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The use of new antigens in the complement-fixation test for acute poliomyelitis

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INTRODUCTION

Hitherto in poliomyelitis complement-fixation tests on human sera the antigenic material has usually been derived from tissue cultures of polioviruses. Despite high infectivity titres, antigens from this source are weak in capacity to fix complement (Selzer, 1958). Concentration of tissue culture antigens improves the complement-fixing power but frequently produces anti-complementary effects. Heating the antigens abolishes the anti-complementary state but often results in loss of homotypic specificity (Le Bouvier, 1955; Schmidt & Lennette, 1956).

When polioviruses Types 1 and 2 were successfully adapted to suckling mice in this laboratory the possibility of improving the complement-fixation test by the use of antigenic extracts from infected suckling mouse brain was envisaged. This publication records the results of investigations of these new antigens.

MATERIALS AND METHODS

Antigens

Antigens were prepared from Mahoney Type 1 and MEF_1 Type 2 polioviruses adapted to suckling mice (Selzer, Sacks & van den Ende, 1952; Selzer & Butchart, 1959). Type 3 has not yet been adapted. The mice were infected when 4–5 days old and all were usually paralysed within 24–36 hr. The harvested brains were treated with acetone and ether, and extracted with saline as described by Casals (1949). The extracts were centrifuged at 10,000 r.p.m. for 10 min. in the No. 40 rotor of a Spinco ultra-centrifuge to remove coarse debris. The supernatant fluid was stored in 1 ml. amounts at $-20°$ C. These antigens were not anticomplementary, and the yield was about 15 ml. per 100 mice.

For some tests the antigens were heated at 56° C. for 30 min. before use.

Control antigens were similarly prepared from normal suckling mouse brain and from suckling mouse brain infected with Blue Tongue virus.

In the complement-fixation tests 1 or 2 units of antigen were used in a volume of 0·1 ml. The unit was determined by 'box' titration using a Type 1 and Type 2 human convalescent polio serum of high potency and type specificity. In the case of Mahoney Type 1 virus, one unit was contained in 0·1 ml. of either a 1 in 8 or 1 in 16 dilution of the original Casals extracts. In the case of MEF_1 Type 2, the corresponding unit was a dilution of either 1 in 32 or 1 in 64.

Sera

Sera were obtained from patients in the City Hospital for Infectious Diseases, Cape Town, during a recent epidemic of poliomyelitis. The first sample from each patient (acute phase serum) was taken on admission, and the second 3–4 weeks later prior to discharge to a convalescent home. It was not possible to get samples several months later for further tests. The majority of these patients had paralytic poliomyelitis, several had meningeal signs only, others had paresis, and some had no signs of involvement of the nervous system. Only two patients had been immunized within the year prior to the onset of the illness (cases 15 and 29).

Control sera were obtained from (*a*) members of laboratory staff whose sera were investigated 2 years previously for neutralizing antibodies, and (*b*) patients suffering from diseases of the nervous system other than poliomyelitis such as cerebral haemorrhage, paraplegia due to spinal tuberculosis, brain tumour, encephalitis of unknown etiology, myopathy, etc.

Complement-fixation tests

All sera were inactivated at 56° C. for 30 min. Serial twofold dilutions of sera were prepared from a dilution of 1:10 up to 1:320. Dilutions below 1:10 were not used because frequently the amount of available serum was small and repeated tests were usually performed on each specimen.

Complement was titrated in the presence of antigen. To 0·1 ml. of each serum dilution was added exactly two units of complement in a volume of 0·2 ml. followed by 0·1 ml. of antigen. Each serum was tested simultaneously against the various antigens including control antigens (for non-specific effect). The test was incubated at 4° C. overnight and then left on the bench for 1 hr. to warm up to room temperature, after which 0·2 ml. of sensitized cells containing equal volumes of 3% sheep cells and 3 MHD of haemolysin were added. This was incubated at 37° C. for 30 min. before readings were taken.

The titre of the serum was expressed as the reciprocal of the highest dilution producing 3+ or 4+ (i.e. 75–100%) fixation with the specific antigen.

Neutralizing antibody content of sera

Whenever sufficient serum was available neutralization tests were performed. Initially a 1:6 dilution of convalescent serum was tested in monkey tissue culture for neutralizing antibodies to 100 TCD_{50} of types 1, 2 and 3 polioviruses. If the results were positive, serial twofold dilutions (up to a maximum dilution of 1:192) of acute and convalescent sera were tested for antibodies using two or three culture tubes per dilution. The antibody titre is expressed as the reciprocal of the highest dilution of serum producing protection.

