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Homogeneous Heavy Substances from Healthy Tissues.

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During the course of work with the ultracentrifuge on the virus responsible for "jaundice"[1] in silkworms, macromolecular substances that sediment with the sharp boundaries indicative of a high degree of molecular homogeneity have been isolated from both healthy and diseased worms.

Recent investigations with the analytical ultracentrifuge[2] have demonstrated the sensitivity of viral proteins to salts in moderate concentrations and the consequent importance of using the mildest possible methods of extraction and treatment when dealing with the materials responsible for viral activity. For the present experiments both the fully diseased and the healthy control worms were preserved until needed in the freezing compartment of an electric refrigerator. Several of the frozen worms—usually 15 to 25 in number—were ground with salt in an iced mortar while in this state. A suspension of this mass in 0.05M saline was filtered through hardened filter paper and clarified first in an ordinary centrifuge and then by being put in the quantity-ultracentrifuge and run up quickly to 9000 r.p.m. and immediately stopped. When the dark but essentially clear solution that resulted was ultracentrifuged for an hour in a field of *ca.* 60,000g, a large, dark pellet was obtained which could be partially resuspended in dilute saline and then purified by successive sedimentations and low-speed centrifugations. After 2 or 3 ultracentrifugations the supernate became colorless and the pellet resuspended to give a yellowish, opalescent solution. The ultracentrifugal diagram prepared by the absorption-method from such a solution is shown in Fig. 1. The diagrams from diseased worms have been somewhat poorer and more diffuse than those from healthy worms but the measured sedimentation-constants have been the same in each case. The purified solution from normal worms does not produce observable symptoms of disease when fed undiluted to healthy worms.

It is interesting to compare the properties of these macromolecules from healthy tissues with those of the various plant and animal

[1] See Glaser, R. W., Virus Diseases of Insects, in *Filterable Viruses*, edited by Thomas Rivers, Baltimore, 1928.

[2] Wyckoff, Ralph W. G., *J. Biol. Chem.*, 1937, **121**, 215.

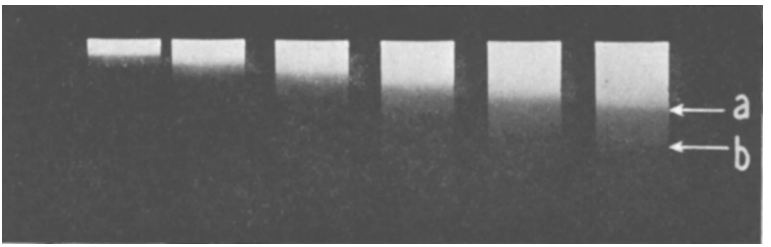

FIG. 1.
Sedimentation-diagram of a macromolecular solution obtained from healthy silkworms. The boundary (a) sediments with $s_{20°} = 53 \times 10^{-13}$, (b) with $s_{20°} = 92 \times 10^{-13}$. Interval between exposures = 5 min. Light source = mercury arc. Filters: bromine plus chlorine cells.

virus-proteins that have been isolated. The healthy substances are unstable and after standing for a short time in the ice-box yield diffuse instead of sharp sedimentation-diagrams; after a couple of days, evidence can no longer be found for large molecules. They are thus less stable than many viral proteins but do not break up more readily than numerous known viruses. Solutions give protein-reactions and absorb strongly in the ultraviolet region below 2700Å, as do proteins. Their analytical sedimentation-boundaries are as sharp as those of viral proteins. These boundaries move with rates that are somewhat smaller but of the same general magnitude as those of the measured viral proteins. Observations on a dozen concordant diagrams (Fig. 1) show that the sedimentative rates are the same for the corresponding substances from healthy and "jaundiced" worms. The measured constants are $s_{20°} = 53 \times 10^{-13}$ and $s_{20°} = 92 \times 10^{-13}$ cm. sec.$^{-1}$ dynes^{-1}. Since these may not be molecules of simple proteins, their weight cannot safely be estimated without determinations of density. Unconjugated proteins sedimenting at these rates would be expected[3] to have molecular weights in the neighborhood of 3 and 6 millions respectively.

Such non-pathogenic macromolecules as these are probably of widespread occurrence. They have already been seen with the ultracentrifuge in extracts of both healthy tissues and tissues inoculated with equine encephalomyelitis[4] and other viruses.

[3] Svedberg, T., *Chem. Rev.*, 1937, **20**, 81.

[4] Wyckoff, Ralph W. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 771.