SEPARATION OF SMALL INFECTIVE COMPONENTS OF MEF_1 POLIOMYELITIS AND HORSESICKNESS VIRUSES BY MIGRATION INTO AGAR GEL

by

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Several of the small viruses which have been analysed in the Spinco preparative ultracentrifuge by the method of POLSON AND LINDER[1] have shown the presence of infective particles of widely different sedimentation constants. Thus the MEF_1 strain of poliomyelitis showed the infectivity to be associated with two components of sedimentation constants 100 *S* and 170 *S* (SELZER AND POLSON[2]). Neurotropic Rift Valley fever which had been passaged intracerebrally for 106 generations in mice followed by 50 passages in chick embryos and a further 9 intracerebral passages in mice showed infective particles of sedimentation constants 175 *S* and 492 *S* (NAUDE, MADSEN AND POLEN[3]) and African horsesickness virus has been shown to have its infectivity associated with particles of sedimentation constants 180 *S* and 476 *S* (POLSON AND MADSEN[4]). Neurotropic yellow fever virus on the other hand is an exception in that it has all its infectivity associated with a particle of sedimentation constant 170 *S* (POLSON[5]).

These differences in sedimentation constants may be interpreted in several ways, *viz.*

(a) The particles may be covered with layers of lipoid of different thickness which would thereby give the particles overall lower densities and consequently different sedimentation constants.

(b) The particles may be of different shapes so that they have different frictional constants and consequently different sedimentation rates.

(c) The particles may have the same shape (possibly spherical) but different diameters.

Most of the animal viruses which have been purified and identified on electron micrographs are spherical or nearly spherical and no case has yet been found which showed rod-shaped particles as some plant viruses do, notably tobacco mosaic virus. Possibility (b) is thus very unlikely.

To decide between possibilities (a) and (c), use was made of their abilities to migrate into agar gels of different concentration. It has been found that the migration of proteins (including viruses) into agar gel depends on the concentration of agar in the gel.

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METHODS

Standardization of the agar gel

By layering a dilute gel over a more concentrated gel an osmotic pressure gradient can be established across the dilute gel. If a watery suspension of protein be placed on the surface of the dilute gel some fluid will be drawn into the gel, and if the pore sizes in the gel are larger than the protein particles the protein will move into the dilute gel with the water. If pigmented protein is used the movement into the gel can be followed by the movement of the colour band. The osmotic pressure gradient across the dilute gel layer can be varied at will by the incorporation of different concentrations of glycerine in the concentrated gel in the lower part of the tube. In the present experiments this was avoided because the proteins were drawn into higher gel concentrations than when glycerine was omitted. Two haemocyanines of different molecular weight were used in the standardization of the agar gel. That from the crayfish *Jasus lalandi* has a particle diameter of 10.5 mμ and a molecular weight of 450,000 (JOUBERT[6]); and that from the whelk *Caminella sincta* has sedimentation constant 100 *S*, a probable molecular weight of 6,600,000 and a particle diameter of 24 mμ (POLSON AND LINDER (*loc. cit.*)). In addition to these, rabbit haemoglobin was also used.

A 5 cm long column of 8% agar dissolved in *M*/15 phosphate buffer of pH 7.2 was allowed to set in each of a series of $^7/_8'' \times 6''$ test tubes. Over these concentrated gel columns a 5 mm thick layer of agar varying in concentration in the different tubes from 0.7% to 8% were placed. A separate set was prepared for each protein tested. A small amount (0.25 ml) of the protein solution under test was placed on the upper surface of the agar in each tube and allowed to remain in contact for three days, the tubes being stored in the cold. The surfaces of the different gels were then washed free of unabsorbed protein and the gels examined for the presence of protein which had penetrated it. It was found that rabbit haemoglobin migrated into the gel to the same extent irrespective of its agar content within the range tested. *Jasus lalandi* haemocyanine moved into those gels which contained less than 7% agar, whereas for the haemocyanine of *Caminella sincta* the limiting concentration was approximately 3%. The relationship between the particle's diameter and the minimum agar concentration which prevents its entrance under the particular conditions of our experiments is probably that shown in Fig. 1. Very dilute agar gels, in which the molecules are far apart, would hold back large particles only and very concentrated gels with their molecules close together would exclude small particles. The following equation can be applied: $Cd = K$ (1)

