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A Dehydrated Bacterial Agglutination Antigen (Bang's Disease).*†

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This is a preliminary report on the use of a dehydrated bacterial antigen for agglutination testing. The studies reported are confined to *Bact. abortus* antigen used in the diagnosis of Bang's disease of cattle.

Preparations of bacterial agglutination antigens for use in macroscopic methods of agglutination testing for disease have universally been made by suspending the bacteria in salt solution. The early workers[1] in this field considered the salt solution as essential for the agglutination phenomenon. This conclusion is known now[2, 3] to be erroneous.

We are studying a method of agglutination testing in which a washed, dehydrated antigen preparation is mixed in measured quantities with fixed amounts of agglutination serum. The bacterial suspension, in distilled water, is evaporated to dryness in petri dishes in the incubator at 37.5°C. A film results which is transparent, amber colored and quite brittle. The antigen is kept in the dehydrated form until needed for use.

A satisfactory method of conveying the dehydrated antigen to the agglutinating serum in the necessary minute measured amounts

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1 Bordet, Jules, *Ann. Inst. Pasteur*, 1899, **13**, 225.

2 Donham, C. R., and Fitch, C. P., *J. Inf. Dis.*, 1933, **53**, 98.

3 Unpublished data.

has been effected by resuspending the antigen in distilled water. By suspending weighed quantities of the dehydrated antigen in definite amounts of water and pipetting this suspension, it is possible to measure precise amounts of the antigen onto a suitable glass plate. The water is again removed by evaporation before an electric fan. The antigen adheres to the glass plate.

Measured amounts of agglutinating serum are mixed with the dried antigen. This can be accomplished conveniently by stirring with an ordinary wooden toothpick. Macroscopic agglutination takes place rapidly, within one to several minutes, when the serum contains specific agglutinins.

It is possible to mix the serum and antigen in varying proportions and thus obtain a system of dilutions which gives agglutination titres comparable to those obtained by other methods of agglutination testing. We are not prepared to recommend definite proportions. Further studies are necessary. However, sufficient data are available to justify the following statements: The dilution system can be arranged so that the titres obtained with dehydrated antigen essentially agree with those obtained by the test-tube and rapid methods in testing most serums. There are, however, occasional serums which do not show agreement in titres when tested by the different methods.

So far the disagreement has always been in one direction, namely some serums cause agglutination of dehydrated antigen that do not cause it when tested with aqueous antigen preparations. Similarly some serums show much higher titres when tested with dehydrated antigen. Testing with dehydrated *Bact. abortus* antigen differentiates between some serums which have appeared to be identical serologically, when tested by the test-tube or rapid methods.

It remains to be shown whether the titres obtained with dehydrated antigen are more or less instructive than those obtained with aqueous preparations of the antigen.

We have accumulated a group of cattle that had, at the time of purchase, positive titres when tested with dehydrated antigen and either entirely negative or suspicious titres by the other methods. Some of these have terminated their pregnancies either by abortion or normal parturition and *Bact. abortus* has been isolated from them. Such animals have subsequently developed definitely positive agglutination titres when tested with aqueous preparations of the antigen. These serums showed positive agglutination titres when tested with dehydrated antigen earlier in the course of the disease than they did when tested with aqueous preparations of the antigen.

Other possible uses of dehydrated antigen which are being studied include: 1. Its use for preparing test fluids for the rapid and test-tube methods of agglutination testing, chiefly for standardizing the bacterial concentration and as a method of preservation over long periods of time. 2. Its use as a product for diagnosing Bang's disease by means of allergy.

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Localized Pain Accompanying Faradic Excitation of the Stomach and Duodenum.

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The following observations, hitherto unreported, were made during the course of experiments designed to test the reaction of the human gall bladder to faradic stimulation of the gastro-intestinal tract.[1]

Eleven medical students cooperated in this undertaking, several of them submitting to repeated experimentation. The device selected for stimulating the gut tract was a standard Rehfuss tube enclosing a copper wire soldered to the metal olive at the end of the tube. The other electrode consisted of a moist pad fastened to the arm of the subject. Through this circuit was sent an induction current that was almost intolerable when tested by the lips, but which was not unbearable when applied to the gut, causing sensations varying from barely perceptible, gnawing sensations, to heartburn and sharp colicky pain. With the aid of fluoroscope and barium meal, it was ascertained that when the olive was pushed against the wall of the stomach the current caused ring contraction of the gut and then increased peristalsis distal to that point. In each case the duration of the current was 10 seconds.

Figure 1 (left) records observations made upon one student on 4 different days. The circles indicate varying positions of the electrode in the gut (as determined by X-rays); the dots the site of the pain area on the abdominal wall. With the subject prone, excitation of the mid-pyloric stomach was localized at the lower middle or left epigastric regions; of the pyloric antrum, at the lower

[1] Boyden, E. A., *Anat. Rec.*, 1933, **55** (Suppl.), 8.