HUMORAL ANTIBODIES AND RESISTANCE OF VACCINATED AND CONVALESCENT MONKEYS TO POLIOMYELITIS VIRUS

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The observation has been made frequently (1–5) that monkeys actively immunized with different preparations of the virus of poliomyelitis while developing humoral antibodies, often do not resist either intracerebral or intranasal inoculations of the virus. In this paper we shall present our experiences with the nasal instillation in monkeys which have passed through clinically perceptible attacks of experimental poliomyelitis and the correlation of the effects produced with humoral antibodies present as quantitatively ascertained. The study has practical significance in determining whether a certain concentration of antiviral bodies in vaccinated monkeys has the same value as regards protection to infection as it has in convalescents.

The question of reinfection in convalescent monkeys has been studied by Flexner (6) in relation especially to second attacks of the disease in children. Contrary to accepted views, he has found that reinfection takes place in nasally instilled monkeys, and that the second attack may sometimes be induced by the same although apparently oftener by a foreign strain of virus; and he has also made tests for the presence of humoral antibodies to both kinds of strains in the reinfected animals (personal communication).

EXPERIMENTAL

Reaction of Convalescent Monkeys to Nasal Instillation of Virus.—As early as 1910, Flexner (7) and later others (8a, 9) showed that monkeys convalescent from experimental poliomyelitis are, with only rare exceptions, resistant to intracerebral inoculation of the same strain of virus.

Monkeys inoculated with the Rockefeller Institute strain of mixed virus (M. V.) which was used in this study rarely recover after the development of paralysis; the collection of a suitable number of convalescents for this investigation was therefore no easy task (*cf.* Flexner (7, 10)). Of the nine monkeys studied, five were originally infected with virus by way of the nose and four by intracerebral injection. The intranasal test for susceptibility consisted of two instillations of virus, 48 hours apart. A 10 per cent suspension in saline was used of a mixture of glycerolated cords from at least four monkeys, paralyzed after nasal infection, and 1 cc. was instilled in each nostril. At least three or four normal monkeys received the same virus suspension by the same route, whenever any of the convalescents were being tested. During this study thirty control monkeys were used and all developed poliomyelitis.

Of the nine convalescent monkeys given one or more series of instillations at monthly intervals, six resisted the intranasal tests and three died in a peculiar manner.

Flexner (11) has shown that the bringing of virus into contact with the nasal membrane is never an indifferent process in monkeys and that both normal and convalescent animals respond to its presence with changes in the cerebrospinal fluid consisting of mononuclear pleocytosis and even of globulin; and this response takes place largely independently of the appearance of obvious clinical symptoms of disease. Certain monkeys are highly resistant to the nasal instillation of virus, but these exceptional animals still react with the changes in the cerebrospinal fluid, from which Flexner concluded that "the refractory state, therefore, resides apparently in the nerve cells, the principal seat of usual virus attack—not in the nervous tissues as a whole." The resistance of the animals in our series was measured by the complete absence of fever or other signs of disease as contrasted with the uniform occurrence of paralysis among the control monkeys in each experiment.

The histories of the three animals which succumbed in an unusual manner are as follows:

Macacus rhesus 1-82, the first of the series to succumb, was completely paralyzed after the first nasal infection. It recovered some function, however, and 3 months later was again submitted to nasal instillation of virus. 4 days after the first instillation and within less than 48 hours after the second, it was found dead; the only signs before death appeared to be increased weakness and subnormal temperature (99.2° and 96.7°F.) on the 2nd and 3rd days. The cause of death was not investigated in this case, because the rapid course did not suggest poliomyelitis.

Subsequently, however, two additional monkeys (Nos. 3-07 and 3-26) died in a similar manner after nasal instillation of virus. Monkey 3-26 developed paraly-

