CASTELLANI'S ABSORPTION TEST.

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The difficulties which have appeared when carrying out this highly specific and valuable test on human sera during and after infection or mixed inoculations have been great. Various and instructive researches have since Castellani's original work been carried out in England, especially by Boycott, Bainbridge and O'Brien, Cummins and Cumming. Because of its utility and aid, not only in the classification of agglutinins, but in the determination of particular strains of a group, an attempt has been made to devise a simple, practical, and reliable method of carrying it out as part of the routine work of the clinical laboratory. The method to be described has been developed from tests made during eighteen months on the blood sera of some 300 bacteriologically diagnosed and on other cases of single or multiple infections, and on inoculated subjects. Comparative agglutination examinations were made on the slide by the method described in a previous paper (see Journal of the Royal Army Medical Corps, April, 1918) before and after the absorption test, to distinguish the action of one or more specific agglutinins from that of co- or hetero-agglutinins.

Harvey and Wood (Journal of the Royal Army Medical Corps, 1910) showed that an excess of micro-organisms other than Bacillus paratyphosus A removed or reduced the amount of detectable specific agglutinin for B. paratyphosus A present in the serum. I have confirmed and extended this observation by proving that in a similar manner supersaturation of a serum with other than the corresponding emulsions removes or reduces the detectable specific agglutinins for B. dysenteriae Shiga, Flexner-Hiss, and Micrococcus melitensis. I was therefore led to experiment with previously determined quantities of the factors employed in the test, and have endeavoured to evolve a method which would include in its technique the utilization of a practically defined amount of micro-organisms and a practically defined agglutinin content of a serum, and find that the following technique gives constant and reliable results.

Technique.—The quantity of micro-organisms employed is one drop (½ minim or ½ of a cubic centimetre) of an emulsion standardized as described in the paper on agglutination.

The dilution of serum employed is that which shows on the slide the onset of agglutination of an emulsion of micro-organisms at approximately one minute of time. [On the same principle a defined quantity of agglutinin in human serum for each emulsion could be tried by the selection of a particular dilution of serum from amongst those showing a positive finding in the agglutination test done by the examination of a series of
increasing dilutions for each emulsion by my three full minutes' test on
the slide or the old-established high-titre method.]

From three drops of this dilution one drop of the stock emulsion
corresponding to the micro-organism of infection (and which has caused
the development of the specific and any co- or hetero-agglutinins), removes
or sufficiently reduces all these agglutinins; while one drop of any other
emulsion that was agglutinated by reason of the presence of co- or hetero-
agglutinins developed during the infection, removes only the respective
c0- or hetero-agglutinin. After absorption by an emulsion, the serum
dilution is re-examined for agglutination on the slide with the fixed time
limit of three full minutes.

Very rarely a particular dilution of serum from a case shows the onset
of agglutination of two or three different bacterial emulsions at approxi-
mately one minute. It is, therefore, practically always necessary to put
up two or three series of sedimentation tubes, each series containing the
desired dilution of serum for one of the emulsions that is to be tested: and
at the same time each of the other agglutinated emulsions is tested in that
dilution.

An example will make clear the technique and the principles of the
method. In the initial time-governed agglutination test on the slide, the
serum of a patient is found to agglutinate three emulsions—B. dysenteriae
Shiga, B. paratyphosus A, and M. melitensis. To carry out the absorption
test on the lines described it is found that respective dilutions of the
serum are to be prepared and that B. dysenteriae Shiga begins agglu-
tinating at approximately one minute in a 1 in 6; B. paratyphosus A in a
1 in 160; and M. melitensis in a 1 in 10 dilution of the serum, then three
series of the sedimentation tubes must be put up. In each tube of the
first series three drops of the serum dilution 1 in 3 is placed; into each of
the second series three drops of the serum dilution 1 in 80; into each of
the third series, three drops of the serum dilution 1 in 5; into one of the
tubes of each series is placed respectively one drop of emulsion of
B. dysenteriae Shiga, B. paratyphosus A, and M. melitensis. To each tube
is then added two drops of sterile formalized saline. That the drop con-
taining the emulsion may contain as nearly as may be practicable the
quantity of saline necessary to make the dilution in the absorption tube
equal to that in the control tube, the pipette, during the dropping of the
emulsion is held at an angle of approximately 67°.

A few drops of each serum dilution corresponding to that in each series
are prepared in three other tubes, and kept each alongside the absorption
test tubes of the series, so that each dilution can be later tested to control
and compare its agglutinability with that of the absorbed serum dilutions
in the other tubes of the same series.

The tubes when ready are gently shaken and capped with plasticine to
prevent evaporation. They are left for twenty to twenty-four hours at
laboratory temperature, or seven to eight hours at 37° C., or for two hours
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at 55° C. The tubes containing emulsion were gently shaken once during these intervals, when left at laboratory or 37° C. temperature.

