THE USE OF OXALATED PLASMA IN THE KAHN AND KOLMER TESTS FOR SYPHILIS

by

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INTRODUCTION

In 1893, Arthus and Pages (Perspectives in Biochemistry, 1938) found that mammalian blood, to which 0.1% potassium oxalate had been added, remained fluid and no clot was formed. Oxalated blood has since been extensively used in quantitative chemical determinations: dextrose, non-protein-nitrogen, urea nitrogen, uric acid, creatinine, protein estimation, phosphate, chlorides, alkali reserve and cholesterol. Osgood (1931) described a "Uniform System of Hematologic Methods for Use with Oxalated Venous Blood", which included hemoglobin estimation, red cell count, platelet count, red cell volume, color index, volume index, saturation index, icterus index, Van den Bergh test, white cell count, smear for differential count, peroxidase test, fragility test and sedimentation rate determination. Because of its wide use in many other tests, it was thought that the use of oxalated blood in the Kahn and Kolmer tests for syphilis might be of interest.

Serum and Plasma

Serum is the amber fluid which results from the clotting of blood and is used in the approved methods for the laboratory diagnosis of syphilis. When blood clots, the fibrin precipitates out, binding together the cellular elements into a mass, which also contains the serum. Serum is expressed by contraction of the clot.

The two theories of clotting are those of Howell and Eagle, (Bodansky, 1938). Howell believes that cephalin (from platelets or tissues) neutralizes heparin (which normally acts as anti-prothrombin)

so that prothrombin in the presence of calcium ions forms thrombin. Thrombin converts fibrinogen into fibrin. Eagle's theory is summarized as follows:

Prothrombin + calcium + platelets --- thrombin
(activating enzyme) (enzyme product)

Fibrinogen + thrombin --- fibrin

According to both theories, calcium is necessary for the conversion of prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin. When potassium oxalate is added to fresh blood, no clotting occurs because the ionized calcium has been changed to the insoluble form of calcium oxalate. No thrombin is formed, and the fibrinogen remains unchanged in the plasma, which is the fluid medium of the blood. Oxalated blood will form a clot when a solution containing calcium ions is added to it.

Plasma contains calcium in the insoluble form of calcium oxalate, and fibrinogen. In serum the ionized calcium remains, while the fibrinogen has been converted to fibrin which formed the supporting meshwork of the clot.

The following is an abstract of material on reagin and the mechanisms of the Kahn and Kolmer tests for syphilis from Eagle's book, "The Laboratory Diagnosis of Syphilis", 1937:

Evidence points to the conclusion that the active substance of syphilitic serum, reagin, is an antibody to the Treponema pallidum. Reagin, like antibodies in general, is a protein. Reagin is always associated with the globulin fraction of serum protein, and is car-

ried down with it quantitatively upon half-saturation with ammonium sulphate. The albumin fraction of syphilitic serum is always Wassermann negative. The sera of syphilitic patients contain widely varying amounts of reagin. The presence of reagin in a patient's serum is determined by serological tests, two of which are those of Kahn and Kolmer.

Mechanisms of the Kahn and Kolmer Tests

The Kahn test reagents are antigen and 0.9% sodium chloride solution. Kahn antigen is a stable cholesterinized alcoholic tissue extract which contains an active lipoid substance. This lipoid material, which is obtained from lean beef heart, is considered to be a phosphatide and is classified with the lecithins. It is soluble in alcohol and insoluble in acetone. Powdered beef heart is extracted with ether to remove fats, fatty acids, sterols and soaps. These substances even in small concentrations would cause the formation of coarse aggregates in negative sera. Cholesterol increases the attraction of the lipoid particles for reagin and also causes the aggregation of smaller lipoid particles into larger ones. This antigen has the striking property of specifically reacting with syphilitic serum. The term antigen is applied solely to its combining affinity for the reagin in syphilitic serum and not to its ability to stimulate the formation of antibodies. This antigen is a haptene; it can be combined with specific antibody, but cannot alone induce their formation in an experimental animal.

When the antigen suspension is added to syphilitic serum the tissue lipoid particles combine, under the conditions of the tests, irreversibly with the reagin to form the antigen-reagin compound, just as any true antigen combines with its specific antibody. Evidence points to the hypothesis that when antigen combines with reagin, the reagin forms a sensitizing layer of insoluble globulin around each particle of the colloidally dispersed antigen dilution. The layer of reagin globulin around the antigenic particles has been shown to be the agent which fixes complement, and sensitizes the antigen particles to aggregation by electrolyte.

