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HOTTA, Susumu

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PROPAGATION AND CYTOPATHIC EFFECTS OF JAPANESE ENCEPHALITIS AND RELATED VIRUSES IN MAMMALIAN CEREBELLAR TISSUE CULTURES[a, b]

Susumu HOTTA and Mikito OHNO

Department of Microbiology,
Kobe University School of Medicine

Humio MIZOGUTI and Hiroshi MUSASHI

Department of Anatomy,
Kobe University School of Medicine

Susumu HOTTA, Mikito OHNO, Humio MIZOGUTI and Hiroshi MUSASHI. *Propagation and Cytopathic Effects of Japanese Encephalitis and Related Viruses in Mammalian Cerebellar Tissue Cultures.* Kobe J. Med. Sci. 13, 1-21, March 1967—— Cerebellar tissues from puppies, kittens and human embryos were cultivated on a cover slip by the roller tube technique, using a culture medium consisting of human cancerous ascitic fluid, chick embryo extract and Gey's balanced salt solution with a high concentration of glucose.

In any of the tissue cultures employed, three strains of mouse-passaged Japanese encephalitis (JE) virus and a strain of Negishi virus, a member of the Russian Spring-Summer encephalitis group, multiplied unequivocally. The highest virus titers in fluid phase were usually obtained 3 to 7 days after inoculation of virus. The grades of multiplication, however, were different from one virus-culture combination to another; for instance, growth of JATH strain JE virus in any of the culture systems was significantly superior to that of K. I. strain JE virus. Under the conditions studied, viral growth in kitten cerebellar tissue cultures was generally lower than that in puppy or human embryo cerebellar cultures. Differences in the nervous tissue culture affinity among different virus strains were evident.

In the virus-infected cultures, the Purkinje cells exhibited definite degeneration. The nuclei, cell bodies, dendrites and neurites, as well as neurofibrils showed characteristic changes revealed clearly by Bodian's sliver impregnation and/or Nissl stain. The alterations proceeded in the course of infection, and the cells were eventually disintegrated. The cytopathic effects were prevented by mixing the viruses with specific antiviral antisera prior to inoculation. In control cultures incubated under disadvantageous conditions such as prolonged incubation without changing medium or incubation at higher temperatures, the Purkinje cells showed certain abnormal appearances, but such alterations were distinguishable from those occurring inevitably in the virus-infected cultures. Virologic and neuropathologic significances of the findings are discussed.

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a Aided by a Grant for Scientific Research from the Ministry of Education of Japan and by the Fund for Virus Research of Hyogo Prefecture.

b Preliminary accounts for part of the work were published in Japanese by Liao (1961)[11], Ohno (1964)[14] and Hotta *et al* (1964c)[6].

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INTRODUCTION

While the *in vitro* cultivation of Japanese encephalitis (JE) and related viruses has been studied from various angles, much of the previous works are concerned with utilization of visceral or non-neural tissues. In the earlier literature there are reports demonstrating the growth of JE virus in embryonic brain tissue cultures from the chick (Kawakita, 1939)[9] or the mouse (Simagosi, 1939)[21]; however, descriptions of the cytopathology of infected culture cells are lacking. Since one major significance of encephalitogenic virus infections is seen in the central nervous system, it is considered of interest to inquire into the infective pattern(s) of nervous tissue cultures by such viruses. This paper will report experiments in which cerebellar tissue cultures from certain species of mammals were employed as cellular substrates to investigate this problem.

MATERIALS AND METHODS

Tissue culture

Tissues used were from 1 to 15-day-old puppies and kittens, and from human embryos obtained by artificial abortion. The cerebellar cortex was minced into fragments, approximately 1 mm^3 in size, which were then washed several times with Gey's balanced salt solution (BSS). Four tissue pieces were planted on a cover slip, about 10×50 mm and 0.17 mm thick, in a clot consisting of heparinized chicken plasma and chick embryo extract. Each coverslip was put in a tube, 15×150 mm in size, and 2 ml of culture medium was added. The medium consisted of 50% human cancerous ascitic fluid, 5% chick embryo extract, and 45% Gey's BSS with 600 mg% glucose and 1,000 u/ml penicillin. It was ascertained that there were no antibodies and/or inhibitors against tested viruses (as indicated below) in the medium. The tubes thus prepared were incubated in a roller apparatus rotating at about 10 revolutions per hour at 37°C. The nutrient fluid was changed usually at 3 day intervals. As to general outlines of culture procedures, descriptions by Lumsden and Pomerat (1951),[12] Pomerat and Costero (1956),[19] Hild (1957),[8] Okamoto (1958, 1959)[15,16] and Musashi (1960)[18] were referred to.

Virus

Viruses applied were JE viruses, Gl strain, of the 240th to 245th mouse brain passages, JATH 260 strain*, of the first to fourth mouse brain passages, and K. I. (K. Inoue) strain*, of the second or third mouse brain passages, as well as Negishi virus*, a member of the Russian Spring-Summer encephalitis group (Ando *et al*, 1952[1]; Okuno *et al*, 1961[17]), of the 7th to 10th mouse brain passages. A 10% homogenate of infected brains was prepared by adding the culture medium. The supernatant fluid obtained from centrifugation at 3,000 rpm for 15 minutes was diluted appropriately with the medium and used as the initial virus suspension.

*Kindly supplied by Dr. A. Oya and Dr. K. Ishii, of the National Institute of Health of Japan.

Inoculation and titration of virus

Culture tubes in which good cellular explants were observed under low magnification were chosen for virus inoculation. After the nutrient fluid was discarded, the cultures were washed several times with Gey's BSS. Two-tenths ml of the virus suspension was introduced into each tube and allowed to cover the cellular explants for 2 hours at 37°C. The cultures were again washed with Gey's BSS and 2 ml of fresh medium was added.

At intervals following the virus inoculation, portions of the culture fluid were collected and pooled. Serial 10-fold dilutions of the pools in Gey's BSS were injected into 2 to 3-week-old mice intracerebrally and LD_{50} titers were determined by the method of Reed and Muench (1938)[20].

For identification of the cultivated viruses, neutralization tests were performed. The infected culture fluid at a given concentration was mixed with antiviral immune rabbit serum of equal volume. After the mixture was held at 37°C for 1 hour and at 4°C for an additional hour, 0.02 ml thereof was injected into mice intracerebrally. Loss of infectivity was the criterion for identifying cultivated agents as respective viruses.

Morphologic examination

At the time of viral titrations, the coverslips were removed from selected tubes and dipped in warmed Gey's BSS for several minutes for washing. They were then fixed in formol-bromide or 95% ethanol and stained by Bodian's silver impregnation or Nissl stain.

The following controls were similarly examined: (i) cultures receiving a normal mouse brain homogenate; (ii) cultures inoculated with virus mixed with specific antiviral antiserum and held at 37°C for 1 hour just prior to the inoculation; and (iii) cultures incubated under disadvantageous conditions such as prolonged incubation without changing medium or incubation at higher temperatures (39-40°C).

RESULTS

Viral growth curve

Typical examples are illustrated in Figs. 1, 2 and 3.

All the viruses tested multiplied in each of the culture systems employed. The highest virus titers in fluid phase were obtained usually 3 to 7 days after inoculation of virus. The grades of viral multiplication, however, differed from one virus-culture combination to another. JATH strain virus multiplied well both in puppy and human embryo cerebellar cultures, whereas growth of K. I. strain virus was inferior to that of JATH strain in all of the culture systems. Differences in the nervous tissue culture affinity among the different JE virus strains were evident. It was also noted that grades of viral growth in kitten cerebellar tissue cultures were generally lower than those in cultures of puppy or human embryo cerebellar tissues.