The tubes are then centrifuged, and a drop of the clear supernatant fluid of each tube of the first series is tested on the slide with a loopful of emulsion of B. dysenteriae Shiga, a drop from each tube of the second series with a loopful of B. paratyphosus A and a drop from each tube of the third series with a loopful of M. melitensis.

Taking the first series, if there is specific agglutinin present in the serum for B. dysenteriae Shiga the emulsion of B. dysenteriae Shiga will be agglutinated at approximately one minute in the fluid from the tube containing B. paratyphosus A, and from the tube containing M. melitensis. If, however, the agglutination of B. dysenteriae Shiga is due to a hetero-agglutinin action developed in a M. melitensis infection, the B. dysenteriae Shiga will be agglutinated only in the fluid from B. paratyphosus A as the emulsion of M. melitensis will remove the hetero-agglutinin for B. dysenteriae Shiga, and that of B. dysenteriae Shiga will remove the agglutinin for itself.

Observations are then made on the second series of tubes to determine the specificity or otherwise of the agglutinin present in the serum for B. paratyphosus A, and on the third series to determine that for M. melitensis.

Simpler tests are more often called for, and the technique modified accordingly.

There is the possibility of pathogenic micro-organisms other than those of the seven groups represented and employed in the initial agglutination tests giving rise to a co- or hetero-agglutinin which might cause agglutination of one of the emulsions employed, but up to the present this has not occurred.

In rare dysentery cases strains approximating in biological characters to typical strains of the B. dysenteriae Flexner-Hiss group have been isolated and found to be agglutinated by the patient's serum and not by specific Flexner-Hiss animal anti-serum. Of these particular strains each absorbed the agglutinin from the patient's serum but did not absorb the agglutinin from the Flexner-Hiss specific animal anti-serum, and the converse held when employing a typical agglutinating strain of B. Flexner-Hiss. This result appears of value in view of the work upon similar strains of B. dysenteriae obtained both in France and in the Mediterranean.

A specific agglutinin may be distinguished from a co-agglutinin in infections with one of the enterica groups; and the presence of specific agglutinins to more than one group may be detected. Again, I was able by this test to distinguish the specific agglutinin for B. aertrycke, developed in nine bacteriologically proven cases, from the agglutinin for a B. paratyphosus B strain due to previous T.A.B. inoculations. An emulsion of B. Gaertner did not remove either agglutinin.

This method also showed that the specific agglutinin for B. typhosus
developed in serum in response to anti-typhoid vaccine inoculation might be temporarily increased by inoculation with anti-paratyphoid vaccine.

In the experiments carried out in developing this method it was found that specific agglutinins for $B.\text{typhosus}$ or $B.\text{paratyphosus}B$ were not reduced by supersaturation with any other emulsion tested than their respective emulsion. This fact permits the use of the supersaturation absorption test to determine if specific agglutinin for $B.\text{typhosus}$ or $B.\text{paratyphosus}B$ is present in a given serum.\(^1\)

**Immediate Absorption Test.**—Occasionally but rarely it is noted in carrying out the initial slide agglutination test that two or three emulsions are agglutinated in such a manner as to indicate that an absorption test may be tentatively carried out on the slide. Useful information may be gained where serological tests are urgently called for.

From my limited experience of this immediate test and from the tentative work, it appears that one should use a dilution of serum that shows the onset of the complete agglutination of the emulsion to be tested at any time within the range of thirty to ninety seconds. The agglutinin content of the serum is not then too high for its necessarily sufficient absorption.

To give an example. In a $M.\text{melitensis}$ infection it was found by the initial slide agglutination test on the serum that $V.\text{cholerae}$ and $M.\text{melitensis}$ emulsions were agglutinated immediately into rather marked flocculi, leaving the serum dilution clear. The highest dilution producing such complete agglutination within thirty to ninety seconds of both emulsions was determined, and in the drops thereof, each containing the agglutinated emulsion, the platinum loopful (two millimetres in diameter) of distilled water, to compensate for evaporation, was added. A similar loopful of the $M.\text{melitensis}$ emulsion was placed in the agglutinated $V.\text{cholerae}$ containing drop, and vice versa. On the re-examination made with the three full minutes time limit the $M.\text{melitensis}$ was agglutinated and the $V.\text{cholerae}$ not agglutinated; that is to say, $V.\text{cholerae}$ was incapable of taking out the specific agglutinin for $M.\text{melitensis}$, while the $M.\text{melitensis}$ removed the hetero-agglutinin for $V.\text{cholerae}$.

By a similar procedure co-agglutinins formed for $B.\text{dysenteriae}$ Flexner-Hiss in $B.\text{dysenteriae}$ Shiga infections, or co-agglutinin formed in $B.\text{typhosus}$ infection (in non-paratyphoid inoculated patients) may be distinguished from specific agglutinins, etc.

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\(^1\)Since writing the above I have employed this test during an outbreak of plague with most satisfactory results.