HERPES SIMPLEX ENCEPHALITIS:

REPORT OF A CASE

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In October, 1951, a patient in a hospital on the Atlantic side of the Canal Zone was diagnosed clinically as having encephalitis. An autopsy, performed at the Board of Health Laboratory, confirmed the clinical impression. Animal inoculations resulted in the recovery of a strain of herpes simplex virus.

Isolation of the herpes virus in encephalitis has been rare. Smith, Lemette and Reames summarized the known cases up to 1941, and they reported the first case in which the virus was isolated and nuclear inclusions were found in the brain. Armstrong recorded a case of suspected lymphocytic choriomeningitis in which a herpes virus was isolated from the spinal fluid. The patient recovered and subsequently developed antibodies against the virus. Three additional cases have since been reported, 1 by Zarafonetis, Smadel, Adams and Haymaker, and 2 by Whitman, Wall and Warren. In each of these cases, the virus was isolated and nuclear inclusions were found.

REPORT OF CASE

Clinical Data

History. A stuporous, 20-year-old white girl was admitted to the hospital on October 5, 1951. She had been well until 5 days before admission, when she developed a frontal headache, which was not relieved by aspirin. She saw her physician who took x-ray films of her sinuses, which were normal. The headache increased in severity, and on October 4 she was compelled to leave work. That afternoon she vomited, and late in the evening had a convulsion. Amytal was administered parenterally and she was taken to the hospital. No history of a herpetic lesion was elicited.

Physical examination. Blood pressure was 115/75, pulse rate 90 and respiratory rate 28 per minute, temperature 101.8. The patient was well developed and well nourished. She responded only to strong stimuli. The skin was clear. The pupils reacted well to light and funduscopic examination was normal. The neck was supple, and Kernig's sign was absent. The chest and abdomen were negative, there was no muscle rigidity, deep tendon reflexes were absent, and no localizing neurologic signs were present.

Laboratory examinations. Serology and urinalysis negative. October 5: X-ray films of skull, negative; hemoglobin 12.5 Gm., white cells 17,100, neutrophils 93, lymphocytes 7; spinal fluid cell count, 55 red cells, pressure normal, chloride 820 mg., protein 31.4 mg., sugar 48.3 mg., globulin negative. October 5 and 9: Blood cultures sterile. October 6: white cells 14,300, neutrophils 79, lymphocytes 21; spinal fluid cell count, 800 white cells, no differential, chloride 800 mg., protein 22 mg., sugar 93.7 mg., no organisms on Gram stain. October 10: white cells 15,500, neutrophils 29, lymphocytes 11.

Course in hospital. Shortly after admission, the patient became mentally clear. However, she began to vomit, developed neck rigidity and her headache increased in severity, radiating to the occiput. No deep tendon reflexes were obtained. The following day she be-

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came drowsy and semistuporous and her temperature rose to 103.2. On October 7, she became disoriented, with short lucid intervals. The following day she was incontinent and completely disoriented. On October 10 her neck was rigid and her temperature fell. On October 11 athetoid movements of the right arm developed. On October 12, her temperature was normal and she became deeply comatose. Her right pupil was dilated and the neck was boardlike in rigidity. She quietly died that evening, on the eighth hospital day and the twelfth day of illness.

Pathologic Findings

Autopsy findings were limited to the brain. The spinal fluid was slightly turbid and the meninges were smooth and hyperemic. The brain weighed 1375 Gm. The convolutions were flattened, the sulci narrowed and there was no surface exudate. The entire brain seemed softer than normal. The left temporal lobe was soft, and there were multiple, small, coalescent petechial hemorrhages over its surface, and these extended into the brain substance. This area had a slight yellowish tinge on sectioning, but no grossly purulent material was seen. A small reddish brown nodule, 1 cm. in diameter, was localized near the surface.

Using sterile precautions, portions of tissue from various parts of the brain were removed and stored at -20 F. Other portions were removed for bacteriologic study. The cerebrospinal fluid was treated in a similar manner.