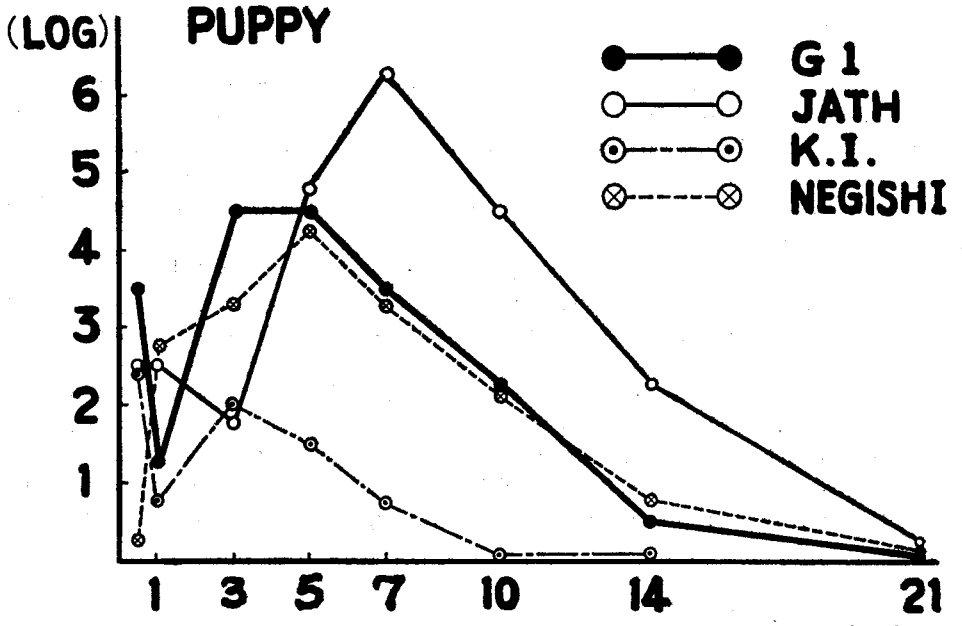

Fig. 1 Viral growth curves in puppy cerebellar tissue cultures. Oridinate indicates virus titer of culture fluid (mouse-intracerebral LD_{50} per 0.02 ml, expressed in logarithmic number), and abscissa indicates period of cultivation in days.

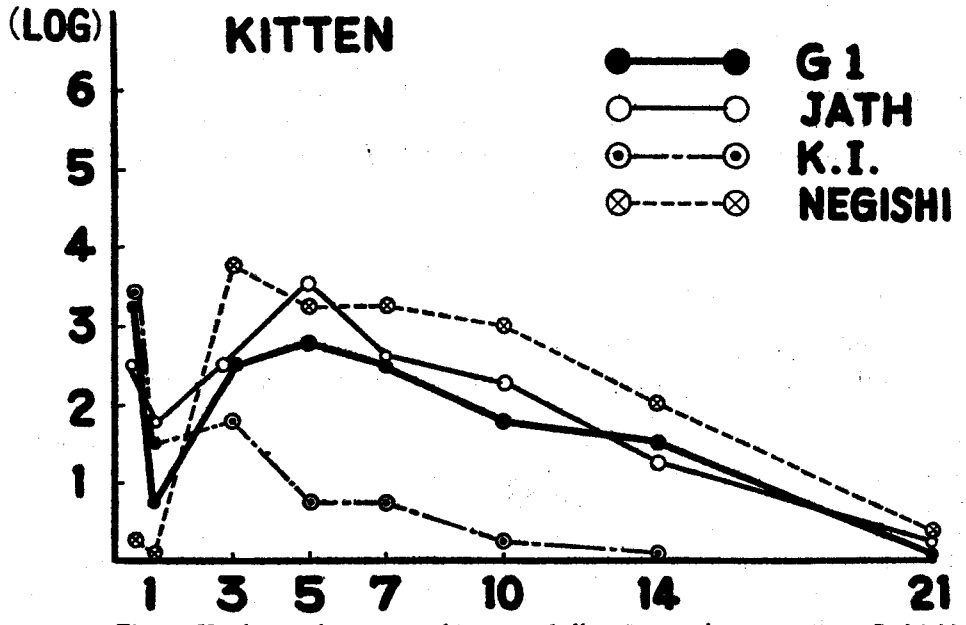

Fig. 2 Viral growth curves in kitten cerebellar tissue cultures. Legends are the same as those in Fig. 1.

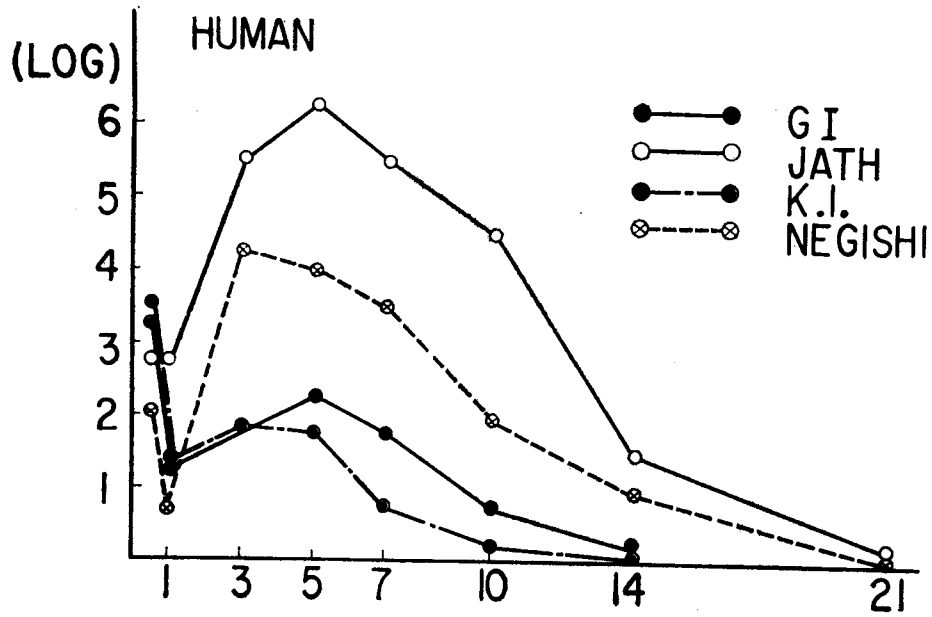

Fig. 3 Viral growth curves in human embryo cerebellar tissue cultures. Legends are the same as those in Fig. 1.

Active viruses incubated at 37°C in roller tubes with culture medium minus living cells disappeared within 48 hours. It was clear that viruses present in the initial inocula did not significantly affect the titers of viruses found in the culture fluids from infected tubes.

The infectivity of culture fluid was lost completely by being mixed with respective antiviral antiserum, therefore, the identification of cultivated viruses was unequivocal.

Morphologic observation

Usually 1 to 2 weeks after the beginning of incubation at 37°C, there is an outgrowth of cells surrounding the original explants. Such outgrown zones consist generally of fibroblastic cells, macrophages and glial cells. Nerve cells are seen only within the area of the flattened original tissue pieces because they have apparently no migratory function. Amoung them the Purkinje cells were chosen as the major subjects of observation in the present work.

The Purkinje cells in tissue culture are characterized by their comparatively large size and can be recognized without great difficulty. The nuclei containing one or two nucleoli are clearly distinguished by the nuclear membrane from the cytoplasm. Silver impregnation by the Bodian method reveals the neurofibrils extending throughout the cell body as well as into the dendrites and neurite. The dendrites are shown to have a number of smaller branches. The neurites are observed to run straight and bend angularly at some places or make smooth round curves. By

