FURTHER EXPERIMENTS WITH THE SOLUBLE ANTIGEN OF THE MEF_1 POLIOMYELITIS VIRUS

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In 1951, Casals, Olitsky & Anslow showed that a suitable antigen for complement-fixation tests could be produced from suckling mouse brains infected with the MEF_1 strain of poliomyelitis virus. This method is now in general use.

In a previous communication from this laboratory (Selzer & Polson, 1954) we reported that there was present in suspensions of brains of infant mice infected with the MEF_1 strain of poliomyelitis virus an antigen—other than the infective virus itself—which accounted for more than half of the specific complement-fixing power of the brain suspensions. The non-infective antigen which appeared to be specific was present in supernatants from which infective virus had been removed by centrifugation and had a particle size of approximately 12 mμ as determined by ultra-centrifugation. Rustigian, Havens & Kolner (1954) have also recently described a non-infective antigen in preparations of MEF_1-infected suckling mouse brains.

In this communication are recorded the results of further investigations on this small non-infective complement-fixing component or soluble antigen.

MATERIALS AND METHODS

Virus

The virus used was the MEF_1 strain of poliomyelitis virus received from Dr P. K. Olitsky and adapted to suckling mice in our laboratory (Selzer, Sacks & van den Ende, 1952). Infected mouse brains were harvested and stored at either 4 or $-20°$ C. until sufficient material had been gathered for individual tests.

Sera

(1) Immune sera were prepared against the virus as it occurs in adult mouse brains (adult virus) and against virus which had been adapted to suckling mice (suckling virus) by more than 100 serial passages.

Serum was prepared by repeated injections of 10% saline suspension of virus-infected brains into mice aged 2 months or more. Groups of 50 to 100 were immunized, each receiving five or six intraperitoneal injections of 0·5 ml. of the virus-infected brain suspension. The injections were given at intervals of 5 days, and the mice bled from the heart 10 days after the last injection.

(2) Another group of mice received a similar course of immunizing injections of the supernatant fluid (soluble antigen) of virus-infected suckling mouse brain emulsion from which the virus had been removed by two successive centrifugations at 30,000 r.p.m. for 110 min.

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(3) Lansing, Leon and Brunhilde immune sera were obtained from the Poliomyelitis Research Foundation, Johannesburg. These were monkey immune sera prepared with tissue culture fluid as antigens.

(4) Blue Tongue immune serum was prepared in the same way as that against the MEF_1 suckling virus.

Preparation of antigens for complement fixation

The antigens were prepared in several ways. In some experiments the infected suckling mouse brains were emulsified in physiological saline with the aid of powdered glass and a mortar and pestle to give a 20 % brain suspension, whilst in others a Waring blender was used and an amount of physiological saline added sufficient to give a 40 % brain suspension. In some experiments the antigen was prepared by the acetone-ether method described by Casals (1949). This method consists of the removal of a considerable amount of lipoid by treatment with acetone and ether at low temperature, and extraction of the dried residue in physiological saline. Gross particulate material is removed by centrifugation at 10,000 r.p.m. and the supernatant used as antigen. This method of preparation does not destroy the infectivity of the virus.

In some experiments saline suspensions of infected mouse brains were simply shaken up with an equal amount of chloroform or ether, the organic solvent and emulsion phases discarded and the dissolved organic solvent removed from the aqueous phase by evaporation under reduced pressure.

Control antigens from normal uninfected suckling mouse brains were prepared in the same ways.

When crude brain suspensions were used as antigens these received preliminary centrifugation at 10,000 r.p.m. for 15 min. to remove gross particulate material. Samples of emulsified suspension were reserved for complement fixation and infectivity tests. For the separation of soluble antigen from infective virus the suspensions were further centrifuged at 30,000 r.p.m. for 100–110 min. and the upper two-thirds of the fluid (SNF) removed. To minimize the amount of infective virus present the SNF received a second and sometimes a third similar centrifugation.

The deposit from the first high-speed centrifugation was resuspended in the original volume of saline, and the suspension recentrifuged at 30,000 r.p.m. for 100 min. The SNF was removed and the deposit treated in the same way a further two or three times.