sis of only the lower extremities following the original intracerebral inoculation,[1] but remained otherwise well and active with normal temperature (about 102°F.) for 1 month, when it was given virus intranasally. The temperature dropped to 100.2°F. the next day, and to 100°F. on the 2nd day, when it appeared sick and seemed to breathe with difficulty; it was given still another nasal instillation of virus and was found dead the following morning. Necropsy revealed no pathological changes in the lungs or viscera. The olfactory bulbs were tested for virus, but contained none, and microscopic examination of sections of the central nervous system revealed only old poliomyelitis lesions. Monkey 3-07 exhibited complete paralysis of the left arm and some weakness of the other extremities as a result of the original intracerebral inoculation but was otherwise well and active with normal temperature (about 102.4°F.) for 5 weeks, when it was submitted to the same test as monkey 3-26. For the next 2 days it exhibited no change either in temperature or in physical condition. On the 3rd day, or 24 hours after the second nasal instillation of virus, its temperature dropped to 100.7°F.; it appeared ill and had difficulty in breathing. On the 4th day it was almost prostrate, temperature 97.6°F., and respiration exceptionally difficult and of irregular rhythm. It was anesthetized and sacrificed at this stage; the lungs and viscera showed no evidence of disease, the central nervous system showed no gross changes, and microscopically there was evidence of only old poliomyelitis lesions. The olfactory bulbs, thalamic region, pons and medulla were tested for the presence of poliomyelitis virus but none was found. It may be pointed out that another convalescent monkey, No. 4-2, was given the same virus suspension simultaneously with monkeys 3-07 and 3-26, but remained entirely well, and that three normal controls developed typical poliomyelitis after the usual course of fever and within the usual time. None of the other convalescent monkeys tested subsequently by the same procedure exhibited any abnormal signs.

From the evidence presented here one cannot attribute the deaths of these three convalescent monkeys to a second attack of poliomyelitis. Although there are reports of certain reactions in convalescent monkeys (such as a rapid rise in temperature after intracerebral inoculation (12) or rapid death after intrasplenic injection of virus (13) which have been interpreted as allergic or anaphylactic manifestations, one cannot be at all certain that such phenomena played a part in the cases just described. At the same time it is difficult to dismiss these three deaths as merely coincidental.

Neutralizing Antibodies in Convalescent Monkeys.—All the convalescent monkeys were bled at monthly intervals after the onset of paraly-

[1] All such operations were made with the aid of deep ether anesthesia.

TABLE I

Serum Antibodies and Resistance to Nasal Infection of Poliomyelitis Convalescent M. rhesus Monkeys

Monkey No.	Route of primary inoculation and extent of initial paralysis	Time since onset of disease	Extent of paralysis at time of test	Antibodies in serum	Result of nasal instillation of virus*
		wks.			
1-82	Intranasal; paralysis of all extremities; almost prostrate	12	Marked residual paralysis but able to sit up	Not tested	Dead 4th day†
3-07	Intracerebral; complete paralysis of left arm and weakness of other extremities; up and about	3.5	Unchanged	Negative	Not tested
		5	"	" ‡	Prostrate with marked, irregular dyspnea—4th day. Sacrificed†
3-26	Intracerebral; paralysis only of lower extremities. Otherwise active	4	"	"	Dead 3rd day†
42	Intranasal; paralysis right arm and weakness of other extremities	4	Almost complete recovery	"	Remained well
		8	" " "	Positive	" "
		12	" " "	"	" "
7-31	Intracerebral; paralysis of all extremities; almost prostrate	4	Improved; can sit up	Negative	" "
		8	" gets about	Positive	Sacrificed 2nd day
7-20	Intranasal; paralysis of left upper extremity only	8	Almost entirely well	Negative	Remained well
		13	" " "	Positive	" "
7-19	Intranasal; complete paralysis of lower extremities and partial paralysis of upper extremities	8	Sits up; improved function of upper extremities	"	" "
		13	" "	"	" "

43	Intranasal; fever and facial paralysis for few days	4	Entirely well	Not tested	" "
		8	" "	Negative‡	" "
		12	" "	Positive	" "
34-15	Intracerebral; widespread partial paralysis	4	Almost complete recovery	Not tested	Resisted intracerebral inoculation
		8	" " "	Positive	Remained well

* Controls not tabulated; see text.

† See text for complete record.

‡ Tests repeated with same result.

sis and prior to reinoculation, in order to determine whether or not they possessed demonstrable antibodies at the time they were tested for resistance to reinfection (Table I).

