ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS*

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In a previous publication (1), it was shown that for each gram of active cord virus, given intracutaneously as an emulsion, 6 cc. of human convalescent serum, injected subcutaneously, was required to protect an animal from paralysis and that some degree of active immunity followed its use. Immunity, unpreceded by symptoms of the disease, was obtained when the serum was given either at the time of the virus administration, or 3 days prior to it, or 3 days subsequently. However, when virus inoculation followed serum administration, it was probably less effective than when it was given simultaneously with, or before, the injection of serum. Monkeys which received virus at the same time as serum, or before it, produced sufficient immunity for 0.95 cc. of their serum to neutralize 0.05 cc. of 5 per cent cord emulsion, containing more than five completely paralyzing doses of virus. Experiments will be described in this paper which were designed to investigate the production of active immunity by mixtures of virus-containing monkey cord and serum from human convalescents, with or without incubation at 37°C., and of cord without serum. The degree of immunity developed is expressed as the number of completely paralyzing doses of virus neutralized by 0.9 cc. of the serum of the treated animals.

Technique

The technique employed was the same as that described in a previous paper (1). Unless otherwise stated, active cord virus, Fl mixed, glycerinated 3 to 8 weeks, and recently collected human convalescent serum, were used.

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EXPERIMENTAL

Experiment 1.—Each of three animals received mixtures of virus and serum, incubated for 2 hours, intracutaneously, Nos. 1-31 and 1-66 receiving 1.5 gm. of cord and 6 cc. of serum while No. 1-65 received 1.5 gm. of cord and 4.5 cc. of serum. Two other animals, Nos. 1-60 and 1-64, received 9 cc. of serum subcutaneously, followed in 3 days by 1.5 gm. of cord, intracutaneously. Monkeys 1-40 and 1-53 received the virus intracutaneously and the serum subcutaneously at the same time; the former was given 1.2 gm. of cord and 7.5 cc. of serum and the latter 1.5 gm. of cord and 9 cc. of serum. Lastly, Monkeys 1-61 and 1-63 received the virus, followed in 3 days by the serum, the former receiving 1.5 gm. of cord and 9 cc. of serum, the latter 1.3 gm. of cord and 7.5 cc. of serum. None of the animals developed any symptoms of poliomyelitis, but three died of intercurrent infection. 2 to 3 months later 0.98 cc. of serum from each animal was tested for protective properties against 0.02 cc. (in Test 1) and against 0.05 cc. (in Test 2) of a 5 per cent emulsion. As the minimal completely paralyzing dose of the virus was 0.01 cc. of 5 per cent emulsion of cord, the tests were made with 2 and 5 minimal completely paralyzing (M.C.P.) doses respectively. One control received incubated mixtures of virus and the serum from Monkey 1-63, which had been given 9 cc. of serum at the time the other animals were inoculated, this being the maximum amount received by the treated animals. A second control received virus and the serum of Monkey 1-48, which had been inoculated with 2 gm. of cord removed from an animal that had been paralyzed 35 days, and whose cord was found, on intracerebral inoculation, to have no infective power.

The course of immunization and the results of the neutralization tests are shown in Table I.

The serum of one animal (No. 1-64), which received serum followed by virus, failed to neutralize, while the serums of the remaining five neutralized 0.02 cc. or 2 M.C.P. doses of virus. Of these five, three serums failed to neutralize while the serum of one monkey (No. 1-63) neutralized 0.05 cc. of cord containing 5 M.C.P. doses of virus. The fifth serum (No. 1-53) either completely, or almost completely, neutralized 5 M.C.P. doses of virus, for it did not prevent the development of symptoms suggestive, but not diagnostic of poliomyelitis. The animal developed irritability, ruffling of the hair and general weakness 24 days later. On the 25th day, although the hind legs appeared weaker than the fore limbs, the cerebrospinal fluid was normal. These manifestations cleared up in 3 to 4 days, and were not diagnostic of poliomyelitis inasmuch as the cerebrospinal fluid was normal and the animal had diarrhea a few days later.

