# A COMPARISON OF COMPLEMENT-FIXATION TITRES IN POLIOMYELITIS-INFECTED TISSUE-CULTURE FLUIDS AND MOUSE BRAINS

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Previous communications from this laboratory (Selzer & Polson, 1954 and Selzer & van den Ende, 1956) reported the presence of a non-infective antigen in suspension of brains of infant mice infected with the $MEF_1$ strain of poliomyelitis. This antigen, which was specific and accounted for more than half the complement-fixing power of brain suspensions, was present in the supernatants from which infective virus had been removed by ultra-centrifugation. The fact that polio tissue-culture fluids fixed so little complement in comparison with infected suckling mouse brains suggested that this soluble antigen was not formed in infected monkey kidney tissue-culture fluids.

In this communication are recorded the results of investigations on polio-infected tissue culture fluids and suckling mouse brains using both Mahoney, Type 1 and $MEF_1$, Type 2 strains.

## MATERIALS AND METHODS

### *Viruses*

The viruses used were: (1) $MEF_1$, Type 2, poliomyelitis virus adapted to suckling mice in this laboratory (Selzer, Sacks & van den Ende, 1952) and then adapted to tissue culture. (2) Mahoney, Type 1, poliomyelitis virus grown in tissue culture.

### *Sera*

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

(2) Further Mahoney and $MEF_1$ immune sera were each prepared from infected monkey kidney tissue-culture fluids by inoculation into 2-month-old mice. A similar course of immunizing injections of 0·5 ml. of undiluted infected tissue-culture fluids was given. The fluids were given a preliminary centrifugation at 3000 r.p.m. for 1 hr. to remove cell debris and the supernatant used for inoculation.

(3) Coxsackie B3 immune serum was similarly prepared from monkey kidney tissue-culture fluids. The preliminary centrifugation was omitted so that non-specific antibodies which may be produced to monkey kidney tissue could be detected.

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(4) Rabies immune mouse serum was prepared in the same way as the sera against Mahoney and $MEF_1$ suckling mouse polio viruses.

All sera were heated at 56° C. for 30 min., and were used in a dilution of 1/20 for complement-fixation tests.

### *Tissue culture*

Trypsin digests of monkey kidney were prepared by the method of Melnick, Rappaport, Banker & Bhatt (1955). The cells were grown in test-tubes, each tube receiving approximately $6 \times 10^5$ cells in 1 ml. of nutrient medium consisting of Hanks' solution with 0·5 % lactalbumin hydrolysate and 5 % calf serum. When the cells were ready for inoculation the serum was omitted from the medium.

Titrations of infectivity of antigens were carried out in tissue culture using serial tenfold dilutions prepared in Hanks' solution. Each dilution was inoculated into three tubes giving an inoculum of 0·1 ml. per tube. The tubes were observed regularly for 8 days for cytopathogenic change and the infectivity calculated by the method of Reed & Muench (1938).

### *Preparation of antigens for complement fixation*

Infected tissue-culture fluids were harvested when the cells showed complete cytopathogenic change, centrifuged at 10,000 r.p.m. for 10 min. to remove cell debris and the supernatant used as antigen.

The infected suckling mouse brain extracts were prepared by the acetone-ether method described by Casals (1949). These antigens were centrifuged at 10,000 r.p.m. for 10 min. to remove coarse debris.

Samples of infected tissue-culture fluids and infected suckling mouse brain antigens were reserved for complement-fixation and infectivity titrations 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 formed the soluble antigen fraction. The rest of the fluid was discarded and the pellet redispersed in a volume of saline equal to the original volume. The resuspended pellet was sometimes centrifuged a second time at 30,000 r.p.m. for 110 min. and the pellet redispersed as before.

Complement-fixation tests were performed by the method of Casals & Olitsky (1950). 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 1/20.

## RESULTS

The results of typical experiments are recorded in Tables 1, 2 and 3.

Table 1 is an example in which the complement-fixing power of sera prepared against $MEF_1$, Mahoney, and Coxsackie B3 tissue-culture viruses and $MEF_1$ suckling mouse brain virus are compared, using $MEF_1$ tissue-culture fluid as antigen. The antiserum prepared against $MEF_1$-infected suckling mouse brain fixed complement less well than that prepared against $MEF_1$ TC virus. Both the original virus suspension and the redispersed pellet showed virtually the same fixation of complement, whilst the complement-fixing activity of the soluble antigen was only detectable in undiluted fluids.

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On the other hand, in $MEF_1$ suckling mouse infected brains (Table 2) not only was soluble antigen present in high titre in the presence of antiserum to extracts of infected mouse brain, but in amounts greater than that of the virus fraction. Antiserum to tissue-culture virus, on the other hand, though fixing complement to high titre with the virus fraction was less active with the soluble antigen fraction, again suggesting that the soluble antigen content in tissue-culture virus too is low.

After several tissue-culture passages the $MEF_1$ virus was still highly pathogenic suckling mice, indicating no apparent change in the virus.

