STUDIES ON THE CARDIOTOXICITY OF STREPTOLYSIN O

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Streptolysin O is one of the numerous exotoxins secreted by the streptococcus of Group A. The best known property of this toxin is the haemolytic effect exerted on mammalian red cells. Haemolysis only occurs when the toxin is in the reduced form. Streptolysin I in the oxidized form is haemolytically inactive, but can be activated by the addition of reducing agents such as sulphydryl compounds.

Streptolysin O is immunologically antigenic, and a high proportion of patients with streptococcal infection show a strong antibody response during convalescence. Administration of streptolysin to animals—for example, rabbits—is regularly followed by synthesis of specific antibody; and both the oxidized and reduced forms combine with specific antibody.

When injected intravenously in adequate doses, streptolysin O produces death in most laboratory animals. Research by Kellner, Bernheimer, Carlson & Freeman (1956) and by Halbert, Bircher & Dale (1961) has shown that the lethal effect of streptolysin may be attributed to its toxic action on the heart. The object of the present investigation was to analyse with different technical procedures the modalities and the mechanism of the cardiotoxic effects of streptolysin O.

METHODS

Streptolysin O has not been obtained in pure form. In these experiments crude or partially purified preparations of streptolysin of different origins and greatly varying haemolytic activity were used. A minimal haemolytic dose of streptolysin ($MHD50$) is defined as the quantity of streptolysin causing lysis of 50% of the fresh rabbit red cells contained in 0.5 ml. of a standardized buffered suspension in 45 min at 37° C. It has been calculated that 1 $MHD50$ represents about 4×10^{-3} μg of protein.

Most preparations of streptolysin were in the oxidized state and were reduced by mercaptoethanol in phosphate buffer at a final concentration not exceeding M/11. Crude streptolysin was dialysed overnight at 4° C before use in experiments *in vitro*.

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The following streptolysins were used:

(1) Crude oxidized, lyophilized streptolysin prepared for us by the Mérieux Institute, Lyon, used for the bulk of the experiments and cited as S^M (titre 10,000 $MHD50$/phial).

(2) Crude naturally reduced lyophilized streptolysin, batch No. 2864, prepared by Dr. Wahl of the Pasteur Institute, Paris, and cited as S^L (titre 1,000 $MHD50$/phial).

(3) Partially purified, naturally reduced streptolysin prepared by Dr. Alouf of the Pasteur Institute, Garches, available only in small quantity and cited as S^{G1} (titre 2,500 $MHD50$/ml.).

(4) Purified streptolysin (a few milligrams only) obtained from Dr. Halbert of the National Institute of Health (U.S.A.) and cited as S^{HAL}. Its properties have been described in detail elsewhere (Halbert, Bircher & Dahle, 1961) (titre 160,000 $MHD50$/mg protein).

The toxic action of streptolysin on heart muscle has been investigated in three different ways: (1) *in vivo*, by a study of the effects of the intravenous injection of streptolysin on the e.c.g.; (2) *in vitro*, by a study of the effect of streptolysin on the contractility and electrical activity of auricular myocardium of animal and human origin and on the isolated perfused rat heart; (3) by a study of the oxygen consumption of auricles from animals receiving a lethal dose of streptolysin.

Cardiotoxicity in vivo

Most of the studies were carried out in both male and female Swiss or Balb mice weighing about 20 g. The lethal dose was established by intravenous injection of the naturally reduced or activated toxin. For electrocardiography, mice were pretreated with anticoagulant—either coumadin (Warfarin, the sodium salt of 3(acetonyl benzyl)-4 hydroxycoumarin) 0.50 mg/20 g the day before, or calcium heparinate (calciparine) 0.1 ml./20 g intravenously 1 hr before—and were then anaesthetized with urethane 20 mg/20 g intraperitoneally. Needle electrodes pinning the animal by the paws were connected to the leads of a Sanborn 150 e.c.g. pre-amplifier. S^M was injected intravenously in doses varying from 500 to 5,000 $MHD50$/20 g. Control experiments were carried out with non-active oxidized S^M and the reducing agent.

Similar experiments were performed on adult Wistar rats.

Cardiotoxicity in vitro

Cardiotoxicity was studied on the following preparations *in vitro*.

A. (1) Isolated right rabbit auricle. After exsanguination of the animal by carotid section, the right auricle was rapidly dissected, care being taken not to injure the nodal area, and plunged in ice-cold Krebs solution, which brings about an immediate arrest of the contractions. It was found that the subsequent recuperation of the spontaneous contraction was more regular after this treatment. The auricle was tied, apex upwards, to a mobile glass organ holder in a 25 ml. bath of oxygenated Krebs solution at 37° C, and its contractions were recorded on a rotating smoked drum.

