

## VIRUSES OF POLIOMYELITIS

### AN IMMUNOLOGICAL COMPARISON OF SIX STRAINS\*

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During the past 5 years we have collected 6 strains of the virus of poliomyelitis and we have observed certain characteristics which differentiate some from the others.

Two of these strains (namely those isolated in New York and Connecticut in 1931) have already been compared with another pair of them representing more widely known strains of the virus (1). It has seemed justifiable, however, to pursue the comparisons further because the strain differences are considerable and poorly understood.

In poliomyelitis there is as yet no standard or recognized method whereby strains of the virus may be classified. Their properties are manifold and there is no indication as to which is of the greatest taxonomic importance. Thus, an outstanding property of a given strain is that of maximum virulence for the monkey on intracerebral inoculation. However, virulence by this route does not necessarily parallel virulence by the nasal or the cutaneous routes, to which latter fact one of our strains bears witness (2). But for purposes of classification, besides comparing virulence by different routes of inoculation, it is obvious that one should use the immunological approach, for it is generally the method of choice in classifying substrains of infectious agents. It has, nevertheless, been evident from the start that certain features of the immunology of experimental poliomyelitis render this approach difficult. It is recognized, for instance, that although

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sera of convalescent monkeys usually effect neutralization, irregularities have been reported (3-5). It is also recognized that the vaccination of monkeys with the virus results not only in the irregular production of immunity to infection, but in the irregular production of humoral antibodies, and, furthermore, that these two responses do not necessarily go hand in hand as the reports, among others, of Olitsky and Cox (6), Sabin and Olitsky (5), Hudson, Lennette and Gordon (7) and Kramer *et al.* (8) bear witness. In spite of these obvious objections one gathers, from Aycock and Kagan (9) and Stewart and Rhoads (10), that the artificial immunization of monkeys appears to afford the best method available for the immunological comparison of strains, and, moreover, it has been successfully used by Kramer (11).

Consequently, following this method we vaccinated monkeys with the virus and later collected the presumably immune serum. This was saved until sera representing each strain were in hand. Then after a lapse of 12 to 18 months the appropriate neutralization tests were done. In the interim the vaccinated animals were tested by a series of intracerebral inoculations for resistance to homologous and heterologous strains. This last took 12 months to complete and the outcome was difficult to interpret, but the results of the neutralization tests seem reasonably clear and as such they form the subject of this report.<sup>1</sup>

#### *Methods*

*"Immunization" or "Vaccination" of Monkeys.*—6 groups of from 2 to 4 monkeys each were inoculated intracutaneously with each of our 6 strains of virus. At least 1 animal was inoculated with live virus and, following the suggestion of Brodie (12), 1 animal with formalinized virus. This was prepared in saline from a 20 per cent suspension of spinal cord ground with sand in a mortar. The suspension was centrifugalized at moderate speed, 1600 R.P.M., for 6 to 8 minutes, and to 1 cc. of the opalescent supernatant fluid 1 cc. of 0.2 per cent formalin (0.08 per cent formaldehyde) in saline was added and the mixture was allowed to stand in the ice box for 24 hours before using.

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<sup>1</sup> The results of the series of intracranial reinoculations appear in Appendix B but not in the body of the paper because they did not yield information of value for the immunological classification. They are presented in graphic form in Appendix B and described briefly there in order to show that they do not furnish data contradictory to the pattern of the neutralization tests.

Live virus was given in saline as a 10 per cent suspension prepared within 2 hours of inoculation.

In each instance 2 cc. of the 10 per cent suspension (either formalinized or live) were inoculated in 10 piqûres into the shaved abdominal skin of *rhesus* monkeys. Each animal being immunized received 2 such doses at an interval of 2 weeks. Samples of blood to be used for testing for the presence or absence of neutralizing substances were taken prior to the 1st vaccinating dose and 2 weeks after the 2nd dose.

*Description of Strains.*—6 strains of the virus of poliomyelitis were used. In describing them we realize that the respective virulence of each does not represent a fixed quantity and that no satisfactory standards of virulence in experimental poliomyelitis have been set up. When certain of our strains had not been passed for several months, their virulence diminished and sometimes massive doses were necessary to recover them.

