A COMPARISON BETWEEN CONVALESCENT SERUM AND NON-CONVALESCENT SERUM IN POLIOMYELITIS*

By MAURICE BRODIE, M.D.

(From the Department of Bacteriology, McGill University, Montreal, Canada)

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The work of Anderson and Frost (1), Peabody, Draper and Dochez (2), Leake (3), Aycock and Kramer (4) and others has shown that the serum of so called normal individuals, who have had no clinical history of poliomyelitis, may neutralize the virus of poliomyelitis. Aycock and Kramer (4), Schultz and Gebhardt (5), found this specific neutralizing power in the serum of most urban adults.

On the grounds that the majority of urban adults are thought to be immune to poliomyelitis, tests had been made for the presence of neutralizing substances in the sera of twenty-nine adults, thirteen of whom had a history of contact with cases of poliomyelitis and all of whom denied a history of clinical symptoms. At the same time, the potency of the serums was compared with that of pooled convalescent serums.

Anderson and Frost (1) were the first to demonstrate neutralizing substance for the virus of poliomyelitis in the serums of non-convalescent adults and Peabody, Draper and Dochez (2), in their studies, found four out of six serums, obtained from individuals with no clinical history of the disease, neutralized the virus. Later Leake (3) made similar observations, but the first extensive study in this direction was undertaken by Aycock and Kramer (4) who, in their series of urban residents, found 69.6 per cent with neutralizing substance and that the serums of 87.5 per cent of the adults of this group neutralized the virus. Likewise, Schultz and Gebhardt (5) showed that, of thirteen so called normal adult serums, 69.1 per cent neutralized the virus of poliomyelitis. Others (6–9) have also demonstrated specific neutralizing substances for the virus of poliomyelitis in the blood serum of non-convalescent adults.

Although the neutralizing power of these non-convalescent serums has not been

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compared with batches of convalescent serum, Shaughnessy, Harmon and Gordon (7) claim that the neutralizing power of serums from normal individuals may equal, or exceed, that of individual convalescent serum. On the other hand, Weyer, Park and Banzhaf (8) did not find any of six adult serums to equal the neutralizing power of either of two individual convalescent serums.

Technique

Cord virus which had been glycerinated 2 to 4 months was used. In order to obtain a fairly even distribution of virus, six or seven segments of each cord specimen were used.

The neutralization test was carried out as follows: 1 cc. of a 5 per cent cord emulsion and 1 cc. of serum were incubated 2 hours (with frequent agitation) and then, after a second period of 8 hours on ice, the mixture was diluted to 5 cc., 1 cc. of which was injected into the frontal lobe of a monkey. In the first test, 1 cc. of undiluted serum was added to 1 cc. of virus, but in the succeeding tests, 1 cc. of serum, diluted 1:2, 1:3, 1:4 and so forth, was added.

EXPERIMENTAL

The average level of neutralizing power of convalescent serum was determined by finding the smallest quantity of each of four batches of serum which would neutralize 80 minimal completly paralyzing (M.C.P.) doses of virus and then dividing the sum of these amounts by four. For the purposes of this work, the M.C.P. dose is limited to the smallest quantity of virus-containing tissue that will cause a complete and rapid paralysis in monkeys weighing 2.5 to 4 kilos, within 13 days. In my experience, less than a completely paralyzing dose either failed to produce symptoms, or at most produced paralysis after a prolonged incubation period. More than a completely paralyzing dose invariably produced severe paralysis. The constancy of these results leads me to believe that monkeys do not vary greatly in their individual susceptibility to the disease.

Experiment 1.—The separate cords of three monkeys and a pool of cords from four monkeys, all removed at the height of paralysis and preserved in glycerine 2 to 4 months, were titrated in order to determine their M.C.P. dose (Table I). Each gave approximately the same M.C.P. dose, which seems to indicate a remarkable constancy, for cord infected with this virus at least, at that stage of the disease. After a further period of 2½ months, the pooled specimen did not show any change in its infectivity.

Experiment 2.—To determine the average neutralizing power of convalescent serum, the virus used to test the convalescent serums was a pooled specimen of

six segments from each of four separate cords and on the above evidence constant results were expected. The virus emulsion, made up from cord tissue of each of these twenty-four segments, was titrated at the beginning and at the completion of the experiments and showed no appreciable change in its strength.

