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Antigens common to Types 1 and 2 poliomyelitis viruses

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INTRODUCTION

A previous communication (Selzer, 1960) described the use of new antigens in a complement-fixation test (CFT) for the diagnosis of acute poliomyelitis in a recent epidemic. During these investigations a few cases appeared in which only one type of virus was demonstrated in the stool, and only homotypic neutralizing antibody in the acute and convalescent sera, yet these sera contained CF antibodies to both Types 1 and 2 polioviruses in significant amounts. In one patient, a substantial rise in the CF titre to both types occurred during the illness. This suggested the existence of virus strains possessing antigens common to both Types 1 and 2 polioviruses. Attempts to prove this are now described.

MATERIALS AND METHODS

Antigens

Precipitating antigens

Precipitating antigens were prepared from Type 1 (Brunenders) and Type 2 (MEF_1) poliovirus tissue culture vaccines which had been rejected because of a small amount of residual live virus. (The titre of the original material was approximately $10^{6 \cdot 5}$ $TCID_{50}$/0·5 ml.) Ninety litres of 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 cell debris and finally ultra-centrifuged at 30,000 r.p.m. for 90 min. in the no. 40 rotor of a Spinco. The very small pellets were redispersed in saline. Thirty litres of Type 2 poliovirus were similarly concentrated a 1000-fold but chloroform treatment was omitted. These antigens were stored at $-20°$ C.

Antigens for complement fixation

Complement-fixation test antigens were prepared from Mahoney, Type 1, and MEF_1, Type 2, polioviruses adapted to suckling mice and the complement-fixation tests were carried out as previously described (Selzer, 1960).

Sera

Human Sera

These were obtained from patients during a recent epidemic of poliomyelitis in Cape Town. The first sample from each patient (acute phase serum) was taken on admission to hospital, and the second 3–4 weeks later prior to discharge to a convalescent home.

Two groups of sera were selected for this investigation. Sera in the first group (patients *Claassens*, *Bardien* and *Modana*) showed significant heterotypic as well as homotypic complement fixation. The second group (patients *Gallies*, *Williams*, *February* and *Mathews*) gave only homotypic or relatively little heterotypic fixation.

Animal sera

Five guinea-pigs and two or three rabbits were immunized with each of the viruses of *Claassens*, *Bardien* and *Modana* and five guinea-pigs each with viruses from *Gallies*, *February*, *Williams* and *Mathews*. The virus after one or two tissue culture passages was mixed with an equal volume of mineral oil adjuvant consisting of 10% Arlacel A in Drakeol and 1 ml. was administered intramuscularly to each animal. This was followed by 0·5–1 ml. of TC virus (without adjuvant) 2 weeks later. Rabbits received a third injection of 1 ml. intraperitoneally after a further 2 weeks. The animals were bled 2 weeks after the last injection.

Control sera

The following were used as controls:

(*a*) Sera from twelve patients suffering from acute poliomyelitis which showed only homotypic CF and neutralizing antibodies.

(*b*) Coxsackie B 1, B 2 and B 3 mouse immune sera prepared from tissue culture virus.

(*c*) One human convalescent serum from a case of Coxsackie B 3 infection.

(*d*) Sera from six patients infected with ECHO 4 virus.

(*e*) Sera from rabbits and guinea-pigs immunized with Type 1 (Stockholm) and Type 2 (MEF_1) polioviruses prepared in tissue culture.

(*f*) Standard poliomyelitis Type 1 and Type 2 monkey immune sera.

Micro Ouchterlony tests

A 1% solution of washed agar (Difco) was prepared in normal saline and 14 ml. portions were allowed to set in 9 cm. Petri dishes containing a trace of Merthiolate powder. For each test six peripheral wells were cut equidistant from each other and from the central well. The wells were 2 mm. in diameter and 5 mm. apart. The serum to be tested was placed in the central well and the antigens in adjacent cups peripherally. The plates were kept in a humidified jar at room temperature and examined daily for 5 days. Any precipitation which occurred during this period was usually detectable within 24 hr.

Neutralizing antibody content of sera

When sufficient serum was available, neutralization tests were performed. Initially a 1:6 dilution of convalescent serum was tested in monkey kidney tissue culture for neutralizing antibodies to 100 TCD_{50} of Types 1 and 2 polioviruses. If the results were positive, serial twofold dilutions (up to maximum dilutions of

1:192 for human sera and 1:1536 for animal sera) 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

Results in human cases of poliomyelitis

Table 1 indicates 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, the results of the precipitin test and the neutralizing antibody titre of the acute and convalescent sera. The presence of precipitating antibodies is indicated by 'plus' signs (+), lines with the maximum sharpness and intensity being recorded as +++.