*McC. Strain, Los Angeles, California, 1934.*—This “human”<sup>2</sup> strain was recovered from the nasopharynx of a child during a mild abortive attack of poliomyelitis; the isolation and first few passages have been described (13). Material used for vaccination and subsequent intracerebral testing of the vaccinated monkeys came from a single monkey (C 6-7, Dec. 3, 1934)<sup>3</sup> and represented the 3rd passage of the strain. The dose (for intracerebral inoculation) was 0.5 cc. of a 10 per cent suspension. The experimental disease produced by this and subsequent passages was definite, though not severe. The incubation period was from 3 to 5 days, the febrile period from 3 to 6 days and the animals seldom developed complete paralysis of all 4 limbs. A few attempts to infect monkeys by the nasal and cutaneous routes proved unsuccessful.

During the interim between preparing sera and performing the neutralization tests this strain died out, and recourse was had to another line of passage which had been carried on in another laboratory.<sup>4</sup>

Eventually 8th passage material (monkey 4-44, Mar. 23, 1936) was used in the neutralization tests. In respect to incubation period and type of experimental disease produced, this passage behaved the same as the earlier ones. The dose employed consisted in equal parts of (a) a 10 per cent virus suspension, and (b) serum to be tested. The mixtures were held for 2 hours in the incubator (37°C.) and inoculated intracerebrally in 0.5 cc. amounts.

<sup>2</sup> There is no standard definition of “human” and “passage” in experimental poliomyelitis. We have taken human to refer to strains not far removed from man and which usually do not lead to death of the monkey; passage to designate those strains which are well established in the monkey and which usually give rise to fatal experimental infection.

<sup>3</sup> In this and similar notations the date, Dec. 3, 1934, refers to the day the cord was harvested.

<sup>4</sup> We are indebted to Dr. Leslie T. Webster of The Rockefeller Institute for Medical Research for this material.

*Wfd. Strain, Los Angeles, California, 1934.*—This human strain was recovered from the medulla and spinal cord of a girl dead on the 6th day of poliomyelitis. Material for vaccination represented the 3rd passage (monkey C 5-5, Nov. 10, 1934). Material for subsequent intracerebral testing of vaccinated monkeys represented the 3rd passage (monkey C 5-5), the 4th passage (monkey C 1-03, July 28, 1935) and the 5th passage (monkey C 1-08, Aug. 14, 1935). Mention (2) has been made of the high intracutaneous infectivity of this strain; on subsequent passage it has produced experimental poliomyelitis more consistently when injected intracutaneously than has been noted with any of our other strains. The dosage used for intracerebral inoculation and the type of experimental disease produced were the same as with the McC. strain.

For neutralization tests 6th passage material was used (monkey C 1-20, Nov. 24, 1935). The dose was the same as in the neutralization test with McC. strain.

*We. Strain, New Haven, Connecticut, 1931.*—This human strain was recovered from the nasopharynx of a child suffering from a mild abortive attack of poliomyelitis; the isolation (14) and early passages (15) have been described. Material for vaccination and subsequent intracerebral testing of vaccinated monkeys represented the 9th passage (monkey C 7-4, Dec. 11, 1934). The experimental disease produced by this strain generally has been mild and characterized by features which recall those of the McC. and Wfd. strains.

For neutralization tests, material from the 9th passage was used (monkey C 1-25, Dec. 14, 1935) in the same dose as with the McC. and Wfd. strains.

*Flexner Strain, New York, 1931.*—This "passage" strain was recovered from the medulla and spinal cord of a fatal human case in the epidemic of 1931 in New York.<sup>5</sup> When used for vaccination the strain was in its 11th passage (monkey C 8-1, Jan. 24, 1935). Material for subsequent intracerebral testing of vaccinated monkeys represented the 11th passage (monkey C 8-1) and the 12th passage (monkey C 1-06, Aug. 4, 1936). The intracerebral test dose was 0.5 cc. of a 0.5 per cent suspension. During the early passages this strain had maintained an intracerebral virulence comparable to that of the We. strain and at that time was considered as a human strain (1, 15), but by the 10th passage the virulence of the Flexner strain became enhanced in that it generally gave rise to extensive paralysis and often ultimately to death of the infected animal.

