THE AGGLUTINABILITY OF BLOOD AND AGAR STRAINS OF TYPHOID BACILLI.

By CARROLL G. BULL, M.D., and IDA W. PRITCHETT.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 24, 1916.)

Gay and Claypole state that two transplantations on 10 per cent rabbit blood agar rendered typhoid bacilli non-agglutinable in an immune serum produced with plain agar cultures.[1] They further state that an immune serum obtained by means of blood agar cultures of the bacilli agglutinated equally well both blood and agar strains and that this serum agglutinated freshly isolated strains which were not agglutinated by an ordinary immune serum, thus rendering an early identification of the strains possible. They hold also that the inagglutinability of recently isolated strains is not due to some property acquired in the living animal, as a simple subculture for two generations on blood agar produces similar effects.

With a view of producing a general agglutinating serum according to this method, we first attempted to render our laboratory strains inagglutinable by growing them on 10 per cent rabbit blood agar. Two generations on blood agar failed to change the agglutinability of these strains. Cultivation on blood was continued and the agglutinability of the strains was tested after each two or three transfers. A detectable difference between the blood and agar strains did not arise, even after twenty-five generations. Since it was possible that our results were due to the particular culture with which we were working, fifty-five other cultures have been collected and subjected to a similar study.

The Strains.

We are indebted to Dr. Homer F. Swift of the Presbyterian Hospital, Dr. F. B. Humphreys of the German Hospital, and Dr. L. M.

[1] Gay, F. P., and Claypole, E. J., *Arch. Int. Med.*, 1913, xii, 621.

Famulener of St. Luke's Hospital for a number of the strains used in this work. We are especially indebted to Dr. Famulener for sending us twenty strains as they were isolated from patients. There were in all twenty-five freshly isolated strains. These were transferred to blood agar as soon as they had been identified. The remainder of the strains had been under artificial cultivation for a number of months.

Technique.

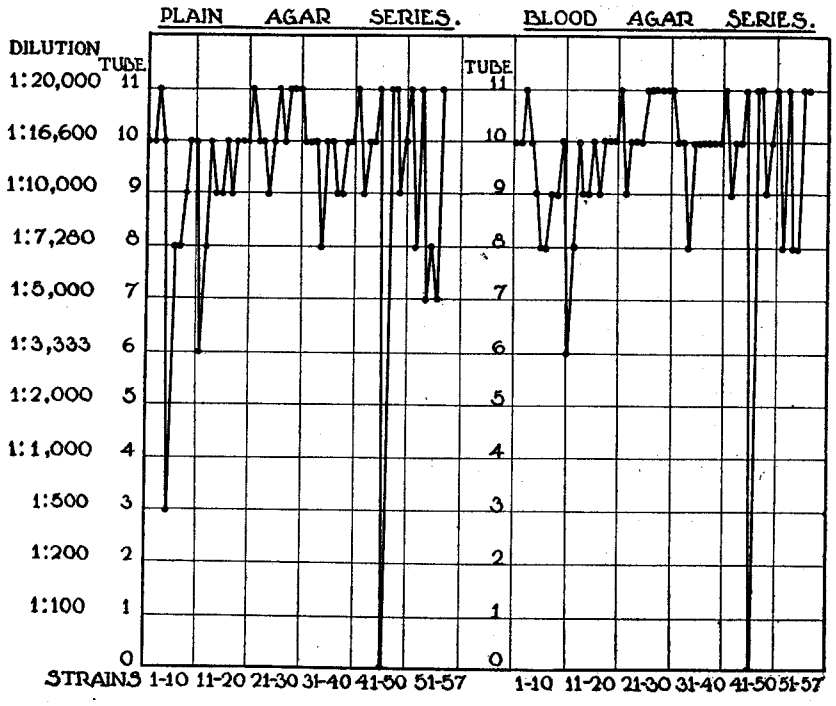
The serum used to test the agglutinability of the various strains was obtained by immunizing rabbits with a typical typhoid bacillus grown on plain agar. It agglutinated the homologous strain in a dilution of 1:20,000. The following procedure was used in making all the tests.