Each batch of pooled convalescent serum represented that of twelve to twenty-eight donors, who had had the disease at least 2 years previously. The results are given in Table II.

TABLE I

Titration of Four Specimens of Cord

(1) Glycerinated Mar. 15, 1931 Titration July 15, 1931 Incubation period—6 days			Pooled specimen		
			(4) Glycerinated June and July, 1931 Titration Sept. 1, 1931 Incubation period—5–7 days		
Monkey No.	Cord	Result	Monkey No.	Cord	Result
	mg.			*mg.*	
1	0.0625	No paralysis	1	0.03125	No paralysis
2	0.125	Paralysis, 13 days	2	0.0625	Paralysis, 23 days, slow course
3	0.250	" 9 "			
4	0.5	" 7 "	3	0.125	Paralysis, 10 days
5	1.0	" 7 "	4	0.250	" 7 "
(2) Glycerinated July 29, 1931 Titration Sept. 18, 1931 Incubation period—13 days			(4) 2½ mos. later		
1	0.03125	No paralysis	1	0.03125	No paralysis
2	0.0625	" "	2	0.0625	Paresis right arm
3	0.125	Paralysis, 7 days	3	0.125	Paralysis, 6 days
(3) Glycerinated Aug. 7, 1931 Titrated Oct. 13, 1931 Incubation period—7 days					
1	0.03125	No paralysis			
2	0.0625	" "			
3	0.125	Paralysis, 7 days			

Averaging the titrated neutralizing power of these four specimens of pooled convalescent serum, representing over a hundred donors, a mean of 0.04 cc. neutralizing 80 M.C.P. doses of virus is obtained. The batches of serum varied considerably in strength, for the weakest showed only 58 per cent of the neutralizing power of the strongest and the remaining two approximated to the arithmetical average figure.

TABLE II

Serum	Cord	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
	mg.	*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*	
1	10	0.2	No paralysis	0.1	No paralysis	0.05	No paralysis	0.04	No paralysis	0.033	No paralysis	0.029	No paralysis	0.025	Paralysis, 21 days
2	10	0.2	" "	0.1	" "	0.05	" "	0.04	" "	0.033	Paralysis, 7 days				
3	10	0.2	" "	0.1	" "	0.05	" "	0.04	" "	0.033	Paralysis, 11 days				
4	10	0.2	" "	0.1	" "	0.05	" "	0.04	Paralysis, 8 days						

Controls

Monkey No.	Cord	Result	Monkey No.	Cord	Result
	mg.			*mg.*	
2-32	0.03125	No paralysis	2-94	0.03125	No paralysis
2-33	0.0625	Paralysis, 23 days, slow	2-93	0.0625	Paresis right arm
2-34	0.125	" 10 " rapid	2-96	0.125	Paralysis, 6 days, rapid
2-46	0.250	" 7 " "			

10 mg. of cord emulsion contains 80 M.C.P. doses.

Experiment 3. The Titration of Normal Adult Serums.—The adult serums were tested by the technique used in titrating convalescent serum. A similar quantity of the same pooled virus was used and the tests were carried out at the same time, so that, when neutralizing substances were demonstrated, the titre could be compared with that of the convalescent serums. Of the twenty-nine adults tested, fourteen had a history of known contact with cases of poliomyelitis, but, in view of the recent work of Kramer and Aycock (10), as one was exposed but 2 weeks prior to the tests, he was listed as a non-contact.

In addition to titrating the virus at the beginning of the experiment and at the time of the last completed set of neutralization tests, an additional control monkey received a mixture of swine serum and virus, in order to show that the serum of an animal, said to be refractory to the virus of poliomyelitis (11), did not contain specific neutralizing substances. The results are given in Table III.

In the first test twenty-two or 76 per cent neutralized the virus, so that, if the protective power is expressed in terms of the average protective power of convalescent serum, seven contained less than two-fifths the neutralizing power of the average for the four convalescent serums. It is possible that in two cases, (Nos. 2 and 19), the virus was partially neutralized by the serums, for the disease ran a much slower course than usual, after incubation periods of 12 and 13 days, respectively.

In the second test nineteen or 65 per cent neutralized, so that three were below three-fifths strength, but equal to two-fifths the strength of convalescent serum. In the third test, two failed to neutralize, and so contained less than four-fifths but equalled three-fifths the strength of convalescent serum, so that seventeen had at least four-fifths the protective power of the average for the four pooled convalescent serums. Of these seventeen, six out of the ten which were tested, equalled the neutralizing power of convalescent serum.

