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 intro:duction 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 antistreptolysin 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.](image)

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.](image)