VIRUS-B INFECTION OF THE CENTRAL NERVOUS SYSTEM OF MONKEYS USED FOR THE POLIOMYELITIS VACCINE SAFETY TEST

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In 1934, Sabin and Wright[1] isolated a virus from the brain and spinal cord of a patient dying of acute transverse myelitis. The patient had been bitten by an apparently normal rhesus monkey and had become ill shortly thereafter. This virus was called "B-virus" * and has been studied quite extensively. Although, initially, there was no success in transmitting the disease to rhesus monkeys, Sabin[2] was later able to infect them with the virus. In 1935, Sabin and Hurst[3] described the pathologic features of experimental Virus-B infection in rhesus monkeys. They found that animals inoculated intracerebrally developed severe meningitis with lesions developing around the penetrating blood vessels. In one of 7 inoculated animals, there were glial and necrotic foci in the white and gray substance of the brain.

A recent report has described the lesions of natural Virus-B infection in monkeys.[5] The lesions in the central nervous system were minimal, consisting of localized involvement of the pons and medulla. There were glial and lymphocytic infiltrations around the nerve roots of the trigeminal and facial nerves and in the nucleus and tract of the descending branch of the trigeminal nerve and in the solitary tract. It is our belief that this histologic pattern may be altered and increased in severity by artificial means. We wish to report on Virus-B infection as we have seen it in the course of examining histologic preparations of central nervous system tissue of monkeys used in the safety test for poliomyelitis vaccine.[6]

MATERIAL AND METHODS

Monkey Safety Test for Poliomyelitis Vaccine

Rhesus (*Macaca mulatta*) or cynomolgus (*Macaca irus*) monkeys, weighing 4 to 8 pounds and in overt good health, were used. Pre-inoculation blood samples were obtained from the femoral vein. Animals were anesthetized with sodium pentobarbital (15 mg. per lb.) given intraperitoneally; 200 mg. of cortisone acetate were given in the left thigh and calf muscles, and 300,000 units of procaine penicillin were injected in the right deltoid muscle region.

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* Various names have been used to designate this virus; among them, B-virus, Virus-B, *Herpesvirus simiae*,[4] herpes B virus. In this paper the term Virus-B will be used.

Poliomyelitis vaccine was inoculated by the combined intraspinal, intracerebral and intramuscular routes as follows: 0.5 ml. into the lumbar enlargement of the spinal cord, 0.5 ml. into the thalamic region of each cerebral hemisphere and 1 ml. into the gastrocnemius muscle of the right leg. Animals were observed daily for 17 to 19 days for signs of poliomyelitis. At the end of this period, the monkeys were sacrificed by bleeding from the heart, and a 10 ml. blood sample was saved as the post-inoculation specimen.

Necropsy Observations

The spinal cords were removed by the dorsal approach. Blocks of tissue from the lumbar and cervical enlargements and the cerebral cortex at the site of injection were removed aseptically and frozen, in the event that virus isolation studies were indicated. The monkeys were then perfused through the arteries with 10 per cent formalin saline solution.

Initially, histologic examination was carried out on tissues from the lumbar and cervical spinal cord. If lesions were found, tissue from the medulla, pons, midbrain, cerebellum and cerebrum were also studied.

Sections were stained with gallocyanin[7] and with azure-eosin.[8]

Virologic Techniques

Virus isolation attempts were made on tissues from all animals with histologic lesions. Sections of lumbar and cervical cord and frontal cortex were pooled, and a 20 per cent suspension was prepared in Eagle's basal medium containing antibiotic agents.

Rhesus monkey kidney cell cultures and rabbit kidney cell cultures were trypsinized according to methods described by Youngner.[9] Growth medium consisted of Medium 199 with 2 per cent calf serum; maintenance medium was Eagle's basal medium. Cultures were washed 3 times with Earle's balanced salt solution before inoculation.

One tenth of a ml. of the tissue suspension was inoculated into both monkey and rabbit kidney cell cultures containing 1 ml. of maintenance medium. Cultures were incubated at 36° C. and observed daily for 14 days, with sub-cultures being made at the end of 7 days. Supernatant fluids from tubes manifesting cell degeneration were transferred to fresh cultures to confirm the presence of a transmissible agent.

