LOSS OF MYOCARDIAL CONTRACTILITY INDUCED IN ISOLATED MAMMALIAN HEARTS BY STREPTOLYSIN O*

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Infections with group A streptococci are considered to be responsible for the development of important structural and functional changes in the hearts of human beings and experimental animals (1, 2), but the manner in which streptococcal infections bring about these changes remains obscure. Of the diverse extracellular products elaborated during the growth of group A streptococci, only two—streptococcal proteinase and streptolysin O—are known to have an effect upon the heart. A single intravenous injection of crystalline streptococcal proteinase has been shown to cause focal necrosis of the myocardium in rabbits, mice, and guinea pigs (3); and solutions containing streptolysin O have been found to induce systolic contracture of the isolated frog heart (4–6). The studies now to be presented demonstrate that a minute quantity of streptolysin O brings about a prompt and irreversible loss of myocardial contractile power when perfused through the isolated hearts of guinea pigs, rabbits, and rats.

Materials and Methods

The general plan of the experiments was as follows. Hearts were removed from guinea pigs, rabbits, and rats, set up in a modified Langendorff apparatus, and perfused through the coronary arteries with an oxygenated salt solution containing glucose. The rate and amplitude of contraction were recorded mechanically on a moving paper strip, and the rate of perfusion was measured by means of a flow-meter. While the heart was beating vigorously, varying amounts of streptolysin O, previously activated with cysteine, were introduced into the perfusion fluid, and the effects on the rate and amplitude of contrac-

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tion and on the rate of perfusion were observed. In control studies, the isolated and perfused hearts were exposed to cysteine, to streptolysin O not activated with cysteine, and to streptolysin O the hemolytic activity of which had been inhibited by means of cholesterol or specific antibody.

Animals.—Normal, male, market-bought guinea pigs weighing 300 to 600 gm. were employed in most experiments. A small number of adult rabbits of mixed breed and a number of male white rats were also used.

Isolation and Perfusion Technique.—The animals were anesthetized with sodium pentobarbital supplemented with ether when necessary. A catheter was tied into the trachea and positive pressure respiration was maintained when the chest was opened. Heparin, 5 mg./kilo, was injected into the inferior vena cava to prevent blood coagulation. A glass cannula was inserted into the first portion of the aorta, and the heart was removed rapidly and set up in a modified Langendorff perfusion apparatus according to the procedure described in detail by Garb, Penna, and Scriabine (7). A fine silk suture was passed through the apex of the heart; one end of the suture was tied to the heart, the other was carried over a small pulley and attached to a writing lever which recorded on a paper strip moving at a constant rate of speed. The amplitude of contraction and the heart rate could thus be measured directly from the tracing. Reduction in the amplitude of contraction was graded on an arbitrary scale from 0 to ++++, in which 0 represents no change from the baseline recording; +, up to 25 per cent reduction; ++, 25 to 50 per cent reduction; and +++ and ++++, 50 to 75 per cent, and more than 75 per cent reduction, respectively.

The perfusion fluid employed had the following composition per liter:

NaCl	7.0 gm.
KCl,	0.42 "
$CaCl_2$	0.24 "
$MgCl_2$,	0.2 "
$NaHCO_3$,	2.1 "
Dextrose,	1.8 "

This fluid was also used as the diluent for all materials injected. The perfusion pressure in all experiments was 52 cm. of water. The temperature of the perfusion fluid was maintained at 37°C., and a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled continuously through the fluid.

The rate of flow of perfusion fluid through the heart was measured at frequent intervals during each experiment by means of a flow-meter incorporated in the apparatus. Since the cannula was inserted into the aorta just above the aortic valve, it was to be expected that most of the fluid would flow through the coronary arteries. To confirm this, an experiment was done in which a guinea pig heart was isolated in the usual fashion, the inferior and superior venae cavae were ligated, and a catheter was tied into the pulmonary artery to collect the effluate from the right side of the heart. More than 97 per cent of the fluid perfused into the aortic cannula escaped from the right side of the heart through the catheter in the pulmonary artery, the finding indicating that practically all the fluid passed through the coronary arteries and was discharged through the coronary sinus into the right auricle or through those Thebesian channels that empty into the right heart. It seems reasonable, therefore, to regard the rate of perfusion as a measure of coronary flow.

