

Blood-cholesterol (modified Sackett's method^{3, 4}) is expressed in mg per 100 cc of whole blood.

Conclusion. Following injection of rabbits with typhoid vaccine, a higher agglutinin-titer against *E. typhosa* was obtained in those with induced hypercholesterolemia† as compared with control rabbits having normal blood cholesterol.

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Macromolecular Components of Untreated and of Formolized Normal Chick Embryo Tissue.*

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Macromolecular substances have been found in healthy tissues of both plants and animals.¹ In experiments associated with studies of the equine encephalomyelitis virus protein² and the non-infectious immunizing principle³ of formalin-treated virus-diseased chick embryos,⁴ we have obtained such substances from normal chick tissue and from the tissue treated with formalin.

Chick embryos incubated for 11 days were harvested, chilled, the heads removed and the bodies ground in a Ten Broeck grinder held in cracked ice. The resulting body mince was made to a 20% suspension and extracted as indicated. Large particles were removed with the angle centrifuge and the resulting extracts fractionated by

³ Sackett, G. E., *J. Biol. Chem.*, 1925, **64**, 203.

⁴ Mirsky, I. A., and Bruger, M., *J. Lab. and Clin. Med.*, 1932, **18**, 304.

† Because cholesterol, *per se*, in increased concentration in the blood might produce false agglutination, the sera of 3 rabbits with marked hypercholesterolemia (700 to 800 mg per 100 cc) produced by the feeding of cholesterol were tested; no clumping was observed.

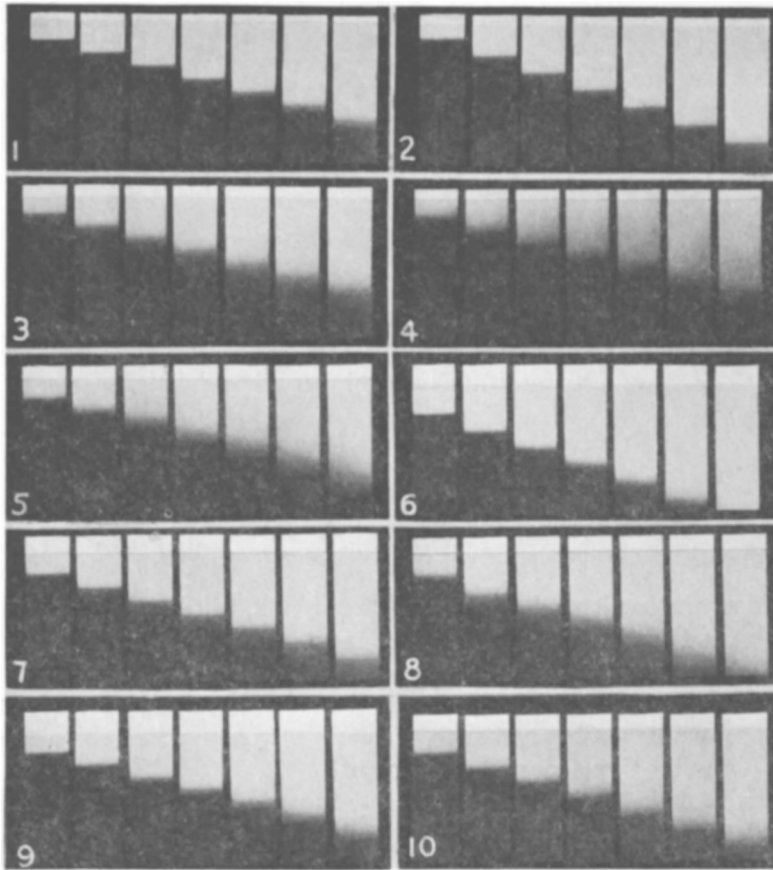
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¹ Wyckoff, R. W. G., *Cold Spring Harbor Symposia on Quantitative Biology*, 1938, **6**, 361.

² Wyckoff, R. W. G., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 771.

³ Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 89.

⁴ Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.



Sedimentation diagrams of macromolecular components from extracts of untreated normal embryo tissue and from the tissue treated with formalin. The ultracentrifugal field employed to obtain the photographs was about 47,000 g and the interval between exposures was 5 minutes.

FIG. 1. Normal untreated tissue extracted in dilute buffer. Pellets dissolved in buffer and photographed after 2 ultracentrifugal cycles.

FIG. 2. Same preparation after 3 cycles showing boundary of increased sharpness.

FIG. 3. Normal untreated tissue extracted with 0.9% saline. Pellets of second cycle dissolved in saline.

FIG. 4. Same preparation after a third cycle. Note homogeneity not increased.

FIG. 5. Formalized tissue extracted with water and pellets dissolved in dilute buffer. Photographed after the second cycle.

FIG. 6. Same preparation after third ultracentrifugal cycle.

FIG. 7. Formalized tissue extracted with 0.9% sodium chloride solution. Second cycle.

FIG. 8. Same preparation after the third cycle. The boundary is more diffuse than after the second cycle.

FIG. 9. Untreated tissue extracted with water and pellets dissolved in water. Second cycle.

FIG. 10. Same preparation after third cycle.

alternate ultracentrifugal runs of 9,000 g for 5 minutes and 67,000 g for 1 to 1½ hours. The products have been studied by ultracentrifugal analysis.

The results observed when extraction was made and the pellets were dissolved with 0.005 M ammonium hydroxide-ammonium chloride buffer pH 8.2 are shown in Figures 1 and 2. After 2 ultracentrifugal cycles, double or diffuse boundaries were present whereas only one of slight diffuseness was seen after the third cycle. Here $s_{20}^{\circ} = \text{ca } 70 \times 10^{-13} \text{ cm sec}^{-1} \text{ dynes}^{-1}$. A similar material was obtained by extraction with glycerin in 50% concentration in the dilute buffer. When extraction was made in 0.9% sodium chloride solution alone or combined with glycerin and the pellets were dissolved in saline, indications of the same component were evident after the second cycle (Fig. 3) but further homogeneity was not attained by additional cycles (Fig. 4). The sharpest boundaries were obtained with material derived by extraction of tissue and solution of the pellets in distilled water (Fig. 9 and 10).

A component with sedimentation pattern indistinguishable from that of the derivative from untreated tissue was obtained from normal tissue to which formalin was added to 0.4% (as in the preparation of chick vaccines⁴). When formalin was added to tissue suspended in water, relatively sharp boundaries with little spread were obtained with the pellets dissolved in dilute phosphate buffer (0.005 M, pH 7.0) (Fig. 5 and 6), and the sedimentation constant was $s_{20}^{\circ} = \text{ca } 70 \times 10^{-13}$. Extraction, formolization and fractionation in 0.9% sodium chloride solution gave a similar product of much less homogeneity (Fig. 7 and 8). The observed sedimentation constant varied somewhat under both conditions, depending on protein concentration, but $s_{20}^{\circ} = \text{ca } 70 \times 10^{-13}$ was always observed with proper pellet dilution and was maintained with increased dilution to extinction of the boundary.

These results demonstrate the presence of a macromolecular protein or a group of such substances of related size in normal chick embryo tissue, persisting in a similar or but slightly altered form on treatment of the tissue with dilute formalin