INFECTION OF RABBITS WITH THE VIRUS OF POLIOMYELITIS.*

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The question whether rabbits succumb to the inoculation of the virus of epidemic poliomyelitis has been disputed. Krause and Meinicke and, later, Lentz and Huntemüller have claimed that rabbits may be infected with the virus, to the effects of which many succumb. On the other hand, Flexner and Lewis, Landsteiner and Levaditi, Römer and Joseph, and Leiner and von Wiesner all failed to produce effects in rabbits by inoculation of tissues of the spinal cord and brain from cases of human and of experimental (monkey) poliomyelitis.

It has now been established beyond peradventure that the monkey is regularly to be infected with the virus of poliomyelitis by means of intracerebral inoculation, with the result that an experimental disease is set up agreeing in all essential clinical and pathological effects with epidemic poliomyelitis in man. Conversely, the inoculation of the virus into the peritoneal cavity, subcutaneous tissue, blood, or other part distant from the central nervous system, is followed much less constantly by infection. The majority of the negative inoculations of the rabbit were made intracerebrally, and the remainder, with rare exception, intraperitoneally. Grown rabbits were usually employed. The inoculated rabbits remained well or succumbed to intercurrent diseases. However, Flexner and

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1*Deutsch. med. Wochschr., 1909, xxxv, 1825; 1910, xxxvi, 693.
2*Ztschr. f. Hyg., 1910, lxvi, 481.
Clark\textsuperscript{5} showed that the poliomyelitic virus would survive for a period of days in the subcutaneous tissues of the rabbit.

Although it has developed that by inoculating portions of the central nervous system of fatal cases of epidemic poliomyelitis into monkeys experimental poliomyelitis is readily produced, it has been found that the cerebrospinal fluid, blood, and other fluids from human cases fail to transmit the infection to these animals. The mesenteric lymph glands were shown by Flexner and Lewis,\textsuperscript{8} in one instance, to harbor the virus.

If, now, we contrast with these facts, that are at present undisputed, the findings of Krause and Meinicke, and Lentz and Huntelmüller, we are confronted with a remarkable discrepancy. These investigators state that the inoculation into rabbits of cerebrospinal fluid, blood, brain, or spleen obtained from human cases of poliomyelitis leads to the death of these animals, and the inoculation of similar tissues obtained from rabbits that have succumbed, into other rabbits, will bring about their death. The results, moreover, were determined in part by the mode of inoculation. When the injections were made into the blood or peritoneal cavity, a greater number of positive results was obtained than when they were made into the brain. Finally, it was ascertained that the effects could be produced in rabbits not only with emulsions of the organs mentioned but also with Berkefeld filtrates prepared from them.

To recapitulate: The monkey is subject to intracerebral inoculation with the central nervous system of human beings succumbing to epidemic poliomyelitis, to which it responds by developing a disease in all respects resembling the human affection. The correspondence includes the microscopical anatomy of the brain and spinal cord. The monkey is little, if at all, subject to inoculation with the cerebrospinal fluid or blood of human cases of the disease. It is claimed by those who have made the successful experiments with monkeys, that rabbits are insusceptible to inoculation with the virus of poliomyelitis, whether derived directly from human cases or indirectly from infected monkeys. Another set of investigators

\textsuperscript{1} Verbal communication.

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claims to have produced a fatal disease in rabbits by inoculating nervous tissue, cerebrospinal fluid, and other materials derived from human cases; in these animals, however, the symptoms of the human affection are not reproduced, and the characteristic histological lesions present in the nervous system of man and the monkey do not occur.

It is obvious, therefore, that, admitting the infection of rabbits to have been produced, it is difficult, if not impossible, to identify the result achieved with epidemic poliomyelitis of human beings or of the monkey. An effort was made to establish the result by retransferring the disease from rabbits to monkeys. Lentz and Huntemüller injected two monkeys with the filtrate of the brain and cord of a rabbit that had succumbed to the injection of cerebrospinal fluid obtained from a recently paralyzed human being, and Krause and Meinicke inoculated two monkeys intraperitoneally with an emulsion of the brain and cord from a rabbit that had been injected from a previously treated rabbit that had succumbed. In no instance did the monkeys develop the characteristic symptoms and lesions of experimental poliomyelitis. The first two monkeys showed so-called recurring transient paralysis at intervals of from two to four weeks. One died of marasmus; the other was etherized during a paralytic attack. The central nervous system failed to show the characteristic lesions, but at most showed "marked injection of vessels, hemorrhages, degenerations and small round cell infiltration of the large ganglion cells." The other two monkeys developed symptoms of nervousness and muscular weakness on the third day after intraperitoneal injection and died on the fourth day. Meinicke⁹ has alluded to the occurrence in these animals of "stasis, hemorrhage, and of diffuse and perivascular infiltration of the central nervous system." The conclusion is inevitable, therefore, that the experimental conditions thus far produced by inoculating the rabbit material into monkeys cannot be identified either with the clinical or pathological effects of experimental poliomyelitis as now known.

