GENERAL AND CARDIOTOXIC ACTION OF O-STREPTOLYSIN

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Two types of streptococcal hemolysins are known, viz., S-streptolysin, which is formed in culture media containing serum, and which does not possess antigenic properties, and O-streptolysin, for the formation of which the presence of serum is not essential.

O-Streptolysin is rapidly fatal to white mice, which die with symptoms resembling those of histamine poisoning [4]. According to some authors [5], no changes in cardiac activity resulted from a single introduction of the product into an isolated frog's heart, but repetition of the dose was followed by cardiac arrest in systole. Other authors [6] found inflammatory changes in the myocardium after parenteral injection of O-streptolysin. Clinical and immunological observations reported by various authors have indicated that in many cases O-streptolysin aggravates the course of streptococcal infection [3].

For all these reasons we thought it would be of interest to make a more detailed study of the toxic properties of O-streptolysin.

EXPERIMENTAL METHOD

Except for experiments involving the use of isolated frogs' hearts, we used concentrated preparations, obtained from filtrates of streptococcal cultures by the method of fractional precipitation with ammonium sulfate (added at first to a saturation of 35% to remove inactive material, and then to 70% to precipitate O-streptolysin). In experiments with isolated frogs' hearts we used preparations made by the method of A.P. Konikov [1]. Titration of O-streptolysin with antistreptolysin was also carried out by the procedure described by this author.

Various doses of O-streptolysin, all in 0.5 ml of solution, were administered by intravenous injection into white mice. Injections of O-streptolysin which had been inactivated by boiling were given simultaneously to control mice.

EXPERIMENTAL RESULTS

All the animals died after receiving doses of 216 units or more, and at least 50% died after receiving 150-180 units. All the animals of the control groups survived the injections. Death was preceded by dyspnea, flow of bloodstained fluid from the nostrils, and rigors. At autopsy, the lungs were found to be edematous, the liver, spleen and kidneys were hyperemic, and blood was frequently found in the urinary bladder. In the great majority of cases death ensued within 10-20 minutes of the injection.

Rabbit antistreptolytic immune serum neutralized the action of O-streptolysin, if given in equivalent amounts. Thus, in one of our experiments we gave 600 units of O-streptolysin, amounting to 3 MLD, together with an amount of antiserum exceeding the neutralizing dose by 10%. All the mice survived injection with this

mixture. This detoxicating action on O-streptolysin was not exhibited by antifibrinolytic or normal rabbit serum.

Some of the sera taken from rabbits immunized with streptococcus vaccine also displayed some neutralizing action.

The specific protective action of antistreptolytic serum was clearly demonstrated in experiments on passive immunization.

To one group of mice we gave injections of 0.5 ml of antistreptolysin, containing 500 units, equivalent to 4500 O-streptolysin units, or 21 MLD. To a second group we gave 0.5 ml of serum from rabbits immunized with streptococcus vaccine. On the following day, the animals of both groups received 3 MLD of O-streptolysin. All the animals of the first group survived, but all those of the second group died.

The kymogram shown in Figure 1 illustrates the action of O-streptolysin on an isolated frog's heart. Introduction into the heart of an O-streptolysin preparation containing 900 units in 1 ml was quickly followed by arrest of the heart in systole. The toxic effect of O-streptolysin was manifested in all the experiments after its first introduction, when given in high dosage. The heartbeat was not restored by washing.

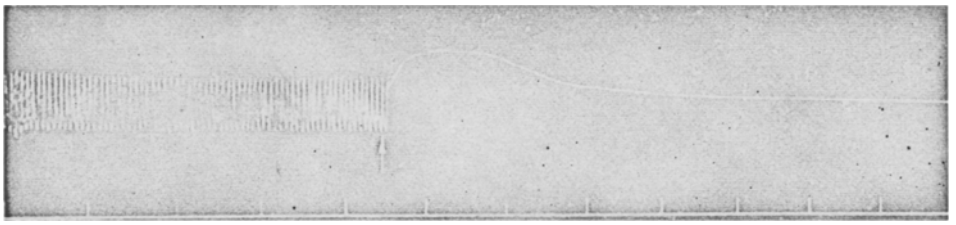

Fig. 1. Effect of a massive dose of O-streptolysin (↑, 900 units) on an isolated frog's heart. Time marker 30 seconds.

Perfusion of the heart with preparations of O-streptolysin containing 20 units in 1 ml was rapidly followed by reduction in the amplitude of the contractions, with subsequent arrest in diastole. Washing out restored the heartbeat.

