THE EFFECT OF POLIOMYELITIS VIRUS ON HUMAN BRAIN CELLS IN TISSUE CULTURE*

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Plates 1 to 4

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Much of the previous work with poliomyelitis viruses and tissue cultures has been concerned with the growth and continued propagation of the viruses. Enders (1) has fully reviewed the literature on the use of tissue cultures for the production and assay of viruses and their antibodies, but little has been written about the effect of viruses on the tissue culture cells themselves.

However, recently papers have been appearing which deal with the effect of the poliomyelitis virus on cells from different animals. Stoler and Gey (2) studied the effect of the poliomyelitis virus, Lansing type, on cells of a human fibrosarcoma strain (A.Fi). They observed that "the cells became rounded, dark, granular and irregular in outline" and were completely destroyed 72 hours after the virus was added. Dulbecco and Vogt (3) have made a detailed study not only of the multiplication of the poliomyelitis virus in the kidney cell of a monkey but also of the effect of the virus on the kidney cell. Barski et al. (4) have recorded in microfilm the effect of poliomyelitis virus III on human connective tissue cells, showing progressive changes and death of the cells. So far the effect of the poliomyelitis virus on nerve cells has not been reported.

During the past 12 years human brain cells, both fetal and adult, have been grown in tissue cultures in this anatomy laboratory. The various types of cells found have been studied and described (5–12). As a result of these studies it seemed reasonable to use some of the better known types of brain cells for experimental work with poliomyelitis virus. The work reported here is limited to the effect of poliomyelitis virus I, Mahoney strain, on human brain cells. Work with poliomyelitis virus types II and III is now in progress and will be reported later. Particular attention has been given to the cytopathological changes induced by the virus infection in the different types of brain cells.

Materials and Methods

The tissue cultures used were made from the brains of stillborn fetuses measuring 95, 115, 165, 210, and 221 mm. (crown-rump), and from the brains of a 2 month old infant and of

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adults 28, 30, 41, and 47 years old. The last tissues were obtained at operations. The parts of the brain most frequently used were the cortex of the frontal and temporal lobes and the cerebellum. Hanging drop cultures were made as individual cells could be followed more readily there than in roller tube cultures in which the infected cells were often lost in manipulating the tubes.

The pieces of brain, after being washed in Gey's solution (13), were put into a nutrient medium (12). It consisted of 6 ml. of Gey's solution and 6 ml. of chick embryo extract (9 to 10 day old embryos) made up with Gey's solution in the proportion of 1 part of chick embryo to 2 parts of Gey's solution. To this mixture were added 6 to 8 drops of human placental serum and 0.001 gm. of penicillin G potassium. In this medium the tissue was cut into pieces about 1 mm. in diameter. These were put on coverslips in a drop of chicken plasma which soon coagulated. They were inverted over depression slides and sealed with a mixture of 1 part paraffin and 6 parts vaseline and incubated at 37.5°C. They were fed every 2 or 3 days with the nutrient medium. Poliomyelitis virus I Mahoney strain was used in titers of 10^3, 10^4, and 10^6 infectious particles per ml. as measured in monkey kidney roller tube tissue cultures.

When there was a good growth of brain cells the coverslip was lifted and all the old nutrient medium was withdrawn with a fine pipette so as not to dilute the loop full of virus which was then added. The coverslip was placed on a culture slide (a flat slide with a glass ring) and sealed. This preparation was placed in a micro slide incubator kept at 37.5°C. Here the effect of the virus on different types of nerve cells, on protoplasmic and fibrous astrocytes, on oligodendroglia, and on macrophages was followed. Photomicrographs were taken when changes were observed.

There were controls for each experiment consisting of tissue cultures of the same age from the same part of the same brain but to them was added only the medium used in culturing the virus without the virus, or virus heated at 60°C. for 30 minutes.

