

loid enthusiasts are prone to explain the clotting of milk and the influence of different milk modifiers in inappropriate colloid terms. Alexander<sup>2</sup> first used colloid terminology in explaining milk clotting and since then many writers have ascribed to "colloidal protection" the influence of such modifiers as gelatin, tapioca, starch, barley gruel, gum arabic, etc. Palmer and Richardson<sup>3</sup> have indicated certain fallacies in Alexander's original claims, but do not offer any alternative theory to account for the commonly observed influence of such milk modifiers.

In a series of experiments, including both *in vitro* and *in vivo* observations, we have found no relation whatever between protective colloid value, as measured by gold number, and influence upon the nature of the milk curd. We have, however, found a direct correlation between the *viscosity* of the modified milk and the fineness of curd produced, and feel warranted in concluding that the term "protective colloid" is not strictly applicable in this connection. The important property, which is involved in producing fine, soft, flocculent curds, appears to be viscosity rather than protective colloid value. For this reason a modifier which best maintains its viscosity unimpaired at body temperature, and under the conditions of gastric digestion may be expected to be most efficacious for the purpose. Tapioca was used as an example of farinaceous material of relatively low protective colloid value (measured by gold number) but of high viscosity. It proved to be an efficient means of obtaining fine soft curds.

This is a preliminary report.

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<sup>1</sup> Brennemann, J., *Arch. of Ped.*, Vol. xxxiv, Feb., 1917.

<sup>2</sup> Alexander, J., *J. Am. Chem. Soc.*, 1910, xxxii, 680-687.

<sup>3</sup> Palmer, L. S., and Richardson, G. A., *Colloid Symposium Monograph*, 1925, iii, 112-134.

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### Identity of Precipitin and Complement Fixing Substances in Syphilitic Sera. I.

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Whether or not different types of antibodies are identical is still one of the classical questions in immunology. In this country, Zinsser<sup>1</sup> is the outstanding champion of the "unitarian" hypothesis.

The identity of precipitins and complement fixing substances in syphilitic sera is being championed in Germany, especially by Jacobstahl.<sup>2</sup> He was the first to show that the precipitate produced by extract antigen and syphilitic serum possesses the property of fixing complement, while the supernatant fluid remaining after removal of the precipitate lacks this complement fixing property.

The question of the identity of complement fixing and precipitin substances in syphilitic serum has recently been indirectly revived through the efforts of several workers to evolve a combined precipitation—complement fixation method in syphilis. Thus, in the case of the Sachs-Georgi test, Kafka,<sup>3</sup> Keining<sup>4</sup> and others have attempted to add complement to the resulting precipitate of this test and carry out a complement fixation reaction. The results, however, have been variable, due undoubtedly to the technical nature of the Sachs-Georgi reaction and the prolonged incubation which it requires.

It was believed that the Kahn test<sup>5</sup> might be suitable for the study of the identity of precipitin and complement fixing substances in syphilitic sera. This test gives immediate precipitation reactions on mixing serum with antigen. By adding the hemolytic system to the serum antigen mixture of the completed test, it was believed that immediate complement fixation reactions might also be obtained. If the complement-fixing and precipitin substances are identical, there should be parallelism between the complement fixation and precipitation results. If not identical, little or no parallelism should exist.

*Procedure of combined precipitation-complement fixation test:* Kahn tests were performed with syphilitic and non-syphilitic sera in the regular manner. After reading the results, 0.15 cc. amounts of the antigen-serum-saline mixture of the third tube of the test, containing about 0.035 cc. serum, were pipetted into a tube and 0.1 cc. (2 units) of complement added. The mixture was placed in the water bath at 37° C. for 30 minutes after which 0.1 cc. (2 units) amboceptor and 0.1 cc. sheep-cell suspension were added, and the mixture again placed in the water bath for about 15 minutes. When the serum and antigen controls showed complete hemolysis, the complement fixation results were read. Complete fixation of complement as well as complete precipitation was read four plus. Weaker reactions were read three, two and one plus, respectively. The usual serum, antigen and anticomplementary controls were employed.

