ISOLATION OF THE VIRUS OF HERPES SIMPLEX AND THE DEMONSTRATION OF INTRANUCLEAR INCLUSIONS IN A CASE OF ACUTE ENCEPHALITIS*

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Although there is an extensive literature pertaining to the virus of herpes simplex as an etiological agent of encephalitis in man, there is no reported case of fatal encephalitis from which the herpes virus has been isolated which has shown intranuclear inclusions in the brain resembling those of herpetic lesions. In the first report of the Mathewson Commission,[1] a summary of the literature concerning the relation of the herpes virus to encephalitis mentioned only 9 instances of the isolation of a virus identified as that of herpes simplex from cases of encephalitis. In 5 of these, the virus was isolated from the brain; in 3, from the spinal fluid; and in 1, from the nasopharynx.

Subsequent to this report, Gay and Holden [2] isolated a virus from the brain of a man who died during an acute exacerbation of chronic encephalitis. They considered it identical with the herpes virus. A second virus, isolated by Gay and Holden [2] from the brain of a laboratory worker, 27 years old, who died following the bite of a monkey, was likewise reported as the virus of herpes simplex. However, it seems that the latter virus, also isolated and described by Sabin,[3] was not the virus of herpes simplex but that now designated as virus B. Also, with brain material from 3 cases of encephalitis, all occurring in children following measles, Gay and Holden [2] produced in rabbits, skin lesions which were in keeping with those of herpes. In none of these 3 cases was the virus identified as that of herpes.

Other investigators [4-8] have reported the presence of an agent which they considered the virus of herpes, although not definitely established as such, in the brain or in the spinal fluid of patients

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with symptoms of encephalitis or meningitis. The methods used in these investigations to demonstrate the presence of herpes virus were the production of an encephalitis in rabbits following the intra-ocular or intracerebral inoculation, or the production of corneal lesions alone following intra-ocular inoculation. The accuracy of the latter method has been criticized by Rupilius and Szekely,[9] who questioned the criteria used for judging a positive corneal reaction for herpes virus. They were able to produce a punctate keratitis, similar to that described by some investigators as a positive reaction for the herpes virus, by inoculation of non-specific solutions.

In addition, the etiological significance of the herpes virus, when found in the brain or spinal fluid, has been questioned because of its occasional demonstration in the spinal fluid of individuals showing no evidence of encephalitis or herpetic eruptions. Such instances, however, are less frequent than is commonly supposed, and according to Doerr and Hallauer [10] latent infections of the central nervous system with herpes virus never have been proven. In 1937 Zurukzoglu [11] summarized the reports of results obtained by a number of investigators with spinal fluid from individuals showing no evidence of encephalitis or herpetic eruptions. In five different investigations, in which a total of 108 spinal fluids were tested for the presence of herpes virus, the results were entirely negative. Zurukzoglu cited the following investigations in which positive results were obtained. The herpes virus was isolated from only 1 of 100 spinal fluids examined by Flexner and Amos. Of 50 fluids examined by Zurukzoglu, 2 gave positive results. Zurukzoglu also tested the spinal fluid from 8 cases, without clinical symptoms of encephalitis, shortly after the occurrence of herpes labialis. Two of these gave evidence, by intra-ocular inoculation of rabbits, of herpes virus in the spinal fluid. The only results reviewed, which differed strikingly from the others, were those of Bastai and Busacca who reported the presence of the herpes virus, demonstrated by intra-ocular inoculation of rabbits, in 18 of 22 spinal fluids from unselected cases.

In addition to these reports of the demonstration of a virus in the brain or spinal fluid, there are a number of reports [12,13] of the demonstration of a virus which was capable of producing keratitis and/or encephalitis by corneal inoculation of rabbits in

the nasopharynx or saliva of patients with clinical symptoms of acute or chronic encephalitis. The etiological significance for encephalitis of the herpes virus thus obtained may be questioned in view of the frequent occurrence of herpes infections and also in view of the demonstration of the herpes virus in the saliva of normal individuals.[14]

In 1933 Dawson [15] reported a case of fatal lethargic encephalitis in a boy 16 years of age. Sections of the brain showed, in addition to minute hemorrhages and perivascular infiltration of lymphocytes, intranuclear inclusion bodies in the cerebral lesions comparable to those seen in herpetic infections. In this instance no etiological agent was isolated. To our knowledge this is the only description in the literature of intracellular changes in the brain lesions of human encephalitis suggestive of those produced by the herpes virus. However, these lesions were believed not to be identical with those of herpes simplex because the nuclear inclusions were never of the very large granular type seen in herpes.