Virus isolation and typing

Viruses were isolated from stools and typed by the usual methods using monkey tissue cultures as the medium.

RESULTS

Results in cases of acute poliomyelitis

Tables 1 and 2 indicate the age of the patient (ranging from 1 month to 36 years); the type of poliovirus isolated from the stool; the time (in days after onset of illness) when acute and convalescent blood samples were taken; the complement-fixing (CF) titre of the acute and convalescent sera and their neutralizing antibody titre. In calculating the date of sampling 3 days have been added if only the date of onset of paralysis was known but not the duration of any previous symptoms, and if the date of onset of paralysis was not known the letter X has been used.

There was no correlation between the clinical picture (meningeal, paralytic or non-paralytic) or the severity of the illness and the complement-fixing titre of the serum. The age of the individual was not a factor, as in some infants under 6 months the titres were as high as in adult cases. In many instances the CF titre of the acute phase serum was negative or low, but in some cases it reached its maximum as early as 3–5 days after the onset of illness (cases 16, 27, 33 and 34). Of interest is the positive CF test in an infant aged only 4 weeks when the acute phase serum was taken (case 38). In many cases the same high CF titre was obtained whether one or two units of antigen were used.

(*a*) *Type* 2 *poliovirus isolated from stool* (10 *cases*)

In cases 1–10 the results of the CF tests correspond with the results of stool investigations and also with the results of neutralization tests on the serum. All ten cases showed a rise in CF antibody in the convalescent serum. The rise varied from twofold to eightfold. In cases 8 and 9 a modified box-titration was done as two units of antigen not only failed to indicate a rise in titre in the convalescent serum but even produced a slight drop. One unit was used in the tests in these two cases as well as in case 10. Crossing (i.e. both homotypic and heterotypic complement fixation) was found in five of the ten cases. In three of these (cases 7, 8 and 9) there were CF antibodies to Type 1 antigen in the acute phase sera but not in the convalescent sera. In one (case 10) the Type 1 CF antibody was present in both acute and convalescent phase sera but showed no rise in titre, whereas that of the Type 2 antibody rose considerably. In the remaining case of crossing (case 2) the heterotypic CF antibody was not present in the acute serum and appeared in the convalescent serum only in an insignificant titre.

The crossing cannot be explained by postulating recent infection with Type 1 poliovirus as there were no neutralizing antibodies to this type. None of these cases had been immunized.

(*b*) *Types* 1 *and* 2 *polioviruses isolated from the stool* (1 *case*)

Case 11 showed a twofold rise in CF titre to both Types 1 and 2 antigen, and it will be noted that both viruses were isolated simultaneously from the stool.

(*c*) *Type* 1 *poliovirus isolated from the stool* (42 *cases*)

Thirty-two patients (cases 12–39 and 45–48) showed the homotypic CF and neutralizing antibodies in the sera, and no 'crossing' in the complement-fixation

Table 1. *Poliomyelitis complement-fixing and neutralizing antibody titres in sera from patients from whom poliomyelitis virus was recovered in stools*