The neutralization test was performed in the same manner as that previously used by Olitsky and Cox (2) for demonstrating antibody in the serum of vaccinated monkeys, whose resistance to infection is now being compared with that of the convalescents. In brief, 0.2 cc. of a Berkefeld N filtrate of a 5 per cent suspension of poliomyelitis cords in saline solution was mixed with 0.8 cc. of the serum, incubated 2 hours at 37°C., and overnight in the refrigerator, and the whole mixture injected intracerebrally in a monkey. The amount of virus in this mixture represented approximately twenty minimal infective doses.

It should be noted that none of the sera obtained from five monkeys had any demonstrable antibodies 4 to 5 weeks after the onset of paralysis; at 2 months the sera of only two of six monkeys tested failed to neutralize, while at 3 months these two also exhibited antiviral bodies. Many of the neutralization tests were repeated several times with the same results; hence it is clear that the development of antiviral bodies in convalescent monkeys is generally quite slow and at times may require as long as 3 months to become demonstrable. Reports of the presence of antibody as early as 36 hours after paralysis (8b) should, therefore, be regarded either as exceptional or as the result possibly of misinterpretation of a single test. Leake (14) reported the absence of neutralizing antibodies in a monkey 1 month after the onset of poliomyelitis, and Aycock and Kramer (15) found no antibody in two convalescent sera obtained 4 to 6 weeks after paralysis, although at 6 months after the disease the sera of these animals neutralized the virus.

Antibody in the Preparalytic Stage.—In a report published after the completion of the above experiments, Jungeblut (9) stated that antibodies appear first during the preparalytic stage, disappear rapidly during the onset of paralysis, and then reappear slowly during convalescence. This conclusion is based on the observation that of the sera of nine monkeys in the preparalytic stage, four completely neutralized 0.2 cc. of a 10 per cent virus suspension, one partially neutralized (as reflected by prolongation of the incubation period for more than 14 days), and the remainder failed to neutralize. When paralysis ensued, however, the monkeys which previously had demonstrable

antibodies now showed none. In an attempt to repeat this finding, the sera of six monkeys in the preparalytic stage were tested against 0.2 cc. of 5 per cent virus filtrate (Berkefeld N), but none of them neutralized.

TABLE II

Titration of Antiviral Substance in Sera of Monkeys Which Resisted or Succumbed to Nasal Infection with Poliomyelitis Virus

Source of serum	Amount of serum added to 0.2 cc. of 5% Berkefeld N filtrate*	Result of test
	cc.	
From three convalescent monkeys which *resisted* repeated attempts at reinfection by way of nose	0.8	Neutralization
	0.4	Partial neutralization?—paralysis after 17 days' incubation
	0.1	Neutralization
	0.025	No neutralization
From three vaccinated monkeys which *succumbed* to nasal instillation of virus	0.8	Neutralization
	0.4	"
	0.1	Partial neutralization?—paralysis after 15 days' incubation
	0.025	No neutralization
From one similarly vaccinated monkey which *resisted* nasal infection on four attempts at monthly intervals but succumbed to an intracerebral injection of 0.5 cc. of 5% virus suspension	0.8	Neutralization
	0.1	"
	0.025	No neutralization
Normal monkey sera	0.8	" "
	0.8†	" "

* Total mixture made up to 1 cc. with saline, when necessary, and after incubation injected intracerebrally in a monkey.

† Only 0.1 cc. of a Berkefeld N filtrate of 5 per cent virus suspension was used in this mixture.

Correlation between Antibody and Susceptibility to Reinfection.—It is evident from the results shown in Table I that convalescent monkeys are resistant to reinfection at a time when their sera contain no demonstrable antibody. By the use of the same test, antiviral bodies were readily detected in the serum of vaccinated monkeys which proved to

be fully susceptible to the nasal instillation of poliomyelitis virus (2). It is clear, therefore, that the difference in resistance between convalescent and vaccinated monkeys is not directly related to the content of antiviral bodies. In order to study further the possible quantitative relationship between the humoral antibodies and resistance to nasal infection, the sera of three convalescent monkeys, bled after the development of demonstrable antibodies, were pooled and titrated simultaneously with the pooled sera of three vaccinated monkeys which failed to resist infection. The serum of another monkey (vaccinated at the same time and in the same manner as the other three) which resisted four different intranasal tests at monthly intervals but succumbed to an intracerebral inoculation of 0.5 cc. of a 5 per cent virus suspension was similarly titrated. Decreasing amounts of the various sera were added to a constant amount of virus, *i.e.*, 0.2 cc. of a Berkefeld N filtrate of a 5 per cent pooled cord suspension, the amount employed in all the other neutralization tests. The results, shown in Table II, indicate no appreciable quantitative difference in serum antibody in monkeys which resisted infection and in vaccinated monkeys which succumbed to the same intranasal test dose of poliomyelitis virus.