Tissue Cultures of Adult Brains

Two experiments were performed using tissue cultures from the frontal lobe of a 47 year old man. In the first experiment the tissue culture was 59 days old when poliomyelitis virus I, $10^{6.5}$, was added at 10.25 a.m. A neuron with 4 long neurites was selected for study (Fig. 1). At 11.25 a.m. (Fig. 2) no effect of the virus was seen. At 12.25 p.m. the upper right neurite had begun to contract (Fig. 3). At 12.58 (Fig. 4) the process was still contracting. By 1.25 p.m. (Fig. 5) the upper right neurite had been absorbed into the cell and the lower right neurite was contracting. At 3.30 (Fig. 6) this lower right neurite was still contracting. Unfortunately the cell was lost when the slide incubator was moved and the explanted tissue floated free in the medium. Fig. 7 shows a group of cells on the same slide 6 hours after the virus had been added. The neurites are contracted and have small swellings along their sides. There are terminal bulbs on 2 of the processes where the protoplasm has not been drawn into the cell body before the cell died. The controls were normal.

Another tissue culture, 63 days old, from the same material shows a neuron (Fig. 8) 24 hours after inoculation. It is an irregular mass of cytoplasm, containing many vacuoles and irregular, contracted neurites. All the other neurons on the same slide were dead, some with their processes contracted, others with their processes extended but granular. The macrophages were also contracted and dead. The controls were normal.

Seven experiments were performed with tissue cultures made from the frontal lobe of a 28 year old woman. Virus I, 10^3 and 10^6 was used. The tissue cultures were of

different ages, from 16 to 56 days old. In two of these experiments in which the cultures were 34 and 36 days old and the titer of the virus was 10^3, there were noticeable changes on the 1st day. In the 34 day old culture a neuron contracted one of its processes 2½ hours after inoculation. After 5 hours it was an irregular mass of cytoplasm with 2 processes extended.

In the experiment using the 36 day old culture one neuron contracted a long process after 5 hours' exposure to virus I, 10^3. Twenty-four hours after inoculation vacuoles appeared in the cytoplasm. Most of the processes were extended. On the 3rd day the cell was dead. There were swellings along its extended neurites. The cytoplasm was granular. The controls were normal. Four other neurons on the same slide reacted in approximately the same way.

It was especially noted in this experiment that the cell body became granular and lost its surface film while the processes remained extended and intact with a surface film, often with granular swellings along their sides. Twenty-two other cells, neurons and astrocytes, were studied in this culture. All were dead on the 3rd day with extended processes and granular cytoplasm.

In another experiment of this series (28 year old woman) a 56 day old culture was inoculated with poliomyelitis virus in a concentration of 10^6 at 10.50 a.m. A large neuron (Fig. 9) was observed as it changed its shape and position during the next 6 hours, but it showed no signs of injury. At 10.25 a.m. the next day its processes were contracting and being drawn into the cell body (Fig. 10). Then very quickly the cell contracted and in 25 minutes it was a mass of granules (Fig. 11). The following day other neurons in the same culture showed vacuoles and were masses of granules. There was no recovery. The controls were in good condition.

In the other four experiments with tissue cultures from the same person the results were similar. The neurons were usually dead 3 days after inoculation, if not sooner. The macrophages and glia cells were also dead while the controls were in good condition. There was no recovery of the cells treated with the poliomyelitis virus.

Using the same virus inoculation of 10^6 on a 34 day old culture from the frontal lobe of a 41 year old person a large neuron was studied. Two hours after the virus was added a long process was contracting. The next day (22 hours later) the neuron was dead; the cell body was a mass of granules but the processes were intact. Five hours later the processes were beaded and granular. In the same field another neuron was granular and dead. Two fibrous astrocytes were alive with extended branched processes. Seventy-two hours after inoculation the macrophages were abnormal with extended active processes or were contracted and dead. The astrocytes and oligodendroglia were dead. The controls were normal.

Tissue Cultures of an Infant's Brain

Tissue cultures made from a piece of the temporal lobe of a 2 month old infant gave good growths of neurons, astrocytes, and oligodendroglia. When the cultures were 18 days old poliomyelitis virus I, in a concentration of 10^4 was added with no immediate effect on the cells. After 24 hours some of the neurons were contracting and drawing their neurites into the cell body. The next day some of the neurites had terminal lobes. These were not always drawn into the cell body before death. There was no recovery. The astrocytes and oligodendroglia were also contracting. The oligodendroglia showed

more cytopathological changes than the astrocytes. After 72 hours most of the cells had contracted and were dead. The controls were in good condition.

