247 (2479)

Researches on the antigenic modifications of bacillus typhosus.

By D. COMBIESCO* (Bucharest).

[*From the Department of Pathology, Johns Hopkins University, Baltimore, Md.*]

Our investigations[1] concerning the vaccination of animals through buccal and cutaneous routes have led us naturally to the study of the antigenic modifications of certain micro-organisms, especially of *B. typhosus* and *B. paratyphosus B* in contact with the body fluids. We shall give on this occasion some of our results.

As early as 1891, Metchnikoff[2] had seen that the vibrio Metchnikovi, grown in the exudate of the immunized individual could form either clumps or give a culture made up of separate organisms, according to the previous environment of the microbe. Later Metchnikoff and Bordet[3] reported that "a vibrion chloreæ (vibrion of eastern Prussia of highly accentuated virulence) was only partially affected by the serum of a horse immunized against cholera, after having been exposed for a certain time to the action of phagocytic protoplasm" *"protoplasma phagocytaire"*. Grassburger and Shattenfroh,[4] in their work on symptomatic anthrax, found that the serum obtained by the injection of virulent exudate agglutinated the microbe growing in this exudate, but not the microbe grown upon artificial medium. Bordet and Sleeswijk,[5] in their work with *B. pertussis,* showed that by changing the nutritive medium, one can create, using parts of the same culture, races distinguished serodiagnostically by their agglutination reactions, incapable of uniting with the same antibodies, and whose injection into animals gives different immune-sera. Gay and Claypole[6] found the same thing for *B. typhosus.* Their

* Introduced by W. L. Holman.

[1] Combiesco, D., *Compt. rend. Soc. de biol.*, 1923, 88, 904; the same, 1923, 89, 634, 637, 640.

[2] Metchnikoff, E., *Ann. Inst. Pasteur*, 1891,, (quoted by Bordet).

[3] Metchnikoff and Bordet, *Ann. Inst. Pasteur*, 1896, x, 1931.

[4] Grassburger and Shattenfroh, *Sitzungsberichten der Kaiserlichen Akademie der Wissenschaft in Wien*, mathem. Naturw. Klasse, 1905.

[5] Bordet and Sleeswijk, *Ann. Inst. Pasteur*, 1910, xxiv, 776.

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

cultures were grown either upon agar-agar or 10 per cent blood agar.

We used, throughout these experiments, the same strain of *B. typhosus;* one that we obtained by the courtesy of Dr. Holman of the Department of Pathology. The cultures were grown on ordinary agar slants for 24 hours at 37° C. and emulsified in physiological salt solution, 10 cc. for each slant. The emulsions were mixed so as to have as nearly as possible the same quantity of organisms in the same volume. One part of this emulsion was used for the inoculation of a series of rabbits. Another part was mixed with oxalated blood of normal rabbits (or the blood of rabbits partially treated as discussed below), and kept for 5 hours at 37° C., then inoculated into another series of rabbits, taking care that the number of microbes injected into each rabbit was equal to that inoculated into the rabbits of the first series. Space does not permit us to give all the details, we will content ourselves for the time being with giving the more conclusive results, shown by the examination of some of the animals used.

Rabbit No. 5 (1850 gm.) received a first intraperitoneal injection of 1 cc. of the emulsion heated one hour at 60°, on the third day the same dose, intravenously, on the fifth day the same dose of living bacteria, also intravenously; this dose being repeated on the 7th and 9th days.

Rabbit No. 3 (2025 gm.) on the same days received the same inoculations as rabbit No. 5; this time, the emulsion of bacteria was exposed to the action of 1 cc. oxalated blood. The first time the blood was that of a normal rabbit; at the second injection the blood from rabbit No. 3 which had received the above mixture two days before was taken—on the third, fourth and fifth inoculations the blood was always taken from rabbit No. 3 or another that had received the same treatment.

Five days after the last inoculation, the serum of rabbit No. 5 agglutinated very well the *B. typhosus* grown on ordinary agar in a dilution of 1:200 (examined after 2 hours at 37° C.). Under the same conditions the serum of rabbit No. 3 did not agglutinate the *B. typhosus* at all.

