A COMPARISON OF COMPLEMENT-FIXING ANTIGENS BY TYPES 1 AND 2 POLIOMYELITIS-INFECTED SUCKLING MOUSE BRAINS

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In previous communications from this laboratory (Selzer & Polson, 1954, and Selzer & van den Ende, 1956), it was reported that ultra-centrifugation of emulsions of suckling mouse brains infected with MEF_1 Type 2 poliomyelitis virus separated a non-infective soluble antigen from infective virus. High complement-fixing titres obtained with the supernatant were considered to be due mainly to the presence of the soluble antigen. This antigen was not found in monkey kidney-tissue cultures of either the MEF_1 Type 2 or Mahoney Type 1 polioviruses (Selzer, 1958) and it was suggested that its absence was probably a factor accounting for the low complement-fixing power of tissue-culture fluids.

Subsequently, Mahoney poliovirus was adapted to suckling mice by mouse-to-mouse passage (Selzer, 1959). This communication compares the results of complement-fixation tests with emulsions of suckling mouse brains infected with the Mahoney and the MEF_1 strains, respectively.

MATERIALS AND METHODS

Viruses

The Mahoney Type 1 poliomyelitis virus had been through nine to-and-fro tissue culture-mouse passages and thereafter adapted to suckling mice by a long series of consecutive mouse-to-mouse passages in this laboratory. The complement-fixation tests with the suckling mouse brain emulsions were performed at various stages of the adaptation up to the seventy-fifth mouse-to-mouse passage. The MEF_1 Type 2 poliovirus adapted to suckling mice in this laboratory had been through 110 mouse-to-mouse passages before complement-fixation tests were done.

Sera

(1) Mahoney and MEF_1 immune mouse sera were prepared against virus which had been adapted to suckling mice. Groups of fifty 2-month-old mice were immunized, each mouse receiving five or six intraperitoneal injections of 0·5 ml. of 10% saline suspension of virus-infected brains at 5-day intervals. The mice were bled from the heart 10 days after the final injection.

(2) Further Mahoney and MEF_1 immune mouse sera were similarly prepared against virus grown in monkey kidney-tissue culture. Undiluted infected tissue culture fluid in 0·5 ml. amounts was used for each injection.

(3) Brunhilde, Type 1, immune monkey serum was provided by the Poliomyelitis Research Foundation, Johannesburg.

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(4) Coxsackie B3 immune mouse serum, prepared by the same method as the sera against Mahoney and MEF_1 suckling mouse polioviruses, was used as a control serum.

Preparation of antigens

Infected suckling mouse brain extracts were prepared by the acetone-ether method described by Casals (1949). The extracts were centrifuged at 10,000 r.p.m. for 10 min. in Model L Spinco, rotor 40, to remove coarse debris. Samples were reserved for complement-fixation tests and the rest spun at 30,000 r.p.m. for 110 min. to separate soluble antigen from infective virus. The upper half of the supernatant fluid constituted the soluble antigen fraction and the remainder of the supernatant was discarded. The pellet was redispersed in a volume of saline equal to the original volume.

Complement-fixation tests

These were performed by the method of Casals & Olitsky (1950). The end-point was defined as the highest dilution of antigen giving 50 % fixation in the presence of a 1/20 dilution of immune serum.

Titrations of infectivity of antigens

These were made in monkey kidney-tissue culture in tubes containing 6×10^5 cells in 1 ml. Hanks' solution. Serial tenfold dilutions of antigen were prepared in Hanks' solution, and 0·1 ml. of each dilution was inoculated into each of three tubes. The tubes were observed for 8 days for cytopathogenic change and the infectivity was calculated by the method of Reed & Muench (1938).

RESULTS

Mahoney antigens prepared from the brains of paralysed mice in early mouse-to-mouse passages fixed either very little complement or none at all, even though the titres of infective virus were equal to or higher than those obtained in later passages. The amount of complement fixed gradually increased as the virus became better adapted to growth in suckling mice.

The results of typical experiments are recorded in Tables 1 and 2. In Table 1 are the results of complement-fixation tests with the antigens of Mahoney virus from infected suckling mouse brains and the various antisera. The original virus suspension (A) and the redispersed pellet (B) fixed appreciably more complement than the soluble antigen (C).

These findings are in sharp contrast to those in Table 2 in which the antigens of MEF_1 virus from infected suckling mouse brains were tested with three antisera. In this instance the soluble antigen fixed more complement than did the redispersed pellet.

The infectivity of the virus fraction in the MEF_1 and Mahoney antigens varied from $10^{5·5}$ to $10^{5·7}$ $TCID_{50}$/ml. The soluble antigen fractions of both strains were non-infective apart from a very occasional titre not exceeding 10^2 $TCID_{50}$/ml.

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There was no cross-fixation between any of the antigens of the Types 1 and 2 poliomyelitis viruses used in these experiments. The complement-fixing titre of the virus fraction purified by centrifugation was similar in both virus types, but there was a big difference in the titres obtained with their soluble antigen fractions.

