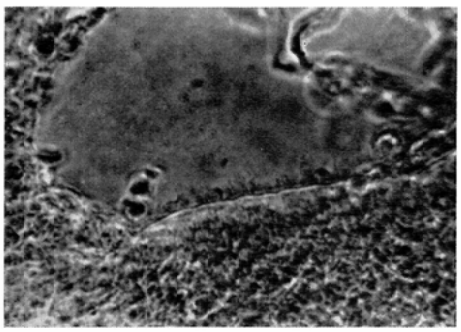


Fig. 2. Mean value of

$$\frac{\text{speed of ciliary motility of exp. after irr.} \,/\, \text{speed of ciliary motility of exp. before irr.}}{\text{speed of ciliary motility of cont. after irr.} \,/\, \text{speed of ciliary motility of cont. before irr.}} \times 100$$

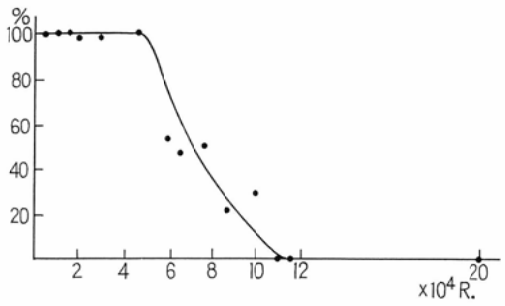
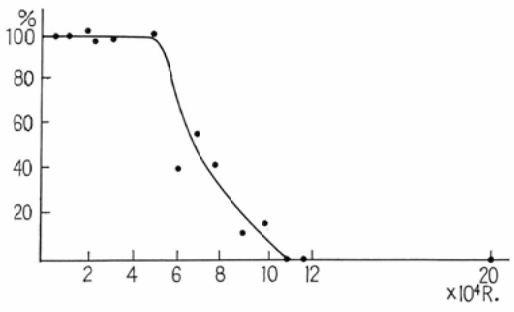



Fig. 3. Percentage of continuation time of ciliary motility of controls with that of experimentals after irr.




## Part 2 Effects of Ionizing Radiations on the Growth Activity of Cultures from Central Nervous Tissue

### Materials and Methods

Materials: The cerebral cortices obtained from the third and lateral ventricular linings of the γ-

irradiated and non-irradiated kittens, which were younger than 1 month of age, and the cerebellar cortices of the γ-irradiated 3 day old ICR-strain mice were used as materials.

Culture Techniques: The culture method employed was the same as that described in part 1.

Irradiation: Co-60 source was used for γ-irradiation of the kittens, the mice and the cultures. The distance from the source to the cultures were 42.6 cm for the in vitro radiation studies and 60 cm for irradiation to the head of the kittens and mice. The dosage of Co-60 irradiation was measured by a Siemens Universal Dosimeter. The intensity for group 1 was 135 R/min. For group 2 the intensity was 69.5 R/min. The temperature of the cultures during irradiation time of 15 minutes to 6 hours fell to between 21°C and 28°C. Cultures of the controls were set at the same temperature exactly. During the exposure time of 15 to 86 minutes to the head of kittens and mice, controls were treated in all ways as the experimental group but not irradiated.

**Experimental Groups:**

Group 1—In vitro

In this series of experiments the cerebral cortices were removed from the third and lateral ventricular linings of the kittens under 1 month of age, placed in a dish containing 10 ml of Gey's balanced salt solution. Then three fragnents, cut approximately 0.5–1 $mm^3$ in the solution, were explanted on to a cover slip of No. 1 thickness in a clot of equal parts of heparinized rooster plasma and tissue extract of chick embryos. A cover slip was then placed in a dish. After this manipulation the cultures were irradiated with doses ranging from 2,000 to 50,000 R. The cover slips with the cultures were then placed in a roller-tube with the fluid nutrient medium, described in part 1. The cultures were maintained in the usual manner. At 7 days in vitro cover slips with cultures were mounted in closed chambers and the growth patterns of the cellular proliferation, migration of cerebral cortices were compared with controls. Controls were of non-irradiated cerebral cortices in all other ways as experimentals.

Group 2-In vivo

Series 1. These experiments were concerned with thd determination of the effects of Co-60 radiation on cerebral cortices in vivo. Kittens in the first month of age were used. They were set in the holder to prevent them from moving, and irradiated with a series of doses ranging from 1,000 to 6,000 R. Then they were sacrificed one hour after irradiation. The cerebral cortices were removed from the third and lateral ventricles and cultured in the usual manner. At 7 days in vitro cover slips with cultures were mounted as described in group 1 and the growth and development was compared with controls.