All additions of streptolysin O and other materials were made through a fine polyvinyl plastic catheter threaded through the apparatus and ending in the cannula about 1 inch above the heart. The total volume of the catheter was 0.5 ml. The outer end of the catheter was fitted with a hypodermic needle to which a luer-lok syringe was attached for injection

purposes. The volume of injected material was 2 ml. in all cases, and each injection was followed immediately by 0.7 ml. of perfusion fluid to flush out the catheter. The total injection time was about 15 seconds. It was estimated from the rate of perfusion that all the injected material flowed through the heart within ½ to 2 minutes following injection. The perfusion fluid was not recirculated, and the exposure of the heart to the injected material was therefore brief.

Streptolysin O.—Most of the experiments to be described were carried out with fractionated culture supernates prepared from 15 liter cultures of the C203S strain of group A streptococcus. After growing the cocci according to the method of Bernheimer, Gillman, Hottle, and Pappenheimer (8), the supernate was concentrated 5- to 8-fold by pervaporation from cellophane sacs in the presence of a small amount of toluene. The concentrate was chilled, saturated with ammonium sulfate, and allowed to stand overnight in the cold. The resulting precipitate was collected on a Buchner funnel and dissolved in a small volume of cold water. The cloudy solution was dialyzed overnight against running tap water and then centrifuged to remove insoluble material. The clear supernate was dialyzed in the cold against 80 per cent saturated ammonium sulfate. To the resulting precipitate was added sufficient 80 per cent saturated ammonium sulfate to bring the volume to 100 ml., or in some instances to 50 ml., after which the material was stored in the cold. As required, aliquots were dialyzed free of ammonium sulfate, a 3- to 4-fold increase in volume occurring during dialysis. The dialyzed material was stable for 4 to 5 days when stored at 4°C. Four different lots of streptolysin O were employed in these studies. Their content of streptolysin O prior to dialysis ranged from 10,000 to 53,000 hemolytic units per ml. Hemolytic activity was measured by the method of Bernheimer (9) modified by preliminary activation with cysteine and by the inclusion of 0.1 per cent bovine serum albumin in the saline-phosphate diluent.

Unless otherwise indicated, the dialyzed streptolysin O preparation was activated immediately prior to use by adding an equal volume of perfusion fluid containing 1 per cent cysteine and adjusted to pH 7.0. After 10 minutes at room temperature the mixture was diluted to the desired concentration of streptolysin O, using 0.1 per cent bovine serum albumin (Armour) in perfusion fluid as the diluent. The bovine serum albumin was added in order to stabilize the activity of dilute solutions of streptolysin O. In a number of preliminary experiments it was ascertained that neither cysteine in the concentration employed, nor 0.1 per cent bovine serum albumin had any significant effect on the rate or amplitude of contraction of the isolated perfused heart.

EXPERIMENTAL

Loss of Contractile Force Induced in the Isolated and Perfused Hearts of Guinea Pigs, Rabbits, and Rats by Solutions Containing Streptolysin O

A number of experiments were first done in which the isolated and perfused hearts of guinea pigs were exposed to solutions containing various concentrations of streptolysin O. All told, the hearts of nineteen normal guinea pigs were removed and set up in the perfusion apparatus; each was given a single injection of streptolysin O in a dosage of 7.5 to 100 hemolytic units, diluted to 2 ml. in perfusion fluid containing 0.1 per cent bovine serum albumin. In every instance in which 15 or more units of streptolysin O was injected, a reduction in the contractile force of the heart, as evidenced by a decrease in the amplitude of contraction, was noted within ½ to 2 minutes following in-

jection. The amplitude of contraction decreased rapidly and progressively during the next 2 to 3 minutes, and the contractions ceased completely or nearly so usually within 5 minutes of the time of injection. In many cases there were no visible ventricular contractions whatever, and only a few flickering beats of the auricles could be seen upon close examination of the heart. The

TABLE I

Effect of Solutions Containing Streptolysin O on the Isolated and Perfused Hearts of Normal Guinea Pigs

Guinea pig No.	Streptolysin O*	Reduction in amplitude of contraction‡
	hemolytic units	
1	100	++++
2	100	++++
3	100	++++
4	100	+++
5	75	++++
6	75	++++
7	75	++++
8	75	+++
9	50	++++
10	50	+++
11	25	++++
12	25	++++
13	15	++++
14	15	+++
15	15	+
16	10	+
17	10	0
18	10	0
19	7.5	0

* Streptolysin O was activated with cysteine just prior to use. All dilutions were made in 0.1 per cent bovine serum albumin in the same fluid as was used for perfusion of the heart.

