AN IMPROVED METHOD OF ADJUSTING COMMERCIAL COLLOIDAL GOLD SOLUTION USED IN THE SERUM COLLOIDAL GOLD TEST

ALFRED REICH, B.S.,
Department of Clinical Pathology

and

PENN G. SKILLERN, M.D.
Department of Endocrinology

THE precipitation of serum colloidal gold by human serum has been reported to be due to the presence of serum gamma globulin.[1,2,3] The serum colloidal gold test, when correlated with other clinical and laboratory findings, has proved of particular value in our institution as an aid to the diagnosis of struma lymphomatosa (Hashimoto's disease).[3,4] Its use in this disease was first reported by Cooke and Wilder,[5] and further discussed by Luxton and Cooke[6] more recently. Although electrophoretic analysis of the serum proteins is the most accurate quantitative measure of an increase in serum gamma globulin, its use is limited to a few large medical centers.

The widespread use of the serum colloidal gold test has been limited by the basic difficulty of adjusting the pH of commercial colloidal gold solutions. The purpose of this report is to present the details of an improved method of adjustment of commercial Lange's colloidal gold solution*, and to review the method of performing the test which is a slight modification of that described by Maclagan.[7]

Method of Adjusting Lange's Colloidal Gold Solution For Use in the Serum Colloidal Gold Test

A. Materials Required

1-a. Lange's colloidal gold solution

2-a. Normal serum

3-a. Positive serum

4-a. One per cent acetic acid solution

5-a. Barbital buffer solution, pH 7.6

Formula:		
Diethylbarbituric acid . . .	0.55	gm.
Sodium barbitone	0.31	gm.
Phenol	0.2	gm.
Distilled water	100	ml.

6-a. Test tube racks

**Lange's Colloidal Gold Solution, manufactured by Magar Chemicals, Inc., Cornwall Landing, New York; and by The Keystone Laboratory, Erie, Pennsylvania.*

7-a. Clean test tubes, 13 mm. by 100 mm.

8-a. Graduated serologic pipets, 5 ml., 1 ml., and 0.1 ml.

B. First Step

1-b. Arrange two rows each of eight test tubes in a rack, and number the tubes in each row *1* through *8*, from left to right.

2-b. Into each tube of the first row, pipet 0.02 ml. of the normal serum.

3-b. Into each tube of the second row, pipet 0.02 ml. of the positive serum.

4-b. To each tube of both rows, add 0.18 ml. of barbital buffer.

C. Acidification of the Colloidal Gold Solution

1-c. Arrange a third row of eight test tubes in another rack, and number the *tubes 1* through *8*, from left to right.

2-c. Deliver to the bottom of each tube, beginning at the left with *tube 1*, the following amount of 1 per cent acetic acid solution, in respective order: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 ml.

3-c. To each tube, add 4 ml. of colloidal gold solution and mix. *Tube 1* now contains 0.0025 ml.; *tube 2*, 0.005 ml.; *tube 3*, 0.0075 ml.; *tube 4*, 0.01 ml.; *tube 5*, 0.0125 ml.; *tube 6*, 0.015 ml.; *tube 7*, 0.0175 ml.; and *tube 8*, 0.02 ml. of 1 per cent acetic acid per ml. of colloidal gold solution.

D. Determination of the Optimal Quantity of Acid for the Test

1-d. Using a graduated pipet, add 1 ml. of acidified gold solution from *tube 1* (*3-c*) to each of *tubes 1* (*4-b*) of the positive and negative serums. Similarly pipet 1 ml. of acidified gold solution from *tube 2* (*3-c*) to each of *tubes 2* (*4-b*) of the positive and negative serums. Continue this process for the remainder of the correspondingly numbered tubes.

2-d. Agitate each *4-b tube* gently and allow to stand at room temperature overnight.

3-d. Read: Select the pair of *4-b tubes* (with corresponding numbers) of which the positive member shows complete precipitation of gold and a water-clear supernatant and its negative counterpart shows either no change or at most a slight color change but no precipitation of gold. When two or more pairs of tubes show these changes, from them is selected the pair having the least amount of acetic acid.

Each tube of the selected pair contains the optimal

quantity of 1 per cent acetic acid per ml. of gold solution for this test.

The adjustment of the commercial colloidal gold solution should be made every two weeks.

Method of Performing the Serum Colloidal Gold Test

When the optimal quantity of 1 per cent acetic acid per milliliter of colloidal gold solution has been determined by the above procedure, seven test tubes are set up for each sample of serum to be examined, including a known normal serum and a known positive serum, if available, as a control. Barbital buffer is added to the first six tubes: 0.36 ml. to *tube 1*, and 0.2 ml. to each of *tubes 2* through *6*. Into the seventh tube pipet 0.34 ml. of 1 per cent sodium chloride solution. To *tube 1*, add 0.04 ml. of the serum to be tested; mix the contents; and transfer 0.02 ml. of the mixture to *tube 2*. Then mix the contents of *tube 2* and transfer 0.2 ml. of the mixture to *tube 3*. This procedure is followed through *tube 6*, from which 0.2 ml. of the mixture is discarded. No serum is added to *tube 7*.

The corrected colloidal gold solution is prepared only immediately before it is to be used. Two large test tubes are employed: the required amount of colloidal gold solution is measured into one tube, and the estimated appropriate quantity of acetic acid is delivered to the bottom of the other. The contents of the two tubes are rapidly mixed by pouring the colloidal gold solution into the acid and then pouring that mixture back into the first tube. One ml. of this mixture is then added to each of the above-mentioned seven tubes; the contents of each tube is shaken and allowed to stand at room temperature overnight. The results of the test are then read.

