the tests. In general, complement fixation tests like the precipitin reactions, showed differences between the various preparations of Bence-Jones protein, a cross-reaction between human serum and the salted-out specimens of non-crystallizable Bence-Jones protein, and a complete difference between the crystalline Bence-Jones protein and blood-serum.

Anaphylactic Reactions.—It was difficult to sensitize guinea pigs to the crystalline Bence-Jones protein, though not to the other preparations, indicating again an antigenic difference in that respect. Guinea pigs were sensitized actively by the intravenous injection of 0.25 c.c. of a 6 per cent. solution of the various Bence-Jones proteins, and by 0.25 c.c. of human serum. Some animals were passively sensitized by the intraperitoneal injection of the antiserum to the crystalline preparation. Three weeks after the first injection of protein or human serum in the series of actively sensitized animals, these guinea pigs were tested in two ways for specific sensitivity. The reaction of the animal as a whole was used when the intoxicating dose was given intravenously or intraperitoneally, and the method of Schultz and Dale was used with the uterine horns of the guinea pigs to provide graphic records of the experiments. These reactions also demonstrated (1) differences between the various Bence-Jones proteins, (2) a mixture of human serum proteins and Bence-Jones proteins in the preparations made in the attempt to salt-out Bence-Jones protein from the urine, and (3) complete difference between the crystalline Bence-Jones protein and human serum. (Demonstration of charts of precipitin and anaphylactic reactions.)

112 (1694)

On the influence of tissue enzymes on the bacteriophage principle.

By ANN G. KUTTNER.

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

I have previously reported before this Society the isolation of a lytic principle active against typhoid and dysentery bacilli obtained by the d'Hérelle technique from the filtrate of a stool from

a typhoid convalescent. The fact that the lytic agent could be transmitted apparently indefinitely in series and that it was only active against vigorously growing bacteria, suggested that the lytic agent might be derived from the bacterial cell itself. My next step was to try to produce a lytic principle from the typhoid bacilli without any interaction with the living animal body.

I proceeded on a theory first suggested by d'Hérelle, but discarded by him, namely, that the so-called phenomenon of d'Hérelle might be due either to an activation of the natural autolysin present in all bacteria, or to the removal of autolysin-inhibiting substance. Once this natural autolysin was liberated, it could in turn liberate an active autolysin from the next generation of bacteria and so on indefinitely.

It seemed possible from the work of Twort and other observers, such as Cantacuzène and Marie, and from the more recent papers of Turro, that tissue extracts might play a part in starting the activities of the autolysin.

I want to report briefly on some preliminary results I have obtained by the action of tissue extracts on typhoid bacilli. Up to the present time, I have obtained lysis of typhoid bacilli, transmittable in series by the action of extracts of two different tissues, namely: small intestine and liver. Both tissues were derived from guinea pigs. In the case of the small intestine, three small intestines from normal guinea pigs were pooled, washed and minced. Without drying the tissue was divided and extracted in different strengths of glycerine. After 11 days extraction in 50 per cent. glycerine at 37° C. some of the supernatant fluid was centrifuged and filtered through a Berkfeld. The addition of a small amount of this filtrate to a young turbid typhoid culture produced a slight amount of clearing as compared with the control. A loop from this tube was plated and then heated at 55° C. The plate showed regular and irregular colonies. The broth fishings of the irregular colonies carry the lytic principle and typhoid bacilli can be dissolved in series starting with a broth fishing of one of these irregular colonies. It has also been possible to transmit the lytic principle from the first tube after heating to kill the resistant typhoid bacilli, and I have obtained lysis of typhoid bacilli in seventh generation removed from the tissue

extract. Small intestine extracted with 25 per cent. glycerine has given similar results. Glycerine extracts of the large intestine and of muscle tissue have so far given negative results.

I prepared the liver extract according to the method used by Turro in preparing tissue extracts. Turro has reported that extracts of leucocytes, muscle tissue, kidneys, pancreas, thyroid, etc., digest bacterial protein. He has worked particularly with anthrax bacilli, but also with cholera and typhoid. He does not in any of the papers that he has published up to this time, connect his results with the phenomenon of d'Hérelle, and does not show that the dissolved bacteria can dissolve new cultures. He states that no special ferment derived exclusively from polynuclear leucocytes is necessary to digest bacteria, but that all tissue cells probably contain such ferments.

The liver extract was prepared in the following way: The liver from a normal guinea pig was minced, shaken up with acetone, dried in vacuo and pulverized. To approximately one gram of liver powder, 20 c.c. of salt were added. To one tube, 40 drops of chloroform were then added and to the other, a small amount of sodium fluoride. The tubes were placed in the incubator for 14 hours. The tube with chloroform was sterile, the tube to which the sodium fluoride was added, was contaminated, both were centrifuged and the latter was filtered through a Berkfeld.

Both these liver extracts dissolve cultures of typhoid bacill and the lytic principle can be transmitted in series from the dissolved culture. The 6th generation from the liver extract has now been reached and the lytic action has increased both in the degree of clearing and in the rapidity of the action. Cultures are not sterilized completely and the two types of colonies, one, the bearer of lytic principle, develop on plating. Control experiments to determine whether it is the action of the glycerine on the bacteria that produce the lysis have been negative. Similar control experiments with chloroform and sodium fluoride have up to the present time not produced a transmittable lytic principle.

These tissue extracts do not appear to be specific, but the range of their action has not yet been determined. Both the intestinal extract and the liver extract are active against several

different strains of typhoid so that the results cannot be attributed to the idiosyncrasies of one strain. The strains have been plated out repeatedly to see if irregular colonies carrying the lytic principle could be obtained from the normal culture, but up to the present time no lytic principle has been isolated without the interaction of tissue enzymes with the typhoid bacilli.

113 (1695)

Growth-determining substances in bacteriological culture media.

By J. HOWARD MUELLER.

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

Some months ago a report was made of a series of experiments based on the observation that, while a peptone-free meat infusion broth would produce abundant growth of hemolytic streptococci, short boiling with charcoal removed this property entirely. The addition of commercial peptone or of a sulphuric acid hydrolysate of certain proteins, such as casein or meat, reactivated the charcoal-treated infusion and heavy cultures of streptococci could be obtained on the mixture, while neither one alone gave the slightest trace of growth. It was shown that the activating material in the protein hydrolysate was precipitated by mercuric sulphate, and that it had not been possible to identify it with any of the amino acids known to be precipitated by this reagent either alone or in combination. It is the purpose of the present communication to describe the further purification of this activating material.

Much of the work has been done using a commercial preparation called "aminoids" in place of an acid hydrolysate of casein. This consists of an enzyme digest of milk proteins continued until the product is biuret free. It has been used simply as an economy of time since in handling large quantities the acid hydrolysis is somewhat cumbersome. Every step in the separation, however, has been checked on an acid hydrolysate and it is believed that there is no essential difference in the factors involved.

In attempting to separate the active material from the mercuric sulphate precipitate, fractional precipitation with the same