It is the purpose of this paper to present a case of encephalitis occurring in an infant 4 weeks old in which intranuclear inclusion bodies, compatible with those of herpes, were found in the cerebral lesions and from which a virus, identical with that of herpes simplex, was isolated from brain tissue. Because of the importance of establishing the identity of the etiological agent, a detailed account of its investigation is given.

Report of Case

Clinical History and Observations. The patient, a white male infant 4 weeks old, was admitted to St. Louis Children's Hospital because of irritability, refusal to nurse, and twitching of the left side of the body.

No family history of significance was obtained. The infant was born at home 1 month prematurely; the delivery was normal. Until 4 days before entry to the hospital he had been breast fed, had developed normally and appeared in good health. At that time, however, it was noticed that he was irritable and fretful, but there was no noticeable fever and no vomiting. Two days before admission to the hospital he began to complain when moved and to scream out suddenly. On the day before admission, twitching of the left arm and leg was first noticed and the child cried continuously. On the day of admission the twitching of the left side of the body continued, the child refused to nurse and had a slight fever.

The patient appeared moderately well nourished; he was listless and was having occasional twitchings of the left arm and leg. The fontanelle was full

and tense. Both ear drums were dull, and the left one bulging. To external examination the eyes were normal except for a slight clouding of the cornea. Examination of the eye grounds disclosed a pale zone about the optic disks; however, a diagnosis of optic atrophy was considered questionable. The tongue and mucous membranes of the mouth appeared normal. The pharynx was not hyperemic. A seborrheic dermatitis was present on the scalp, but no other abnormalities of the skin were observed. The white blood cell count was 18,000; the red blood cell count, 4,100,000; and the hemoglobin content of the blood, 11 gm. per 100 cc. The temperature on admission was only slightly elevated and later dropped to subnormal levels.

As a result of the first lumbar puncture, bloody, sterile fluid was obtained. Several other attempts were made to obtain spinal fluid from the cisterna and from the ventricles, and a small amount of blood-tinged cisternal fluid was obtained on one occasion.

The child's illness steadily progressed. The fontanelle became more and more tense; the convulsive movements of the extremities continued; and there was a generalized convulsion on the day after admission to the hospital. Death occurred on the fifth hospital day. A clinical diagnosis of acute encephalitis was made.

Postmortem Examination

A complete autopsy revealed no macroscopic abnormalities other than those in the brain. On gross examination of the brain the leptomeninges appeared hyperemic; and in the region where the intraventricular punctures had been made there were a few small clots of blood, but no extensive hemorrhage. Material was taken from the frontal cortex for animal inoculation. Nothing unusual was observed when a cut was made through this portion of the brain; however, the entire brain was unusually soft, even for that of a young infant. Unfortunately, only the cerebellum and the brain stem were saved for microscopic study.

The microscopic study of organs other than the brain revealed nothing abnormal.

Sections from the pons, medulla, and cerebellum were stained with hematoxylin and eosin, phloxine-methylene blue, and with bacterial stains. There was an extensive inflammatory process involving the brain tissue and, to some extent, the meninges. In certain areas the meninges were entirely normal, while in others, always in association with changes in the underlying brain tissue, there was an infiltration of cells concentrated about blood vessels. These cells were chiefly lymphocytes and larger mononuclear cells. Only an occasional polymorphonuclear leukocyte was seen. The walls of the meningeal blood vessels were not damaged.

