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A precipitin test for acute poliomyelitis and for assessing antibody response to oral poliovaccine

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INTRODUCTION

The phenomenon of antigen-antibody precipitation in gels has been used in poliovirus studies by Le Bouvier (1957) and Grasset, Bonifas & Pongratz (1958), but a precipitin method has not yet been adopted in general routine use as a diagnostic test for acute poliomyelitis. The established diagnostic methods entail the isolation of poliovirus from stools and the demonstration of a rising neutralizing antibody titre in sera. These procedures can be carried out only in a virus laboratory requiring highly trained personnel, and results may not be available for several weeks.

This publication describes a precipitin test using potent non-infectious antigen, which can be performed in any laboratory and which usually gives results within 24 hr. Details are presented of the use of the method as a diagnostic test for acute poliomyelitis and for studying serum antibody responses to a trivalent oral Sabin-type poliovaccine prepared in South Africa and recently administered on a nation wide scale.

MATERIAL AND METHODS

Sera

For assessment of the precipitin method as a diagnostic test, sera were obtained from 50 patients in the City Hospital for Infectious Diseases, Cape Town, during a recent epidemic of poliomyelitis. The patients ages ranged from 1 month to 26 years. The majority had paralytic poliomyelitis; the others had paresis or only meningeal signs or no clinical evidence of nervous system involvement. The first sample of blood was usually taken on admission ('acute phase serum') and the second sample 3–4 weeks later ('convalescent serum'). Control sera were obtained from 14 patients in Groote Schuur Hospital, Cape Town, who had nervous-system disease other than poliomyelitis (e.g. cerebral haemorrhage, tumour, hemiplegia, encephalitis of unknown etiology, Guillain-Barre syndrome, paraplegia due to spinal tuberculosis, metastatic carcinoma or myopathy), from seven patients infected with ECHO 4 or Coxsackie B3 virus, and from animals immunized with ECHO 1, 10 or 13 or Coxsackie B1 or B4 viruses.

For assessment of the precipitin method for evaluation of the antibody response to oral poliovaccine, sera were obtained from two groups as follows:

(*a*) Ninety students of the University of Cape Town Medical School. The majority of these had received a monovalent (Type 1) oral poliovaccine $6\frac{1}{2}$ months previously. The initial blood samples were drawn from all immediately prior to their taking the first dose of the trivalent (Types 1, 2 and 3) oral poliovaccine prepared and issued in South Africa in 1961. The majority were bled again at weekly intervals for the ensuing 3 weeks, some were bled at the end of the 4th week and final samples were taken from all between the 5th and 7th weeks. Sera were not tested thereafter because the students then received the second dose of trivalent vaccine.

(*b*) Two hundred and forty non-European schoolchildren of a poor community near Cape Town. Their ages ranged from 7 to 14 years. None had ever been given Salk vaccine, but all had received the monovalent (Type 1) oral poliovaccine $4\frac{1}{2}$ months previously. The single sample of blood from each of these schoolchildren was drawn prior to their taking trivalent oral vaccine.

All sera from groups *a* and *b* were inactivated at 56° C. for 30 min. Not more than 0·1 ml. of serum was needed for the precipitin test.

Antigens

Precipitating antigens were prepared from Type 1 (Brunenders), Type 2 (MEF_1) and Type 3 (Saukett) poliovirus tissue culture Salk-type vaccines. The titre of the original material was approximately $10^{6\cdot5}$ $TCID_{50}$/0·5 ml. Ninety litres of formalin-inactivated Type 1 virus were concentrated 1000-fold by pervaporation, purified by two treatments with chloroform (Polson & Hampton, 1957), spun at 10,000 r.p.m. for 10 min. to remove all cell debris, and finally centrifuged for 90 min. at 30,000 r.p.m. in the No. 40 rotor of a Spinco ultracentrifuge. The tiny pellets were redispersed in saline. Thirty litres of Type 2 and of Type 3 polioviruses were similarly concentrated 1000-fold, but the chloroform treatment was omitted. All antigens were stored at $-20°$ C.

