THE BEHAVIOR OF THE VIRUS OF YELLOW FEVER IN MONKEYS AND MICE

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Communicated May 15, 1931

The earlier efforts to find a laboratory animal that would react in any way to inoculation with yellow fever resulted only in repeated failures. Now it appears, rather unexpectedly, that several distinct species are readily susceptible. Stokes, Bauer and Hudson[1] opened an important field of work by the discovery that the rhesus monkey is a very satisfactory animal for the study of this disease. One of the most interesting observations in this new field of work was made by Theiler[2] who showed that white mice die regularly after an intracerebral inoculation of the virus of yellow fever. Mice injected in this manner develop a fatal encephalitis but the liver remains normal whereas the disease in man and monkeys is characterized by extensive necrosis of the liver. Furthermore, Theiler noted that the virus, after repeated passages in mice, loses its virulence for monkeys upon injection in the usual manner into the peritoneum. This loss of infectivity for the monkey obviously makes it necessary to determine whether the strain of yellow fever which was originally inoculated into the mice may have become contaminated or supplanted by some unknown virus existing in a latent condition in stock mice or monkeys. Theiler provided evidence that the encephalitis in mice is due to yellow fever by showing that the serum of patients convalescent from yellow fever affords a measurable degree of specific protection. Subsequently, the observations of Theiler have received full confirmation notably in the laboratories of the International Health Board.

The question of the identity of the disease, producing encephalitis in mice without necrosis of the liver is of fundamental importance in any study of the etiology of yellow fever. Direct proof that the infection in mice is yellow fever was sought in the following experiments by attempting to restore its virulence for monkeys. Also, a series of cross immunity tests were carried out. A typical strain of yellow fever maintained in monkeys and mosquitoes and producing necrosis of the liver was compared with the "mouse virus" which is this same strain of virus modified by repeated passage from brain to brain of mice unless it has inadvertently been supplanted by some unknown virus.

Intracerebral Injection of Monkeys.—A suspension of infective mouse brain which was avirulent for monkeys upon subcutaneous or intraperitoneal inoculation, was injected into the left anterior frontal lobe of the brain of a monkey (*Macacus rhesus*). On the sixth day after injection,

fever developed (104.5°F.) accompanied by an almost complete paralysis of the right side of the body suggesting focal growth of the virus in the brain. Death occurred on the seventh day. The gross anatomical findings were not remarkable. The subcutaneous tissues were distinctly yellow, the liver was red and firm, the stomach and spleen were normal in appearance. Twenty cc. of blood were withdrawn from the heart of this animal just before death and injected intraperitoneally into a normal monkey. No symptoms developed and death occurred $5^1/_2$ weeks later. The autopsy failed to show any cause of death.

Another monkey was injected intracerebrally with infective mouse brain and became suddenly semi-comatose on the fifth day after injection. Mosquitoes (*Aëdes aegypti*) were given an infective feeding at this time. On the following day the monkey was in a dying condition and was sacrificed. The liver was extensively degenerated and occasionally fine acidophilic granules were noted in the nuclei, but there was no necrosis. A normal monkey was bitten by 26 of these mosquitoes, 17 days after their infective feeding but the monkey remained well and susceptible.

Serial Inoculations in Monkeys.—Serial subinoculations in four monkeys were carried out by injection of infective brain to brain, the first monkey of this series being injected with the brain of a monkey dying after intracerebral injection of mouse virus. The infective brain for these inoculations was taken from monkeys either at the moment of death or from animals which were sacrificed in a dying condition, that is, when coma had developed, accompanied by a subnormal temperature such as 94°F. In this series of animals, paralysis developed simultaneously on both sides. There was marked encephalitis but no necrosis of the liver. The first monkey injected with infective monkey brain showed the typical gross changes of yellow fever, namely, well-marked jaundice, extensive black hemorrhage into the stomach and a very yellow liver. It appeared as though the virulence of this mouse strain might be quickly restored to its normal condition by passage through monkeys, but on the contrary, the subsequent animals in this series did not show changes either in the gross or microscopically that were in any way comparable to the ordinary reaction of monkeys to yellow fever.

After passage through 4 monkeys this virus was injected into the brain of 6 mice and all died within 7 to 8 days.

Cross Immunity Tests.—In the course of these experiments five monkeys were inoculated into the brain with infective mouse brain and all died or were sacrificed in a dying condition, usually in 6 or 7 days after injection. This finding enables one to carry out cross immunity tests in two directions. Normal monkeys were injected intraperitoneally with the mouse strain and subsequently tested for immunity by the injection of infective

monkey blood. Conversely, monkeys immune to the typical virus of yellow fever were inoculated intracerebrally with mouse virus.

Intraperitoneal Injections.—Three normal monkeys were injected intraperitoneally with infective mouse brains and all remained apparently well. Two of these monkeys received 2 subsequent injections of mouse brain virus. These three monkeys were then injected with the typical monkey virus and two developed a sharp febrile reaction for one day only, after an incubation period of 3 and of 4 days, respectively. All three remained in excellent condition without any apparent signs of illness whereas 3 controls died typically of yellow fever.

Intracerebral Injections.—Four monkeys which had been immunized to the typical virus of yellow fever were injected intracerebrally with a suspension of infective mouse brain. Two of these animals remained perfectly well whereas their controls died, the sections of the brain showing a definite encephalitis. One of the immunes died on the 7th day after injection as a result of a bacterial abscess which developed at the site of inoculation in the brain. The control of this animal died at the beginning of the fourth day after injection. Histologically, degenerative changes were seen in the liver; the brain showed diffuse encephalitis complicated by a suppurative meningitis. It would appear that this animal was infected by the mouse virus and that death was hastened by an intercurrent meningitis. The fourth of these immune monkeys died 6 days after injection of a typical virus encephalitis. The liver was normal. It is noteworthy that this animal had received no immunizing injection for a period of one year and 8 and $^2/_3$ months.