‡ Reduction in amplitude of contraction in this and in succeeding tables was graded on an arbitrary scale from 0 to ++++, in which 0 represents no change; +, up to 25 per cent reduction; ++, 25–50 per cent reduction; +++, 50–75 per cent reduction; and ++++, greater than 75 per cent reduction.

The guinea pigs in this experiment weighed 300 to 425 gm.

loss of contractile power induced by streptolysin O was not spontaneously reversible, for no recovery in the amplitude of contraction was observed even in those preparations in which perfusion was continued for as long as 75 minutes after injection. The results of these experiments are summarized in Table I, and a photograph of a typical tracing is shown in Fig. 1.

The rate of perfusion through the coronary arteries decreased sharply following injection of streptolysin O, particularly in those cases in which large doses were given. The flow began

to increase again within 1 to 2 minutes, and in many instances returned almost to the pre-injection levels within 5 minutes (see Fig. 2). In no case, however, did recovery of contractile force accompany the reestablishment of coronary flow. The relationship between loss of contractility and reduced coronary flow will be considered in more detail further on.

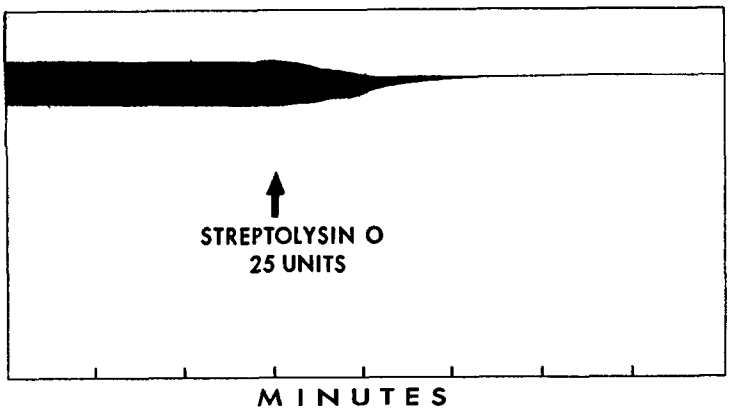

FIG. 1. Photograph of a record showing the effect of a solution containing streptolysin O on the amplitude of contraction of the isolated and perfused heart of a normal guinea pig. Each division on the time scale represents one minute.

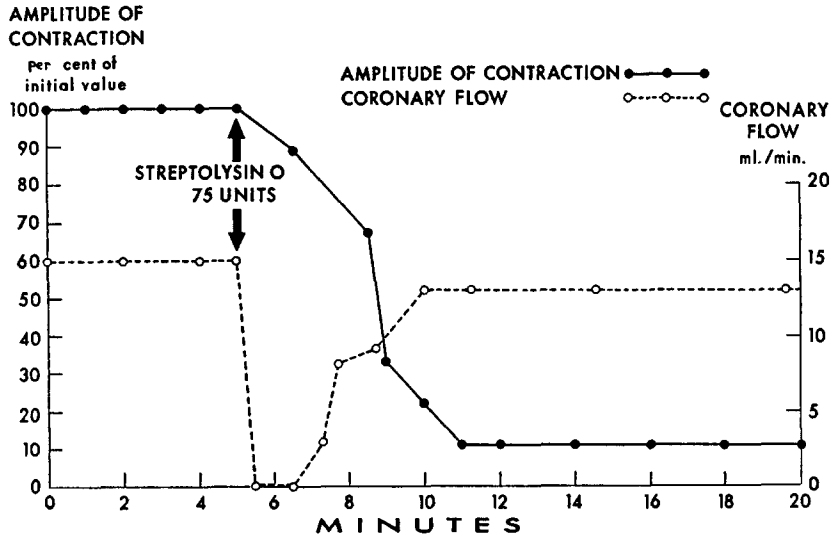

FIG. 2. The effect of a solution containing streptolysin O on the amplitude of contraction and rate of coronary flow of the isolated and perfused heart of a normal guinea pig.

In some cases there was a slight decrease in the heart rate, and occasionally also, arrhythmias occurred; these changes in rate and rhythm were transitory, however, and usually appeared toward the end of the reaction, just before contractions ceased entirely.