Kahn antigen is mixed with 0.9% sodium chloride solution to give the antigen suspension used in the tests. When this antigen suspension is added to normal human sera, the particles remain discrete and give no visible aggregation. In syphilitic sera there is a visible aggregation, because the reagin forms a sensitizing film around the lipoid particles of the antigen suspension, causing them to form aggregates in a solution of suitable electrolyte concentration. In the absence of salts the surface charge around each particle causes the particles to repel each other. When this charge is depressed below a critical level as it is in weak solutions of electrolytes, the sensitizing films can cohere upon impact and the visible aggregates are the criterion of a positive test.

These aggregates consist chiefly of the lipoid particles, but also contain the firmly bound reagin which forms a film adherent

to the particles. If these aggregates, in syphilitic serum, are washed with salt solution they show no further reactivity with syphilitic serum. These aggregated particles when heated to 100° C. for a few seconds again become discrete and regain their ability to combine with reagin. This indicates that the lipoid is present in the precipitate and that the reactive groups on the surface are blocked by the reagin with which they have combined. The destruction of the reagin film by a few seconds' heating at 100° C. is further evidence for its protein character. Injection of the lipoid particles into guinea pigs sensitizes them to human sera protein, showing that the aggregates contain protein.

Reagents used in the Kolmer test are:

1. 0.85% sodium chloride solution
2. Antigen
3. Hemolysin
4. 2% suspension of sheep blood cells in .85% sodium chloride solution
5. Complement

The Kolmer antigen, like the Kahn antigen, is an alcoholic, cholesterinized tissue extract. It is used in such dilute solution that when added to syphilitic serum no visible aggregation is formed. An indicator system is necessary to show when the antigen is combined with the reagin of syphilitic serum.

Hemolysin and sheep cells are used as the indicator system. Hemolysin is rabbit serum from an animal which has been immunized

against sheep blood cells. Like all antibodies, the active component of hemolysin is associated with the globulin fraction of the serum protein. Hemolysin has the property of sensitizing the sheep cells' suspension so that they are hemolysed when sufficient active complement is added. When cells and hemolysin are mixed together there is no hemolysis unless complement is added.

Complement is the term applied both to fresh guinea pig serum and to the hemolytic substance which it contains. It is the most important single reagent in the complement-fixation test. It is found in fresh serum of man and animals and its activity varies with individuals and species. Fresh guinea pig serum is used because it is a readily available source of satisfactory complement.

Complement has not been purified and its chemical nature is rather complex and at present unknown. Extensive study for over thirty years has as yet given no clue to its intimate structure or mode of action. Six fractions of complement have been described, each supposed to be a functionally distinct entity:

1, and 2. Midpiece and Endpice. Sachs and Altmann (1906) found that hydrochloric acid precipitated the euglobulin of fresh serum. After centrifugalization the supernatent fluid was devoid of hemolytic activity. The precipitate was inactive when redissolved in salt solution. If the two fractions were recombined the mixture was as hemolytic as the original serum, similarly diluted. Dialysis and carbon dioxide have a similar "splitting action" on complement. The labile fraction of complement which is carried down with the euglobulin precipitate has

been termed midpiece, while the fraction which remained in the supernatent fluid has been termed endpiece.

3. Coca found that a suspension of yeast cells destroyed the activity of complement with the midpiece and endpiece remaining active. He postulated a third yeast-inactivable component of complement which resisted heating to 56° C. unlike the midpiece and endpiece.

4. Gordon, Whitehead and Wormall found ammonia and ammonia salts to have a specific destructive effect on complement which was not due to inactivation of either midpiece or endpiece. They termed this ammonia sensitive principle the "fourth component of complement", distinct from midpiece, or fraction inactivated by yeast. Toda and Mitsuse found that this principle could be removed by chloroform, ether, or cadmium chloride and was associated with the albumin fraction of the serum. E. Maltsuer (1935) found that ammonia inactivated complement could be reactivated by incubation with a leucocyte suspension for 2 hours at 37 C.