In general, the changes seen in the sections from the three parts of the brain were alike, but less severe in the sections from the cerebellum. Perivascular infiltration of cells like that occurring in the meninges was conspicuous within the brain substance. The vessel walls, however, appeared normal except that the lining endothelial cells were often unusually large. There were focal accumulations of cells having either round or elongated nuclei: some with indistinctly outlined cytoplasm, others with sharply outlined cell margins (Fig. 1). These focal accumulations of cells closely resembled the focal lesions, largely of microglial origin, seen in other types of virus encephalitis. In some instances they were associated with degenerating nerve cells. In other microscopic fields a more diffuse inflammatory reaction of mononuclear cells occurred, involving, in some instances, groups of large ganglion cells which showed stages of degeneration. Isolated degenerating nerve cells surrounded by a cluster of small mononuclear cells were also seen. In sections from both the pons and medulla, areas of necrosis were present which were slightly larger than a low power field (16 mm. objective). This necrosis was not localized about vessels. In fact, the vessels were remarkably well preserved, even in the necrotic zones. One necrotic area had undergone liquefaction (Fig. 2); in the others there were many large fat-holding phagocytes and remnants of necrotic brain tissue.

In the cerebellum the Purkinje cells appeared normal except in localized areas where they, together with the adjacent small nerve cells, were undergoing necrosis.

Intranuclear Inclusions. The degenerative changes in individual nerve cells were especially interesting. Some of these cells showed pyknotic nuclei and deeply stained, shrunken cell bodies; others were poorly outlined and showed varying degrees of karyolysis. Many cells, however, showed more specific nuclear changes in the form of intranuclear inclusions which varied somewhat in appearance. In some nuclei the chromatin was marginated at the nuclear membrane about an acidophilic central body (Figs. 3 and 4). The smaller of these central bodies were four to five times the usual size of a nucleolus. Occasionally, there was a fine network of light blue-staining material which radiated from the acidophilic central body to the basophilic margin. In other

cells a clear area surrounded the acidophilic inclusion and at times a small, basophilic nucleolus was seen in addition to the marginated chromatin. Many of the inclusions conformed in shape to the general contour of the nucleus which contained them. Other nuclei had a different appearance. In these there was a very deeply stained margin of chromatin arranged in dots, while the rest of the nucleus was completely occupied by a material which stained lilac with phloxine-methylene blue and was often definitely granular (Figs. 5 and 6). Nuclear changes of both types were interpreted as intranuclear inclusion bodies corresponding to forms seen in known herpetic lesions.

Isolation of the Virus

A piece of cortex was ground without abrasive in a small amount of nutrient broth. After light centrifugation, 0.03 cc. of the supernatant fluid was inoculated intracerebrally into 6 Swiss mice. On the third day after inoculation, 4 of the 6 mice were found dead and the remaining 2 were observed in convulsions. The brains of these mice were removed aseptically, cultured, and passed to other Swiss mice, each of which received 0.03 cc. of a 10 per cent dilution of the brain material; these animals died or were killed, after being observed in convulsions, on the third day following inoculation. The infective agent has been maintained in Swiss mice until the present, and the brains of mice have been used as the source of material for studying its characteristics.

To determine the nature of the infectious agent, aerobic cultures of the human brain were made in dextrose infusion broth and both aerobic and anaerobic cultures of the infected mouse brains were made in broth and on blood agar. No organisms were grown. Sections from the human brain and from the brains of experimental mice, stained by the MacCallum-Goodpasture method, were studied for bacteria. None could be demonstrated. The infectious agent was therefore considered to be a virus and, as a matter of convenience, was designated "R. T." virus.

Infected mouse brains from early passages were stored in 50 per cent glycerin in Locke's solution at 5° C. After 5½ months the virus from the second mouse brain passage remained active, apparently retaining its original infectivity; a 10 per cent emul-

sion inoculated intracerebrally killed mice in slightly less than 3 days.

The filtrability of the virus was tested as follows. A 10 per cent suspension of infected mouse brain was made in 2 per cent normal horse serum broth, centrifuged at 2000 r.p.m. for 5 minutes in a horizontal centrifuge, and the supernatant fluid further diluted to 1 per cent with horse serum broth. This suspension was put through two new Berkefeld N candles, prepared, after washing, by filtering 30 cc. of 2 per cent normal horse serum broth through each. Neither filtrate was infectious for mice by intracerebral inoculation, while the unfiltered 1 per cent brain suspension was still infectious when diluted tenfold, all of 4 Swiss mice dying following intracerebral inoculation.

On a number of occasions, when the same procedure was carried out using nutrient broth as a diluent and for preparing Berkefeld N and V filters, no virus was demonstrated in the filtrates. These results are not surprising in view of the known difficulty in the filtration of herpes virus.

