EXPERIMENTS WITH THE VIRUS OF POLIOMYELITIS*

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(Received for publication, March 3, 1930)

I. Attempted Production of Poliomyelitis in Rabbits

The majority of the experiments reported in this communication are concerned with the attempted production of poliomyelitis in rabbits.

Since Landsteiner and Popper produced acute anterior poliomyelitis in monkeys in 1909, attempts to produce the disease in rabbits have been numerous. The results in most instance have been entirely negative although several investigators have reported paralyses and typical or atypical lesions through several generations of young rabbits. Some recent workers, Fairbrother (1) and Harmon, Shaughnessy and Gordon (2), give comprehensive summaries of the literature, making an extended review unnecessary here. Fairbrother reported his own negative results with intracerebral, intravenous and intraperitoneal injections of young rabbits and his inability to adapt the virus to the rabbit by brain passage. Harmon, Shaughnessy, and Gordon using intraperitoneal and intracerebral injection of young rabbits also had completely negative results.

The ease with which the viruses of vaccinia (Noguchi (3)) and of herpes (Levaditi (4), Gay and Holden (5)) are adapted to the testicle of the rabbit suggested to us that testicular injection and passage might be a favorable method for adapting the poliomyelitis virus to the rabbit organism. The apparent symbiosis of vaccinia and poliomyelitis viruses in the monkey's skin reported by Thomsen (6), suggested an additional factor which might possibly aid in the adaptation.

The following experiments were carried out combining these two suggested aids to adaptation.

* Under a grant from the Milbank Fund for the study of infantile paralysis.
† Work partly completed under tenure of a Fellowship in Medicine from the National Research Council.
Rabbits of about 1500 to 2000 gm. were used for the injections and passages, except in one series of experiments where very young rabbits were used as indicated below. Small or medium sized Macacus rhesus monkeys were used. The poliomyelitis virus used is a moderately virulent strain supplied by Dr. Aycock. The virus, as injected, consisted of the supernatant of a centrifugalized 20 per cent saline sand-ground suspension of monkey cord (less often brain) obtained in the first few days of paralysis. The vaccine virus was a 20 per cent centrifugalized suspension of a Noguchi testicular virus harvested at 4 days.

Attempts were first made to produce poliomyelitis in the rabbit by direct testicular injection of poliomyelitis virus suspension and of mixtures of equal parts of poliomyelitis and vaccine viruses. 1 cc. of the poliomyelitis virus suspension was injected into both testicles of ten rabbits; from eight of which the testicles were removed at periods varying from 1 to 7 days for further passages. In two the testicles were left undisturbed. Locally only a moderate inflammatory reaction occurred. Symptoms occurred in only one animal—a paralysis of the forelegs on the seventh day. The cord of this animal showed only patches of round cell infiltration which were present also in control cords. Three rabbits were injected into each testicle with 2 cc. of the mixture of equal parts of poliomyelitis and vaccinia viruses. None of these animals developed any symptoms; the testicles showed only the usual vaccinal reactions.

Attempts were made to adapt the virus to the rabbit by serial passage through several testicles with final injection into the brain.

20 per cent virus emulsion was injected into a rabbit's testicle which was removed aseptically under anaesthesia in 4 days, ground up with saline and reinjected into the testicle of a new animal. The virus was passed in this manner through four animals. The testicle of the fourth animal was injected into the 4th ventricle of a fifth. No symptoms occurred in the final animal or in any animal of the series. A similar series with final intracerebral injection was also negative. A serial testicular passage through four animals at 3 day intervals in which each injected testicle was injected intracerebrally into a test animal also gave entirely negative results. Mixtures of equal parts of poliomyelitis and vaccinia viruses were tested for adaptation in this same manner in two series without development of symptoms in any animal. The vaccinia virus used had been previously shown not to produce encephalitis.

Incidental to the attempted production of immune serum reported below, a number of rabbits were given repeated combined intraperitoneal and intradermal injections of poliomyelitis virus and of poliomyelitis and vaccine virus mixtures.
Six were given ten 1 cc. intraperitoneal injections and ten 0.1 cc. intradermal injections of poliomyelitis virus during a period of 5 weeks. One rabbit was given twenty such combined injections during 10 weeks. No paralyses or significant symptoms occurred in any.

In a second group nine rabbits were given ten 2 cc. intraperitoneal and ten 0.2 cc. intradermal injections of the poliomyelitis-vaccine virus mixtures. One was given twenty such injections. No significant symptoms occurred in this group.