Experiments with Tissue Cultures of Fetal Brains

Experiments were performed with tissue cultures made from fetuses measuring 115, 210, and 221 mm. crown-rump. In one experiment, poliomyelitis virus I was added to a 17 day old culture made from the 115 mm. (CR) fetus. Several neurons were present in this culture. One hour later the long neurites of a large neuron began contracting. Five and one-half hours after inoculation the neuron had withdrawn all its processes and contracted into an irregular mass of cytoplasm. It was lost when the slide was moved since it was not attached to the coverslip by processes. The controls were normal. In a second experiment poliomyelitis virus I was added to a 54 day old culture made from the frontal lobe of a 221 mm. (CR) fetus. Half an hour later the fine tips of the long processes of a neuron were contracting as the processes were being withdrawn into the cell body. Five hours later the neuron was an irregular mass of cytoplasm without processes. The controls were normal.

In a third experiment a 17 day old culture of the cerebral hemisphere of a 210 mm. (CR) fetus contained macrophages, astrocytes, and oligodendroglia, but no neuron. Virus I, 10^4, was added on October 26, 1954. On October 27 nearly all the cells were active and appeared normal. On October 28 some of the macrophages were dying but the glia cells were in good condition. On October 29 most of the macrophages were abnormal, their processes were elongated and beaded (Figs. 12 to 14) instead of being broad and veil-like. The astrocytes and oligodendroglia had contracted and were dead (Figs. 15 and 16). By October 30 all the cells were dead or abnormal while the controls were active and in good condition.

Effect of Poliomyelitis Virus Type I and Its Antiserum on Tissue Cultures

In these experiments a loopful of monkey antiserum for type I poliomyelitis virus, or of normal monkey serum, was first added to the tissue cultures and this was followed immediately by a loopful of poliomyelitis virus I. This antiserum and the normal monkey serum were used in a dilution of 1 to 25 in Parker's medium 199(14). Material for the tissue cultures came from the frontal lobe of a 30 year old person (22 day old culture) and from the cerebellum of a 95 mm. (CR) fetus (10 day old culture). Tissue cultures from a neuroblastoma were also used. In all cases the tissue culture cells lived and continued to migrate out from the explanted tissue in the cultures protected by the antiserum. In one case the culture was in excellent condition 11 days after the antiserum and virus had been added. Controls, in which the virus alone, or monkey serum plus virus, was added, showed after 3 days that the tissue culture cells were dead or abnormal. Other tissue cultures grew well in the normal monkey serum.

Effect of Poliomyelitis Virus Type I, Heated to 60°C. for 30 Minutes, on Tissue Cultures

Poliomyelitis virus type I with a titer of $10^{6.5}$ was heated to 60°C. for 30 minutes and inoculated into 10 day old tissue cultures from the cerebral hemisphere of a 95 mm. (CR) fetus and into 27 day old cultures from the cerebellum of a 165 mm. (CR)

fetus. At the end of 3 days the cultures were in good condition. The controls, tissue cultures treated with the same virus unheated, were dead or abnormal. Other cultures fed at the same time with Parker's medium 199(14) alone were in excellent condition.

DISCUSSION

From the foregoing experiments it can be seen that the fetal brain cells were uniformly more sensitive to poliomyelitis virus type I than were the adult brain cells. Furthermore in the tissue cultures the effect of the virus on the neurons followed a certain definite pattern though there was great variation in the timing of the reaction of the neurons to the virus. This variation does not seem to have been dependent on the age of the tissue cultures, which varied from 16 to 63 days, nor on the general condition of the cultures as in all the experiments only those in good condition were used. It appeared to depend on the type of the cell, the state of the individual cells themselves, and the size of the infecting dose of the virus. All the cells of a given type on one slide reacted in much the same way at the same time.