In several experiments the isolated and perfused hearts of guinea pigs were given repeated small injections of streptolysin O in doses of 5 to 10 units each. The effects of successive small doses upon the contractile force of the heart appeared to be cumulative, but the induction of a substantial decrease in myocardial contractile force under these conditions required a larger total quantity of streptolysin O than had been observed in single dose experiments.

A number of experiments were next performed in order to learn whether the effect of streptolysin 0 on myocardial contractility was limited to the guinea pig heart or whether the hearts of other mammals would be affected as well.

TABLE II

Effect of Solutions Containing Streptolysin O on the Isolated and Perfused Hearts of Normal Rabbits and Rats

Rabbit No.	Streptolysin O*	Reduction in amplitude of contraction
	hemolytic units	
1	750	++++
2	200	++++
3	100	++++
4	50	+++
5	25	+
Rat No.		
1	75	++++
2	50	++++
3	25	++++
4	10	+++
5	10	+++
6	5	++++
7	2	+

* Streptolysin O was activated with cysteine just prior to use and diluted in perfusion fluid containing 0.1 per cent bovine serum albumin.

The rabbits in this experiment weighed 2,900 to 3,300 gm., and the rats, 275 to 500 gm.

Accordingly, the hearts of five normal rabbits, and those of seven normal white rats, were removed and set up in the perfusion apparatus. Each rabbit heart was exposed to a single injection of streptolysin O in a dose of 25 to 750 hemolytic units while each rat heart was exposed to 2 to 75 units. In every case, except with the smallest doses employed, there resulted prompt and striking reduction in the amplitude of contraction (see Table II and Fig. 3), the sequence of events being almost precisely the same as that observed previously with the guinea pig hearts. The heart of an additional rat, not shown in the table, did not respond to 100 hemolytic units but underwent failure upon exposing it to a second dose of 100 units.

It was apparent from these studies that solutions containing streptolysin O were extremely toxic to the isolated perfused hearts of guinea pigs, rabbits,

and rats, and that brief exposure of such hearts to relatively low concentrations of the lysin resulted in a striking reduction in contractile force.

Evidence That the Cardiotoxic Factor Is Streptolysin O

The streptolysin O solutions employed in the foregoing experiments, although highly concentrated and purified to a considerable degree, were nevertheless known to contain other substances in addition to streptolysin O. Studies were therefore undertaken to determine whether the active cardiotoxic principle was in fact streptolysin O, or some other agent, or agents, contained

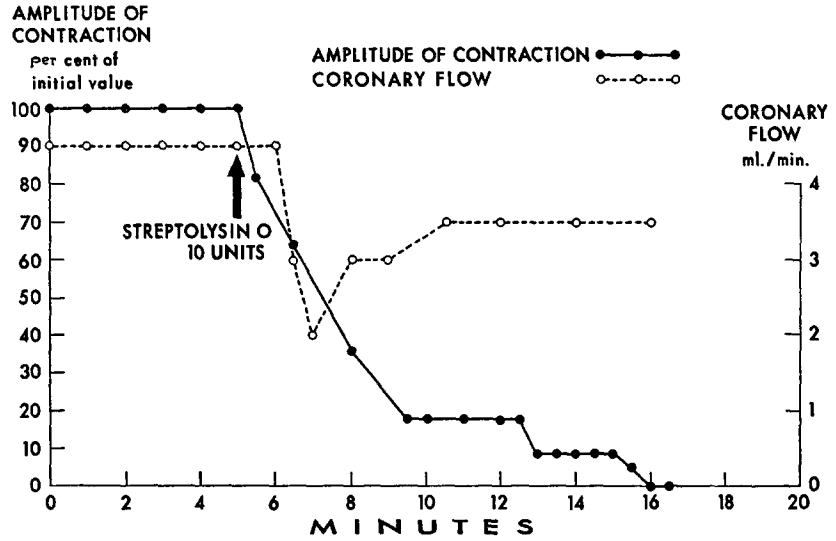

FIG. 3. The effect of a solution containing streptolysin O on the amplitude of contraction and the rate of coronary flow of the isolated and perfused heart of a normal rat.

in the solutions. In these investigations advantage was taken of the fact that the hemolytic activity of streptolysin O is known to require activation by cysteine or other sulfhydryl compounds, and that the hemolysin can be inhibited by cholesterol and by sera containing specific antibody.

