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# Macromolecular Components of Chick Embryo Tissue Diseased With the Virus of Equine Encephalomyelitis.*

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Chick embryo tissue diseased with virus of equine encephalomyelitis (Eastern strain) has been studied in relation to the homogeneous macromolecular substances recently obtained from normal tissue[1] and the results observed are described here.

Eleven-day chick embryos were inoculated with virus and harvested when moribund. The heads were removed and the body tissue was ground without diluent in chilled Ten Broeck grinders. Extraction, ultracentrifugal fractionation and analysis were similar to those of the previous studies.[1]

When the diseased tissue mince was extracted in 20% suspension with a mixture of equal parts 0.9% sodium chloride solution and glycerin at pH 8.5 and the pellets were dissolved in 0.9% saline (the sequence employed by Wyckoff[2] for isolation of the equine encephalomyelitis virus protein[3]) the component with $s_{20}^{\circ} =$ ca $250 \times 10^{-13}$ cm $sec^{-1}$ $dynes^{-1}$ was observed in the second or third ultracentrifugal cycle (Fig. 1 and 2). This product was infectious to the order of $10^{14}$ mouse units per gram, and the result corroborated previous findings.[3]

On the other hand, when extraction was made with water, no evidence of this material was seen; instead, only boundaries with $s_{20}^{\circ} =$ ca $70 \times 10^{-13}$ cm $sec^{-1}dynes^{-1}$ were obtained (Fig. 3) as from non-diseased tissue.[1] Infectivity to the order $10^{14}$ was likewise associated with this product.

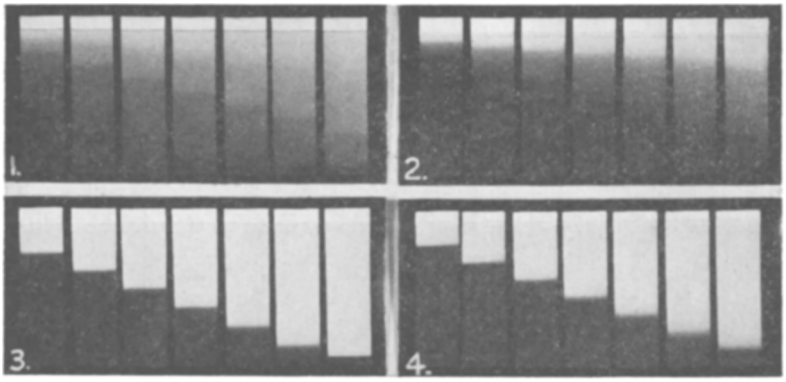
Fractionation of 0.9% saline extracts of virus-diseased tissue formolized to 0.4% yielded pellets which, on solution in saline, gave diffuse boundaries indicative of $s_{20}^{\circ} =$ ca $70 \times 10^{-13}$. Formolization and extraction with water at pH 7.0 gave a product with relatively sharp boundaries (Fig. 3). These derivatives of 0.9% saline and of

* This work was aided by a grant from Lederle Laboratories, Pearl River, N. Y.

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

2 Wyckoff, R. W. G., personal communication.

3 Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 771.




Sedimentation diagrams of ultracentrifugal fractions of virus-diseased chick embryo tissue.

FIG. 1. Product from diseased tissue extracted in mixture of equal parts 0.9% sodium chloride solution and glycerin. Photographed at 3-minute intervals in an ultracentrifugal field of 17,000 g after third fractionation cycle. $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$ cm sec.$^{-1}$ dynes$^{-1}$.

FIG. 2. A similar highly infectious preparation showing the light, diffuse boundary indicative of the normal tissue component $s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$ cm sec.$^{-1}$ dynes$^{-1}$.

FIG. 3. Material from water extract of diseased tissue photographed at 5-minute intervals in a field of 47,000 g. $s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$ cm sec.$^{-1}$ dynes$^{-1}$. Infectious titre $10^{14}$ mouse units per gram.

FIG. 4. Component from water extract of formolized diseased tissue photographed in field of 47,000 g. $s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$ cm sec.$^{-1}$ dynes$^{-1}$.

water extraction were both highly antigenic, protecting guinea pigs against 1000 mouse infectious units of virus given intracerebrally. Thus far this material from formolized virus-diseased tissue is indistinguishable by ultracentrifugal analysis from the non-immunizing product from formalin-treated normal tissue.[1]

The product $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$ has not been obtained from non-diseased tissue.[1] Extracts of low salt content from both diseased and normal tissue yield products similar in quantity and sedimentation pattern ($s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$) that are apparently identical and unrelated to $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$. Unstable in solutions of salt concentration necessary for demonstration of $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$, the presence of this normal component is indicated only in the very diffuse pattern associated with $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$ (Fig. 2). Further work is necessary to explain the high infectivity of $s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$ from diseased tissue, though this may be due to the presence of quantities of $s_{20}^{\circ}$ = ca 250 $\times$ $10^{-13}$ too small for ultracentrifugal detection.

The presence of $s_{20}^{\circ}$ = ca 70 $\times$ $10^{-13}$ in formalin-treated normal tissue as well as formalin-treated virus-diseased tissue raises the question of the significance of the component previously described as

$s_{20}° = ca\ 60 \times 10^{-13}$ associated with the immunizing principle.[4] Although $s_{20}° = ca\ 70 \times 10^{-13}$ from formolized diseased tissue is consistently highly antigenic, the actual immunizing principle involved may be unrelated to and only non-specifically associated with it.

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## Glucuronic Acid Produced by Surviving Slices of Liver from Animals Poisoned with Phosphorus or Chloroform.

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It has been previously demonstrated[1] that surviving liver slices shaken in the Warburg apparatus in a saline phosphate medium (pH :7.4) produce conjugated glucuronic acids in presence of borneol, menthol or phenol. This production is significantly increased by the addition of sodium-lactate or pyruvate. The present investigation is concerned with the glucuronic acid synthesis by liver slices excised from guinea pigs poisoned with phosphorus or chloroform. Phosphorus poisoning was produced by the subcutaneous injection of 0.75 mg of yellow phosphorus (dissolved in olive oil) per 100 g animal weight. Other guinea pigs were injected with 0.03 to 0.035 cc of chloroform per 100 g animal weight on 2 successive days, and the livers were removed 3 days after the first injection. The glucuronic acid production of slices of these livers was determined with and without addition of sodium-lactate. The method was described in previous publications.[1]

As a result of phosphorus poisoning the addition to liver slices of lactate produced no significant increase in either the oxygen uptake or the glucuronic acid production. Histologically these livers showed fatty infiltration, swelling of the nuclei and, in the terminal stages of the poisoning, diffuse nuclear destructions and no signs of regeneration. The addition of lactate to liver slices poisoned with chloroform resulted in an increase of the oxygen uptake and of the

[4] Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 89.

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[1] Lipschitz, W., and Bueding, E., *Proc. XVI Internat. Physiol. Cong.*, Zurich, 1938, 120; *J. Biol. Chem.*, 1939, **129**, 333.