Table 1. *Poliomyelitis complement-fixing, precipitating and neutralizing antibodies in the sera of patients*

Patient	Polio type virus in stool	Days after onset	CF test* Type 1	CF test* Type 2	Precipitin test Type 1	Precipitin test Type 2	Neut. test† Type 1	Neut. test† Type 2
Claassens	2	11	40	0	+++	+++	—	48
		24	40	40	0	+	0	192
Bardien	2	4	80	640	+++	+++‡	—	12
		21	20	160	+++	+++‡	0	>192
Modana	1	*X*	20	0	+	0	6	0
		X+22	160	80	+++	+++	24	0
		X+61	40	0	+++	+++	24	0
Gallies	1	8	0	0	+++	0	24	—
		23	10	0	+++	0	192	0
Williams	1	5	80	0	+++	0	96	—
		23	80	0	+++	0	96	0
February	1	6	80	0	+++‡	0	192	—
		21	160	20	+++‡	0	192	0
Mathews	2	3	0	0	0	+++	—	6
		21	10	80	0	+++	0	96

* 0 at the starting dilution of 1/10.
† 0 at the starting dilution of 1/6.
‡ 2 bands.
X, day of onset not known.

The results with the sera of the individual patients are considered below:

Claassens showed an impressive rise in CF antibody titre to the homologous poliovirus isolated from the stool (Type 2), but there was an equally significant though stationary CF antibody titre to Type 1 poliovirus. Precipitating antibodies were present to both Types 1 and 2 polio antigens (Fig. 1), Type 2 showing at least two lines of precipitation. However, only the homotypic neutralizing antibody was present. In the convalescent serum only homotypic precipitating antibody was detected and the response was poor.

Bardien also showed a striking CF antibody titre to both Type 1 and Type 2 antigens in the acute phase serum, precipitating antibodies to both antigens (with two bands of precipitation to Type 2 antigen) and only homotypic neutralizing antibody in the sera.

Modana, from whom polio virus, Type 1, was isolated from the stool samples, showed only homotypic neutralizing antibody in the three samples of blood examined. The second and third samples, however, showed a significant rise in CF and precipitating antibodies to both types of virus. The results of the precipitin tests are shown in Fig. 2.

Gallies and Williams. In the sera of these patients only homotypic CF, precipitating and neutralizing antibodies were detected.

February and Mathews had relatively only low titre heterotypic CF antibody as compared with the homotypic level. Only homotypic precipitating and neutralizing antibodies were found.

Table 2. *Poliomyelitis complement-fixing, precipitating and neutralizing antibodies in the sera of animals immunized with the viruses from* Claassens, Bardien *and* Modana

Animals		CF test*		Precipitin test		Neut. test†	
		Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
Claassens (Type 2 isolated)							
Guinea-pig	6	0	1280	+ + +	+ + +	0	> 1536
	7	0	640	+ + +	+ + +	0	> 1536
	8	0	320	0	+ + +	0	384
	9	0	320	0	+ + +	0	384
	10	0	320	+ + +	+ + +	0	96
Rabbit	*C* (*a*)	0	40	0	+ + + ‡	0	384
	C (*b*)	0	80	+ + +	+ + +	0	> 768
	C (*c*)	0	160	+ +	+ + +	0	768
Bardien (Type 2 isolated)							
Guinea-pig	1	0	640	0	+ + +	0	96
	2	0	160	+ +	+ + +	0	1636
	3	0	320	0	+ + +	0	192
	4	0	320	0	+ + +	0	24
	5	0	> 1280	+ + +	+ + +	0	> 1536
Rabbit	*B* (*a*)	0	80	0	+ + +	0	384
	B (*b*)	0	40	0	+ + +	0	192
Modana (Type 1 isolated)							
Guinea-pig	11	80	0	+ + +	0	768	0
	12	40	0	+ + +	0	768	0
	13	20	0	+ +	0	384	0
	14	80	0	+ + +	0	96	0
	15	20	0	+ +	0	96	0
Rabbit	*M* (*a*)	0	0	0	0	48	0
	M (*b*)	Anti-complementary		+	0	192	0
	M (*c*)	0	0	+ +	0	192	0

* 0 at the starting dilution of 1/10.
† 0 at the starting dilution of 1/3.
‡ 2 lines of precipitation.

