BOOK REVIEWS

THE KAHN TEST. A Practical Guide. By R. L. Kahn, M.S., Sc.D.

(Second Notice.)

DR. KAHN has not only described very fully the technique of his test, but has discussed at some length the phenomena of precipitation in syphilis.

The value of an antigen is largely, if not entirely, dependent on its lipoid content, and for every antigen there is an optimum lipoid concentration; the quality of the lipoids also affects the sensitiveness of an antigen and may account for the variations between different specimens. Every antigen has a "titre," that is, an optimum salt solution: antigen ratio, and it is only when it is used in this ratio that the best results are obtained. Similarly, there is an optimum ratio between the amount of antigen suspension and serum which gives the greatest precipitation; in other words, there is a "zone" of precipitation bounded by zones of no precipitation. Precipitation is hastened by shaking—most likely by mechanically bringing together the serum—antigen substances and by hastening collision between the lipoid particles. It is in this respect that the Kahn reaction differs from most of the other precipitation tests.

The requirements for optimum precipitation are:

1. Optimum concentration of antigenic lipoids.
2. Proper physical state of the antigen suspension.
3. Correct quantitative relation between serum and antigen suspension.
4. Shaking.
5. Minimum total dilution of antigen suspension-serum mixture.

Kahn appears to favour the view that precipitins and complement-fixing substances are identical; his experiments showed that if complement was added to a completed positive Kahn test (serum-antigen-suspension mixture) it was fixed as proved by the absence of haemolysis when sensitised cells were added. In 1,800 sera he obtained 93 per cent. agreement.

In a theoretical consideration of the mechanism of precipitation the author postulates a set of conditions in which the lipoids are:

1. Highly dispersed in the original alcoholic extract of heart muscle.
2. Aggregated in the antigen-salt-solution mixture.
4. Reaggregated if the serum is syphilitic.

He regards it as probable that the syphilitic antibody becomes adsorbed on to the surface of the particles of the lipoids in the antigen, and permits these particles to reunite into visible aggregates. As to
BOOK REVIEWS

whether the reaction is a true immunity one or not he retains an open mind.

Turning to the subject of the choice and preparation of an antigen, the author discusses the effect of preliminary ether extraction before alcoholic extraction, and points out that excessive as well as deficient preliminary ether extraction of powdered heart muscle will produce an antigen of reduced sensitiveness. Ethereal extract added to a given antigen within limits will increase its sensitiveness, and has been named "sensitising reagent." It is interesting also to note that the addition of cholosterolised alcohol has the same effect, whilst addition of the two in suitable amounts has the greatest effect of all. In this way an antigen may be made as sensitive as desired.

On the other hand, an antigen may be reduced in sensitiveness if necessary—also in three different ways. The whole test depends on the standardisation of the antigen: each, when prepared, should be tested for sensitiveness and corrected to a "standard." Great stress is laid on this, since only in this way can uniform results be obtained.

A number of interesting facts have been elicited in experiments: it would appear that the reacting substances in both sera and spinal fluids are associated with the globulin and not the albumin fraction; sera heated to 56°C. for thirty minutes give more marked precipitation than unheated sera; within well-defined limits, acids tend to produce precipitation, whilst alkalies tend in the opposite direction.

Having dealt with the phenomena of precipitation the author sets out his technique in considerable detail. Apparatus should be standardised if consistent results are to be obtained, and all tubes, vials and pipettes are minutely described with actual measurements. An ingenious shaking machine is described which holds the racks of tubes firmly and gives the requisite (275-285) number of oscillations per minute with a stroke of 1½ inches. In practice, the reaction is a very simple one: only three reagents being required, viz., serum, salt solution, and antigen. The first two require no comments. The antigen is prepared by extraction of powdered ox-heart with alcohol (after a preliminary extraction with ether).

When prepared an antigen requires "standardising," which consists of three steps:

1. Titration to determine the amount of salt solution to be added to it.
2. Determination of sensitiveness.
3. Correction to standard when sensitiveness is not similar to that of standard antigen.

These are somewhat complicated procedures requiring standard antigen for comparison purposes, but, since the latter is on the market (though somewhat expensive), need deter no one from undertaking the test.

In order to obtain uniform results, it is absolutely essential that the standardisation of an antigen should be conscientiously carried out; where this is not possible, it is better to purchase the antigen.

For the routine test serum and antigen-suspension are mixed in the proportions of 3 : 1, 6 : 1 and 12 : 1, each test requiring three tubes. The tubes are shaken for three minutes, and the results read after adding given amounts of salt solution to each tube.

It will thus be seen that the test is extremely simple to carry out:
no special apparatus is required, and shaking may be carried out by hand.

