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NEW METHOD FOR THE QUICK RECOGNITION OF THE CHOLERA VIBRIO AND THE TYPHOID BACILLUS

By Max Gruber and Herbert E. Durham

One of us (G.) has already reported[1] briefly in No. 9 of this periodical of March 3, that the blood serum (and the peritoneal lymph) of a guinea pig immunized against cholera has a very striking specific effect--previously overlooked or not appreciated--on the cholera vibrio, likewise the serum of an animal immunized against typhoid on the typhoid bacterium, etc.

If one adds the appropriate protective serum to the floating suspension of the agar culture of one of the mentioned types of bacteria, the bacteria are seen to conglutinate into big balls, and the spontaneous movement comes to a standstill. These effects are connected in the closest manner with the protective action of the sera. They are the consequences of the fact that the bacteria become sticky under the impact of the antibodies contained in the immune serum. Consequently, we label the specific substances of the immune sera conglutinants or agglutinins.[2]

Highly effective immune sera still produce marked agglomerating reactions in astonishingly large dilutions. But if the process is to occur quickly and fully, one must use larger (higher) serum concentrations. The higher the concentration selected, the more surprising the result. If a highly effective serum is available, one can achieve complete agglomeration and retardation in movement within a short time, by adding 5%, 1%, or even less serum to the floating suspension of the fully virulent bacteria. It is not necessary

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1- The first report was made on January 3, 1896 by Durham before the Royal Society in London. On February 28, Gruber gave a lecture on the whole problem with demonstrations in the Royal Viennese Society of Physicians.

2- The label used earlier "Glabrificin" is based on a lapsus calami.

to use the mircroscope in order to observe the effect of the serum. The agglomeration of the bacteria can already be recognized with the unaided eye from the fact that the uniform clouding of the fluid soon becomes flocculent,the flocculi become increasingly large and drop to the bottom, whereby the fluid becomes completely clear. The whole process which takes place after adding the immune serum to the bacterial floating suspension, resembles in every respect the gradual elimination of a flocculent precipitate from a salt solution after adding a precipitant.

Extensive experiments have taught us that cholera vibrios and typhoid bacteria react without exception in the manner described to the influence of their corresponding immune sera. Up to now we have tested 20 cholera vibrio generations of the most varied origin with the aid of many different kinds of immune sera concerned, and have always had the same success.

How do these highly effective immune sera behave vis-a-vis other types of bacteria?

If the immune serum is added to the floating suspension of foreign agar cultures, the result may be quite different.

The most effective cholera serum has no agglomerating effect at all on numerous vibrios, e.g., on the V. proteus of Finkler-Prior, V. Metschnikovi, V. danubiens, the luminescent vibrio in the Brinks (Rumpel) case, etc. Similarly, the most effective typhoid serum is completely ineffective against numerous types of coli bacteria.

The protective power of the serum in animals fails completely vis-a-vis all such insensitive bacteria.

We have already pointed out in all our earlier publications that the effect of the immune sera is not sharply specifically limited, as might appear to be the case after such experiments. The cholera serum has a more or less strong agglutinating effect on vibrios which apparently are not derived from the cholera vibrio; this, to mention only a few examples, on the luminescent vibrios raised by Rumpel in the Oergel, Elvers cases, etc.

The cholera serum has the same or almost as strong an effect on V. Ivanoff, on the V. "Seine-Versailles, Sanarelli's and on the V. Berolinensis, as on the vibrios accepted as genuine, from the intestine of cholera patients. The question therefore is whether or not the three mentioned types of vibrios

are to be classed with the cholera vibrio. In regard to the first two types, most experts assume the former (V. Ivanoff), and for the Berolinensis the latter.

In all cases in which the cholera serum has an agglutinating effect on bacteria, it also has a protective action in the animal body, and the degree of protection increases in proportion to the intensity of the effect in vitro. The cholera serum protects against V. Ivanoff, V. Seine-Versailles and V. Berlinesnsis just as much as against the known cholera vibrios.

The effect of the typhoid serum is generally speaking more sharply limited specifically than that of the cholera serum. But we have recently found bacteria which are similar to typhoid, but are certainly not typhoid bacilli, and yet are affected quite strongly by the typhoid serum.

Thus, a few days ago one of us (G.) came to know a type of bacillus in the Bac. enteritidis of Gartner, which is agglutinated in a completely typical manner by the concentrated typhoid serum, although it acidifies the litmus when strongly within 24 hours and forms gas in sugar agar,i.e., is specifically different from the typhoid bacillus, as is generally assumed. To be sure, quantitative differences still appear here in regard to the effect of the typhoid serum on the Bac. enteritidis, and the effect on genuine typhoid bacilli, which is plainly obvious in testing serum dilutions.

These observations which refute the assumption of a strong specificity in the effect of the cholera and typhoid serum are in accord with the fact that various foreign immune sera and perhaps even normal sera have a distinct agglomerating effect on cholera vibrios and typhoid bacteria.