	Patient	Age	Polio Type virus in stool	Days after onset	Poliomyelitis antibody titres in CF test* Type 1	CF test* Type 2	Neut. test† Type 1	Neut. test† Type 2	Neut. test† Type 3
1	V.Ja.	6 mo.	2	10	0	0	–	24	–
				25	0	40	0	96	0
2	N.Ma.	1½ yr.	2	3	0	0	–	<6	–
				21	10	80	0	96	0
3	J.Jo.	7 mo.	2	8	0	0	.	.	.
				38	0	20	.	.	.
4	L.Nq.	1 yr.	2	12	0	0	–	–	–
				31	0	20	0	+	0
5	P.Nt.	7 mo.	2	10	0	80	–	–	–
				24	0	160	0	+	0
6	N.Me.	10 mo.	2	6	0	0	.	.	.
				24	0	20	.	.	.
7	C.Fr.	1 yr.	2	8	20	10	0	+	0
				22	0	80	0	+	0
8	V.Mh.	1¼ yr.	2	12	10	10‡	–	96	–
				28	0	20	0	96	0
9	P.Ne.	1 yr.	2	8	20	20‡	–	>192	–
				26	0	40	0	>192	0
10	W.Cl.	2 yr.	2	11	40	0‡	–	48	–
				24	40	40	0	192	0
11	A.Ma.	1 yr.	1, 2	3	80‡	20	24	12	96
				20	160	40	24	24	192
12	M.Mz.	10 mo.	1	7	40	0	48	—	>192
				25	80	0	48	0	>192
13	T.Mo.	1 yr.	1	6	80	0	192	–	–
				23	160	0	192	0	0
14	F.Wi.	1½ yr.	1	5	40	0	24	–	–
				24	80	0	96	0	0
15	L.Xa.	1 yr	1	18	80	0	96	–	–
				24	80	0	96	0	0
16	Y.Wi.	1½ yr.	1	5	80‡	0	96	–	–
				23	80	0	96	0	0
17	V.Sa.	6 mo.	1	3	40	0	<6	–	–
				23	80	0	48	0	0
18	J.St.	1½ yr.	1	8	40	0	192	–	–
				21	40	0	192	0	0
19	M.Ar.	1 yr.	1	10	10	0	48	–	–
				23	20	0	48	0	0
20	P.Ap.	10 mo.	1	7	40	0	192	–	–
				24	80	0	>192	0	0
21	W.Mg.	11 mo.	1	6	80	0	192	–	–
				23	160	0	192	0	0
22	M.Sn.	1 yr.	1	8	160‡	0	192	–	–
				25	160	0	192	0	0
23	J.Ma.	10 mo	1	6	20	0	96	–	–
				24	40	0	96	0	0
24	G.Ba.	3 mo.	1	X	0	0	48	–	–
				X+18	80	0	192	0	0
25	R.Ri.	6 mo.	1	6	0	0	24	–	–
				24	80	0	192	0	0
26	L.Ma.	6 mo.	1	14	0	0	24	–	–
				25	10	0	24	0	0
27	B.No.	1 yr.	1	3	80	0	48	0	0
				24	80	0	192	0	0
28	A.Da.	5 mo.	1	4	0	0	24	–	–
				23	80	0	192	0	0
29	M.Pe.	9 mo.	1	9	0	0	96	–	–
				23	20	0	192	0	0

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Table 1. (*cont.*)

	Patient	Age	Polio Type virus in stool	Days after onset	Poliomyelitis antibody titres in CF test* Type 1	CF test* Type 2	Neut. test† Type 1	Neut. test† Type 2	Neut. test† Type 3
30	B.Sa.	9 mo.	1	5	40	0	>192	–	–
				25	80	0	>192	0	0
31	A.Ge.	1¼ yr.	1	5	0	0	6	–	–
				23	40	0	192	0	0
32	K.Ja.	5 mo.	1	6	0	0	+	–	–
				22	80	0	+	0	0
33	A.Fr.	2 yr.	1	5	80‡	0	48	–	>192
				22	80	0	96	0	>192
34	C.Ad.	11 mo.	1	5	320	0	96	–	–
				23	160	0	192	0	0
35	B.Dr.	8 mo.	1	9	40	0	+	–	–
				22	80	0	+	0	0
36	J.Da.	2 yr.	1	7	40	0	+	–	–
				24	40	0	+	0	0
37	E.Ga.	10 mo.	1	8	0	0	24	–	–
				23	10	0	192	0	0
38	I.Jo.	1 mo.	1	4	20	0	24	0	0
				23	40	0	192	0	0
39	W.Pr.	3 yr.	1	7	20	0	6	0	0
				30	80	0	192	0	0
				48	80	0	>192	0	0
40	G.Fe.	1½ yr.	1	6	80	0	>192	–	–
				21	160	20	>192	0	0
41	E.v.Ro.	4 yr.	1	8	160‡	80	96	24	96
				26	160	80	192	36	192
42	L.Ja.	1¼ yr.	1	8	160	40	192	–	–
				24	160	20	192	0	0
43	D.Ko.	1¼ yr.	1	7	160	10	–	–	–
				23	160	0	+	0	0
44	I.Wi.	1 yr.	1	14	160	10	<6	–	–
				24	160	0	48	0	0
45	D.Mo.	4 mo.	1	6	0	0	+	0	0
				22	80	0	+	0	0
46	A.Ho.	1½ yr.	1	10	80	0	+	–	–
				29	80	0	+	0	0
47	G.Da.	1 yr.	1	4	40	0	24	–	–
				23	80	0	48	0	0
48	M.Ro.	36 yr.	1	5	80	0	96	>192	>192
				Died	–	–	.	.	.
49	N.Mo.	2 yr.	1	X	20‡	0	6	0	>192
				X+22	160	80	24	0	>192
				X+61	40	0	24	0	>192
50	M.v.Sc.	5 yr.	1	7	160‡	160	96	192	192
				Died					
51	E.Ha.	1 yr.	1	17	160‡	80	–	–	–
				24	160	40	+	0	0
52	A.d.Cr.	5 yr.	1	4	40‡	20	24	192	24
				22	80	20	96	>192	24
53	P.Ca.	26 yr.	1	13	160‡	40	0	–	–
				27	160	40	48	0	0
54	B.Sa.	1¼ yr.	3	8	0	0	–	–	+
				23	0	0	0	0	+
55	S.Sm.	6 yr.	3	13	0	0	–	–	–
				27	0	0	0	0	+
56	C.d.Jo.	7 yr.	3	27	0	0	0	0	+