For neutralization tests, 15th passage material was used (monkey 4-91, May 9, 1936). The dose consisted of 0.5 cc. of equal parts of (a) 1 per cent virus, and (b) serum to be tested (10 per cent virus used June 1 proved too strong).

*Aycock Strain, Vermont, 1921.*—This well known and virulent passage strain was received in 1931.<sup>6</sup> It was recovered in the fall of 1921 from a case in Vermont and has been subjected to many more passages in the monkey than any of the

<sup>5</sup> This strain was received by us in 1932 through the kindness of Dr. Simon Flexner of The Rockefeller Institute.

<sup>6</sup> This strain was received through the kindness of Dr. W. L. Aycock of The Harvard Medical School.

strains mentioned above. The exact number of passages is not known, "perhaps a hundred" (16). The experimental disease which followed its use was severe and generally fatal and, in this respect, was similar to that produced by the Flexner and Park strains.

For vaccination, material from monkey C 8-2 (Jan. 29, 1935) was used. For subsequent intracerebral testing of vaccinated monkeys, material from C 8-2 and from the next passage (monkey C 1-07, July 29, 1935) was used in a dose of 0.5 cc. of a 0.5 per cent suspension.

For the neutralization tests material from another passage was used (monkey 4-89, May 7, 1936). The dose consisted of 0.5 cc. of equal parts of (a) 1 per cent virus suspension and (b) serum to be tested; as in the Flexner strain.

*Park Strain.*—This highly virulent passage strain<sup>7</sup> was received in 1931 when it had already been subjected to many passages in the monkey. It is a mixed strain and so the date of its primary isolation and the actual number of passages is unknown. It caused extensive paralysis of almost all infected monkeys and there were very few survivors.

For vaccination, material from monkey C 8-0 (Jan. 22, 1935) was used. For subsequent testing of vaccinated monkeys, material from C 8-0 and from the next and the 2nd following passages (monkey C 1-05, July 30, 1935, and monkey C 1-21, Nov. 19, 1935) was used. The dose varied from 0.5 cc. of 10 per cent to 0.5 cc. of 0.1 per cent suspension. For the neutralization tests, material from a later passage was used (monkey 5-10, June 9, 1936). The dose consisted of 0.5 cc. of a mixture of equal parts of (a) 0.2 per cent virus and (b) the serum to be tested.

Comparative neutralization tests with human sera have been reported already wherein the We., Flexner, Aycock and Park strains were designated respectively, W, F, Aycock and M (1).

*Experimental Animals.*<sup>8</sup>—*Macacus rhesus* monkeys of 2 to 3 kilos were used throughout for the production and testing of the antisera. As is not unusual in experimental poliomyelitis certain animals were used more than once. As regards the propriety of doing this, it is pertinent to state that daily temperature readings were taken for 4 weeks from the day of inoculation and also that 2 observers independently exercised the monkeys daily for as long as the animals were in stock, which frequently covered a period of months. It seemed unlikely, therefore, that a mild attack of the experimental disease would pass unnoticed. However, this did happen to us once before (1)<sup>9</sup> and apparently happened again in the case of monkeys 4-71 and 4-93 of this series (Appendix A). Accordingly special mention of monkeys used more than once appears in Appendix A, in a footnote.

*Preservation of Material.*—The spinal cords and brain stems bearing the stock

<sup>7</sup> This strain was obtained through the kindness of Dr. W. H. Park and Dr. E. R. Weyer, Bureau of Laboratories, Department of Health, City of New York.

<sup>8</sup> All operations and inoculations were done under full ether anesthesia.

<sup>9</sup> Paul and Trask (1), page 461.

strains were kept in 30 cc. of 50 per cent glycerine in 2 ounce soft glass bottles, at first stoppered with rubber, but later during the period of the neutralization tests, with aluminum screw caps. In the first 9 months of this study Merck's blue label glycerine made up in saline was used; in the second 9 months this was replaced by Kahlbaum's glycerine diluted to 50 per cent in distilled water. The stocks of virus and serum were kept in a refrigerator held between 2° and 4°C. Serum samples were kept without preservative in small glass bottles capped with "no-air" rubber stoppers.

