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## Agglutination phenomena with diphtheria antitoxin.

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In search for an in vitro test for diphtheria antitoxin the following observation was made: (*a*) When an emulsion of the diphtheria bacillus, Park 8, is mixed with diphtheria antitoxin, allowed to stand at 37° C. for 1 hour, centrifuged, washed with saline and re-suspended, it is no longer agglutinated by diphtheria-agglutinating serum. (*b*) The organisms sensitized in this way are inagglutinable by acid agglutination.

In this test for the inhibition of acid agglutination the cells are suspended in a buffer solution which gives a maximum agglutination with unsensitized cells. The point of maximum agglutination for acids varies somewhat depending on the culture and the buffer mixture, but for phthalate mixtures (Clark and Lubs) diluted 1–1 with distilled water it is about $P_H$ 4.2 for a three or four-day broth culture of the Park 8 strain.

To determine the specificity of the test for diphtheria antitoxin, experiments were carried out along four different lines.

1. The following sera were used in place of diphtheria antitoxin and the test carried through: normal human serum, positive T.B. human serum, normal guinea pig, concentrated tetanus antitoxin, fresh antitoxic serum, normal horse, normal sheep, normal rabbit.

In the above experiment there was inhibition of agglutination when the cells had been sensitized with antitoxic serum and with tetanus antitoxin; and when the other sera had been used there was agglutination. A guinea-pig test with the tetanus antitoxin showed that it contained diphtheria antitoxin, and it was subsequently discovered that this tetanus antitoxin was from a horse which had previously been used for the production of diphtheria antitoxin.

When diphtheria bacilli are sensitized with diphtheria agglutinating serum instead of antitoxin there is inhibition of agglutination

when the cells are subsequently subjected to serum agglutination but acid agglutination is not inhibited.

A possibility which was kept in mind was that the effect with antitoxin might be due to the presence of agglutinoids in antitoxic serum. In an attempt to check this, agglutinating serum diluted (1–10) was heated 75–80° C. for 1 hour and subsequently used in the test; both with acid and serum agglutination there was no inhibition. One might conclude from this experiment that the agglutinins were destroyed by the high temperature and that no agglutinoids were produced. Up to the present attempts to produce agglutinoids from agglutinins have not been successful.

2. An emulsion of diphtheria organisms incubated with a mixture of antitoxic serum and diphtheria toxin in suitable quantities is subsequently agglutinated both by acid and by diphtheria-agglutinating serum, whereas if the toxin is replaced by an equal volume of broth the cells are not agglutinated. The conditions for this experiment are limited by two factors: there must be sufficient antitoxic serum to sensitize the cells, and an excess of toxin.

Diphtheria organisms sensitized with antitoxin are rendered agglutinable by mixing with diphtheria toxin, centrifuging, washing with saline and re-suspending.

Diphtheria toxin has this same neutralizing effect on diptheria-agglutinating serum. From this one might conclude that diphtheria toxin contains agglutinogens besides true toxin.

3. In an attempt to apply the test quantitatively two general methods were used: Organisms were sensitized with progressive dilutions of antitoxic sera and the limit of inhibition read as the end point; and mixtures of serum and toxin after standing 1 hour at room temperature were added to sensitized cells directly, and after dialyzing—it had been found that toxin dialyzes through parchment paper—and the dialysate mixed with sensitized cells which were subsequently tested for agglutinability.

Neither method gave results which were uniformly consistent with guinea-pig experiments. Using the first method some results were obtained which were parallel to the guinea-pig tests, in other cases the results were reversed, *i.e.*, in some cases two sera which by Ehrlich's method gave, *e.g.*, 300 Å.U. and 150 Å.U. per c.c.

respectively showed by this test that the second contained more antitoxin than the first.

In this connection it is well to recall that according to the work of Roux,[1] Danysz,[1] Momont[1] and Cruveilhier,[2] the results obtained with antitoxic sera by Ehrlich's method do not always parallel those obtained by the French method—a method which has at least the merit that its results are based on animal experiments, the conditions of which correspond in a measure to those which obtain in the actual treatment of the disease. These workers claim that in some cases the results are not only not parallel but may even be the reverse of each other. On the other hand according to the work of Marx[3] the two methods give parallel results. No comparison between the test described here and the French method has yet been carried out.

4. It would be of interest to know whether washed diphtheria organisms do actually take up antitoxin. A carefully controlled experiment to test this was carried out. Organisms which had been washed several times with saline were mixed with a known amount of antitoxin, allowed to stand for 1 hour at room temperature, centrifuged and the supernatant liquid drawn off. A guinea-pig test with an appropriate amount of toxin showed a fall in antitoxic content of the mixture. The difficulty with this experiment is that it is not known when the cells are sufficiently washed. It is proposed to repeat this experiment in the following way: Wash diphtheria cells several times and then wash again batches of these cells and test the first and progressively washed lots to see whether a point is reached where the drop in antitoxin reaches a constant.

## SUMMARY.

Both acid and serum agglutination of Park 8 strain of *B. diphtheriæ* are inhibited when the organisms are first sensitized with diphtheria antitoxin; when diphtheria-agglutinating serum is used instead of antitoxin, serum agglutination is inhibited but not acid agglutination.

This inhibition phenomenon with antitoxin is specific, at least for the Park 8 strain.

[1] Abstract in the "Bacteriology of Diphtheria"; Nuttall, Graham, Smith, 1913, 525.

[2] Cruveilhier, *Ann. Inst. Pasteur*, xix, 1905, 249.

[3] Marx, *Zeit. f. Hygiene*, 1901, xxxviii, 372.

It is not definitely established whether it is a test for antitoxin; it is possible that the phenomenon may be due to agglutinoids, or to a diphtheria antibody not noted heretofore.

Nicolle, Debains and Césari[4] have described a qualitative test for toxin and antitoxin which is based on a precipitin reaction. It is evident from the results above that while the test may be specific for the diphtheria bacillus and for the other organisms used by them, it should be subjected to further investigation before it can be accepted as specific for toxin and antitoxin as defined by Ehrlich's guinea-pig test.

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## Action of some purin derivatives on the isolated bronchus.

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In connection with a study of the effects of various drugs on the isolated bronchi of pigs the authors studied a number of purin derivatives. The effects of caffein or trimethyl xanthin in doses of 1–20 mgm. in 25 c.c. of Locke's solution gave the following results; small doses produced no effect on bronchial muscle or occasionally a very slight constriction. After large doses of caffein a little relaxation of the normal bronchial preparations was noted. When, however, such bronchial preparations were first brought into a state of high tonus or contraction, as for instance on treatment with muscarin, the relaxing effect of a subsequent dose of caffein was much more marked. On the whole, however, the results obtained indicated that caffein has a very weak dilator effect on the bronchus.

Following experiments with caffein, observations were made on the effects of theobromin or 1–3 dimethyl-xanthin and theocin or 3–7 dimethyl-xanthin. It was found that both dimethyl-xanthins produced much greater broncho-dilatation than trimethyl-xanthin or caffein. The authors were unable to obtain a mono-methyl xanthin but they did study the effects of xanthin itself. Although

[1] Nicolle, Debains and Césari, *Comp. rend.*, 1919, clxix, 1433.