* 0 at the starting dilution of 1/10.
† 0 at the starting dilution of 1/6.
‡ 1 unit antigen used in the test.
\+ Neutralizing antibody present in a dilution of 1/6 insufficient for titration.
– Not done.

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tests. In one (case 48) no convalescent serum was available as the patient died. Of the remaining 31 cases all except 7 showed a rise in CF antibody titre in the convalescent sera. The rise ranged from twofold to as high as 0–80 in a group of patients (cases 24, 25, 28, 32 and 45) all less than 6½ months old. In the seven cases which did not show a rise the CF titre ranged from 40 to 160 in the acute sera.

Eight patients (cases 41–44 and 50–53) showed crossing. This was negligible and transient in two (cases 43 and 44), but considerable in the others. A prior recent infection with Type 2 poliovirus could explain this finding in three (cases 41, 50 and 52) who were children showing neutralizing antibodies to Types 1 and 2 poliovirus, but this explanation is untenable in the remaining three patients (cases 42, 51 and 53). Case 53 showed non-specific fixation in a serum dilution of 1/10 to Blue Tongue Virus antigen.

Table 2. *Poliomyelitis complement-fixing and neutralizing antibody titres in sera from patients from whom no virus was recovered or no stool examined*

					Poliomyelitis antibody titres in				
			Polio virus in stool	Days after onset	CF test*		Neut. test†		
	Patient	Age			Type 1	Type 2	Type 1	Type 2	Type 3
57	E.Sc.	1½ yr.	No stool	X	20	40	0	<6	0
				X+23	20	160	0	>192	24
58	S.Jo.	11 mo.	None	6	20	0	+	–	–
				25	20	0	+	0	0
59	R.Ba.	5 mo.	None	14	80	0	192	–	–
				28	80	0	192	0	0
60	R.Wh.	28 yr.	No stool	9	40	0	12	0	24
				Died					
61	I.Ba.	36 yr.	No stool	5	80	40	24	>192	0
				Died					
62	Y.Eb.	8 mo.	None	10	0	0	0	0	0
				26	0	0	0	0	0
				105	0	0	0	0	0

* 0 at starting dilution of 1/10.
† 0 at starting dilution of 1/6.
\+ Neutralizing antibody present in a dilution of 1/6.
– Not tested.

Two patients (cases 40 and 49) showed a rise in titre to both Type 1 and Type 2 CF antibodies but only Type 1 virus was isolated from the stool. Although these children were in wards with other patients from whose stools both type viruses were isolated, in neither was there any neutralizing antibody to Type 2 poliovirus to suggest a recent infection and neither had been immunized.

(*d*) *Type* 3 *poliovirus isolated from stool* (3 *cases*)

In three patients (cases 54–56) there was no fixation of complement with unheated Types 1 and 2 antigens, but this series is inadequate to exclude the possibility of crossing with these antigens.

(*e*) *No virus isolated or no stool received for examination* (6 *cases*)

These patients (cases 57–62) were clinically cases of poliomyelitis with severe or moderately severe paralysis, and none had been immunized. Case 57 showed a

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fourfold rise in Type 2 poliomyelitis CF antibody titre in the convalescent serum, a slight rise in Type 3 neutralizing antibody and a very considerable rise in Type 2 neutralizing antibody. In view of the degree of rise in Type 2 CF and neutralizing antibodies it is considered that Type 2 poliovirus was the causal agent. In cases 58 and 59 there was no rise in CF titre, but the CF test result suggests Type 1 polio infection which was confirmed by the neutralizing antibody result. Cases 60 and 61, both of whom died, gave a positive CF test to Type 1 poliovirus with the only available samples of serum. The result in the former patient suggests Type 1 infection, which also appears to be the responsible virus in case 61, although crossing makes assessment a little more difficult.