*Neutralization Tests.*—As already stated, samples of serum were obtained from all vaccinated monkeys prior to the administration of the 1st vaccinating dose and 2 weeks after the 2nd. Neutralization tests were run on the postvaccinal samples of serum with the 6 stock strains of virus. Ordinarily, for each strain, we used 1 postvaccinal serum sample selected from a monkey vaccinated with live virus. As the work progressed and larger animal quarters were available, we included 2 postvaccinal (live and formalinized) samples of serum for each strain.

The technique employed was similar to that which we have previously described (1). It may suffice to say that the 6 strains of virus were used in the doses mentioned above. Samples of cord and brain stem from a single monkey were weighed, ground in sand with the appropriate volume of saline solution and the suspension was centrifuged in the angle centrifuge at 1500 R.P.M. for 5 minutes. The supernatant fluid was mixed with equal parts of undiluted monkey serum. The mixture was placed in an incubator at 37°C. for 2 hours and monkeys were subsequently inoculated intracerebrally with 0.5 cc. amounts. Daily temperature readings were made for a period of 4 weeks and all animals were exercised daily as long as they remained in the laboratory.

In each series of tests 2 controls were included, a protected control in which pooled human convalescent serum (collected October, 1931) was mixed with the virus, and an unprotected control in which normal monkey serum (representing the prevaccinal bleeding of 1 monkey) was mixed with the virus. The normal monkey serum chosen, represented the first bleeding from an animal which later was vaccinated with the strain of virus to be used in the current test. Some attempt at titration of the virus in decimals of the test dose was included in 7 of the 8 tests. The diluted virus was set up with equal volumes of the normal monkey serum.

In the experiment of Jan. 17 with the We. strain, where the results were seriously clouded by the occurrence of bacillary dysentery and again with the experiment of June 1 with the Flexner strain, when the protected control came down, the series of tests was repeated.

The results of our neutralization tests have been expressed as follows: + signifies that neutralizing properties were present in the serum, in that the test animal did not develop the experimental disease within the 4 week period of observation; — signifies that neutralizing properties were absent, in that the animal developed the experimental disease within an incubation period not more than 5 days longer than that of the unprotected control. If the incubation

period was longer than this, the result was recorded by  $\pm$  to signify partial neutralization. Thus the results were generally easy to classify but there was one exception; in the experiment of June 1. Monkey 4-71 developed fever without other signs of experimental poliomyelitis and subsequently resisted another intracerebral inoculation of active virus. In Appendix A this is shown as possible experimental poliomyelitis.

#### RESULTS

*Comparative Neutralization Tests.*—In Table I and in the appendices (where more details appear) the results of the neutralization tests are presented. They are arranged so that the strains appear in 3 groups which are designated Old, 1931 and 1934. The Old group includes the Park and Aycock strains. The 1931 (eastern) group includes the Flexner and We. strains and the 1934 (western) group includes the Wfd. and McC. strains. By noting the results listed under the experiments of May 11 and July 13, it may be seen that the Aycock and Park strains were compared by means of 8 pairs of tests; in 5 pairs the results were identical and in 3 pairs they were somewhat different, but not completely so (2;  $\pm$  vs. +; 1;  $\pm$  vs. -). In general, therefore, the Old strains resembled each other. This statement is fortified by inspection of the table which reveals that the Old strains were more closely related to each other than to any of the 4 other strains. Further, it may be noticed that the Old strains were well neutralized by the homologous and 1934 antisera. In fact the Old strains were more frequently neutralized than were those of the other groups. This is worth noting because both the Aycock and Park strains were especially virulent for monkeys and the effective neutralization shown here suggests that immunological differences between the groups rest on a qualitative immunological basis.

The 2, 1931 strains were compared by 6 pairs of tests shown in Table I. The results were identical in 4 pairs, fairly close in 1 pair and dissimilar in 1 pair. Again, however, inspection of the table shows that the outstanding feature is that the 1931 strains appear more closely related to each other than to any other strains. That this relationship was independent of the mere virulence of the strains is indicated in Appendix A, where the titrations show that the Flexner was considerably more virulent than the We. strain. In support of this is the fact that when the Flexner strain was used as

5 per cent virus on June 1, the protected control and all monkeys except 4-71 came down with experimental poliomyelitis.