The reading of results is usually quite simple—positive reactions are indicated by the presence of definite particles suspended in a transparent or opalescent medium, whilst in negative ones the medium is free from visible particles. In a proportion of cases, however, it is difficult to say whether definite particles are present or not. Considerable practice is required in border-line cases. The author uses the naked eye only, and suggests the use of the concave mirror of a microscope when the reflection of the particles in a thin layer of the fluid is well shown; the reviewer has found the use of a slit lamp (as used in the Dreyer-Ward Sigma test) and hand lens a valuable aid in all cases not very obviously positive.

It will be readily seen that the performance of the test is both simple and rapid. Since, however, in the measuring of a large number of very small amounts of reagents fatigue may induce inaccuracy, it would appear that the employment of some "dropping" method similar to the Donald method as used in the W.R. (No. 1 Method, M.R.C. Special Reports Series, No. 14) saves time and maintains accuracy by avoiding fatigue.

Results are recorded as ++ + + , ++ + , + + , + , ± , − ; ++ or more is considered positive, + and ± doubtful, and − negative.

In addition to the ordinary routine test on serum a number of modified reactions are described.

The test may be made quantitative by dilution of the serum and the results recorded according to the amount of the dilution; a presumptive procedure using a highly sensitive antigen and only one tube per test is useful where a high degree of delicacy and speed are required, and is especially suitable in testing sera from cases of syphilis under treatment and for tests of cure; a micro-procedure using much smaller quantities of reagents is useful where only very small quantities of sera are available, and especially in testing chancre fluid. It is when spinal fluids require to be tested that the value of the Kahn test is somewhat diminished in comparison with the Wassermann.

Except in the case of strongly positive spinal fluids, it is necessary to concentrate them before testing. To a given quantity of fluid an equal amount of saturated ammonium sulphate is added. The globulins thus precipitated are removed by centrifugation and redissolved in saline and put up with a specially titrated antigen. It will be seen that the procedure is much more complicated and time consuming than is the case with sera. It is claimed that results at least equal to the W.R. are obtained.

Dr. Kahn has produced a book primarily intended for laboratory workers, but one that should be read by all clinicians who desire to understand the interpretation of the reaction. No detail has been omitted, and anyone conversant with ordinary laboratory methods can easily carry out the whole procedure once he has grasped the contents of the book. Several excellent plates illustrate the apparatus required, whilst tables are given in almost every case showing the various steps taken in standardising antigen, arriving at its titre and setting up the test. The whole subject is clearly and simply set out. There is at excellent bibliography.

As to the value of the Kahn test there can be no two opinions. In
has proved itself, in comparison with other precipitation or flocculation reactions and with complement-fixation reactions, both highly sensitive and remarkably specific. Moreover, it is easy to perform and rapidly carried out. Whether it should be relied on alone is a matter of opinion. Most workers prefer to use it in conjunction with some form of complement-fixation reaction; this appears to be the ideal, since it would appear that it is rather more sensitive than most forms of Wassermann, especially in treated cases of syphilis; whereas, on the other hand, its specificity has in rare instances been called in question. Further, in the case of spinal fluid the procedure is rather laborious, and it is at least doubtful if it is quite the equal of the best complement-fixation reactions. It should be pointed out that the author has not attempted to produce the most sensitive test possible, but has aimed at a somewhat conservative one in order to attain the maximum of specificity. When sensitivity is the principal object, the presumptive procedure should be adopted. It is doubtful how far the laborious and complicated procedure of standardising an antigen is necessary; where the reaction is to be used alone it is certainly necessary, but when it is used in parallel with some other method, either precipitation or complement-fixation, it does not seem to be so essential, though in any case it is wise to compare a “home-made” antigen with standard antigen before taking it into routine use. Fortunately the latter can now be purchased in this country, though it is somewhat expensive.

T. E. O.


(Second Notice.)

‘This book,’ as stated by the author, ‘is essentially a summary of the clinical and laboratory investigations which I have made ... in serum diagnosis by complement-fixation, not only in syphilis, but likewise in the wide field of the bacterial, protozoal and metazoal diseases of human beings and the lower animals, and in the identification of blood and seminal stains, the detection of meat and milk adulterations and other soluble albumins.’

Part I. deals with the underlying principles of serum haemolysis and complement-fixation.

Haemolysins, also known as sensitisers and amboceptors, may be divided into natural and immune. The former are of importance not only in relation to blood transfusion but also in complement-fixation, since some workers have relied on these in performing modifications of the Wassermann reaction; they also sometimes play a part in the ordinary Wassermann test, since they may be present occasionally in such comparatively large amounts as to tend to produce a falsely negative reaction. Immune haemolysins, on the other hand, are produced by injecting an animal with the erythrocytes of an unrelated species, and it is this variety which is employed in carrying out the Wassermann reaction; it is highly specific for its corresponding or homologous antigen of erythrocytes. The active antigenic constituent of erythrocytes appears to lie mainly in the protein of the stroma,