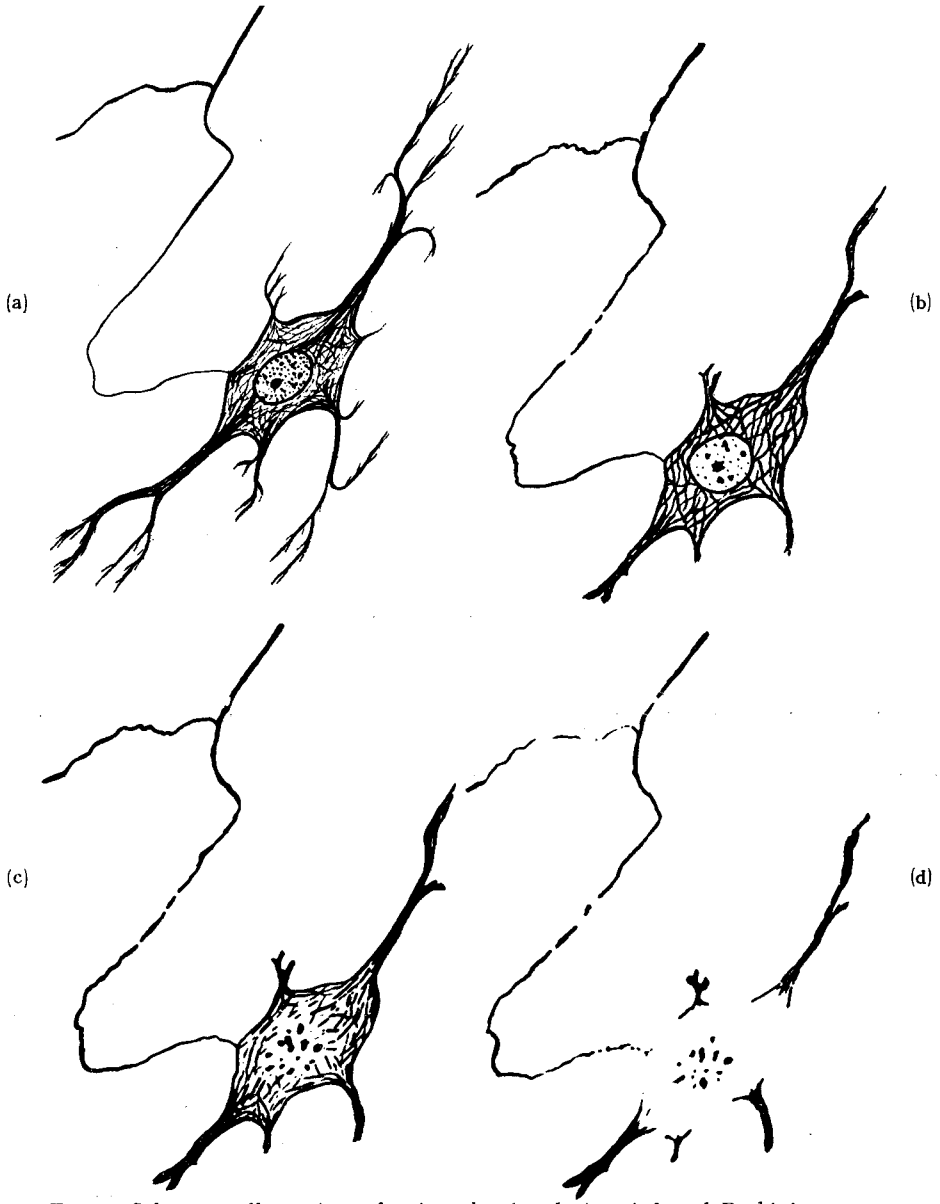

Fig. 4 Schematic illustrations of uninoculated and virus-infected Purkinje cells in tissue culture.
(a) Uninoculated cell.
(b) Virus-infected cell in early stage of degeneration.
(c) Virus-infected cell in middle stage of degeneration.
(d) Virus-infected cell in advanced stage of degeneration.

the Nissl method, the tigroid substances are visible.

In the virus-infected cultures, the Purkinje cells exhibited definite degeneration. The characteristic changes revealed by the Bodian stain were as follows: (i) In the early stage of degeneration, usually 3 to 5 days after virus inoculation, the nuclei changed their original staining properties and contained unusual granules. The neurofibrils in both cell bodies and dendrites stained irregularly and roughly. Branches of the dendrites were shortened or reduced in number. The neurites showed abnormal appearances such as breaking, swelling, serpentine turning, unusual branching, *etc.* (ii) In the middle stage of degeneration, usually 5 to 7 days after virus inoculation, the above-mentioned changes proceeded in degree and extent. (iii) In the advanced stage of degeneration, usually 7 days or more after virus inoculation, the nuclei were disintegrated into masses of debris and the neurofibrillar patterns usually seen in non-infected "normal" control cultures disappeared. The dendrites became rudimentary. The neurites were broken and could not be completely traced.

In Nissl-stained preparations, the tigroid substances lost their original staining ability, and vacuoles of various sizes were seen in the cytoplasm.

These changes were not produced in cultures receiving a normal mouse brain homogenate nor in those inoculated with the viruses mixed with specific antisera prior to inoculation.

In cultures subjected to incubation without changing culture medium or incubation at 39–40°C, the Purkinje cells showed certain degenerative pictures. Such alterations, however, proceeded fairly slowly so that the complete destruction of cells was rather rare during the observation periods lasting 3 or 4 weeks. A general tendency was, moreover, that the damage to nuclei induced by the non-specific effects was comparatively mild, seldom undergoing the severe destruction as observed inevitably in the virus-infected cells.

Plate-Figs. 1 to 22 inclusive depict typical examples of the findings revealed both in virus-infected and control cultures. Schematic illustrations of the virus-induced changes are shown in Figs. 4 a, b, c and d.

DISCUSSION

In spite of the recent technical developments in the *in vitro* cultivation of central nervous cells, application of the methods to the study of "neurotropic" viruses has still been limited. Hogue *et al.* (1955, 1958)[4,5] reported effects of polioviruses upon human brain cells in tissue culture; some of the morphologic changes they observed were contraction of the cell processes and granular appearance of the cell bodies. Fernandes and Pomerat (1961)[2] inoculated rabies virus into puppy cerebellar tissue cultures and demonstrated inclusion bodies in the cytoplasm of the Purkinje cells.

In the present experiments, cerebellar tissue cultures from puppies, kittens and human embryos were inoculated with JE and Negishi viruses. The viruses tested multiplied in each of the culture systems used. However, the affinities between viruses and nervous tissue cultures, as judged by the titers of virus in fluid phase, were different among the different virus strains employed. Our previous work

(Hotta *et al.* 1964b)[7] indicated that grades of JE virus multiplication in cultures of various human tissues differed significantly among different virus strains. It was also reported by another investigator (Oya, 1963)[18] that grades of JE virus multiplication in mouse brain were different among different strains of virus. These data well suggest natural variations of the pathogenicity of JE virus. Problems of whether there is a correlation between the grade of multiplication of JE viruses in nervous tissue culture and the "neurotropism" of the same viruses *in vivo* deserve further considerations.

The Purkinje cells in virus-infected cultures exhibited characteristic alterations after being exposed to the viruses. Some of the distinct pictures revealed by the Bodian and/or Nissl stains were: disintegration of nuclei, disappearance of neurofibrils and tigroid substances, and various deformities of dendrites and neurites, followed by the eventual destruction of the whole cells. Association of the noted changes with the infection of viruses was indicated by the following facts: (i) The morphologic changes appeared and proceeded in accordance with the increase of viruses in tissue cultures; (ii) the cytopathic effects were completely prevented by mixing the viruses with specific antiviral antiserum prior to inoculations; (iii) no similar lesions were found in control cultures which received a normal mouse brain homogenate; and (iv) although certain abnormal appearance were observed in some non-infected Purkinje cells, especially in those subjected to disadvantageous conditions, such alterations were distinguished from the changes noted inevitably in the virus-infected cells.

It is cited in this connection that JE-specific immunofluorescence is clearly demonstrated in the Purkinje cells of JE-infected puppy cerebellar tissue cultures (Yoshida and Hotta, 1966).[22] In this case, however, the fluorescence was detected in the cytoplasm and the fibers (dendrites and neurites), but not in the nuclei, which showed profound degeneration by Bodian stain. Further studies are required to investigate the significance of nuclear damage in the process of JE virus infection in tissue culture.

No conclusive evidence has yet been given in the present work as to whether there are any difference(s) in the degenerative patterns produced in each virus-culture combination. Apparently the changes observed in each case were essentially the same, judging by the Bodian and Nissl pictures. A tendency was noted, on the other hand, that the degeneration induced by the Negishi virus was more acute than that by any of the JE viruses tested. Clarification of this problem might throw light upon a facet of the "neurovirulence", or, more accurately, "neuronovirulence" of encephalitogenic viruses.

The results obtained here prompted us to examine the susceptibility of dogs to JE virus. In separate experiments by us, it was found that 20 to 200-day-old dogs, inoculated intracerebrally with mouse-passaged Gl stain JE virus, developed characteristic signs indicating central nervous system involvement, and that distinct histopathologic changes together with specific immunofluorescent reactions were shown in the brain tissues, in which increase of active virus was unequivocally demonstrated (Hotta *et al.* 1964a[6]; Kuromaru *et al.* 1966[10]). This can be considered as an example in which virologic aspects observed in tissue cultures from animals of a particular species are correlated directly with those noted in the body of the same

host. Parallel studies *in vivo* and *in vitro*, using dogs and dog cerebellar tissue cultures, may be useful for elucidating pathologic mechanisms underlying JE infection.

ACKNOWLEDGMENT

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REFERENCES

1. ANDO, K., KURATSUKA, K., ARIMA, S. HIRONAKA, N., HONDA, Y. and ISHII, K.
Kitasato Arch. Exp. Med. 1952, *24*: 49–61.
Studies on the viruses isolated during epidemic of Japanese B encephalitis in 1948 in Tokyo area.

2. FERNANDES, M. V. and POMERAT, C. M.
Z. Zellforsch. 1961, *53*: 431–437.
Cytopathogenic effects of rabies virus on nervous tissue *in vitro*.

3. HILD, W.
Z. Zellforsch. 1957, *47*: 127–146.
Observations on neurons and neuroglia from the area of the mesencephalic fifth nucleus of the cat *in vitro*.

4. HOGUE, M. J., MCALLISTER, R., GREENE, A. E. and CORIELL, L. L.
J. Exp. Med. 1955, *102*: 29–36.
The effect of poliomyelitis virus on human brain cells in tissue culture.

5. HOGUE, M. J., MCALLISTER, R., GREENE, A. E. and CORIELL, L. L.
Am. J. Hyg. 1958, *67*: 267–275.
A comparative study of the effect of the poliomyelitis virus types 1, 2 and 3 on human brain cells grown in tissue culture.

