object of this report is to supply evidence that the small particle in adapted MEF_1 poliomyelitis is capable of being cultured independently of the larger-sized virus particle.

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SUMMARY

By means of a combined process of migration into agar gel and ultracentrifugation, the 24 mμ component of MEF_1 poliomyelitis virus adapted to sucklings has been separated from the 30 mμ particles with which it is normally associated, and grown in adult as well as in suckling mouse brains. The progeny of this separated variant in adult and suckling mice is composed of the 24 mμ particles only. This virus is probably a real variant as it "breeds truly" in the host, *i.e.* the suckling mouse.

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THE ELECTROPHORETIC MOBILITIES OF ADAPTED MEF_1 POLIOMYELITIS VIRUS AND ITS SOLUBLE ANTIGEN

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In a previous communication it was reported that the greater part of the complement-fixing ability of an extract of MEF_1-infected suckling mouse brains was due to a substance of smaller size than that of the infective virus particles (SELZER AND POLSON[1]). From its rate of sedimentation in the preparative Spinco ultracentrifuge the diameter of the small component was estimated at 12 mμ. It was further shown that the infectivity was carried by particles of two different sizes. One to ten percent of the infectivity was associated with particles of 24 mμ and the remainder had a

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diameter of approximately 29 mμ. The existence of the smaller infective units was confirmed by later experiments in which separation of the two infective particles was achieved by migration into agar gel of such a concentration that it would allow the entrance of the 24 mμ but exclude the 29 mμ particles (POLSON[2]). Further proof for the existence of the 24 mμ particle was recently obtained when we succeeded in isolating and propagating it in adult as well as suckling mice, the propagated virus being composed of infective particles of 24 mμ only (POLSON AND SELZER[3]).

The relationship between the virus and its soluble antigen is still obscure. The latter could be a matrix substance or a cellular by-product of specific nature produced during the development of the virus, but which does not enter the constitution of the virus, or it could be specific virus material produced in excess of that required for the synthesis of the virus particles. Evidence of a virus component of a size similar to that of the soluble antigens has recently been found by HAMPTON[4]. By desiccating purified MEF_1 virus and resuspending it in water HAMPTON found that the virus particles dissociated into uniformly-sized spheres of diameters approximately 10–15 mμ. These particles were no longer infective and had lost most of their complement-fixing power with specific mouse immune serum. At least in their serological reactivity, therefore, they appear to be different from MEF_1-soluble antigen. It must, however, be borne in mind that MEF_1-soluble antigen may during the process of dissociation undergo changes, such as denaturation or loss of receptor sites, which alter its immunological reactivity.

Not only has it been found that particles of the same size as soluble antigen can be obtained by dissociation of purified virus particles, but it appears that the electrophoretic mobility of soluble antigen and infective virus are the same. This suggests, though it does not prove, that soluble antigen may in fact form an integral part of the virus. It is the purpose of this paper to record the electrophoresis experiments carried out with the MEF_1 virus and its soluble antigen as they occur in the brains of virus-infected suckling mouse brains.

EXPERIMENTAL

Electrophoresis of MEF_1 poliomyelitis virus

The virus derived from infective suckling mouse brains was partially purified by repeated differential ultracentrifugations in the Spinco preparative centrifuge. The virus was suspended in 20% normal rabbit serum in buffer and dialysed overnight against phosphate buffer of pH 8.2, and ionic strength of 0.1. The mixture of virus and serum was electrophoresed and samples taken of the contents of the electrophoresis tube according to the technique of POLSON, JOUBERT AND HAIG[5]. The virus content of the samples was determined by intracerebral titration in 3–4 weeks old mice, using an inoculum of 0.03 ml and groups of 6 mice per dilution. The virus titres expressed as $\log_{10}$ LD 50 per inoculum were plotted against the position where the samples were taken. The position of the samples could also be related to the position of the various components present in the serum as recorded in the serum electrophoresis diagram. In addition, the position of the haemoglobin boundary could be easily determined. In Fig. 1 the results of 4 experiments are presented graphically. In all 4 experiments the content of the ascending column was sampled. One set of results obtained with samples from the descending column is also recorded.

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Taking the position of the virus boundary to be in the region of rapid rise in virus titre, the results show that this corresponds to that of a component with the electrophoretic mobility, under the particular experimental conditions employed, of haemoglobin or a component that moves at a slightly slower rate than β-globulin. In the descending column small amounts of virus corresponding to approximately 0.1% of the original remained in the globulin components. This was probably virus adsorbed to the walls of the electrophoresis tube which was dislodged when the samples were taken.