Since most of the reported successful attempts to produce the disease in rabbits have been made with animals somewhat younger than those used in the above work, a number of experiments were carried out using animals weighing from 500 to 800 gm.

Three animals were injected intracerebrally with 0.3 cc. of a mixture of equal parts of 20 per cent poliomyelitic monkey cord and 20 per cent normal rabbit testicle. The normal testicle was added to the virus because of the apparent enhancement of the virus in monkeys by this tissue as reported below, and because of the enhancement of vaccine virus by testicular tissue reported by Duran-Reynals (11). One of this group showed salivation and a spastic condition of the hind leg in 28 days but recovered. The other two died within 30 days without definite symptoms.

At different times 20 per cent poliomyelitic monkey cord was injected into the testicles of three adult rabbits. These were removed in 24 hours, ground and injected intracerebrally into three groups of young animals in 0.2 and 0.3 cc. amounts. A total of 14 animals were injected in the three groups. Of these one showed definite flaccid paralysis of the hind legs in 28 days. An emulsion of the brain and cord of this animal injected intracerebrally into a monkey produced no symptoms. Three of the animals, two of which died, showed other symptoms including spastic leg conditions, salivation, convulsions and postural abnormalities. The brain and cord of one of the animals showing salivation and convulsions produced no symptoms when injected intracerebrally into a monkey. Seven of the animals died without symptoms, other than diarrhea, at periods from 5 to 27 days. The brain and cord of one of these injected into a monkey produced no symptoms. The remainder of the animals were still living and showed no symptoms after 2 months.

The brains and cords of some of the above animals showing definite symptoms were injected intracerebrally into seven young rabbits. Of these two showed spastic leg conditions at 13 and 28 days respectively. Two died without showing symptoms at 21 and 30 days. Three survived 2 months without symptoms.

Brains and cords of animals having shown symptoms were also passed through the testicles of an adult rabbit for a 24 hour period and again injected intracerebrally into young animals. Four were injected, none of which showed symptoms. Two died without symptoms at 3 days and 23 days.
A control group of young rabbits consisting of seven injected intracerebrally with normal rabbit testicle and five uninoculated was observed through the same period as the above. Many of the symptoms seen in the injected group were seen in the control group. Deaths without observable symptoms were just as numerous in this group—in fact only one animal survived of the seven inoculated with normal testicle. The only differences between the two groups were: first, definite flaccid paralysis occurred in the experimental group which never occurred in the control group; and second, the possibly greater proportion of definite symptoms to the number of deaths without symptoms seen in the virus injected group.

These results in young rabbits, especially in regard to the occurrence of symptoms in control animals, are in striking agreement with the results just reported by Harmon, Shaughnessy and Gordon.

To exhaust the possibilities of producing the disease in rabbits, the gastro-intestinal route of infection was tried.

Three rabbits were given 8 cc. doses of 20 per cent poliomyelitis virus by stomach tube on 2 successive days. In one of these a sterile irritation of the meninges was produced by the injection of 0.3 cc. broth into the 4th ventricle at the time of the second virus dose. Since Burner and Conseil (7) found that chloral or opium injections increase the susceptibility of the brain to vaccine virus, another was given large subcutaneous injections of injectable opium with each virus dose. No symptoms were produced in any of these animals.

Although no disease could be produced in rabbits by testicular injection and passage the survival time of the virus in the rabbit’s testicle was determined.

In other rabbit tissues the virus has been found to survive varying lengths of time: subcutaneous tissues several days, (Flexner and Clark (8)); anterior chamber 23 days, (Levaditi and Danulesco (9)) and in the brain 4 days but not 7, (Amoss (10)). 1 cc. of a 20 per cent poliomyelitic cord emulsion was injected into a rabbit’s testicle and 1 cc. of an emulsion of this testicle made with 5 cc. saline 24 hours later, was injected intracerebrally into a monkey and produced no symptoms. This procedure was repeated with negative results on three occasions. That the virus was not destroyed by the tissue in vitro was shown by injection of a mixture of virus with fresh rabbit testicle in the same proportions. There was an apparent enhancement of the virus action by this mixture similar to that noticed by Duran-Reynals (11) with vaccinia. In spite of this enhancing action, the virus does not survive 24 hours in the testicle. Testicles injected 4 days previously with poliomyelitis virus also gave negative results on injection into monkeys. Similar experiments with poliomyelitis-vaccinia virus mixtures gave no evidence that the vaccine virus lengthened the period of survival.
While poliomyelitis virus causes no disease in rabbits and does not retain its pathogenicity for monkeys when passed rapidly through rabbits' testicles, it was considered possible that the virus so treated might retain its antigenic properties. Virus passed through 2, 3, and 4 rabbits' testicles at 4 day intervals, and repeatedly injected intraperitoneally and intradermally into monkeys produced no immunity. Rabbits' testicles injected with virus from 1 to 7 days previously, injected into 3 monkeys intraperitoneally and subcutaneously in 6 injections at 3 day intervals, gave no immunity. Similar experiments with poliomyelitis-vaccinia mixtures were likewise negative.