For comparison with these observations, one monkey immune to typical yellow fever was injected into the brain with the standard virus using infective monkey blood. This animal remained well.

Pathology. Brain.—Histologically, the brains of the monkeys inoculated intracerebrally with the mouse strain of virus usually showed, in varying degree, definite evidence indicating a virus encephalitis. Occasionally nuclear inclusions were seen in the necrotic ganglion cells which were similar but not altogether identical with the inclusions found in infective mouse brains. These examinations were very kindly made by Dr. E. W. Goodpasture.

Liver.—Of five normal monkeys injected into the brain with mouse virus, none showed lesions of the liver comparable to the changes which occur in man or in monkeys dying of typical yellow fever. In one of these monkeys, a moderate amount of necrosis of the liver was found, and in another, the liver was normal. In the remaining three monkeys the liver showed moderate degenerative changes consistent with the earlier changes seen in yellow fever but by no means diagnostic and quite unlike the extensive necrosis seen in monkeys dying in the usual manner.

Of the four monkeys inoculated serially into the brain with infective monkey brain, the first showed marked fatty infiltration of the liver without any degeneration; in the next two, the liver was normal and in the fourth, there was degeneration without necrosis.

Of all the monkeys which received intracerebral injections, there was only one in which hemorrhage occurred into the stomach.

Two control monkeys were injected into the brain with the typical virus of yellow fever using 0.05 cc. of infective monkey blood. In both of these animals, definite necrosis occurred in the liver but no necrosis nor encephalitis was seen in sections of the brain.

Question of Immunization in Man.—Considerable evidence has gradually accumulated which indicates that a mild or even inapparent infection with yellow fever affords much more protection than any injection of killed virus. It is noteworthy that rhesus monkeys can be injected with impunity with the living mouse virus. The monkey appears to be even more susceptible than man to yellow fever and sooner or later the question may come up concerning the suitability of the mouse virus for protective inoculation in man under special circumstances. Such a suggestion would require careful control in two directions, namely, whether the injection of mouse virus in man would be free from danger and whether it would afford adequate protection. Unfortunately the accidental infections of laboratory workers with the mouse strain have not been fully recorded. The symptoms appear to be very mild. However, it is not established that the apparently mild infections without jaundice or albuminuria are free from danger.

Under any normal conditions in the endemic zones of yellow fever it is clear that reliance should be placed on measures for mosquito control. In recent years serious outbreaks of yellow fever have occurred under conditions which did not permit prompt control by anti-mosquito measures. Under special circumstances, it is conceivable that the inoculation of a strain of very low virulence might be attended with less hazard than is involved in the risk of spontaneous infection with a very potent strain of yellow fever.

Summary.—A strain of virus from a yellow fever monkey maintained by repeated passage from brain to brain of mice till it no longer infected monkeys (*M. rhesus*) by ordinary routes of injection was compared with the original strain of yellow fever maintained in monkeys and mosquitoes. Monkeys inoculated into the brain with a suspension of infective mouse brain developed regularly a typical fatal encephalitis but without characteristic changes in the liver. Blood of such animals proved non-infectious for monkeys, in two experiments, one test being made by direct inoculation and the other by means of mosquitoes.

Direct inoculation from brain to brain through a series of 4 monkeys failed to restore the virulence of the strain; obviously this experiment by

no means exhausts the possibilities of restoring the mouse strain of virus to its original condition in which it produces a fatal infection in monkeys after intraperitoneal injection with extensive necrosis of the liver. Such a restoration of virulence would be important both for theoretical and practical reasons.

Two monkeys injected intracerebrally with infective blood of monkeys died characteristically of yellow fever with necrosis of the liver and without the development of encephalitis.

Tests for cross immunity were carried out by injecting normal monkeys intraperitoneally with mouse virus and subsequently testing them with the typical virus of yellow fever. Also monkeys immunized to typical yellow fever were injected intracerebrally with infective mouse brains. Cross protection was very well marked though it was not entirely complete. The intraperitoneal injection of infective mouse brains proved to be a very convenient method for immunizing monkeys against a typical potent strain of yellow fever.

The results of these cross-immunity tests are entirely consistent with the interpretation that the virus in mice is yellow fever and there is no indication that it is contaminated by any secondary virus. However, the amount of data available at present is not overwhelming and there is no urgent need for drawing any altogether final conclusion. In the meantime, a more detailed investigation of these immunological findings is in progress.

* This work was supported by generous grants from the DeLamar Mobile Research Fund.

[1] Stokes, A., Bauer, J. H., and Hudson, N. P., *Amer. Jour. Trop. Med.*, **8**, 103 (1928).

[2] Theiler, M., *Ann. Trop. Med. and Parasit.*, **24**, 249 (1930). *Ibid.*, **25**, 69 (1931)

THE HEAT OF COMBUSTION OF METHYL ALCOHOL[1,2]

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Read before the Academy, April 28, 1931

Within the past few years the heat of combustion of methyl alcohol has been the subject of much discussion on the part of those interested in the reaction involving the synthesis of methyl alcohol from carbon monoxide and hydrogen. Because of its industrial importance, the equilibrium conditions for this reaction have been studied by many investigators, whose data have been more or less concordant.

Several years ago Kelley[3] calculated the entropy of methyl alcohol from his calorimetric measurements of the heat capacity, and combining this with the heat of formation, calculated the free energy of formation of