

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.

The author wishes to express his gratitude to Dr. Edwin J. Cohn for supplying the purified protein fractions used in this study, which were prepared under contract with the Office of Scientific Research and Development upon recommendation by the Committee on Medical Research.

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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.

beta and gamma globulins.

The electrophoretically pure protein fractions prepared by Dr. Edwin J. Cohn¹⁰ presented an opportunity to study the effects upon colloidal gold flocculation of pure human albumin, gamma globulin and the fraction containing alpha and beta globulins.

Method. Increasing concentrations of pure human albumin, gamma globulin and the fraction containing alpha and beta globulins in amounts varying between 0.005 and 1.0 g% were added to normal spinal fluids and to spinal fluids from patients with general paresis. The albumin and the alpha and beta globulins were isoelectric while the gamma globulin was in salt form. Ten experiments were performed with the normal spinal fluids and 5 with the spinal fluids of patients with paresis. The colloidal gold test was performed in the usual manner by the addition of 2.5 cc of colloidal gold solution to 0.5 cc of spinal fluid in serial dilutions of 1:10, 1:20, 1:40 to 1:5120. 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—red blue, 2—orchid, 3—blue, 4—light blue, and 5—colorless.

Results. The addition of pure gamma globulin to normal spinal fluid produced a typical paretic colloidal gold curve—5555432100. Complete flocculation in the first tube was noted when 0.020 g% of gamma globulin was added to normal spinal fluid. The degree of flocculation increased propor-

tionately with the increased concentration of gamma globulin. The addition of 0.20 g% of gamma globulin caused complete flocculation in 7 of the 10 dilutions. (Table I.)

The addition of pure human albumin to the spinal fluid of a patient with general paresis converted the paretic colloidal gold curve to normal. A strongly positive paretic colloidal gold curve was made to appear normal by the addition of 0.8 g% of albumin. The inhibition of colloidal gold flocculation was progressive as the concentration of albumin increased from 0.05 to 0.8 g%.

Summary. The addition of pure gamma globulin to normal spinal fluid produced a typically paretic colloidal gold curve. The degree of flocculation was proportional to the amount of gamma globulin added. Similar concentrations of the fraction containing alpha and beta globulins did not affect the colloidal gold reaction. Albumin inhibited colloidal gold flocculation. The addition of pure human albumin to the spinal fluid of a patient with paresis converted the paretic curve to normal. Since the gamma globulin is increased in the spinal fluid of patients with paresis,¹¹ the paretic colloidal gold curve probably depends upon the relative increase in gamma globulin.

The author is greatly indebted to Dr. Edwin J. Cohn for supplying the purified protein fractions used in this study, which were prepared under contract with the Office of Scientific Research and Development upon recommendation by the Committee on Medical Research.

⁹ Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

¹⁰ Cohn, E. J., *Chem. Rev.*, 1941, **28**, 395.

¹¹ Kabat, E. A., Moore, D. H., and Landow, H., *J. Clin. Invest.*, 1942, **21**, 571.

TABLE I.
Effect of Purified Protein Fractions on the Colloidal Gold Curve of Normal and Paretic Spinal Fluid.

Amt of protein added (g%)	Normal spinal fluid			Paretic spinal fluid. Albumin
	γ globulin	α and β globulins	Albumin	
0	0000000000	0000000000	0000000000	5553210000
.005	2211000000	0000000000	0000000000	
.01	2221110000	0000000000	0000000000	
.02	5221100000	0000000000	0000000000	
.03	5522110000	0000000000	0000000000	
.04	5553211000	0000000000	0000000000	
.05	5555432100	0000000000	0000000000	5543210000
.10	5555554210	0000000000	0000000000	5321000000
.20	5555555433	0000000000	0000000000	4211000000
.60				1110000000
.80				0000000000