Series 2. In this experiment the effect of radiation on central nervous system was quantitatively examined. ICR-strain mice of 3 days of life employed. The mice were set in the holder made of paraffin and irradiated in the same way as series 1. The cerebellar cortices were removed and cultured in the usual manner. The culturegrowth was estimated according to the method which was similar to that described by previous author[29]. On the 1st and the 5th day of culture, the outline of the cutures were drawn by means of a projectoscope. Then the surface areas enclosed by each successive outline were measured with a planimeter and the increase in the surface area of cultures was estimated as follows. The surface areas of the cultures were measured on the 1st (A) and the 5th (B) day and their increase were determined by B/A and compared with that of non-irradiated controls. At 7 days in vitro a part of cover slips with cultures were mounted and others were mounted at 16 days in culture as described in group 1 and their pat-

terns of cellular proliferation and migration of the cerebellar cortices in culture were compared with controls.

## Observations and Results

Group 1. Irradiation in vitro.

The doses chosen arbitrarily were 2,000, 3,000, 4,000, 5,000, 6,000, 8,000, 10,000, 30,000 and 50,000 R. A total of 15 tissue fragments of cerebral cortices were used at each level, with the same number of controls.

Growth pattern of cerebral cortices from kittens in tissue culture: The general features of the growth pattern and the grown cellular elements were similar to those described by the previous investigators[15)16)24)28)35)]. Glial elements have migrated usually in this stage of culture. Most glial elements were astrocytes provided with so many slender processes which had little membranous structure along them, so that the feltworks of the fibers gave the appearances of loosely knittend meshes of fine laces. Besides these active elements there were a large amount of cells which showed less tendency to migrate and remained in the original explants. These cultures contain the true nervous elements and non-nervous elements composed of fibroblasts and perineuronal glial elements. Since it is not always possible to distinguish exactly between fibroblasts and glial satellite cells in tissue culture these two cell types were not classified and considered as perineuronal satellite elements in this investigation. Most of neurons remained in the center of the explants.

Growth pattern of cerebral cortices from kittens irradiated in vitro: The outgrowth of perineuronal satellite elements of cerebral cortices from kittens were unaffected by irradiation with doses below 10,000 R. At a dose of 30,000 R the outgrowth was significantly less extend and dense than those of controls. Marked changes were observed at 50,000 R level and neither growth nor cellular migration of any type was observed. The outgrowth was completely inhibited. Fig. 4. shows the cell types and growth densities of typical cultures that were present in 7 day culture.

Group 2. Irradiation in vivo.

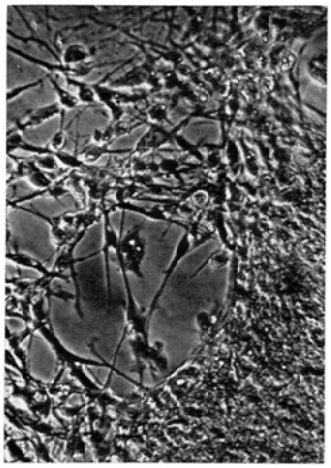
Series 1. The doses chosen were 1,000, 2,000, 3,000, 4,000, 5,000 and 6,000 R. A total of more than 20 tissue fragments of cerebral cortices were used at each level with approximately the same number of those to act as controls.

Growth pattern of cerebral cortices from kittens irradiated in vivo and cultured: At doses below 2,000 R the perineuronal satellite elements grew out of the explants and attained the same density as controls. At 3,000 R there was marked decrease in extent and density of cell populations. At 4,000 R there found almost complete inhibition in the outgrowth. Cerebral cortices removed from kittens having received 5,000 R and 6,000 R were incapable of any growth or of cellular migration in these conditions of culture. They appeared completely necrotic and there were some of cellular debris surrounding each colony. Fig. 5. indicates the degree of growth and the cellular types that were present in 7 day culture.