EXPERIMENTS WITH RABBITS.

The unsettled status of the problem outlined led to a review of the ground. As available samples of virus we had the MA and K material which has been under constant observation by Drs. Flexner, Lewis, and Clark for nearly two years. This virus, in the form of filtrates prepared from the central nervous system of monkeys, has not diminished perceptibly in activity and is uniformly effective by intracerebral inoculation in monkeys in doses of 0.1 cubic centimeter or less.

The effects produced in rabbits are not very definite. Death tends to occur from the seventh to the eleventh day. Just before death, motor weakness and convulsions have been noted. Our first successful experiment belongs to this class. Seventeen rabbits varying in weight between 450 and 1,250 grams were inoculated either intravenously or intraperitoneally with a monkey filtrate in doses ranging between five and twenty cubic centimeters. On the fifth day after the intraperitoneal injection of the twenty cubic centimeters of the filtrate, one rabbit, apparently perfectly well previously, suddenly developed general convulsive seizures and great motor weakness and died within an hour. The post mortem examination was negative. Cultures and film preparations were made from the blood and organs, but no bacteria were discovered, and no gross lesions of the organs were found. The sixteen rabbits left were observed over a period of two months and remained apparently well.

In repeating and continuing the experiments with rabbits, only young animals weighing from 350 to 550 grams were employed. The brain and spinal cord of a poliomyelitic monkey were mixed with saline solution in the proportion of 1 to 8, thoroughly rubbed up, shaken mechanically for half an hour, and centrifugalized, and the supernatant fluid was passed through a Berkefeld filter. Of the filtrate, from 2.5 to 35 cubic centimeters were injected intravenously or intraperitoneally into the rabbits. In carrying forward the inoculation from rabbit to rabbit, filtrates and emulsions of the brain and cord were chiefly used, but in some instances the spleen, liver, or defibrinated blood was employed. Usually the brain and cord were rubbed up with eighty cubic centimeters of saline solu-
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CHART I.

I = inoculation; D = death; V = intravenous injection; P = intraperitoneal injection; C = intracerebral inoculation.

Unless otherwise specified, rabbit cord and brain were used.
tion and treated as stated above for the monkey tissue; and the spleen and liver were injected as emulsions. The results of the experiments are arranged in graphic form in Chart I.

The chief data observed may be stated as follows: There is no appreciable immediate effect produced by the injections, and nothing is noticeable for some days thereafter. When the animals succumb, death occurs between the eighth and the fifteenth day. It was not possible to determine in all instances the symptoms immediately preceding death, as this might take place during the night. When death occurred during the day the symptoms observed set in suddenly. General convulsions occurring in paroxysms, and, as a rule, tonic in character, attracted attention. The animals became more or less rigid and opisthotonic. Less often the convulsions were clonic, or both types were combined. In the interval between the seizures, more or less pronounced general weakness was evident. Death usually took place in from ten minutes to half an hour after the onset of symptoms. The post mortem examination showed merely a marked hyperemia of the cerebral cortex and no other striking condition of the nervous system or other organs. The histological examination, which was carried out most fully with the central nervous system, yielded nothing definite and characteristic. The typical perivascular infiltrative lesions of the blood vessels of the spinal cord and medulla, the degeneration of the ganglion cells, and the edema, hemorrhage, and cellular infiltration of the gray and white matter, such as are always readily seen in the human and monkey tissues, were wholly absent from the rabbit specimens.