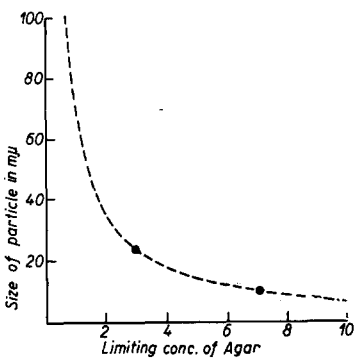

Fig. 1. Relationship between size of particle and limiting concentration of agar. (Lowest concentration of agar preventing entrance of particle into it.)

In this equation d is the diameter in mμ of the particle and C the limiting concentration of the agar. K is a constant. The value of K calculated from the results obtained with the two haemocyanines is approximately 70. Equation (1) enables one to calculate the approximate limiting concentration of agar for the smaller animal viruses.

RESULTS

MEF_1 poliomyelitis

From equation (1) it was calculated that the 170 *S* (30 mμ) particle of MEF_1 virus would be held back by 2.5% agar. To test this the virus from 20 infected suckling mouse brains was extracted in 20 ml 10% rabbit serum saline and partially purified by differential ultracentrifugation. The final pellet was dispersed in 1 ml 10% rabbit serum saline and 0.25 ml of this suspension was placed in each of 4 tubes prepared with a top layer of 2.5% agar and the usual bottom layer of 8% agar. They were left in the refrigerator for 3 days and during this period nearly all the fluid diffused into the agar. The surface of the agar was thoroughly washed by impinging a light jet of 10% serum saline on to it. After four such washings the tubes were inverted and left to drain. The 2.5% agar layers were then scooped out and ground up in 10 ml serum

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saline with the aid of a Ten Broeck grinder. The agar was centrifuged off at 10,000 r.p.m. for 10 minutes and the supernatant fluid mixed with an equal amount of *Caminella sincta* haemocyanine used for particle size determination by the ultracentrifugation method. After centrifugation at 20,000 r.p.m. for 100 minutes in the

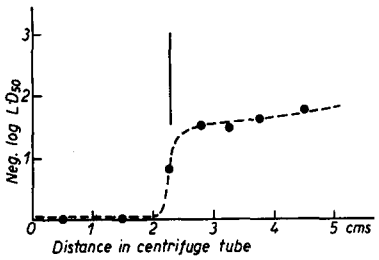

Fig. 2. Sedimentation diagram of component with sedimentation constant 100 *S* isolated from MEF_1 poliomyelitis by migration into 2.5% agar. The solid vertical line represents the position of the haemocyanine (*C. sincta*) boundary. Centrifugation at 20,000 r.p.m. for 100 minutes.

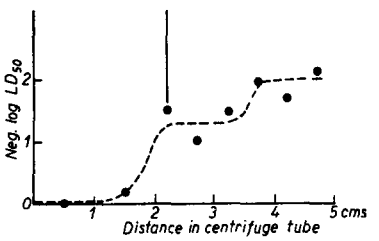

Fig. 3. Sedimentation diagram of MEF_1 poliomyelitis virus isolated by migration into 1.5% agar. The solid vertical line represents the position of the haemocyanine (*C. sincta*) boundary. Centrifugation at 20,000 r.p.m. for 100 minutes.

Model L Spinco preparative ultracentrifuge successive layers were removed and the virus content of each determined by titration in mice. Fig. 2 records the sedimentation diagram of the virus and shows the position or the haemocyanine boundary. There is a single sedimentation boundary corresponding to the virus particle of 100 *S*. No evidence is found of the 170 *S* particle normally also present in material not previously subjected to diffusion into 2.5% agar.