Case 62 presented clinically with bulbar paralysis. No virus was isolated from the stools in either monkey kidney tissue culture or in suckling mice. The CF tests were negative and the failure to develop any neutralizing antibody to any of the three types of poliovirus over a period of $3\frac{1}{2}$ months must surely exclude the diagnosis of poliomyelitis. No neutralizing antibodies were demonstrable to Coxsackie A7 virus. To date the etiological agent of this patient has not been established.

Results using heated antigens

A third of the sera were tested with the antigens which had been heated at 56° C. for 30 min. Heating did not tend to broaden the reaction except in one instance of a Type 3 infection in which it produced a positive test to a titre of 40 to Type 2 antigen with both the acute and convalescent sera. Heating resulted in a slight loss of potency of the antigens, except in only two instances in which it produced a substantial increase in CF titre of the acute serum.

Results in controls

Ten staff members known to have neutralizing antibodies to all three types of polioviruses 2 years ago were tested for CF antibodies. None had CF antibodies to Type 2 poliovirus and only two had CF antibodies to Type 1 and the titre was low (10).

The sera of sixteen patients, aged from 21 to 70 years, showing neurological disease other than poliomyelitis were investigated. The clinical diagnoses included myopathy, the Guillain–Barré syndrome, paraplegia due to metastatic tumour deposits or osteitis of the spine, hemiplegia due to a variety of causes and myositis. Eleven did not show CF antibodies to either Types 1 or 2 polioviruses. Of the five cases giving a positive CF test, one was positive to Blue Tongue antigen and the result must therefore be considered non-specific. This patient also had a positive Wassermann reaction. Two patients, aged 11 and 18 years respectively, with encephalitis of undetermined etiology, gave a positive CF test to a titre of 10 to Type 1 poliovirus and in addition one of these cases had a titre of 10 to Type 2. One patient, aged 33 years, in whom the clinical diagnosis was myositis, had a CF antibody titre of 20 to Type 1. In the fifth positive case the serum was taken during the recent epidemic of poliomyelitis from an African male, aged 32 years, with ataxia due to basilar impression of undetermined etiology. Clinically polio-

myelitis was not suspected and a stool was not submitted for virus studies. His serum had a significantly high CF antibody titre and had neutralizing antibodies to all three polioviruses. Unfortunately further blood samples were unobtainable as he left for a remote part of the country.

All five cases with positive CF antibody tests also had neutralizing antibodies in their sera.

COMMENT

In this series of 62 patients clinically diagnosed as acute poliomyelitis stools were examined in 59 cases with recovery of poliovirus in 56. Type 1 virus was found in 42 and Type 2 in 10 cases. There was one case with both Types 1 and 2 viruses and three cases with Type 3 virus in the stool.

Complement-fixation tests using the antigens derived from suckling mouse brain infected with either Type 1 or Type 2 poliovirus showed the presence of homotypic CF antibodies in 100 % of the 52 cases with Type 1 or Type 2 poliovirus in the stool. Homotypic neutralizing antibodies were present in all sera available for the test in this group of patients. In the one case with both Types 1 and 2 in the stool both CF antibodies were present in the serum.

In 15 cases of the group with Type 1 or Type 2 infection the initial complement-fixation tests on acute phase sera were performed between the 3rd and 5th day of illness and in 12 (80 %) of these the test was positive in titre ranging from 20 to 320 with the majority 40 to 80. A further 15 cases of this group were tested on the 6th or 7th day with the finding of 11 (73 %) positives in similar titre range. This finding of a positive CF test in 23 out of 30 cases (76 %) within the first week of illness is in contrast to reports that CF antibody is slow in appearing and may take weeks to reach its maximal level in tests using antigens derived from polio infected tissue cultures (Lennette & Schmidt, 1957).

A negative test in the initial sample of serum was found in 15 (28·5 %) out of the 53 cases with Type 1 and/or Type 2 virus in the stool. It is noteworthy that six of these negatives were in the group of 10 cases of Type 2 infection, whereas the series of Type 1 cases gave only 21 % negative results in the initial complement-fixation test. In no case was the test performed with a dilution of serum less than 1/10. On the subsequent test of the convalescent serum every case which gave an initial negative result was found to be positive with antibody titre of 40 or 80 in the majority and a titre of 10 in only two cases.

A rise in CF antibody titre was found in 31 of 45 cases of Type 1 or Type 2 infection whose sera were re-tested between the 3rd and 4th weeks of illness. The degree of rise in cases other than those initially negative was twofold in all instances with the majority reaching titres of 80 or 160. Of the 14 cases which did not reveal a rise only one dropped (from 320 to 160) and the 13 stationary tests had already had titres of 40–160 and the initial tests were performed in the 2nd week of illness in nine instances.