II. Attempted Production of Poliomyelitis Neutralizing Antibodies in Rabbits

Attempts were next made to produce poliomyelitis neutralizing antibodies in rabbits by the injection of poliomyelitis virus suspensions and of poliomyelitis-vaccinia mixtures. While horses and sheep have been used rather frequently in attempts to produce specific serum, the only previously recorded attempt with rabbits is that of Tsen (12) who was unable to obtain any evidence of a neutralizing antibody in the serum of rabbits given repeated subcutaneous injections of poliomyelitis virus.

In our experiments three series of rabbits were given repeated intraperitoneal and intradermal injections of poliomyelitis virus and of poliomyelitis-vaccinia mixtures. In the first series three rabbits were given 10 combined intraperitoneal and intradermal injections of poliomyelitis cord during a period of 5 weeks—1 cc. intraperitoneally and 0.1 cc. intradermally; 3 were given 10 injections of poliomyelitis-vaccinia mixtures—2 cc. intraperitoneally and 0.2 cc. intradermally, and 3 controls were immunized only with vaccinia virus. The rabbits were bled from the heart 14 days after the last injection and the sera from each group pooled and tested for neutralizing antibodies. 0.5 cc. serum was mixed with 0.5 cc. 5 per cent virus supernatant, incubated 2 hours at 37°C. and left in the ice box over night. The mixtures were then injected intracerebrally into monkeys. Contradictory results were obtained with two separate tests of the sera of the first series. In one case the serum from the rabbits immunized by the poliomyelitis-vaccinia mixtures protected and the serum from the rabbits immunized by the poliomyelitis alone did not; in the other test the results were reversed. On account of these irregular results, two more series of rabbits were immunized. Five were given 10 intraperitoneal and intradermal injections of poliomyelitis suspensions in the same doses as before
and 7 were given the 10 injections of the virus mixtures as before. Two other rabbits were given 20 injections over a period of 10 weeks—one of poliomyelitis suspensions and the other of poliomyelitis-vaccinia mixture. Neutralization tests on the sera from these two series were carried out at the same time. The sera from the larger series were pooled into small groups and a total of 9 monkeys tested by the injection of serum plus 5 per cent virus mixtures from the two series.

None of the sera in these later series showed any neutralizing power—all the monkeys succumbing to typical poliomyelitis.

Despite the irregular results with the first two tests, it seems certain that an anti-poliomyelitis serum cannot be produced in the rabbit. Definite precipitin reactions have been obtained with normal and poliomyelitis monkey brain material with these sera and it is possible that the irregular apparent neutralizing action is due to the fixation of virus by this precipitation.

III. Infection and Immunization of Monkeys by the Gastro-Intestinal Route

Recent reports of probable milk-borne epidemics by Aycock (13) and the epidemiological studies by Kling (14) indicating that the disease may be water-borne, have made it desirable to reconsider the question of infection by the gastro-intestinal tract. Schultz (15) has recently reported negative results by feeding infected milk to monkeys and has given a brief summary of the literature. Landsteiner and Levaditi (16) were unable to immunize by the gastro-intestinal route.

In our first experiment a small monkey after fasting 18 hours was given 15 cc. of bile by stomach tube. 24 hours later 15 cc. of 20 per cent poliomyelitis virus were given by the same method and at the same time an irritation of the meninges was produced by the injection of 0.3 cc. sterile saline into the 4th ventricle. No symptoms developed. 51 days later the bile and virus injections were repeated to determine if any sensitization had developed—likewise without results. 23 days after this injection the animal was tested for immunity by intracerebral injection and developed a typical, though extremely mild, poliomyelitis. The control developed a severe, fatal type of the disease.