The pooled serum of non-contacts had at least two-fifths but less than three-fifths the average neutralizing power of convalescent serum and the pooled serum of contacts had at least three-fifths but less than four-fifths the average neutralizing power of convalescent serum.

Experiment 4.—The seven serums which had not been worked out in Experiment 3, which had at least four-fifths the neutralizing power of convalescent serum, were tested to determine whether or not they equalled the neutralizing power of convalescent serum. In addition, six of the normal serums which at

TABLE III

Blood donor No.	Age	History of contact	Cord emulsion	Test 1		Test 2		Test 3		Test 4	
				Serum	Result	Serum	Result	Serum	Result	Serum	Result
	yrs.		*mg.*	*cc.*		*cc.*		*cc.*		*cc.*	
1	31	None	10	0.1	Paralysis, 8 days						
2	42	"	10	0.1	" 12 "		(Slow course)				
3	27	"	10	0.1	" 12 "						
4	35	"	10	0.1	No paralysis	0.07	Paralysis, 13 days				
5	24	"	10	0.1	" "	0.07	" 14 "				
6	41	"	10	0.1	" "	0.07	No paralysis	0.05	Paralysis, 13 days		
7	33	"	10	0.1	" "	0.07	" "	0.05	" 8 "		
8	15	"	10	0.1	" "	0.07	" "	0.05	No paralysis	0.04	Paralysis, 10 days
9	21	"	10	0.1	" "	0.07	" "	0.05	" "	0.04	" 9 "
10	21	"	10	0.1	" "	0.07	" "	0.05	" "		Not complete
11	23	"	10	0.1	" "	0.07	" "	0.05	" "		" "
12	23	"	10	0.1	" "	0.07	" "	0.05	" "		" "
13	26	"	10	0.1	" "	0.07	" "	0.05	" "		" "
14	19	"	10	0.1	" "	0.07	" "	0.05	" "	0.04	No paralysis
15	21	"	10	0.1	" "	0.07	" "	0.05	" "	0.04	" "
16	19	2 wks.	10	0.1	Paralysis, 11 days						
17	54	Yes	10	0.1	" 8 "						
18	30	2–3 mos.	10	0.1	" 11 "						
19	32	2–3 " and previously	10	0.1	" 13 "		(Slow course)				
20	30	2–3 "	10	0.1	No paralysis	0.07	Paralysis, 12 days				
21	37	2–3 " and previously	10	0.1	" "	0.07	No paralysis	0.05	No paralysis	0.04	Paralysis, 7 days
22	28	2–3 " " "	10	0.1	" "	0.07	" "	0.05	" "	0.04	" 11 "
23	35	6–8 wks.	10	0.1	" "	0.07	" "	0.05	" "		Not complete
24	24	2 mos. and previously	10	0.1	" "	0.07	" "	0.05	" "		" "

25	30	2–3 mos.	10	0.1	No paralysis	0.07	No paralysis	0.05	No paralysis			Not complete
26	25	3 mos. and previously	10	0.1	" "	0.07	" "	0.05	" "	0.04		No paralysis
27	28	3 " " "	10	0.1	" "	0.07	" "	0.05	" "	0.04		" "
28	31	Experimental disease	10	0.1	" "	0.07	" "	0.05	" "	0.04		" "
29	35	" "	10	0.1	" "	0.07	" "	0.05	" "	0.04		" "
		Pooled contacts	10	0.1	" "	0.07	" "	0.05	Paralysis, 13 days			
		Pooled non-contacts	10	0.1	" "	0.07	Paralysis, 21 days					

Controls

Monkey No.	Cord emulsion	Result	Monkey No.	Cord emulsion	Result
	mg.			*mg.*	
2-32	0.03125	No paralysis	2-96	0.03125	No paralysis
2-33	0.0625	Paralysis, 23 days, slow course	2-93	0.0625	Paresis right arm
2-34	0.125	" 10 " rapid course	2-94	0.125	Paralysis, 6 days, rapid course

One monkey received 0.25 mg. of cord and 0.9 cc. of swine serum—paralysis 7 days. 10 mg. of cord = 80 M.C.P. doses.