As proven in a previous communication (1), virus following serum

TABLE I

Monkey No.	Cord	Serum	Method	Result	Neutralization Test 1				Neutralization Test 2			
					Serum from Monkey No.	Cord in 5% emulsion	Serum	Result	Serum from Monkey No.	Cord in 5% emulsion	Serum	Result
	gm.	*cc.*				*cc.*	*cc.*			*cc.*	*cc.*	
1-31	1.5	6	Incubated 2 hrs., injected intracutaneously	Died, intercurrent infection								
1-66	1.5	6	" "		166	0.02	0.98	No paralysis	166	0.05	0.95	Paralysis, 8 days
1-65	1.5	4.5	" "	Died, 5 wks., intercurrent infection								
1-60	1.5	9	Serum subcutaneously. Virus intracutaneously 3 days later	Died, 10 wks.								
1-64	1.5	9	" "		164	0.02	0.98	Paralysis, 7 days	164	0.05	0.95	Paralysis, 8 days
1-40	1.2	7.5	Virus intracutaneously. Serum subcutaneously, at same time	Died, intercurrent infection, 10 wks.	140	0.02	0.98	No paralysis	140	0.05	0.95	Paralysis, 8 days
1-53	1.5	9	" "		153	0.02	0.98	" "	153	0.05	0.95	*
1-61	1.5	9	Virus intracutaneously. Serum subcutaneously, 3 days later		161	0.02	0.98	" "	161	0.05	0.95	Paralysis, 12 days
1-63	1.3	7.5	" "		163	0.02	0.98	" "	163	0.05	0.95	No paralysis

Monkey No.	Treatment	Serum from Monkey No.	Cord emulsion 5%	Serum	Result
			cc.	*cc.*	
1-48	2 gm. of cord tissue, intracutaneously	—	0.005	0.995	No paralysis
		1-48	0.01	0.99	Paralysis, 7 days
1-63	9 cc. convalescent serum	1-63	0.01	0.99	" 13 "

* Possible mild attack of poliomyelitis.

administration is less effective than when virus and serum are given simultaneously, or when virus is given before serum. Although Nos. 1-61 and 1-63 were treated in the same manner and although No. 1-61 received more virus than No. 1-63, yet it developed a poorer immunological response to the virus, which suggests that some animals respond to antibody formation better than others.

The antibody is evidently a specific response to the virus, for the control animal (No. 1-48) which received 2 gm. of cord tissue, in which no virus was demonstrable, failed to produce any antibody.

Only one and possibly two out of six animals neutralized 0.05 cc. of 5 per cent cord emulsion containing 5 M.C.P. doses of virus. In other experiments (1), better immunological responses were obtained with smaller quantities of cord and serum, for two out of three monkeys neutralized 0.05 cc. of 5 per cent emulsion, containing more than 5 M.C.P. doses of virus, while the third gave partial neutralization.

The smaller antibody response in these experiments may have been due to a decrease in the activity of the antigen, resulting from a loss of infectivity or to more complete neutralization by the use of a stronger serum. On the other hand, as already pointed out, animals may vary in their immunological responses and so the animals used in Experiment 1, owing to a high incidence of intercurrent infection, may not have responded to the antigen as well as those monkeys used in the previously reported experiments (1). In subsequent experiments information was sought for the elucidation of these possibilities.

Experiment 2.—The strength of the serum used in the previously reported experiments, and that being used in the present experiment, were compared by determining the smallest amount of each, which would neutralize 80 M.C.P. doses of virus. The virus, which was titrated at the beginning and at the end of the experiment, maintained its potency.

The results are given in Table II.

It is evident then that the serum used in Experiment 1 had less neutralizing power than the serum used in the previously reported experiments (1), and so the serum was not responsible for reducing the strength of the antigen.