5. Toda and Mitsuse described a fraction associated with the albumin fraction which could be removed by benzene.

6. Gordon and Hoyle reported that sterile guinea pig serum kept at room temperature for a month could be reactivated by adding an equal volume of fresh guinea pig serum prevbusly heated at 56 C. to destroy its thermolabile constituents.

Eagle does not believe that these fractions necessarily represent functionally distinct entities. P. Schmidt believes that complement may be a single labile substance variously affected by dif-

ferent physical and chemical manipulations.

Complement deteriorates rapidly with age, especially in the temperature range of 30-55 C. Fresh serum kept at 0 C. may show only 20-30% loss in complement activity in twenty-four hours; at 20-25 C. there may be 50% loss in 12 hours; while at 56 C. there is 95% decrease in less than five minutes. This destruction at 56 C. involves both midpiece and endpiece and is utilized in the inactivation of human sera to inhibit its variable complement activity prior to the addition of a constant quantity of fresh guinea pig serum in all tests. Prolonged shaking causes the diminution of complement activity. It is most stable at pH 7. Complement is destroyed at any reaction more acid than pH 5.0, or more alkaline than pH 8.5; it is relatively unstable at pH 5.5-6.5, and 8.0-8.5. Relatively slight concentrations of salts with bivalent cations (barium chloride, calcium chloride) inhibit the activity of complement without necessarily destroying it. Oxalated, citrated, or heparinized plasma contains complement which is as active as serum.

Complement has the property of hemolysing sensitized cells, and loses this property in the presence of its specific antibody. The property of hemolysing sensitized cells disappears in the presence of an antigen and its specific antibody. Complement cannot act until it has been bound, "fixed" by the antigen-antibody combination. "This susceptibility of complement to fixation by an antigen-antibody compound, as distinct from either the antigen or antibody alone, is one

of its characteristic and identifying properties."

The active hemolytic principle does not dialyze through a colloidon membrane, and appears to be constantly associated with the serum protein, being carried down with it upon saturation with ammonium sulphate, and disappears whenever the protein is irreversibly coagulated. The active principle is readily absorbed.

In the Kolmer test two antigen-antibody systems, antigen lipoid-reagin and hemolysin-sheep cells, are in competition for the possession of complement. The antigen-serum mixture is given the first chance. The ability of the reagin-antigen combination to fix complement is influenced by electrolyte concentration, pH, temperature, and duration of incubation. Following the incubation of the antigen-serum-complement mixture, sensitized cells (equivalent parts of hemolysin and 2% suspension of sheep cells) are added to allow hemolysis to take place in those tubes in which complement remains free.

If the serum contains syphilitic reagin, the antigen lipoid-reagin combination fixes complement and there is no hemolysis of the sensitized cells. In the serum from a non-syphilitic individual the complement is free to act on the sensitized cells with hemolysis as the visible result. Hemolysis indicates that there is free complement with no significant complement fixation and that the serum contains no reagin. The sensitized cells serve only as an indicator for the presence of unbound complement.

Review of Literature

Osgood (1940) states that serologic tests for syphilis on oxalated blood are accurate if done within twenty-four hours.

Eagle (1937) advises against the use of oxalated plasma in the complement fixation tests because it is sometimes anticomplementary due to excess anticoagulant and because a heavy precipitate of heat coagulated fibrinogen is formed upon inactivation.

Gregory (1936) performed over 3000 complement-fixation tests on oxalated plasma with entirely satisfactory results. A series of comparative tests on serum and plasma gave identical results. He found that oxalated specimens had a tendency to become anticomplementary on standing overnight and advised that inactivation be delayed until just before the tests are performed, or that the inactivation be repeated. With the observation of this precaution he found anticomplementary results to be rare. Gregory found that centrifugalization after inactivation removed the precipitated fibrinogen and gave a clear serum.

Suzumu Watanabe (1919) found that 0.001 grams of sodium oxalate per cubic centimenter of guinea pig blood had but very slight and almost negligible influence upon complement activity as shown by comparison with the serum of the same blood. 0.004 grams of sodium oxalate per cubic centimeter of blood had a very distinct inhibitory effect upon complement.

Watanabe (1919) performed Wassermen tests on the oxalated

plasmas and sera of eighteen persons. The sera and plasmas of six persons yielded negative results, while twelve showed positive reactions of similar degree in both plasmas and sera. In five cases the plasmas yielded stronger reactions than the corresponding sera.