TABLE IV

Blood donor No.	Age	History of contact	Cord emulsion	Test 4		Test 5	
				Serum	Result	Serum	Result
	yrs.		*mg.*	*cc.*		*cc.*	
10	21	None	2.5	0.04	Paralysis, 5 days		
11	23	"	2.5	0.04	" 12 "		
12	23	"	2.5	0.04	No paralysis		
13	26	"	2.5	0.04	" "	0.033	Paralysis, 8 days
14	19	"	Neutralized in last experiment			0.033	" 11 "
15	21	"	"	"	" " "	0.033	No paralysis
23	35	6–8 wks.	2.5	0.04	Paralysis, 10 days		
24	24	2 mos. and previously	2.5	0.04	" 6 "		
25	30	2–3 mos.	2.5	0.04	No paralysis		
27	28	3 mos. and previously	Neutralized in last experiment			0.033	Paralysis, 11 days
28	31	Experimental disease	"	"	" " "	0.033	" 15 "
29	35	" "	"	"	" " "	0.033	No paralysis
Convalescent Pool 5			2.5	0.04	Neutralized	0.033	Paralysis, 30 days

Controls

Monkey No.	Cord emulsion	Result	Monkey No.	Cord emulsion	Result
	mg.			*mg.*	
3-05	0.0625	Paralysis, 6 days	3-08	0.015625	Paralysis, 23 days
3-04	0.125	" 6 "	3-07	0.03125	" 6 "
3-02	0.250	" 7 "	2-83	0.0625	" 6 "
			2-62	0.125	" 6 "
			2-65	0.125	" 5 "

2.5 mg. of cord = 80 M.C.P. doses.

least equalled the average potency of the convalescent serums were selected and tested further. As an additional comparison, a fifth sample of pooled convalescent serum was included in the experiment.

The virus used in this experiment had four times the infectivity of that used in the previous experiment, the M.C.P. dose being 0.03125 mg., so that 80 M.C.P. doses were represented in 2.5 mg. of cord. The results are shown in Table IV.

Convalescent serum, Pool 5, containing the serums of twenty donors, had the same neutralizing power as the average of the other four pools.

Of the seven serums, which showed in the last test at least four-fifths the neutralizing power of convalescent serum, only three equalled the neutralizing power of convalescent serum. Combining the results of Experiments 3 and 4, nine out of the twenty-nine serums tested at least equalled the neutralizing power of convalescent serum. Of the six of these tested further (Test 5), two showed higher neutralizing power than the average of the convalescent serums.

The results may be expressed as follows:

Potencies of 7 serums were	below	2/5	the	average	of	the	convalescent	serums		
3	"	equalled	2/5	"	"	"	"	"	"	
2	"	"	3/5	"	"	"	"	"	"	
8	"	"	4/5	"	"	"	"	"	"	
9	"	at least equalled		"	"	"	"	"	"	

Summary

In 7 cases	0.1	cc.	of	serum	failed	to	neutralize	80	M.C.P.	doses	of	virus
" 3 "	0.07	"	"	"	"	"	"	80	"	"	"	"
" 2 "	0.05	"	"	"	"	"	"	80	"	"	"	"
" 8 "	0.04	"	"	"	"	"	"	80	"	"	"	"
" 9 "	0.04	"	"	"	neutralized			80	"	"	"	"

Two out of six of these nine showed higher neutralizing power since 0.033 cc. of serum neutralized 80 M.C.P. doses of virus and five pooled convalescent serums gave an average figure of 0.04 cc. of serum neutralizing 80 M.C.P. doses of virus.

DISCUSSION

Of twenty-nine serums, the largest series of non-convalescent urban adults tested for antibody, twenty-two or 76 per cent showed definite protective power against poliomyelitis virus. Two other serums appeared to give partial neutralization, since in the animals receiving these serums the disease ran a much slower course after incubation periods of 12 and 13 days, respectively; therefore, in this series, the incidence of specific neutralizing substances against the virus of poliomyelitis, is approximately 80 per cent, which agrees with the figures obtained by Aycock and Kramer (4).

Out of 16 non-contacts tested, 12 had at least two-fifths, 10 had at least three-fifths, 8 had at least four-fifths and 4 had at least equal the average of the neutralizing power of the convalescent serums. Of the 13 contacts tested, 10 had at least two-fifths, 9 had at least three-fifths, 9 had at least four-fifths and 5 had at least equal the neutralizing power of convalescent serum. In this series then, known contacts and

avowed non-contacts were equally represented in the serums of low potency, but in the serums of higher potency the known contacts predominated slightly. The pool of the serums of known contacts was of slightly higher value than the pool of the serums of non-contacts, but they were respectively a little above and a little below half the strength of the average obtained for pooled convalescent serum.