Experiment 3.—1 gm. and less of the cord used for the previously reported experiments (1) produced infection when administered intracutaneously. In order

TABLE II

Serum	Virus emulsion 5%	Test 1		Test 2		Test 3		Test 4		Test 5		Test 6		Test 7	
		Serum	Result	Serum	Result	Serum	Result	Serum	Result	Serum	Result	Serum	Result	Serum	Result
	cc.	cc.		cc.		cc.		cc.		cc.		cc.		cc.	
Experiment 1	0.2	0.2	No paralysis	0.1	No paralysis	0.05	No paralysis	0.04	No paralysis	0.033	Paralysis, 7 days				
Previously reported experiments	0.2	0.2	" "	0.1	" "	0.05	" "	0.04	" "	0.033	No paralysis	0.03	No paralysis	0.025	Paralysis, 21 days

Controls

Monkey No.	Virus emulsion 5%	Result	Monkey No.	Virus emulsion 5%	Result
	cc.			cc.	
2-32	0.000625	No paralysis	2-94	0.000625	No paralysis
2-33	0.00125	Paralysis, 23 days	2-93	0.00125	Paresis right arm
2-34	0.00250	" 10 "	2-96	0.00250	Paralysis, 6 days
2-46	0.005	" 7 "			

0.2 cc. of 5 per cent virus emulsion = 80 M.C.P. doses of virus.

to determine if the cord being used for the present experiments had equal infectivity, each of five animals received 1 gm. of cord. Four different lots of cord, either as single or pooled specimens, were used. A sixth animal received 1.2, a seventh 1.5 and an eighth 2 gm. of cord.

The results are given in Table III.

At least 2 gm. of cord intracutaneously were necessary to produce infection. Less than this amount failed to infect small young animals as well as older and larger animals. The different cord specimens

TABLE III

Monkey No.	Cord No.	Variations in stage and type of disease	Cord amount	Result
			gm.	
1-93	17, 115, 117, 179, 186, 191	Average incubation period 7.8 days	1	No paralysis
2-00	17, 115, 117, 179, 186, 191	Autopsied immediately after complete paralysis	1	" "
2-09	177, 191, 199, 204, 206	Average incubation period 5.9 days	1	" "
2-23	218	Infected with 80 M.C.P. doses of virus. Long period from onset of paralysis to prostration	1	" "
2-30	218	Autopsied immediately after complete paralysis	1	" "
2-12	188	14 hrs. between complete paralysis and autopsy. Infected with 1 M.C.P. dose of virus	1.2	" "
2-51	218		1.5	" "
2-60	191, 199, 204, 206	Short interval from onset of paralysis to prostration	2	Paralysis, 5 days

were obtained from animals in which the course of the disease varied and from the results obtained it appears that no influence is exerted (1) by long and short incubation periods, (2) by long and short intervals between the onset of paralysis and complete paralysis, (3) by 1 to 14 hours interval between complete paralysis and autopsy, (4) by the animals furnishing the cord specimens receiving 1 or 80 infective doses of virus. Moreover, specimens which had been glycerinated as little as 4 and 19 days respectively, were used, whereas in the earlier experiments the virus had been glycerinated 6 to 16 weeks.

It is obvious then, that at the time of the present experiments, the cord was less infective than that which was used the year previously. Therefore, it is quite possible that the immunological response is a function of cord infectivity and that the weaker cord produced less immunity than the stronger, when used in the same manner.

Experiment 4.—The minimal completely paralyzing intracerebral dose of three of these cord specimens (two pooled cords and one single specimen) was deter-