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Results in animals immunized with the viruses isolated from the stools

Table 2 shows the results of the tests on sera from guinea pigs and rabbits immunized with the viruses obtained from *Claassens*, *Bardien* and *Modana*. In all the animals the CF antibody was highly specific, as was also the neutralizing antibody. The precipitating antibodies in the case of *Claassens* were positive for both Type 1 and Type 2 antigens in five of the eight animals immunized (Fig. 3); in the case of *Bardien* two of the five guinea-pigs were positive to both antigens, but in *Modana* there was no cross-precipitin reaction in any of the animals.

Table 3. *Poliomyelitis complement-fixing, precipitating and neutralizing antibodies in the sera of animals immunized with the viruses isolated from* Gallies, Williams, February *and* Mathews.

		CF test*		Precipitin test		Neut. test†	
Animals		Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
Gallies (Type 1 isolated)							
Guinea-pig	35	10	0	+ +	0	+	0
	36	40	0	+ + +	0	+	0
	37	40	0	+ + +	0	+	0
	38	10	0	+ + +	0	+	0
	39	40	0	+ + +	0	+	0
Williams (Type 1 isolated)							
Guinea-pig	31	10	0	+ +	0	+	0
	32	0	0	+ +	0	+	0
	33	10	0	+ +	0	+	0
	34	40	0	+ +	0	+	0
February (Type 1 isolated)							
Guinea-pig	26	10	0	+ + +	0	+	0
	27	20	0	+ + +	0	+	0
	28	10	0	+ + +	0	+	0
	29	0	0	+ + +	0	+	0
	30	0	0	+ + +	0	+	0
Mathews (Type 2 isolated)							
Guinea-pig	21	0	160	0	+ + +	0	+
	22	0	80	0	+ + +	0	+
	23	0	40	0	+ + +	0	+
	24	0	80	0	+ + +	0	+
	25	0	320	0	+ + +	0	+

* 0 at the starting dilution of 1/6.
† Only 1/6 dilution tested.

Table 3 shows the results on sera of guinea-pigs immunized with the viruses obtained from patients in whom the CF, precipitating and neutralizing antibodies were all homotypic, except for some low titre heterotypic CF antibodies found in the convalescent sera of *February* and *Mathews*. All the immunized animals showed a homotypic response only.

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Results in controls

Twelve patients suffering from acute poliomyelitis showed only homotypic CF and neutralizing antibodies and the precipitins too were homotypic only.

Coxsackie B1, B2 and B3 mouse immune sera, the human serum from a convalescent Coxsackie B3 infection and the sera from six patients suffering from ECHO 4 infection all failed to show precipitating antibodies to the polio antigens.

Sera from animals immunized with Stockholm Type 1 and MEF_1 Type 2 viruses and the standard Type 1 and Type 2 monkey sera showed only homotypic precipitating antibodies.

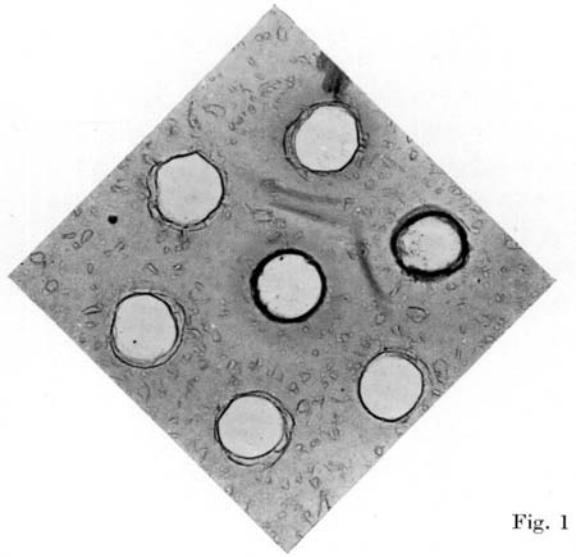
COMMENT

The sera of the patients *Claassens*, *Bardien* and *Modana* have CF and precipitating antibodies to both Type 1 and Type 2 antigens although all three show only homotypic neutralizing antibodies. The fact that the lines of precipitation cross (Pl. 1, Figs. 1 and 2) indicates the presence of two different antigen-antibody systems. In a previous communication (Selzer, 1960) it was shown that certain human sera capable of neutralizing either Type 1 or Type 2 polioviruses (but not both) could fix complement in the presence of either virus. In explanation it was suggested that certain strains of the virus might share one or more common or very similar antigens. This suggestion is now supported by the results of the very specific gel precipitin tests with sera from the patients *Claassens*, *Bardien* and *Modana* which contain precipitating antibodies for Type 1 and Type 2 polioviruses. It is of interest that some of the animals immunized with viruses from these patients produced both homotypic and heterotypic precipitating antibodies but only homotypic CF antibodies. It is unlikely that the precipitin reactions of the animal antisera were due to non-specific monkey kidney tissue culture products, first because the lines of precipitation with Type 1 and Type 2 cross, indicating the presence of two different systems, and secondly because animals immunized with other viruses such as Stockholm, Type 1, and MEF_1, Type 2, polioviruses grown in tissue culture gave highly specific results and the sera from animals immunized with the Coxsackie viruses grown in tissue culture failed to give lines of precipitation with the polio antigens.