Response of Laboratory Animals to the Virus

Mice. As already stated, mice succumbed in 3 days to intracerebral inoculation of 10 per cent emulsions of infected mouse brain; and the virus, maintained by mouse passage, was uniformly infectious by this route in dilutions as great as 10^{-4}. Following subcutaneous inoculation of as much as 0.25 cc. of a 10 per cent emulsion of infected mouse brain, an occasional mouse developed paralysis, but the great majority remained well.

After intracerebral inoculations of the virus the mice frequently showed little or no evidence of illness until they began to have active muscular twitchings or generalized convulsions. Death in severe convulsions usually followed shortly thereafter.

Lesions were not found in organs other than the central nervous system. In sections of the brain the most conspicuous lesion was a meningeal exudate extending into perivascular spaces within the brain. In some areas the exudate was composed of well preserved, small lymphocytes and slightly larger mononuclear cells. Other fields showed a partially necrotic meningeal exudate, in the debris of which lymphocytes and mononuclear cells, together with polymorphonuclear leukocytes, were still recogniz-

able. In the mouse brain, inclusions in the nerve cells have been but rarely observed.

Guinea Pigs. Of 8 guinea pigs inoculated intracerebrally with 0.15 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 6 remained well and 2 died. One of the latter, which died on the eighth day following inoculation, was autopsied. Microscopic sections of the brain showed a slight cellular infiltration in the meninges and perivascular spaces similar to that seen in the less severe reactions in the mouse brains. No intracellular inclusions were observed in several sections.

The corneae of 2 guinea pigs were scarified and rubbed with a 10 per cent emulsion of infected mouse brain. In each animal, beginning on the fourth day, a slight opacity of the cornea appeared. This opacity decreased after the seventh day, and neither animal showed other symptoms.

Three guinea pigs, inoculated intradermally with 0.2 cc. of a 10 per cent emulsion of infected mouse brain, developed, on the fifth day thereafter, small inflammatory nodules at the site of inoculation. After the ninth day these nodules regressed and the animals remained well.

Rabbits. Three rabbits, when inoculated intradermally, developed no lesions. Four rabbits were inoculated intracerebrally with 0.3 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain. Of these 4 animals, 2 remained well. A third showed convulsions on the twelfth day, and at that time an opacity of the cornea had also developed. The fourth was found dead on the fifth day.

Five rabbits were inoculated on the scarified cornea with approximately 0.1 cc. of a 10 per cent emulsion of infected mouse brain. Only 1 of these animals remained normal. Each of the other 4 developed an opacity of the cornea which appeared 6 to 11 days after inoculation and gradually spread to involve the entire cornea. In one instance, extensive ulceration of both the cornea and the conjunctiva occurred. One of the rabbits was killed and the eye removed for study as soon as the opacity appeared. In the other 3 animals which developed a keratitis, partial paralysis of the posterior extremities occurred 2 to 3 days following the first appearance of the keratitis. In 2 of these animals this paralysis was unilateral, on the same side as the infected

cornea. In the third, both posterior extremities were involved. One of these paralyzed animals, in which the corneal opacity did not appear until the eleventh day and the paralysis not until the twelfth day, survived. The other 2 were killed when the paralysis was first observed, on the ninth and tenth days respectively.

Microscopic sections of 3 eyes, presenting well developed opacities of the cornea, showed a keratitis with an infiltration of polymorphonuclear leukocytes in the cornea and an extensive infiltration, composed of mononuclear cells and polymorphonuclear leukocytes, in the adjacent conjunctiva and the ciliary bodies. In the section of 1 eye the external half of the cornea and the adjacent conjunctiva was completely necrotic. Sections from the eye removed at the time of the first appearance of opacity showed only a slight inflammatory reaction, limited to the edge of the cornea and to the immediately adjacent conjunctiva. The epithelium covering the cornea of the latter eye was still intact, and at the edge of the cornea the epithelial cells were enlarged and undergoing proliferation. Several mitotic figures were seen. However, no intranuclear inclusions were observed.

