normally, an additional ventricular filling takes place or at least the intra-ventricular pressure is increased. This operates to lengthen the duration of the systolic ejection phase and through this the length of the entire systole. When the synergic auricular contractions are in abeyance as in auricular fibrillation, the ventricles either fill less efficiently or at least the initial tension is not as high, in spite of a considerable venous pressure in the veins.

Summary: 1. The duration of ventricular systole and its ejection phase are influenced at corresponding heart rates by the diastolic filling and the initial pressure of the ventricles, as demonstrated by recent experimental work.

2. The duration of these periods in hearts with auricular fibrillation is shorter than normal at corresponding heart rates, even in spite of the higher venous pressures present.

3. These intervals increase when the heart reverts to a normal mechanism.

We conclude, therefore, that these observations give probable if indirect evidence of the dynamic importance of auricular systole in the normal heart beat in man.

159 (2119)

Kahn precipitation test for syphilis—improved procedure.

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The precipitation test for syphilis proposed by the author[1] called forth favorable comment from a number of investigators (Keim and Wile,[2] Herrold,[3] Young,[4] Ide and Smith,[5] Holmes[6]

[1] PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 182; *Arch. Derm. and Syphil.*, 1922, v, 570 and 734, vi, 332.

[2] *J. Amer. Med. Ass'n.*, 1922, lxxix, 870.

[3] *J. Amer. Med. Ass'n*, 1922, lxxix, 957.

[4] *J. Amer. Med. Ass'n*, 1922, lxxix, 1674; *Amer. J. of Public Health*, 1923, xiii, 96.

[5] *Arch. Derm. and Syphil.*, 1922, vi, 770.

[6] *J. Mo. State Med. Ass'n*, 1922, xix, 479.

and Levin[7]). Since the publication of the preliminary studies, however, several observations have been made which led to some changes in the original procedure; also, a modified technic has been evolved which is employed as a check on the original one. The improved method discussed in the following resumé embraces both the original and modified procedures.

THE ANTIGEN

Preparation of Alcoholic Extract: Beef heart is freed from fat and fiber in the usual manner and passed several times through a meat grinder. It is then spread on a platter and dried by means of a revolving fan. The dried plates are broken up into small particles and ground in a mortar or coffee grinder. The ground muscle is then extracted with ether at ice-box temperature until supernatant ether is free from coloring matter. Between three and four ether extractions will bring this about. At the end of the final extraction, the ether is filtered off and the ground muscle dried for some hours at room temperature until free from ether odor.

Given quantities of dried material are placed in Erlenmeyer flasks. A quantity of 95 per cent. alcohol equivalent to five times the amount of dried material is added to each flask. The extraction is carried out in the ice-box from nine to ten days. After that period, 10 c.c. of supernatant extract is pipetted into a large test tube and the color compared either with some antigen that is known to give good results or with the following approximate color standard:

1. A solution is prepared containing 0.5 gm. potassium bichromate ($K_2Cr_2O_7$) in 100 c.c. distilled water (permanent standard solution).

2. One c.c. of this solution is mixed with 75 c.c. distilled water.

3. Ten c.c. of solution 2 is measured into a tube of the same size containing the extract to be tested and the colors compared.

If the amount of coloring matter in the new extract is weaker than in the standard, room temperature or incubator temperature for some hours or overnight may be resorted to until coloring matter is brought up to that contained in the standard. If the

[7] *J. Kans. State Med. Ass'n*, 1923, xxiii, 4.

color of new extract is the same or more intense than the standard, the extraction may be considered completed. The extract is now filtered off and is kept in the dark at room temperature as stock solution. This extract will keep for at least a year and possibly for many years.

Antigen prepared from ground heart muscle kept for some weeks or months does not give as sensitive results as that prepared from material freshly ground and dried. It has furthermore been observed that the non-specific sediments in negative sera following incubation of serum and antigen are due to impurities in the extract. These impurities are caused, in most cases, by excessive contact of the extract with cork or rubber stoppers.

The use of an alcoholic non-cholesterinized antigen for this test has been discussed in previous studies. Further investigations are under way. At present, in routine work, the use of a cholesterinized antigen only is recommended and the procedures discussed below apply to this type of antigen.

Cholesterinization of Antigen: A given amount of extract is measured into an Erlenmeyer flask and a quantity of cholesterin added to render it a 0.4 per cent. solution. The cholesterin is dissolved by warming in a water bath with gentle rotation. The solution is then filtered to remove impurities and is ready for use.