Activation by Cysteine.—The hearts of two normal guinea pigs were removed and set up in the perfusion apparatus. One was exposed to 2 ml. of a solution containing 750 units of streptolysin O that had not been activated with cysteine; the other was given twice this dose, likewise unactivated. The only effect observed in either case was a slight reduction in coronary flow. Each heart was then given 2 ml. of a solution containing 75 units of streptolysin O activated with cysteine, and loss of contractile force ensued promptly in both cases (see Table III). Control experiments showed that cysteine alone in the concentrations used had no effect on the isolated heart.

Inhibition by Cholesterol.—A fine suspension containing 1 mg. cholesterol per ml. was pre-

pared according to the method of Cohen, Schwachman, and Perkins (10). To 4.5 ml. of the cholesterol suspension were added 0.2 ml. of cysteine-activated streptolysin O, 0.5 ml. of 1 per cent bovine serum albumin, and 0.05 ml. of 1 per cent cysteine solution. Another mixture identical with the first but lacking cholesterol was prepared at the same time. The amount of streptolysin O added in each case was such that the final concentration was 300 hemolytic units in 2 ml. of the mixture. Both mixtures were allowed to stand at 20°C. for 5 minutes; they were then centrifuged briefly and the clear supernates tested in the isolated and perfused hearts of two guinea pigs. The solution containing the cholesterol-treated streptolysin was administered first and was found to have no effect on the amplitude of contraction, whereas the solution containing comparable amounts of streptolysin O but no cholesterol caused a precipitous decrease in the amplitude of contraction within 2 to 3 min-

TABLE III

Effect of Activation with Cysteine, and Incubation with Cholesterol and Specific Antibody on the Cardiotoxic Action of Solutions Containing Streptolysin O

Guinea pig No.	Streptolysin O*	Treatment of Streptolysin O prior to injection	Reduction in amplitude of contraction
	hemolytic units		
20	750	Not activated	0
	75	Activated	++++
21	1500	Not activated	0
	75	Activated	++++
22	300	Cholesterol	0
	300	No cholesterol	++++
23	300	Cholesterol	0
	300	No cholesterol	++++
24	75	Antibody, 20 units	0
	75	No antibody	++++
25	375	Antibody, 50 units	0
	75	No antibody	++++

* Streptolysin O was activated with cysteine unless specifically stated to the contrary.

Antibody—horse antistreptolysin globulins, 20,000 units per ml., diluted to stated concentration in perfusion fluid.

utes (see Table III). It is of interest that the cholesterol-treated streptolysin O solution in each case caused a moderate reduction in the rate of coronary flow.

Inhibition by Antiserum.—Equal parts of horse antistreptolysin globulins[1] diluted to contain 20 Todd antistreptolysin units per ml. (11) and a solution of cysteine-activated streptolysin O containing 75 hemolytic units per ml. were mixed and permitted to stand at 20°C. for 5 minutes. Two ml. of this mixture administered to the isolated and perfused heart of a normal guinea pig had no effect on the amplitude of contraction, though there was a moderate reduction in coronary flow. This injection was then followed by 2 ml. containing 75 units of the same streptolysin O solution but having no antibody. Reduction in amplitude of contraction ensued promptly (see Table III). In another experiment, 375 hemolytic units of streptolysin O incubated with 50 units of antibody was likewise found to be ineffective, but a second injection containing 75 units of the same streptolysin O solution without anti-

[1] Antistreptolysin globulins, 20,000 units per ml. Batch No. 7, 19-9-47. Serum Institute, Carshalton, England.

body caused a marked decrease in contractile force. In control experiments, normal horse serum diluted to the same extent as the antibody preparation did not interfere with the cardiotoxic activity of streptolysin O.

Halbert, using agar diffusion techniques, has obtained evidence for the presence of erythrogenic toxin in the concentrated streptolysin O preparations used in these studies (12). Hence the effect of highly purified erythrogenic toxin on the isolated heart was investigated. In addition, experiments were done with streptolysin O produced by a group C streptococcus and with streptolysin O purified by electrochromatographic means.

Erythrogenic Toxin.—The erythrogenic toxin, kindly supplied by Dr. A. M. Pappenheimer, Jr., was prepared from *Streptococcus pyogenes* Strain 594B according to the method of Hottle and Pappenheimer (13), and contained one million skin test doses per ml. Administration of as much as 300,000 skin test units of erythrogenic toxin to the isolated guinea pig heart had no effect on the heart rate, amplitude of contraction, or the rate of coronary flow.