In the brain of the rabbit which died on the fifth day after intracerebral inoculation a most intense inflammatory and necrotizing process was seen. The meninges showed an extensive exudate of lymphocytes, mononuclear cells, and polymorphonuclear leukocytes which was partially necrotic. In the outer part of the cortex of the cerebrum, at times continuous with the meninges, there were large areas of early necrosis, in and about which there was an infiltration of many polymorphonuclear leukocytes. In many nerve cells adjacent to these areas of necrosis, conspicuous large acidophilic inclusions were seen within the nuclei. These inclusions varied considerably in size, some completely filling the nucleus except for a deep-staining margin of chromatin. The staining of the inclusions with phloxine-methylene blue varied from light pink to lilac. In sections from the other 3 brains, 1 of a rabbit inoculated intracerebrally and 2 of animals inoculated on the scarified cornea, a well preserved meningeal exudate of lymphocytes and mononuclear cells was seen. In addition there were perivascular infiltrations of lymphocytes, focal accumulations of irregularly shaped mononuclear cells resembling those seen in the human brain, and occasional small, localized areas of

degenerating brain tissue, usually adjacent to the meninges. Nerve cells containing intranuclear acidophilic inclusions occurred, but these were far less numerous than in the brain of the rabbit dying on the fifth day.

Rats. Of 12 rats inoculated intracerebrally with 0.06 to 0.08 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 5 died 3 to 5 days following inoculation. The brains of 3 of these were examined microscopically. There occurred an infiltration of mononuclear cells in the meninges similar to that seen in the other species but with little necrosis of the exudate. Within the brain substance were seen changes comparable to those in the rabbit brains, but less severe. Intranuclear inclusions, though not numerous, were found in the nerve cells, always in areas where an inflammatory reaction was present.

Monkeys. Three rhesus monkeys, inoculated intracerebrally, remained well.

Chick Embryo. The virus was inoculated on the chorioallantoic membrane of the developing chick embryo. Large, discrete, opaque foci were observed when the membranes were examined on the second and third days following inoculation. Microscopically, the membranes showed a marked proliferation of the ectodermal layer of cells. Many of these cells contained intranuclear acidophilic bodies, and the lesions resembled, in general, those described for the virus of herpes simplex.

Neutralization of the Virus

Neutralization tests were carried out in mice with the R. T. virus and with a known herpes simplex virus (Rockefeller Institute H. F. strain). The serums used were an immune serum prepared by inoculating rabbits with the R. T. virus and two serums prepared by immunizing chickens (one with a known herpes virus (H. F. strain), and the other with a herpes virus modified by passage in the chicken embryo*). The technic used in the neutralization test was as follows: A 10 per cent suspension of virus was prepared by grinding infected mouse brains with a requisite amount of broth. After light centrifugation, the supernatant fluid was removed and serial tenfold dilutions in broth were made. Virus

* We are indebted to Miss Katherine Anderson, of the Department of Pathology, Vanderbilt University, for supplying us with the immune chicken serums.

dilutions of 10^{-2}, 10^{-3}, and 10^{-4} were used with undiluted immune serum. To 0.2 cc. of each virus dilution was added 0.4 cc. of serum. The mixtures were incubated for 2 hours at 37° C. and then injected intracerebrally in 0.03 cc. amounts into groups of 4 Swiss mice. The animals were watched daily during an observation period of 3 weeks for evidence of infection. Table I shows

TABLE I

Cross Neutralization Tests with Herpes Simplex Virus (Rockefeller Institute H. F. Strain) and with the R. T. Virus

Serum	Virus	Duration of life of test mice		
		Dilution of virus used in serum mixtures		
		10^{-2}	10^{-3}	10^{-4}
		days	*days*	*days*
Rabbit, immune to R. T. virus	R. T. virus	1, 12, S*, S	S, S, S, S	S, S, S, S
Rabbit, normal	R. T. virus	1, 4, 5, 5	5, 6, 6, 7	11, 13, 15, S
Rabbit, immune to R. T. virus	Herpes simplex	5, S, S, S	S, S, S, S	S, S, S, S
Rabbit, normal	Herpes simplex	4, 5, 5, 6	4, 6, 7, S	11, 19, S, S
Chicken, normal	R. T. virus	4, 4, 4, 6	7, 7, 10, 12	9, 11, S, S
Chicken, herpes (H. F.) immune	R. T. virus	5, 6, 6, 11	11, 12, S, S	13, S, S, S
Chicken, herpes (modified) immune	R. T. virus	6, 7, 10, S	S, S, S, S	S, S, S, S
Chicken, normal	Herpes simplex	3, 3, 3, 4	6, 7, 7, S	5, S, S, S
Chicken, herpes (H. F.) immune	Herpes simplex	6, 8, 9, S	S, S, S, S	5, S, S, S
Chicken, herpes (modified) immune	Herpes simplex	6, 7, 7, S	S, S, S, S	10, S, S, S