TABLE IV

Pool 1			Pool 2		
Cord specimens 17, 115, 177, 179, 186, 191 Time of glycerination 1–16 mos.			Cords 191, 199, 204, 206 Time of glycerination 2–4 mos.		
Monkey No.	Cord in 5% emulsion	Result	Monkey No.	Cord in 5% emulsion	Result
	cc.			*cc.*	
2-11	0.00250	No paralysis	2-32	0.000625	No paralysis
*1-68	0.0050	Paralysis, 11 days, left leg	2-33	0.00125	Paralysis, 23 days
2-04	0.01	Paralysis, 5 days	2-34	0.00250	" 10 "
2-05	0.01	" 9 "	2-35	0.005	" 7 "
1-99	0.02	" 6 "			
Cord 218 Time of glycerination 2 mos.			Pool 2 repeated		
2-01	0.000625	No paralysis	2-94	0.00625	No paralysis
2-02	0.00125	" "	*2-93	0.00125	Paresis right arm
2-03	0.0025	Paralysis, 6 days	2-96	0.00250	Paralysis, 6 days

* Paralysis did not extend.

mined by finding the minimal quantity of each, which would cause a complete paralysis within 24 hours after an inoculation period of less than 13 days in monkeys weighing 2.5 to 4 kilos.

The results are shown in Table IV, where it will be seen that the cord virus of Pool 1, containing specimens which had been glycerinated a considerable time (up to 16 months), produced infection with 0.005 cc., but the minimal completely paralyzing dose was 0.01 cc., and that the M.C.P. dose of Pool 2 and Virus 218 was 0.0025 cc. of a 5 per cent emulsion. Nevertheless, these specimens would not infect in a

dose of 1 gm. of cord intradermally and when accompanied by 6 cc. of convalescent serum gave a poor immunity response. The results of the published experiments (1) have shown that 1 gm. of cord and 6 cc. of serum, in various combinations, produce considerable immunity if the virus in the cord is of such a strength that 1 gm. or less infects, when administered intracutaneously.

Experiment 5.—In order to determine the immunological response to a gram of cord of known strength, Monkeys 2-09 and 2-30 each received intracutaneously a gram of Cords 2 and 3 respectively, approximately 8000 M.C.P. doses of virus. Likewise No. 1-93 received a gram of pooled cord Virus 1, approximately 2000 M.C.P. doses of virus. At the same time Monkey 1-75 was given 1 gm. of cord and 6 cc. of serum and No. 1-97 1 gm. of cord and 7 cc. of serum, the cord being given intracutaneously and the serum subcutaneously.

The course of immunization and the results of the neutralization test are shown in Tables V, *a*, and V, *b*.

TABLE V, *a*

Monkey No.	Immunization		Serum	Neutralization Test 1			
	Cord amount	M.C.P. dose virus		Serum from Monkey No.	Cord in 5% emulsion	Serum	Result
	gm.				*cc.*	*cc.*	
2-09	1	0.0025	0	2-09	0.01	0.9	No paralysis
2-30	1	0.0025	0	2-30	0.01	0.9	" "
1-93	1	0.01	0	1-93	0.01	0.9	" "
1-97	1	0.01	7				
1-75	1	0.01	6	1-75	0.01	0.9	Paralysis, 12 days
				Convalescent monkey	0.1	0.9	No paralysis

Controls

Monkey No.	Cord amount of 5% emulsion	Result
	cc.	
2-40	0.000625	No paralysis
2-51	0.00125	" "
2-52	0.00250	Paralysis, 7 days

TABLE V, *b*

Neutralization Test 2				Neutralization Test 3				Neutralization Test 4			
Serum of Monkey No.	Cord in 5% emulsion	Serum	Result	Serum of Monkey No.	Cord in 5% emulsion	Serum	Result	Serum of Monkey No.	Cord in 5% emulsion	Serum	Result
	cc.	*cc.*			*cc.*	*cc.*			*cc.*	*cc.*	
2-09	0.01	0.9	No paralysis	2-09	0.02	0.9	Paralysis, 12 days	2-09	0.02	0.9	Paralysis, 11 days
2-30	0.01	0.9	" "	2-30	0.02	0.9	No paralysis	2-30	0.03	0.9	" 17 "
1-93	0.01	0.9	" "	1-93	0.02	0.9	" "	1-93	0.03	0.9	" 11 "
1-97	0.00250	0.9	Paralysis, 5 days								
1-75	0.00250	0.9	Paralysis, 6 days								
								Convalescent monkey	0.05	0.9	Paralysis, 7 days