Burdon (1932) found that less time was required to secure the laboratory reports when plasma was used in place of serum. Citrated plasma was secured more rapidly and easily than serum. 0.1 cubic centimeter of 10% sodium citrate solution per cubic centimeter of blood was used as anticoagulant. The plasma and serum of 361 individuals were tested by the Kline method. Kahn tests were performed on 318 of the same serum and plasma specimens. A comparison of the results of the Kline tests showed following:

Absolute agreement	72.55%
Relative agreement	25.79%
Disagreement (6 cases)	1.66%

A comparison of the results of the Kahn tests on serum and plasma showed the following:

Absolute agreement	89.32%
Relative agreement	8.48%
Disagreement (7 cases)	2.2%

In the cases of disagreement, only one was regarded as a false positive, in which both tests on the plasma were positive, while the corresponding serum was negative in both tests.

In all other cases the positive plasma reactions in both tests were in closer accord with the clinical condition of the patient than

the negative or doubtful reactions of the corresponding sera. The plasma showed a consistent tendency to give a stronger reaction than the corresponding sera.

Burdon says "Comparison of results with clinical data indicates that the marked sensitivity of the plasma may be distinctly advantageous in the diagnosis of early syphilis and in instances where the test is employed as a therapeutic guide." Burdon believes that the use of plasma in tests for syphilis deserves further trial.

Marie Strube Slawson, in an unpublished paper, reported the results of 130 Wassermann and Kahn tests on oxalated plasma and the corresponding sera. The results of the plasma tests agreed with those of the serum in every case. Two serum specimens were anticomplementary, while three of the plasma specimens were anticomplementary. In at least one instance the age of the plasma (48 hours) might have accounted for its being anticomplementary.

Israelson and Bojevskaja (1929) found that citrated and oxalated plasma could be used in the complement fixation tests. The results of active Wassermann tests on citrated plasma were in 90.7% agreement with the inactive Wassermann method. Their results showed that active Wassermann tests on citrated plasma had a greater sensitiveness than, and a specificity equal to, the inactive Wassermann method.

Warburg (1922) considered the addition of oxalate to blood as analogous to the addition of an equivalent amount of hypertonic saline solution.

EXPERIMENTAL METHODS

The effect of potassium oxalate on the activity of complement was studied. The complement titration (U.S.P.H.S., 1940) was carried out in two series of tubes, containing equal amounts of the reagents. In one series, the reagents were added to tubes containing 2 milligrams of potassium oxalate. After incubation at 37 degrees C. for one hour, both series of tubes showed the exact unit of complement to be the same. In this experiment the potassium oxalate (2 milligrams in 3 cubic centimeters of solution) was of a higher concentration than in the tests using oxalated plasma, in which there was less than .4 milligrams in 1.7 cubic centimeters of solution.

Plasma from well mixed whole blood, which was inactivated at 56° C. for 30 minutes was hemolyzed.

Specimens of whole blood to which 2 milligrams of potassium oxalate per cubic centimenter had been added as anti-coagulant were secured from the University of Oregon Medical School Out-patient Clinic Laboratory. Most of these specimens were from patients in Multnomah County Hospital, who were under treatment for a variety of conditions. Specimens were also obtained from patients in the out-patient clinic and in Doernbecher Children's Hospital. No attempt was made to secure specimens from any one selected group of patients.

Blood drawn one day was kept in the refrigerator over-night

and the following day, eighteen to twenty-four hours later, the plasma was separated from the cells. The whole blood specimens were centrifuged for ten minutes and then placed in a water bath at 55-56° C. for two minutes. (It was necessary only that the level of the water be above that of the cells.) This caused the blood cells to congeal slightly, and when cooled, to room temperature for several minutes the plasma was readily separated from the cells by pouring it into other tubes. This was found to be a simpler and easier method than using pipettes, and had the additional advantage of allowing practically all of the plasma to be secured. This method was of particular value when there was but a small amount of plasma.

Inactivation of the plasma at 55 to 56° C. for two minutes caused precipitation of the fibrinogen, but inactivation was completed before the specimens were centrifuged. The specimens were centrifuged for twenty minutes which gave a clear plasma with the fibrinogen packed into a compact mass. The clear plasma was poured off into other tubes, obviating the use of pipettes.