Fig. 3 records the results of a similar experiment in which the upper gel layer contained only 1.5% agar. The normal sedimentation pattern indicating the presence of virus of 2 particle sizes was obtained.

It was shown experimentally that it was not simply the grinding of a virus suspension in the presence of 2.5% agar gel which gave rise to the altered sedimentation diagram. A sedimentation diagram of a virus suspension treated in such a way is recorded in Fig. 4. From these experiments it is concluded that the slower sedimenting component of MEF_1 poliomyelitis virus is a particle of smaller diameter than the component with a sedimentation constant of 170 *S* and that its slow sedimentation is not due to a higher lipoid content.

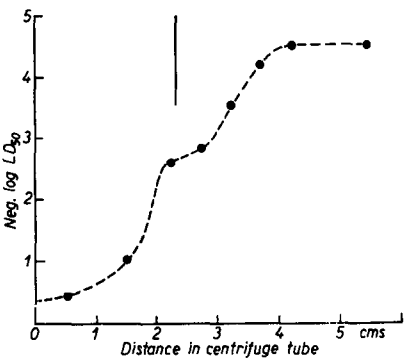

Fig. 4. Sedimentation diagram of MEF_1 poliomyelitis virus extracted from infective material ground up in the presence of 2.5% agar. The position of the haemocyanine (*C. sincta*) boundary is indicated by the solid vertical line. Centrifugation at 20,000 r.p.m. for 100 minutes.

Neurotropic African horsesickness virus

From equation (1) it can be calculated that the 50 mμ particle of horsesickness virus which has a sedimentation constant of 476 *S*, would be held back by agar gel with a

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concentration of approximately 1.5%. The partially purified virus of the strain Vryheid obtained from 20 infected adult mouse brains was suspended in 1 ml 10% rabbit serum saline and allowed to migrate into 1.5% agar gel under similar conditions as was done with the MEF_1 virus. The extract of the 1.5% agar gel was ultracentrifuged

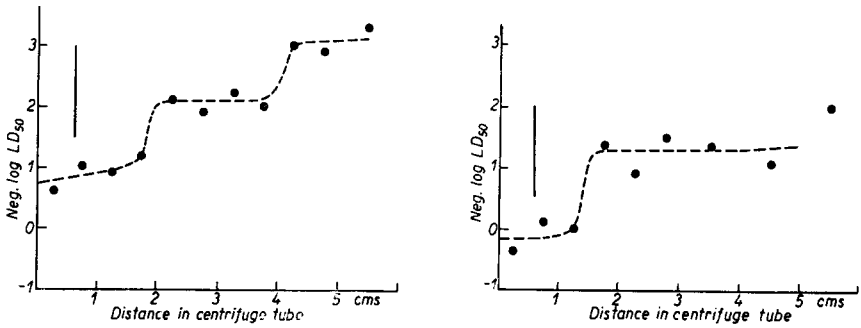

Fig. 5. (a) Sedimentation diagram of unfractionated Horsesickness virus. (b) Sedimentation diagram of Horsesickness virus isolated by migration into 1.5% agar. The positions of the haemocyanine (*C. sincta*) boundaries are indicated by the solid vertical lines. Centrifugation at 11,000 r.p.m. for 100 minutes.

at 11,000 r.p.m. for 100 minutes in the presence of *Caminella sincta* haemocyanine (sedimentation constant 100 *S*). As a control, material not subjected to migration into agar was also ultracentrifuged. Figs. 5(a) and (b) record the results of a typical experiment. Components of both the main particle sizes are present in the untreated material and only the component with a sedimentation of 180 *S* is present in the material recovered from the gel. Unlike the sedimentation diagrams obtained with the MEF_1 virus suspensions (Figs. 2 and 3) those obtained with horsesickness virus suspensions show considerable amounts of virus above the two sedimenting boundaries. Attempts to isolate a still smaller component by migration into gel of higher concentration were not satisfactory on account of the low titre of the apparently smaller component with consequent large losses during recovery from the agar. An additional not previously recognised component was found on ultracentrifuging the material isolated with 1.5% get at 20,000 r.p.m. for 100 minutes. The results of such an experiment in which *Caminella sincta* haemocyanine was present as reference are shown in Fig. 6. Like the slower sedimenting component of MEF_1 virus this component has a sedimentation constant of approximately 100 *S*.