Repeated perfusion with the same preparation, after washing out the heart three or four times, as was done by Bernheimer and Cantoni [5], did not cause arrest of the heart in systole.

In these experiments neither the first nor the second subthreshold dose of O-streptolysin caused more than a certain retardation of the action of the heart, with its subsequent arrest in diastole. Washing out the heart restored its action (Figure 2).

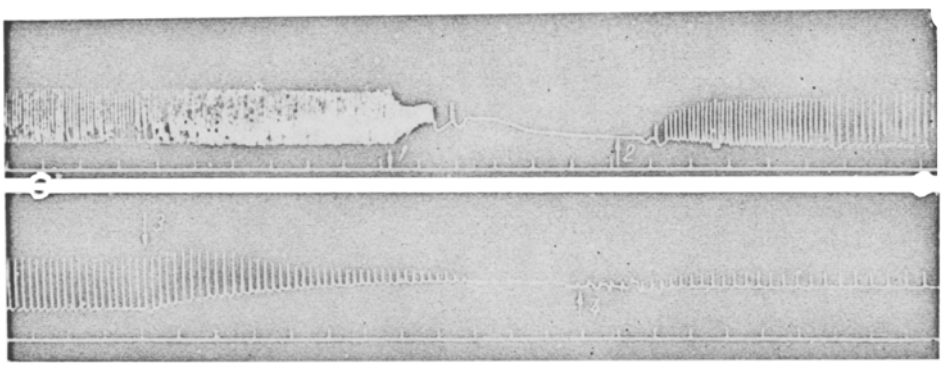

Fig. 2. Effect of repeated small doses of O-streptolysin on an isolated frog's heart: 1,3) introduction of 20 units of preparation; 2,4) washing out with Ringer solution. Time marker: 30 seconds.

Numerous repetitions of the experiment gave the same result. We were not able to observe any biphasic action of O-streptolysin, or any sensitizing action of the first dose, as reported by the above authors.

No perceptible effect on the heartbeat followed administration of preparations of O-streptolysin inactivated by boiling.

We have not been able to trace any references in the literature to the action of O-streptolysin on the isolated heart of mammals. Yet the results of such investigations could be of more value in the study of the pathogenesis of some streptococcal infections than are those obtained using the heart of cold-blooded animals. The kymogram of Figure 3 illustrates the effect of introducing 7000 units of O-streptolysin into the perfusion stream; a period of acceleration of short duration was followed by spastic contraction of the myocardium, and by arrest in systole. Relaxation of the heart muscle could not be achieved by prolonged perfusion with the nutrient medium.

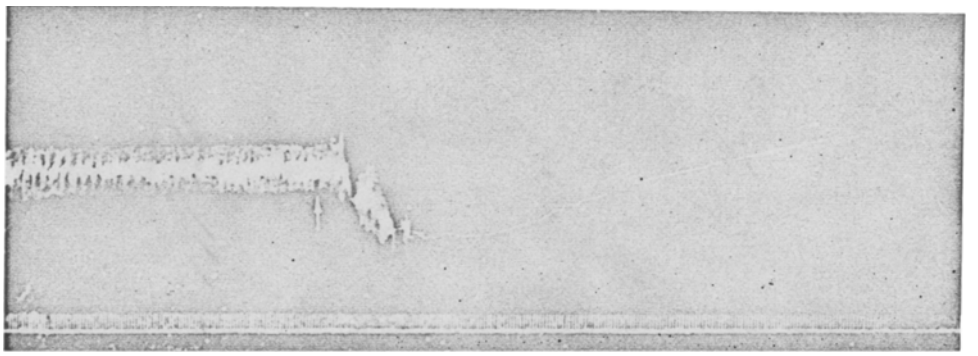

Fig. 3. Effect of O-streptolysin (7000 units) on an isolated cat's heart. Time marker: one second.

These effects were not seen when O-streptolysin inactivated by boiling was introduced into the perfusion fluid. The boiled preparation caused a considerable, though transient, increase in the amplitude of the heartbeat, after which it continued to contract normally for a long time, although more feebly than before boiled O-streptolysin was given. The kymogram of Figure 4 was recorded during one of these experiments. We repeated the introduction of boiled O-streptolysin (1500 units) three times. After each introduction of boiled preparation an increase in amplitude of the heartbeat was seen, without subsequent arrest of the heart.