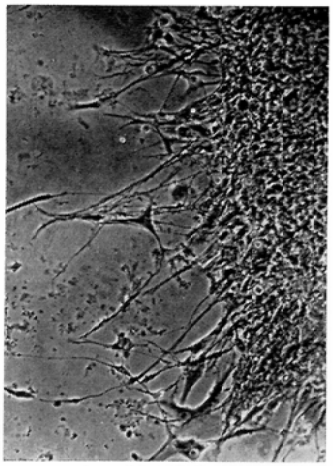
Series 2. Since marked inhibition of growth activity of the cultures of cerebral cortices from kittens receiving irradiation were found at doses of more than 3,000 R, the dose of 3,000 R was chosen. A total of 33 tissue fragments of cerebellar cortices were used with 39 of those to act as non-irradiated controls.

Growth pattern of cerebellar cortices from ICR-strain mice in tissue culture: The general features of the growth pattern and grown cellular elements were almost the same as those reported by the previous

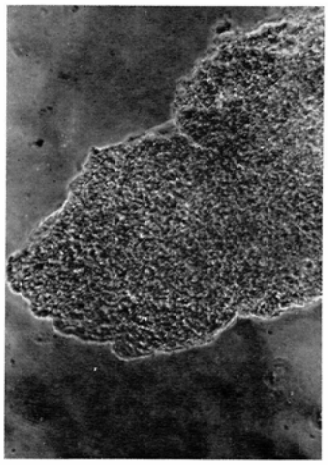
Fig. 4. The outgrowth of the cultures of the cerebral cortices from kittens younger than 1 month old irradiated in vitro after explantation. Phase contrast photographs of living unstained cerebral cortices. × 100



