mammals. The object of this study was first, to repeat the earlier experiments of Minkowski; second, to find out if slight changes of method would perhaps serve to show a synthesis of uric acid; and third, to consider the problem experimentally in relation to a man suffering from chronic gout. In gout it is possible that an abnormal synthesis of uric acid occurs, and also, since the uricolytic powers of the gouty organism are less active than normal, a synthesis masked in a normal person might be evident in a person suffering from gout.

The results of the experiments may be briefly summarized as follows: when lactic acid is administered to a normal man who has been fed on a purin free diet, there is no resulting increase of uric acid in the urine, even when the amounts of lactic acid are very large —*i. e.*, 20 grams in a dose. In a dog on a purin free diet (milk, eggs, and rice), following the hypodermic injection of lactic acid, and of lactic acid and urea, there was in both instances a slight increase in the percentage of total nitrogen excreted as uric acid. The absolute amounts were also slightly increased as were those of allantoin. These figures are difficult to interpret and we are not prepared to assert without further investigation that there is a synthesis of uric acid in the manner described.

In a case of chronic gout the effects of the lactic acid and urea were entirely obscured by the irregularity of nitrogen excretion; periods of nitrogen retention and excretion making it impossible to estimate the effects of the treatment.

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Some critical considerations on the serum diagnosis of syphilis.

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In its application to the detection of syphilis antibody the Bordet-Gengou phenomenon of complement fixation has received but little consideration in its quantitative aspect. As will presently be pointed out it is only by respecting the quantitative relations of all reagents concerned that the test becomes reliable and delicate. Even with an adequate quantity of antigen, blood cell suspension and the patient's serum the detection of the antibody

by means of complement fixation may or may not be successful according to whether or not appropriate amounts of hemolytic amboceptor and complement are employed. A large excess of either one of these two reagents can prevent the test from revealing the presence of the antibody. While it is easy for a serologist to see why complement should be used in definite and uniform quantity, not every worker seems to be conscious of the disturbing effects which are exerted by an excess of the amboceptor. In view of the overlooking of certain principles of hemolysis by most of the investigators of the present time a brief consideration of this particular subject seems to be advisable. For the sake of convenience I take the example of the antisheep hemolytic amboceptor for illustrating the influence of an excessive amount of the amboceptor upon the phenomenon of complement fixation. The effects exerted by the excessive sensitization is two-fold. The first effect is to augment gradually the activity of guinea-pig's complement by increasing doses of the amboceptor, until a maximum is reached. Thus in the presence of one unit of amboceptor 0.1 c.c. of the complement is usually required to produce complete hemolysis. By using four, eight and twenty units of the amboceptor the same effect is obtainable with $\frac{1}{3}$, $\frac{1}{5}$ and $\frac{1}{10}$ of the 0.1 c.c. of complement respectively. For this reason it is impossible to demonstrate a partial fixation of complement by using more than several units of the amboceptor, and when several units are employed the test suffers in delicacy. The second effect is still more disturbing than the first. It depends upon partial dissociation of the complement from its combination with the antigen and antibody compound. A quantity of syphilis antibody just sufficient to fix 0.1 c.c. of the complement against two units of the amboceptor is no longer efficacious to hold back the complement from partial liberation against the influence brought on by more than four units of the amboceptor. The fixation of the complement by two and three units of syphilis antibody respectively is also quite ineffective to prevent hemolysis when ten and twenty units of the amboceptor are added. Under these conditions the test fails to indicate the presence of any syphilis antibody although it is really present. When eight units of syphilis antibody are employed the fixation of complement becomes so firm that twenty units of the amboceptor can no longer bring about its liberation.

From the foregoing it becomes at once evident that any system of the complement fixation test in which definite and appropriate amounts of these two vitally important reagents are not employed is not delicate and accurate enough to be a reliable diagnostic measure.

Referring to the method of Wassermann I may state that it has all the disadvantages arising from the presence of unknown, but often considerably large amounts of natural antisheep amboceptor contained in human serum. Wassermann was quite unaware that the natural antisheep amboceptor is capable of being reactivated by guinea-pig's complement, and hence he recommended the use of two units of immune antisheep amboceptor of the rabbit with the view of obtaining complete hemolysis. The reactivability of the natural amboceptor by this complement has been discovered since by Bauer who, in turn, proposed to utilize the natural amboceptor and dismiss the use of the immune amboceptor. By systematic examination of more than 100 specimens of human sera in regard to the content of natural antisheep amboceptor I found that it varies from almost none to as many as twenty units in 0.1 c.c., the quantity usually employed for each tube in the fixation test. Thus the method of Wassermann is destined to give unreliable and inaccurate reactions. Bauer's modification is just as inaccurate as the original as it relies upon unknown amounts of the natural amboceptor alone.

