INTRADERMAL VERSUS SUBCUTANEOUS IMMUNIZATION OF MONKEYS AGAINST POLIOMYELITIS.

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Early experiments of Flexner and Lewis (1), Levaditi and Landsteiner (2), and Römer and Joseph (3) showed that monkeys once recovered from poliomyelitis are immune to subsequent intracerebral inoculations of poliomyelitis virus. This immunity was apparent no matter how slight had been the symptoms of the initial infection. The refractory state was of long duration and was absolute within the limit of infecting doses employed. These same workers noted that sera of convalescent monkeys, when mixed with poliomyelitis virus *in vitro*, rendered the material, otherwise infective, inactive when introduced intracerebrally in test animals.

These observations became the basis for numerous efforts to immunize animals against experimental poliomyelitis. Flexner and Lewis (4) injected monkeys subcutaneously with living active virus, beginning with a dose of 0.05 cc. This amount was given daily for four days and the series was repeated twice with a four day rest period between each individual set of injections. After the last interval the animals received on successive days 0.1, 0.5, and 1.0 cc. of virus, and after one month 5.0 cc. A week's rest period then followed, after which time the animals were tested intracerebrally with 2.0 cc. of fresh Berkefeld filtrate of poliomyelitis virus. Control monkeys which received 0.1 to 0.01 cc. of a similar filtrate intracerebrally developed typical poliomyelitis, whereas the vaccinated animals remained free from symptoms.

Levaditi and Landsteiner (5) attempted to immunize monkeys by a single subcutaneous inoculation of 0.5 cc. of virus suspension previously heated to 50°C. for 30 minutes. They failed to produce any immunity by this treatment. In another experiment glycerinated virus was heated to 50°C. for 2 hours; this heated virus was still active in producing disease when inoculated intracerebrally, but did not infect when given daily in subcutaneous doses of 2 cc. each over a period of one month. Nine days after the last subcutaneous injection two treated monkeys were tested intracerebrally. One of these showed slight prodromal symptoms of

poliomyelitis and the second gave no evidence of disease, whereas the control developed typical poliomyelitis.

Kraus (6) attempted the attenuation of poliomyelitis virus by phenolization and found that virus treated by 1 per cent phenol was rendered ineffective in four days, even when inoculated subdurally. He then endeavored to immunize monkeys by subcutaneous injection of 5 to 10 cc. of virus treated with varying concentrations of phenol for different periods of time. Of fifteen animals subjected to intracerebral test inoculations, twelve were immune. It is interesting to note that three animals immunized with virus treated with 1.5 per cent phenol for five days—a procedure calculated by Kraus to render virus inactive—were completely protected. In a second communication Kraus (7) reports results on two animals, one of which received 5 cc. of fresh virus cord emulsion subcutaneously, followed fifteen days later by 6 cc. of 0.5 per cent phenolized cord; a second monkey received 6 cc. of 0.5 per cent phenolized cord. Ten days later both monkeys resisted a test intracerebral inoculation with paper filtrate, whereas a control developed the typical disease.

Olaf Thomsen (8) gave monkeys daily sub-infective inoculations subcutaneously for twelve days and subsequently at weekly intervals, 0.06, 0.2, 0.4, 1.0, and 2.0 cc. of virus suspension. All animals were then resistant to intracerebral test but the author states that every animal showed symptoms such as excitement, tremor, and ataxia, during immunization. A second group was treated, using considerably smaller immunizing doses; of this series no animal showed symptoms during the immunizing procedure, yet all resisted test inoculation. The initial immunizing dose in this second series was only one hundredth of the estimated intracerebral infecting dose.

Zappert, Wiesner, and Leiner (9) attempted to immunize four monkeys by means of subcutaneous injections of gradually increasing doses of active virus emulsions. During the immunization, two of the animals died of intercurrent infection, one of typical poliomyelitis, and one of a supposedly marantic type of the disease. They attempted to induce an artificial immunity in one animal by the use of phenolized virus. The monkey developed the disease during the treatment designed to immunize against it.

Flexner and Amoss (10) described a so-called immunizing strain of poliomyelitis virus. To free a contaminated glycerinated brain from organisms, the tissue was immersed in 0.5 per cent phenol for a few hours and replaced in glycerine. This procedure was repeated once. The animals were subsequently infected with 1.0 cc. of 10 per cent suspension, their disease running an average eleven day course. In a series of passages the virulence of the strain decreased until a point was reached where the monkeys showed very few symptoms,—such as ataxia, tremor, and slight convulsive seizures—recovered, and were subsequently immune to strong virus.