Ouchterlony tests

A solution of washed agar (Difco) was prepared according to the method of Pereira & Allison (1959) and 14 ml. portions were allowed to set in 9 cm. Petri dishes. A central well and six peripheral wells all equidistant were made in the gel with a cutter. The wells were 2 mm. diameter and 5 mm. apart. Test sera were placed in the peripheral wells and the antigen in the central well. The plates were kept in a humidified jar at room temperature and observed daily for 4 days. Lines of precipitation were usually detectable within 24 hr. and were graded from $\pm$ to $+++$ according to sharpness and intensity.

Neutralizing antibody tests

Sera in dilution 1/6 were tested in monkey-kidney tissue culture for neutralizing antibodies to 100 TCD_{50} of Types 1, 2 and 3 polioviruses. In positive cases serial twofold dilutions (up to 1/192 dilution of sera from patients with acute polio-

myelitis, and 1/800 or 1/1600 dilution of sera from students) were tested, using two or three culture tubes per dilution. The antibody titre is expressed as the reciprocal of the highest dilution of serum giving protection.

RESULTS

Patients with acute poliomyelitis and controls

Table 1 shows the results of the precipitin test on acute phase and convalescent sera from the 50 patients with acute poliomyelitis. Precipitation bands which appeared in the agar gels, almost invariably within 24 hr., have been recorded on a grading of sharpness and intensity from a minimum weakly positive ± to a maximum strongly positive + + +.

In the group of 37 patients with Type 1 poliovirus in stools, the Type 1 precipitin test was positive in the acute phase sera of 34 cases (91·7 %) and positive in all the convalescent sera (100 %). The lines of precipitation were usually sharp and intense + + or + + +, and in only two patients was it weakly positive ±. Positive results were obtained in sera taken as early as 3 days after onset of illness. In all patients the test indicated the presence of homotypic precipitins, that is, it was positive for the type virus isolated from stool, and the corresponding neutralizing antibody was present in the sera. In addition, the presence of heterotypic precipitins ('crossing') was shown by the test in three cases (patients 29, 33 and 34). Double bands of precipitation with homologous antigen appeared in the tests on both acute phase and convalescent sera of eight patients and in only the acute phase sera of four cases.

The tests in the nine cases with Type 2 poliovirus in the stool showed the presence of homotypic precipitins in seven out of the eight (87·5 %) acute phase sera tested and in all nine convalescent sera (100 %). The sharpness and intensity grading was + + or + + + in 14 of the 16 homotypic precipitation bands, the remaining two being +. There were also heterotypic bands in six cases, the grading being evenly distributed from + to + + +. Double homotypic bands appeared in four tests.

In the one patient with Types 1 and 2 polioviruses in stool, the precipitin test was positive for Types 1, 2 and 3 viruses and neutralizing antibodies to all these types were present in the sera.

In all three patients with Type 3 poliovirus in stool the precipitin test was positive. The test in one of these cases showed homotypic precipitation only, but in the other two there was crossing with Type 1 virus. One of these patients had previously been infected with virus of this type.

The precipitin test was negative in all the control patients (who had not received the oral vaccine) and in animals immunized against ECHO and Coxsackie viruses.

Individuals who received oral poliovaccine

In the group of 90 students whose sera were tested for precipitins and neutralizing antibodies before and after taking a single dose of trivalent oral poliovaccine there were 49 (54 %) who developed precipitins to at least one type during the

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Table 1. *Poliomyelitis precipitins and neutralizing antibodies titres in sera from patients suffering from acute poliomyelitis*