Neutralization tests were performed in rabbit kidney cell cultures against approximately 100 $TCID_{50}$ with specific anti Virus-B serums.

Results

During the period 1957 to 1959, the spinal cords of 6,300 monkeys were examined, and 26 were found to have lesions of various types. In 5 of the 26, the lesions were quite similar. Attempts were made to isolate agents from the spinal cords in all 26 monkeys. This was successful only in the 5 cases which had similar histologic alterations. The agents recovered from these 5 monkeys were all identified as Virus-B by specific type of cytopathogenic effect, neutralization by Virus-B antiserums (4 cases) and, in one instance, by rabbit inoculation.

Histologic Observations

There was a diffuse encephalomyelitis in all 5 monkeys. In the lumbar cord, there was severe necrosis, most marked in the area of inoculation

trauma (Figs. 1 and 2). The architecture of the lumbar cord was destroyed at one or more levels in all the animals. There was a lymphocytic infiltration of the meninges; the nerve roots surrounding the lumbar levels were infiltrated by neutrophils and there was focal demyelinization (Fig. 3).

In the thoracic and cervical cord, damage was much less severe. There were lymphocytic infiltrations around penetrating blood vessels and small glial foci in the gray and white substance (Fig. 4). Neurons were not damaged in the cervical levels, whereas, in the lumbar cord, the necrotizing process had destroyed them.

Lesions were quite extensive in the medulla and pons. At these levels the lesions were midline in all cases and there was marked edema of the tissue with necrosis and a diffuse glial infiltration. The perivascular spaces were densely infiltrated by lymphocytes.

In the pons, lesions were characteristically in the region of the floor of the fourth ventricle. There was a diffuse softening; glial infiltration involved the vestibular nuclei, the medial longitudinal fasciculi, and the nuclei of the spinal tracts of the fifth cranial nerves. This process seemed to extend into the fourth ventricle so that in 4 of the 5 cases there was loss of the ependymal lining (Fig. 5) and in one case there was an inflammatory infiltration within the ventricle itself.

In the midbrain there were small glial foci in the midline and around the aqueduct of Sylvius. Glial infiltrates were also noted in the thalamus, putamen and caudate nuclei. Small scattered glial foci were seen in the temporal and parietal cortex in 2 monkeys. The hippocampus, occipital cortex, amygdaloid and hypothalamus were free of lesions.

Serologic Examination

Pre- and post-inoculation serums were available from 2 monkeys for antibody studies. Both animals had neutralizing antibodies in the pre- and post-inoculation serums at a dilution of 1:256, as determined in the tissue culture neutralization test with one of the Virus-B isolates.

Discussion

In studies by Sabin[2] and others[5,10–14] it has been found that many apparently healthy monkeys have neutralizing antibodies to Virus-B. The antibody levels have varied greatly. Sabin and Hurst[2,10] found levels varying from 1:20 to 1:500. Keeble, Christofinis and Wood[5] tested the serums of 100 rhesus monkeys aged 1 to 2½ years and found that 17 had antibodies equal to or greater than 1:8 when tested against a recent Virus-B isolate. In addition, isolation of this virus from normal monkey kidney cell cultures has occurred on several occasions.[12,14] Thus

it would appear that this virus can remain latent in monkey tissues for long periods of time.

Recently, Keeble and co-workers[5] reported on natural Virus-B infection in rhesus monkeys. These animals had vesicular lip lesions and ulcers of the tongue which healed within 7 days. In 5 of 17 animals Virus-B was isolated from the lesions. The central nervous system tissues of 12 of these 17 were examined histologically, and lesions were found in 10. These, as stated previously, consisted of localized involvement of the trigeminal and facial nerve roots and the solitary tract. Similar central nervous system alterations have been seen in our laboratory previously and have been incorporated in the tabulation of lesions (Category 1) found in the course of the safety testing of monkeys up to 1955.[6] Since virus isolation was not attempted prior to 1955, a specific viral diagnosis cannot be made. Recent attempts to demonstrate intranuclear inclusions in this tissue have been unsuccessful.