Ten days after the last injection the animals were bled again; the examination made under the same conditions showed: for rabbit No. 5, a clear agglutination titre of 1:10,000, with limit of 1:40,000. For rabbit No. 3, absence of agglutination at dilution of 1:20, and a slight agglutination at 1:100 (giving the

same appearance as in rabbit No. 5 at 1:40,000). We repeated the agglutination experiments but this time we made an emulsion of agar cultures, not in salt solution as above, but in citrated plasma of the normal rabbit, or the plasma defibrinated by shaking. The emulsion was kept at 37° C. for 3 hours before being added to serum dilutions. The results: the serum of rabbit No. 3, which does not agglutinate the bacteria cultivated on ordinary agar and emulsified in physiological salt solution, agglutinates very well up to 1:10,000, the same microbe when it has been affected by plasma. We worked with this plasma in dilution 1:5 and 1:10, and observed differences with the different solutions as we will discuss.

It is quite easy to find normal plasma which does not give any agglutination and such plasma naturally must be used in such studies.

Another group of animals received only two inoculations under the skin at 7 day intervals. Some of the animals "a" 0.5 and 1.0 of the emulsion of living bacteria; the others "b" the same amount of emulsion after exposure to normal oxalated rabbit blood for 5 hours at 37°. Ten days after the last inoculation the serum of rabbits "a" agglutinated very well (1:200 to 1:400) the bacteria grown on agar and emulsified in physiological salt solution, the serum of rabbits "b" either did not agglutinate at all, or at a dilution of 1:20, a very slight agglutination that one need not consider. The same experiment made with the emulsion in oxalated plasma or defibrinated plasma of normal rabbits in the dilutions mentioned above gave the following results:

1. The serum of rabbits "a" agglutinates the microbes affected by the plasma but with an intensity varying according to the whole plasma, or different dilutions of the plasma or one or the other plasma. For instance: the serum of certain rabbits agglutinated better the microbes emulsified in the oxalated plasma, diluted to 1:10, than the microbe in the same undiluted plasma. One agglutinates very well the bacterial emulsion in the oxalated plasma, and not at all, or very poorly, the bacteria emulsified in the defibrinated plasma.

2. The serum of rabbits "b" under the same conditions, agglutinates very well the organisms that are exposed to the influence of the plasma, showing, however, a certain variation according to the plasma and the dilution of plasma used. The

serum of normal rabbits, and physiological salt solution did not agglutinate the modified bacteria at all.

So under certain conditions, antigenic modifications of typhosus are evident.

248 (2480)

Researches on the antigenic modifications of bacillus paratyphosus B.

By D. COMBIESCO* (Bucharest).

[*From the Department of Pathology, Johns Hopkins University, Baltimore, Md.*]

In this series of experiments we have sought to determine whether *B. paratyphosus B* undergoes the same antigenic modifications as *B. typhosus*[1] when it is influenced by the fluid of the organism; in this particular case in contact with the oxalated blood of the normal rabbit.

In these experiments, emulsions of 24 hour agar cultures were heated at 60° C. for one hour. Six rabbits, "a", received two subcutaneous injections of this vaccine 7 days apart (0.5 and 1.0 cc.). Four rabbits, "b", were inoculated, time and quantity the same, with emulsion that had been kept in contact for 5 hours at 37° C. with oxalated blood of normal rabbits. Five days after the last injection the four rabbits of series "b" and two of series "a" were bled. The serum of rabbits "a" agglutinated the original *B. paratyphosus B* after 24 hours at 37° C. in dilutions of 1:100 to 1:200. That of rabbits "b", under the same conditions, either did not agglutinate at all, or in a dilution of 1:20, which has no significance. Ten days after the last inoculation the animals were again bled, and we tested their agglutinins as before. The serum of rabbits "a" agglutinated in a dilution of 1:200 to 1:400, while that of rabbits "b" agglutinated in dilution 1:20, or not at all.

---

* Introduced by W. L. Holman.

[1] Combiesco, D., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 490.