* S = Mouse remained well 21 days.

the results of the cross neutralization tests between a strain (H. F.) of known herpes simplex virus and the R. T. virus. It is readily apparent that immune serum to each virus was capable of neutralizing both viruses to the same extent and that the two viruses are immunologically identical.

DISCUSSION AND SUMMARY

A child, 4 weeks old, was brought to the hospital because of irritability, refusal to nurse, and twitchings of the left side of the body, and died on the fifth hospital day after a progressive accentuation of the cerebral symptoms. From the brain tissue taken at autopsy, a virus was isolated in mice which has been identified as that of herpes simplex.

During life the child's spinal fluid was sterile and no microörganisms were cultivated in dextrose infusion broth inoculated with an emulsion of the brain tissue. Furthermore, continued attempts to cultivate bacteria from the brains of mice, which died following the inoculation of infectious material originally derived from the human brain, have yielded negative results. The fact that we have been unable to show that the virus will pass through either Berkefeld N or V filters does not militate against its identification as herpes simplex, which is known to be filtrable only with difficulty.

The virus was highly infectious for mice following intracerebral inoculation, but only slightly so following subcutaneous inoculation. Rats, guinea pigs, and rabbits were also susceptible to infection with the virus. In these three species, an encephalitis resulted from intracerebral inoculation; rabbits, however, appeared more susceptible by this route than did guinea pigs and rats.

Following corneal inoculation in the guinea pig, an opacity developed which regressed. In the rabbit, however, the opacity may progress to ulceration of the cornea; and in three out of four animals showing corneal lesions a paralysis of the extremities developed. Intracutaneous injection of three guinea pigs was followed in each instance by the appearance of small nodules in the skin at the site of inoculation. No lesions developed when rabbits were injected intradermally.

Discrete, opaque foci appeared on the surface of the chorio-allantoic membrane of chicken embryos when they were inoculated with the virus.

Histological study of sections from the pons, medulla, and cerebellum of the brain of the child disclosed a meningo-encephalitis, the most conspicuous features of which were areas of necrosis, perivascular cellular infiltration, focal and diffuse inflammatory reaction, and nerve cell degeneration with intranuclear inclusions similar to those seen in known herpetic lesions.

Similar intranuclear inclusions were present in the brains of infected rabbits and rats. None was observed in the sections of the guinea pig brains studied, and few in those of mice. Inclusions were also present in large numbers in the proliferative lesions of the chick chorio-allantoic membrane. In all of the

experimental animals the cerebral lesions were essentially the same, although varying in intensity.

Cross neutralization was complete in tests performed with known herpes virus and antiserums. These results, together with those of the histological study and with the susceptibility of experimental animals, lead to the conclusion that the agent isolated is herpes simplex virus.

The work of Dodd, Johnston, and Buddingh,[16] and of Burnet and Williams,[17] has definitely shown the importance of aphthous stomatitis in children as a primary herpetic infection. It seems, therefore, that infants and children are highly susceptible to infection with herpes simplex virus, and one might anticipate that involvement of the central nervous system would be most likely to occur at this age. However, a review of the literature shows that this is the first report of a case of encephalitis from which herpes simplex virus was isolated in which the etiological significance of the virus has been established by the demonstration of typical herpetic inclusions in the human brain tissue.