The lesions we have described were found in cortisone-treated animals after intraspinal, intrathalamic and intramuscular inoculation of inactivated poliomyelitis vaccine. It would appear that the use of cortisone and the intraspinal inoculation trauma caused the reactivation of a latent Virus-B and the development of the severe lesions that we have described. In support of this is the presence of neutralizing antibodies to Virus-B in the serums of two monkeys which were obtained before and after the inoculation of vaccine.

It is of interest that the lesions described in this report are similar to those found in two fatal human cases.[15,16] The reaction of humans who are markedly susceptible to this infection thus seems to be similar to that in cortisone-treated traumatized monkeys which are naturally somewhat resistant.

Since Virus-B infection is a serious and often fatal disease in man,[2,15–18] considerable caution should be used when handling potentially infected tissues. Virus isolation procedures are required on the central nervous system tissues of safety test monkeys showing histologic lesions.[19] Therefore, if the possibility of Virus-B infection is suggested histologically, laboratory personnel can be advised accordingly.

Summary

Lesions of Virus-B infection of the central nervous system of monkeys used in safety tests for poliomyelitis vaccine have been described. These lesions, in contrast to the lesions reported in natural Virus-B infection, are severe and extensive throughout the central nervous system. It seems apparent that these lesions are related to reactivation of latent Virus-B by the intraspinal inoculation of vaccine and by the use of

cortisone. It is important to recognize these characteristic lesions so that virologists can be warned of the dangers of handling infected tissues.

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LEGENDS FOR FIGURES

Illustrations were prepared from tissues stained with gallocyanin.

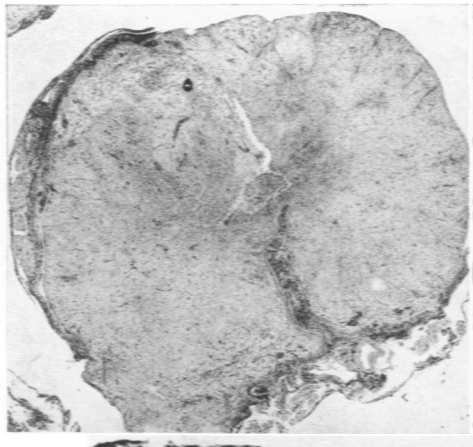
FIG. 1. Lumbar spinal cord of a monkey inoculated intraspinally with poliomyelitis vaccine. There is loss of architecture and necrosis of the entire section. Virus-B was recovered from this tissue. × 9.

FIG. 2. Lumbar spinal cord of a monkey inoculated intraspinally with poliomyelitis vaccine. This is a higher magnification of an area in Figure 1. The extensive necrosis is clearly seen. × 285.

FIG. 3. Nerve roots surrounding the lumbar spinal cord illustrated in Figure 1. There is infiltration by neutrophils, necrosis and demyelinization. × 105.

FIG. 4. Cervical spinal cord of a monkey inoculated with poliomyelitis vaccine. This is the cervical cord of the same monkey shown in Figure 1. There is a small glial infiltration in the anterior horn but no evidence of chromatolysis. × 105.

FIG. 5. Pons of a monkey inoculated with poliomyelitis vaccine. There is loss of the ependymal lining of the fourth ventricle with necrosis and an inflammatory infiltration just below and invading the ventricle. Virus-B was recovered from central nervous system tissue in this monkey. × 105.