Abramson and Gerber (11) treated emulsions of brain and cord of poliomyelitic monkeys for four hours with 0.5 per cent formaldehyde; this material was infective

when introduced subcutaneously in monkeys. They then endeavored to immunize by heated virus. On five successive days monkeys were injected with cord emulsion; the emulsion was heated to 55°C. for thirty minutes the first and second day, to 45°C. for thirty minutes the third day, to 37°C. for thirty minutes the fourth, and was used without preliminary heating on the fifth day. The dose was 5 cc. on each day. Three weeks after treatment the animals were bled and their sera tested for its power to neutralize virus. Of eight sera, three neutralized, four led to prolonged incubation period, and one failed. Intracerebral tests indicated that five of the treated monkeys were resistant to three to six minimum lethal doses of virus, whereas three proved susceptible.

In another series of three monkeys, Abramson and Gerber gave daily injections subcutaneously of 5 cc. of 10 per cent cord emulsion previously heated to 55°C. for one hour. On intracerebral test three weeks later, all developed poliomyelitis; of the three sera tested, one monkey showed no symptoms and two a delayed incubation period but eventually developed the disease.

McKinley and Larson (12) inoculated monkeys intracerebrally with 0.15 cc. of filtrate of a mixture of 5 per cent emulsion of castor oil soap and virus emulsion. The animals remained well and later resisted intracerebral inoculation of 0.7 cc. virus filtrate. Four more monkeys received 4 cc. of the virus-soap mixture intraperitoneally; none developed poliomyelitis, whereas a control with virus alone became paralyzed in a typical manner. Eleven days after the intraperitoneal virus-soap treatment, all four monkeys were tested intracerebrally; three remained well and one developed poliomyelitis.

The largest and most varied series of tests of poliomyelitis immunization is that of Aycock and Kagan (13). These investigators attempted to immunize with virus attenuated by various methods. The old experiments of Kraus with phenolized virus were repeated using material treated with 1.0, 0.75, 0.50, and 0.25 per cent phenol. The mixtures were kept for seven days in the icebox. Monkeys were then given four injections every other day of from 8 to 10 cc., beginning with the 1.0 per cent phenolized virus, and ending with the 0.25 per cent. Of four animals so treated, two became paralyzed during the process of vaccination, one failed to resist intracerebral test inoculation, and one resisted. In a second experiment monkeys were injected subcutaneously with virus cords dried over caustic potash from one to twenty-six days. Two of six monkeys became paralyzed during treatment, two failed to show protection on intracerebral inoculation, and two proved resistant. Next, virus cord was exposed to different glycerol-water dilutions (5 to 50 per cent glycerol) for seven months at ice box temperature. Monkeys were injected daily subcutaneously, beginning with virus from 5 per cent glycerol and ending with 50 per cent glycerol. Three animals developed paralysis during immunization; three failed to resist an intracerebral test; one resisted. In another group, virus in agar was introduced subcutaneously in eight animals; the total virus emulsion given ranged from 20 to 96 cc. of 5 per cent suspension in

from three to seventeen injections; two animals became paralyzed during treatment, two failed to show subsequent immunity, and two resisted.

In a fifth experiment virus was introduced intracutaneously in from 1 to 2 cc. amounts but was distributed in 0.05 cc. blebs, thus making from twenty to forty piqures each day of inoculation. The total amount of virus injected ranged from 5 to 76 cc. in six to forty-three inoculations, given during a period ranging from fifteen days to five months. Twelve monkeys were used; one became paralyzed during treatment; one failed to resist intracerebral inoculation; ten resisted one intracerebral test, but of these, two failed to withstand a second such test. Serum from eight resistant monkeys neutralized virus twenty-one times; one monkey's serum protected in one test, although the animal itself was not immune to intracerebral test inoculation.

From the review of the literature, it is apparent that the results of experiments designed to immunize monkeys against poliomyelitis have been inconclusive. Two facts stand out clearly; first, that it is impossible to protect monkeys by the use of killed virus, and second, that a definite though inconstant resistance to poliomyelitis can be brought about by the intradermal and subcutaneous introduction of the living virus. It was therefore deemed advisable to compare the results of the two routes of inoculation in order to gain information as to their relative efficacy. The following experiments were carried out with this point in view.

Experimental.