Other Streptolysin O Preparations.—Streptolysin O was obtained from a culture of group C streptococcus, Strain H46A, according to the procedure described by Rowen and Bernheimer (14); the streptolysin O activity of the lot employed was 167,000 hemolytic units per ml. In addition, streptolysin O from a culture of group A streptococcus was fractionated electrochromatographically (15); the fraction tested had 15,000 hemolytic units per ml. Both preparations of streptolysin O when injected into isolated guinea pig hearts in doses of 50 to 150 units caused a sharp decrease in amplitude of contraction and complete loss of contractile force within a few minutes. By contrast, fractions from the same electrochromatographic separation which had no hemolytic activity had no effect on the isolated heart.

It is evident from these experiments that the agent responsible for the loss of contractile force of the isolated and perfused heart resembled streptolysin O in that it required prior activation with cysteine and it was inhibited completely by cholesterol and by specific antibody. Moreover, streptolysin O prepared from a group C streptococcus and streptolysin O fractionated electrochromatographically were both active. Erythrogenic toxin, one of the known impurities in the streptolysin O solutions, was completely inactive. Taken together, the findings provide strong evidence that streptolysin O itself is the active cardiotoxic factor.

The Effect on Coronary Flow of Solutions Containing Streptolysin O

The injection of solutions containing streptolysin O into the isolated and perfused hearts of guinea pigs, rabbits, and rats was followed in almost every case by a sharp drop in the rate of coronary flow, often to zero. In some cases, particurarly when larger doses of streptolysin O were given, the coronary flow dropped precipitously to zero and remained there. In most cases, however, the reduction was transitory, lasting but a minute or two; the flow then rose progressively, but did not quite reach the preinjection levels (Figs. 2 and 3). There appeared to be no direct relationship between the reduction in rate of

coronary flow and the reduction in the amplitude of contraction, for in those instances in which the coronary flow returned toward normal there was no corresponding increase in the amplitude of cardiac contraction. Moreover, a moderate drop in coronary flow also occurred when the cardiotoxic action of streptolysin O was inhibited by cholesterol or by antiserum even though there was no reduction in the amplitude of contraction. In order to obtain further evidence that the effect of streptolysin O solutions on ventricular contractility was not secondary to the reduction in coronary flow, several experiments were carried out in which the flow of perfusion fluid to the isolated heart was clamped off completely for 5 minutes and the effect on contractile force studied.

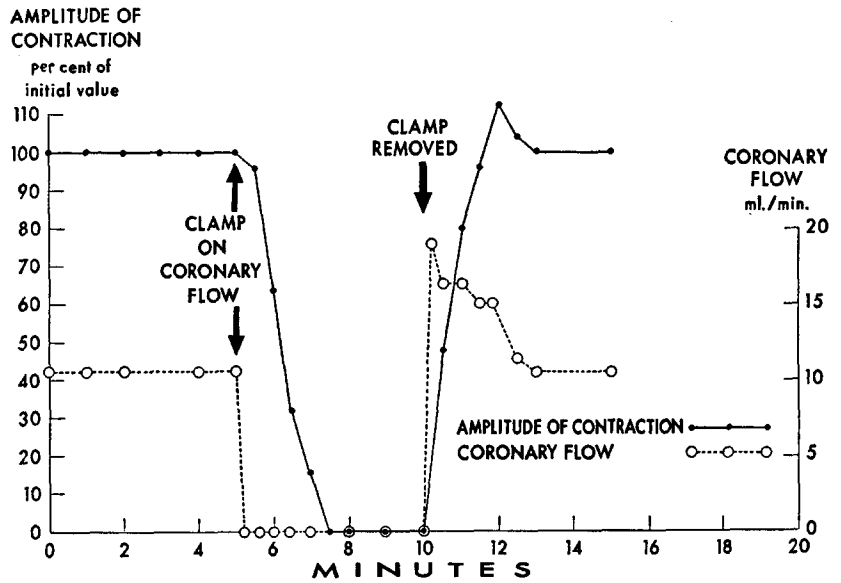

FIG. 4. The effect of clamping off the coronary flow completely for 5 minutes on the amplitude of contraction of the isolated and perfused heart of a normal guinea pig.