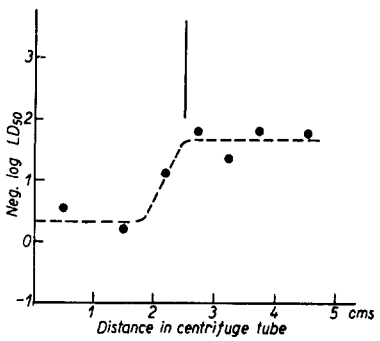

Fig. 6. Sedimentation diagram of Horsesickness virus isolated by migration into 1.5% agar gel and ultracentrifuged at 20,000 r.p.m. for 100 minutes. The vertical solid line indicates the position of the reference haemocyanine (*C. sincta*).

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SUMMARY

From the foregoing experiments it is concluded that the slower sedimenting components of MEF_1 poliomyelitis and African horsesickness viruses are definitely smaller particles than the infective components with higher sedimentation constants. They were capable of migrating into agar gel which held back the components with higher sedimentation constants. The possibility that their differing sedimentations on centrifugation depend on factors other than size, such as lipoid content, can therefore probably be excluded. In the case of horsesickness virus an additional infective component with a sedimentation constant of approximately 100 *S* was shown. This is additional to the two components with sedimentation constants of 180 *S* and 476 *S* which have been previously identified (POLSON AND MADSEN (*loc. cit.*)).

RÉSUMÉ

Les résultats décrits permettent de conclure que les constituants à sédimentation lente du virus de la polyomyélite MEF_1 et du virus Africain du cheval sont des particules nettement plus petites que les constituants infectieux à constantes de sédimentations plus élevées. Ils peuvent migrer dans un gel d'agar qui retient les constituants à constantes de sédimentations élevées. Il n'est donc pas possible que leurs sédimentations différentes après centrifugation soient dues à des facteurs autres que la taille, tels que la teneur en lipoïde. Dans le cas du virus du cheval, les auteurs ont observé un constituant infectieux supplémentaire dont la constante de sédimentation est d'environ 100 *S*. Ce constituant s'ajoute aux deux constituants de constantes de sédimentations respectives 180 *S* et 476 *S* qui ont été antérieurement identifiés (POLSON ET MADSEN (*loc. cit.*)).

ZUSAMMENFASSUNG

Aus den obenangeführten Versuchen wird die Folgerung gezogen, dass die langsamer sedimentierenden Virus-Komponenten der MEF_1 Poliomyelitis und der afrikanischen Pferdesterbe entschieden kleinere Partikel darstellen, als die infektiösen Komponenten mit höheren Sedimentierungskonstanten. Sie waren imstande, in einen Agar-Gel hineinzuwandern, durch welchen die Komponenten mit höheren Sedimentierungskonstanten zurückgehalten wurden. Es kann daher mit Wahrscheinlichkeit behauptet werden, dass die beim Abschleudern festgestellten verschiedenen Sedimentierungsgeschwindigkeiten der Grösse und nicht anderen Faktoren, wie dem Lipoidgehalt, zuzuschreiben sind. Im Falle des Pferdesterbevirus wurde eine weitere infektiöse Komponente mit einer Sedimentierungskonstante von ungefähr 100 *S* gefunden. Die Letztere kommt also noch zu den beiden früher identifizierten (POLSON UND MADSEN (*loc. cit.*)) Komponenten mit Sedimentierungskonstanten von 180 *S* und 476 *S* hinzu.

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