Eight monkeys (Table I) were immunized by the intracutaneous route, following in general the procedure of Aycock and Kagan. The injections were made biweekly and the total amount of a single day's dosage (1.5 to 2.0 cc. of 5 per cent glycerolated virus) was distributed in some twenty small blebs. The duration of the immunizing period was variable, lasting from three to five months. The total amounts of virus administered ranged from 42 to 66 cc. Before intracerebral test inoculation, all animals were bled in order to test their sera for virus-neutralizing power. The test inoculations were made with fresh virus injected intracerebrally in doses of 0.5 cc. of 5 per cent suspension. During the immunization period all animals were observed daily in order to detect possible abortive symptoms of disease and were exercised to bring out masked weaknesses.

Eight more monkeys (Table II) were treated in an analogous fashion but received their immunizing virus subcutaneously instead of intracutaneously. The amounts of virus used and the time intervals were comparable with those of the intracutaneous series, and bleedings and test inoculations were done in the same manner. Both tests for active immunity and for passive serum protection were rigorously controlled. The results in the two series are best seen in the tables.

DISCUSSION.

The primary purpose of this series of experiments was to determine whether the intradermal or the subcutaneous introduction of poliomyelitis virus was most effective in protecting monkeys against virus inoculation. Reference to Tables I and II shows that the degree of immunity produced is strikingly in favor of the intradermal method. Of the eight animals subjected to that procedure, all but one showed slight symptoms of the disease when tested by intracerebral inoculation of an amount of virus sufficient to cause characteristic poliomyelitis in the controls. No animal, however, developed more than the mildest abortive symptoms, such as tremor or excitement. No definite paralysis developed in any instance, and no subsequent muscle atrophy was observed. These results are sharply at variance with those of intracerebral inoculation of the group of monkeys treated by subcutaneous inoculation of virus. Four of the eight animals of this series developed typical poliomyelitis which progressed to prostration in two instances, and to well-marked paralysis in the other two. The remaining four animals proved to be completely refractory to the intracerebral tests.

During the process of immunization, the animals were closely observed to determine whether or not they developed an abortive form of poliomyelitis which might explain the subsequent immunity to the disease. Wickman (14), during the Swedish epidemic of 1905, noticed a considerable number of cases in man, which showed slight, transient symptoms, without developing the outspoken disease. Caverly (15), in the Vermont epidemic of 1904, saw six children with fever, nausea, and convulsions, whose illness never progressed further. Medin (16) also observed such abortive cases. Aycock (17) mentions the possibility that mild attacks of poliomyelitis are responsible for the development of immunity. In view of these observations we were on the alert to detect slight symptoms referable to the treatment. However, no deviation from the normal was discovered. Subcutaneous inoculation of virus has in our experiments on eight animals failed to produce the disease, although it has given rise to poliomyelitis in the hands of others (Flexner and Lewis, Aycock, and Olaf Thomsen). As evidence of the relative safety of intradermal inoculation of virus, in experiments to be reported, as much as 16 cc. of virus suspension

TABLE I.

Intradermal Immunization.

Monkey	Immunization		Total virus inoculated, 5 per cent suspension	Strain	Intracerebral test			Result of test		Serum neutralization	
	Begun	Ended			Date	Amount	Strain	Tested animal	Control	Test	Control
			cc.								
1	9/21/27	12/27/27	42	M.A.	Died of tuberculosis during immunization						
2	9/21/27	1/19/28	66	M.A.	1/19/28	0.5 cc. 5 per cent suspension	M.A.	No symptoms	Typical poliomyelitis Prostrate in 6 days	Pooled neutralized	Typical poliomyelitis Prostrate in 11 days
3	9/21/27	1/ 3/28	48	M.A.	1/19/28	0.5 cc. 5 per cent suspension	M.A.	Slight excitement			
4	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Slightly slow	Typical poliomyelitis Prostrate in 6 days	Neutralized	Typical polio
5	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Tremor and ataxia; weak deltoid		Neutralized	Typical polio
6	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Slightly slow		Neutralized	Typical polio

7	6/ 8/28	11/ 1/28	56	Aycock	11/20/28	0.5 cc. 5 per cent suspen-sion	M.A.	Slow and excited	Typical polio-myelitis Paralyzed in 8 days	Neutral-ized	Typical polio
								Excitement and tremor			
8	6/ 8/28	11/ 1/28	56	Aycock	11/20/28	0.5 cc. 5 per cent suspen-sion	M.A.			Neutral-ized	Typical polio

TABLE II.