The standard Kahn test, U.S.P.H.S. 1940, and the simplified Kolmer test, U.S.P.H.S. 1940, were performed on these plasma specimens. The results were compared with those obtained by the clinic laboratory with Kahn and Kline tests on the corresponding sera. Different antigen was used in the tests on plasma from that which was used in the tests on sera. The plasma and sera used in these tests were in some cases not obtained at the same time. In most cases, however,

they were either secured at the same time or within a few days.

In a few specimens inactivation for two minutes caused coagulation of the plasma. It could be readily seperated from the cells, however, and poured into another tube. The final centrifugation gave a very clear specimen in a short time.

503 Kahn tests were run on the oxalated plasma from 411 patients, the results of which are given in Tables 1--6. Three series of Kolmer tests were run. In the first series of 73 specimens no results are given because 37 specimens were anticomplementary. The results of 101 specimens in the second series are given in Table 7. The results of 214 specimens in the third series are shown in Tables 8 and 9.

TABLE 1.

Results of 503 Kahn tests on oxalated plasma from 411 patients, with results obtained by Kahn and Kline tests on the corresponding sera.

Number of Tests	Plasma Kahn	Serum Kahn	Serum Kline
392	- - -	- - -	-
25	- - -	- - -	±
2	- - -	- - 1	-
4	- - ±	- - -	±
7	- - ±	- - -	-
2	- - 1	- - -	±
1	- ± -	- - 1	-
1	- ± ±	- - -	1+
2	- ± ±	- - -	-
2	- ± 1	- - -	-
3	- - 1	- - -	-
2	- 1 1	- - -	-
1	- - 2	- - -	-
1	- - 2	- - -	±
1	- - 1	- - -	1+
1	- - 1	- - -	2+
1	- - -	- - -	3+
1	- 2 2	- 1 2	±
1	- 1 3	- - -	-
1	- - -	- 1 2	2+
3	- 1 2	- - -	-
1	± 1 2	- - -	±
1	- ± 2	- - -	-
1	- 2 2	- - -	-
1	- 2 3	- - -	-
1	1 3 3	- 2 3	1+
1	- 4 4	- - 4	2+
1	1 ± ±	3 3 3	-
1	3 3 3	- - -	-
1	3 3 1	- - -	±
1	2 4 4	- - -	-
29	4 4 4	4 4 4	4+
2	4 4 4	4 4 4	3+
1	4 4 4	2 4 4	3+
1	4 4 4	2 3 4	3+
1	4 4 4	1 4 4	3+
1	2 4 4	1 3 4	3+
1	2 4 4	3 4 4	4+
1	2 4 4	4 4 4	4+
1	2 4 4	1 3 3	2+
1	- 4 4	4 4 4	4+
503			

TABLE 2.

Results of 486 Kahn tests on plasma which are in agreement with the Kahn and Kline tests on the corresponding serum.

Number of Tests	Plasma	Serum	
	Kahn	Kahn	Kline
392	- - -	- - -	-
25	- - -	- - -	±
2	- - -	- - 1	-
7	- - ±	- - -	-
4	- - ±	- - -	1+
1	- ± -	- - 1	-
3	- - 1	- - -	-
3	- - 1	- - -	1+
1	- ± ±	- - -	-
2	- ± ±	- - -	1+
2	- ± 1	- - -	-
2	- 1 1	- - -	-
1	- - 2	- - -	-
1	- - 2	- - -	±
1	- 2 2	- 1 2	±
29	4 4 4	4 4 4	4+
2	4 4 4	4 4 4	3+
1	4 4 4	2 4 4	3+
1	4 4 4	2 3 4	3+
1	4 4 4	1 4 4	3+
1	2 4 4	4 4 4	4+
1	2 4 4	3 4 4	4+
1	2 4 4	1 3 4	3+
1	2 4 4	1 3 3	2+
1	- 4 4	4 4 4	4+
486			

TABLE 3.

Results of 13 Kahn tests on plasma which are in relative agreement with the Kahn and Kline tests on the corresponding serum. (positive or negative in one, doubtful in another.)