A homotypic CF antibody response without crossing was found in 37 (71 %) of the 52 cases with Type 1 or Type 2 virus in the stool. Five of the heterotypic results occurred in the smaller series of 10 cases of Type 2 infection, but the hetero-

typic antibody was transient or in lowest titre except in one instance with a stationary titre of 40. The specificity of the complement-fixation test was higher (76 %) in the larger group of cases with Type 1 infection, in which there were 10 cases of crossing. In the latter the heterotypic antibody response was either transient, diminishing or stationary. In four of these cases the results could be explained by postulating an anamnestic response to antibody present from a previous heterotypic infection. In the remaining six cases lacking neutralizing antibody to Type 2, the only feasible explanation is that offered by several authors, namely, that certain strains of poliovirus share common antigens. In the one case of mixed Types 1 and 2 infection the CF antibodies to both viruses were present.

In the entire series of poliomyelitis cases investigated only three yielded Type 3 poliovirus in the stool. This type virus has not yet been adapted to suckling mice. The CF tests with antigens from Types 1 and 2 polio-infected mice did not show any heterotypic fixation, but the number of cases is insufficient for evaluation.

In the six cases in which either stool was not received or virus was not isolated in the specimens sent for examination, the results of the complement-fixation tests supported the clinical diagnosis of poliomyelitis in five instances. Two of these cases died before repeat serum tests were made. In two cases the CF antibody response was homotypic and in titre of 20 and 80 respectively but remained stationary. In the remaining case the response was crossed. In all five cases, however, complement-fixation test data, in conjunction with the neutralizing antibody test results, appeared to justify naming the responsible type virus.

In 26 control cases there were seven positive CF tests and all had neutralizing antibodies. One of the positive responses was non-specific, four had a titre of 10, one a titre of 20, and the remaining positive control patient appeared to have had a recent poliovirus infection.

Heating the antigens did not tend to broaden the reaction and produced only a slight loss of potency except in two acute phase sera in which the finding of a substantial increase of CF titre was in agreement with the reports of others (Le Bouvier, 1955; Schmidt & Lennette, 1956).

The antigens derived from polio-infected suckling mouse brain appear to be of distinct value in the diagnosis of acute poliomyelitis by complement-fixation tests. They also appear to be superior to antigens of polio-infected tissue culture fluids in detection of CF antibodies. Positive tests are recognizable earlier in the disease and titres are higher. No instance of false negative result has been found in this series. In all cases, the result was homotypic, although in some cases there had been a milder heterotypic reaction.

SUMMARY

A complement-fixation test for acute poliomyelitis using unheated antigens derived from suckling mouse brain infected with poliovirus Type 1 or Type 2 is described.

The results of tests in 62 patients clinically diagnosed as cases of acute poliomyelitis in a recent epidemic and in 26 controls are recorded.

The CF tests were positive in 100 % of 53 cases with poliovirus Type 1 and/or

Type 2 in stool. A positive result was obtained in 23 (76 %) of 30 cases whose sera were examined in the first 7 days of illness.

Negative tests of the initial serum samples were found in 15 (28·5 %) of 53 cases, but all these became positive in titres of 40 or 80 on testing of convalescent serum.

In 31 (69 %) of 45 cases whose sera were re-tested between the 3rd and 4th weeks of illness the CF antibody levels rose, reaching titres of 80 or 160 in most instances. Of the remaining 14 cases only one dropped in insignificant degree (from titre 320 to 160) and the 13 stationary results had been positive in titres of 40–160 on initial tests most of which were performed in the 2nd week of illness.

Homotypic CF antibody response without crossing was found in 37 (71 %) of 52 cases with Type 1 or Type 2 virus in stool. In the cases of crossing the heterotypic antibody response was either transient, diminishing or stationary in all and in only low titre in most instances.

In 26 control cases there were seven positive CF tests, but one of these was non-specific, five were in lowest titres, and one case appeared to have had recent poliomyelitis infection.

Heating the antigens did not broaden the reaction. It caused only slight loss of potency except in two cases in which the CF titre increased substantially.

The antigenic preparation described appears to be superior to antigens of other origin in the diagnosis of acute poliomyelitis by complement-fixation tests, as positive tests are recognized earlier in the illness and the titres are higher. Homotypic results were obtained in all cases and no instance of false negative occurred in this series.

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