Subcutaneous Immunization.

Monkey	Immunization		Total virus inoculated, 5 per cent suspension	Strain	Intercerebral test			Result of test		Serum neutralization	
	Begun	Ended			Date	Amount	Strain	Test animal	Control	Test	Control
			cc.								
9	10/27/27	1/27/28	45.9	M.A.	2/11/28	0.5 cc. 5 per cent suspension	M.A.	No symptoms	Prostrate 9th day	Pooled neutralized	Paralyzed 17th day
10	10/27/27	1/27/28	42.8	M.A.	2/11/28	0.5 cc. 5 per cent suspension	M.A.	No symptoms			
11	10/27/27	1/27/28	38	M.A.	2/11/28	0.5 cc. 5 per cent suspension	M.A.	No symptoms			
12	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Prostrate 10th day	Prostrate 11 days	Neutralized	Typical polio
13	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Paralyzed 18th day. Recovered		Serum contaminated	
14	3/ 5/28	5/21/28	42	M.A.	5/31/28	0.5 cc. 5 per cent suspension	Aycock	Prostrate 9th day		Typical polio	

15	6/ 8/28	10/16/28	56	Aycock	11/20/28	0.5 cc. 5 per cent suspension	M.A.	No symptoms	Prostrate in 7 days	Not done		
16	6/ 8/28	10/16/28	56	Aycock	11/20/28	0.5 cc. 5 per cent suspension	M.A.	Paralyzed on 9th day. Recovered		Neutralized	Typical polio	

has been given intracutaneously at one time without producing symptoms, while 0.005 cc. of Berkefeld filtrate of virus of the same strain inoculated intracerebrally consistently produced characteristic poliomyelitis in six days.

The question of the degree of protection conferred by the treatment proved to be an extremely interesting one. It has often been observed that different strains of poliomyelitis virus vary markedly in their power to produce the disease in susceptible animals. We therefore attempted to detect degrees of immunity by testing animals by intracerebral inoculation, not only with virus of the strain with which they had been immunized, but also with other strains. Thus monkeys treated with the M.A. strain of virus were tested with a fairly recent virus isolated in Vermont by Aycock, and animals immunized with Aycock strain were tested with the M.A. virus. The difference in the results is well-marked; monkeys treated in exactly the same way proved totally resistant to the relatively weak M.A. virus and not totally immune to the stronger Aycock virus. A group of three animals immunized with M.A. strain is described in Table III. All withstood subsequent intracerebral inoculation with both M.A. and Aycock virus but one of the three developed typical poliomyelitis on inoculation with a very active pooled, mixed virus derived from material of the original M.A. and K. strains which had been preserved in glycerol since 1920 (18, 19, 20).

That the immunity induced in the monkeys in these experiments is relative only, is more strikingly shown by tests employed to determine the power of the sera to neutralize the virus. The results of these determinations are shown in Table IV. The sera of Monkeys 1, 2, and 3 were pooled in one and those of 9, 10, and 11 in a second group. These two mixed sera neutralized, as was to be expected, since on intracerebral test the monkeys had proved resistant. Moreover, Sera 6, 7, and 8, derived from monkeys which had presented definite symptoms of poliomyelitis on intracerebral inoculation, were found also to neutralize completely a small, though ample dose, approximately 50 M.L.D. of a highly active virus filtrate of the pooled mixed virus strain. The results of the neutralization tests of sera 12 and 16 are especially significant. Although the monkeys from which they had come had proved ordinarily susceptible to intracerebral

TABLE III.

Results on Reinoculation.

Monkey	Method of immunization	Immunization ended	Intracerebral test			Result	
			Date	Amount	Strain	Test animal	Control
First intracerebral inoculation							
2	Intradermal M.A.	1/19/28	6/11/28	0.3 cc. 5 per cent suspension	Aycock	No symptoms	Prostrate on 30th day
9	Subcutaneous M.A.	1/27/28	6/11/28	0.3 cc. 5 per cent suspension	Aycock	No symptoms	
10	Subcutaneous M.A.	1/27/28	6/11/28	0.3 cc. 5 per cent suspension	Aycock	No symptoms	
Second intracerebral inoculation							
2	Intradermal M.A.	1/19/28	12/ 5/28	0.2 cc. 5 per cent suspension	Pooled mixed virus	No symptoms	Prostrate on 7th day
9	Subcutaneous M.A.	1/27/28	12/ 5/28	0.2 cc. 5 per cent suspension	Pooled mixed virus	Prostrate on 12th day	
10	Subcutaneous M.A.	1/27/28	12/ 5/28	0.2 cc. 5 per cent suspension	Pooled mixed virus	No symptoms	

TABLE IV.