Number of Tests	Plasma	Serum	
	Kahn	Kahn	Kline
1	- - 1	- - -	2+
1	- - -	- - -	3+
1	- - -	- 1 2	2+
1 (D.C.)	- ± 2	- - -	-
3	- 1 2	- - -	-
1	± 1 2	- - -	±
1	- 1 3	- - -	-
1 (G.G.)	- 2 2	- - -	2+
1	- 2 3	- - -	-
1	1 3 3	- 2 3	1+
1	- 4 4	- - 4	2+
13			

TABLE 4.

Results of 4 Kahn tests on plasma which show disagreement with the Kahn and Kline tests on the corresponding serum.

Number of Tests	Plasma	Serum	
	Kahn	Kahn	Kline
1 (W.H.)	3 3 3	- - -	-
1 (V. M.)	3 3 1	- - -	±
1 (G.G.)	2 4 4	- - -	2+
1 (A.B.)	- ± ±	3 3 3	-
4			

TABLE 5.

Comparison of Kahn Tests on Serum and on Plasma.

Agreement			Relative Agreement	Disagreement
-	±	+		
448	1	39	11	4
97.0%			2.2%	0.8%

TABLE 6.

Comparison of Results of Kahn Tests on Plasma with Kline Tests on the corresponding Serum.

Agreement			Relative Agreement	Disagreement
-	±	+		
447	2	39	13	2
97.0%			2.6%	0.4%

Explanation for Tables 5 and 6:

Agreement: negative, doubtful or positive in both serum and plasma.

Relative agreement: negative or positive in one, doubtful in the other.

Disagreement: negative in one, positive in the other.

TABLE 7. (Series II)

Results of 101 Kolmer tests on oxalated plasma from 96 patients, with results obtained by Kahn and Kline tests on the corresponding serum.

Number of Tests	Plasma Kolmer	Serum Kahn	Serum Kline
76	-	- - -	-
3	-	- - -	+
2	4+	4 4 4	4+
1	4+	2 4 4	4+
2	4+	- - -	-
1	2+	4 4 4	4+
1	2+	- - -	1+
1	1+	- - -	1+
3	1+	- - -	-
1	-	3 4 4	4+
1	-	3 3 3	-
1	-	- - 3	1+
1	-	- - -	4+
1	-	- - -	1+

4 anticomplementary
2 slightly anticomplementary

Comparison of the results of the Kolmer tests on the plasma with the Kahn tests on the serum, shows the following:

Agreement in 85 tests - - - - - - - - - - - - - - 84.15%

Relative agreement in 2 tests - - - - - - - - - - 1.98%

Disagreement in 8 tests - - - - - - - - - - - - - 7.92%

Slightly anticomplementary in 2 plasmas - - - - 1.98%

Anticomplementary in 4 plasmas - - - - - - - - - 3.96%

Comparison of the results of the Kolmer tests on the plasma with the Kline tests on the serum, shows the following:

Agreement in 89 tests - - - - - - - - - - - - - - 88.11%

Relative agreement in 2 tests - - - - - - - - - - 1.98%

Disagreement in 4 tests - - - - - - - - - - - - - 3.96%

Slightly anticomplementary in 2 plasmas - - - - 1.98%

Anticomplementary in 4 plasmas - - - - - - - - - 3.96%

TABLE 8 (Series III)

Results of 214 Kolmer tests on plasma from 148 patients, with the results of the Kahn and Kline tests on the corresponding serum.

Number of Tests	Plasma Kolmer	Serum Kahn	Serum Kline
179	-	- - -	-
7	-	- - -	+
8	4+	4 4 4	4+
1	4+	3 4 4	4+
1	4+	- 1 4	3+
1	4+	- - -	3+
2 (1 slightly anti-complementary)	4+	- - -	2+
2	4+	- - -	-
3	3+	- - -	1+
1	2+	- - -	1+
2	1+	4 4 4	4+
5	1+	- - -	1+
1	-	- - -	1+
1 slightly anti-complementary	-	- - -	-

Comparison of the results of Kolmer tests on plasma with the Kahn tests on serum shows the following:

Agreement in 198 tests - - - - - - - - - - - - 92.5%

Relative agreement in 6 tests - - - - - - - - 2.8%

Disagreement in 9 tests - - - - - - - - - - - - 4.2%

Slightly anticomplementary in 1 plasma - - - - 0.47%

Comparison of the results of Kolmer tests on plasma with the Kline tests on serum shows the following:

Agreement in 208 tests - - - - - - - - - - - - 97.2%

Relative agreement in 3 tests - - - - - - - - 1.4%

Disagreement in 2 tests - - - - - - - - - - - - 0.93%

Slightly anticomplementary in 1 plasma - - - - 0.47%

TABLE 9 (Series III)

Results of Kolmer tests on plasma which show relative agreement or disagreement with the Kahn and Kline tests on the corresponding serum.

