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Improved Sequence for Rapid Consistent Purification of Equine Encephalomyelitis Virus Protein.*

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The procedure described here for purification of equine encephalomyelitis virus protein[1] yields consistently a product of high molecular homogeneity. Advantage is taken chiefly of the following principles: (1) prolonged extraction to aid in eliminating the normal chick tissue component;[2] (2) extraction, fractionation and solution of the protein in a balanced salt solution, mammalian Ringer,[3] instead of 0.9% NaCl or buffer salt solutions; (3) filtration of crude extracts with celite to remove mucoid and colloid materials; and (4) aggregation or partial precipitation of the protein in slightly acid medium prior to the first ultracentrifugal cycle.

Diseased embryo tissue ground in the cold in the usual way,[2] is suspended in 4 times its volume of normal Ringer's solution made to pH 9.0 with NH_4OH without buffer salts. After extraction at 5°C for 72 to 96 hours, gross material is eliminated in the angle centrifuge. To each 100 cc of the turbid supernatant fluid, 5 g of No. 512 Celite Filter Aid (Johns-Manville Co., N. Y.) are added and the suspension is filtered with suction through a 1 to 2 mm mat of No. 503 Celite. Standard celite then added to the filtrate, 2 g per 100 cc, is filtered off through a mat also of standard celite. Sometimes the latter step is repeated to obtain an entirely clear filtrate.

The filtrate is acidified to pH 6.5 with 0.2 N HCl, and 120 cc of it distributed immediately in 8 collodion tubes is spun at 17,000 g for 45 minutes. The pellets are taken up in 30 cc of Ringer's solution. In 2 tubes, the solution is spun at 17,000 g for 5 minutes. The supernatant fluid diluted to 60 cc is ultracentrifuged 30 minutes at 67,000 g and the resulting pellets are taken up in 15.0 cc of Ringer fluid.

* This work was aided by the Dorothy Beard Research Fund and by a grant from Lederle Laboratories, Pearl River, New York.

1 Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 462.

2 Sharp, D. G., Taylor, A. R., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 459.

3 Bayliss, W. M., *Principles of Physiology*, Longmans, Green and Co., Ltd., London, 4th ed., page 211.

This is spun 15 minutes at 15,000 g and the supernatant fluid diluted to 30 cc. From this volume, the protein is sedimented at 67,000 g for 30 minutes. The 2 pellets are dissolved in 1.5 to 3 cc of Ringer's solution, pH 9.0 depending on yield.

The character of the product with respect to homogeneity can be judged by the photometric tracing in Fig. 1. Practically all of the normal chick component[2] is eliminated in the first ultracentrifugal cycle and no evidence of it has been observed after the second cycle. Similar loss of colloid occurs in the process of filtration and in the first and second ultracentrifugal cycles.

Only slight loss of infectivity occurs in filtration, and no evidence is seen of damage to the protein by short exposure to pH 6.5. Precipitated at pH 6.5 the purified protein redissolves to its initial homogeneity and infectivity. Acidification facilitates sedimentation and separation of the protein from the normal chick component in the first relatively low speed cycle and greatly increases the final yield of the homogeneous product, ca 0.4 mg per gram of diseased embryo

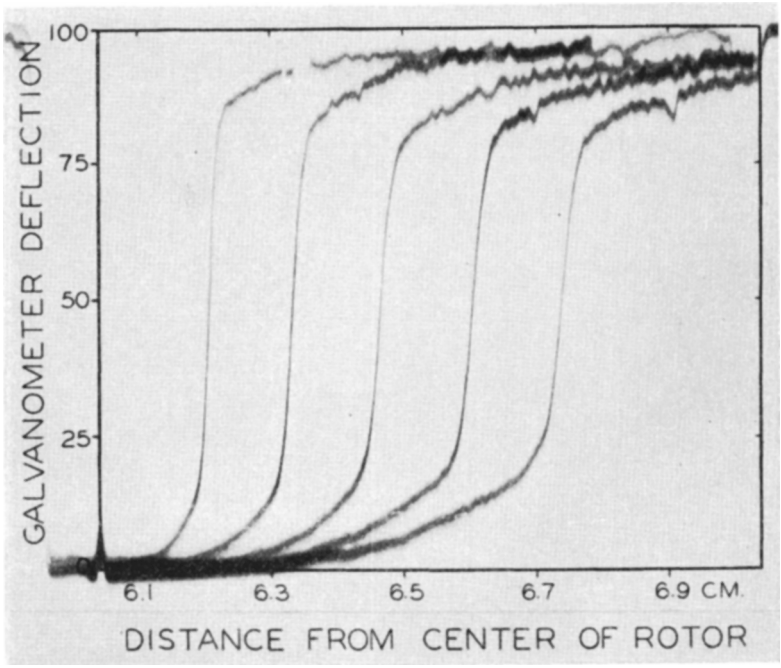

FIG. 1.
Photometer curves from sedimentation diagrams of a typical preparation of Eastern strain equine encephalomyelitis virus protein purified by the method described. The protein concentration was 1.8 mg per cc, the interval between curves 5 minutes and the ultracentrifugal field 17,000 g.

tissue. In Ringer's solution, at a protein concentration of 1 to 1.5 mg per cc, infectivity does not diminish within 7 days at 5°C and the sedimentation pattern is unchanged for periods of several weeks. In 0.9% NaCl or Ringer-buffer salt solution (NH_4OH-NH_4Cl, 0.05 M), however the protein loses much infectivity in 96 hours. In the latter solution the boundary may remain intact for 3 weeks when infectivity has diminished 6 decimal dilutions or more.

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Immunization with Non-Infectious Formalin Derivative of Purified Equine Encephalomyelitis Virus Protein.*

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Previous ultracentrifugal studies[1] of crude equine encephalomyelitis chick vaccines[2] have failed to yield definite evidence of the character of the immunizing principle. Information concerning its probable nature has been sought in the present work by study of the purified virus protein treated with formaldehyde.

Purified protein (Eastern strain),[3] dissolved in Ringer's solution, pH 8-9, 2.0 mg per cc, was treated with various concentrations of CH_2O. Before exposure to CH_2O, one m.i.u.[4] of protein was $10^{-13.5}$ g. When the concentration of CH_2O was less than 0.01 M, inactivation was not always complete in 2 weeks, and tests for immunizing capacity were not made. With other concentrations of CH_2O used, inactivation was complete in the time shown in Table I, as judged by the failure of $10^{-5.5}$ g of protein to infect mice.

The effect of CH_2O on the protein molecules was somewhat similar

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1 Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 462.

2 Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.

3 Taylor, A. R., Sharp, D. G., Finkelstein, H., Beard, D., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 648.

4 Cox, H. R., and Olitsky, P. K., *J. Exp. Med.*, 1936, **63**, 745.