VIRUS III ENCEPHALITIS.

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Plates 12 to 14.

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In 1923 Rivers and Tillett (1) described reactions in rabbits which were considered to be induced by the virus of varicella. Subsequently, however, in Rivers' (2, 3) laboratory and also in Swift's (4, 5), it was found that the reactions were caused not by the virus of varicella but by an unknown virus indigenous to rabbits which had been accidentally encountered in the work on chicken-pox and rheumatic fever. Inasmuch as the spontaneous disease caused by the virus has not been recognized, no name other than Virus III has as yet been given this active agent. The term is used merely for convenience and designates the third strain of the virus encountered with which most of the work happens to have been conducted.

The character of Virus III lesions and the presence of acidophilic nuclear inclusions (3) in the injured tissues led Rivers and Tillett to do cross-immunity experiments to determine what relationship, if any, Virus III bears to herpetic virus (2). None was found. Furthermore, in 1924, rabbits were inoculated intracerebrally with Virus III to determine if it was capable of producing an encephalitis (2). A temperature above 104°F., which persisted for a week and which was much more marked in the experimental than in the control animals, was the only abnormality noted. Because of the mild reaction no further study of Virus III encephalitis was made at that time.

From the time of discovery of Virus III, early in 1923, until September, 1926, when emulsions of tissues containing the virus were frozen, desiccated, sealed in tubes, and stored on ice, testicular passages (approximately 300) were made at intervals of 3 or 4 days. In January, 1928, the dried virus was removed from the ice box and testicular passages were resumed. In the course of some experiments,
intracerebral inoculations with the virus were made in rabbits and signs of encephalitis, which were followed by death in a number of instances, were observed. It is with this encephalitis caused by Virus III that the present paper deals.

EXPERIMENTAL.

Methods and Materials.—In sealed tubes on ice, frozen and desiccated Virus III (6) retains its activity indefinitely. Its activity is also maintained for at least 6 weeks if infected testicular emulsions are mixed with equal amounts of glycerol, sealed, and stored on ice. Experiments, however, were always conducted with fresh material. Either emulsions of infected testicles or brain emulsions containing the virus served this purpose. The emulsions were prepared by grinding the infected tissues with sand in a mortar and then adding enough Locke's solution to make a 20 per cent suspension. To free the material from sand, centrifugation at low speed for 1 minute was employed. 2,000 gm. rabbits were used. 0.2 cc. of an emulsion was the amount chosen for intracerebral or for intradermal inoculation, and 1.0 cc. for intratesticular inoculation. Even though it does not so appear in the text-figures, at least two rabbits were inoculated each time the virus was passed. In working with Virus III this is necessary because an immune animal is occasionally encountered which results in the loss of the virus. The sterility of all tissues was tested by means of aerobic and anaerobic cultures. All operations were performed under ether anesthesia. Tissues for histological studies were fixed in Zenker's fluid and stained either with eosin-methylene blue or by Giemsa's method.

In Text-figs. 1 to 3 are outlined the methods of procedure employed in the study of Virus III encephalitis. Although the majority of intracerebral inoculations was made with testicular virus, it will be seen in Text-fig. 1 that the virus propagated itself through three successive intracerebral passages, causing in each instance definite signs of encephalitis, e.g., tremor, ataxia, irritability, circling, salivation, retention of urine, generalized tonic and clonic contractions of the skeletal muscles, or paralysis. From the text-figures it will also be observed that potent testicular virus did not produce signs of encephalitis in every rabbit of certain series and that the results of intracerebral inoculations varied considerably in the different series of animals, i.e., at times all the rabbits died, while on other occasions none showed signs other than a pathological increase in temperature. There is no adequate explanation for this remarkable variation in activity of the virus when inoculated intracerebrally.
TEXT-FIG. 1. Outline of procedure employed in the study of Virus III encephalitis. T indicates site of inoculation (testicle) and also organ emulsion (testicle) used for next passage of virus. B indicates site of inoculation (brain) and also organ emulsion (brain) used for next passage of virus. + indicates occurrence of definite clinical signs of encephalitis. – indicates absence of clinical signs of encephalitis other than fever. Rabbit T 1 was inoculated intratesticularly with glycerolated Virus III 5 passages removed from an animal inoculated with the desiccated material that had been stored on ice more than a year.

TEXT-FIG. 2. Symbols employed in manner similar to that in Text-fig. 1. Rabbit T 25 was inoculated intratesticularly with glycerolated Virus III from Rabbit T 1.

TEXT-FIG. 3. Symbols employed in manner similar to that in Text-fig. 1. Rabbit T 30 was inoculated intratesticularly with desiccated Virus III that had been stored on ice more than a year.
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Rabbit B 5.

March 6. Inoculated intracerebrally with 0.2 cc. of fresh testicular Virus III from Rabbit T 4.