On microscopic examination all sections of the cerebral cortex showed a severe inflammatory reaction, the temporal lobe being most involved. The brain stem, cerebellum and basal nuclei were not involved. The meninges were edematous and infiltrated with all types of inflammatory cells. Within the brain substance, there was marked perivascular cuffing with lymphocytes and plasma cells. There were multiple foci of necrosis, which were replaced by neutrophils and large foamy macrophages. Numerous small hemorrhages were seen. The nerve cells were greatly reduced in number and showed varying degrees of degeneration.

Multiple sections were stained with Giemsa, and with phloxine methylene blue. In 1 section, a few nuclei were found that contained purple, round, glassy appearing bodies. Because these were scarce, and because we could not duplicate the finding in other sections, we felt that they might be artifacts, although their appearance was like that of the nuclear inclusions seen in herpetic lesions.

Portions of tissue and spinal fluid were cultured in this laboratory for bacteria and proved to be sterile. Similar specimens were sent to the Gorgas Memorial Laboratory in Panama.

Isolation of the Virus

On October 17, 1931, a transverse section of the left temporal lobe of the patient (J. L.) and a tube containing approximately 10 ml. of slightly cloudy spinal fluid were received in the laboratory. Both specimens had been maintained in a deep freeze compartment at approximately -20 F. for 4 days. Small pieces of the brain tissue were ground in a mortar with sufficient physiologic salt solution to make a 10 per cent suspension. The supernatant fluid after light centrifugation was used to inoculate laboratory animals as follows: 1. Marcon melon was injected intracerebrally with 1 ml. and subcutaneously with 1 ml.; 1 albino rabbit intracerebrally with 0.3 ml.; 2 guinea pigs intracerebrally with 0.15 ml. each; and 8 white Swiss mice intracerebrally with 0.03 ml. each. Eight Swiss mice were also each inoculated intracranially with 0.03 ml. of the spinal fluid. Cultures of the inoculums were bacteriologically sterile. The temperature of the larger animals was taken once daily.

The rhesus monkey and the 2 guinea pigs remained asymptomatic throughout a 30 day observation period. The 8 mice injected with spinal fluid also remained well and were not immune when tested later with the virus recovered from brain tissue. The rabbit showed fever beginning on the fifth day after inoculation. On the ninth day it showed light tremors and weaving motions of the head, and on the tenth day was sacrificed when markedly ill with tremors and paralysis of the right hindleg. Three of the 8 mice inoculated with the suspension of brain tissue died on the sixth day after injection, and 2 others were noted to be ill with ruffled fur, arching of the back and ataxic gait at the same time. These were sacrif-
ficed for histologic preparations and passage as were also 2 others that developed symp-
toms on the eighth and ninth days after inoculation, respectively. The eighth mouse re-
mained asymptomatic. The virus was established without difficulty in mouse brain and
has been maintained for 9 serial passages to date. The incubation period has varied from
3 to 10 days and the mortality from 80 to 100 per cent, the incubation period and mortality
being more constant in the later passages. Glycerinated virus produced a higher mortality
and severer symptoms than fresh material.

Five rabbits inoculated with passage virus by scarification of the cornea all showed
cloning of the cornea on the second to third day after inoculation, followed by marked
keratoconjunctivitis. Fever appeared on the fifth or sixth day and lasted 2 or 3 days, after
which there was spontaneous recovery without the development of encephalitis.

Inoculation of the chorio-allantoic membrane of 11-to-12-day-old chick embryos with
mouse brain virus resulted in the appearance of numerous discrete grayish white plaques
about 1 mm. in diameter on the second and third days after inoculation. Nuclear inclusion
bodies as described by Dawson could be demonstrated in impression smears prepared from
the membrane and stained with Giemsa.

Sections of mouse and rabbit brain stained with hematoxylin and eosin revealed mening-
aeal exudation, perivascular infiltration with round cells and nerve cell degeneration of
varying degrees of intensity. Lesions were more pronounced in rabbit than in mouse brain.

 Cultures of all passage material were consistently negative for bacteriologic growth,
and impression smears revealed no parasites.