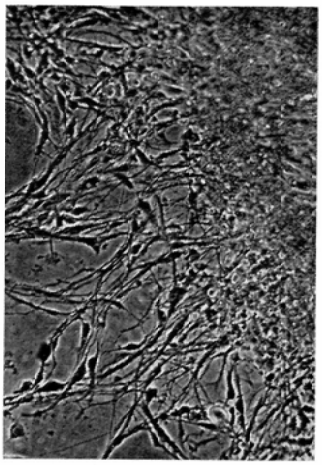
10,000 R



30,000 R



50,000 R



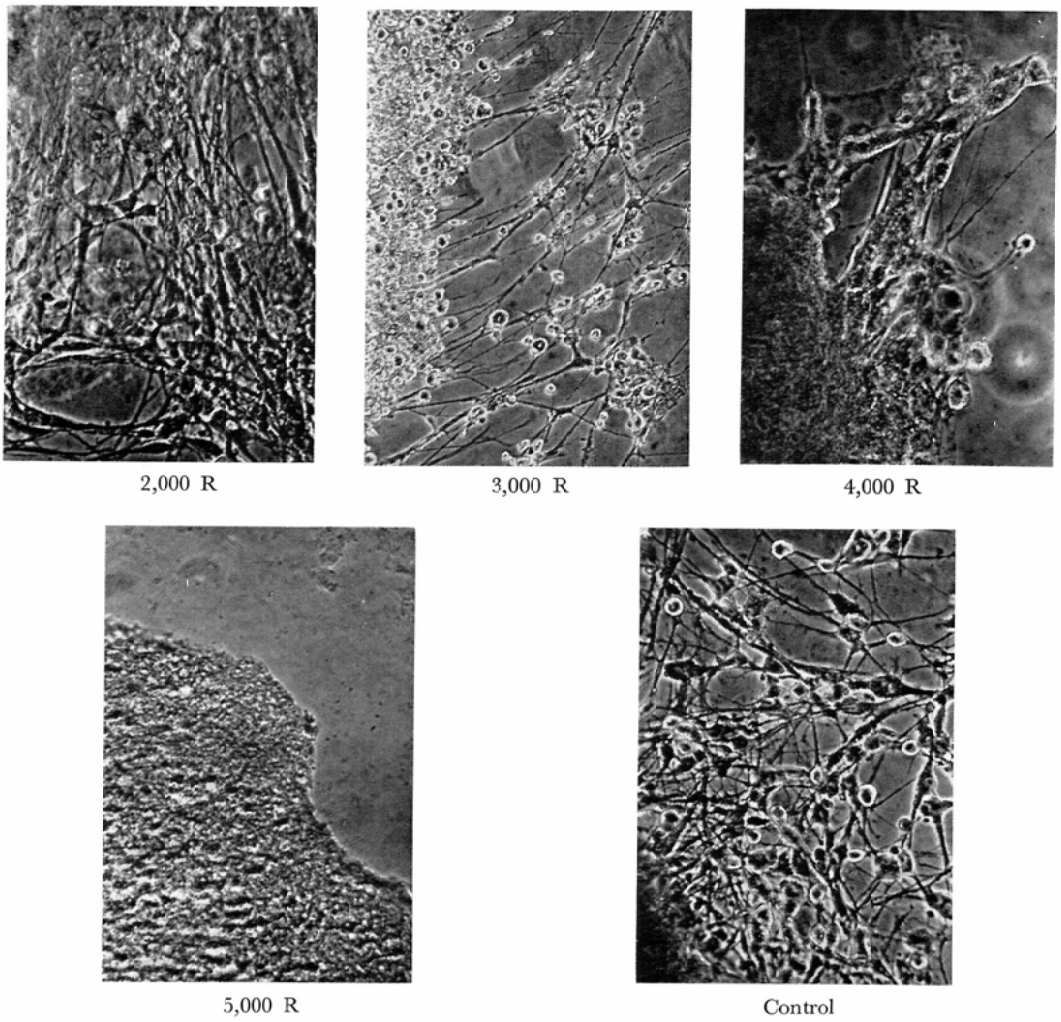
Control

investigators[5)23)24)27)31)40)].

Outgrowth of cerebellar cortices from ICR-strain mice irradiated in vivo and cultured: the outgrowth of perineuronal satellite elements was less extend and dense than those of controls.

The culture-growth was estimated according to the method described previously. The increase in the surface areas was compared statistically with that of controls. Statistical analysis of the 5th day growth rate in cultures of cerebellar cortices obtained from ICR-strain mice having received 3,000 R to the head showed that the increase in extent and density of cellular populations in the outgrowth of an experimental group was significantly less than that of controls. Fig. 6. shows the degree of growth and the cellular types that were present in 7 day culture. Fig. 7. indicates the neurons of cerebellar cortices from 3 day old ICR-strain mice irradiated to the head with 3,000 R, living 16 days in vitro. Fig. 8. shows the mean value of the growth rate at 5 days in vitro of cerebellar cortices removed from the mice irradiated with 3,000 R.

Fig. 5. The growth densities of cerebral cortices from kittens irradiated to the head with 2,000, 3,000, 4,000 and 5,000 R and then cultured.
Phase contrast photographs of living unstained cerebral cortices. × 100



2,000 R　3,000 R　4,000 R

5,000 R　Control

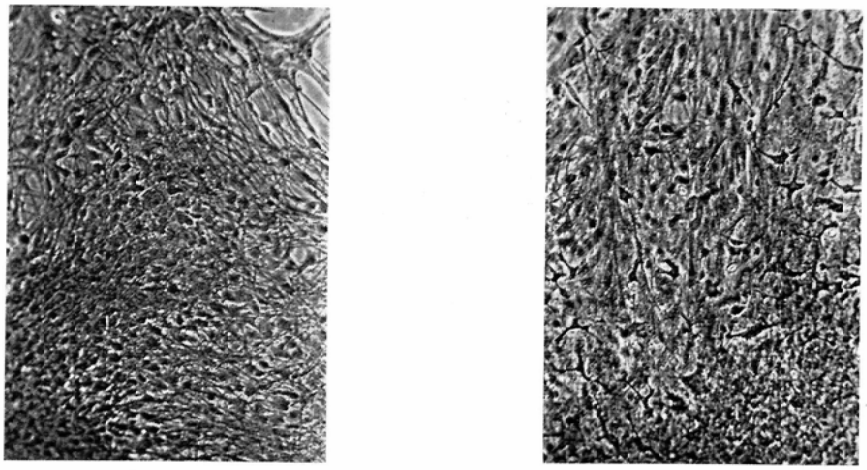
Fig. 9. indicates the frequency distribution of the growth rate at 5 days in culture of cerebellar cortices removed from the mice irradiated with 3,000 R.

## Discussion

The evaluation of the effects of radiations on any norma tissues is difficult, and this is particularly so for the central nervous system. Many of investigators have studied the effects of ionizing radiations on the central nervous system both in vivo and in vitro.