Section through Hippocampal Region.—Slight general thickening of pia-arachnoid with cellular infiltration consisting of many endothelial leucocytes, a few lymphocytes, and rare polymorphonuclear cells. Occasional acidophilic nuclear inclusion (Fig. 7) in endothelial leucocytes. In places, fixed endothelial cells are prominent and rarely contain inclusions; few mitotic figures; slight perivascular infiltration of penetrating vessels. Generalized meningeal hyperemia with a moderate amount of hemorrhage. Few nuclear inclusions in cells that probably are arachnoidal fibroblasts. Hemorrhage in wall of third ventricle, accompanied by very slight reactive changes. Nuclear inclusions in cells of ependyma (Fig. 6) and choroid plexus. In foci in hippocampus are numerous typical nuclear inclusions; the cellular degeneration is associated with very little, if any, reaction.

Section through Cerebellum.—Meningeal lesions similar to those described above; marked cortical hemorrhage. Nuclear inclusions in outer cells of molecular layer. Pycnotic and fragmentary degeneration of nerve cells of granular layer (Fig. 5). Marked hyaline necrosis of Purkinje cells with pycnosis and chromatolysis of nuclei (Fig. 1). No inclusions found in Purkinje cells.

Rabbit B 6.


Section through Hippocampal Region.—Diffuse meningitis; some edema, hemorrhage, and fibrin. Nearly all of the cells are endothelial leucocytes; occasional typical nuclear inclusion. Nothing of importance observed in hippocampus.

Section through Cerebellum.—Meningeal reaction similar to that described above.
Some diffuse and marked focal degeneration of Purkinje cells as indicated by the striking oxyphilic reaction involving both the nucleus and the cytoplasm; chromatolysis, karyorrhexis, and a fading out of the Purkinje cells together with adjacent cells of the molecular layer. Throughout the degenerating areas the lack of a significant degree of inflammatory response is noteworthy.

Rabbit B 7.

March 12. Inoculated intracerebrally with 0.2 cc. of fresh brain Virus III from Rabbit B 6.

Rabbit B 8.

March 12. Inoculation similar to that of Rabbit B 7.

Sections through the Point of Inoculation and also through the Hippocampus.—Diffuse meningitis—the cells chiefly endothelial leucocytes; a slight generalized invasion of peripheral cortical tissue both over the surface of the brain and also beneath the ependyma. Some perivascular thickening due to endothelial cells. Numerous characteristic nuclear inclusions in endothelial leucocytes and some in arachnoidal fibroblasts, especially in those applied along large vessels. Hyaline degeneration of scattered nerve cells in periphery of cortex. About the site of inoculation, hemorrhage, spongy degeneration of the parenchyma, and a confused cellular picture are observed. All cells are swollen; nuclei are reduced to peripheral chromatin rings with central inclusions. No other structure is sufficiently preserved to enable absolute identification of specific cell type, but it appears that all structures contain inclusions—endothelial, nerve, and glia cells. Marked degeneration of hippocampal cells; swollen nuclei; numerous typical nuclear inclusions.
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Rabbit B 29.

April 19. Inoculated intracerebrally with 0.2 cc. of fresh testicular Virus III from Rabbit T 26.

April 20. Temperature 104.8°; animal wild. April 21. Temperature 104.0°; animal wild. April 22. Temperature 105.4°; animal wild. April 23. Temperature 104.5°; tremor. April 24. Temperature 103.0°; marked tremor and ataxia; circling to left; head pulled to left; salivation; twitching of muscles around mouth and of fore legs; occasional generalized tonic and clonic contractions of the skeletal muscles. Animal sacrificed for histological studies. Brain perfused with Zenker's fluid.

The usual meningitis with more polymorphonuclear cells than usually seen. Isolated Purkinje cells show typical hyaline necrosis and nuclear chromatolysis. Nuclear inclusions in ependymal cells lining fourth ventricle and in neighboring glia cells.

Very severe degenerative changes in hippocampus (Fig. 4) with nuclear inclusions in almost every cell (Fig. 2) and extensive spongy degeneration in fiber layers. Slight perivascular hemorrhage. Perivascular infiltration (Fig. 8) consisting of polymorphonuclear cells, lymphocytes, and endothelial leucocytes.

Spongy degeneration and necrosis of nerve cells with typical nuclear inclusion in thalamic region. Epithelial cells of choroid plexus contain nuclear inclusions.

Meningitis with some of the cells showing nuclear inclusions extends to the cervical cord. Slight involvement of periphery of cord and of perivascular sheaths.

Rabbit B 35.

May 19. Inoculated intracerebrally with 0.2 cc. of fresh testicular Virus III from Rabbit T 31.