The results indicate that certain virus strains have complement-fixing antigens which are common to both Type 1 and Type 2 polioviruses; others have precipitating antigens common to both, whilst others again show both complement-fixing and precipitating antigens. The CF and precipitating antigens are therefore probably distinct from each other and from antigens giving rise to neutralizing antibodies. The latter is probably strictly type specific.

SUMMARY

During a recent epidemic of poliomyelitis three viruses isolated (one Type 1 and two Type 2) elicited both Type 1 and Type 2 CF and precipitating antibodies in the patients whilst only the homotypic neutralizing antibodies were present. These viruses inoculated into animals produced specific CF and neutralizing antibodies,



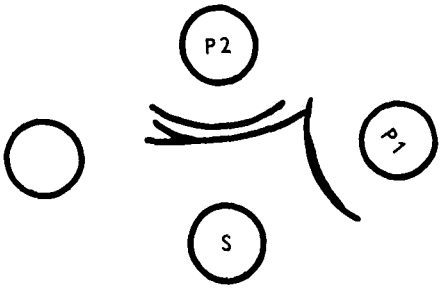
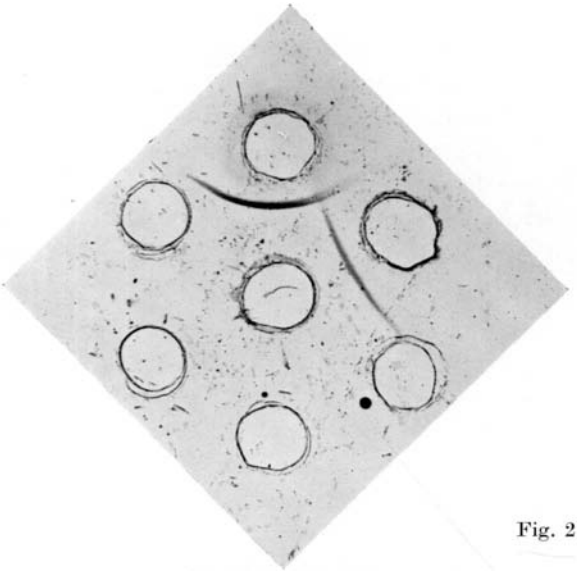


Fig. 1



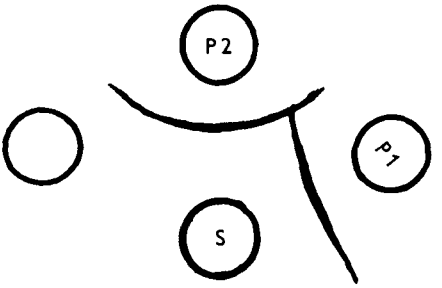
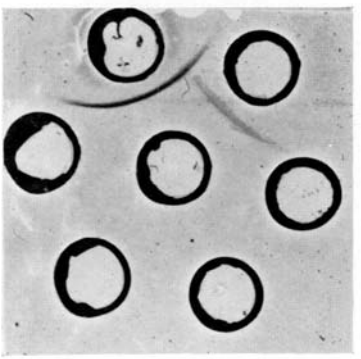


Fig. 2



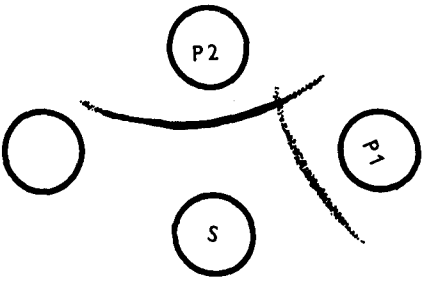


Fig. 3

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but precipitating antibodies to both types were present in a significant number of animals. These findings suggest that certain polioviruses have complement-fixing and precipitating antigens common to both Type 1 and Type 2 viruses.

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EXPLANATION OF PLATE

Fig. 1. *Claassens* (patient). Enlarged photograph and drawing of the precipitin reaction.

Fig. 2. *Modana* (patient). Enlarged photograph and drawing of the precipitin reaction.

Fig. 3. *Claassens* (guinea-pig 7). Photograph and drawing of precipitin reaction. P 1 = poliovirus Type 1; P 2 = poliovirus Type 2; S = serum.

(All photographs enlarged five times.)