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Macromolecular Component of Chick Embryo Tissue Diseased with Western Strain Equine Encephalomyelitis Virus.*

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A macromolecular component has been isolated[1] by ultracentrifugation from chick embryo tissue diseased with the Eastern strain of equine encephalomyelitis virus (E.S.). This protein, within the limits of tests thus far made, behaves as the virus and is specific[2] to the virus-diseased embryo tissue, though it is separable with difficulty from the lighter normal tissue component with $s_{20}° = \text{ca } 70 \times 10^{-13}$ cm sec^{-1} $dynes^{-1}$.[3] In the present paper are described ultracentrifugal studies of extracts of chick embryo tissue diseased with Western strain virus (W.S.) from which a macromolecular protein possessing the properties of this strain has been isolated.

In a typical experiment, diseased tissue was extracted 24 hours at about 5°C in 4 times its volume of 0.15 M NaCl solution made to pH 8.5 with NH_4OH. Cleared of tissue debris by angle centrifugation, the extract was ultracentrifuged in 8 15-cc tubes at 67,000 g for 30 minutes. The 8 pellets were taken up in 60 cc water, pH 8.5 with NH_4OH, and the resulting suspension was spun in 4 tubes at 6000 g for 5 minutes. The supernatant fluid was then spun in 4 tubes at 17,000 g for 30 minutes. A specimen from the 4 pellets was dissolved in 0.2 M NaCl solution adjusted to pH 9.0 with NH_4OH for examination in the analytical ultracentrifuge (Fig. 1), and the remainder was taken up again in 60 cc water for repetition of the cycle of 6000 g and 17,000 g. The final pellets were dissolved in 0.2 M NaCl solution for ultracentrifugal analysis (Fig. 2).

The sedimentation diagram after the second cycle showed the indistinct, diffuse boundary (Fig. 1, a) of persisting $s_{20}° = \text{ca } 70 \times 10^{-13}$ and the more prominent, somewhat diffuse boundary (Fig. 1, b) of a heavier material. After the third cycle, only the latter

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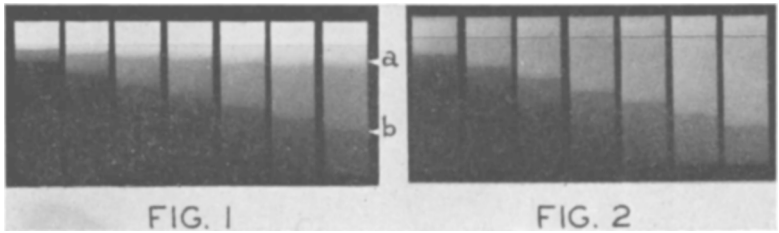

FIG. 1 FIG. 2

The ultracentrifugal field employed to obtain the photographs was about 17,000 g and the interval between exposures was 3 minutes.

FIG. 1. Photograph of preparation obtained in the second fractionation cycle. The indistinct boundary at a indicates residual normal tissue component s_{20}° = ca 70×10^{-13} cm sec^{-1} dynes^{-1} and the more prominent boundary b is that of the specific heavy component.

FIG. 2. The same preparation photographed after the third cycle. The boundary of only the heavier component with s_{20}° = ca 273×10^{-13} cm sec^{-1} dynes^{-1} is present.

was seen (Fig. 2). The average constant for this material in third cycle pellets has been s_{20}° = ca 273×10^{-13} cm sec^{-1} dynes^{-1}.

The sedimentation constant of the W.S. protein, the identity of which was established by serological test, has been the same as that of the E.S. protein which, in our experience has given s_{20}° = ca 273×10^{-13} cm sec^{-1} dynes^{-1}. The W.S. protein is infectious to the order of 10^{13} mouse units per gram as compared with the order 10^{14} for E.S.[2] The yields of W.S. and E.S. proteins are similar, namely 0.5 to 1.0 mg per gram of tissue, indicating comparable concentrations in the respective diseased embryos.

In many respects the W.S. and E.S. proteins behave alike. Both can be obtained consistently free of contaminating salt-sensitive[2] s_{20}° =ca 70×10^{-13} by prolonging tissue extraction in 0.15 M NaCl solution for 1-3 days. The sharpest boundaries, however, are seen when fresh extracts are fractionated immediately, but here s_{20}° = ca 70×10^{-13} frequently persists through repeated cycles. Most of the inhomogeneous colloid regularly associated with the proteins can be eliminated also by careful fractionation, but preparations relatively free of it have always shown slightly diffuse boundaries.

In the experiment described here, water was used in the second and third fractionation cycles. Thus far we have been unable to photograph either W.S. or E.S. proteins in water while s_{20}° = ca 70×10^{-13} is seen regularly under these conditions. Either no boundary or a very diffuse shadow is seen where boundaries of the W.S. and E.S. proteins should appear. Pellets purified in saline and yielding sharp boundaries in saline show no boundary when the salt content is lowered to 0.05 M NaCl or less. This finding is a possible explanation of the high degree of infectivity associated with water

purified $s_{20}° = ca\ 70 \times 10^{-13}$ from diseased embryos.[2] In such water preparations the E.S. and W.S. proteins appear to be present but because of factors not yet clear, boundaries are not obtained.

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Specific Chemotherapy of Experimental Staphylococcus Infections with Thiazol Derivatives of Sulfanilamide.

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Experimental and clinical experience has shown that staphylococcus infections are either uninfluenced, or at best, slightly affected by sulfanilamide, sulfanilyl dimethyl sulfanilamide;[1, 2] by prontosil;[3] by p-Amino-benzene-sulphonyl-4-aminobenzenesulphonedimethylamide (uleron);[1, 2] di-(p-formylaminophenyl) sulphone and di-(p-butylaminophenyl) sulphone.[4] On the other hand, sulfanilyl sulfanilamide and sulfapyridine seemed to have some curative effects on experimental staphylococcus infections in mice.[2, 5]

The relative ineffectiveness of present chemotherapeutic compounds against staphylococcic infections stimulated researches for compounds of greater efficiency against this organism.

Three new thiazol derivatives of sulfanilamide have recently been developed. 2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamidomethylthiazol (sulfamethylthiazol), and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol) are analogues of sulfapyridine.

Sulfathiazol and sulfamethylthiazol have since been described by Fosbinder and Walter.[6] Like sulfapyridine the thiazol compounds are equivalent to or superior to sulfanilamide against *Streptococcus hemolyticus* and the various strains of pneumococci, but unlike either of these preparations they exert a marked effect upon staphylococci, both *in vitro*[7] and *in vivo*.

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