AD NUMBER

AD866753

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 19 FEB 1972. Other requests shall be referred to the Army Biological Laboratory, Attn: SMUFD-AE-T, Fort Detrick, MD 21701.

AUTHORITY

SMUFD, per d/a ltr, dtd 18 Feb 1972

AD 866753

TRANSLATION NO. 2613

DATE: 3/10/70

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Wiener Klinische Wochenschrift, 1897 T-772-2

From the State Institute for the Production of Diphtheria Serum.

Director: Prof. R. Paltauf

On the Specific Reactions in Germ-Free Filtrates of Cholera, Typhus and Pest Bouillon Cultures Produced by a Homologous Serum.[1]

By Dr. Rudolf Kraus, Assistant at the Institute

Issaeff and Ivanoff, Gruber and Durham, and Pfeiffer acquainted us with the process of agglutination with a reaction, which proved to be of far-ranging significance for medical bacteriology.

The main value of this reaction is that we have a method which does not follow the exactness of a chemical reaction.

This reaction, aside from proving the specificity of the pathogenic micro-organisms, represented the starting point of a new systematology in bacteriology and founded the serum diagnostic.

Recent works on agglutination have been concerned with the practical utilization of this reaction. Our knowledge on the nature of the agglutination has not been advanced significantly since the discovery of the reaction.

In the beginning Gruber had formed the idea that the coverings of the bacteria swell up under the influence of the specific serum, become glutinous and become rolled up together by means of their glutinous surfaces. Gruber thought that the swelling up was a form of preparation for the micro-organisms to attack the bactericidal material of the organism.

This assumption of Gruber's, however, does not hold up to be entirely true. Nevertheless, Roger[2] recently succeeded in observing the rising up of Oidium albicans under the influence of the serum, and the changes in typhus assumed by Gruber could not be found. The agglutinated bacilli absorb color material well, they reveal no changes in formation, their flagellae become very discernible. The agglutinated bacilli also breed continuously.

Bordet[3] conceived the agglutination as a process that could be explained through molecular attraction.

---

[1]Delivered as a temporary report in the Royal Society of Physicians on 30 April 1897.

[2]Roger, Revue generale de sciences, 1896.

[3]Bordet, Annales de l'Institut Pasteur, 1896, No. 4.

Agglutination was until then viewed as a vital function of the micro-organism. One thought that the serum could be effective only on live bacteria.

Then Widal[4] proved that the reaction could also occur with dead typhus bacilli. The dead typhus bacilli were precipitated likewise at 56° in vitro by the typhus serum and agglutinated just as the living ones. The bacteria killed off by a higher temperature are not suitable for the reaction.

The fact that the dead typhus bacilli can also be agglutinated, means a step forward for the theory of agglutination and for the practice of serum diagnostic.

I can confirm the validity of Widal's information on typhus and cholera.

Widal's discovery and the discovery of E. Buchner[5] on fermentation with zymosis (which with high pressure liquifies yeast) form the origin for the following tests.

The two above-mentioned scientists suspected that the solutions of any dissolved bacterial bodies could yield specific reactions with a specific serum just as the cultures themselves.

The tests were made chiefly with cholera.

For special reasons the tests were conducted mostly with germ-free filtrates of the cholera bouillon cultures.

Different kinds of cholera bouillon cultures were filtered by bacterial filters (Pukal). The clear filtrates were tested by vaccination as well as by leaving the filtrates alone at 37° until steril

After they were proven to be sterile, various amounts of the sterile cholera serum (goat serum) were added and placed in the incubator (37°).

Within 24 hours one could observe the following:

The filtrates, which were transferred with a specific serum, became opaque and formed small flakes that settled on the bottom. Deposits formed and the liquid above the deposits became perfectly clear.

The opacity of the liquid can be weak or strong. The deposits can be in large or small amounts. Just exactly why the reaction is so unpredictable has not yet been discovered. Sometimes the filtrates yield no reaction at all even when taken in large amounts and even when a great deal of serum is added. As regards the appearance and the composition of the deposits, the deposits are gray-white, brown, rather coherent,

---

[4] Widal, La Semaine medicale, 1897, No. 5.

[5] E. Buchner, Reports of the German Chemical Society, 1897.

can be shaken, then clouds the bouillon and dissolves again very quickly.

The chemical test of the deposits, which I carried out in the chemical laboratory of Dr. Freund, reveals that these deposits consist of two protein bodies one of which gives off an alkali albuminate reaction and the other gives off a peptone reaction.

In order to determine with certainty the specificity of the deposits, control tests were made with filtrates, which precipitated with a specific serum, with a heterologous serum.