6. HOTTA, S., KUROMARU, S., FUNASAKA, K. and MIZOGUTI, H.
Acta Neuropathol. 1964a, *3*: 494–510.
Experimental infection of dogs with Japanese B encephalitis virus.

7. HOTTA, S., OHYAMA, A., HIROSE, N. and OHNO, M.
J. Japan. Assoc. Infect. Dis. 1964b, *38*: 124–132.
Tissue culture studies on Japanese B encephalitis virus. Ⅳ. Viral growth curve in cultures of human tissues. (in Japanese with English summary).

8. HOTTA, S., OHYAMA, A., MUSASHI, H., OHNO, M. and LIAO, C. C.
J. Japan. Assoc. Infect. Dis. 1964c, *38*: 277–291.
Tissue culture studies on Japanese B encephalitis virus. V. Growth and cytopathogenicity of JBE and related viruses in cultures of mammalian central nervous tissues. (in Japanese with English summary)

9. KAWAKITA, Y.
Japan. J. Exp. Med. 1939, *17*: 211–225.
Cultivation *in vitro* of the virus of Japanese encephalitis.

10. KUROMARU, S., FUNASAKA, K., YASUI, Y., HOSOMI, T., MIZOGUTI, H. and HOTTA, S.
Psychiat. Neurol. Japon. 1966, *68*: 138–139.
Immunofluorescent studies on experimental infection of dogs with Japanese encephalitis virus (Report I). (in Japanese)

11. LIAO, C. C.
Bull. Kobe Med. College, 1961, *21*: 916–937.
The *in vitro* cultivation of cerebellum tissues with special reference to the infection of cultivated nervous cells with Japanese B encephalitis virus. (in Japanese with English summary)

12. LUMSDEN, C. E. and POMERAT, C. M.
Exp. Cell Res. 1951, *2*: 103–114.
Normal oligodendrocytes in tissue culture.

13. MUSASHI, H.
Contributions from Dept. of Anatomy, Kobe Med. College, 1960, *11*: 1–28.
In vitro cultivation of the nerve cell. (in Japanese)

14. OHNO, M.
Bull. Kobe Med. College, 1964, *26*: 351–369.
Growth curves and cytopathogenic effects of encephalitis viruses in central nerve tissue cultures. (in Japanese with English summary)

15. OKAMOTO, M.
Z. Zellforsch. 1958, *47*; 269–287.
Observation on neurons and neuroglia from the area of the reticular formation in tissue culture.

16. OKAMOTO, M.
Proc. 15th Japan. Med. Congr. 1959, V: 579–587.
Tissue culture of the central nervous system. (in Japanese)

17. OKUNO, T., OYA, A. and ITO, T.
Japan. J. Med. Sci. & Biol. 1961, *14*: 51–59.
The identification of Negishi virus: A presumably new member of Russian Spring-Summer encephalitis virus family isolated in Japan.

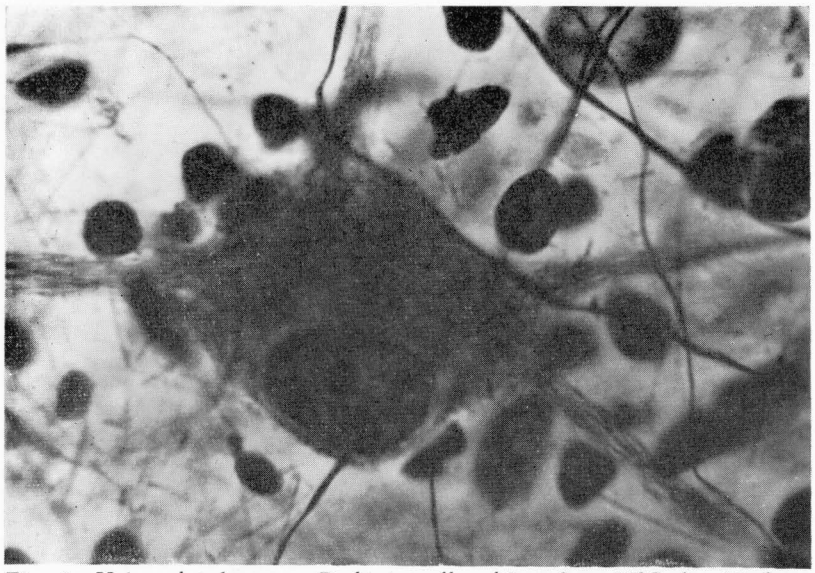
18. OYA, A.
Progress in Virology, ed. Institute for Virus Research, Kyoto University, 1963, pp. 53–72.
Infection and immunity caused by Japanese encephalitis virus. (in Japanese)

19. POMERAT, C. M. and COSTERO, J.
Am. J. Anat. 1956, *99*: 211–247.
Tissue cultures of cat cerebellum.

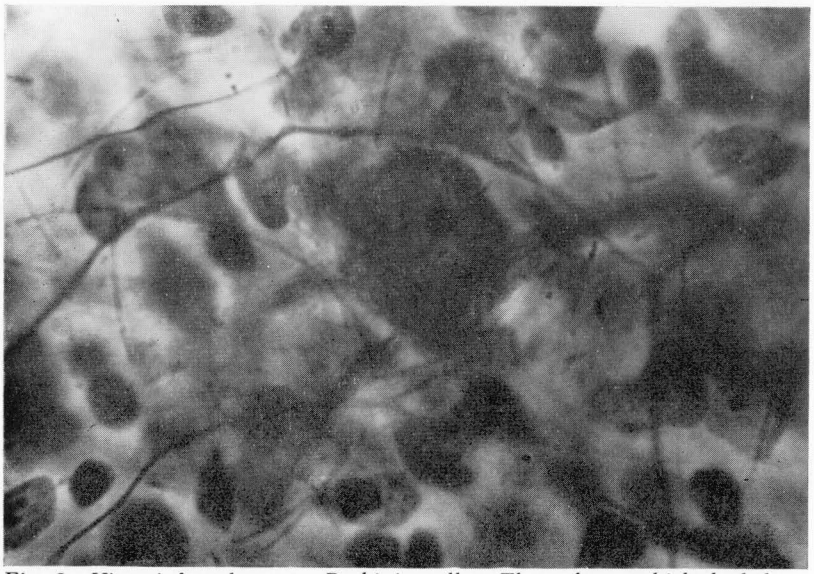
20. REED, L. J. and MUENCH, H.
Am. J. Hyg. 1938, *27*: 493–497.
A simple method of estimating fifty per cent endpoints.

21. SIMAGOSI, H.
Osaka Igakkai Zasshi, 1939, *38*: 419–433.
Über den Massen-Gewebekultur des Encephalitisvirus japanisches Typus. (in Japanese with German summary)

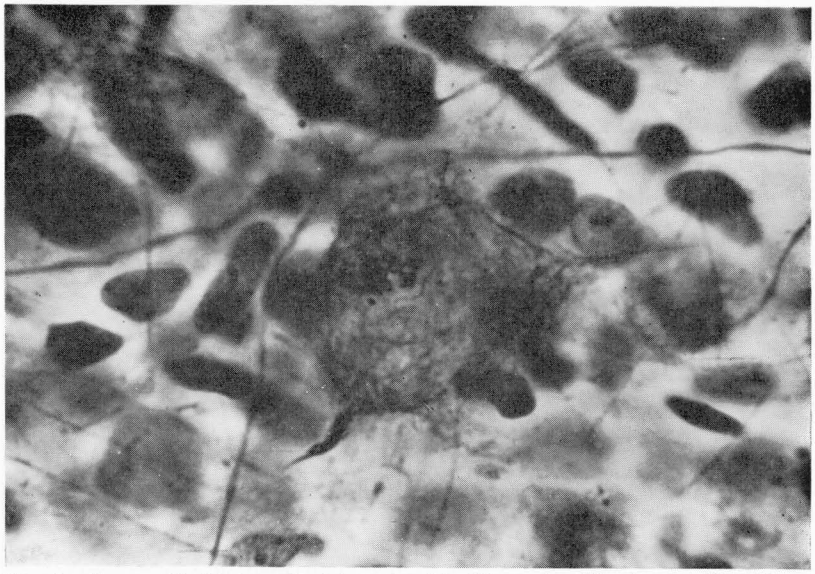
22. YOSHIDA, J. and HOTTA, S.
Japan. J. Microbiol. 1966, *10*: 183–188.
Immunofluorescence of Japanese encephalitis virus infection *in vitro*: Localization of viral antigen in canine cerebellar tissue cultures.