DISCUSSION

In view of the recently accumulated evidence which indicated that the majority of monkeys, treated with preparations of active virus, are not rendered resistant to nasal instillations of poliomyelitis virus in spite of the fact that they develop readily demonstrable serum antiviral bodies (1, 2, 3, 5), it was desirable to examine by similar methods the resistance and serum antibodies of monkeys recovering from a distinct paralytic attack of the experimental disease.

In recent years investigators who found vaccinated monkeys with serum antibodies and without resistance to intracerebral or intranasal infection with poliomyelitis virus postulated a certain "tissue immunity" as distinct from humoral immunity (1). It was not clear, however, to what extent variations in the quantitative level of antibodies in the serum could account for the difference in susceptibility or resistance to infection. Thus it may have been supposed that convalescent monkeys and those of the vaccinated ones which resisted infection might have had a larger amount of serum antibodies.

In the present investigation, nine convalescent monkeys were tested for susceptibility to infection with poliomyelitis virus by way of the nose. Three of these monkeys succumbed with unusual signs, but careful postmortem study eliminated a second attack of poliomyelitis as the cause of death; the remaining six successfully resisted repeated instillations of virus which in each case produced poliomyelitis in all the control monkeys. Of particular interest was the observation that convalescent monkeys were resistant to reinfection before antiviral bodies were demonstrable in their serum, and that the sera of all the monkeys tested several times 4 to 5 weeks after paralysis contained no demonstrable antibody; all monkeys, however, finally developed antibodies—some of them at 2 months and others not until 3 months after the onset of paralysis. It should be pointed out that by the use of the same test, vaccinated monkeys have been shown to contain readily demonstrable serum antibody at 5 to 6 weeks after the first inoculation without, however, exhibiting any resistance to the same amount of virus instilled intranasally (2). It was furthermore demonstrated that the serum of convalescent monkeys, when antibody finally appeared in it, was no more potent than that of the susceptible, vaccinated monkeys. It is interesting to compare these results with some of those recently reported by Jungeblut (9). His studies differed from these in that the virus was injected intracerebrally. He showed that of twenty-three convalescent monkeys studied at different times after the onset of paralysis, all resisted reinoculation with large doses of virus (no peculiar deaths of the type described here were reported) and this resistance was apparent long before the appearance of antibodies in the serum. It appears, therefore, that the resistance of convalescent monkeys to reinfection with the same strain of virus by either the intracerebral or intranasal routes cannot be correlated with the demonstrable presence of antiviral bodies in the blood. In order to avoid misleading generalizations from this observation, it should be recalled that different viruses may act differently in the same host, and that even the same virus may vary in this respect in two distinct hosts. Thus, the virus of equine encephalomyelitis readily and rapidly induces serum antibodies in *Macacus rhesus* monkeys, most of which do not become resistant to intracerebral inoculation of the virus (16), while in the guinea pig even completely inactivated, formolized vaccines give rise to an ex-

traordinary resistance to intracerebral inoculation (1000 M.I.D. or more) with very little or no antibody in the serum (17).

CONCLUSIONS

1. Monkeys convalescent from a paralytic attack of poliomyelitis develop humoral antibodies slowly; in the present series their first appearance in most was at 2 months and in some not until 3 months after the attack.

2. Convalescent monkeys display resistance to reinfection with the same strain by the nasal route long before antibodies become demonstrable in their serum, in this respect differing from many vaccinated monkeys whose serum neutralizes the virus, while they remain susceptible to nasal infection.

3. When antibodies appear in the serum of resistant convalescent monkeys, they are not quantitatively greater than in the serum of vaccinated monkeys which succumb to infection. As regards resistance to infection, humoral antibodies, therefore, do not have the same significance in vaccinated as in convalescent poliomyelitis monkeys.

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