Table 1. *Complement-fixation tests with the $MEF_1$, Type 2 virus grown in monkey kidney tissue culture, and immune sera prepared against $MEF_1$ suckling mouse virus and $MEF_1$, Mahoney aud Coxsackie B3 tissue-culture viruses*

| Antisera to | Antigen dilutions $\frac{1}{2}$ | $\frac{1}{4}$ | $\frac{1}{6}$ | $\frac{1}{8}$ | $\frac{1}{12}$ | $\frac{1}{16}$ | $\frac{1}{24}$ | Serum control | Virus titre |
|---|---|---|---|---|---|---|---|---|---|
| **A. Original tissue culture fluid as antigen** | | | | | | | | | |
| $MEF_1$ suckling virus | 4 | 4 | 4 | 1 | 0 | 0 | 0 | 0 | |
| $MEF_1$ TC virus | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | |
| Mahoney TC virus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $10^{7.5}$ |
| Coxsackie B3 TC virus | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | . | |
| **B. Virus fraction separated by ultra-centrifugation as antigen** | | | | | | | | | |
| $MEF_1$ suckling virus | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | |
| $MEF_1$ TC virus | 4 | 4 | 4 | 4 | 3 | 2 | 0 | 0 | |
| Mahoney TC virus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $10^{6.7}$ |
| Coxsackie B3 TC virus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | . | |

C. Soluble antigen fraction as antigen

| Antisera to | $\frac{1}{1}$ | $\frac{1}{2}$ | $\frac{1}{3}$ | $\frac{1}{4}$ | Serum control | Virus titre |
|---|---|---|---|---|---|---|
| $MEF_1$ suckling virus | 0 | 0 | 0 | 0 | 0 | |
| $MEF_1$ TC virus | 2 | 0 | 0 | 0 | 0 | |
| Mahoney TC virus | 0 | 0 | 0 | 0 | 0 | $10^{2.5}$ |
| Coxsackie B3 TC virus | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | . | |

Similar results were obtained repeatedly with Mahoney tissue-culture fluids as antigens, viz. poor fixation of complement with the original tissue-culture fluid and pellet, despite high infectivity titres, and no fixation of complement with the 'soluble antigen' (Table 3). The slight fixation of complement in the presence of Coxsackie B3 and $MEF_1$ immune sera was due to non-specific monkey kidney products. These sera were specifically prepared to detect antibodies to such products, so that one could distinguish specific fixation in the presence of infective virus from fixation obtained in the presence of disintegrated cells acting as antigen.

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

| Antisera to | Antigen dilutions 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | Serum control | Virus titre |
|---|---|---|---|---|---|---|---|---|---|---|
| **Exp. 1** | | | | | | | | | | |
| *A. Original extract as antigen* | | | | | | | | | | |
| $MEF_1$ suckling virus | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | $10^{5.7}$ |
| $MEF_1$ TC virus | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | |
| Rabies 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 | 4 | 1 | 0 | 0 | 0 | 0 | $10^{5.7}$ |
| $MEF_1$ TC virus | 4 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | |
| Rabies 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 | 3 | 0 | 0 | 0 | $10^{2.0}$ |
| $MEF_1$ TC virus | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Rabies suckling virus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | . | |
| **Exp. 2** | | | | | | | | | | |
| *A. Original extract as antigen* | | | | | | | | | | |
| $MEF_1$ suckling virus | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | $10^{5.7}$ |
| $MEF_1$ 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 | $10^{5.7}$ |
| $MEF_1$ 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 | 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 | . | |

## COMMENT

From the above results it would appear that there is little or no soluble antigen in the fluids obtained from polio-infected monkey kidney tissue cultures, and this may account for the persistently low fixation of complement despite the high infectivity titres of these fluids. It also raises the question of the nature of the so-called soluble antigen. Is it an integral part of the virus? It is unlikely to reside at the surface because antibodies to soluble antigen and whole virus are evidently different. It

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Table 3. *Complement-fixation tests with Mahoney, Type 1 virus grown in tissue culture and immune sera prepared against Mahoney suckling mouse virus and Mahoney, $MEF_1$ and Coxsackie B3 tissue culture viruses*

| Antisera to | Antigen dilutions $\frac{1}{2}$ | $\frac{1}{4}$ | $\frac{1}{6}$ | $\frac{1}{8}$ | $\frac{1}{12}$ | $\frac{1}{16}$ | $\frac{1}{24}$ | Serum control | Virus titre |
|---|---|---|---|---|---|---|---|---|---|
| **A. Original tissue culture fluid as antigen** | | | | | | | | | |
| Mahoney suckling virus | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mahoney TC virus | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | |
| Coxsackie B3 TC virus | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $10^{6\cdot5}$ |
| $MEF_1$ TC virus | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | . | |
| **B. Virus fraction separated by ultra-centrifugation as antigen** | | | | | | | | | |
| Mahoney suckling virus | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mahoney TC virus | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | |
| Coxsackie B3 TC virus | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | $10^{7\cdot0}$ |
| $MEF_1$ TC virus | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | . | |

C. Soluble antigen fraction as antigen

| Antisera to | $\frac{1}{1}$ | $\frac{1}{2}$ | $\frac{1}{3}$ | $\frac{1}{4}$ | Serum control | Virus titre |
|---|---|---|---|---|---|---|
| Mahoney suckling virus | 0 | 0 | 0 | 0 | 0 | |
| Mahoney TC virus | 0 | 0 | 0 | 0 | 0 | |
| Coxsackie B3 TC virus | 0 | 0 | 0 | 0 | 0 | $10^{4\cdot2}$ |
| $MEF_1$ TC virus | 0 | 0 | 0 | 0 | 0 | |
| Antigen control | 0 | 0 | 0 | 0 | . | |

may, however, be a specific product of virus-cell interaction, which is not a constituent of the virus but a by-product only formed to any extent in certain actively metabolizing tissues such as suckling mouse brain.

## SUMMARY

Ultra-centrifugation of emulsions from suckling mouse brains infected with the $MEF_1$ strain of poliomyelitis virus separates a non-infective antigen, or soluble antigen, from infective virus. This antigen is responsible for most of the complement fixation and explains the high titres obtained. On the other hand, the same virus, and also Mahoney, Type 1 poliomyelitis virus, grown in monkey kidney tissue culture, fail to produce this soluble antigen, and this is probably a factor in the low complement-fixing titres obtained in tests with these fluids.

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