Patient	Age	Polio type virus in stool	Days after onset of illness	Precipitins T1	Precipitins T2	Precipitins T3	Neutralizing antibodies T1	Neutralizing antibodies T2	Neutralizing antibodies T3
B.G.	3 mo.	1	×	+ +	0	0	96	—	—
			× + 18	+ +	0	0	> 192	—	—
B.D.	1½ yr.	1	4	+ +	0	0	> 192	—	—
			24	+ +	0	0	> 192	0	0
C.P.	26 yr.	1	13	+ + +	0	0	> 192	—	—
			27	+ + +	0	0	> 192	0	0
C.G.	3½ yr.	1	13	+ + +	0	0	> 192	—	—
			25	+ + +	0	0	> 192	0	0
D.R.	1¼ yr.	1	10	+ + +	0	0	> 192	—	—
			24	+ + +	0	0	192	0	0
D.A.	5 mo.	1	4	+ +	0	0	48	—	—
			23	+ + +	0	0	> 192	0	0
D.M.	3 yr.	1	8	+ +*	0	0	> 192	—	48
			28	+ +*	0	0	> 192	0	96
de C.A.	5 yr.	1	4	+ + +	0	0	48	> 192	48
			24	+ + +	0	0	192	> 192	48
de L.A.	1½ yr.	1	3	0	0	0	24	—	—
			22	+	0	0	192	0	0
du T.T.	1¾ yr.	1	3	+	0	0	192	—	—
			23	+ + +	0	0	> 192	0	0
F.G.	1½ yr.	1	6	+ + +*	0	0	> 192	—	—
			21	+ + +*	0	0	> 192	0	0
G.E.	10 mo.	1	8	+ + +*	0	0	48	—	—
			23	+ + +	0	0	> 192	0	0
G.A.	1¼ yr.	1	5	+ + +	0	0	12	—	—
			23	+ + +	0	0	> 192	0	0
H.E.	1 yr.	1	17	+ + +	0	0	> 192	—	—
			24	+ + +	0	0	> 192	0	0
J.K.	5 mo.	1	6	±	0	0	12	0	—
			22	+ + +	0	0	> 192	0	0
J.I.	1 mo.	1	4	0	0	0	40	—	—
			23	+ + +	0	0	> 192	0	0
J.L.	1¼ yr.	1	8	+ + +*	0	0	> 192	—	—
			24	+ + +	0	0	> 192	0	0
K.S.	1¼ yr.	1	5	+ +	0	0	> 192	—	—
			25	+ +	0	0	> 192	0	0
K.R.	2¼ yr.	1	10	+ +*	0	0	> 192	—	192
			26	+ +*	0	0	> 192	0	96
L.F.	1 yr.	1	5	+ + +*	0	0	96	—	—
			26	+ + +	0	0	> 192	0	0
L.J.	6 yr.	1	18	+ +*	0	0	48	192	—
			38	+ +*	0	0	192	192	0
M.L.	6 mo.	1	14	±	0	0	> 192	—	—
			25	±	0	0	> 192	0	0
M.S.	1 yr.	1	9	+ +*	0	0	> 192	—	—
			24	+ +*	0	0	> 192	0	0
N.L.	1½ yr.	1	5	+ +	0	0	192	—	—
			23	+ +	0	0	192	0	0

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Table 1 (*cont.*)