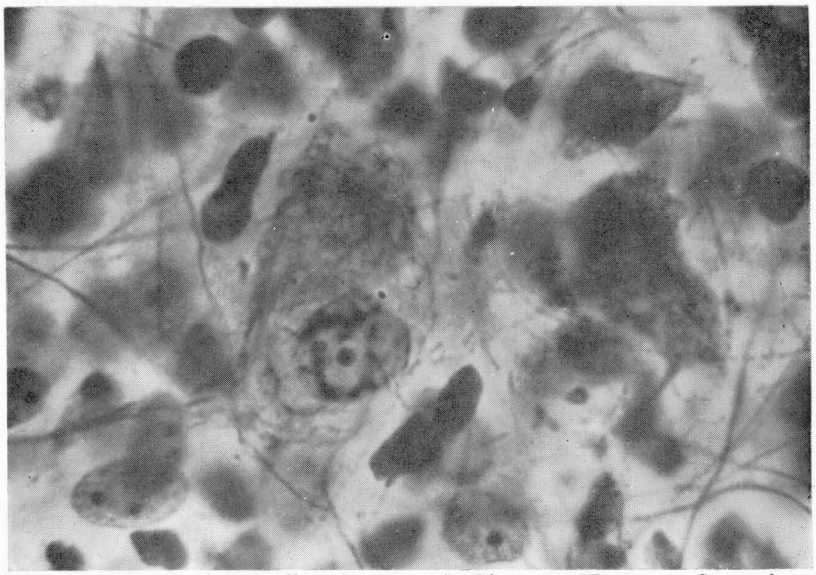
Plate–Fig. 1. Uninoculated puppy Purkinje cell cultivated at 37°C for 15 days. 100×10, oil immersion.
Networks of fine neurofibrils are seen in the cell body which looks apparently homogeneous. The neurofibrils extend into the dendrites. The nucleus looks granular and contains a dark-stained, clear-defined nucleolus. Nuclei of smaller sizes are probably of the glial origin.



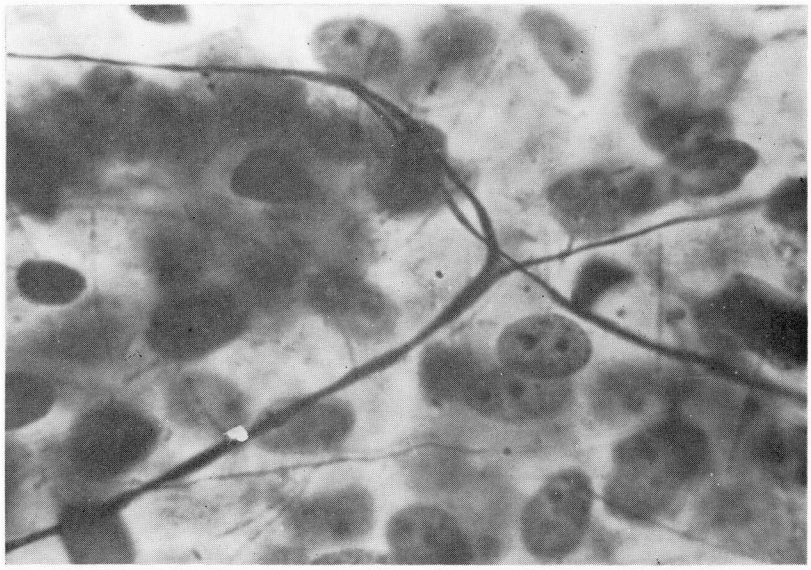
Plate–Fig. 2. Virus-infected puppy Purkinje cell. The culture which had been incubated for 10 days was inoculated with Gl strain JE virus. Three days after the virus inoculation. 100×10.
Early stage of degeneration. The neurofibrils both in the cell body and dendrites stain more roughly than in uninoculated control cells. Branches of the dendrites are shortened and reduced in number.



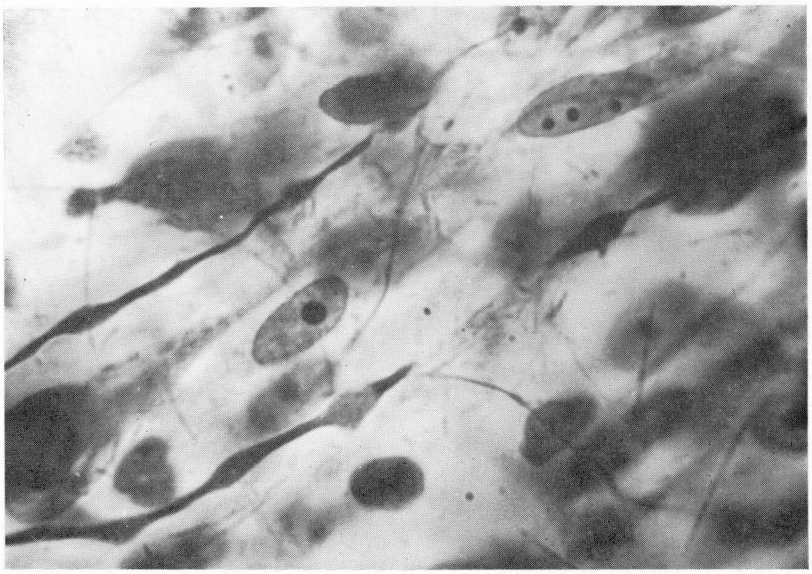
Plate–Fig. 3. Puppy Purkinje cell infected with Gl strain JE virus. Five days after the virus inoculation. 100×10.
Middle stage of degeneration. The neurofibrils are irregular. The nucleus and nucleoli are disintegrated into granular masses. The dendrites are shortened and thin, and their smaller branches have disappeared.



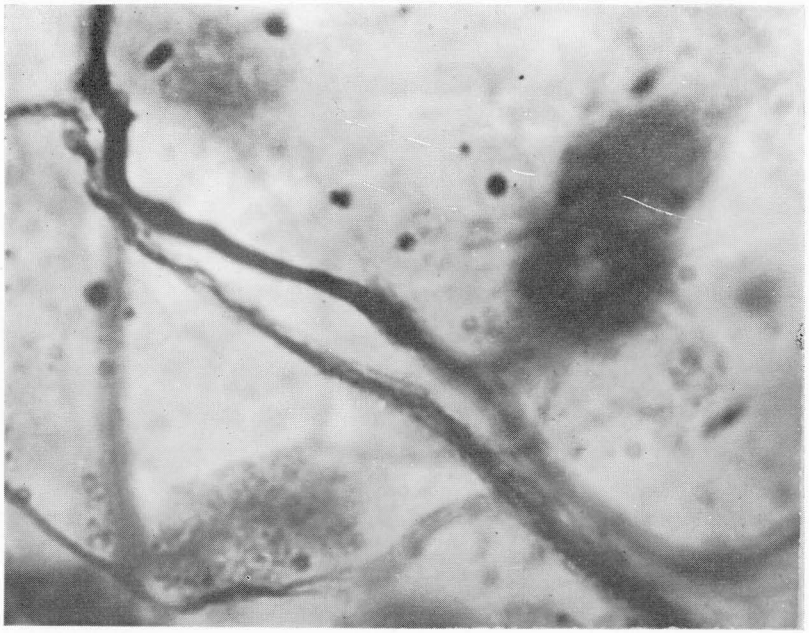
Plate–Fig. 4. Puppy Purkinje cells infected with Gl strain JE virus. Seven days after the virus inoculation. 100×10.
Advanced stage of degeneration. Two highly disintegrated Purkinje cells are seen.

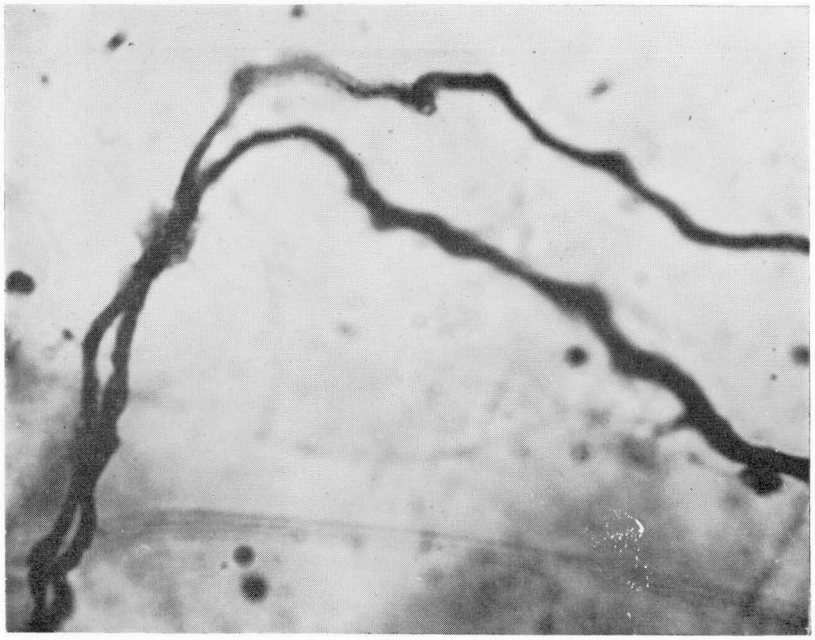


Plate–Fig. 5. Uninoculated puppy cerebellar culture. 100×10.
The neurites run straight, bend angularly or make smooth round curves.