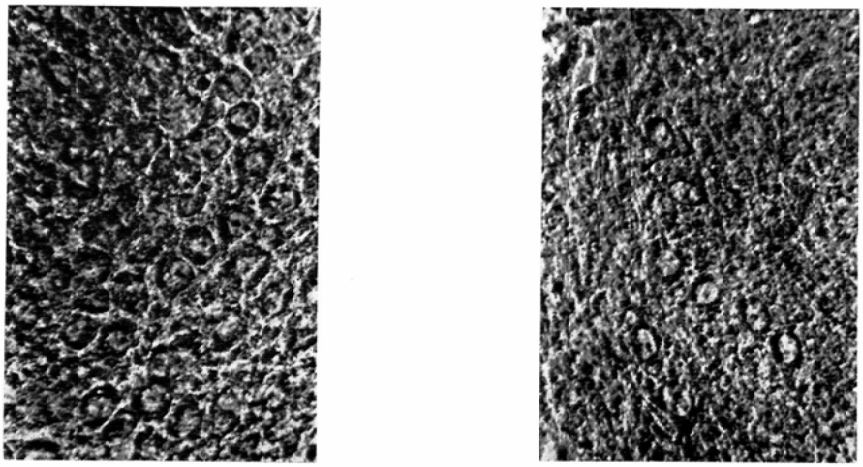
Clemente et al concluded that in an evaluation of neurological disorders resulting from ionizing radiations to the brain, it is apparent that consideration must be given to the degeneration of astrocytes and the impairment of the blood-brain barrier as factors involved in producing neuronal injury[9]. The astrocyte is a kind of neuroglia. Therefore, investigation with regard to the effects of ionizing radiations on neuro-

Fig. 6. The growth densities of cerebellar cortices from ICR-strain mice irradiated to the head with 3,000 R and then cultured. Phase contrast photographs of living unstained cerebellar cortices. × 100



3,000 R　　Control

Fig. 7. Neurons of cerebellar cortices from ICR-strain mice irradiated to the head with 3,000 R and then cultured. Note completely unaffected neruons. Phase contrast photographs of living unstained cerebellar cortices. × 400

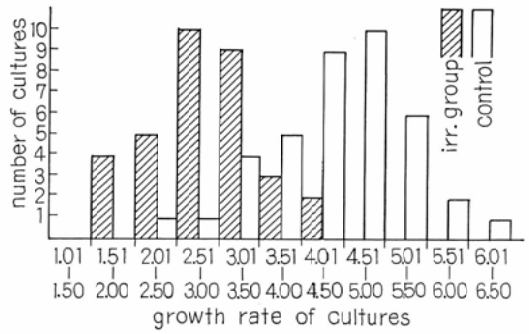


3,000 R　　Control

Table 1. Mean value of growth rate at 5 days in culture of cerebellar cortices removed from 3 day old ICR-strain mice irradiated with 3,000 R.

| | number of cultures | mean value of of growth rate |
|---|---|---|
| control | 3 9 | 4.39 ± 0.141 |
| irr. group | 3 3 | 2.84 ± 0.108 |

Fig. 8. Frequency distribution of growth rate at 5 days in culture of cerebellar cortices removed from 3 day old ICR-strain mice irradiated with 3,000 R.




glia is important in researching the radiation-induced disturbances of central nervous system. A number of investigators have reported the sensitivity of the neuroglia[8)7)-9)11)14)22)34)]. Ependyma cells are considered to be a most primitive glial element[17)]. Its ciliary motion may have some significant effect on the movement of cerebrospinal fluid within the brain ventricles[17)]. Though the ciliary activity of the ependyma cells was sensitive to lack of oxygen or marked changes in pH in the medium, the cells in culture were found to be astonishingly resistant to some unphysiological conditions[15)17)]. Their tolerance was observed to be far greater than neuron to adding a chemical substance such as alcohol to the medium[15)17)].

In this investigation the sensitivity of ciliary activity of ependyma cells to γ-irradiation was studied and it was found that no marked alteration of the ciliary movements were produced below 50,000 R. Surprisingly the ciliary movements of a part of ependyma cells were still observed at a dose of 100,000 R, though they were retarded and the continuation time was shorter than that of controls. Possibility was considered in this experiment that the rapid recovery of the ciliary activity might occur in the period between the end of exposure and observation. In order to resolve the question whether the ciliary activity was unaffected during exposure or affected during irradiation but recovered rapidly before observation, the under microscopical observation during Ra-226-irradiation was made and no manifestation of alteration in ciliary activity was found during exposure below 22,000 R. Thus the ependyma cells in culture were found to be astonishingly resistant to γ-irradiation.

Hild pointed out the ability of rapid recovery of affected ciliary activity of the ependyma cells[17)]. In this experiment, however, the ciliary activity was unaffected not only at the time of observation but also during irradiation at least below 22,000 R.