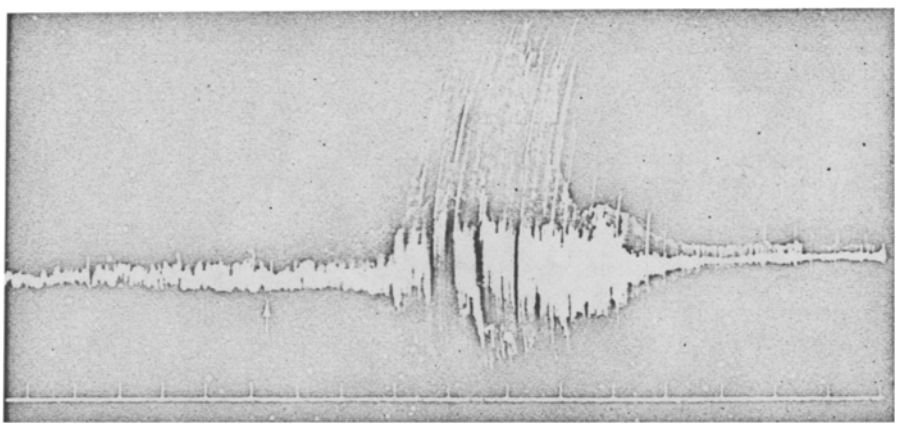

Fig. 4. Effect on an isolated cat's heart of 25 mg of boiled O-streptolysin ↑ Time marker: 30 seconds.

The specificity of action of O-streptolysin was also shown in experiments with erythrogenic toxin and with fibrinolysin. Introduction of large doses of these preparations (15,000 units and 2 ml, respectively) into the perfusion fluid did not cause any appreciable changes in the activity of the isolated heart.

In the next series of experiments we examined the effect of repeated small (subthreshold) doses of O-streptolysin. The first dose caused strengthening of the heartbeat, of short duration; it was followed by a gradual restoration of the normal rhythm. The same effects followed the second dose. The third dose caused arrest of the heart in systole.

The toxic action of O-streptolysin on the isolated cat's heart was abolished by previous addition to it of an equivalent amount of antistreptolytic serum. Introduction of such mixtures into the perfusion fluid affected the rhythm and the strength of the cardiac contractions, but did not lead to arrest of the heart.

Sera obtained from rabbits immunized against other streptococcal antigens (erythrogenic toxin, fibrinolysin, streptococcal vaccine) had no similar effect on the toxicity of O-streptolysin, with the exception of one batch of antifibrinolytic serum.

We examined the effect of O-streptolysin on the isolated heart of animals which had previously been immunized against this toxin. The results were not clear-cut, probably because of the differences in the degree of immunity of different individuals. In some of the experiments the effect of O-streptolysin in no way differed from that found for the heart of nonimmunized animals. In other experiments, the action of O-streptolysin was weaker; the myocardial spasm normally supervening after arrest of the heart did not appear.

Our study of the toxic properties of preparations of O-streptolysin on animals confirm published reports of its general toxic action on white mice. However, published figures for the minimum lethal dose for mice vary within very broad limits – from 10 units [4] to 4000 units [5]. Our value for the minimum lethal dose falls between these extremes.

These differences may be due to differences in sensitivity of mice of different strains to the toxic action of O-streptolysin. It may, however, be that the differences between the values for the minimum lethal dose reported by the various authors depend on the degree of purification of the preparations used by them, since they may contain different amounts of other streptococcal products, the introduction of which, into the organism, is not necessarily without effect. At the same time, there can be no doubt that O-streptolysin exerts a specific toxic action, not dependent on the possible presence of other streptococcal products. This is shown by the observation that strictly equivalent amounts of antistreptolytic serum neutralized its toxic action.

Arrest of an isolated cat's heart after repeated administration of O-streptolysin seems to have been the result of its cumulative action, since the total amount introduced in these experiments equalled the dose required for manifestation of the typical effects of this toxin. The specificity of the cardiotoxic action of O-streptolysin was established in experiments with other streptococcal toxins, and by experiments involving its neutralization by antistreptolytic immune serum and by other antistreptococcal immune sera.

SUMMARY

O-streptolysin has a general toxic effect and causes quick death when injected intravenously in white mice.

The cardiotoxic effect of O-streptolysin was studied in experiments on isolated hearts of frogs and cats. It was demonstrated that O-streptolysin brings about cardiac arrest and contraction of the heart muscle. However, streptococcal antigens do not give such an effect on the isolated heart. The toxic action of O-streptolysin may be neutralized by the specific immune serum. The influence of O-streptolysin on the isolated heart of immune animals differed from its action on the hearts of normal animals in a number of experiments.

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