3678

Antigens in Culture Filtrates of Bacterium Enteritidis.*

SARA E. BRANHAM.

From the Department of Hygiene and Bacteriology, the University of Chicago.

In earlier experiments[1] it was found that Berkefeld filtrates of synthetic medium cultures of *B. enteritidis* were good antigens, stimulating the production of agglutinins, precipitins, complement-fixing antibodies, and possibly antitoxins when injected into rabbits. When these filtrates were concentrated by evaporation *in vacuo*, the products obtained were better antigens than suspensions of *B. enteritidis*. Protein could not be demonstrated in this material obtained by evaporation, but when the salts were dialyzed away, and the contents of the dialyzing sac were also concentrated *in vacuo*, the non-dialyzable residue gave very faintly positive reactions for tryptophane and histidine, and a faintly positive ninhydrin reaction. Our interest was roused in the potent antigenic substances evidently present in these bacterial culture filtrates, and a study of their nature was undertaken.

Cultures of *B. enteritidis* in the synthetic medium previously described[1] were incubated for 7 to 10 days, and then filtered through Berkefeld N filters. The clear filtrates were then evaporated to dryness *in vacuo* at a temperature not exceeding 40° C. The residue, having the consistency and appearance of brown sugar, was dialyzed in a special adaptation of the apparatus devised by Hanke and Koessler[2] until it was free from demonstrable salts.

The non-dialyzable material within the parchment bag was easily separated into three crude fractions. One of these consisted of a small quantity of fine white powder which apparently contained a considerable amount of carbohydrate. This fraction has already been briefly described,[3] and further studies are being made upon it. A second fraction was a brownish water-soluble material which, although it gave none of the usual protein reactions, probably contained some protein, since it had a nitrogen content of 6 to 7 per cent. The third fraction was brownish in color, was water-insoluble, and gave a good biuret, as well as positive vanillin and Millon tests, but negative diazo and ninhydrin reactions. This fraction contained about 9 per cent of nitrogen. The nitrogen determinations were made with the micro-Kjeldahl apparatus and with the

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

technique described by Koch.[4] Calculations of total nitrogen were made on an ash free basis.

From 100 liters of the culture filtrates, about 2 grams of the water-soluble fraction, and about 1 gram of the water-insoluble fraction have been obtained.

The toxic principle previously described as present in culture filtrates of *B. enteritidis*[5] is in the water-insoluble fraction. Solutions of this material, made in N/10 NaOH and subsequently neutralized with HCl, when injected intravenously into rabbits, produced all of the symptoms described in this earlier paper. Different lots of the material varied greatly in toxicity, just as the strength of the poison varied in different filtrates of the same strain of *B. enteritidis*. One sample was so potent that 0.1 mg. killed a rabbit weighing 2 kilograms within 2 hours; other samples required much larger doses; some produced only transient dyspnea and diarrhea with the amounts given.

These two protein-containing fractions are good antigens. Rabbits were immunized by 3 series of 3 injections given intravenously at 48 hour intervals, with a week's rest after each series. The total amounts received by the animals varied from 15 to 25 milligrams.

The sera obtained from the rabbits immunized with the water-soluble fraction agglutinated *B. enteritidis* in dilutions of 1 to 4000. They also agglutinated *B. paratyphosum B* (Schotmüller type) and *B. dysenteriae* (Shiga type) in dilutions of 1 to 400, and *B. coli* 1 to 40.

Sera obtained from the rabbits immunized with the water-insoluble fraction agglutinated *B. enteritidis* in dilutions of about 1 to 1000. They agglutinated *B. typhosum* (Rawlings) and *B. dysenteria* (Shiga) completely in dilutions of 1 to 600, *B. paratyphosum B* (Schotmüller type) and *B. suipestifer* in dilutions of 1 to 300, and *B. coli* in 1 to 100. *B. paratyphosum A*, *B paratyphosum B* (Aertrycke type), and *B. dysenteriae* (Flexner) were not agglutinated by any of these antisera, which were prepared with materials obtained from a single strain of *B. enteritidis*.

It would seem from these agglutination tests that these two protein-containing fractions do not possess the strict specificity of the carbohydrate-containing material. This group relation is also indicated in precipitin tests, in which varying dilutions of saline solutions of these two fractions were tested for reactivity with anti-sera prepared by immunization of rabbits with the original unconcentrated filtrates, with *B. enteritidis*, *B. paratyphosum A*, *B. paratyphosum B* (Aertrycke type), *B. paratyphosum B* (Schotmüller type), *B. suipestifer*, *B. typhosum*, and *B. dysenteriae* (Shiga).

Positive reactions of varying intensity were obtained with a number of these sera, although they were in no case so pronounced as with the antisera for *B. enteritidis* or its culture filtrates.

Although both of these fractions apparently possess elements in common with other members of the colon-typhoid group, they seem to be antigenically distinct from each other; *i. e.*, antisera obtained with the water-soluble fraction was precipitated by the water-soluble fractions in relatively high dilutions, but by the water-insoluble fraction was very slightly precipitated in dilutions of 1 to 10. Since these crude fractions have not been purified, it would be surprising if some cross reactions did not occur.

The relation of the antigenic activity of culture filtrates to the proteins contained in these crude fractions, and the relation of these proteins to the colon-typhoid group as a whole, cannot be determined until the materials are purified.

This is a preliminary report.

[1] Branham, S. E., and Humphreys, E. M., *J. Infect. Dis.*, 1927, xl, 516.
[2] Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1925, lxvi, 495.
[3] Branham, S. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 349.
[4] Koch, F. C., *J. Lab. and Clin. Med.*, 1926, xi, 774.
[5] Branham, S. E., *J. Infect. Dis.*, 1925, xxxvii, 291.

3679

Comparison of Electromotive Effect of Concentration on Tissues, Proteins and Other Substances.

R. BEUTNER, P. O. HUTH AND C. K. BUSH.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville.

Previous work in this laboratory[1] led us to conclude that proteins cannot be the essential cause of animal electricity. We then investigated the relation of salt concentration to electromotive forces which is characteristic for tissues. This seemed particularly important in relation to the recent work of Mond.[2]

Uninjured green plants show the greatest electromotive effect of the concentration, and exhibit also a striking regularity.[3] With animal organs, such as excised muscle, etc., this effect is much smaller and variable owing to unknown conditions, *but it is almost always in the same direction, viz., the dilute solution is on the positive side.* Mond,[2] working in Höber's laboratory, has tried to match