Graded dilutions of the serum were made with physiological salt solution. The dilutions ranged from 1:100 to 1:20,000. 1 cc. of the different dilutions was transferred to small test-tubes and one drop of bacillary emulsion was added to each, and also to a salt solution control. The emulsions of the bacilli were made by adding from 4 to 5 cc. of salt solution to 24 hour cultures on blood agar and plain agar slants, respectively. The blood and agar cultures of each strain were always tested on the same day and with the same serum dilutions. A special effort was made to have the emulsions from the corresponding blood and agar cultures of the same thickness. The tests were incubated for 2 hours at 37° C. and allowed to stand at room temperature for 2 hours before the results were read. A second reading was made the following morning. There was no difference in corresponding blood and agar cultures at the two readings. The controls were never agglutinated.

Results of the Agglutination Tests.

Fifty-seven strains were grown on blood agar for twenty-five generations and tested for agglutinability at varied intervals according to the technique just described. Only slight differences between the corresponding blood and agar strains were detected. The blood cultures were often more agglutinable than the agar cultures. There

was, however, considerable variation of the individual strains (both blood and agar cultures), and one inagglutinable strain was encountered. This strain was irregular in other particulars and will be considered more in detail below. The results are presented graphically in Text-figs. 1 and 2.

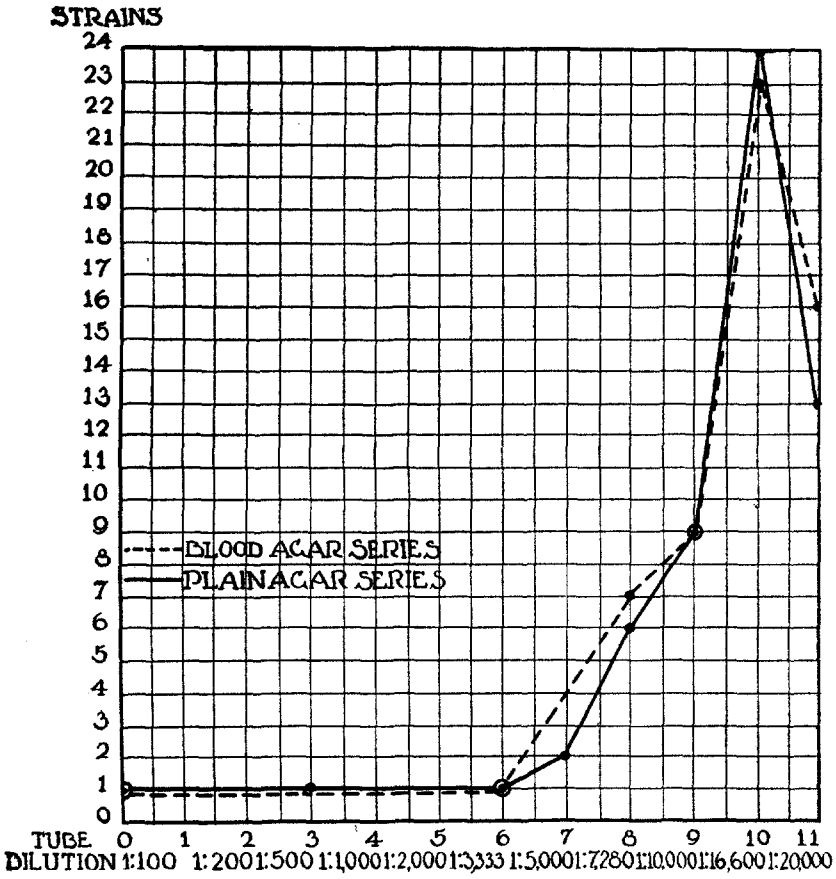


TEXT-FIG. 1. This figure shows the individual variations among the fifty-seven different strains. The dots on the abscissæ represent the individual strains from 1 to 57, in the order in which they were tested. The highest dilutions in which the strains agglutinated are represented by the ordinates.

The Irregular Strain.