May 20. Temperature 102.8°; animal seems normal. May 21. Temperature 104.9°; animal seems normal. May 22. Temperature 104.0°; sick. May 23. Temperature 104.0°; worse; tremor. Tremor persisted until May 26, when the rabbit was sacrificed. The brain was perfused with Zenker's fluid. This animal showed no signs of encephalitis except fever, tremor, and a tendency to stand rigidly in one position for long periods of time.

Meninges show lymphocytic, endothelial and plasma cell infiltration, fibrin, and hemorrhage. Nuclear inclusions in endothelial leucocytes and fixed endothelium. Perivascular lymphocytic infiltration along penetrating vessels. Hyaline necrosis with nuclear karyorrhexis of many Purkinje cells. Nuclear inclusions in small nerve cells interspersed between Purkinje cells, in glia cells, and in endothelial leucocytes. Spongy degeneration of associated fiber layer.

Extensive spongy degeneration with nuclear inclusions in nearly every cell in the hippocampus. Some inclusions in peripheral cortical glia cells and in invading endothelial leucocytes.

A few typical inclusions in glia cells in periphery of cervical cord. Slight endothelial cell infiltration.
In addition to Virus III encephalitis this brain also showed lesions of the spontaneous encephalitis described by Wright and Craighead (7).

From the results of the experiments described above it is obvious that the active agent used is capable of producing an encephalitis in rabbits. The question naturally arises, however, as to whether the virus now under investigation is the one originally encountered 5 years ago, or whether it has become contaminated by another virus, e.g., vaccine virus or herpetic virus.

Relation of Present Virus III to Original Virus III.

Repeated experimental passages of the virus in animals that are occasionally spontaneously infected make it impossible to say definitely that the original strain of Virus III has not been contaminated by a new strain of the active agent. A fortunate circumstance, however, enabled us to demonstrate that the present Virus III is identical with, or at least similar to, the original Virus III. In 1925 Rivers and Pearce (8) found that the transplantable rabbit neoplasm described by Pearce and Brown (9) is infected with Virus III and that the virus persists in the tumor and is regularly passed from rabbit to rabbit with each successive transfer of the tumor. In view of these facts, in order to establish a relationship between the present Virus III and the Virus III of 1925 it was only necessary to determine whether the tumor rabbits are refractory to the active agent now being used. For this purpose Dr. Pearce supplied 6 rabbits that had shown good growths of the tumor.

6 tumor rabbits were inoculated intradermally and intracerebrally respectively with 0.2 cc. of a fresh testicular emulsion containing virus of the same generation as Rabbit B 6 (Text-fig. 1). As controls, an animal which had recovered from encephalitis caused by our virus was inoculated in a similar manner, and 2 normal stock animals received intradermal and intratesticular inoculations. The results showed that the recovered animal and the 6 tumor rabbits had no reaction in the skin and evidenced no signs of encephalitis, while the normal animals had a very marked reaction in the skin and a high fever.

From the results of the above experiment one is justified in concluding that in all probability the virus now being used is identical with the original Virus III.
Consideration of Possible Contaminants.

The next question to arise dealt with the possibility that the emulsions containing Virus III were contaminated by the virus of vaccinia, rabies, or herpes.

Vaccine Virus.—One can be quite positive that the emulsions containing Virus III do not also contain vaccine virus, inasmuch as Dr. Pearce’s tumor rabbits, not immune to vaccine virus, are completely protected against the activity of our emulsions. Furthermore, no Guarnieri bodies were observed in cells injured by our active agent.

Rabic Virus.—In making repeated passages in rabbits, it is not inconceivable that one might rarely encounter the virus of rabies. This possibility is very remote. Moreover, Dr. Pearce’s tumor animals are immune to our virus, even when inoculated intracerebrally. If rabic virus were a contaminant, her animals, although immune to Virus III, would die of rabies. This did not occur, nor were Negri bodies found in the brains of rabbits dying of Virus III encephalitis.

Herpetic Virus.—The clinical and pathological picture presented by our animals at times so closely resembles that caused by herpetic virus that one naturally would like to know whether our emulsions are contaminated by the virus of herpes.

5 rabbits were chosen; 1 was a normal stock animal, the other 4 had recovered, 4 to 8 weeks previously, from Virus III encephalitis. Each animal was inoculated intracerebrally with 0.2 cc. of a brain emulsion containing H. F. herpetic virus. All of the rabbits showed the usual signs of herpetic encephalitis and were dead within 7 days.

The above experiment clearly indicates that our virus is not contaminated by herpetic virus.

DISCUSSION.

Virus III is an active, filterable agent indigenous to rabbits (2, 5). It undoubtedly causes a natural infection in these animals, yet the spontaneous disease has not as yet been recognized. As previously shown (1–3), the virus under experimental conditions produces a high fever and characteristic lesions in the cornea, testicles, and skin. Furthermore, within epithelial and endothelial cells of these lesions
acidophilic nuclear inclusions, similar to those seen in varicella and herpes, occur (3).