Normal human serum was also added in different amounts to the cholera filtrate typhus, coliserum (goat serum), normal horse serum, diphtheria-, cholera- and antistreptococcus serum (horse serum), and never could precipitates be observed, as those that develop in cholera filtrates with the adding of cholera serum. The different control tests also remained perfectly clear at 37° for 24 hours.

From these tests I concluded in the report so far available that in the germ-free filtrates of the cholera bouillon cultures specific could occur with the adding of specific serum (obtained from the immunization with cholera agar-cultures). This reaction is just as specific as agglutination is with living or dead cultures.

Whether the substance in the filtrates, which are precipitated with the specific serum, are the destroyed products of the bacterial bodies, I could not determine at that time so it was merely written off then as a probability.

The following test described below provides direct proof of this assumption.

Cholera bodies, part of which were out of agar-cultures, and part of which were obtained by means of filtration of bouillon cultures, were mixed with powdered glass and placed under an atmospheric pressure of 300.

The pressed mass was thinned out with the alkali bouillon and filtered with a bacterial filter. The filtrate was tested for sterility and later cholera serum was added to it as well as to filtrates from the cholera bouillon cultures.

In the course of 24 hours at 37° one could observe the clouding and the formation of the precipitates.

The same result could be attained by a modification of the test.

Cholera cultures (two days old) were scraped off from the agar-plates, at 37° and left alone until dry and then dissolved in a weak alkali bouillon. The solution was filtered through a bacterial filter. The sterile solution was mixed with cholera serum.

The result was the same as with the filtrates from the cholera bouillon cultures and from the pressed culture masses.

This test therefore proved that the substances of the filtrates of the cholera bouillon coltures precipitated out with cholera serum are viewed as the direct material of the vibrio bodies.

It was thereby proved simultaneously that the specificityof the serum exists not only for the formed bacteria (living or dead), but also for the bacterial bodies placed in the solution.

The works of Widal[6], Levy and Bruns[7] can be utilized as additional evidence for the latter conclusions. Widal and later Levy and Bruns could immunize animals with the germ-free filtrates from the typhus and cholera bouillon cultures and develop a serum with agglutinative qualities, such as those that had been obtained with agar-cultures. It was likewise proved here that the filtrates contained substances which might cling to the bacterial bodies.

The same specific reactions as occurred with cholera serum in the germ-free filtrates from the cholera bouillon cultures could also be observed with the typhus serum in the germ-free filtrates from typhus bouillon cultures and with the plague serum in the filtrates from the plague bouillon cultures.

The cloudiness and the precipitation are similar to those described with the cholera filtrates. The reaction occurs sometimes stronger and sometimes weaker , and can also even fail to take place. The conditions for this behavior could not be determined at that time. The control test turned out negative with heterologous kinds of serum.

I used plague culutres to make an analogous test to the one with the cholera bodies to identify the precipitable substances in the filtrate .

The plague culture (two days old) was scraped off from the agar-plate, dried at 37°, and dissolved in a weak alkali bouillon. The solution was filtered by a pukal and the sterile solution replaced by plaque serum (horse serum).

The result was the same as with cholera.

It should be pointed out in this respect that to produce this cloudineness and precipitation larger amounts of serum are necessary than for agglutination cultures. I could not determine the quantative relation. The amount of decomposed products of the bacterial bodies is in all probability very unequal, dependent perhaps on the virulence of the culture, the age and the degree of alkalinity of the bouillon. This might possibly be a reason for the variability of the intensity of the deposits (precipitates) and for the possible failure of the reaction to take place.

---

[6] Widal and Sicard , Annales de L'Institut Pasteur, 1897. cit. according to Levy

[7] Levy and Bruns , Berliner Klinische Wochenschrift, 1897, No. 23.

Another work will attempt to prove whethe the changing, frequently lacking, toxicity of the cholera filtrates can be drawn into a causal relationship with it.

Prof. Paltauf[8] attempted on the basis of these tests to explain the mechanics of the agglutination of stationary micro-organisms.

Whether the filtrates of toxin-producing bacteria with the adding of a specific serum (obtained with toxins) are able to form precipitates, should be determined by the following test. Germ-free filtrates of diphtheria bouillon cultures were mixed with the anti-toxi serum (horse serum) in varying amounts. After 24 hours at 37°the filtrates with a specific serum remained perfectly clear.

The control samples of the filtrates also yield no reaction with the adding of the cholera, typhus, coliserum(goat's serum), antistreptococcus serum (horse serum).

These tests allow the following conclusions to be drawn :

1. In germ-free filtrates from cholera, typhus, and plague bouillon cultures specific deposits occur when homolcgous serum is added.

2. The substances, which precipitated out of the filtrates with a homologous serum, belong to the bacterial bodies.

3. Germ-free toxins (diphtheria toxin) yield no specific reactions when homologous antitoxins are added.