We are convinced, however, that filtrates prepared from the nervous tissues of monkeys having experimental poliomyelitis are not wholly innocuous to young rabbits. Individual susceptibility plays a large part in this species of animal. But certain individuals succumb in a remarkable manner to the injections. Filtrates and emulsions prepared from these rabbits are capable of producing identical effects in still other rabbits. We have not succeeded in accomplishing an indefinite transfer of the experimental disease through rabbits, but we have transmitted it through a series of six rabbits. Whether this represents the limit of transfer with the
particular virus which we have used cannot be stated. It is, of course, possible that the last or seventh series contained no single susceptible individual.

However this may be, the disease thus produced in rabbits cannot be recognized as poliomyelitis. It is significant that it can be produced by Berkefeld filtrates as well as by emulsions of organs which have regularly been found devoid of bacteria. There is one way in which to determine whether these rabbits carry, even if they do not necessarily succumb to, the poliomyelitic virus; namely, to retransfer it to monkeys and thus to reproduce the typical disease and lesions in them. This effort had not been made successfully before. In our experiments, we were dealing with samples of virus that regularly produce characteristic effects in the monkey. We made three such transfers from the second, fourth, and sixth rabbit-passages, all being successful. The injections consisted of filtrates of the brain and spinal cord of the rabbits, prepared in the manner stated above. All the monkeys belonged to the *Macacus rhesus* species.

*Monkey No. 1.*—On March 9, inoculated intracerebrally (ether anesthesia) with 4 c.c. of the filtrate from the second rabbit passage. No symptoms until March 14, when the animal was noted to be somewhat excitable. On the next day, a tremor of the head was present. March 15, flaccid paralysis of the arms and legs appeared. March 17, death.

The post mortem and histological findings were characteristic of experimental poliomyelitis.

*Monkey No. 2.*—On April 1, inoculated intracerebrally with 3 c.c. of the filtrate from the fourth rabbit passage. No symptoms until April 10, when excessive nervousness was noted. April 11, tremor of head present. April 12 flaccid paralysis of arms and legs. April 20, death.

The post mortem and histological findings were characteristic of experimental poliomyelitis.

*Monkey No. 3.*—On April 26, inoculated intracerebrally with 2.5 c.c. of the filtrate from the sixth rabbit passage. April 29, nervousness and excitability were noted. April 30, paralysis of arms and legs. May 2, death.

The post mortem and histological findings were characteristic of experimental poliomyelitis.

**DISCUSSION.**

The experimental results given above would, it seems, leave no doubt that the virus of poliomyelitis can be propagated in certain

**These findings have been described in detail by Flexner and Lewis (Jour. Exper. Med., loc. cit.) and will not be repeated here.**
individual rabbits, at least through a limited series of inoculations. They also show that the virus is not confined to the central nervous system in these animals, but occurs, perhaps in equal amount, in other organs,—certainly in the liver and spleen. This distribution coincides with the absence of localized lesions in any organ in the rabbit, as far as has been discovered. Perhaps a finer study of the histology of the organs may reveal definite lesions of this new experimental disease. But it is certain that nothing resembling the characteristic lesions of the central nervous system in human beings and monkeys has appeared in the rabbit as a result of the inoculation of the strains of poliomyelitic virus thus far employed to infect them. Whether the virus can be adapted to the rabbit so as to bring out the special lesions of localization cannot be predicted. But it would appear that not all strains of the virus can be transmitted successfully even to a small fraction of individuals of all varieties of domesticated rabbits. In this way, probably, is to be explained the failure of Römer and Joseph\(^\text{11}\) to secure any result whatever in their large series of tests.

There is still another aspect of the poliomyelitis question that is affected by these experimental results. It is now established that the virus of the disease can survive and probably even be propagated in a domestic animal that does not show any of the peculiar symptoms of epidemic poliomyelitis as it occurs in man. Conceivably, therefore, some animal, in relation to man, may act as a reservoir for the virus, which only occasionally and under special and peculiar circumstances transmits it to human beings. That such animal reservoirs of pathogenic microorganisms exist in nature has been conclusively proven by the establishment of the connection between infected goats and Malta fever;\(^\text{12}\) and rendered probable in another instance, by reason of the finding that the wild antelope, without itself exhibiting obvious evidences of disease, can readily be infected with the trypanosome of sleeping sickness, from which Glossina may become infected.\(^\text{13}\)

\(^{11}\) Münch. med. Wehnschr., 1910, lvii, 2685.