Shaughnessy *et al.* (7), comparing individual convalescent and so called normal adult serums, claimed that the latter had as a rule better neutralizing power than the former. It is quite likely, however, that, in many of their neutralization tests, the virus tissue used was not active, since eight of fourteen controls did not contract paralysis with as much as 1 cc. of a 5 per cent emulsion (12). This widespread distribution of neutralizing substances amongst adults and the high titre in more than half of them might result either from exposure to the virus of poliomyelitis, and subsequent immunity through a subclinical attack of the disease, or it may occur as a natural maturation with age. There is considerable epidemiological and experimental evidence to suppose that the former mechanism may be partly responsible for the development of the neutralizing substances in these adult serums. Frost (13), who first suggested this hypothesis, emphasized the differences in the age distribution of the disease in urban and rural districts and concluded that an immunity, which was directly proportional to concentration of population, developed with age. In addition, Aycock (14) has shown that the age distribution is similar to that of measles and diphtheria and believes that subclinical infection accounts for the widespread immunity in adults against the virus of poliomyelitis. As further evidence it has been shown that the incidence of neutralizing substance against the virus of poliomyelitis parallels the Schick test and that the incidence of this neutralizing substance increases with age according to concentration of population, (Aycock and Kramer (4)).

The work here described confirms the high incidence of antibody amongst urban adults and shows that a good proportion have a high degree of neutralizing ability. It has also been shown that the incidence of more potent serum is greater amongst contacts, the majority of whom had frequent exposure to the virus, than amongst non-contacts, which supports the hypothesis that exposure is a factor in bringing about immunity.

The Clinical Use of This Serum

To meet an emergency, serum was collected, for therepeutic purposes, from eleven of the seventeen normal persons showing high protective power in their serum, on the grounds that these serums seemed to be quite as strong as the average convalescent serum, and that larger quantities could be obtained from adults than from convalescent patients, the majority of whom are children.

Over 8 litres of blood was collected and the pooled serum was used in the treatment of sixteen early cases which occurred at the height of the epidemic. In Table V a comparison is made between these and twenty-five cases, which occurred at approximately the same time and which were treated with convalescent serum.

TABLE V

Serum	No. cases	Total serum	No paralysis		Mild paralysis		Severe paralysis		Deaths	
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
		cc.								
Convalescent	25	21–25	20	80	2	8	3	12	1	4
		4–50								
Selected adult	16	7–25	13	81.2	0	0	3	18.8	1	6.3
		9–50								

The series is small and difficult to interpret, but the rapid fall of temperature and the amelioration of symptoms, in the cases which did not develop paralysis, was equally evident with the serum from both sources.

Three cases which developed paralysis following non-convalescent serum and five which contracted paralysis after the administration of convalescent serum, did not show any fall of pulse or temperature or any symptomatic improvement after the intravenous administration of serum.

In these early cases of poliomyelitis, there is a similarity in the responses to convalescent and high titre non-convalescent serums. Zingher (15) reported satisfactory results, for a small series of pre-paralytic poliomyelitis patients, with large quantities of pooled untested serum from normal adults.

The supply of convalescent serum, children being the usual source,

will not always meet demands in an epidemic. The present observations indicate that the supply can be considerably augmented by serum of tested adults. Furthermore, by obtaining serum from tested adults, the donor list is reduced and there is a considerable amount of time saved. In order to obtain 13.5 litres of convalescent serum for the 1931 epidemic in Montreal, 200 bleedings were necessary and a donor list of 150 persons was used.

CONCLUSIONS

1. Of twenty-nine so called normal urban adults tested, seven had less than two-fifths, three had two-fifths, two had three-fifths, eight had four-fifths and nine had a neutralizing power at least equal to the average of five batches of pooled convalescent serum.

2. Known contacts and avowed non-contacts were equally represented in the serums of low potency, but in the serums of higher potency, the known contacts predominated.

3. The pooled serums of known contacts and of non-contacts were respectively a little above and a little below half the strength of the average obtained for pooled convalescent serum.

4. Two series of early cases of poliomyelitis, the one treated with normal serum of proved protective power, the other with convalescent serum, showed no advantage of one type of serum over the other.

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