	Patient	Number of tests	Plasma	Serum	
			Kolmer	Kahn	Kline
	R.S.	1	4+	- - -	3+
	R.S.	1	4+	- 1 4	3+
(F)	G.G.	2	4+	- - -	2+
	W.S.	3	3+	- - -	1+
	W.S.	1	2+	- - -	1+
	W.S.	2	1+	- - -	1+
	G.A.	2	1+	4 4 4	4+
(?)	3 patients	3	1+	- - -	1+
(F)	2 patients	2	4+	- - -	-
(?)	1 patient	1	-	- - -	1+

Three cases, R.S., W.S., and G.A., were under treatment for syphilis. 6 plasma specimens within two weeks on W.S. showed results varying from 1+ to 3+. The serum tests on G.A. were done some time before the tests on the plasma.

Four of the results, which are marked (F), must be regarded as false positives.

No remarks can be made on the results of the 4 Kolmer tests which are marked (?).

The false positive results may possibly be related to fever. Several cases showing a false positive test with plasma were quite ill and had a fever.

In the first series of Kolmer tests 73 specimens were run, 21 of which were anticomplementary and 16 slightly anticomplementary. Preserved complement less than one week old, containing .3 gram of sodium chloride per cubic centimeter, was used in this series. Two full units of complement were added to each tube. The saline solution used contained 8.5 grams sodium chloride and 1 milligram magnesium sulphate per liter.

In the second and third series two and one-half units of fresh complement diluted 1:40 were added to each tube. The complement was hyperactive, having a titer of .2 to .25 cubic centimeter of 1/30, which Kolmer, U.S.P.H.S. 1940, advises using in a minimum titer of .3 cubic centimeter 1/30 dilution. The saline solution used contained 8.5 grams sodium chloride per liter.

In the second series of Kolmer tests 101 specimens were run, 4 of which were anticomplementary and 2 slightly anticomplementary. One group of 31 specimens was inactivated at 56 degrees C. for thirty minutes, placed in the refrigerator overnight, and reinactivated the next day for fifteen minutes at 56 degrees C., immediately before being used in the tests. Of this group 1 was anticomplementary, while 2 were slightly anticomplementary. Of 32 specimens which were inactivated immediately after the plasma was separated from the cells and just before use in the test, none were anticomplementary. In a group of 17 specimens which were placed in the refrigerator overnight and inactivated at 56 degrees C. for thirty minutes the next day before use in the tests, one was anticomplementary. Another group of 20 specimens

was inactivated at 56 degrees C. for thirty minutes immediately after cells and plasma were separated and used in the tests. One specimen was anticomplementary.

The third series of 209 specimens were inactivated for fifteen minutes at 55 degrees C. just before the tests were performed. 90 specimens were inactivated just after the plasma was separated from the cells. One was found to be slightly anticomplementary. 80 specimens were kept in the refrigerator 24 hours after plasma and cells were separated. 32 specimens were kept in the refrigerator for 4 days, one of which was slightly anticomplementary. 7 specimens were kept at room temperature for 24 hours. A comparison of the results for specimens inactivated for 30 minutes, Series II, Table 7, and those inactivated for 15 minutes, Series III, Table 8, shows that those inactivated for 15 minutes gave better agreement with tests on serum.

In one case (L.G.) with no clinical evidence of syphilis, there was agreement between the results of the tests on plasma and serum, when they were at first positive, and later negative.

In Table 3, the results of 13 Kahn tests on plasma, which show relative agreement with the tests on serum, are given. In 2 specimens there was agreement between the Kahn tests, while the results of the Kahn tests on plasma showed only relative agreement with results of the Kline tests on the serum. In 3 cases, in which the serum tests were negative, the Kahn tests on plasma were doubtful, while other plasma tests on these same cases were negative and in agreement with the

results of the serum tests. Another case (G.G.) will be discussed below. One case (D.C.) had a high fever and was suspected of having infectious mononucleosis. The serum tests, which were negative, were done when the patient was in good health. No definite remarks can be made in regard to the other results showing relative agreement.