The usual pattern of the reaction of the neuron to the poliomyelitis virus was first the contraction of the tips of the long processes and then the withdrawal of these long processes into the cell body. Even here there was variation. Sometimes the processes were completely drawn into the cell body and sometimes a bulb formed at the tip of a process which was then partly or completely drawn into the cell. Later the cell body became granular, lost its surface film, and became a loose mass of granules surrounding the nucleus. These gradually drifted apart and disappeared in the medium.

Sometimes the neurites or processes were not withdrawn and would remain intact long after the cell body had gone to pieces. Eventually the processes became granular, developed small nodules which finally became a string of fine granules that gradually disappeared. In some cases the processes broke into fragments which then became granular and disintegrated. This disintegration of the cell and in some cases the sudden contraction of the cell body after long exposure to the virus corresponds to the reaction of the monkey kidney cell to poliomyelitis virus described by Dulbecco (3).

In many of the neurons vacuoles developed; this has been observed in normal adult neurons (12) and is a sign that the cell is not functioning normally.

The astrocytes and oligodendroglia usually were slower in reacting to the virus, the first effect of the virus being noticed 48 to 72 hours after inoculation. Eventually they too succumbed to the virus.

The macrophages were also affected by the virus but were not as susceptible as the neurons. Here again there was great variation in the time of reaction to the virus, some being affected in 24 hours and others being alive but abnormal after 72 hours. Eventually they all died from the effects of the virus. In

all the experiments which were followed through 3 or 4 days there was no recovery of the cells. The controls were uniformly in good condition.

It seems reasonable to conclude that the observed changes were due to infection with the poliomyelitis virus since they were prevented by heating the virus to 60°C. for 30 minutes or by the addition of specific antiserum.

Contraction of neurons was observed within ½ hour after infection in some of the fetal tissue cultures. This suggests the presence of a preformed toxic substance rather than the results of an active infection and additional studies are being carried out.

SUMMARY

Poliomyelitis virus I, Mahoney strain, affected human brain cells grown in tissue cultures usually causing death of the cells in 3 days. The neurons reacted in different ways to the virus, some died with their neurites extended, others contracted one or more of their neurites. Terminal bulbs were frequently formed at the tips of the neurites when they were being drawn into the cell body. The final contraction of the cell body and the change into a mass of granules were often very sudden. Vacuoles often developed in the neuron. There was no recovery.

Astrocytes, oligodendroglia, and macrophages were affected by the virus but not as quickly as the neurons.

The age of the tissue culture was not a factor when the cells were in good condition.

The age of the individual donor of the brain tissue was a factor; the fetal brain cells appeared to be more sensitive to the virus than the adult brain cells. The fetal neurons often reacted ½ hour after inoculation while the adult neurons reacted more slowly, 2 to 24 hours after inoculation.

All these changes seemed to be caused by virus infection because they were prevented by specific antiserum or by preheating the virus.

