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Ultracentrifugal Concentration of a Homogeneous Heavy Component from Tissues Diseased with Equine Encephalomyelitis.

RALPH W. G. WYCKOFF.

From the Rockefeller Institute for Medical Research, Princeton, N. J.

With the demonstration of the existence of a heavy protein in plant tissues diseased with tobacco mosaic virus[1] it became inevitable that similar proteins should be sought in other kinds of virus-infected tissues. Most viruses are more or less unstable towards the chemicals which would naturally be used in efforts to concentrate and purify them. The air-ultracentrifuge[2] has, however, furnished a new tool[3] which has been successful in extracting and purifying several of the less stable virus proteins causing plant diseases[4] and in preparing a similar substance[5] from virus-induced rabbit papillomas (Shope). The same type of ultracentrifugal examination has now been made of tissues diseased with the virus of equine encephalomyelitis.*

As in the previous ultracentrifugal concentrations an angle-centrifuged tissue suspension was ultracentrifuged for one to one and a half hours in a maximum field of ca 50,000 times gravity. All but a trace of the virus activity was thus sedimented. The translucent pellet formed at the bottom of the tube by this ultracentrifugation was resuspended in a suitable solvent and the aggregated colloidal matter thrown down by another low speed centrifugation. Ultracentrifugation of this solution furnished a smaller but cleaner pellet. On occasion the purification of this pellet was carried further by a repetition of the cycle of centrifugation, ultracentrifugation and resuspension. The virus of equine encephalomyelitis is not thermostable; it rapidly becomes non-infectious at room temperature and cannot be relied upon to preserve its activity for long periods of time in the icebox. Therefore all manipulations, including the ultracentrifugations, were carried out without delay and in the cold.

1 Stanley, W. M., *Science*, 1935, **81**, 644; *Am. J. Botany*, 1937, **24**, 59.

2 Beams, J. W., and Pickels, E. G., *Rev. Sci. Instr.*, 1935, **6**, 299; Biscoe, J., Pickels, E. G., and Wyckoff, R. W. G., *J. Exp. Med.*, 1936, **64**, 39; Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 503; 1937, **65**, 565; Wyckoff, R. W. G., and Lagsdin, J. B., *Rev. Sci. Instr.*, 1937, **8**, 74.

3 Wyckoff, R. W. G., *Proc. Am. Phil. Soc.*, 1937, **77**, 455.

4 Stanley, W. M., and Wyckoff, R. W. G., *Science*, 1937, **85**, 181.

5 Beard, J. W., and Wyckoff, R. W. G., *Science*, 1937, **85**, 201.

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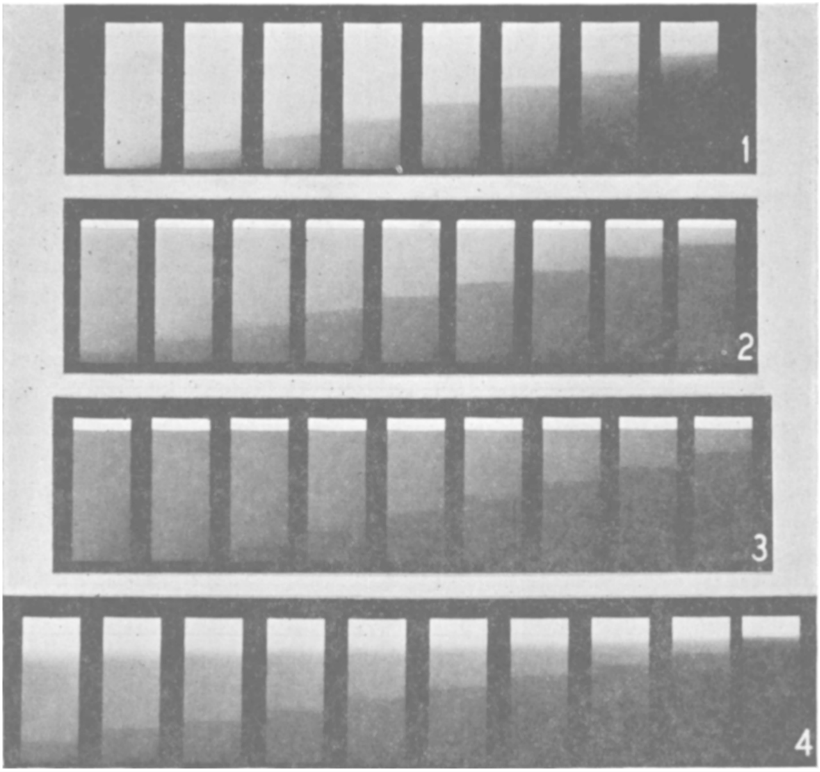