We have recently observed that different lots of alcoholic extract are capable of holding in solution different amounts of cholesterin. Thus, one extract appears to be saturated on adding 400 mgm. of cholesterin per 100 c.c., whereas another extract is capable of holding as much as 600 mgm. in solution, and even more. The probable explanation for this is the varying lots of extract, although possessing approximately the same color range, may contain different amounts of lipoid. One may then expect that an extract comparatively poor in lipoids will be capable of dissolving more cholesterin before reaching the saturation point than one which is rich in lipoids. This suggested a method for standardizing the lipoid content of antigens for this test aside from the approximate standardization of these antigens by means of a color range discussed above. The method consists in adding sufficient cholesterin to given alcoholic extracts to bring them to the saturation point at a given temperature.

An alcoholic extract is capable of holding in solution considerably more cholesterin at incubator than at room temperature.

If we choose the latter temperature as our standard, we may find that extract A becomes saturated on adding to it 400 mgm. of cholsterin per 100 c.c.; extract B reaches saturation with 500 mgm., and extract C is capable of holding in solution as much as 600 mgm. of cholesterin per 100 c.c. before reaching the saturation point. These three cholesterinized extracts according to our preliminary experiments, approach one another in sensitiveness. Considerably more work will have to be done in this connection. We may find that to replace the lack of non-cholesterin lipoids in a given extract with cholesterin, may lead to difficulties. A number of extracts prepared from different heart muscles are now being tried out and the results with detailed method of standardization of antigen will be reported in a forthcoming paper. It is touched upon here in order to indicate to workers interested in this test, a clue by which one may overcome the variable elements which enter in the alcoholic extraction of different lots of beef hearts.

At present we recommend standardizing the alcoholic extract by means of an approximate color standard as indicated above and cholesterinizing it by adding 400 mgm. per 100 c.c. This amount of cholesterin approaches the saturation point at room temperature of most extracts and is giving good results in our routine work in this laboratory and, to our knowledge, in other laboratories where this test is employed as a regular procedure.

It is well to cholesterinize amounts of extract which will be likely to last for about a month or two only. Such extracts show a tendency to become slightly less sensitive on prolonged standing.

If a given extract is incapable of holding in solution 400 mgm. of cholesterin per 100 c.c. at room temperature, the mixture should be kept in the incubator in the dark and the tendency of the crystalization of the cholesterin will thus be avoided.

PROCEDURE I (ORIGINAL).

Principle: The mechanism of this procedure is believed to be as follows. The antigen consists of a highly concentrated solution of apparently specific lipoids. Approximately the smallest amount of salt solution (0.85 per cent. NaCl) is added to a given amount of antigen which will result in an opalescent mixture. This renders the mixture unstable with reference to precipitation as indicated by the fact that it will, in practically all cases, become

turbid on standing or in the cold. Since the mode of adding salt solution to antigen markedly affects the final mixture, a method is indicated for this purpose in which tubes of a given calibre are employed; the aim being to produce mixtures which, although clear, are on the verge of precipitation. The employment of a minimum amount of salt solution furthermore eliminates the inhibitory effect of excessive amounts of this solution on specific precipitation of serum and antigen. Assuming therefore that we are dealing with an unstable antigen salt-solution mixture and assuming further that the reacting substances of syphilitic serum are probably also relatively unstable, as indicated by the quantitative fluctuations of these substances during the course of the disease, one might expect that mixing these two unstable substances would result in precipitation.

Dilution of Antigen for Tests: The following method of diluting the antigen with salt solution is somewhat simpler than that described in the earlier communications:

1. The amount of antigen required for the tests is measured into an agglutination tube of about 0.8 cm. diameter.
2. Three times the amount of salt solution is added to a similar tube.
3. The saline is poured into the antigen tube with reasonable rapidity and the mixture is immediately poured back into the original antigen tube.
4. This mixture, which is opalescent and shows no signs of turbidity, is now ready for use, although there is no harm in further mixing back and forth.

The Test: Three-tenths c.c. of serum, previously inactivated for one-half hour at 56° C., is measured into a small tube and 0.05 c.c. of antigen-salt solution mixture is added to it, and shaken for about a minute or more. Known positive and negative sera form the controls. The tubes are observed for spontaneous reactions and the final results are read after overnight incubation at 37° C. Best results are obtained with sera that are clear and to which sheep cells (for removal of natural amboceptor) have not been added.

Increasing Sensitiveness of Test: If an antigen, after testing with a number of syphilitic sera, appears to lack sensitiveness, the following simple steps will help overcome this difficulty:
1. The salt solution is chilled by keeping it in the ice-box before

mixing with antigen. This renders the final antigen-salt solution mixture somewhat less stable than mixtures prepared with salt solution kept at room temperature. 2. Instead of mixing 3 parts of salt solution with 1 part of antigen, 2.5 parts of salt solution are mixed with 1 part of antigen. This increases the instability of the final mixture with reference to precipitation. We have not had occasion to use less than 2.5 parts of salt solution with 1 part of antigen in our work.