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DESCRIPTION OF PLATE

Plate 13

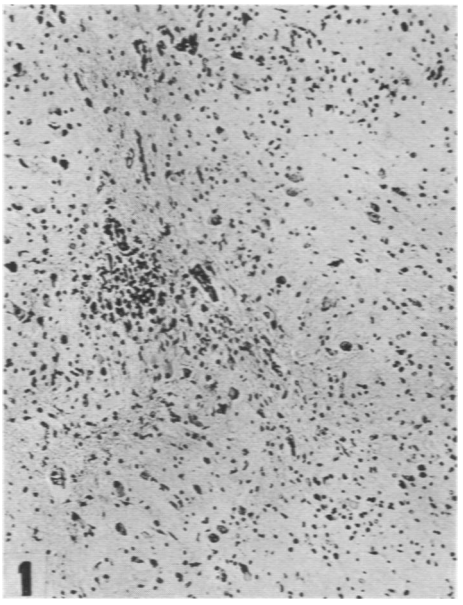
Fig. 1. Focus of proliferated glia in pons. This and all other figures were made from sections of human brain. × 100.

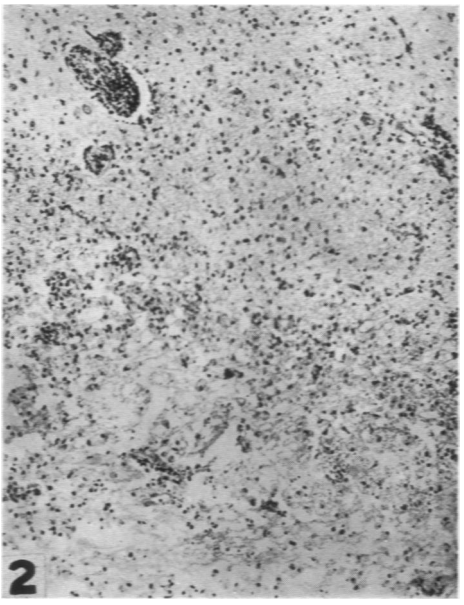
Fig. 2. Area of necrosis and inflammation in pons. Perivascular infiltration of lymphocytes. × 100.

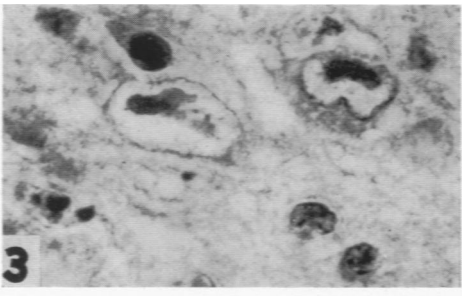
Figs. 3 and 4. Nerve cells in pons showing large intranuclear inclusions separated from nuclear membranes by clear zones. × 720.

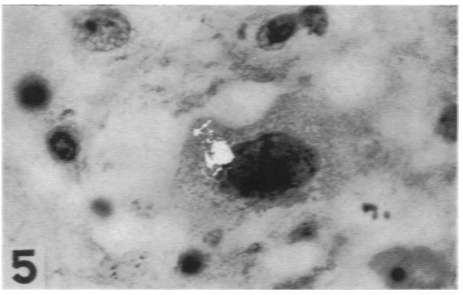
Fig. 5. Nerve cell in pons showing large granular inclusion filling the nucleus. × 720.

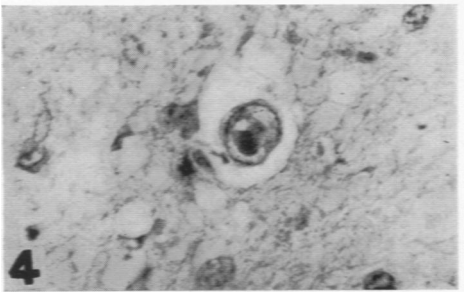
Fig. 6. Nerve cell in cerebellum showing a large granular inclusion filling the nucleus. Dots of nuclear chromatin on the nuclear membrane. × 720.

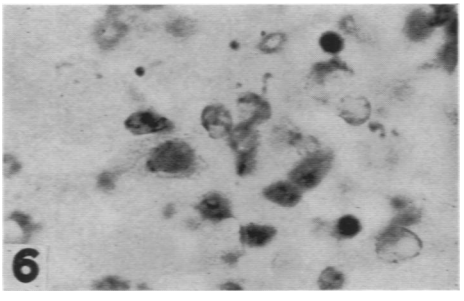












Smith, Lennette and Reames

Herpes Simplex and Acute Encephalitis