Patient	Age	Polio type virus in stool	Days after onset of illness	Precipitins T1	Precipitins T2	Precipitins T3	Neutralizing antibodies T1	Neutralizing antibodies T2	Neutralizing antibodies T3
O.P.	1¼ yr.	1	6	++*	0	0	96	—	—
			25	++	0	0	> 192	0	0
P.M.	9 mo.	1	9	+++	0	0	192	—	—
			23	++	0	0	> 192	0	0
P.W.	3 yr.	1	7	++	0	0	6	—	—
			30	+++	0	0	> 192	0	0
P.J.	1¼ yr.	1	5	0	0	0	48	—	—
			24	+++	0	0	> 192	0	0
Q.A.	2 yr.	1	8	++*	0	±	> 192	—	> 192
			24	+++*	0	±	> 192	0	> 192
R.R.	6 mo.	1	6	++	0	0	48	—	—
			24	+++	0	0	> 192	0	0
S.B.	1 yr.	1	7	++*	0	0	192	—	—
			27	+++*	0	0	> 192	0	0
S.J.	1½ yr.	1	8	+++	0	0	> 192	—	—
			21	+	0	0	> 192	0	0
V.R.E.	4 yr.	1	8	+++*	0	0	192	192	96
			26	+++*	0	+	> 192	192	> 192
V.S.M.	5 yr.	1	7	+++	+++	++	192	> 192	> 192
			Died						
W.J.	1½ yr.	1	5	±	0	0	> 192	—	—
			24	+++	0	0	> 192	0	0
W.I.	1 yr.	1	14	+++	0	0	> 192	—	—
			24	+++	0	0	> 192	0	0
W.Y.	1½ yr.	1	5	+++	0	0	192	—	—
			23	+++	0	0	192	0	0
B.C.	2 yr.	2	7	+++	+++*	0	—	> 192	—
			24	+++	+++*	0	0	> 192	0
C.W.	2 yr.	2	11	+++	+++**	0	—	96	—
			24	0	+	0	0	192	0
J.V.	6 mo.	2	10	0	+++	0	—	48	—
			25	0	+	0	0	192	0
M.N.	1½ yr.	2	3	0	+++	0	—	6	—
			21	0	+++	0	0	192	0
M.V.	1¼ yr.	2	12	++	+++	0	—	192	—
			28	++	+++	0	0	192	0
N.P.	1 yr.	2	8	0	0	0	—	> 192	—
			26	+	+++*	0	0	> 192	0
S.G.	2¼ yr.	2	6	+	++	0	96	> 192	—
			23	+	+++	0	96	> 192	0
W.J.	1½ yr.	2	5	++	++	0	> 192	> 192	> 192
			44	++	++	0	192	> 192	> 192
W.C.	1 yr.	2	32	0	+++	0	0	> 192	0
M.A.	1 yr.	1 & 2	3	+++	+	0	192	> 192	96
			20	+++	+++*	++	96	> 192	> 192
B.V.	3 yr.	3	29	++	0	++	> 192	0	> 192
D.J.C.	7 mo.	3	27	+	0	+	0	0	> 192
S.B.	3 yr.	3	8	0	0	+	—	—	48
			23	0	0	+	0	0	> 192

* double bands; — not done; × not known.

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Table 2. *Development of precipitins in students given the trivalent oral poliovaccine*