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EXPLANATION OF PLATES

PLATE 1

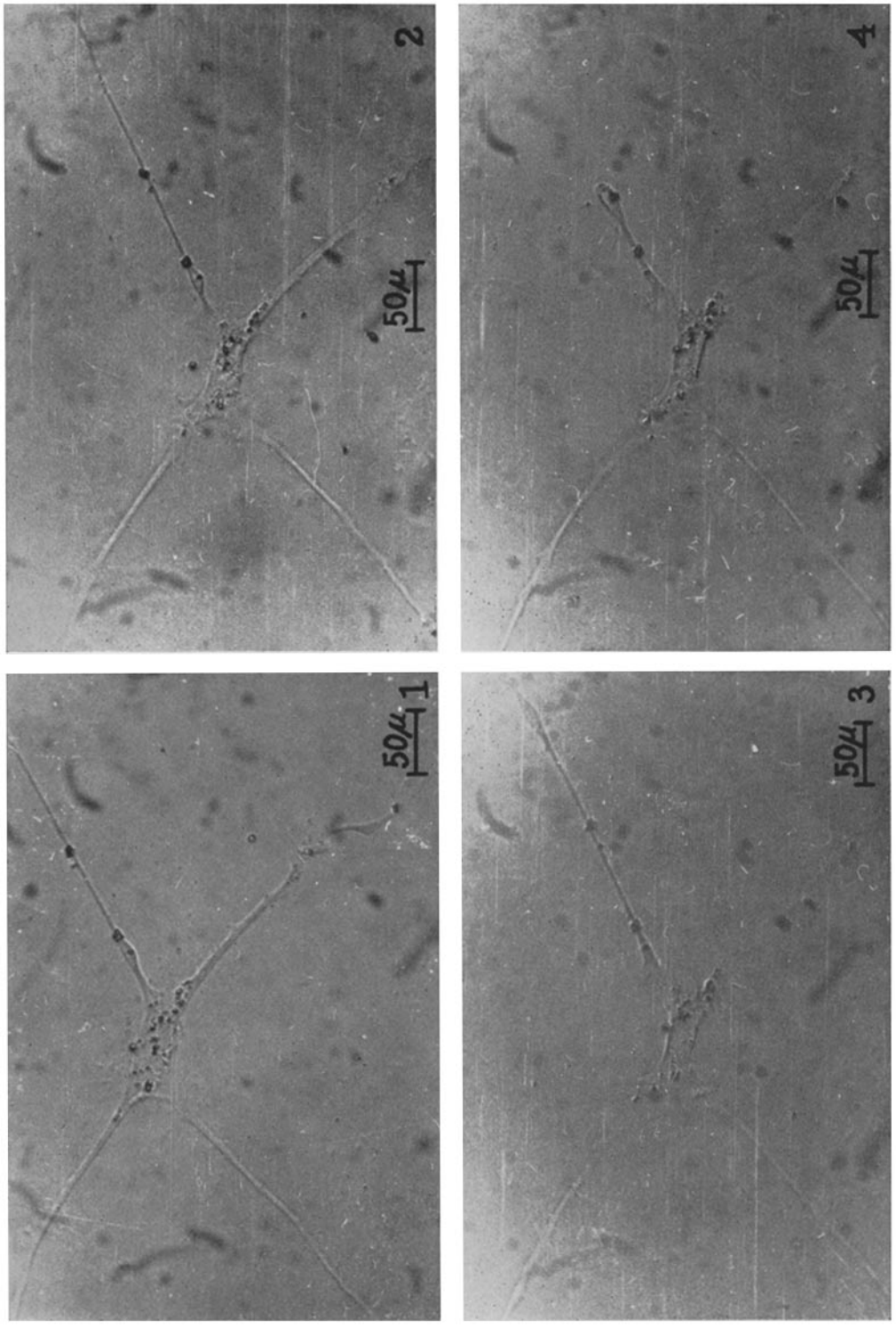
FIGS. 1 to 6. A large neuron from the frontal lobe of a 47 year old man in a 59 day old tissue culture. Poliomyelitis virus I added 10.25 a.m.

FIG. 1. 10.25 a.m. Just after virus was added. No effect.

FIG. 2. 11.25 a.m. No effect.

FIG. 3. 12.25 p.m. Upper right neurite is contracting.

FIG. 4. 12.58 p.m. Upper right neurite still contracting.



(Hogue *et al.*: Effect of poliomyelitis virus on human brain cells)

PLATE 2

FIG. 5. 1.25 p.m. Upper right neurite has been absorbed into the cell. Lower right neurite is contracting.

FIG. 6. 3.30 p.m. Lower right neurite still contracting.

FIG. 7. 4.25 p.m. Group of degenerating brain cells. Terminal lobes at the tips of 2 neurites.

FIG. 8. Large neuron in a 63 day old culture of the same material, 24 hours after poliomyelitis virus was added. Cytoplasm is contracted and full of vacuoles.