Pomerat et al reported that characteristic of the neuronal response to radiation was the individual variation of sensitivity and at 41,000 R some neurons were found to be ffected[32)]. It seems, therefore, that a comparison of these results reveals the greater resistance of ependyma cells than some neurons to γ-irradiation in vitro.

Though the ependyma cells in culture were observed to be resistant to irradiation, it was demonstrated by Brownson that they were not so resistant to irradiation in vivo[8)]. As described above, the ependyma cells in vitro are sensitive to lack of oxygen or marked changes in pH in the medium[15)17)]. This unphysiological condition in vitro seems to be equivalent to that induced by disturbance of blood supply in vivo Therefore, in an evaluation of neurological disorders resulting from irradiation, it seems that consideration

must be given to the circulatory disturbance as a great factor involved in producing injury of ependyma cells.

In this experiment (part 2) the observation was made of the growth activity of cultures of cerebral and cerebellar cortices from kittens and ICR-strain mice exposed both in vivo and in vitro with doses ranging from 1,000 to 50,000 R. The inhibition in the growth activity of the cultures from kittens irradiated with doses ranging from 3,000 to 6,000 R was observed on the 7th post irradiation day. This phenomenon was also observed on the 5th post irradiation day in the cultures from ICR-strain mice exposed with 3,000 R. It appears, therefore, that the early effect of radiation on the perineuronal satellite elements is suppression. In the experiments of series 2 in group 2, at a dose of 3,000 R, the inhibition in the growth activity of perineuronal satellite elements of cultures from the mice cerebellar cortices was observed, while no marked alteration was found in neurons. These results may lead to conclution that the sensitivity of perineuronal satellite elements is generally greater than that of neurons to in vivo irradiations.

In the experiment of group 1. no manifestation of alteration was found in the growth activity of cerebral cortices from kittens irradiated in vitro with doses below 10,000 R. This result reveals that the sensitivity of cerebral cortices from kittens to γ-radiation is far greater in vivo than in vitro.

Arnold et al have pointed that initially there was inhibition of glia, recovery and terminally intense gliosis and the degree of glial reaction was directly related to the rate of delivery and intensity of irradiation[3]. With regard to the early effects of radiation, their concludion is almost the same as that led from this experiments. Brownson reported that in the irradiated monkey no measureable alteration of neuron-neuroglial relationship of cerebral cortex was observed, while, in the controls a significant increase in percentage of neurons with satellite cells was found with increasing age of animal[7]. This evidence seems to correspond to the results in this experiments which showed the greater sensitivity of the perineuronal satellite elements to irradiation than neurons.

Arnold et al concluded from their experiment used monkeys that the effects of X-rays on the central nervous system are direct effects, and are not secondery to vascular occultion[4]. On the other hand, Goldring has pointed out that a comparison of the growth activity of spinal ganglia in tissue culture from chick embryos irradiated both in vivo and in vitro revealed the greater sensitivity of spinal ganglia to X-irradiaiton in vivo than in vitro[13]. He suggested that the factor of the circulatory system is involved in the effects of radiations on the central nervous system.

In this experiment the changes in growth pattern of cerebral cortices in group 1 irradiated in vitro after explantation may be considered the direct effects of the irradiation. The perineuronal satellite elements of the cultures of the cerebral cortices were remarkable in their ability to withstand a massive dose of radiation before their growth pattern was significantly changed. The inhibition in the growth activity of the cultures of cerebral cortices from kittens and of cerebellar cortices from ICR-strain mice in group 2 irradiated in vivo suggested a systemic effect containing not only direct effect but also indirect effect of the irradiation.

The indirect effect was reported to be produced as a result of injury to the blood supply by Spear[36]. Such results were described by Strangeways et al and were also agreed with this investigation[37].

The experimental results using cerebral cortices of kittens and cerebellar cortices of ICR-strain mice suggest, as Goldring pointed out, that the indirect effect of radiation involving the disturbance of the circulatory system play a role in the occurrence of injury to the central nervous system.

**Summary**

1) Radiosensitivity of the central nervous system in tissue culture was studied in young kittens by measurement of radiation-induced alteration of the ciliary motility of their ependyma cells.

No alteration of the ciliary activity of ependyma cells was observed in tissue culture irradiated with less than 50,000 R, while, various degrees of alterations were found at doses ranging from 60,000 to 100,000 R. At a dose of 200,000 R arrest of ciliary motility and ependyma cell necrosis were observed. Under microscopical observation during Ra-226-irradiation no alteration of the ciliary activity was found below 22,000 R.

