

Animal charcoal and globulin (horse serum) were used to adsorb the substances possessing antigenic action from the filtrates. When animal charcoal was added, the antigenic properties were removed, and could not be recovered by extraction of the charcoal. The adsorption with globulin was a more complicated procedure. The technique was briefly as follows: The original antigen was dialyzed and horse serum, one part to twenty of antigen, was added and allowed to stand in the incubator half an hour. The globulin was then precipitated by passing purified CO₂ gas free from HCl through the mixture for one half an hour at 37° C. The globulin precipitate was collected and by shaking it with alcohol the antigenic substances were extracted. The alcoholic extract was concentrated in a vacuum.

The preliminary dialysis of the culture filtrate eliminated practically all of its anticomplementary action. The adsorption with globulin removed the antigenic substances from the culture filtrates and the aqueous extractions so that they were easily obtained in greatly purified and concentrated form.

In preliminary studies the antigens which are used in the diagnosis of syphilis by complement-fixation were also purified and concentrated by similar methods. This method thus allows more precise study of many phases of infection and immunity than has hitherto been possible.

101 (1561)

Observations on the immunization of rabbits with single strain and combined multiple strain vaccines.

By **W. C. NOBLE, JR.** and **RUTH A. THOMAS.**

[From the Department of Bacteriology, New York University (University and Bellevue Hospital Medical College).]

Previous to 1916, the Army and the National Guard were immunized with typhoid vaccine. During the late summer and early autumn of that year, numerous cases of paratyphoid fever developed among the troops along the Mexican border, and the Army medical authorities therefore felt it desirable to substitute a triple vaccine, of typhoid bacilli combined with the paraty-

phoids A and B, for the single strain typhoid vaccine then being issued. Similar experience with paratyphoid in the British Army in Flanders in the same year had also resulted there in the adoption of a combined vaccine.

This immediately brought up the question as to the basis for the use of combined vaccines for prophylactic inoculation and led to the initiation, in the winter of 1917, of the experiments here presented. Other workers in this field had preceded us. Castellani¹ in 1903, showed that on injecting an animal with two different organisms at the same time, agglutinins were produced for both, and that the amount of agglutinin for each of the two organisms was about the same as in those animals inoculated with but one type of organism only. Subsequently he stated that as many as six different types of bacteria might be combined in a single vaccine with this same result, but that if more than six types were combined, a diminished amount of agglutinin for each type resulted. The problem has been attacked by other investigators, who have followed the methods of Castellani, by comparing the agglutinin formation when single and combined vaccines were employed. As we have long known that agglutinin formation is not a real index of the degree of immunity, it has seemed desirable that further study of the problem should be made, in observing the relative amounts of bacteriolysins produced.

Four series of from four to five rabbits each, were immunized; one series was immunized with combined triple vaccine of typhoid and paratyphoid bacilli A and B; three other series with single strain vaccines,—one series each with typhoid, paratyphoid A, and paratyphoid B. At the beginning and end of the experimental period, the blood serum of each rabbit was tested for specific and group bacteriolysins and also for agglutinins. In the series immunized with the single strain typhoid vaccine, all the animals show a sharp rise in immunity, but an interesting paradox is to be observed in the excessive production of the (group) para A lysin beyond the specific typhoid lysin (the highest lytic dilution for para A being 1-700,000, for typhoid 1-75,000; and for para B 1-400.) In the series immunized with para A vaccine, the highest lytic dilution for the homologous strain is 1-20,000; for typhoid it

¹ Castellani, A., *Zeitschrift f. Hyg.*, 1902, 40, p. 1.

is 1-3,000; and for para B 1-300. The third series immunized with para B shows its highest lytic dilution with a heterologous strain, typhoid (1-950); while for para A and para B the corresponding dilutions are 1-380 and 1-350 respectively.

The fourth series of rabbits was immunized with combined triple vaccine, and the degree of immunity reached for each organism (as measured by the highest lytic dilution) would appear to be lower than the degree of immunity obtained for the same organism by inoculation with the single strain vaccine. This result was not wholly anticipated, from the previous work of Castellani¹ and also of Davison.² The latter in a series of very completely worked out experiments with agglutinins, reports that the combined vaccine when injected, gives for each organism as good and usually a greater immunity response than if it had been injected alone. Davison, and likewise Bull³ have also observed some tendency in respect to the heterologous strains to lead to an added formation of the specific agglutinin, a phenomenon somewhat comparable to that of "summation" in muscle contraction. Our results with bacteriolysins, in a small series of animals, if confirmed, would *appear* to differ from Davison's observations with agglutinins. Further experimentation along these lines is desirable, and it is our hope to amplify our own work with improved methods.

102 (1562)

Studies on intestinal implantation of *Bacillus acidophilus*.

By HARRY A. CHEPLIN and LEO F. RETTGER.

[From Yale University, New Haven, Conn.]

B. acidophilus (Moro) is a common inhabitant of the intestinal tract of the albino rat and of man. Ordinarily it is present in very small numbers, however, and often may escape detection.

Diet exerts a profound influence on the character of the intestinal flora. Lactose and dextrin, when fed in sufficient amounts, bring about a marked transformation in bacterial types.

¹ Ibid. and Centralbl. Bakt., 1909, Ab. I, 52, p. 92.

² Davison, W. C., *Arch. of Inter. Medicine*, 1918, XXI, 437.

³ Bull, C. G., *Jour. Exp. Med.*, 1916, XXIII, 419.