Of course, the value of the serological test as a diagnostic tool is considerably diminished by these findings. But the test by no means becomes completely worthless thereby from the diagnostic point of view. Although there may be no thorough going qualitative differences, yet sufficiently significant quantitative differences exist in most cases in regard to the effect of the immune sera on their specially corresponding bacteria and on foreign bacteria, so as to be able to make use of them for diagnosis.

The reaction can be carried out in various ways.

According to the modification developed by one of us (Durham), one proceeds in such a way that the floating suspension of one loopful (2-4 mg) of recent (fresh) agar culture of the bacterial group to be examined is mixed in 1/2 ccm bouillon with a dilution of 10 mg of the highly effective immune serum concerned in 1/2 ccm bouillon (repetition) and then one determines by observing with the naked eye or in a microscopic examination whether or not complete agglutination takes place.

If one is working with cholera vibrios or typhoid bacilla, a complete cessation of movement and complete agglomeration must be detectable microscopically after 10-15 minutes, and complete precipitation of the bacteria and complete clarification of the fluid must take place within one hour.

According to more recent experiments of the other one of us (G.) the reaction is carried out most readily as follows:

A strong guinea pig is immunized to as high a degree as possible. If the immunization takes place through intraperitoneal injection of dead cultures, one can achieve a remarkably efficacious and long lasting immunity within 4-6 weeks. The immunity must be intensified to such a degree that the peritoneal lymph or the blood serum of the animal which is added at room temperature to an equal floating suspension volume from the specifically associated bacterial group brings about complete cessation of movement and complete agglomeration within the first minute.

A pure agar culture is made of the bacteria generation to be examined, and a floating suspension of the 10-20 hours vegetation is prepared, by carefully suspending approx. 2-4 mg. (a small platinum loop full) in 1 ccm sterile bouillon. The agar used for the culture should have a dry surface, and the vegetation mass must be carefully triturated with a drop of bouillon before it is dissolved in the fluid, because otherwise one would introduce small pieces and clots in the floating suspension from the beginning.

Then a drop of the immune lymph or the immune serum is put on a cover glass, an equally large drop of the bacteria floating suspension is deposited next to it, both drops are

mixed, and the cover glass is laid on the hollow ground slide.[3]

.Then if the conglutination of the vibrios is absent under the influence of the cholera serum, it is completely certain that the cholera vibrio is not present. Likewise, it is out of the question that the typhoid bacillus is involved, if the suspected rod-shaped bacilli remain isolated and mobile in spite of the effect of the typhoid serum.

We are concerned just as little with the cholera vibrio or the typhoid bacillus if only a part of the individuals continues to move spontaneously in the serum mixture, and if active motion of single individuals or whole conglomerations is to be observed after expiration of a quarter of an hour, even if only on the spot.

If one is in doubt whether an entirely completed reaction or an incompleted reaction-even if only in traces- is involved, one can make the test more exacting by leaving the preparation for 1/4 -1/2 hour in the incubator. If the reaction was incomplete, a larger number of specimens and conglomerations resume spontaneous movement, whereas in the perfect raction everything remains quiescent.

While the negative reaction permits a completely reliable diagnosis, this is not the case with positive results from the reaction, as just indicated. In that case, the diagnosis "cholera vibrio" or typhoid bacillus has only a greater or lesser plausibility in its favor (for itself) according to the circumstances, and one must then endeavor to obtain additional characteristic features.[4] It has already been pointed out above that the experiments on animals are likewise valueless in such cases.

If the test is for typhoid bacilli, one must by all means prepare first of all a pure culture of the suspected rod-shaped bacilli, a process which apparently will become much easier by means of the Elsner method.

3- Of course, no preservatives may be added to the serum.

4- Perhaps the serum test can be developed into a reliable differentiation process if the quantities are taken into consideration more precisely. Dr. K Landsteiner is working on such experiments.

The definitive investigation (testing) of the vibrios will also have to be carried out with a pure culture. Meanwhile, a preliminary test can be conducted here also even before the production of the pure culture, which may be useful in examing cases where cholera is suspected.

A pre-culture is prepared from the suspected stool or feces according to the method adopted so successfully by Koch. After a few hours, a thin cover of vibrios had formed on the surface of the fluid. A drop from the highest layer of the fluid is extracted and is mixed - after one is sure that active vibrios are present in it - with a drop of a peritoneal lymph taken recently from the animal or an immune serum and their effect is observed. If immediate complete cessation of movement and complete agglomeration takes place, the diagnosis "cholera vibrio" certainly has significant plausibility. If the reaction is completely absent, which is often difficult to observe under the circumstances, "cholera vibrio" would be eliminated. If the reaction remains incomplete, the diagnosis must remain in doubt until the examination of the pure cultures, since a mixture of various vibrio types may be present in the pre-culture.

Thus, by using our method, one will be able to obtain a pretty dependable bacteriological judgement concerning a cholera suspected case under favorable circumstances, already within the first 6-10 hours.