	Pre-vaccine						Post-vaccine																	
	Precipitins			Neutralizing antibodies			Precipitins															Neutralizing antibodies		
	Types			Types			1st week Types			2nd week Types			3rd week Types			4th–5th weeks Types			4th–7th weeks Types			Types		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	□	.	.	.
2	.	●	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	□	.	.	.
4	.	.	.	+	+	+	.	.	.	.	.	.	.	●	□	.	●	□	△	●	□	.	.	.
8	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	△	.	.	△	.	.	.	.	.
10	.	●	□	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
11	.	.	.	+	+	+	.	.	.	.	.	.	△	.	.	△	.	.	△	.	.	.	.	.
13	△	.	.	+	+	+	.	.	.	△	●	□	△	●	□	△	●	□	△	●	□	.	.	.
14	.	.	.	+	○	+	.	.	.	.	●	.	△	●	.	△	●	.	△	●	.	+	+	+
15	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
17	.	.	.	+	+	+	.	.	.	.	.	.	.	●	□	.	●	□	.	●	□	.	.	.
18	.	.	.	+	+	○	.	.	.	.	●	.	.	.	.	.	.	.	.	.	.	+	+	+
20	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	●	.	.	●	.	.	.	.
21	.	.	.	+	+	+	△	.	.	△	●	□	△	●	□	△	●	□	△	●	□	.	.	.
22	△	.	.	+	+	+	.	.	.	△	.	.	△	.	.	△	.	.	△	●	.	.	.	.
23	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
25	.	.	.	○	+	+	.	.	.	.	●	.	△	●	.	△	●	.	△	●	.	+	+	+
26	.	.	.	+	+	+	.	.	.	.	●	□	.	●	.	.	●	.	.	●	.	.	.	.
28	△	.	.	+	+	+	.	.	.	.	●	□	.	●	.	.	●	.	.	●	.	.	.	.
29	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
30	.	.	.	+	+	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	.	.	.
34	.	.	.	○	+	+	.	.	.	.	●	.	.	●	.	.	●	.	.	●	.	○	+	+
35	.	.	.	+	○	+	.	.	.	.	●	□	.	●	□	.	●	□	△	●	□	+	+	+
37	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	●	.	△	●	□	.	.	.
41	.	.	.	+	+	+	.	●	.	△	●	.	.	●	.	.	●	.	.	●	.	.	.	.
43	.	.	.	+	○	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	+	+	+
44	.	.	.	○	○	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	○	+	+
46	.	.	.	○	+	+	△	.	.	△	.	.	△	.	.	△	.	.	△	.	.	+	+	+
47	.	.	.	+	+	+	.	.	.	.	●	.	.	●	.	.	●	.	.	●	.	.	.	.
48	.	.	.	○	+	○	.	.	.	△	.	.	△	.	.	△	.	.	△	.	.	+	+	○
51	△	.	.	+	+	+	.	.	.	△	●	□	△	●	□	△	●	□	△	●	□	.	.	.
57	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	□	.	.	.
58	.	●	.	+	+	+	.	.	.	.	.	.	△	●	.	△	●	.	△	●	.	.	.	.
59	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	△	.	.	△	.	.	.	.	.
61	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
62	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
66	.	.	.	+	○	○	.	.	.	.	●	□	△	●	□	△	●	□	△	●	□	+	+	+
71	.	.	.	○	+	+	.	.	.	.	.	.	△	.	.	△	.	.	△	.	.	+	+	+
72	.	.	.	+	+	+	.	.	.	△	.	.	△	.	.	△	.	.	△	.	.	.	.	.
74	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
76	.	.	.	○	+	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	+	+	+
78	.	.	.	+	+	+	.	.	.	.	.	.	△	●	.	△	●	.	△	●	.	.	.	.
81	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	△	.	.	.	.	.
82	.	.	.	+	+	+	.	.	.	.	.	.	△	.	.	△	.	.	△	.	.	.	.	.
83	.	.	.	+	+	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	.	.	.
84	.	.	.	+	○	+	.	.	.	.	●	□	.	●	□	.	●	□	.	●	□	+	+	+
86	.	.	.	○	+	+	.	.	.	.	.	.	.	●	.	△	●	.	△	●	.	+	+	+
87	△	.	.	+	○	+	.	.	.	△	●	.	△	●	.	△	●	.	△	●	.	+	+	+
88	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	△	●	.	△	●	.	.	.	.
90	.	.	.	+	+	○	.	.	.	.	.	.	.	●	□	.	●	□	.	●	□	+	+	+

Precipitins: △ = Type 1; ● = Type 2; □ = Type 3. Neutralizing antibodies: + present; ○ absent.

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post-vaccine observation period of 7 weeks. This includes eight who had precipitins in pre-vaccination sera but whose post-vaccination tests showed either intensification of precipitation bands from + to + + + or development of additional bands. Details of results in the 49 students are presented in Table 2. The results of precipitin tests done at precisely 4 and 5 weeks post-vaccination appear in one column, and the results in students who came for blood sampling at irregular intervals during the 4th–7th weeks are given in another column.

Precipitins developed during the 1st week in three students, 2nd week in 23, 3rd week in 12, 4th and 5th weeks in five, and between the 4th and 7th weeks in 19 students.

Precipitins to two types of poliovirus developed in 20 students (in 12 instances *during the 2nd week) and to all three types in seven students (in two cases during* the 2nd week). Four students developed fleeting precipitins which appeared during the 2nd week only (students 18, 26, 28 and 41).