A series of exposures showing the sedimentation of the homogeneous heavy component extracted from equine encephalomyelitis-diseased tissue. These pictures were taken every five minutes through a thin layer of the solution lying in a centrifugal field of about 15,000g. The short ultraviolet light used for photography was largely absorbed in the bottom heavy component-containing solution; the liquid above the boundary, freed of heavy particles or molecules, was more transparent. The time rate of descent of the sharp boundary measures the sedimentation constant of the light-absorbing substance.

FIG. 3.

Photographs of the solution of the 2 preceding figures after standing somewhat more than a day at room temperature. The heavy component has nearly all disappeared and the heightened contrast between air bubble and liquid above the boundary is indicative of a further increase in the decomposition products.

FIG. 4.

Photographs of another preparation, less pure than that giving Fig. 1. The lower and sharper boundaries are due to the heavy component; the upper diffuse boundaries, which during the series move about one-fourth of the way down the cell, are due to the lighter inhomogeneous material devoid of virus activity.

Both the original suspensions and those resulting from the ultracentrifugations were examined with an analytical ultracentrifuge arranged for photography according to the classical absorption method of Svedberg. A sharp sedimentating boundary of the kind to be expected from a homogeneous heavy protein or other component of a comparable uniformity of particle size was observed when 100 cc. of the especially infectious material obtained by one method of virus cultivation was reduced to 1 cc. through 2 ultracentrifugal steps (Fig. 1). This heavy component was short-lived. After standing for a few hours, less of it appeared in the sedimentation picture (Fig. 2); only a trace was to be seen after a day or so (Fig. 3). Its disappearance corresponds to the breakdown of the heavy substance; the increase in "unsedimentable" material in passing from Figures 1 to 3 demonstrates that its fragments were small and still ultraviolet-absorbing.

If the experiment was not carried out rapidly in the cold and with a definite sequence of suspending fluids the heavy component either was not found or there were present other non-homogeneous, absorbing materials (Fig. 4) in the final concentrated suspension. The nature and origin of such diffusely sedimenting lighter substances are not entirely clear. Similar products have been encountered in the examination of other virus-diseased tissues under conditions which suggest that they arise through disintegration of either the diseased tissue or of the virus itself. Repeated tests have demonstrated that in the present experiments their amount cannot be related to the amount of virus activity; several totally inactive preparations contained nothing else. None of these boundaries, neither the sharp nor the diffusely sedimenting ones, was found when similar healthy tissues were subjected to control ultracentrifugation.

A precise sedimentation constant for the heavy component has not been determined because its solutions have always been somewhat impure. The highest values, $s_{20°}$ = ca 245×10^{-13} cm. sec^{-1} dynes^{-1}, are probably nearly correct. In less pure preparations where the heavy component sedimented through solutions of lighter proteins, the apparent lowering of s supplied a useful index of the relative amount of this heavy component. From its sedimentation constant, which is of the same order of magnitude as that of the papilloma protein,[5] it is clear that if the particle is spherical and has about the density found for proteins and for the smaller viruses, its molecular weight will be around 25 million; the diameter of such a particle would be about 40 $\mu\mu$.