Example. If four samples of serum are to be examined, 28 test tubes and 28 ml. of colloidal gold solution are required. However, to compensate for an expected loss of small amounts of the colloidal gold solution during such procedures as transference, 30 ml. of the solution is prepared. If the optimal quantity of 1 per cent acetic acid solution has been found to be 0.0075 ml. per ml. of gold solution, 0.22 ml. of 1 per cent acetic acid solution is needed: $30 \times 0.0075 = 0.22$. The procedure, as described above, entails delivering 0.22 ml. of acid into one large test tube, and then rather rapidly adding 30 ml. of Lange's colloidal gold solution to it.

Grading of results. Each of the six tubes for each sample of serum is graded on a scale of *5* to *0*, as is standard practice for colloidal gold chloride tests. Complete reduction, *5*, results in colorless supernatant fluid and pronounced precipitation of the colloidal gold; whereas, no reduction, *0*, is indicated by the unchanged red color of the gold solution and no precipitation. The supernatant fluid of the first three tubes of a test with normal serum may show a very slight violet-to-blue color, or no color change at all, the remaining three tubes usually showing no reduction. The seventh tube should show complete reduction of the

gold solution, because of the well-established fact that a ratio of 1.7 to 5 of 1 per cent sodium chloride solution to Lange's colloidal gold solution will completely reduce the latter.

An abnormal serum colloidal gold curve is obtained when there is complete reduction in the first two tubes and at least a slight reduction in the third tube (551000 to 555555), because of elevated gamma globulin. The remaining three tubes usually become progressively nonreactive.

Comment

The above-described methods of adjusting the commercial colloidal gold solution and of performing the test have made the serum colloidal gold test a useful aid in the diagnosis of struma lymphomatosa. The accuracy of evaluation has been increased by comparing results with a control sample of normal serum in which the gamma globulin value is known to be near 0.80 gm. per 100 ml. (normal 0.60 to 0.91 gm. per 100 ml.) as determined by electrophoretic protein analysis. The performance of the test on known normal serum as well as on suspected abnormal serums provides an additional check on the method.

The value of the test is confirmed by the recent report of Skillern and associates[3] which states that of 26 patients having struma lymphomatosa, 22 had an increased level of gamma globulin; and of the 22 patients, 21 had an abnormal serum colloidal gold test. Many other diseases that cause an elevated gamma globulin also will result in an abnormal colloidal gold test. Thus, the value of the test is limited by its nonspecificity and the result should be correlated with other clinical and laboratory findings. The elevation of the serum gamma globulin in struma lymphomatosa was first reported by Fromm and associates.[8a,b]

The pathologic physiology of this increased gamma globulin in struma lymphomatosa is unknown. We at first thought that it might be due to the effects of thyroxine deficiency, but its absence in 80 to 90 per cent of patients with primary hypothyroidism without goiter is somewhat against this, as also suggested by Luxton and Cooke.[6] Owen and McConahey[9] have reported that the abnormal hormone known to be secreted by the thyroid in struma lymphomatosa has all the properties of thyroglobulin. Whether the breakdown of this continuously secreted thyroglobulin is responsible for the elevated gamma globulin is in the realm of further speculation at present.

Summary

An improved method of adjusting commercial colloidal gold solution (Lange's) using known normal and abnormal serums is presented and a modification of the method of performing the serum colloidal gold test is described. The test is a simple, inexpensive diagnostic aid whereby an increase in serum gamma globulin is qualitatively measured. The test in our institution has been useful, but is not as accurate as the more difficult Tiselius electrophoretic serum protein determination as an aid in differential diagnosis of struma lymphomatosa.

References

1. Gray, S. J.: Colloidal gold reaction of blood serum in diseases of liver. Arch. Int. Med. **65**: 523-544, March 1940.
2. Kabat, E. A., Hanger, F. M., Moore, D. H., and Landow, H.: Relation of cephalin flocculation and colloidal gold reactions to serum proteins. J. Clin. Invest. **22**: 563-568, July 1943.
3. Skillern, P. G., Crile, G., Jr., McCullagh, E. P., Hazard, J. B., Lewis, L. A., and Brown, H.: Struma lymphomatosa: primary thyroid failure with compensatory thyroid enlargement. J. Clin. Endocrinol. **16**: 35-54, Jan. 1956.
4. Skirpan, P., Reich, A., and Crile, G., Jr.: Serum colloidal gold test—an aid to diagnosis of struma lymphomatosa. Am. J. Clin. Path. **25**: 1274-1278, Nov. 1955.
5. Cooke, R. T., and Wilder, E.: Letter to the Editor: Hashimoto's struma lymphomatosa. Lancet **1**: 984, May 8, 1954.
6. Luxton, R. W., and Cooke, R. T.: Hashimoto's struma lymphomatosa: diagnostic value and significance of serum-flocculation reactions. Lancet **2**: 105, July 21, 1956.
7. Maclagan, N. F.: Preparation and use of colloidal sols as diagnostic agents. Brit. J. Exper. Path. **27**: 369-377, Dec. 1946.
8. (a) Fromm, G. A., Lascano, E. F., Bur, G., and Escalante, D.: Chronic nonspecific thyroiditis. Rev. Asoc. méd. argent. **67**: 162-170, May 15, 1953.
(b) Fromm, G. A., Lascano, E. F., and Enriori, C.: Estruma linfoideo (enfermedad de Hashimoto). Endocrinología **1**: 86, 1950.
9. Owen, C. A., and McConahey, W. M.: Serum "thyroglobulin" in Hashimoto's thyroiditis. Presented at Meeting of American Goiter Association, May 1956. J. Clin. Endocrinol.: In Press.