2) Radiosensitivity of the central nervous system in tissue culture was studied by periodic measurements of surface areas and of the extent as well as density of cell populations in the outgrowth of the cultures. Changes in extent and density in the outgrowth of the cultures of cerebral cortices from non-irradiated young kittens irradiated in vitro with 2,000–10,000 R after explantation were not observed at 7 days in culture. However, the marked decrease was found in the cultures irradiated with more than 30,000 R.

3) Inhibition of the growth activity was observed in cultures of cerebral cortices from young kittens irradiated to the head with more than 3,000 R.

4) Statistically significant decrease was observed in the growth rate of the cultures of cerebellar cortices from young ICR-strain mice irradiated to the head with 3,000 R.

In this experiment it was demonstrated that cerebral cortices are astonishingly radioresistant in vitro, while they are not resistant to in vivo irradiation. It is apparent, therefore, that the indirect factors are much more important as compared with direct effect of radiation in the occurrence of disturbance of the cerebral cortices. It seems that consideration must be given to the vascular damage as an indirect factor involved in producing radiation injury in the central nervous system.

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**References**

1) 阿部英也：位相差顕微鏡による上衣細胞の細胞学的研究*Recent Advance in Research of the Nervous System. 8, 663–678, 1964.
2) Armel, K.: Über des Verhalten der Ependymzellen der Ratte in der Gewebekurtur. z. f. mikr-anat. Forsch. 69, 305–334, 1962.
3) Arnold, A. and Bailey, P.: Alterations in the glial cells following irradiation of the brain in primates. Arch. Path. 57, 383–391, 1954.
4) Arnold, A. and Bailey, P.: Changes in the Central Nervous System following irradiation with 23 MEV X-rays from the Betatron. Radiology. 62, 37–46, 1954.
5) Blinzinger, K.: Elektronenmikroskopische Untersuchungen am Ependym der Hirnventrikel des Goldhamsters. Acta neuropath. 1, 527–532, 1962.
6) Bornstain, M.B. and Murray, M.R.: Serial Observations on Patterns of Growth, Myelin Formation, Maintenance and Degeneration in Cultures of New-Bone Rat and Kitten Cerebellum. J. biophys. biochem. Cytol. 4, 499–504, 1958.
7) Brownson, R.H.: The effect of X-irradiation on the perineuronal satellite cells in the cortex of aging brains. Exper. Neurol. 19, 407–414, 1960,
8) Brownson, R.H.: Changes induced in the rat central nervous system following cumulative exposure to X-