(2) Strips of human auricular myocardium from both right and left auricles, were obtained from patients undergoing heart operations. The strips were plunged at once in cold Krebs solution, rapidly mounted in the oxygenated Krebs bath at 37° C, and stimulated with supramaximal stimuli from a rectangular wave stimulator, at the rate of. 1/sec. The contractions were recorded on a smoked drum.

(3) Biauricular preparations of the rat heart. Adult white rats weighing about 250 g were anaesthetized with ether. The heart was removed, the auricles were dissected under magnifying binocular lenses in oxygenated Krebs medium at 37° C, and mounted in an organ bath of 25 ml. oxygenated Krebs at 37° C between two heart clips, one on each auricular apex, and the contractions were recorded on a Sanborn 150 carrier pre-amplifier using transducer model FTA-1-1.

B. Action potentials of the spontaneously beating right rabbit auricle, mounted apex upwards as in A (1), were recorded as follows: A fine light insulated copper wire soldered to the base of a metallic heart clip attached to the apex of the auricle served as one electrode, the second being a thicker silver wire twisted around the organ holder near the point of fixation of the auricle; the two electrodes were connected to a Sanborn twin-viso apparatus. The action potential

was recorded at appropriate intervals during several hours by moving the auricle out of the liquid medium for a few seconds (not more than 10) ; the organ was re-immersed immediately afterwards.

C. Perfusion of the isolated rat heart. Male white rats (200–300 g) were anaesthetized with urethane 200 mg/100 g intraperitoneally and heparin 2.5–5 mg/200 g was then injected in the dorsal vein of the penis. The heart was removed as rapidly as possible, mounted by the aorta on a plastic cannula adapted to the perfusion apparatus, and perfused with Krebs solution at 37° C at a constant hydrostatic pressure of 50 cm ; a mixture of 95% oxygen and 5% carbon dioxide was constantly bubbled through the perfusion bottles. Contractions were recorded on a Sanborn carrier pre-amplifier using transducer model FT-100-1.

The e.c.g. of the perfused heart was recorded simultaneously. The contact electrodes, initially metallic, were placed one on the right auricle, the other on the left ventricle. In later experiments brush electrodes were used for recording by a system of radio emitter and receiver connected to the Sanborn.

Streptolysin O was injected into the plastic perfusion tube at a level from which it took on average 14 sec to reach the heart. A small volume, usually 0.2 ml. and not more than 0.5 ml., was injected. Control experiments were carried out with oxidized streptolysin, streptolysin neutralized by antibody, antibody alone and reducing agent alone.

Oxygen consumption of auricular myocardium

This was studied on preparations of both auricles of mice dissected free of extraneous tissue under magnifying lenses. Dissection was initially carried out in oxygenated Krebs solution at 37° C, but for the bulk of the experiments it was performed in Krebs solution without oxygen at 4° C on a platform surrounded by ice. Auricles from control mice and mice killed with streptolysin were placed in Warburg flasks containing 2.5 ml. of Krebs solution in the main chamber and 0.2 ml. of 20% potassium hydroxide in the central well and kept in the cold room before use. Each flask, containing one preparation, was immediately connected to a micromanometer, plunged in the water bath at 37° C and oxygen passed for 10 min. After equilibration for 10 min, readings were made every 10 min for at least 4 hr, maximal agitation (160 movements/min) being maintained. At the end of the readings, the auricles were removed from the flasks, wiped carefully and at once weighed individually. A summation graph was made for each and its oxygen consumption expressed in μ/mg of fresh tissue/hr.

RESULTS

Lethal action of streptolysin O

General symptoms. The lethal dose of streptolysin O as well as the symptoms developed varied somewhat with the preparation of streptolysin. In the case of S^M, a single dose of 500 $MHD50/20$ g killed all the animals in about 3 min ; with ten times this dose, the animals succumbed in 45 sec (Table 1). Death was preceded by convulsions. Another significant symptom was a pink frothy discharge from the nose and marked respiratory distress. The autopsy of the animals showed massive thrombosis in the chambers of the heart and in the great veins, and signs of acute pulmonary oedema. With larger doses the heart was found at systolic standstill.

These observations do not furnish conclusive evidence as to the cause of death. Investigations were therefore extended in order to verify the cardiotoxic properties of streptolysin I and to establish the mechanism of the lethal action of this toxin. They included electrocardiographic recordings *in vivo* as well as the study of the effect of the toxin on isolated cardiac muscle strips *in vitro*.

Electrocardiographic study. The e.c.g. of mice in our experimental conditions was found to be quite similar to that of man. Lead 2 was found to indicate the highest voltage. The mean heart rate was 509 beats/min with somewhat large variations (360–720).