Table 1. *Complement-fixation tests with the Mahoney virus in suckling mouse brains and immune sera to Mahoney, MEF_1, and Coxsackie B3 viruses in suckling mouse brains, Mahoney virus in tissue-culture fluids and Brunhilde monkey immune serum*

Antisera to...	Serum dilutions $\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	Serum control
Experiment 1								
A. Original extract as antigen								
Mahoney suckling virus	4	4	4	4	4	0	0	0
Mahoney TC virus	4	4	4	4	4	2	0	0
Brunhilde virus	4	4	4	4	4	4	4	0
MEF_1 suckling virus	0	0	0	0	0	0	0	0
Coxsackie B3 suckling virus	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0
B. Virus fraction purified by centrifugation as antigen								
Mahoney suckling virus	4	4	4	4	4	2	0	0
Mahoney TC virus	4	4	4	4	3	0	0	0
Brunhilde virus	4	4	4	4	4	4	1	0
MEF_1 suckling virus	0	0	0	0	0	0	0	0
Coxsackie B3 suckling virus	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	—
C. Soluble antigen fraction as antigen								
Mahoney suckling virus	4	4	1	0	0	0	0	0
Mahoney TC virus	4	0	0	0	0	0	0	0
Brunhilde virus	3	3	2	1	0	0	0	0
MEF_1 suckling virus	0	0	0	0	0	0	0	0
Coxsackie B3 suckling virus	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	—
Experiment 2								
A. Original extract as antigen								
Mahoney suckling virus	4	4	4	4	4	4	1	0
Antigen control	0	0	0	0	0	0	0	—
B. Virus fraction purified by centrifugation as antigen								
Mahoney suckling virus	4	4	4	4	4	1	0	0
Antigen control	0	0	0	0	0	0	0	—
C. Soluble antigen fraction as antigen								
Mahoney suckling virus	4	3	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	—

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DISCUSSION

Although the successful adaptation of the Mahoney strain of Type 1 poliovirus to suckling mouse brain has led to the demonstration of a soluble non-infective complement-fixing antigen in brain extracts of such infected mice, the titre of this antigen is very much lower than that obtained with the soluble antigen prepared from infected suckling brains of the similarly adapted MEF_1 Type 2 strain. It may

Table 2. *Complement-fixation tests with the MEF_1 virus in suckling mouse brains and immune sera to MEF_1 and Mahoney viruses in suckling mouse brains and MEF_1 virus in tissue-culture fluids*

Antisera to...	Antigen dilutions $\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	Serum control
Experiment 1									
A. Original extract as antigen									
MEF_1 suckling virus	4	4	4	4	4	4	1	0	0
MEF_1 TC virus	4	4	4	4	3	1	0	0	0
Mahoney suckling virus	0	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	—
B. Virus fraction purified by centrifugation as antigen									
MEF_1 suckling virus	4	4	4	3	0	0	0	0	0
MEF_1 TC virus	4	4	4	3	0	0	0	0	0
Mahoney suckling virus	0	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	—
C. Soluble antigen fraction as antigen									
MEF_1 suckling virus	4	4	4	4	4	1	0	0	0
MEF_1 TC virus	4	0	0	0	0	0	0	0	0
Mahoney suckling virus	0	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	—
Experiment 2									
A. Original extract as antigen									
MEF_1 suckling virus	4	4	4	4	4	4	3	0	0
Antigen control	0	0	0	0	0	0	0	0	—
B. Virus fraction purified by centrifugation as antigen									
MEF_1 suckling virus	4	4	4	2	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	—
C. Soluble antigen fraction as antigen									
MEF_1 suckling virus	4	4	4	4	4	4	0	0	0
Antigen control	0	0	0	0	0	0	0	0	—

well be that the higher concentration of soluble antigen obtained with the MEF_1 strain is related to the much longer series of mouse-to-mouse passages to which this strain has been subjected as compared with the Mahoney—110 against 75—and that with further passage of the latter the development of the soluble antigen will improve.

Both MEF_1 and Mahoney strains show higher complement-fixation titres with the adapted, partially purified virus from suckling mouse brains than with the

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purified virus fractions from tissue-culture fluids, despite the higher infectivity titres of the latter. Infected mouse brains contain high proportions of dead virus and it is suggested that it is the *dead virus that contributes substantially to the increased complement-fixation titres* obtained with the antigens prepared from the suckling brains.

SUMMARY

Good fixation of complement was obtained with the infective virus fraction of the Mahoney Type 1 poliomyelitis virus which had been adapted to suckling mice. The presence of a soluble antigen, specific to Type 1 virus, was also demonstrated but was small in amount.

The infective virus fraction of the suckling mouse adapted MEF_1 Type 2 poliomyelitis virus produced as good fixation of complement as did Type 1, but the fixation of complement by the MEF_1 soluble antigen was far greater than that obtained with the Mahoney strain.

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REFERENCES

Casals, J. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 339.
Casals, J. & Olitsky, P. K. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 315.
Reed, L. & Muench, H. (1938). *Amer. J. Hyg.* **27**, 493.
Selzer, G. (1958). *J. Hyg., Camb.*, **56**, 254.
Selzer, G. & Butchart, M. (1959). *J. Hyg., Camb.*, **57**, 285.
Selzer, G. & Polson, A. (1954). *Biochim. biophys. Acta*, **15**, 251.
Selzer, G. & van den Ende M. (1956). *J. Hyg., Camb.*, **54**, 1.

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