The amplitude of contraction declined progressively following application of the clamp, and contractions ceased entirely within 2 to 3 minutes. When the clamp was released, however, the contractions resumed immediately, and the amplitude of contractions increased rapidly, always attaining the baseline level and sometimes even exceeding it. An example of such an experiment is given in Fig. 4. In the case of the streptolysin O injections, by contrast, in no instance was there comparable recovery of amplitude of ventricular contraction despite the return of coronary flow almost to the preinjection level. The observations suggest that solutions of streptolysin O contain one or more substances that are capable of causing marked spasm of the coronary arteries. This effect, however, appears to be independent of the effect of streptolysin O on cardiac contraction.

DISCUSSION

Streptococcal preparations such as those employed in these studies are known to contain a number of high molecular weight substances not all of which have been isolated and identified. Among these substances is an agent here shown to bring about striking loss of myocardial contractile power when perfused through the isolated hearts of guinea pigs, rabbits, and rats. This agent is nondialyzable, requires activation by the sulfhydryl compound cysteine, is inhibited by a small amount of cholesterol, and is neutralized by antistreptolysin globulins but not by normal serum. The only extracellular product of streptococcal growth known to possess all these properties is the oxygen-labile hemolysin, streptolysin O. Furthermore, four different batches of streptolysin O having widely different hemolytic activities were found to have the same effect on the isolated and perfused heart when diluted to the same number of hemolytic units. Finally, studies of streptolysin O preparations fractionated electrochromatographically showed that those fractions having streptolysin O activity brought about loss of contractile power of the isolated heart, whereas those devoid of streptolysin O activity were not effective in this regard. It can therefore be concluded that the agent affecting contractility of the myocardium is either streptolysin O or a substance indistinguishable from it by present criteria.

The effect of streptolysin O on the mammalian heart as here demonstrated differed in one important respect from that on the frog heart noted in previous investigations (4, 5). Toxicity for the frog heart was evident only when the streptococcal preparation was administered in two doses. The first dose released an inhibitor from the heart, while the second acted on the heart, now deprived of its inhibitor, to cause systolic standstill. In experiments with guinea pig hearts, it was never possible to demonstrate a requirement for two administrations of streptolysin O even though a number of attempts to do so were carried out by repeatedly exposing the same heart to subtoxic concentrations. The guinea pig heart therefore appears to differ from that of the amphibian in lacking a protective inhibitor against the action of the toxin.

The major effect of streptolysin O observed in these studies was a striking and irreversible reduction in the force of cardiac contraction, indicating that the site of action was primarily the heart muscle itself. Whether this reduction in myocardial contractile force comes about because of interference with a metabolic pathway essential for the production of energy, or by alteration of cell membranes, or by changes in the contractile elements within the muscle, or by other means cannot be determined at the present time. In addition to their effect on the myocardium, the solutions injected caused a marked though transitory decrease in the rate of coronary flow. It is likely that the solutions contained one or more agents, besides streptolysin O, capable of inducing spasm of the coronary arteries; for slight to moderate reductions in coronary flow

were also observed in those experiments in which streptolysin O was inhibited by cholesterol or by specific antibody.

It is noteworthy that administration to the isolated guinea pig or rat heart of solutions containing as little as 15 hemolytic units of streptolysin O caused unequivocal manifestations of toxicity. Since 15 hemolytic units represents not more, and probably considerably less, than 1 μg. of streptolysin O, and since the period of time for the test material to traverse the heart was brief—some 30 to 120 seconds—it is evident that streptolysin O is an exceedingly potent cardiotoxic agent.

SUMMARY

When solutions containing minute quantities of streptolysin O, previously activated with cysteine, were added to a salt and glucose medium that was being perfused by way of the coronary arteries through the vigorously beating isolated hearts of guinea pigs, rabbits, and rats, the amplitude of myocardial contraction decreased abruptly, often to zero. The effect was usually accompanied by a diminution in the flow of perfusion fluid, probably owing to spasm of the coronary arteries. The diminution in coronary flow was transitory, however, lasting as a rule only 2 or 3 minutes, while the loss of myocardial contractile power was permanent and irreversible. No decrease in myocardial contractility was obtained when the streptolysin O was not previously activated with cysteine, or when the streptolysin O was inhibited by cholesterol or by specific antibody, the findings pointing to streptolysin O as the active cardiotoxic agent.

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