Controls

Monkey No.	Cord emulsion 5%	Result	Monkey No.	Cord emulsion 5%	Result
	cc.			*cc.*	
3-05	0.00125	Paralysis, 6 days	3-08	0.0003125	Paralysis, 23 days
3-04	0.00250	" 6 "	3-07	0.000625	" 6 "
			2-83	0.00125	" 6 "

General Findings

In neutralization Test 1, the serums of all except No. 1-97 were tested against 0.01 cc. of 5 per cent cord, containing 4 M.C.P. doses of virus. At the same time 0.9 cc. of monkey convalescent serum was tested against 0.1 cc. of 40 M.C.P. doses of virus. The serum of all three animals which received 1 gm. of cord, alone, neutralized 4 M.C.P. doses of virus, while the serum of No. 1-75, which received 6 cc. of serum along with the gram of cord, failed to do so. The monkey convalescent serum neutralized 40 M.C.P. doses of virus.

Tests 2, 3 and 4 were carried out with a cord whose M.C.P. dose is 0.000625 cc. of 5 per cent emulsion. In Test 2 the serums of Nos. 1-97 and 1-75, which received virus and serum, were tested against 0.00250 cc. of 5 per cent cord emulsion, containing 4 M.C.P. doses, while Nos. 2-09, 2-30 and 1-93 were tested with 0.01 cc. or 16 M.C.P. doses. In Test 3, Nos. 2-09, 2-30 and 1-93 were tested against 0.02 cc. or 32 M.C.P. doses of virus, while in Test 4, No. 2-09 was again tested against 0.02 cc., while Nos. 1-93 and 2-30 were tested against 0.03 cc. or 48 M.C.P. doses of virus. The monkey convalescent serum, which neutralized 40 M.C.P. doses of virus in Test 1, was tested against 0.05 cc. or 80 M.C.P. doses of virus in Test 4.

In Test 2, the serum of No. 1-75, which received 1 gm. of cord and 6 cc. of serum, again failed to neutralize 4 M.C.P. doses of virus and, likewise, the serum of No. 1-97, which received 1 gm. of cord and 7 cc. of serum, failed to neutralize the same quantity of virus. The serums of Nos. 2-09, 2-30 and 1-93, each of which received a gram of cord without serum, neutralized 16 M.C.P. doses of virus.

In Test 3, the serum of No. 2-09 failed to neutralize, while the serums of Nos. 2-30 and 1-93 neutralized 32 M.C.P. doses of virus.

In Test 4, the serum of No. 209 again failed to neutralize 32 M.C.P. doses of virus. The serum of No. 1-93 failed to neutralize 48 M.C.P. doses, while that of No. 2-30, in all probability, almost neutralized 48 M.C.P. doses, inasmuch as paralysis took place after a prolonged incubation period (17 days). The specimen of convalescent monkey serum, which, in Test 1, neutralized 40 M.C.P. doses of virus, failed to neutralize 0.05 cc. of cord, containing 80 M.C.P. doses of virus.

The fact that the serum of No. 2-09 neutralized 0.01 cc. and on two

occasions failed to neutralize 0.02 cc. of the same emulsion, points to the accuracy of the neutralization test.

Monkey 2-09 failed to show as much antibody response as No. 2-30, although they both received the same treatment, (1 gm. intracutaneously of a cord whose M.C.P. dose was 0.0025 cc. of a 5 per cent emulsion), indicating, as had already been pointed out in Experiment 1, that animals may vary in their immunological responses.

It is of interest to note that the serum of Monkey 2-30, which received approximately one-half a skin-infective dose, showed nearly as much immunity as a convalescent monkey. The serum of the convalescent monkey neutralized 40 to 80 M.C.P. doses and that of Monkey 2-30 neutralized 32 to 48 M.C.P. doses.