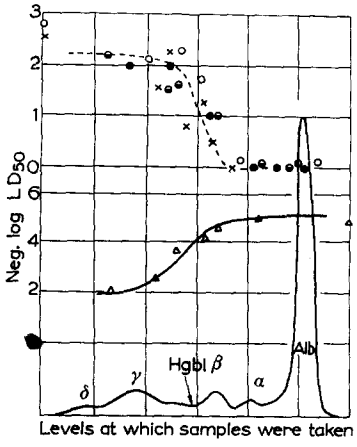

Fig. 1. Showing virus content of samples taken at various levels in electrophoresis column. Top curve, those taken in ascending limb; middle curve, those from descending limb. The bottom curve shows the electrophoretic pattern of rabbit serum under the conditions of the experiments.

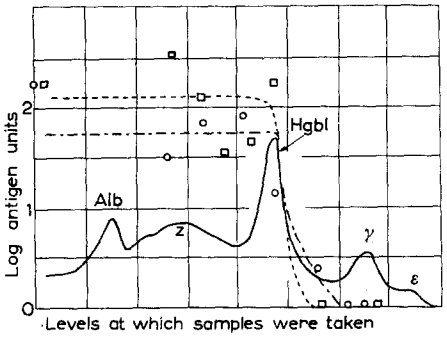

Fig. 2. Showing amount of complement-fixing antigen of samples taken at various levels in the descending limb of an electrophoresis column after electrophoresis of MEF_1 poliomyelitis soluble antigen. The bottom curve shows the electrophoretic pattern of the suspension subjected to electrophoresis. Z: Broad peak between the albumin and haemoglobin levels, which contains α- and β-globulins as well as brain proteins.

Electrophoresis of the soluble antigen of MEF_1 poliomyelitis virus

The soluble antigen used in the electrophoresis experiments was obtained from 60 infective suckling mouse brains extracted in 20 ml phosphate buffer of pH 8.2 and freed of active virus by ultracentrifugation. This extract, which contained the major portion of the complement-fixing power, was dialysed against the phosphate buffer for 2 days and subjected to electrophoresis, using the method of simultaneous electrophoresis of POLSON[6]. Normal rabbit serum diluted to contain 0.5% total protein in phosphate buffer at pH 8.2 was used for comparison. After electrophoresis the column was cut up into a number of samples, care being taken to avoid unnecessary dilution with the buffer. That such dilution did not occur on sampling could be verified by photometric measurements of the haemoglobin content of those samples from the electrophoresis column in which haemoglobin was present at the end of the electrophoresis run. The levels at which the samples were taken could be clearly shown with recordings taken by the Lamm Scale method after each sample. The amount of soluble antigen present in each sample was determined by complement fixation using mouse immune serum. The amount of antigen present is expressed as log units —this being the $\log_{10}$ of the reciprocal of the highest dilution giving 50% fixation in the presence of 1/20 mouse immune serum. The method used was that described

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by CASALS AND OLITSKY[7]. The results of two experiments are recorded in Fig. 2. The method employed allows of sampling only from the descending column and not from both as in experiments recorded in Fig. 1. The positions at which the samples were taken could be related to those of the various proteins of blood and brain origin present in the original material as shown in the electrophoresis pattern in Fig. 2. The peaks that were identified were those of albumin, haemoglobin and γ-globulin, see Fig. 2. The broad component between the albumin and haemoglobin peaks contains the major portion of the brain components as well as those of α- and β-globulins. In experiments performed on extracts of suckling mouse brains, which had been thoroughly washed in buffer prior to emulsification, these components were present in greatly reduced amounts. It is evident that the position of rapid rise in soluble antigen content was again in the region of the haemoglobin component. On comparing the position occupied by the MEF_1 virus boundary and that of the soluble antigen with reference to haemoglobin and other proteins present it is clear that the virus and soluble antigen have identical electrophoretic mobilities at pH 8.2.

Electrophoresis experiments were not performed in buffers of higher hydrogen ion concentration because faint precipitates, presumably of euglobulin, were formed during dialysis and during electrophoresis of the rabbit serum virus mixture at such pH's. Similar precipitates were also noticed at lower pH values in the crude soluble antigen. These precipitates, which were particularly noticeable below pH 7, acted as adsorbents for the virus and soluble antigen and therefore made electrophoretic analyses impracticable.

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SUMMARY

Electrophoretic studies on emulsions of MEF_1 virus-infected suckling mouse brain, indicate that the three components which can be detected by physico-chemical and biological means, namely, the soluble antigen, as well as the 24 mμ and the 29 mμ infective particles have identical electrophoretic mobilities at pH 8.2. This finding may be of significance in determining whether soluble antigen forms an integral part of the MEF_1 virus particle or not. Furthermore the material that has been employed in which "soluble antigen" and virus are not as vastly different in dimensions as is the case with larger viruses, may be particularly useful in the elucidation of problems concerned with the formation and structure of viruses.

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