ALPHA-AGGLUTININ FOR COLIFORM BACILLI IN DIAGNOSTIC AGGLUTINATING SERA.

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The report by Stamp and Stone (1944) of the occurrence of an antigen common to several strains of coliform bacilli, which they designate alpha-antigen, and of the finding that agglutinin for these bacilli may be contained in the sera of certain rabbits, has been confirmed by Fairbrother (1944), who demonstrated the agglutinin in a proportion of the diagnostic sera issued by the Emergency Vaccine Laboratory. He suggested that only rabbits whose sera were free of this agglutinin should be used in the production of diagnostic sera.

Strains of some of these non-lactose-fermenting or late-lactose-fermenting bacilli may grow as colourless colonies on desoxycholate-citrate agar (Pulvertaft, 1943), and since this and other selective media may be more heavily inoculated than was the case with the older indicator media like MacConkey's and Litmus Lactose Bile Salt Agar, such strains are now more commonly encountered. Slide agglutination of alpha-containing strains is rapid and striking, and only a low titre of agglutinin is required. For example, a serum prepared against Bact. dysenteriae Sonne, which was found to contain alpha-agglutinin to a titre of 1 : 25, gave more rapid and striking slide agglutination of an alpha-paracolon suspension than of the Sonne smooth phase suspension against which it had been prepared and for which the titre was 1 : 125.

In the water-bath in Dreyer tubes it was found that agglutination of alpha-paracolon strains was rapid and intermediate in speed and in size of floccules between H and O agglutinations. Slide agglutination was readily given by sera of which the titre after four hours at 50° C. was 1 : 20, and weakly by sera with a titre of 1 : 10. Titres after four hours at 37° C. were usually slightly lower than those seen after four hours at 50° C., but overnight incubation at 37° C. gave the same results as after four hours at 50° C.

Alpha-agglutinin in Rabbit Sera.—Sera from 200 rabbits were examined, covering the years 1937-44. In each case the last available bleed was used, except for a few rabbits from which bleeds were not available, when test bleeds were made. Of these sera, 36 or 18 per cent had a titre of 1 : 20 or over, i.e. they would give ready slide agglutination of an alpha-paracolon culture. The titres of these sera, to which had been added an equal volume of glycerin as a preservative, were as follows. One serum reacted in dilutions up to 1 : 1,280, 2 up to 640, 4 to 320, 16 to 160, 7 to 80, 6 to 40 and a further 12 to 1 : 20. It is apparent that with some sera the titre given by an alpha-containing strain of coliform bacillus might equal or even exceed the titre for the suspension it was intended to agglutinate.

In order to determine whether diagnostic sera could be prepared exclusively in rabbits whose sera were free of alpha-agglutinin, further investigations were carried out. It was found that agglutinin appeared haphazard among the various breeds of rabbit used. Alpha-positive rabbits were distributed fairly evenly throughout the seven-year period studied. The appearance of alpha-agglutinin was not related to the type of inoculum, nor could it be related to periods of ill-health in any rabbit. A long series of sera was available from some rabbits and these were titrated for alpha-agglutinins, commencing at a dilution of 1 : 12₄. Some representative results are given.
Rabbit 16/42. *Flexner II (W).*—Bleed No. 1 (4.6.42) = 0, No. 2 = 0, No. 3 = 12½ trace, No. 7 = 100 trace, No. 8 = 50, No. 10 = 25 trace, No. 11 = 25 trace, No. 13 = 0, No. 14 (22.6.44) = 0.

Rabbit 36/41. *Flexner III (Z).*—Bleed No. 2 (28.11.41) = 25 trace, No. 8 = 12½ trace, Nos. 9, 10, 12 = 0, No. 15 = 25 trace, No. 16 = 12½ trace, No. 18 = 100, No. 20 = 200, No. 21 = 400, No. 22 = 200, No. 23 = 200 trace, No. 24 (4.6.43) = 100.