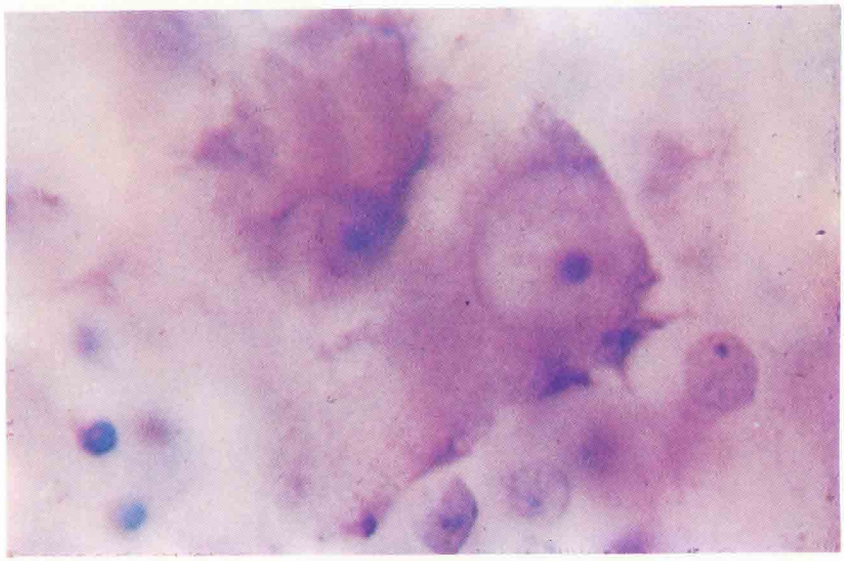


Plate–Fig. 6. Puppy cerebellar culture infected with Gl strain JE virus. Seven days after the virus inoculation. 100×10.
Serpentine turning, breaking, swelling, and abnormal branching of the neurites are seen.

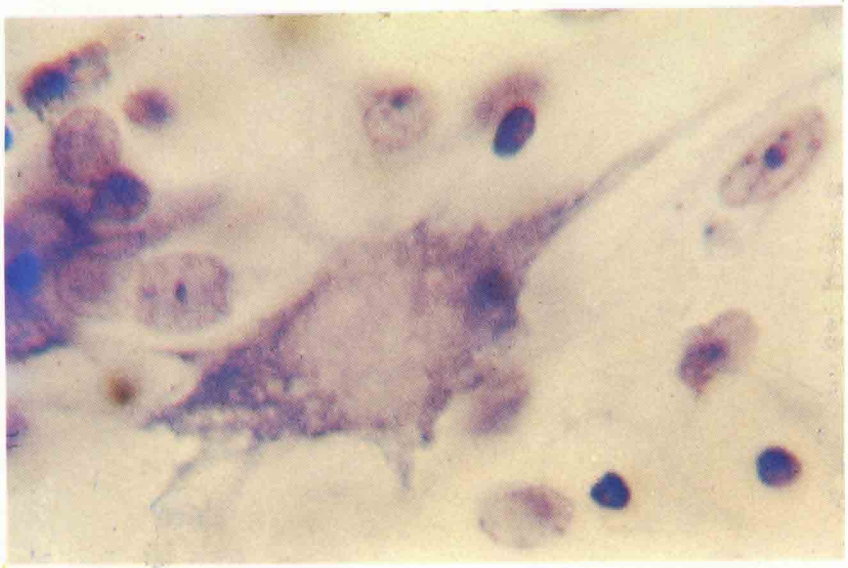




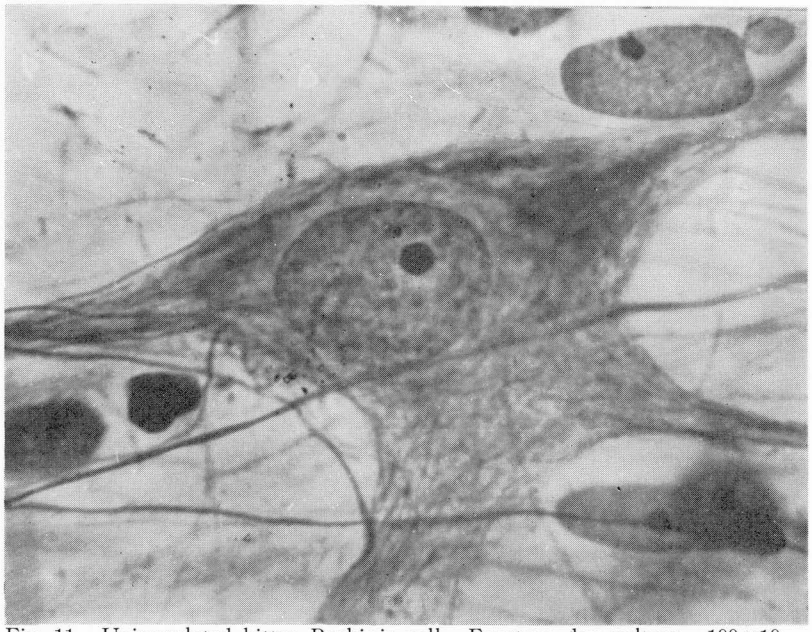
Plate–Figs. 7 and 8. Puppy cerebellar cultures infected with Gl strain JE virus. Five days after the virus inoculation. 100×10.
Neurites exhibit degenerative alterations, such as serpentine turning, nodule formation, swelling, "worn rope"–like appearance, *etc.*



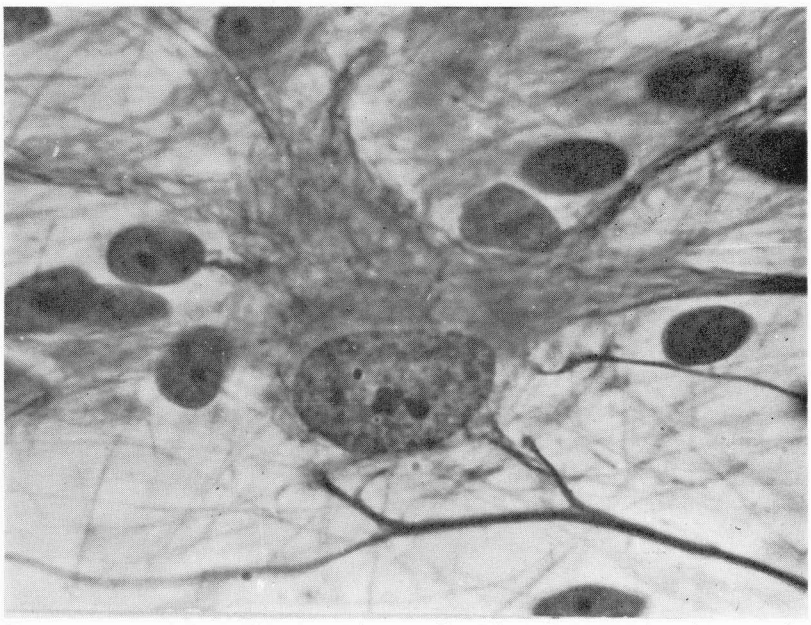
Plate–Fig. 9 Uninoculated puppy Purkinje cells. Nissl stain. 100 × 10.
The nucleus and nucleolus are clearly defined. Tigroid substances are intensively stained.



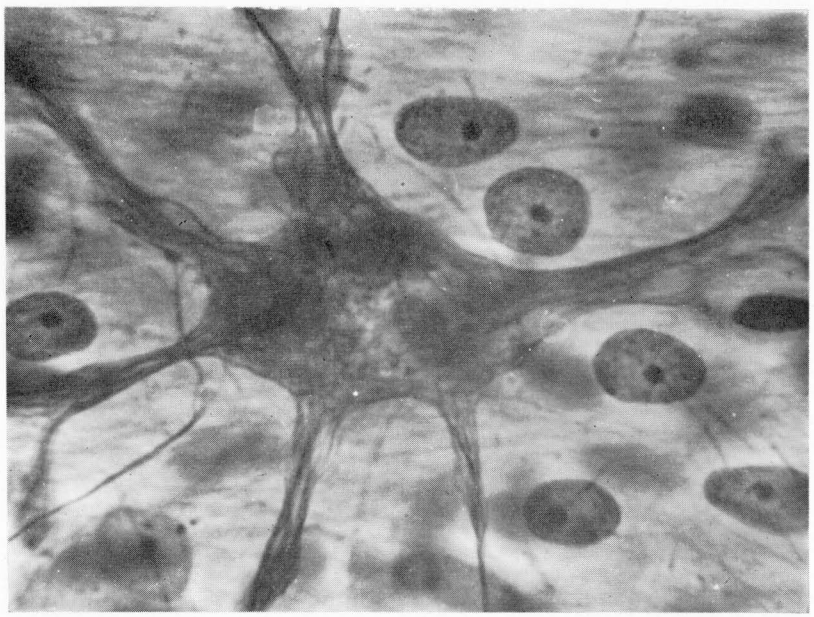
Plate–Fig. 10 Puppy Purkinje cells infected with Gl strain JE virus. Five days after the virus inoculation. Nissl stain. 100 × 10.
The nucleus and nucleolus have disappeared. The staining ability of tigroid substances is lost or reduced in degree. A number of vacuoles are seen in the cytoplasm.