Prior to ingestion of trivalent vaccine, neutralizing antibodies to all three types of poliovirus were present in 69 of the 90 students. Thirty-four (50 %) of these 69 failed to develop precipitins in the post-vaccination observation period. Seven of the 21 students who did not have all three neutralizing antibodies in initial samples failed to develop the deficient neutralizing antibodies and corresponding type precipitins. In the group of 49 students who did develop precipitins as described above (Table 2), neutralizing antibody titres rose eightfold or more in 75 %, and more than sixteenfold in 40 %, after taking vaccine. There were 16 who lacked neutralizing antibodies to one or more types before taking trivalent vaccine; 12 of these 16 students (including one of three who lacked antibodies to two types) developed the deficient neutralizing antibodies and corresponding precipitins. Student no. 18 who initially lacked neutralizing antibody to Type 3 virus developed it after taking the vaccine but the homologous precipitins did not appear. He did develop precipitins to Type 2 virus, however, and the titre of neutralizing antibodies to this type rose from 1/50 to 1/400. Student no. 34 remained deficient in Type1 neutralizing antibody only, but Type 2 virus apparently multiplied in his gastro-intestinal tract. In student no. 44 neutralizing antibodies to Types 1 and 2 were absent initially; he developed precipitins to both types, but neutralizing antibodies to Type 2 only. In the whole series of students this was the only one who developed precipitins without the appearance of the corresponding neutralizing antibody.

In the series of 240 schoolchildren who were tested 4½ months after taking monovalent (Type 1) oral vaccine the precipitin test was positive for Type 1 virus in 193 (80 %), but in only 11 (5 %) were the lines of precipitation sharp and intense + + or + + + . Fourteen (6 %) had precipitins to Type 2 and in only one of these was the precipitation band intense. Two (1 %) had precipitins to Type 3. The first 100 children were also tested for neutralizing antibodies. These antibodies to Types 1 and 2 were present in 100 % and to Type 3 in 98 %.

COMMENT

The precipitin method was found to be very effective as a diagnostic test. It was positive in all 50 patients with acute poliomyelitis. Positive results were also obtained in schoolchildren and students who had taken monovalent oral polio-vaccine $4\frac{1}{2}$ or $6\frac{1}{2}$ months previously, but in only 5 % of these were the precipitation bands graded strongly positive + + or + + +, whereas in 46 (92 %) of patients with acute poliomyelitis the grading was + + or + + + usually in both tests. Furthermore, the phenomenon of double precipitation bands appeared in 25 sera from 16 patients with acute poliomyelitis but was not found in any sera from the other subjects investigated. The conclusion is that sharp and intense precipitation lines or pairing of lines strongly indicates very recent infection with poliomyelitis virus.

Negative results were obtained in every test of sera from all the control series of patients with nervous-system disease other than poliomyelitis and patients infected or animals immunized with Coxsackie or ECHO viruses. There were initially negative results in four patients with acute poliomyelitis whose sera were tested within 8 days of onset of illness. However, six out of a total of eight poliomyelitis patients tested as early as 3 or 4 days after onset of illness and 23 out of 25 tested between the 5th and 8th day gave positive results, and all sera taken from the acute poliomyelitis patients later than the 8th day, including repeat samples from previously negative patients, were positive. These findings indicate that, although a negative or only weakly positive test result may be obtained in the very earliest stage of acute poliomyelitis, it is very unlikely that a patient has poliomyelitis if the precipitin test is not positive in the 2nd week of illness. Since precipitation bands almost always appear within 24 hr. of setting up the sera in agar gels, by the criterion of time, the precipitin test compares very favourably with other diagnostic procedures for acute poliomyelitis. An advantage of the precipitin method is that the test can be carried out by workers who do not require training in techniques of handling infectious virus material as the antigens and test sera used in this technique are inactivated.

In all the patients with acute poliomyelitis the positive tests indicated the presence of precipitins for the type virus isolated from stool. A test, however, may be positive for other types not isolated from the stool. This phenomenon of 'crossing' appeared in 12 patients in this series. In six of these the finding could be ascribed to known previous recent infection with heterotypic virus which was no longer being excreted. In others the crossing could be explained by the hypothesis that polioviruses of different types may have common antigens (Selzer & Larsson, 1961). If crossing is present the type virus responsible for the present illness may still be identified in tests where double precipitation bands appear because it seems that double lines occur only with homotypic precipitins. The tests of 16 poliomyelitis patients in this study showed double bands only with the homotypic virus and there was no case in which double bands of heterotypic precipitins occurred.