irradiation. J. Neuropath. exp. Neurol. 20, 206–218, 1961.
9) Clemente, C.D. and Holst, E.A.: Pathological changes in neurons, neuroglia and blood-brain barrier induced by X-irradiation of head of monkeys. Arch. Neurol. and Psychiat. 71, 66–79, 1954.
10) Costero, I. and Pomerat, C.M.: Cultivation of neurons from the adult human cerebral and cerebellar cortex. Am. J. Anat. 89, 405–467, 1951.
11) Davidoff, L.M., Dyke, C.G. and Elsberg, C.A.: The Effects of Radiation applied directly to the Brain and Spinal Cord. Radiology, 31, 451–463, 1938.
12) Egana, E. and Velarde, M.I.: A comparative study and $\beta$-irradiated rats: "Comparative Neurochemistry"—Proceedings of the 5th international neurochemistry symposium. Austria, 10–15 june 1962. 275–278, Pergamon press, New York, 1964.
13) Goldring, I.P.: The Effects of X-rays on the Growth of Spinal Ganglia from 6- and 12-day Chick Embryos in Tissue Culture. Radiat. Res. 5, 390–403, 1956.
14) Hicks, S.P. and Montgomery, P. O'B.: Effects of acute radiation on the adult mammalian central nervous system. Proc. Soc. Exper. Biol. and Med. 80, 15–18, 1952.
15) Hild, W.: Ependyma cells in tissue culture. Z. Zellforsch. 46, 259–271, 1957.
16) Hild, W.: Observations on neurons and neuroglia from the area of fifth nucleus of the cat in vitro. Z. Zellforsch. 47, 127–146, 1958.
17) Hild, W.: Response of oligodendrocytes to serotonin: Biology of Neuroglia. Windle, W. E., Editor. 180–190, Thomas Illinois U.S.A. 1958.
18) Kim, S.: Neurons in the tissue culture. Arch. Hist. Jap. 23, 401–429, 1963.
19) Kim, S.: Neurons in the tissue culture. Observations on Terminal Boutons in Cultures of Mammalian Central Nervous Tissue. Arch. hist. jap. 25, 371–386, 1965.
20) Levi, G. and Meyer, H.: Nouvelles recherches sur le tissu nerveux cultive in vitro. Morphologie, croissance et relations reciproques des neurons. Arch. Biol. (Liege). 52, 133–278, 1941.
21) Lyman, R.S., Kupalov, P.S. and Scholz, W.: Effects of roentgen rays on the central nervous system. Arch. Neurol. & Psychiat. 29, 56–87, 1933.
22) Miqel, J. and Haymaker, W.: Astroglial reactions to ionizing radiation: with emphasis on glycogen accumulation.: Progress in brain research. Vol. 15, Biology of Neuroglia. Carrea, R., Editor. 89–114, Elsevier publ., New York, 1965.
23) Mizuno, N., Kim, S. and Okamoto, M.: The Study on the Embryonal Granula Cells of the Cerebellum. I. Identification in the Tissue Culture. Arch. hist. jap. 23, 185–211, 1962.
24) Mixuno, N. and Okamoto, M.: Some Observations on the Astrocytes Cultured in Vitro: 'Flattened Astrocyte' and Pinocytosis. Arch. hist. jap. 24, 347–367, 1964.
25) Nagase, K.: Effect of X-irradiation upon the affinity of rabbits central nervous tissue for protoporphyrin-III. Mie-Igaku. 4, 1–24, 1960.
26) Ogi, D.: Studies on the cellular effects by radiations: 1. Effect on the cilia movements of β-ray irradiation of human and rabbit ciliar epithel cells in vitro. Nipp. Acta Radiol. 19, 819–831, 1959.
27) Okamoto, M.: The method of cultivating the dissociated neuron of the central nervous tissue. The Kobe Journal of Medical Sciences. 3, 287–295, 1957.
28) Okamoto, M.: Observations on the neurons and neuroglia from the area of the reticular formation in tissue culture. Z. Zellforsch. 47, 269–287, 1958.
29) Parker, R.C.: Methods of tissue culture. 3rd edition, 267–283, Paul B. Hoeber, New York, 1961.
30) Perse, D.M., Murphy, W.T. and Parsons, J.A.: Acute radiation changes in the Purkinje cells. A study of C3H mice and patient subjected to fractionated ionizing radiation. Radiology 70, 855–859, 1958.
31) Pomerat, C.M. and Costero, I.: Tissue culture of the cat cerebellum. Amer. J. Anat. 99, 211–248, 1956.
32) Quastel, M.R. and Pomerat, C.M.: Irradiation of cells in tissue culture. Z. Zellforsch. 59, 214–223, 1963.
33) Quimby, E.H.: Dosage culculations with radioactive materials. Physical foundations of radiology, 3rd edition, Glasser, O., Editor. Hoeber, New York, 1961.
34) Roisin, L. and Rugh, R.: Neuropathologic investigations of the X-irradiated embryo rat brain. J. Neuropath. exper. Neurol. 21, 219–243, 1962.
35) Saito, H.: Tissue Culture of the Auditory Structures in the Central Nervous System. Pract. Otol. Kyoto. 59, 527–541, 1966.
36) Spear, F.G.: The Biological Effects of Penetrating Radiations. Brit. Med. Bull. 4, 2–10, 1946.
37) Strangeways, T.S.P. and Fell, H.B.: A study of the direct and indirect action of X-rays upon the tissues of the

embryonic fowl. Proc. Roy. Soc. B. 102, 9–29, 1927.
38) Sueoka, O. and Okamoto, M.: Granule Cells of the Mouse Cerebellum Cultured in Vitro: Their Identification, Degeneration and Perikaryal Myelin. Arch. hist. jap. 27, 117–130, 1966.
39) Vogel, F.S.: Effects of high dose γ-irradiation on the brain and on individual neurons: Response of the nervous system to ionizing radiation. Haley, T. and Snider, R.S., Editor, 249–260, Akademic Press, New York, 1962.
40) Yamamoto, T.: Central Nervous Tissue Cultivated in Vitro by Okamoto's Dissociation Method. Arch. hist. jap. 20, 355–389, 1960.