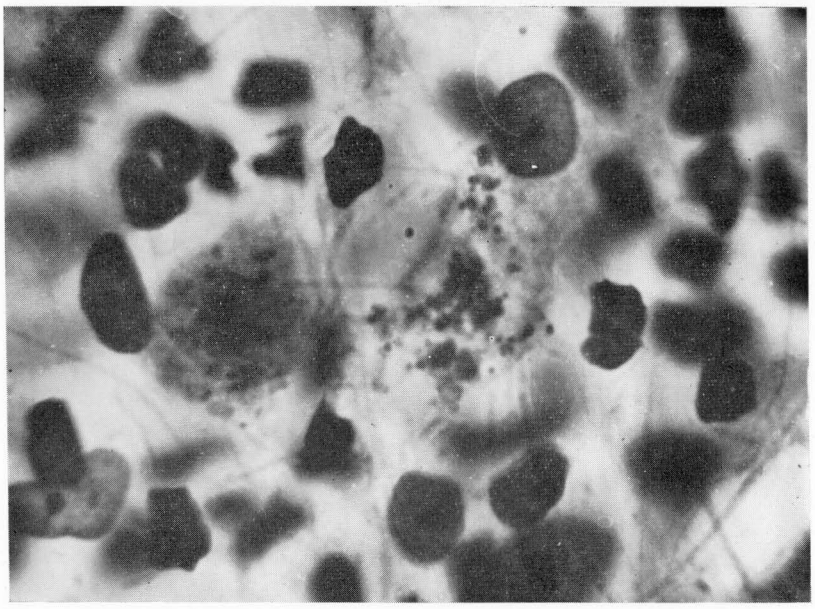
Plate–Fig. 11. Uninoculated kitten Purkinje cell. Fourteen–day–culture. 100 × 10.
General aspects are essentially the same as those of puppy Purkinje cells.



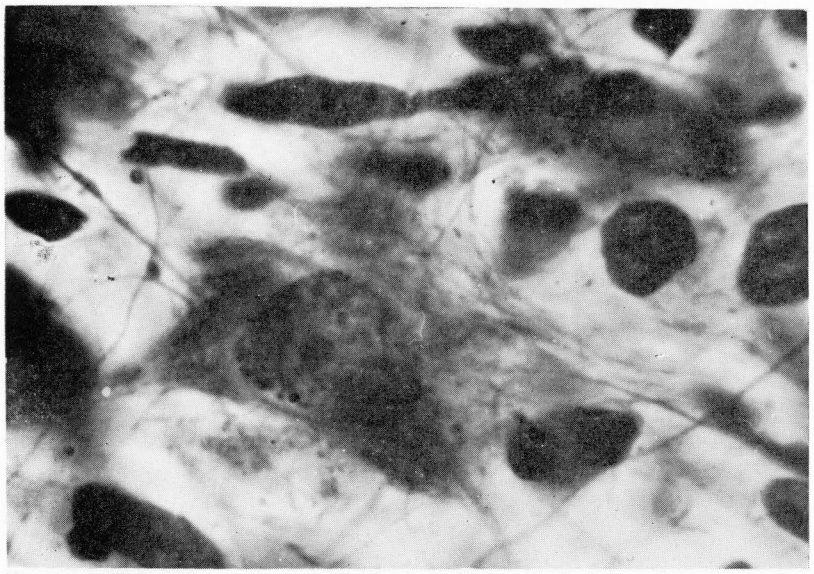
Plate–Fig. 12. Virus–infected kitten Purkinje cell. The culture incubated previously for 10 days was inoculated with Gl strain JE virus. Five days after the virus inoculation. 100 × 10.
Early stage of degeneration.



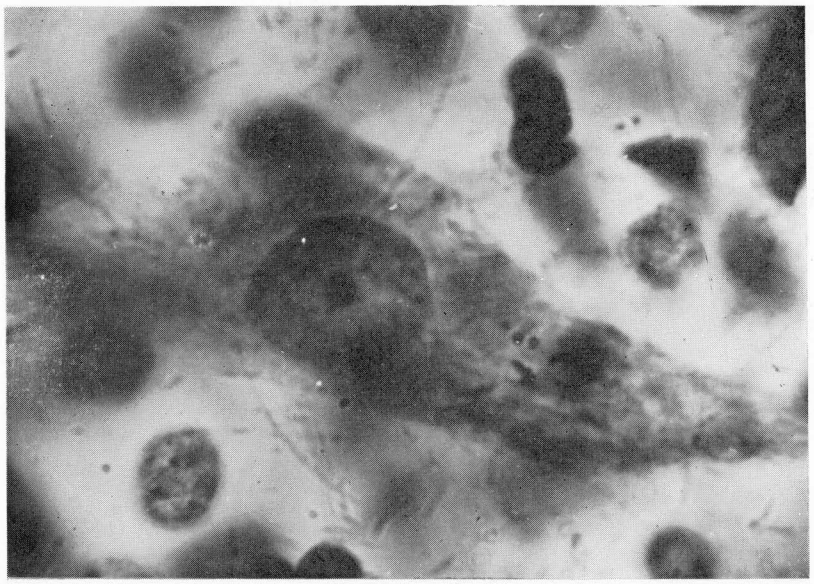
Plate–Fig. 13. Virus–infected kitten Purkinje cell. Specimen from the same group of Plate–Fig. 12. Seven days after the virus inoculation. 100×10. Middle stage of degeneration.



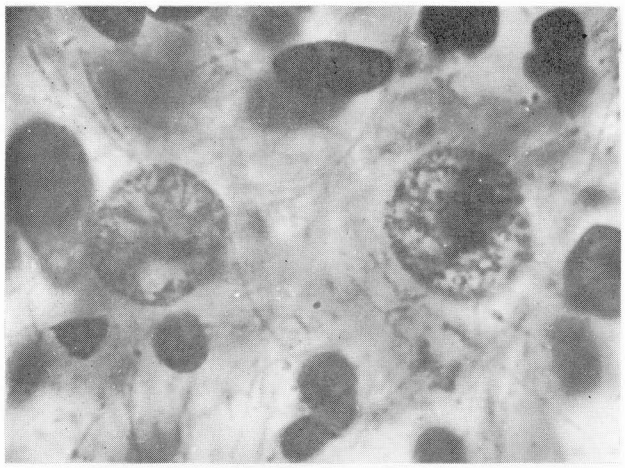
Plate–Fig. 14. Virus–infected kitten Purkinje cells. Specimen from the same group of Plate–Fig. 12. Seven days after the virus inoculation. 100×10. Advanced stage of degeneration.



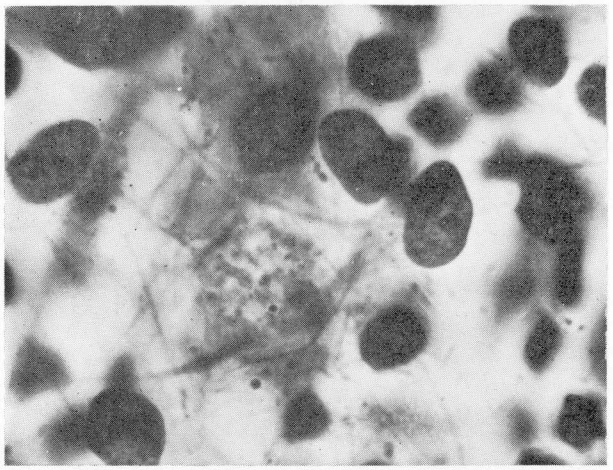
Plate–Fig. 15. Uninoculated human Purkinje cell in cerebellar culture from an 8–month–old foetus. Fourteen–day–culture. 100 × 10.
Human neurofibrils in tissue culture stained by Bodian's method look more slender and stain more pale than in those of canine or feline cells.