The 2, 1934 strains had 6 pairs of tests in common. The results were the same in each pair of tests and as far as these experiments are

TABLE I  
*Results of Neutralization Tests*

Immune sera Monkeys vacci- nated with	Vaccine	Vaccinated monkeys	Strains of virus					
			Old		1931		1934	
			Aycock	Park	Flexner	We.	Wfd.	McC.
			May 11	July 13	June 19	Jan. 17; Feb. 21	Jan. 9	Apr. 22
Old	Aycock	C 8-7 F.	+	+	+			
	C 8-2	C 9-8 L.	±	+	-	+	+	+
	Park	C 6-2 F.		+	-			
	C 8-0	C 9-4 L.	+	+	-	-	-	-
1931	Flexner	C 8-8 F.		-	-			
	C 8-1	C 9-9 L.	-	-	+	+*	-	-
	We.	C 8-4 F.		-	-			
	C 7-4	C 9-3 L.	±	-	-	-*	-	-
1934	Wfd.	C 8-9 F.	+	+	-		+	+
	C 5-5	C 1-01 L.	+	+	-	-	+	
	McC.	C 8-3 F.		-	-			
	C 6-7	C 9-2 L.	±	+	-	-†	+	+

F., formalized vaccine; L., live vaccine.

-, no protection.

+, protection.

±, partial protection.

+\* -\*, test repeated, result confirmed.

-†, test repeated, result first -, second ±.

} Results of neutralization tests.

concerned the 2, 1934 strains may be considered immunologically identical.

Table I also shows the immunological interrelationship of the groups. It can be seen that, in respect to the Old antisera, the 1931 and 1934 groups behaved similarly, but that they differed in their own cross neutralization tests. Furthermore, these groups appear com-

pletely different if the results with the We. sera are eliminated from consideration. This seems a fair thing to do because the We. serum in 2 tests gave no protection against the homologous strains.<sup>10</sup> Therefore in this connection it should be regarded as normal monkey serum.

The 1931 and Old groups were related by the results with the good Aycock and the ineffective We. antisera and separated by the results with the Park, Flexner, Wfd. and McC. antisera. The 1934 group and Old group were related by all antisera except the Park. Thus the 1934 strains were found to be more closely related immunologically to the Old than were the 1931 strains. There is an added interest in this relationship because Kessel, Van Wart, Fisk and Stimpert (17) reported that a California strain isolated in 1935 was related, with merely minor immunological variations, to the well known M.V. virus of The Rockefeller Institute. It is to be noted that the Flexner strain we used was not the well known M.V. virus, which is a passage strain of many years standing, whereas ours was of relatively recent origin. Thus our results, although not exactly comparable, are not in disagreement with those of Kessel *et al.* (17).

*Comparison of the Strains as Effective Antigens.*—It can be seen in Table I that some antisera gave rise to protection in most of the tests, whereas some other antisera were generally ineffective. Thus the Aycock antisera were good whether prepared by live or formalinized vaccine. Contrariwise the We. antisera were poor after either method of preparation. The Wfd. and McC. strains were moderately broad, while the Flexner and Park strains were narrow in their antigenicity. Naturally these apparent differences in antigenic efficiency could depend entirely on the individuality of the vaccinated monkeys, but where the results with the live and formalinized vaccines were mutually corroborative the outcome was likely attributable to the virus. Thus it seems probable that the Aycock strain was an unusually effective antigen. This recalls the report of Flexner and Amoss (18) and of Rhoads (19) on special immunizing strains of the virus.

<sup>10</sup> The partial protection in the test, on May 11, of the Aycock virus and We. antiserum was an inconsistency in the neutralization tests which is impossible to explain with the data at hand.