This strain was brought to us by Dr. Amoss from the laboratory of Hygiene, Vermont State Board of Health. It had been recently isolated from the stool of a clinical case of typhoid and was typical according to the cultural methods ordinarily used to identify typhoid bacilli. We had not observed any irregularities in the strain until

it was found to be non-agglutinable with the immune serum used in the routine agglutination tests already described.



TEXT-FIG. 2. In this figure composite curves of the agglutinability of the blood and agar strains are given. The dilutions are represented by the abscissæ and the number of strains agglutinating in the different dilutions is represented by the ordinates.

Agglutinability.—In the routine tests the Vermont strain failed to agglutinate in a 1:100 dilution of the serum that agglutinated a number of other strains in a 1:20,000 dilution. It was later found that a dilution containing 50 per cent serum would not agglutinate

either the blood or agar cultures of this strain. It was agglutinable by fresh normal rabbit serum in a dilution of 1:5. It agglutinated in the circulation of normal rabbits as readily as other strains. A homologous serum produced by immunizing a rabbit with agar cultures agglutinated both the agar and blood cultures actively. This serum agglutinated typical strains of bacilli but not in as high dilutions as the homologous strains.

It is of interest to note the differences exhibited by the natural and acquired agglutinins. An immune serum that had stood on ice for several days failed to agglutinate the Vermont strain in 50 per cent serum, while normal rabbit serum agglutinated it in a 1:5 dilution. This indicates a lack of specificity on the part of natural agglutinins and shows further that natural agglutinins disappear spontaneously as the serum ages. The same normal serum agglutinated other strains as high as 1:20, hence the Vermont strain was naturally of lower agglutinability.

Cultural Characteristics.—In Hiss' carbohydrate serum-water media the following reactions occurred: Dextrose gave acid, no gas; levulose, acid, no gas; dextrin, acid, no gas; mannite, maltose, galactose, lactose, and saccharose gave no acid, no gas. Litmus milk gave slight acidity. Gelatin was not liquefied. Growth on potato media was typical. It produced indol as readily and as abundantly as *Bacillus coli*.

It is seen that the fermentation reactions of the Vermont strain are irregular, no acid being produced in galactose, mannite, or maltose. Control tests were run with other strains and typical reactions occurred in all the media.

The most radical discrepancy is the production of indol. This reaction has been accepted as a highly dependable test in differentiating typhoid bacilli from other closely related organisms ever since it was first used for this purpose by Kitasato.[2] Andrejew claims that a number of strains with which he worked produced indol.[3] Telle and Huber have more recently tested a number of strains and they obtained only negative results.[4] These authors believe that

[2] Kitasato, S., *Z. Hyg.*, 1889, vii, 515.

[3] Andrejew, P., *Arb. k. Gsndhtsamte.*, 1910, xxxiii, 363.

[4] Telle, H., and Huber, E., *Centr. Bakteriol., 1st Abt., Orig.*, 1911, lviii, 70.

Andrejew's results were due to faulty technique. All text-books on bacteriology teach that typhoid bacilli seldom or never produce even a trace of indol when tested by a standard technique. Therefore, the production of indol by supposed typhoid bacilli must be considered as a radical irregularity and their identity as seriously questioned.

Protection Tests.—Rabbits that have been immunized with typhoid bacilli are highly resistant to intoxication with this organism. They withstand, as a rule, from thirty to forty lethal doses of the living bacilli. The Vermont strain was highly toxic for rabbits and we decided, therefore, to test the resistance of rabbits which had been immunized with a typical strain for this strain. It was found that the rabbits would withstand from fifty to seventy-five lethal doses of the irregular strain.

The resistance of the typhoid immune rabbits to intoxication by the irregular strain either proves the typhoidal nature of the strain or indicates a marked non-specificity of the toxic substances derived from such organisms. However, the protection tests combined with cross-agglutination seem to establish the Vermont strain as a true typhoid bacillus.

SUMMARY.

Cultivation on 10 per cent rabbit blood agar did not affect the agglutinability of fifty-seven strains of typhoid bacilli.

The authors were unable to confirm the observations of Gay and Claypole on the variation in agglutinability caused by cultivating the typhoid bacillus on blood agar.

A typhoid bacillus showing irregularity in fermentation, agglutination, and indol production is described.