Eighteen hundred sera, of which over a third came from syphilitic patients, were tested in this manner. It was found that 93 per

TABLE I.  
Comparison of Precipitation (Kahn) and Complement Fixation Results with  
1864 Sera.

Precipitation reactions	No. of examinations	Complement fixation reactions.		
		Positive	Doubtful	Negative
Positive	647	596	31	20
Doubtful	110	22	64	24
Negative	1107	17	12	1078

Percentage of agreement between the two methods: Complete, 93.24; relative, 4.77; disagreement, 1.98.

Positive reaction = + + + +, + + + or + +.

Doubtful reaction = + or ±.

Complete agreement = positive or negative with both methods.

Relative agreement = positive or negative with one method and doubtful with the other.

cent of the sera gave complete agreement, being either positive or negative in precipitation and complement fixation procedures, 5 per cent of the sera gave relative agreement (Table I) while 2 per cent gave complete disagreement.

An attempt was then made to carry out quantitative precipitation-complement fixation studies.<sup>5</sup> Thus, after completing a quantitative Kahn test on a given syphilitic serum, 0.15 cc. amounts of the antigen-serum-saline mixtures from each tube were pipetted into a series of other tubes, complement added, incubated for 30 minutes and the complement fixation test completed as in the previous experiments. Of 80 sera tested in this manner, it was found that in practically all cases the highly potent sera showed an equally large number of precipitin and complement fixing substances, while the weakly potent sera showed a small number of such substances. The variations occurring were believed to be due to difficulties inherent in the hemolytic system. These studies are still being continued.

This study throws light on the probable identity of complement fixing and precipitin substances. It also appears that the complement fixation results obtained by adding the hemolytic system to completed Kahn tests would compare favorably with many of the Kahn-Wassermann studies reported in the literature. The combined procedure saves much time and material as compared with other complement fixation tests and might prove a popular method in laboratories where the Wassermann is used in conjunction with the Kahn test. Such a procedure should also be desirable when presenting to students the subject of precipitation and complement fixation with syphilitic sera.

*Summary:* We studied the identity of precipitin and complement fixing substances in syphilitic sera by adding the hemolytic system to

completed Kahn tests to determine whether sera giving positive precipitation reactions would give positive complement fixation reactions. Of 1800 sera tested, it was found that 98 per cent gave either identical or approximate results with the precipitation and complement fixation methods. The remaining 2 per cent showed disagreement. This is believed to be due to technical factors rather than to lack of identity of the "antibodies." The work is being continued with a view of reducing this variation.

It is believed that the combination of the hemolytic system with the Kahn test might offer a desirable complement fixation procedure in place of the Wassermann test in laboratories where it is employed parallel with the Kahn test.

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<sup>1</sup> Zinsser, H., "Infection and Resistance," 3rd Edition, McMillan, New York, 1923, p. 317.

<sup>2</sup> Jacobstahl, E., Chapter in Carl Bruck: Serodiagnose der Syphilis, Julius Springer, Berlin, 1924, p. 305.

<sup>3</sup> Kafka, V., *Dtsch. med. Wochenschr.*, 1921, xlvii, 269.

<sup>4</sup> Keining, E., *Dtsch. med. Wochenschr.*, 1921, xlvii, 157.

<sup>5</sup> Kahn, R. L., "Serum Diagnosis of Syphilis by Precipitation," Williams and Wilkins, Baltimore, 1925, p. 148.

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#### Relation of Internal Secretion of Ovaries to Cholesterol Metabolism.

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Pig ovary follicle fluid injected into normal female cats caused a decrease in the blood cholesterol. This decrease did not occur in male cats. Ovariectomized cats showed an increase in the blood cholesterol. This increase can be reduced by the injection of pig ovary follicle fluid and also by ovarian extracts shown to be active by the vaginal smear method. This work shows that the ovarian hormone regulates the cholesterol content of the blood. Ovarian extract injected into male animals does not influence the cholesterol content of the blood. The results are to be made use of as a chemical method of standardizing the activity of the ovarian hormone in extracts.

This is a preliminary report.