## DISCUSSION

The discussion will be limited to the results of the cross neutralization tests, for they concern the major aims of the paper which are to measure and define differences between certain strains of the virus of poliomyelitis. Such differences as those of intracerebral or intranasal virulence have, of course, been long recognized, but it is a relatively recent (1, 11, 15, 17, 20-23) observation that so called immunological differences exist and it is upon these that we have laid special emphasis.<sup>11</sup> Whether these immunological properties of the virus of poliomyelitis are actually more fundamental than other measurable properties is a question we cannot answer, but in this report they have been selected for purposes of strain differentiation and classification. In this respect we believe our results are informative, not only in the differentiation and classification of strains but also on the nature of the differences between them. Most prominent is the fact that, on the basis of these reactions, those strains which have been subjected to the greatest number of monkey passages and which we had termed passage strains (1), do not stand in sharp contrast to groups of more recently isolated strains which we had termed human strains. There is no evidence in these studies to show that the immunological make-up of the strains has been warped, so to speak, by adaptation to the host. Similarities seem to exist between strains isolated from the same epidemic rather than between those which had about the same number of passages. That is, the immunological reactions tend to group together our 2 strains isolated from the eastern epidemic of 1931 in contrast to the 2 strains isolated from the western epidemic of 1934, which also fall together. Furthermore, there is no indication that the immunological reactions of our 2 strains of 1931 (which have had more passages in the monkey than those isolated in 1934) more nearly simulate those of the older strains (Park and Aycock) which have had most passages of all. This is

<sup>11</sup> Recently, Dr. B. F. Howitt (*Science*, 1937, 85, 268), commenting on various differences between strains of the virus of poliomyelitis, stated that she had recovered a peculiar strain from the epidemic in Sacramento, California, during the winter of 1934. The new strain differed immunologically from one isolated in the summer of 1934 in San Francisco and also differed from her passage strain obtained from the New York City Board of Health.

contrary to a suggestion we once made when we had worked with but one pair of human strains (1).

There seems little doubt that some epidemiological application is to be found in the immunological differences. In fact, significant results have been obtained in neutralization tests in which multiple strains were used with human sera (1, 15, 23). Consequently, it would seem that the present conception of the relation of human immunity to the epidemiology of poliomyelitis, which has been constructed on the basis of neutralization tests performed with human sera and but one strain of the virus, will have to be modified in the light of these newer results. In this connection it is of some interest that our sample of pooled convalescent sera, obtained from adults during the 1931 epidemic in New England, neutralized all of our 6 strains. It should be noted again that the convalescent sera were collected from adults, but this brings up many questions too numerous and too complex to warrant discussion here. However, one point is obvious: that, in an effort to clarify the subject, the extent to which antibodies specific for a local strain are present in a given population should be contrasted with the presence of antibodies for a foreign strain, and *vice versa*. It would seem more than probable that there would be some overlapping of these antibodies, but such an experiment would also probably help towards explaining some of the paradoxes now existent in the subject of the relation of neutralizing antibodies to clinical and subclinical immunity to poliomyelitis.

#### CONCLUSION

Qualitative immunological differences exist between early passage strains or so called human strains of the virus of poliomyelitis.

These differences show a relationship to the epidemic source of the virus and are exemplified in this study by 4 strains isolated in different years during an eastern and western epidemic of poliomyelitis.

APPENDIX A  
Protection Tests Done Jan. 9 to July 13, 1936, with Six Strains of Virus of Poliomyelitis and Their Antisera

Vaccinated monkey	Serum samples	Date of bleedings	Strain and passage	Old		1931			1934	
				Aycock	Park	Flexner	We.	Wfd.	McC.	
				4-89 nIV	5-10 nVIII	4-91 XV	C 1-25 IX	C 1-20 VI	4-44 VIII	
		1935		0.5% virus\$ May 11	0.1% virus July 13	5% virus June 1	0.5% virus June 19	5% virus Jan. 17	5% virus Jan. 9	5% virus Apr. 22
C 8-7 F.	1 2	Feb. 27 Mar. 28	Aycock C 8-2 nIII	4-95 +	5-34 +	*4-82 - 4.8.K.26	*4-93 +			
C 9-8 L.	1 2	June 12 July 10		4-98 - 4.5.D.6 4-94 ± 19.22.K.25	5-35 +	*4-75 - 11.12.K.34	5-13 - 6.8.K.15	4-14 0 Dys.D.9	4-08 +	4-73 +
*C 6-2 F.	1 2	Jan. 30 Feb. 28	Aycock C 8-0 nIII	4-93 +	5-36 +		*4-95 - 8.11.K.20			
†C 9-4 L.	1 2	Mar. 27 Apr. 24	Park C 8-0 nIII		5-50 - 7.10.D.21 5-37 +	*4-83 - ?7.R.	5-14 - 4.6.K.18	4-11 0 Dys.D.9	4-01 - 6.12.K.21	4-76 - 6.11.R.
C 8-8 F.	1 2	Feb. 28 Mar. 28	Flexner C 8-1 XI		5-38 - 6.9.D.44	5-01 - 6.9.D.68 5-05 - 5.12.D.24	5-15 - 12.14.D.14 *4-64 - 8.9.R.	*4-12 - 4.8.R.		
†C 9-9 L.	1 2	June 26 July 24		*4-59 - 8.8.K.17	5-39 - 9.11.D.16	5-04 - 11.13.D.63	4-12 +	4-32 +	4-04 - 6.10.K.10	4-78 - 5.9.K.14