It may be seen from the above data that the virus isolated from the brain tissue of the
patient (I. L.) corresponded in animal pathogenicity with the virus of herpes simplex.
However, final identification demanded cross-immunity studies. For this purpose, we
solicited and received from Dr. Charles Armstrong, National Institutes of Health, Beth-
esda, Md., a strain of herpes simplex virus (No. 1166, in mouse brain). This strain was
considerably more virulent for mice than the patient's strain here described, as it regularly
causcd death of all mice injected intracerebrally in 3 days, preceded briefly by intense
hyperirritability and convulsions.

Twelve mice surviving an intracerebral injection with 0.03 ml. of the I.L. strain after
previous immunization with the same virus were tested 1 month later by the intracerebral
injection of the 1166 strain of herpes simplex virus received from Dr. Armstrong. One died
on the fourth day after inoculation, 2 on the fifth day and the remaining 9 survived during
a 30-day period of observation.

In a reverse cross-immunity experiment, 12 mice surviving an intracerebral injection
with the 1166 strain of herpes simplex virus given after a period of immunization—carried
out by a series of subcutaneous and intraperitoneal injections with the same virus—were
finally injected intracerebrally with the I.L. strain. All survived throughout a 30-day
observation period, whereas 12 controls similarly inoculated all died within 4 to 8 days.

These experiments demonstrated the immunologic similarity of the 2 virus strains. The
higher mortality among the I.L. immune mice tested with the 1166 strain of herpes virus
may be attributed to the much greater virulence of the latter strain for mice. However, the
longer incubation period in those dying is indication of a certain degree of protection.

DISCUSSION

This 20-year-old patient entered the hospital with a 5-day history of symp-
toms and signs related to the central nervous system. No definite localizing signs
developed, and she died of a fulminating illness of 12 days' duration. At autopsy,
the brain was somewhat softer than normal and there was a focal area of softening
in the left temporal lobe. The tissue sections revealed a severe diffuse en-
cephalitis. There were suggestive nuclear inclusions in one section. A virus iden-
tified as a strain of herpes simplex by cross immunity studies was isolated from
the left temporal lobe.
Curiously enough, this is the only case of encephalitis other than those associated with poliomyelitis that has been observed at the Board of Health Laboratory since its establishment in 1904, during which time over 18,000 autopsies have been performed.

A comparison between this case and 3 of the 4 cases mentioned above was undertaken. Because the case reported by Smith and his associates occurred in an infant, it was omitted. Striking similarities were noted in each of the 4 other cases. The disease occurred in previously healthy young adults, all in the third decade of life. It was ushered in by a severe headache, which increased in severity for several days before hospitalization was sought. Following admission, the course was uniformly rapid and terminated fatally in from 8 to 12 days after the onset of symptoms. In no case was there a history of a herpetic eruption.

At autopsy, each brain was described as soft, and had a focal area of softening, occurring in the temporal lobe twice and the occipital lobe twice. The general histologic pattern was similar, with focal necroses, perivascular cuffing and small hemorrhages. In the previously reported cases, nuclear inclusions were found in considerable numbers. In our case, we were unable to find definite inclusions; in one slide, we found suggestive inclusions, but were not able to duplicate this finding, and therefore believed that it might be a staining artifact.

In each of the cases a strain of herpes simplex virus was isolated from brain tissue.

In our case, the possibility that the virus was merely incidental must be considered, inasmuch as definite nuclear inclusions were not found. However, in view of the close similarity between these 4 cases, it appears that this is a valid case of encephalitis caused by herpes simplex virus.

It is suggested that a careful attempt to isolate the herpes virus should be made in those isolated cases of encephalitis that have a fulminant course lasting about 10 days, and in which there is gross focal softening of the brain.

SUMMARY

A case of encephalitis in which a herpes simplex virus was isolated is reported. A detailed account of the isolation is given. The similarity between this case and previously reported cases is noted, both from the clinical and pathologic aspects. In our patient typical nuclear inclusions were not observed.

REFERENCES