

the choline esterase concentration of the chorionic villous tissue would be much greater than that of the blood.

The results of this study suggest that the presence of choline esterase is a generalized phenomenon and is not necessarily related to the presence of nerve elements.

Summary. 1. The choline esterase activity of 25 perfused human placentæ was determined by the method of Glick. 2. The choline esterase contained in 1 mg tissue is able to decompose at 35°C in the average $6/10^3$ γ acetylcholine per sec.

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Mechanism of the Colloidal Gold Reaction of Blood Serum in Liver Disease.

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When a colloidal gold suspension is added to certain dilutions of blood serum from patients with liver disease flocculation of the colloidal gold occurs in one or more of the first serial dilutions.^{1,2} The colloidal gold reaction of blood serum in liver disease has proven a sensitive method of detecting early liver involvement according to Loew and Noth,³ Mateer *et al.*,⁴ and Sweet, Gray and Allen.⁵

Flocculation of the colloidal gold by serum from patients with liver disease does not depend primarily upon a quantitative increase in globulin or upon an inverted albumin-globulin ratio.^{1,2} It was suggested by the author¹ that the mechanism of this reaction in liver disease might depend upon a qualitative variation within the serum globulins rather than upon a quantitative change in the total serum globulin.

Electrophoretic studies of the serum proteins in all types of liver disease, both acute and chronic, revealed that the most characteristic and consistent alteration of the serum proteins was an increase in the gamma globulin

associated with a decrease in serum albumin.⁶

Since globulin promotes colloidal gold flocculation and albumin protects the colloidal suspension from flocculation⁷ these studies were undertaken to investigate the effect of the purified protein fractions, prepared by Dr. Edwin J. Cohn,⁸ upon the serum colloidal gold reaction.

Method. Increasing amounts of electrophoretically pure human gamma globulin, albumin, and a purified fraction containing alpha and beta globulins were added to normal blood serum. The serum was then diluted 1:350 with 0.9% sodium chloride and the blood serum colloidal gold reaction was performed in the usual manner. Five cc of properly acidified colloidal gold were added to each of 3 tubes containing 1 cc of serum in final dilutions of 1:3500, 1:7000 and 1:14,000. The tubes were allowed to stand at room temperature and were read after 24 hours. Color changes were expressed by the following numbers: 0—red orange, 1—reddish blue, 2—orchid, 3—blue, 4—light blue, and 5—colorless. Complete flocculation in one or more of the 3 tubes designates a positive test. The usual negative reaction is 222 or 332, and the positive test is 532, 553, or 555.

¹ Gray, S. J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 470.

² Gray, S. J., *Arch. Int. Med.*, 1940, **65**, 524.

³ Loew, E. R., and Noth, P., *Proc. Am. Physiol. Soc.*, 1941, 176.

⁴ Mateer, J. G., Baltz, J. I., Marion, D. F., Hollands, R. A., and Yagle, E. M., *Am. J. Digest. Dis. and Nutrition*, 1942, **9**, 13.

⁵ Sweet, W. H., Gray, S. J., and Allen, J. G., *J. A. M. A.*, 1941, **117**, 1613.

⁶ Gray, S. J., and Barron, E. S. G., *J. Clin. Invest.*, in press.

⁷ Reznikoff, P., *J. Lab. and Clin. Med.*, 1922, **8**, 92.

⁸ Cohen, E. J., *Chem. Rev.*, 1941, **28**, 395.

Results. The addition of 0.2 g% of electrophoretically pure human gamma globulin to normal blood serum produced a positive colloidal gold test—532—which increased in intensity as the concentration of gamma globulin was increased to 1.0 g%. At this point all 3 tubes show complete flocculation—555.

Similar amounts of the purified fraction containing alpha and beta globulin were added to normal blood serum and produced no effect whatever on the blood serum colloidal gold reaction.

Pure albumin inhibited the serum colloidal gold reaction. The addition of 0.5 g% of albumin to normal blood serum inhibited the serum colloidal gold reaction from 322 to 111. Increasing the concentration of albumin to 1.0 or 2.0 g% produced complete inhibition of the serum colloidal gold reaction—000.

No significant alteration of pH was observed in the blood sera containing the purified

protein fractions.

Summary. The addition of pure gamma globulin to normal blood serum produced a positive serum colloidal gold reaction. The purified fraction containing alpha and beta globulins produced no effect. Albumin inhibited the serum colloidal gold reaction. Since the most characteristic and consistent alteration of the serum proteins in liver disease is a decrease in serum albumin and a relatively large increase in gamma globulin it is suggested that the mechanism of the colloidal gold reaction in liver disease depends upon a relative increase in the gamma globulin of the blood.

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Studies on the Mechanism of the Spinal Fluid Colloidal Gold Reaction.

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Zsigmondy¹ laid the foundation for the diagnostic use of the colloidal gold reaction by observing that "certain colloids, especially proteins" prevented the precipitation of colloidal gold suspensions by electrolytes. Lange² found that proteins within certain dilutions did not prevent but actually caused precipitation. The mechanism of the colloidal gold reaction has been the subject of much investigation. Numerous workers^{3,4,5} have concluded that the globulin content of the spinal fluid is the determining factor in the precipitation of colloidal gold and that albumin protects the

colloidal suspension from precipitation. Others^{6,7} have suggested, moreover, that the individual globulin fractions played an important role in the precipitation of colloidal gold.

It has been impossible, heretofore, to study the effect of the individual globulin fractions on colloidal gold precipitation because these fractions could not be obtained in pure form. Tiselius⁸ has shown that the precipitation ranges of the globulin fractions overlap one another grossly and that the euglobulin, pseudoglobulin and albumin fractions prepared by the usual methods of fractional precipitation⁹ are mixtures of albumin, alpha,

¹ Zsigmondy, R., *Ztschr. f. anal. chem.*, 1901, **40**, 697.

² Lange, C., *Berl. klin. Wchnschr.*, 1912, **49**, 897.

³ Felton, L. D., *New York State J. Med.*, 1917, **105**, 1170.

⁴ Weston, P. G., *Am. J. Syph.*, 1919, **3**, 266.

⁵ Cruickshank, J., *Brit. J. Exp. Path.*, 1920, **1**, 71.

⁶ Reznikoff, P. J., *Lab. and Clin. Med.*, 1922, **8**, 92.

⁷ Mellanby, J., and Anwyl-Davies, T., *Brit. J. Exp. Path.*, 1923, **4**, 132.

⁸ Tiselius, A., *Biochem. J.*, 1937, **31**, 313, 1464.