C 8-4 F.	1	Feb. 6	We. C 74 IX	5-40 - 6.8.D.8	5-08 - 7.8.K.14	5-26 - 4.8.R.	4-16 - 5.9.K.9 4-10 - 4.10.R.	4-38 - ?10.D.18 *4-15 - 6.12.K.18	4-02 - 5.12.R.	4-77 - 4.8.R.
	2	Mar. 7								
C 9-3 L.	1	Mar. 27	*4-58 ± 12.13.D.16	5-41 - 4.10.R.	5-06 - 5.7.K.34	5-27 - 6.8.D.15	4-10.R.	4-38 - ?10.D.18 *4-15 - 6.12.K.18	4-02 - 5.12.R.	4-77 - 4.8.R.
	2	Apr. 23								
†C 8-9 F.	1	Mar. 14	4-97 +	5-44 +	*4-70 - 4.8.K.21	5-17 - 9.9.K.19	4-13 - 5.11.R.	4-33 0 Dys.D.19	*1-1 - 6.11.R. 4-05 +	4-74 +
	2	Apr. 11								
†C 1-01 L.	1	July 10	4-96 +	5-45 +	*4-71 ?? 2.R.	5-18 - ?10.K.19	4-13 - 5.11.R.	4-33 0 Dys.D.19	4-07 +	4-74 +
	2	Aug. 7								
C 8-3 F.	1	Feb. 6	*4-50 ± 27.28.D.28	5-46 - 9.10.D.11	*4-74 - ?9.K.16	*4-86 - 7.9.D.17	4-09 - ?9.R.	*4-18 ± 16.21.R.	4-03 +	4-79 - 5.10.R. 4-75 +
	2	Mar. 7								
†C 9-2 L.	1	Mar. 27	McC. *C 6-7 III	5-47 +	*4-73 - 7.14.K.15	5-19 - ?9.D.65	4-09 - ?9.R.	*4-18 ± 16.21.R.	4-03 +	4-79 - 5.10.R. 4-75 +
	2	Apr. 23								

D., died; K., killed; R., recovered; Dys., dysentery; Tbc., tuberculosis; R.W., remained well. III, 3rd passage; nIII, 3rd passage at our hands, etc.  
 +, protection; ±, partial protection; -, no protection; 0, monkey lost; ?, irregular fever; ??, possible experimental poliomyelitis.  
*Example.*—4-98 - } Monkey 4-98, no protection. Fever 4th day. Paralysis 5th day. Died 6th day.  
 4.5.D.6 }

§ Note that final dilutions of virus are given.

\* Previously used in experimental poliomyelitis.

† Previously used in experimental measles.