Rabbit 18/41. *Flexner V (P.119).*—Bleed No. 2 (7.8.41) = 0, Nos. 9, 10, 11, 13 = 0, No. 14 = 12½ trace, No. 18 = 12½ trace, No. 20 = 0, No. 21 = 12½ trace, No. 24 (20.6.44) = 0.

The next two rabbits for which results are quoted received injections of the same suspensions on the same days over a period of thirteen months.

Rabbit 22/43. *Para C-O.*—Bleed No. 1 (11.6.43) = 0, No. 2 = 12½ trace, No. 3 = 0, No. 4 = 12½ trace, No. 5 (6.7.44) = 50.

Rabbit 23/43. *Para C-O.*—Bleed No. 1 (11.6.43) = 0, No. 2 = 25, No. 3 = 400, No. 4 = 800 trace, No. 5 (6.7.44) = 800.

These results show that preliminary selection of alpha-free rabbits does not guarantee the production of sera free of the agglutinin, and that these agglutinins may appear and disappear at any time during the life of a rabbit. Fortunately it has been found extremely easy to absorb alpha-agglutinins with small doses of a suitable suspension. The dosage of a absorbing suspension is comparable to that used in absorbing an unwanted Salmonella H factor, and is very much less than the dose required for a somatic factor like Salmonella O or Flexner group antibody. For example, a serum having a titre for alpha-suspensions of 1 : 1,280 was completely absorbed by a dose of $2 \times 10^8$ per c.c. A Salmonella H serum with a group titre of 1 : 1,280 required a dose of $1 \times 10^6$ per c.c., while absorption of the group agglutinin from a Flexner type serum of which the group titre was 1 : 640 required $2 \times 10^6$ per c.c., or ten times that of the alpha-suspension. Even if two or three times the dose of alpha-suspension required for total absorption is added to a serum, the whole suspension undergoes coarse flocculation, and after standing overnight the clear serum can be decanted, leaving only a small deposit which can be centrifuged to recover the remaining serum, or the whole batch may be passed through a clarifying filter.

As a matter of future policy, the selection of rabbits whose sera are free of alpha-agglutinin is regarded as a useless procedure when it is intended to use the same rabbits for serum production over a period of years. The titration and absorption of individual bleeds is too laborious and time-consuming. As each pool of serum is prepared for issue, it will be tested by slide agglutination and in the water-bath from a dilution of 1 : 10 with a suitable suspension. The absorbing dose required for sera found to contain the agglutinin can be roughly calculated from the titre, allowing 50 per cent excess to ensure complete absorption. For the suspension now in use the dose is $4 \times 10^8$ for each c.c. of serum to be absorbed if the alpha-titre is 100, higher or lower titres requiring proportionate doses. Although this dose is in excess of that actually needed ($2.5 \times 10^8$) the suspension will undergo complete flocculation.

**Summary.**

1. Agglutinin for alpha-antigen, which is common to several strains of coliform bacilli, is found in the sera of many rabbits, and its presence cannot be related to a particular breed of rabbit, to the inoculum used or to periods of ill-health in the rabbits.

2. This agglutinin gives rise to striking slide agglutination of paracolon bacilli containing the antigen, even when the titre is low (1 : 20), and in a few cases its titre may be as high as that for the strain against which the serum has been prepared. It may therefore give rise to diagnostic errors. Since these paracolon bacilli are able to grow on some of the selective media, they appear to be more commonly encountered now than when indicator media only were available. These results emphasize that the commonly-used procedure, to which objections can also be raised on other grounds (Francis, 1944) of picking colonies directly from a primary plate for identification by slide agglutinations, not otherwise confirmed, is liable to give fallacious results in the bacteriology of feces.
(3) The agglutinin may appear at any time during the life of a rabbit and may subsequently disappear. Selection of rabbits free of the agglutinin does not guarantee the subsequent production of alpha-negative sera.

(4) It is desirable that sera prepared for use against alimentary pathogens should be tested for the presence of the agglutinin, which should, if present, be absorbed as a routine. This is to be done with all such sera produced at the Emergency Vaccine Laboratory in future.

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REFERENCES.

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