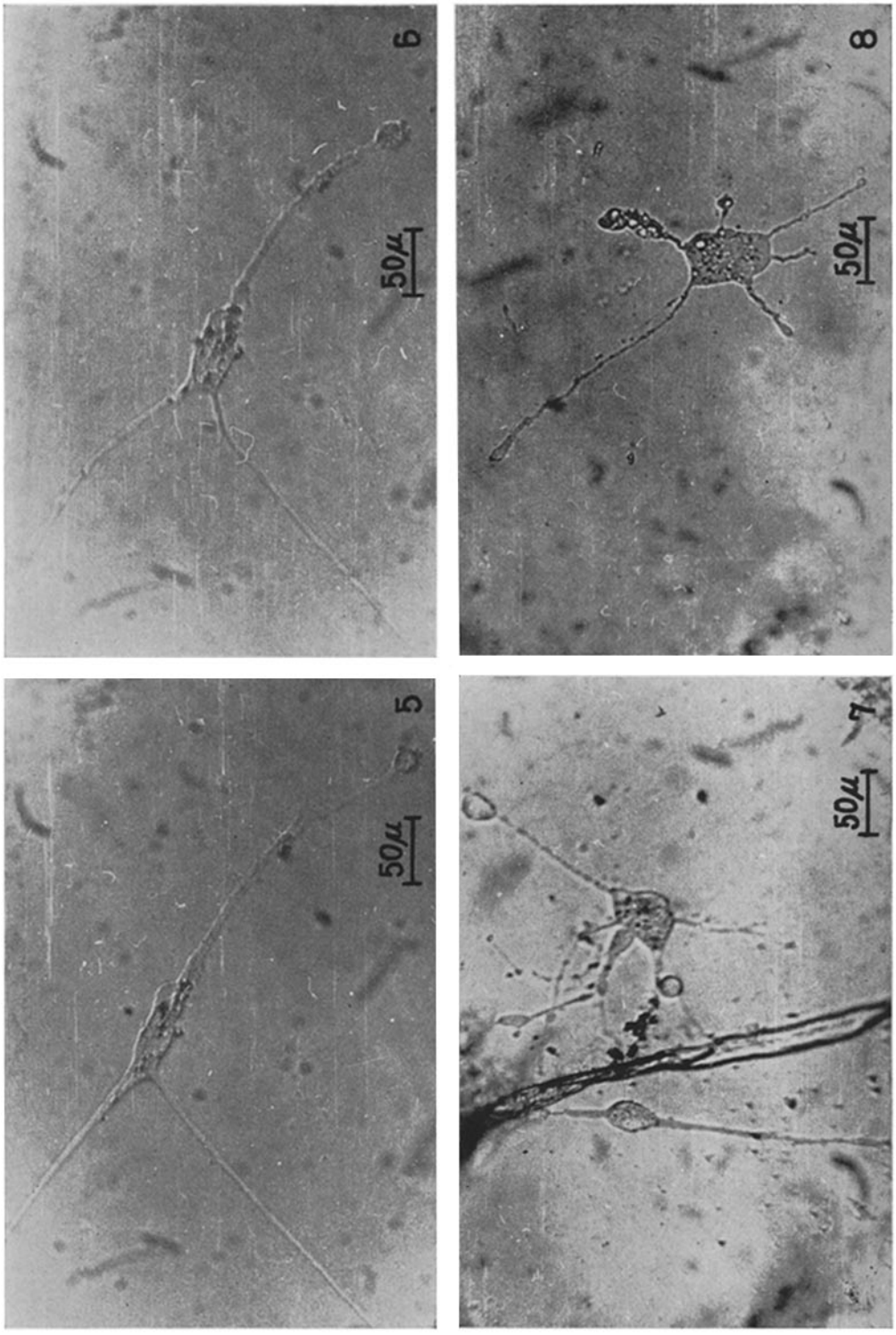


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PLATE 3

FIGS. 9 to 11. Tissue culture from frontal lobe of a 28 year old person. Culture 56 days old when inoculated, virus I, 10^6.

FIG. 9. Six hours after inoculation. No effect.

FIG. 10. Twenty-three hours and 35 minutes after inoculation. Neurites are contracting.

FIG. 11. Twenty-five minutes later (24 hours after inoculation) neuron is dead; a mass of granules.

FIGS. 12 to 16. Tissue cultures from cerebral hemisphere of 210 mm. (CR) fetus. Culture 20 days old. Virus I, 10^4.

FIG. 12. Abnormal macrophages with elongated beaded processes. Three days and 5 hours after inoculation.