The important thing to keep in mind is that the antigen-salt solution mixture used in the tests must show no signs of turbidity. An antigen mixture showing even slight turbidity will be likely to give false weak reactions.

PROCEDURE II (MODIFIED)

Principle: The mechanism of this procedure is believed to be somewhat different from that of procedure I. Antigen and salt solution are mixed in such proportions that the major part of the lipoids is precipitated in a very fine state. The precipitate is obtained free from other elements by centrifugation. On resuspending this precipitate in salt solution, there results a milky but opalescent mixture. This mixture shows no trace of visible precipitate but undoubtedly consists of a suspension of lipoid particles in a very fine form, and when mixed with serum is apparently capable of combining readily with the specific elements of the latter. (The application of this principle to other antigens will be discussed in forthcoming studies).

Dilution of Antigen for Tests:

1. A given amount of antigen (depending on number of tests) is measured into a small tube and an equal amount of salt solution is added to it either from a pipette or from another tube.

2. This is mixed and centrifuged for about five minutes—until the supernatant fluid is practically clear and a white precipitate is settled on the bottom of the tube.

3. The supernatant fluid is poured off and discarded and the amount replaced with salt solution. (*Ex.*: 0.5 c.c. antigen is mixed with 0.5 c.c. saline and centrifuged. Supernatant fluid is poured off and 1 c.c. saline added).

4. On mixing, the precipitate is redissolved in the salt solution,

forming a milky, opalescent mixture with no trace of a precipitate. This mixture is then ready for use.

The Test: Three-tenths c.c. of serum, previously inactivated for one-half hour at 56° C., is measured into a small tube and 0.05 c.c. of new antigen mixture added to it. The tube is shaken for about a minute. Practically all tubes will become slightly cloudy but those showing spontaneous reactions will show definite precipitations in clear serums. The final reading in this case also is taken after overnight incubation at 37° C.

THE READING OF RESULTS

The results are read in accordance with the following scale:

1. One or more large clumps = ++++.
2. Large sized flocculi = +++.
3. Moderate sized flocculi or granules = ++.
4. Small flocculi or granules = +.
5. Very small flocculi or granules = ±.

It is recommended in reading the results that all tubes showing the presence of definite clumps or heavy precipitates in both procedures be first picked out and set aside in a special rack. These are the definitely positive reactions (++++ and +++) and can be read with very little difficulty. The remaining tests carried out with Procedure I are read as follows: 1. Slant the tube to such an extent that it is almost horizontal. This causes the fluid to spread into a thin layer. 2. Hold the slanted tube some inches above the level of the eyes. 3. Focus against some dark object such as the lower part of a window shade. 4. Observe whether the thin layer of fluid is entirely clear or has fine particles evenly distributed.

All remaining tubes of Procedure II receive one c.c. of salt solution each and rack is gently shaken and permitted to stand about ten minutes. The negative tests show opalescence while the positive tests show the presence of precipitates. With some antigens this procedure is unusually sensitive and doubtful (±) reactions may safely be considered negative. Salt solution may occasionally be used with advantage also in diluting some tests carried out with Procedure I.

The results of the two methods outlined check very closely. In isolated cases where there is disagreement, the average finding of the two methods is taken as the final result.

Although the final results are read after overnight incubation, it will be found that the strongly positive serums either react spontaneously after adding antigen or show the presence of definite precipitates after several hours incubation. From fifteen to seventeen hours is more than ample for incubation. Prolonged incubation beyond these hours is to be avoided. An element which will give false weak reactions particularly after prolonged incubation is the employment of tubes which will permit considerable evaporation of the serum during the incubation period. Agglutination tubes having an inner diameter of about 0.8 cm. will be found to give best results.

We have not found it necessary to employ sterile salt solution. Chemically clean but not sterile precautions are required in this test.

STATUS OF IMPROVED PROCEDURE.

The combination of the two steps outlined, together with proper negative and positive controls, forms in our experience a more dependable test than that originally described.

160 (2120)

Dilution of antigen for Wassermann test.

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In the previous communication, the author described a method for preparing antigen for the Kahn Precipitation Test and showed (Procedure II) that the sediment formed on mixing and centrifuging equal quantities of antigen and salt solution may be redissolved in salt solution and employed in the above test for syphilis. The question came up whether the same sediment taken up in salt solution may not be used as an antigen in the Wassermann test and the following experiments were carried out accordingly.