Several observations may be made on the use of the precipitin method in studying

antibody response to ingestion of oral vaccine. The test results in the series of 240 schoolchildren indicated that precipitins, unlike neutralizing antibodies, are present for only a limited time after infection. Practically all of 100 children tested for neutralizing antibodies had them to all three types of poliovirus. Type 1 precipitins were present in 80 % of the total series of children, but 95 % of these gave only weakly positive ± or + precipitation and these were due to feeding monovalent (Type 1) oral poliovaccine 4½ months previously; the incidence of Types 2 and 3 precipitins was negligible.

In 54 % of the 90 students who took trivalent oral vaccine, positive precipitin test results indicated multiplication of virus in the gastro-intestinal tract. Precipitins appeared in a few cases as early as the 1st week after taking the vaccine, but the 2nd week was the commonest time for tests to become positive. Many tests appeared positive during the 4th–7th week but these were probably due to infection contracted from fellow-students excreting virus rather than from ingestion of vaccine.

Of the students with positive precipitin tests after taking trivalent vaccine 54 % showed multiplication of at least two types and 12 % showed multiplication of all three types poliovirus. This suggests that it is advantageous to administer repeated doses of trivalent oral vaccine rather than monovalent vaccine in three separate doses.

With the appearance of precipitins in the students there was a substantial rise in neutralizing antibody titre, usually greater than eightfold and frequently greater than sixteenfold, even in cases where the precipitins were fleeting. In one student only precipitins developed without the appearance of corresponding neutralizing antibodies; no adequate explanation can yet be given for this finding.

The precipitin tests remained negative in 50 % of 69 students who had neutralizing antibodies to all three types poliovirus prior to taking the trivalent vaccine, indicating failure of the vaccine virus to multiply in the gastro-intestinal tract in these cases.

SUMMARY

A description is given of a precipitin test, using potent non-infectious antigens, which can be performed in laboratories not specializing in virology. Trial of the method as a diagnostic procedure gave positive results in 100 % of a series of 50 patients with acute poliomyelitis. Precipitation lines, almost always sharply defined and intense, were obtained in 89 % of 33 sera within 3–8 days of onset of acute poliomyelitis and in all sera after the 8th day. In positive cases precipitation bands usually appeared within 24 hr. after starting the test. In all instances precipitation was homotypic with the type virus isolated from stools. Double bands appeared in very recent cases but only with homotypic precipitins. Single heterotypic lines appeared in 12 cases due to previous infection or perhaps due to infection by virus strains with common antigens. All control patients with nervous system disease other than poliomyelitis or with infection by Coxsackie or ECHO viruses and all control test animals gave negative results. Of 240 poor-community schoolchildren who had taken monovalent oral poliovaccine 4½ months previously, 80 % had precipitins for Type 1, but less than 5 % showed marked

reactions. The sera from the remainder gave only weakly positive results. Contrasting with transience of precipitins, neutralizing antibodies persisted in nearly 100% of children tested.

The precipitin test was used to study antibody response to ingestion of trivalent oral vaccine (prepared and issued by the Poliomyelitis Research Foundation, Johannesburg). More than half (54%) of 90 students who received the vaccine had positive precipitin-test results; most often these results appeared during the 2nd week after ingestion. In this positive group 54% showed multiplication of at least two types and 12% showed multiplication of all three types poliovirus.

I am deeply grateful to the medical students of the University of Cape Town for their enthusiastic co-operation. I would also like to thank Dr J. Gear for the Salk vaccine used for the preparation of the precipitating antigens and Professor Kipps for his constant interest and encouragement.

Note. Only since this article was in the proof stage was I aware of the work of Eggers & Sabin (1961) whose phase contrast microprecipitin tests with poliovirus antigens gave results very similar to those reported in the present paper.

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