The differential centrifugation method used to separate virus antigen from soluble antigen was also applied to extracts made by the Casals method (Table 1).

Complement-fixation tests were performed according to the method described by Casals & Olitsky (1950). All sera were heated at 56° C. for 30 min. before use. The end-point was defined as the highest dilution of antigen giving approximately 50 % fixation in the presence of immune serum added in a dilution of 1/10 or 1/20, depending on the concentration at which it was not anticomplementary.

The infectivity of various samples tested by complement fixation was determined by intracerebral titrations in 3 to 4-week-old mice. Samples of serial tenfold

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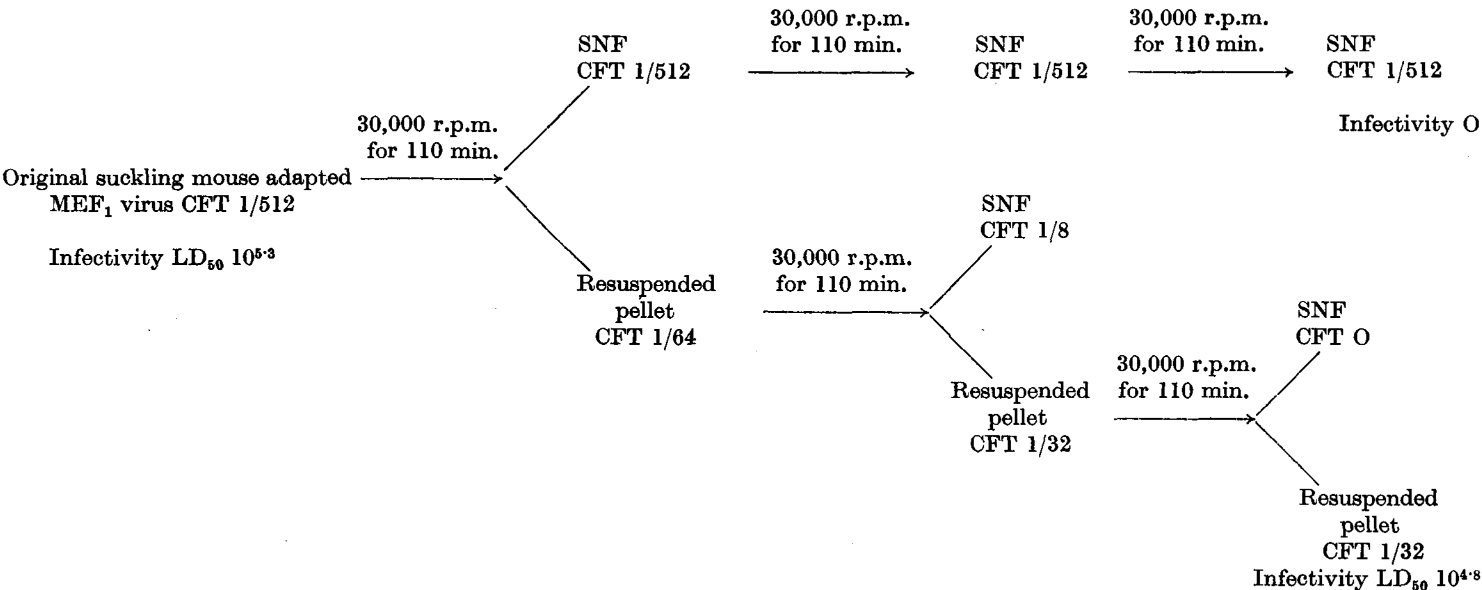
Table 1. *Comparison of the complement-fixing titres of supernatant fluids and deposits after ultracentrifugation of acetone-ether prepared MEF_1 suckling mouse poliomyelitis virus*



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dilutions were injected in 0·03 ml. doses into groups of six mice. The mice were observed daily for 21 days for the development of paralysis and death. Infectivities are recorded as LD_{50} calculated by the method of Reed & Meunch (1938).

Complement-fixation tests were also performed with antigens prepared from the brains of adult mice (3–4 weeks old) infected with the suckling-adapted MEF_1 virus, and the antiserum to the suckling virus.