Serum Neutralizations.

No.	Treatment		Test		Result	Neutralization				Result	
	Dose	Virus	Date	Virus		Virus	Virus treatment	Route	Amount serum	Test	Control
						Intradermal					
1	42	M.A.	Died intercurrent infection			*cc.* 0.3 M.A. Pooled	2 hrs. incubator. Overnight icebox	Icer.	*cc.* 0.9	No symptoms	Typical polio
2	66	M.A.	1/19/28	M.A.	No symptoms						
3	48	M.A.	1/19/28	M.A.	Slight excitement						
4	42	M.A.	5/31/28	Aycock	Slow	0.1 M.V.		Icer.	0.9	No symptoms	Typical polio
5	42	M.A.	5/31/28	Aycock	Tremor, ataxia, weak deltoid	0.1 M.V.		Icer.	0.9	No symptoms	Typical polio
6	42	M.A.	5/31/28	Aycock	Slow	0.1 M.A.		Icer.	0.9	No symptoms	Typical polio
7	56	Aycock	11/20/28	M.A.	Slow and excited	0.3 M.A.		Icer.	0.9	No symptoms	Typical polio
8	56	Aycock	11/20/28	M.A.	Excitement and tremor	0.2 M.V.		Cist.	0.8	No symptoms	Typical polio

Subcutaneous

9	45	M.A.	2/11/28	M.A. 0.5	No symptoms	0.3 Pooled	2 hrs. incubator. Overnight icebox	Icer.	0.9	No symptoms	Typical polio
10	42	M.A.	2/11/28	M.A. 0.5	No symptoms						
11	38	M.A.	2/11/28	M.A. 0.5	No symptoms						
12	42	M.A.	5/31/28	Aycock 0.5	Prostrate	0.1 M.V.		Icer.	0.9	No symptoms	
13	42	M.A.	5/31/28	Aycock 0.5	Paralyzed	0.1 M.V.		Icer.	0.9	Died intercurrent disease. Serum contaminated	
14	42	M.A.	5/31/28	Aycock 0.5	Prostrate	0.2 M.V.		Cist.	0.8	Late polio	Typical polio
15	56	Aycock	11/20	M.A. 0.5	No symptoms	Not done					
16	56	Aycock	11/20	M.A. 0.5	Paralyzed	0.2 M.V.		Cist.	0.8	No symptoms	Typical polio

inoculation of the active pooled virus, their sera neutralized the same potent material. It is interesting to note that in one instance of the 16 animals tested did the serum fail to exhibit neutralizing power.

The results of the serum neutralization tests show that degrees of immunity to poliomyelitis virus exist not only in monkeys but suggest that the same condition exists in man. The employment of relatively small doses of filtrate of a virus strain, whose potency is quite constant, brings out degrees of specific protection in monkeys. Such variations would have been totally obscured by the ordinary means of determining immunity by the intracerebral inoculation of considerable amounts of suspension of virus possessing varying degrees of infective power. It is conceivable that the past failures of certain efforts to induce immunity in monkeys may be explained, not by the inadequacy of the methods employed, but rather by the overwhelming inoculation which the animal was required to withstand, doubtless far greater than that to which any human would be exposed. The intracerebral test inoculation particularly, with its associated damage to nervous tissue, makes demands upon the immune reaction of an animal many times greater than that arising in any natural method of infection.

CONCLUSIONS.

1. The introduction of considerable amounts of living, active poliomyelitis virus into the skin and subcutaneous tissue of monkeys protects the animals against intracerebral inoculations of similar virus material.

2. The degree of protection conferred by intradermal is greater than by subcutaneous injection.

3. During intradermal and subcutaneous inoculations, no local or general pathological signs were observed.

4. The degree of protection produced by the immunization methods used is not absolute, since a percentage of the inoculated monkeys respond to intracerebral injections of highly potent virus.

5. The sera of the animals inoculated intradermally or subcutaneously neutralized poliomyelitis virus *in vitro*, irrespective of the result of intracerebral inoculation, in all except one instance.

6. The power of the serum of treated monkeys to neutralize virus

in vitro is a more delicate test of immunity than is the intracerebral inoculation.

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