TABLE VI

Monkey No.	M.C.P. dose	Course of immunization		Neutralization in M.C.P. doses of virus
		Cord	Serum	
		gm.	*cc.*	
2-09	0.0025	1	—	16
2-30	0.0025	1	—	32–48
1-93	0.01	1	—	32
1-97	0.01	1	7	Less than 4
1-75	0.01	1	6	" " 4
Monkey convalescent serum				40–80

These results confirm those of Experiment 1, in showing that the cord in use during these experiments failed to produce much immunological response when used in the proportions of 1 gm. of cord and 6 cc. of serum. The cord without the serum, however, produced considerable immunity. Table VI summarizes the immunological responses to a gram of two specimens of cord, of which the M.C.P. doses were 0.0025 cc. and 0.01 cc. of a 5 per cent emulsion, respectively. The neutralizing power of the serums of these animals is compared with that of convalescent serum.

DISCUSSION

The work described in this paper indicates that an appreciable active immunity can be produced with the virus of poliomyelitis in

either of two ways: either by intradermal inoculation with a subinfective dose of the virus, when it was found that one-half a skin-infective dose conferred immunity; or by various combinations of virus and human convalescent serum, when it was found that one skin-infective dose given intracutaneously, accompanied by a subcutaneous dose of convalescent serum (in the proportions of 0.03 cc. to 80 M.C.P. doses of virus, which proved just sufficient to protect against intracerebral infection) also conferred immunity. The administration by the skin route of the incubated virus-serum mixture, in the same proportions as is innocuous intracerebrally, gave a small degree of immunity. This suggests that the neutralization is incomplete in these proportions; that is to say, some amount of virus remains which is not discernible by intracerebral inoculation in the amounts used, but when 200 times the quantity is inoculated intracutaneously, sufficient virus remains to immunize. The observation that the administration of the serum 3 days previous to the virus prevented immunization and that half a skin-infective dose of virus intracutaneously with one and one-half times the calculated necessary amount of serum subcutaneously, at the same time, or 3 days later than the virus, produced an appreciable immunity, though of lesser degree, indicates that the different routes of administration of serum and virus in different sites play a part in the result obtained. This procedure necessarily prevents the complete action of the serum on the virus and allows of the escape of some virus to immunize the animal. This view is supported by the observation that a subcutaneous dose of one and a half times the amount of serum necessary, allowed the development of measurable though slight immunity. The serum used in this experiment was three-quarters the strength of the other serum used (1) but the proportions were determined by intracerebral protection tests.

It appears then, to immunize effectively with serum-virus mixtures, only just sufficient serum should be used to protect the animal against paralysis.

The known variation in the infectivity of different cord specimens and the known variation in the protective power of individual (2–6), or of pools of convalescent serum (7), requires the titration of each to arrive at a suitably adjusted mixture for immunization.

Since monkey convalescent serum is very much weaker than human

convalescent serum, the detection of immunity in monkeys is liable to be missed unless small amounts of virus are used for test purposes. In the work reported here, it was necessary to use as little as 2 M.C.P. doses of virus (0.00125 cc. of 5 per cent cord suspension) and in strong immunity 32 M.C.P. doses of virus (0.02 cc. of 5 per cent cord suspension) was as much as was neutralized by 0.9 cc. of monkey serum. With human serum, on the average, 80 M.C.P. doses of virus (0.1 cc. of 5 per cent cord suspension) are neutralized by 0.04 cc. of serum (7). It is possible that other workers (8, 9, and others) have failed to detect immunity in monkeys by requiring the neutralization of too large a dose of virus, expecting something comparable to the human conditions.

CONCLUSIONS

1. A single intracutaneous inoculation with a subinfective dose of the virus of poliomyelitis produces considerable immunity.

2. Virus-serum combinations produce an appreciable immunity, providing just sufficient serum is used to protect the animal from paralysis. If there is an excess of serum, the degree of immunity is considerably reduced.

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