Neutralization tests

Tenfold dilutions of MEF_1 suckling virus were prepared in saline. To a unit volume of each dilution an equal volume of the immune serum diluted 1/5 was added. The mixtures were allowed to stand at room temperature for half an hour, after which each was injected in 0·03 ml. dose into groups of six mice aged 3–4 weeks.

RESULTS

The results of typical experiments are recorded in Tables 1 and 2. It was found that a higher titre of soluble antigen was obtained by the Casals method than simple centrifugation of a crude 20 % saline emulsion of infected brain, but this depends simply on the smaller volume of saline used for the extraction in the Casals method. Table 3 records the results obtained with an extract prepared by the Casals method from 100 MEF_1-infected suckling brains, the extraction of the dry lipoid-free material having been made in 40 ml. of saline, and of a simple saline extract of an equal weight of infected brains blended for 2 min. in a Waring blender in 40 ml. saline. The yield of soluble antigen is approximately the same by both methods.

The complement-fixing titre of the soluble antigen prepared by simple saline extraction is not altered by simple ether or $CHCl_3$ treatment nor does heating the soluble antigen prepared by the Casals method at 56° C. for $\frac{1}{2}$–$1\frac{1}{2}$ hr. alter its complement-fixing power.

The results recorded in Table 1 show that the complement fixation with the soluble antigen fraction of a suspension prepared by the Casals method is significantly greater than that of the virus fraction which had been subjected to repeated centrifugation. In contrast, the virus fraction contains practically all the original infectivity, whereas the soluble antigen is apparently free of infective virus. Similarly in most experiments with saline emulsions the infective titre of the original suspension was $10^{5\cdot5}$ whilst the soluble antigen prepared by centrifugation contained no detectable virus.

In Table 2 are recorded the results of a typical experiment in which the complement-fixing power of sera prepared against adult and suckling MEF_1 viruses are compared. The specificity of such immune sera has been proved repeatedly by their lack of reaction in the presence of antigens prepared from brains of normal uninfected sucklings, or antigens prepared against Blue Tongue or other viruses.

From the results in Table 2 it appears that antiserum to the adult virus fixes complement in the presence of the virus fraction only, and not in the presence of the soluble antigen fraction. Similarly, Lansing immune monkey serum failed to

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react with soluble antigen but did fix complement in the presence of the virus fraction.

Neither Leon nor Brunhilde immune monkey sera fixed complement in the presence of the MEF_1 virus fraction or its soluble antigen.

Table 2. *Complement fixation tests with the* MEF_1 *suckling virus prepared by the acetone-ether method with suckling and adult* MEF_1 *mouse immune sera and Lansing, Leon and Brunhilde monkey immune sera*

(All sera were used in 1/20 dilution.)

Antigens and sera	Antigen dilutions 1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	Serum control
A. Original extract as antigen									
Serum *v.* suckling virus	4	4	4	4	4	4	3	tr.	0
Serum *v.* adult virus	4	4	4	4	0	0	0	0	0
Lansing immune monkey serum	4	4	4	4	0	0	0	0	0
Leon immune monkey serum	0	0	0	0	0	0	0	0	0
Brunhilde immune monkey serum	0	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	.
B. Virus fraction purified by repeated centrifugation as antigen									
Serum *v.* suckling virus	4	4	4	2	0	0	0	0	0
Serum *v.* adult virus	4	4	4	0	0	0	0	0	0
Lansing immune monkey serum	4	4	4	0	0	0	0	0	0
Leon immune monkey serum	0	0	0	0	0	0	0	0	0
Brunhilde immune monkey serum	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									
Serum *v.* suckling virus	4	4	4	4	4	4	0	0	0
Serum *v.* adult virus	0	0	0	0	0	0	0	0	0
Lansing immune monkey serum	0	0	0	0	0	0	0	0	0
Leon immune monkey serum	0	0	0	0	0	0	0	0	0
Brunhilde immune monkey serum	0	0	0	0	0	0	0	0	0
Antigen control	0	0	0	0	0	0	0	0	.

Attempts were made to demonstrate soluble antigen in the brains of adult mice infected with the suckling virus. The results were of doubtful significance, but appear to indicate the presence of a small amount of soluble antigen in such brains.