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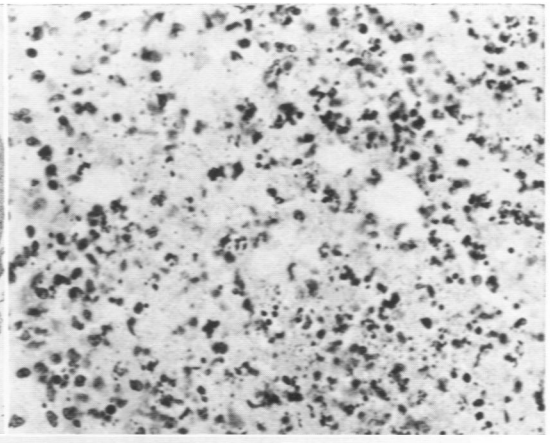

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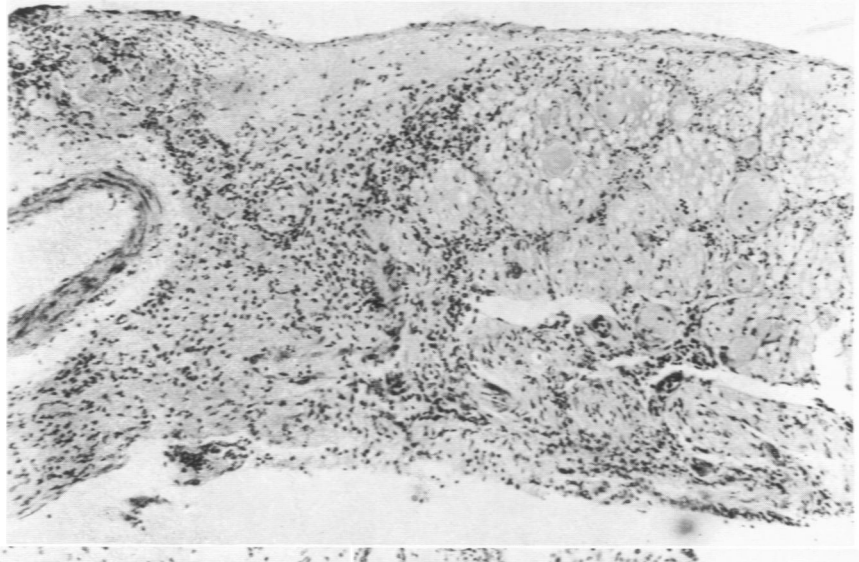

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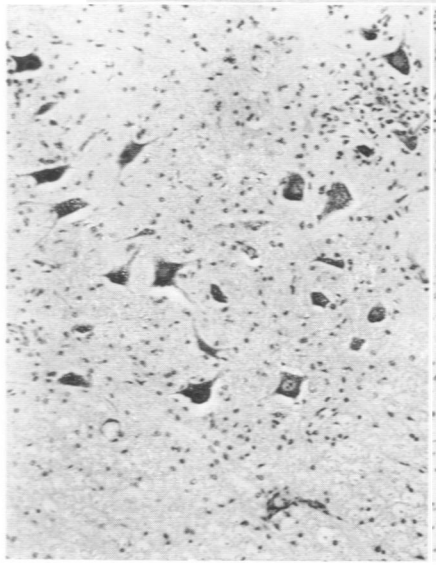

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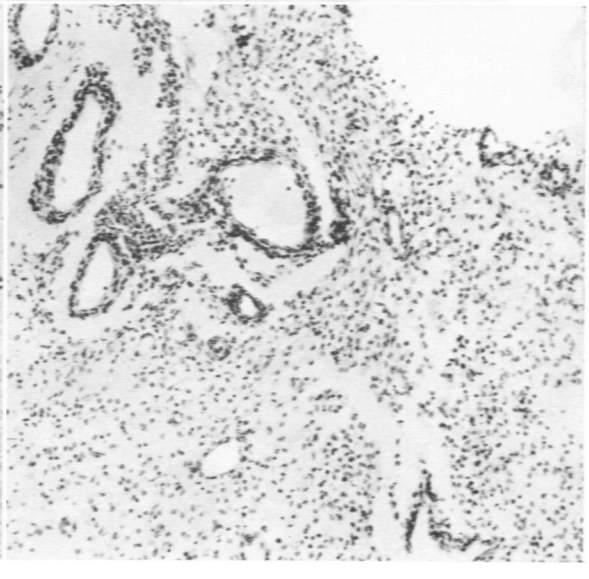

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