APPENDIX A—(Concluded)

	Old		1931			1934	
	Aycock	Park	Flexner	We.	Wid.	McC.	
Pooled human convalescent serum Oct. 23, 1931	4-89 nIV	5-10 nVIII	4-91 XV	C 1-25 IX	C 1-20 VI	4-44 VIII	
	0.5% virus§ May 11	0.1% virus July 13	5% virus June 1 0.5% virus June 19	5% virus Jan. 17 5% virus Feb. 21	5% virus Jan. 9	5% virus Apr. 22	
1/10	4-99 +	5-49 +	5-03 ± 15.18.D.64	4-15 +	4-06 +	4-82 +	
	4-67 8.10.D.15 4-08 8.9.D.9	5-51 13.14.R.	5-02 6.6.K.16	4-22 0 Dys.D.18		4-80 7.10.K.15	
Titration: Decimal test doses, 0.5 cc. intracerebrally:	4-63 5.11.K.18 4-64R.W.	5-48R.W. 5-53R.W.	4-32 6.9.R. 4-39 6.9.K.11	4-21R.W. 4-40 0 Tbc.D.28			
		5-52R.W.	5-24R.W.				

*Further Notes on Certain Monkeys Shown in Appendix A*

In the case of the We., Flexner, Aycok and Park strains, fresh monkeys were used in the entire series of passages which furnished stocks of virus; with the McC. and Wfd. strains, some monkeys had been used before. For the McC. strain, monkey C 6-7 having failed to respond to the intranasal instillation of McC. virus on Oct. 23, 1934, received on Nov. 21, 1934, the same strain intracerebrally, became paralyzed and died Dec. 3, 1934, when the cord was harvested. For the Wfd. strain, monkey C 5-5 had failed to sicken after the intracerebral inoculation on Aug. 22 and Sept. 26, of non-infective material collected July, 1934, in California. On Oct. 30, 1934, the Wfd. strain was inoculated intracranially and on Nov. 10 C 5-5 was prostrate, killed with ether and the cord harvested.

Five of the 12 monkeys vaccinated for the purpose of producing antisera had previously been used in a study in experimental measles. Of these 12 monkeys, C 6-2 (vaccinated with formalinized Park virus), was the only one which previously had received material connected with poliomyelitis. This monkey had failed twice to respond to intracerebral inoculations of preserved monkey cord, presumably non-infective, from the 1931 and 1934 epidemics. Serum of C 6-2 was used in 2 tests.

In the 83 neutralization tests of the various sera (exclusive of titrations) we employed 65 fresh monkeys and 18 monkeys which had previously resisted the inoculation of serum virus mixtures, presumably non-infective. Of the 18 monkeys, 14 proved to be satisfactory because they developed the typical experimental disease in the tests; 2, 4-18 and 4-50, were probably satisfactory because they developed the experimental disease, but only after a prolonged incubation period, and 2, 4-71 and 4-93, proved that they had been unsatisfactory test animals by resisting subsequent intracerebral inoculation with active virus although they had remained free of symptoms during the neutralization test. However, this does not seriously affect the general results, since the experiment including 4-71 was discarded because the protected control came down, and since the result with 4-93 could be eliminated without affecting the direct comparison of the 1931 and 1934 groups of virus.

Histological examination of all monkeys furnishing the stocks of virus revealed the typical lesion of poliomyelitis in the medulla and in the cervical, dorsal and lumbar levels of the spinal cord. This was true also of all monkeys shown in the appendix in so far as 21 of them were examined histologically after experimental poliomyelitis.



*Explanatory Note for Appendix B*

Appendix B illustrates the series of vaccinations and intracerebral reinoculations. It can be seen that 3 vaccinated monkeys became paralyzed as a result of the mere vaccinations and that the monkeys in general were not solidly immunized by intracutaneous vaccination. Thus, in the course of the intracerebral tests 12 of 14 vaccinated monkeys and 1 of 3 vaccinated and infected monkeys developed experimental poliomyelitis with paralysis. In 8 instances this followed tests with strains homologous to the vaccine and in 6 instances followed strains heterologous to the vaccine. The totals do not tally because C 8-4 became paralyzed twice; once after the homologous strain and 5 months later, after a heterologous strain.

It may be noted further, that of 5 vaccinated and 2 vaccinated and infected monkeys which proved to be immune to their homologous strain, 6 were also immune to the other strain of their own group (*i.e.* Old; 1931 and 1934; groups established on the basis of the cross neutralization tests). Of these 6 group immune monkeys, 4 subsequently were found to be susceptible to a strain of one of the other 2 groups. Therefore these results seem to substantiate the immunological classification described in the text, but their value is nullified because of the lack of controls for the interval of time which elapsed between vaccination and infecting intracerebral inoculation.

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