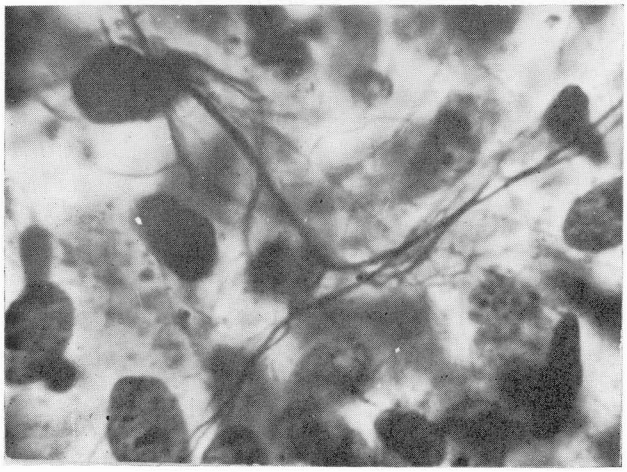
Plate–Fig. 16. Virus–infected human Purkinje cell. The culture previously incubated for 10 days was inouculated with Gl strain JE virus. Three days after the virus inoculation. 100 × 10.
Early stage of degeneration. Neurofibrillar pattern is irregular.



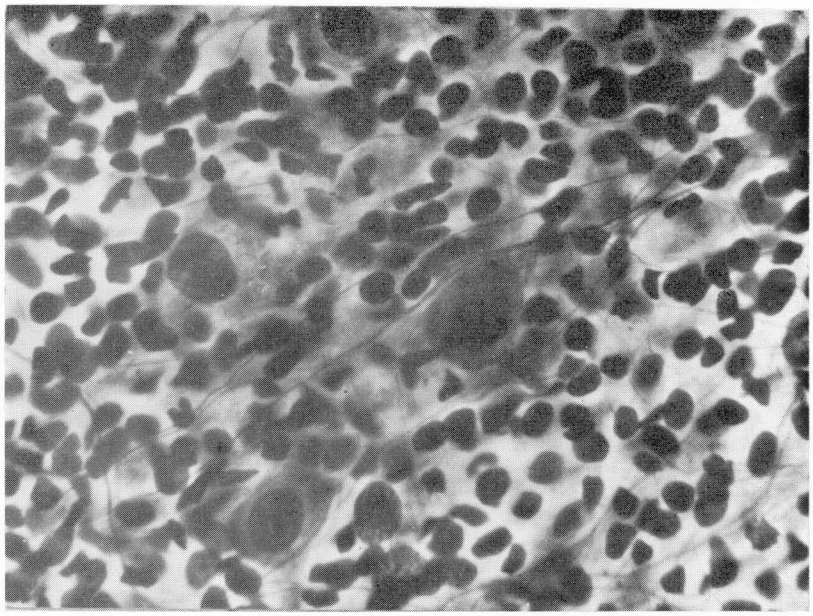
Plate–Fig. 17. Virus–infected human Purkinje cells. Another portion of the same culture shown in Plate–Fig. 16. 100×10.
The nuclei undergo marked degeneration, containing disintegrated nucleoi and granules of irregular shapes.



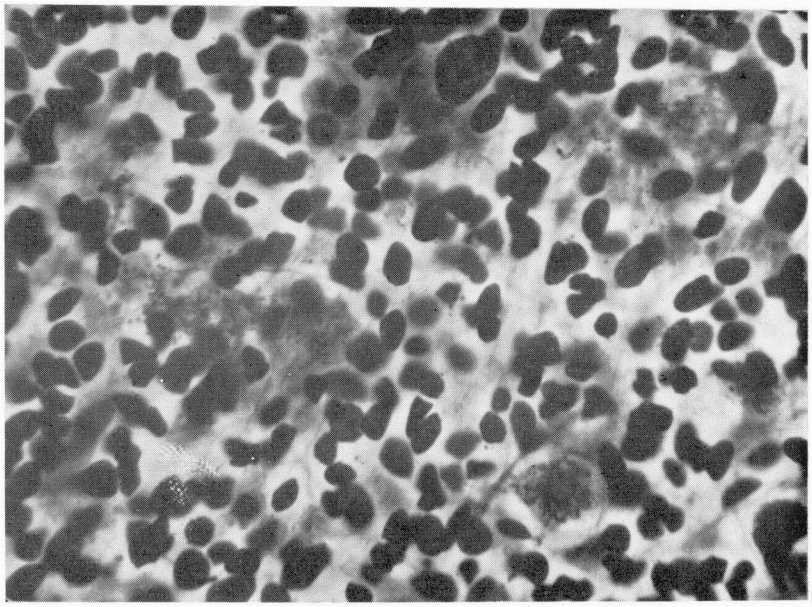
Plate–Fig. 18. Virus–infected human cerebellar culture. Specimen from the same group of Plate–Fig. 16. Five days after the virus inoculation. 100×10.
Advanced stage of degeneration. The cell body disappeared, and the nucleus has been disintegrated into masses of debris.



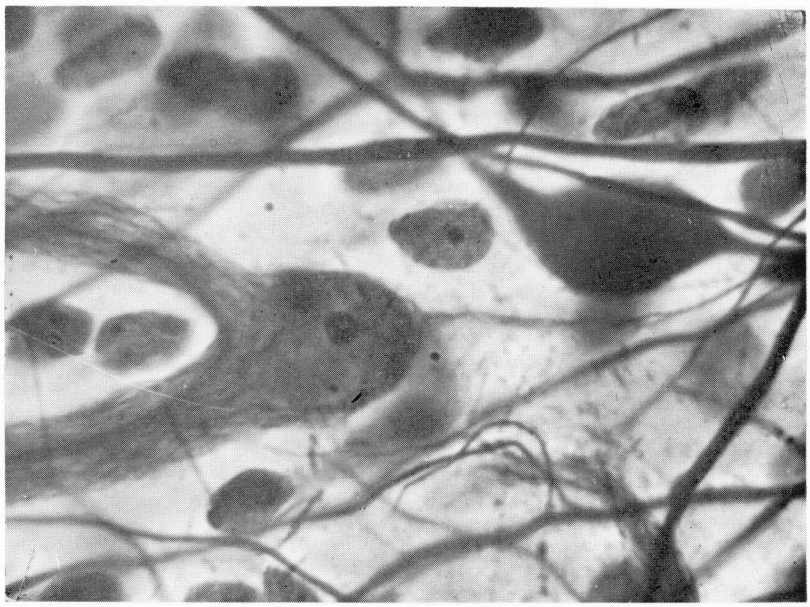
Plate–Fig. 19. Virus–infected human cerebellar culture. Specimen from the same group of Plate–Fig. 16. Five days after the virus inoculation. 100×10.
Neurites are split into finer fibers, looking like "worn ropes".

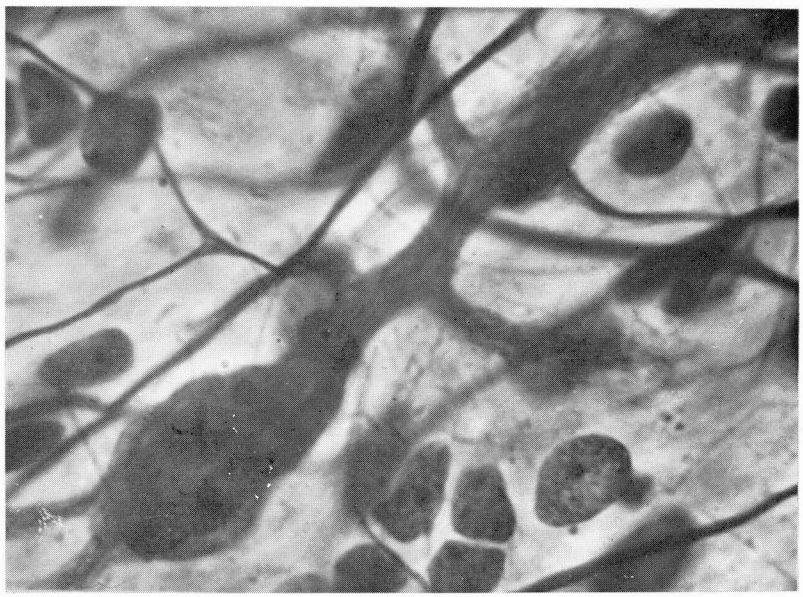


Plate–Fig. 20. Puppy cerebellar culture inoculated with a mixture of Gl strain JE virus and its immune rabbit serum. 40×10.
Purkinje cells show no recognizable alteration.



Plate–Fig. 21. Puppy cerebellar culture inoculated with a mixture of Gl strain JE virus and non-immune control serum. 40×10.
Purkinje cells exhibit distinct degeneration.





Plate–Figs. 22 and 23. Puppy cerebellar cultures incubated at 37°C for 17 days without changing culture medium. 100×10.
While dendrites and neurites exhibit abnormal appearances such as shortening, loss of branches, swelling, nodule formation, *etc.*, their degrees are comparatively milder than those noted in the virus-infected cultures. Nuclear structures are rather well maintained.