The studies described in the present paper clearly indicate that Virus III at times is capable of inducing in rabbits an encephalitis which clinically and pathologically closely resembles that caused by herpetic virus. The most interesting fact disclosed by the present work, however, is that the ability of the virus to produce visible evidences of encephalitis seems to vary greatly from time to time (Text-figs. 1 to 3). No adequate explanation of this striking feature is now available. The question as to whether the frequent passages of the virus under experimental conditions, with an occasional freezing and desiccation or storage in glycerol, have altered its activity cannot be answered at present.

The histopathology of experimental Virus III meningoencephalitis in rabbits resembles in part that of herpetic encephalitis. The two diseases are pathologically similar in that both produce a "chronic" type of meningitis, characterized by lymphocytic, plasma cell, and endothelial cell infiltration (Fig. 7); the perivascular sheaths of penetrating vessels may be distended by similar cells (Fig. 8). In both diseases the hippocampal region is profoundly involved; nerve cells, glia cells, and endothelial leucocytes contain characteristic acidophilic nuclear inclusions (Fig. 2); nerve cells undergo hyaline degeneration and seem to disappear rapidly, leaving a spongy, reticulated zone of ground substance (Figs. 2, 4). The adjacent fiber laminae likewise present a soft, spongy appearance and a few polymorphonuclear and endothelial leucocytes infiltrate the region.

In Virus III encephalitis the next most prominent lesion occurs in the Purkinje cell layer of the cerebellum (Figs. 1, 5). These large cells undergo hyaline necrosis accompanied by nuclear chromatolysis, pycnosis, and karyorrhexis; no inclusions have been observed in their nuclei. Inclusions, however, frequently occur in the smaller nerve cells, in glia cells, and in reacting endothelial leucocytes in the immediate vicinity. The necrosis and disappearance of Purkinje cells leave a zone of spongy degeneration between the granular and molecular layers of the cerebellum. In some animals extensive pycnotic degeneration of nerve cells in the granular layer of the cerebellum (Fig. 5) also occurs. Some of the peculiar clinical manifestations of
the disease are probably due to the cerebellar lesions, but in view of multiple foci of brain involvement one should be cautious in relating the clinical picture to lesions in different anatomical foci.

Meningeal edema and hemorrhage, small hemorrhages at the site of inoculation, and foci of hemorrhage in the deep pontine region further complicate the picture. In some rabbits superficial cortical and subependymal encephalitis was noted; here one finds a spongy degeneration, mitosis of glia and endothelial cells, and infiltration by endothelial leucocytes.

It seems that no type of cell escapes involvement. Inclusions have been seen in nerve cells, glia cells, fixed and mobile endothelial cells, arachnoidal fibroblasts, ependymal cells, and in cells of the choroid plexus. Often one cannot distinguish between a glia cell and an infiltrating endothelial leucocyte because of the loss of characteristic nuclear structure; involved nuclei are reduced to a central, often elongated, inclusion surrounded by a clear zone limited externally by a narrow ring of altered chromatin.

The presence of inclusions, the disappearance of cells and ground substance giving rise to the peculiar soft, spongy, reticular appearance, and the minimal amount of inflammatory reaction agree well with the findings in encephalitis caused by other filterable viruses.

SUMMARY.

Virus III, an active, filterable agent indigenous to rabbits, under experimental conditions produces, in addition to lesions in the cornea, skin, and testicles, an encephalitis which is at times quite similar to that induced by herpetic virus. Virus III and herpetic virus, however, are not immunologically related.

REFERENCES.


EXPLANATION OF PLATES.

**PLATE 12.**

**FIG. 1.** Cerebellum. Hyaline necrosis of Purkinje cells. Zenker; eosin-methylene blue. × 500.

**FIG. 2.** Hippocampus. Cellular degeneration; abundant inclusion bodies in nuclei. Zenker; eosin-methylene blue. × 1000.

**FIG. 3.** Site of inoculation. Large granular nuclear inclusion in nerve cell. Zenker; eosin-methylene blue. × 1500.

**PLATE 13.**

**FIG. 4.** Necrosis of hippocampal cells; spongy degeneration of fiber layers; hyperemia and slight cellular reaction. Zenker; eosin-methylene blue. × 115.


**PLATE 14.**

**FIG. 6.** Inclusions in ependymal cells lining third ventricle. Zenker; eosin-methylene blue. × 1000.

**FIG. 7.** Endothelial reaction in cortical meninges. Three nuclear inclusions, a, b, c. Zenker; Giemsa. × 850.

**FIG. 8.** Cortex showing perivascular infiltration. Zenker; eosin-methylene blue. × 130.
Photographed by Louis Schmidt.

(Rivers and Stewart: Virus III encephalitis.)
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