The antiserum prepared in mice against the soluble antigen freed of infective virus by repeated centrifugation contained no antibodies demonstrable either by complement fixation or neutralization tests.

COMMENT

From the above results it would appear that the soluble antigen is responsible for most of the complement fixation which occurs with MEF_1-infected suckling mouse brain emulsions and the homologous immune serum prepared in mice. Deposits from such emulsions can be washed until the supernatant fluid is free of complement-fixing antigen. The deposit then still contains practically all the infective virus but relatively little complement-fixing power.

There can be little doubt that the complement fixation with soluble antigen is

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specific. The MEF_1 mouse immune sera do not fix complement in the presence of normal brain emulsions nor in the presence of emulsions of brain from suckling mice infected with other viruses, such as that of Blue Tongue. Furthermore, the MEF_1 antigen does not fix complement in the presence of antiserum prepared in monkeys against Types I and III poliomyelitis viruses.

The absence of infectivity of the soluble antigen from mice is proof that a contaminating virus picked up during serial passage is not responsible for the complement fixation.

Table 3. *Complement-fixation with the MEF_1 suckling virus antigens prepared by* (1) *the acetone-ether method and* (2) *saline extraction*

	Antigen dilutions																
	(1) Acetone-ether								(2) Saline								Serum
Antigens	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	control
A. Original extract	4	4	4	4	4	4	2	tr.	4	4	4	4	4	4	4	0	0
Original antigen control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. Virus fraction purified by centrifugation	4	4	4	4	0	0	0	0	4	4	4	4	4	0	0	0	0
Virus antigen control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. Soluble antigen fraction	4	4	4	4	4	4	2	0	4	4	4	4	4	4	0	0	0
Soluble antigen control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

It is particularly striking that the antiserum to the adult virus fixes complement only in the presence of crude brain emulsions or virus separated from the latter by ultracentrifugation, but not with the soluble antigen freed of infective virus. The antiserum to the suckling virus, on the other hand, though fixing complement to approximately the same extent as antiserum to the adult virus if the virus fraction is used as antigen, shows far better fixation with soluble antigen.

It must therefore be concluded that soluble antigen is absent from the brains of adult mice infected with the MEF_1 virus, or that it is present in such a form or amount that it does not stimulate antibody formation or that, if present, it is serologically distinct from that occurring in the brains of suckling mice infected with the same virus. The fact that soluble antigen can be detected, though in small amounts, in extracts of the brains of adult mice infected with suckling virus supports the conclusion that the difference is, at least in part, due to a change induced in the virus itself during its adaptation to the suckling.

It is striking that both infective virus and soluble antigen occur in larger amounts in suckling brains than in brains of adult mice infected with the MEF_1 strain of poliomyelitis virus. It is tempting to conclude that the soluble antigen formed in suckling mouse brains is either a specific product of cell-virus interaction which does not form a constituent of the virus itself and which is not formed or is masked when the virus not adapted by repeated passage in sucklings multiplies in

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the brains of adult mice, or alternately, that it is part of the virus particles formed in excess of the amount required for the complete or mature virus present. The amount of soluble antigen present and its physical dispersion could account for its ready detection by complement-fixation tests in suckling brain emulsions. Either of these hypotheses would, however, require confirmation when quantitative methods, at present not available, can be applied.

The fact that repeated injections of soluble antigen (freed of virus) into mice have failed to elicit any detectable antibodies may mean that the soluble component responsible for specific complement fixation is in the nature of a hapten. On the other hand, the bulk of the material present in the immunizing injections may have been insufficient to elicit detectable antibody formation. In this connexion it is of interest that Henle (1953) has stated that the soluble antigen found in influenza virus infected tissues is incapable of eliciting antibody formation.

SUMMARY

By ultracentrifugation of emulsions of brains from suckling mice infected with the MEF_1 strain of poliomyelitis virus, infective virus can be separated from a non-infective soluble antigen. The soluble antigen which remains in the supernatant fluid is serologically specific and is responsible for most of the complement fixation shown by such brain emulsions.

Soluble antigen is not demonstrable in the brains of adult mice infected with a strain of the virus which has not been adapted to sucklings.

The soluble antigen is heat stable and resists treatment with organic lipoid solvents such as acetone, ether and chloroform.

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