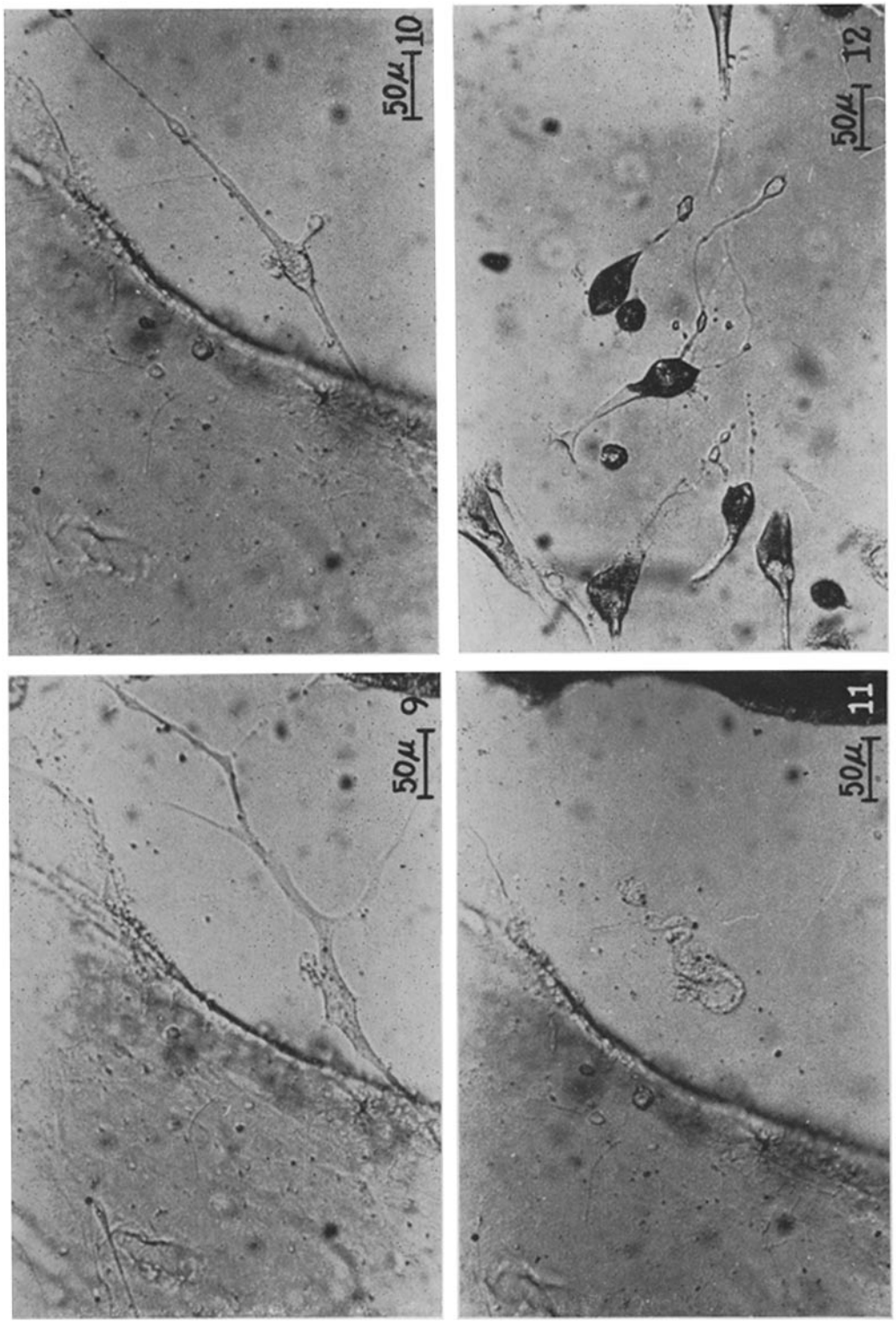

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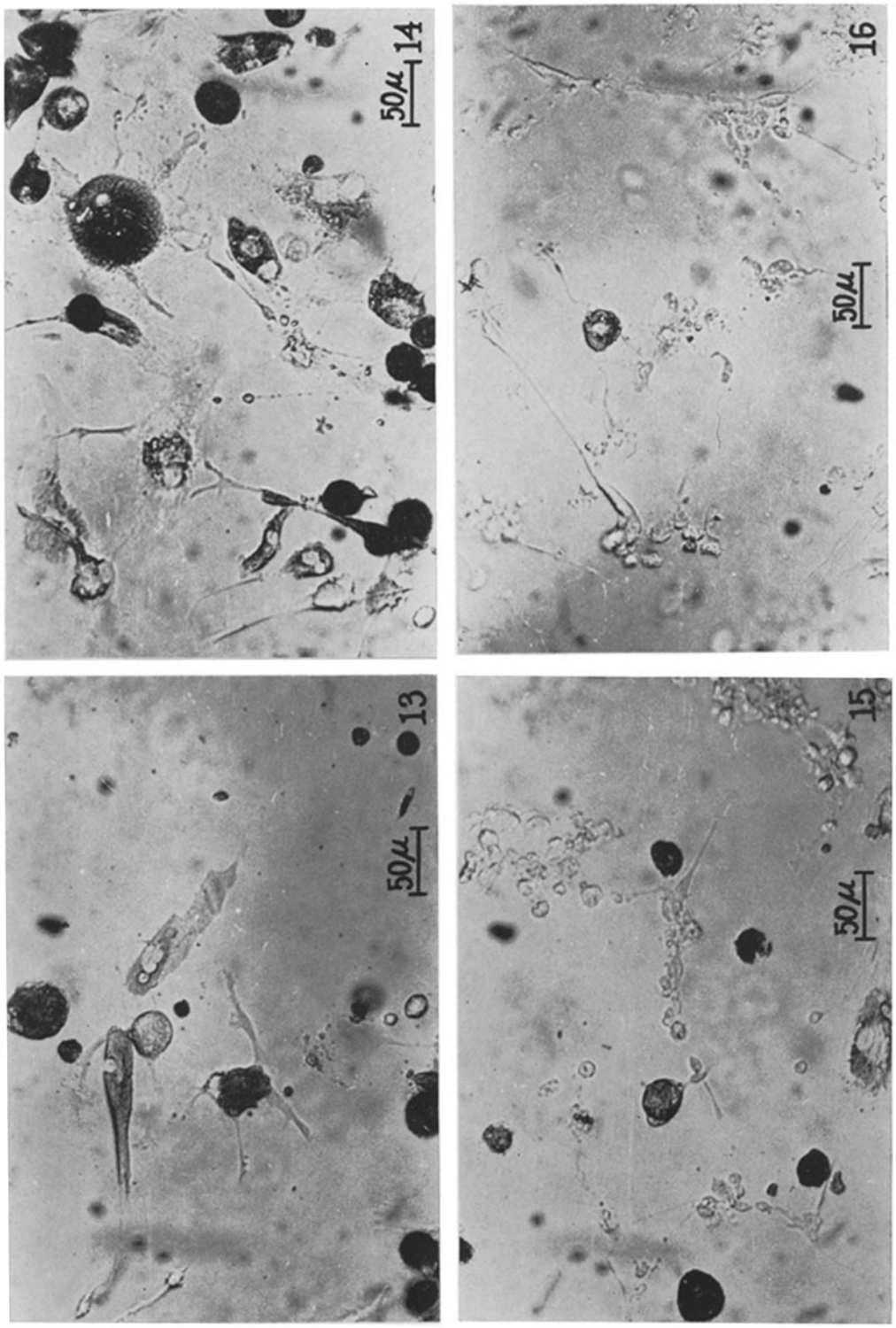
PLATE 4

FIG. 13. Abnormal macrophages with elongated processes. Three days and 3 hours after inoculation.

FIG. 14. Different sized abnormal macrophages three days and 5 hours after inoculation.

FIG. 15. Contracted macrophages and glia cells. 3 days and 3 hours after inoculation.

FIG. 16. Group of dead glia cells and macrophages. 3 days and 5 hours after inoculation.



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