The method which I have recently perfected is also a complement fixation test and differs from the Wassermann method in employing an antihuman hemolytic system instead of an antisheep hemolytic system. Thus the human blood corpuscles are to be hemolysed by means of an antihuman amboceptor prepared in the rabbit and the complement of the guinea-pig. I use two units of the amboceptor for each tube. In this new system the danger of introducing any uncalculated amount of the hemolytic amboceptor is absolutely excluded. As the fixation test is carried out with definite and uniform amounts both of the complement and the amboceptor, the results obtained with different specimens and at different occasions are all comparable with one another. The sharpness of the reaction enables one also to follow the fluctuation of the antibody content even to a fraction of one unit.

Just as I had finished my experiments Tschernogubow published an article in which he stated that he successfully employed an antihuman amboceptor in combination with human complement. As he made no statement as to the source and strength of his antihuman amboceptor no judgment can be made on his method. It is rather striking to observe that the amount of the amboceptor he employed was 0.25 c.c. for each tube, in contrast to 0.002 in my method. It may not be entirely out of place to mention here some essential reasons why I use guinea-pig's serum as complement.

According to my observations the amount of complement in human serum varies considerably in different individuals. In the majority of specimens 0.1 to 0.03 c.c. of the fresh serum contain about enough complement to produce complete hemolysis with ten units of the amboceptor, while the same quantities do not cause any marked hemolysis when two units of the amboceptor are employed. Thus ten units of the amboceptor are to be used as a necessary amount for utilizing human serum as complement. Now in regard to the quantity of each specimen of human serum to be used for complement it is essential to determine the exact strength by a preliminary titration, because if we use some excess of complement the test turns out completely negative. It appears probable that human complement, like that of the rabbit, is not very sensitive to the fixing action of the antigen-antibody of syphilis. In this respect guinea-pig's complement is excellent The method which Tschernogubow recommended is to collect a few drops of a patient's blood in saline solution and use the suspension both for the complement and corpuscles at the same time. But, this does not permit one to make any estimation of the complement content of the blood. Moreover, there is no *direct* way of ascertaining whether any inhibition which may be observed with a given specimen is due to the anticomplementary property of antigen alone or to the combined action of the antigen and syphilis antibody, because the complement and antibody exist in the same serum side by side, if this latter is present at all. Again the relying upon human complement makes impossible the testing of any specimen of blood which has been allowed to stand for several days, as the activity of complement rapidly diminishes and

finally disappears under these conditions. His method is not applicable to cerebrospinal fluid.

In contrast to the use of human complement the use of guinea-pig's serum does not possess one of the disadvantages enumerated. The quantity of complement is always uniform and definite. The human complement does not affect the reaction, because the amount present is too trifling to be of any influence. It is possible moreover to employ for the tests an old specimen of blood, dried or moist, by my method, since guinea-pig's complement is used. Unlike Tschernogubow's the present method enables one to repeat the test in case of need.

Before leaving the subject I would like to point out certain advantages from the technical standpoint of the method which I recommend. The quantity of patient's serum[1] required for the test is only two *capillary* drops (one for each of the two tubes) and no preliminary inactivation at 56° C. is necessary. The hemolytic indicator is readily prepared from the patient or a normal person by mixing the blood with physiological salt solution in the ratio of one drop of the blood to 4 c.c. of the saline solution, 1 c.c. of such suspension being used for each tube. The antigen, complement and amboceptor can be used either in liquid or in dried form. This latter, as prepared on filter paper slips, can be preserved permanently under ordinary conditions at room temperature and be employed in place of the corresponding liquid reagents. In this simple form the test should have a wide application to the sero-diagnosis of syphilis and as a measure and control of the efficient treatment of the disease.

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On nitrogenous metabolism in chronic nephritis.

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The present work represents the results of observations on the character of the nitrogenous metabolism in a patient who was

[1] In case of cerebrospinal fluid use 0.2 c.c. for each tube.