In a second experiment three monkeys were given 4 intrastomachic injections of 10 cc. of 20 per cent virus emulsion preceded 24 hours previously by 10 cc. of ox bile. All the injections were made after at least 18 hours fasting and no food was given for an hour afterwards. The 4 injections were made during a period of 18 days. One monkey died 12 days after the last inoculation as a result of parasitic infestation, and showed no poliomyelitis symptoms or lesions in the cord.
The two remaining monkeys showed no symptoms and were tested for immunity by intracerebral injection of 1 cc. of 20 per cent virus cord 20 days after the last stomach injection. Neither showed any symptoms although three other monkeys inoculated at the same time with the same virus succumbed to severe typical poliomyelitis. On a second test inoculation of 1 cc. of 20 per cent virus 26 days after the first, one of the monkeys developed typical poliomyelitis in 8 days, the other showed only a mild and very much delayed weakness of the legs and still survives without paralysis 42 days after the test inoculation. In this case again several other animals inoculated at the same time with equal amounts of the same virus succumbed to rapid typical poliomyelitis.

The attempts to infect by the gastro-intestinal route were decisively negative in spite of irritation of the tract by bile. Whether or not immunization can be produced by this route is not so decisive, but these experiments give at least a strong suggestion that this may be possible.

IV. No Concentration of Virus in the Cellular Elements of the Blood

The virus of poliomyelitis has not been regularly found in the blood stream of monkeys after the usual methods of infection. Clark, Fraser and Amoss (17) were able to infect by the intracerebral injection of 4 cc. of defibrinated blood taken at the beginning of paralysis on the seventh day after intracerebral injection, but this was in only one case out of ten, and other workers have reported negative or only occasionally positive results. Recently Smith (18) has reported that vaccinia virus may be detected more constantly in the blood stream by separating the white cells, to which the virus is fixed, from the antibody-containing serum. An attempt was made to determine if this is also true for poliomyelitis.

Blood was taken from the hearts of several monkeys at various stages of infection with poliomyelitis. Heparin was added and the buffy coat separated by slow centrifugalization. The white cells from 10 cc. samples of blood, in all cases mixed with a very large proportion of red cells, were injected intracerebrally into monkeys. Blood was taken from three different monkeys at four different stages—from the first appearance of tremors on the seventh day after injection to the stage of final prostration and complete paralysis 11 days after injection. Intracerebral injection of the cells from these four samples into four monkeys produced no symptoms in any, giving no evidence that virus is present in any greater amount in concentrated cell suspensions than in whole blood.
V. Immunization by Neutralized Virus

Römer and Joseph (19) reported that an intracerebral injection of neutralized virus protected against subsequent injections, but Flexner and Lewis (20) were not able to produce immunity by neutralized virus.

A monkey was given 1 intracerebral injection of 1 cc. of a mixture of equal parts of convalescent monkey serum and 5 per cent virus and 4 subcutaneous injections of a like amount of convalescent serum and 20 per cent virus during a period of 30 days. An intracerebral injection of 1 cc. of 20 per cent virus 10 weeks after the last injection produced typical acute poliomyelitis, giving no evidence of any immunization by the serum-virus mixtures.

SUMMARY

1. Efforts to adapt the virus of poliomyelitis to the rabbit organism and to produce poliomyelitis in rabbits by testicular injection and by brain injection after testicular passage produced no evidence that the virus could be adapted in this manner. Suggestive symptoms produced in very young rabbits were duplicated in non-specifically treated and in uninoculated controls. The admixture of a vaccine virus, adapted to the rabbit organism, with the poliomyelitis virus in similar injections and passages did not aid the adaptation. The virus of poliomyelitis did not survive 24 hours in the rabbit testicle—whether alone or mixed with vaccine virus.

Repeated intraperitoneal and intradermal injection of poliomyelitis virus and of poliomyelitis and vaccinia virus mixtures produced no disease in rabbits. Massive doses of concentrated virus by stomach tube in conjunction with meningeal irritation produced no symptoms in rabbits.

2. No neutralizing substances against poliomyelitis virus could be produced in rabbits by the repeated intraperitoneal and intradermal injection of poliomyelitis virus or of poliomyelitis-vaccinia virus mixtures.

3. Although attempts to infect monkeys by intrastomachic injections, after bile irritation of the mucosa, were entirely negative, evidence was obtained that repeated intrastomachic injection after bile irritation may produce an appreciable degree of immunity.
4. No evidence could be obtained that the cellular elements of the blood contain the virus in any greater proportion than the whole blood.

5. One attempt to immunize by neutral virus-serum mixtures was entirely negative.

BIBLIOGRAPHY