The results of the 4 Kahn and 9 Kolmer tests on plasma, which show disagreement with the results of the tests on serum, are given in Tables 4 and 9.

In one case (G.G.) with no clinical evidence of syphilis the serum tests were: Kahn - - -, Kline -, and then later, Kahn - - -, Kline 2+. The Kahn tests on plasma were - 2 2, and 2 4 4. The Kolmer tests on the plasma were 4+ and 4+. The results on the plasma were consistently positive, but must be regarded as false positives.

In case (A.B.) the results of tests on the serum were: Kahn 3 3 3, Kline negative and quantitative Kolmer negative. A Kahn test on the plasma was negative, in agreement with the Kline and Kolmer tests on the serum. The positive results of the other two Kahn tests on plasma, in which the serum tests were negative, must be regarded as false positives.

SUMMARY

Whole blood, to which 2 milligrams of potassium oxalate per cubic centimeter had been added as an anticoagulant, was centrifuged and then placed in a water bath at 56 degrees C. for two minutes. This inactivation for two minutes caused the blood cells to congeal slightly, so that the plasma could be poured into other tubes.

After the regular inactivation, for fifteen to thirty minutes, the plasma contained precipitated fibrinogen. Centrifuging for twenty minutes caused the fibrinogen to form a compact mass, from which the clear plasma could be separated by pouring it into other tubes. This method was used by Burdon. The fibrinogen does not appear to be as readily thrown down by centrifuging, as Burdon found to be the case with citrated plasma.

Plasma, containing fibrinogen, could be stored for four days in a refrigerator, without showing any effect on the results of either the Kahn or Kolmer tests. Plasma which was used in the Kahn tests could be stored in the refrigerator after inactivation, and then be reinactivated for ten to fifteen minutes before being used in the tests. Plasma which was used in the Kolmer tests seemed to give the best results if it was inactivated for only fifteen minutes, and separated from the precipitated fibrinogen, just before use in the tests.

The standard Kahn test was performed on 503 plasma specimens, while the simplified Kolmer test was used in 214 different plasma speci-

mens. The results of these tests on the plasma were compared with the results of the Kahn and Kline tests on the corresponding serum specimens. The results of the serum tests were obtained from the Outpatient Clinic Laboratory, where different antigen was used from that which was used in the tests on plasma.

Comparison of the results of the tests on plasma with those on the corresponding serum is given below.

1. Kahn tests on plasma and serum

   Agreement (in 488 tests)- - - - - 97.0%

   Relative agreement (in 11 tests)- -2.2%

   Disagreement (in 4 tests)- - - - - 0.8%

2. Kahn tests on plasma and Kline tests on serum

   Agreement (in 488 tests) - - - - 97.0%

   Relative agreement (in 13 tests)- -2.6%

   Disagreement (in 2 tests)- - - - - 0.4%

3. Kolmer tests on plasma and Kahn tests on serum

   Agreement (in 198 tests) - - - - -- -92.5%

   Relative agreement in 6 tests - - 2.8%

   Disagreement in 9 tests - - - - - 4.2%

   Slightly anticomplementary in 1 plasma - - - - - - - - - - - - - 0.47%

4. Kolmer tests on plasma and Kline tests on serum

   Agreement in 208 tests - - - - - 97.2%

   Relative agreement in 3 tests - - 1.4%

   Disagreement in 2 tests - - - - - 0.93%

   Slightly anticomplementary in 1 plasma 0.47%

CONCLUSIONS

1. Oxalated plasma containing fibrinogen and free from blood cells, may be kept for several days without any change in its reagin content, or becoming anticomplementary.
2. Inactivation of the plasma causes the fibrinogen to form a precipitate which can be readily removed by centrifugation.
3. Plasma, after the fibrinogen has been removed, is as clear as serum.
4. Clear oxalated plasma is no more anticomplementary than is serum, if it is inactivated for only fifteen minutes, and separated from the fibrinogen precipitate, just before it is used in the complement-fixation tests.
5. Clear oxalated plasma may be used in the Kahn and Kolmer tests for syphilis in place of serum.

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