0.16 to 1.81 on the 3rd day after inoculation, while the highest M/L index in any animal of this group was 5.55.

An increase in monocytes has been reported in many diseases—tuberculosis, syphilis, virus III disease of rabbits, and small-pox. The suggestion has already been made that the proliferative phase of the general reaction is the feature common to these different diseases which may be associated with or responsible for the increased numbers of circulating monocytes. A decrease in the number of lymphocytes has also been reported in many diseases, but so far as we know in no disease has the decrease been so marked nor has it occurred in so short an interval as three days or less after inoculation.

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A Rapid Combined Slide Precipitation and Complement Fixation Test for Syphilis.

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The reaction between syphilitic serum and antigen manifests itself either in the formation of a precipitate or in fixation of an added complement. The fixation process is a prolonged one, requiring one hour or more, while the precipitation occurs almost immediately. Since in the syphilitic sera the same principle is accepted to be responsible for both the fixation of complement and flocculation, the reason for such a difference in the course of both reactions may depend on the concentration of the ingredients. In the various modifications of the Wassermann test the syphilitic serum, antigen and complement are used in diluted form, while the newest precipitation tests (Kahn, Citochol, Kline, Rosenthal) employ undiluted serum and concentrated antigen. It occurred to us that it might be possible to obtain a rapid fixation of complement by using the ingredients in the same concentration as in the precipitation tests. At the same time such a procedure would permit the combination of flocculation and complement fixation in one test.

Keining,[1] Kafka,[2] Kahn, Landau and McDermott,[3] in an attempt

[1] Keining, E., *Deut. med. Woch.*, 1921, **47**, 157.

[2] Kafka, V., *Deut. med. Woch.*, 1921, **47**, 269.

[3] Kahn, R. L., Landau, T. L., McDermott, E., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 775.

to combine complement fixation and flocculation added complement to a mixture of serum and antigen after the formation of a precipitate had already taken place. They then allowed an incubation of ½ to 1 hour for the fixation of the complement as in the classical Wassermann test.

In our preliminary experiments we learned that the mixture of syphilitic serum and antigen was frequently unable to fix the complement if the latter was added at the end of precipitation. We, therefore, concluded that the syphilitic serum must first be mixed with the complement and only then receive the antigen. In many controls we found that the presence of this complement in a mixture of syphilitic serum and antigen, even in amounts exceeding those used in our test, in no way interfered with the precipitation. In adopting the use of undiluted serum for complement fixation it was necessary to take into account that some human sera, when undiluted, have marked anticomplementary properties and inhibit the action of complement even in the absence of an antigen. This source of error can be eliminated, however. It is well known that the fixation of complement by an antigen-antibody complex is irreversible. The fixed complement cannot be liberated by subsequent dilution with saline. On the other hand, a complement inhibited by an anticomplementary serum regains its activity after dilution with saline (Bordet and Gay[4]). Therefore, in our test we add saline to the mixture of serum, complement and antigen after the precipitation takes place. As an additional safeguard to counteract the inhibitory properties of undiluted serum every specimen which is anticomplementary with one unit is tested subsequently with 2 units of complement. In the Wassermann tests the amount of complement used varies depending on its strength. In our test we found it more advisable to use a constant amount of complement in order to obtain a uniform dilution of every specimen of serum which is going to be examined. The adjustment of the hemolytic system is made by varying the concentration of red cells. Thus, for a very active complement a 5% suspension of red cells is used, and for a weak one a 2% or 1% suspension. A preliminary titration determines the optimal concentration of red cells.

The Test. 1. *Ingredients.* (a) The sera to be examined are inactivated at 56° for ½ hour. (b) Antigen: The antigen, as previously described,[5] consists of a mixture of equal parts of an alco-

[4] Bordet, J., and Gay, F. P., *Annales Pasteur*, 1908, **22**, 625.

[5] Rosenthal, L., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 61.

holic ether insoluble beefheart extract and a 2% solution of cholesterol in acetone. The antigen is diluted* with saline in the following manner: 1 cc. of antigen is pipetted into one test tube (4x5/8″) and 1.8 cc. of physiological salt solution into another. The contents of these 2 tubes are mixed by pouring from one into the other 8 times. The resulting emulsion is allowed to stay at room temperature for 10 min. to ripen and must be used within 30 min. (c) Hemolytic system: Guinea pig complement, antisheep hemolytic amboceptor (dilution containing 3 units per 1 cc.) and sheep red blood cells, as in usual Wassermann test.

II. *Glassware.* Glass slides (3¼x4″) mounted with 6 thin metal rings (1″ inside diameter). The rings are dipped in hot paraffin and placed on the slide. For measuring small quantities of complement and antigen we use graduated pipettes, whose pointed end is secured by hot paraffin in the hub of a stainless steel needle (Vim, gauge 25). Each drop of serum from this needle corresponds to 0.01 cc. and each drop of antigen to 0.005 cc.

III. *Procedure.* 1. Use 2 rings for each specimen. 2. Pipette 0.2 cc. of serum into each ring. 3. Add one drop of complement into each ring. 4. Add 2 drops of antigen emulsion into the first ring. Leave the second ring without antigen for control. 5. Rotate slide on a flat surface for 5 min. 6. Examine under the microscope (magnification 80) for formation of clumps in positive sera. 7. Dilute contents of every ring with 0.2 cc. of saline. 8. Pipette into every ring 0.1 cc. of sensitized red cells. 9. Slightly shake the slide and place into incubator at 37° for 15 min. 10. Examine under the microscope (magnification 80). In positive sera the red cells are unchanged and often clumped together, while in negative sera, as well as in controls, no red cells are to be seen as a result of complete hemolysis. With some experience it is easy to judge by the amount of unchanged red cells the degree of inhibition of hemolysis.

The test was performed on 165 syphilitic sera and 220 normal sera, and gave results corresponding with those obtained independently by the Wassermann and Rosenthal precipitation test.

* Originally for the precipitation test for syphilis we used the antigen in undiluted form. Subsequently in collaboration with Dr. Rein we adopted the dilution of antigen with saline.