In order to eliminate the effects of intra-vascular and intra-cardiac thrombosis, observed after the intravenous injection of S^M, animals were pretreated, as described above, with two different types of anticoagulant in doses high enough to prevent intravascular clotting. Pretreatment with anticoagulant did not prevent death of the animal after intravenous streptolysin and only lengthened mean survival time.
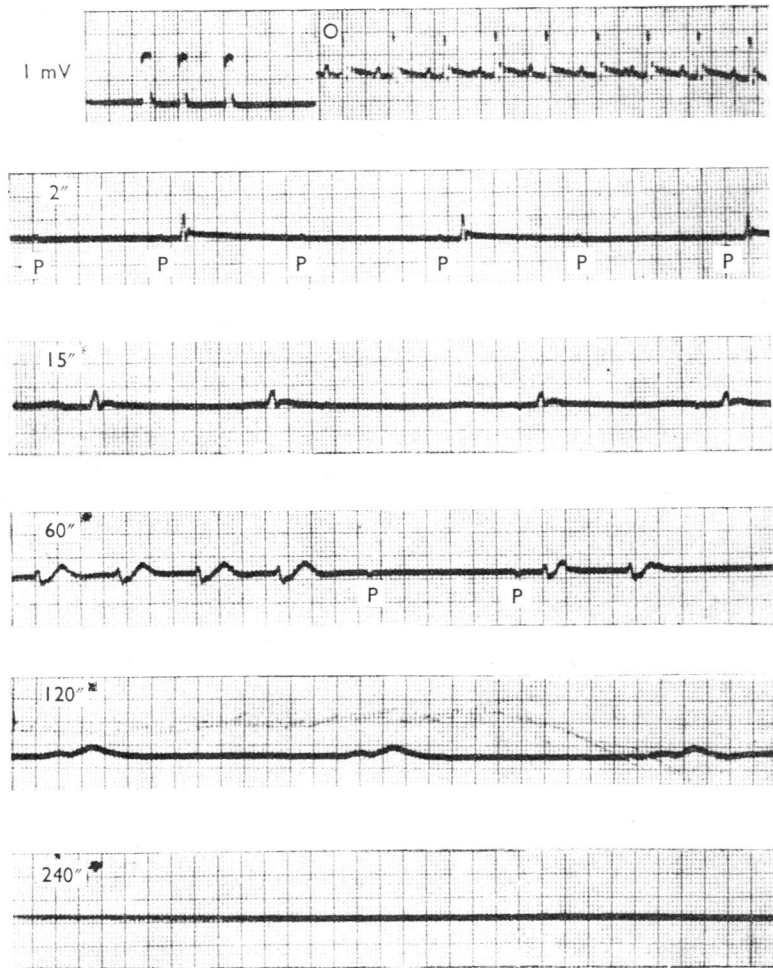

Fig. 1. Effect of intravenous injection of activated streptolysin Mérieux on the e.c.g. of the Balb mouse (dose: 5,000 $MHD50/20$ g). Section of a continuous tracing taken before (O) and at 2, 15, 60, 120 and 240 sec after the injection. Lead 2 ; speed 100 mm/sec.

The e.c.g. of pretreated mice receiving a large lethal dose of streptolysin showed marked alterations. With a dose of 5,000 *MHD*50/20 g the effects were instantaneous. Immediate and intense bradycardia was followed in some cases by temporary arrest of the heart. Reappearance of electrical activity a few seconds later showed continuing bradycardia, atrio-ventricular block and great reduction of voltage. The *P* waves and the *QRS* complex, flattened out and deformed, disappeared progressively. Fibrillation occurred in some cases. All electrical activity of the heart ceased in about 5 min after the injection (Fig. 1).

With smaller doses, 1,000–2,000 *MHD*50/20 g, electrical arrest occurred after longer and more variable intervals (6–18 min or more). Bradycardia, reduction of voltage and widening of the *QRS* complex and the *T* wave were seen to occur more progressively. Atrio-ventricular block and dissociation, temporary or persistent, was observed in nearly all cases.

These observations point to a direct toxic effect of streptolysin on the heart. The reduction of voltage and alteration of the *QRST* complex suggest an effect on the ventricular muscle itself, while atrio-ventricular block and dissociation would implicate involvement of the conducting tissue. This conclusion is confirmed by the studies *in vitro* on the isolated auricle as well as on the perfused rat heart.

Effects of streptolysin O on the isolated heart muscle strip

The *in vitro* effect of streptolysin O on auricular myocardium has been studied as regards contractile force and rhythm as well as regards the changes in the action potential of the isolated right auricle. The isolated right auricle of the rabbit and the bi-auricular preparation of the rat heart, beating spontaneously in oxygenated saline medium, showed a marked decrease in the force of contraction after the administration of crude (S^M, S^G) or pure (S^HAL) streptolysin in a concentration of 50–200 *MHD*50/ml. or more. Apart from some slowing there was generally little effect on the rhythm. Small strips of human
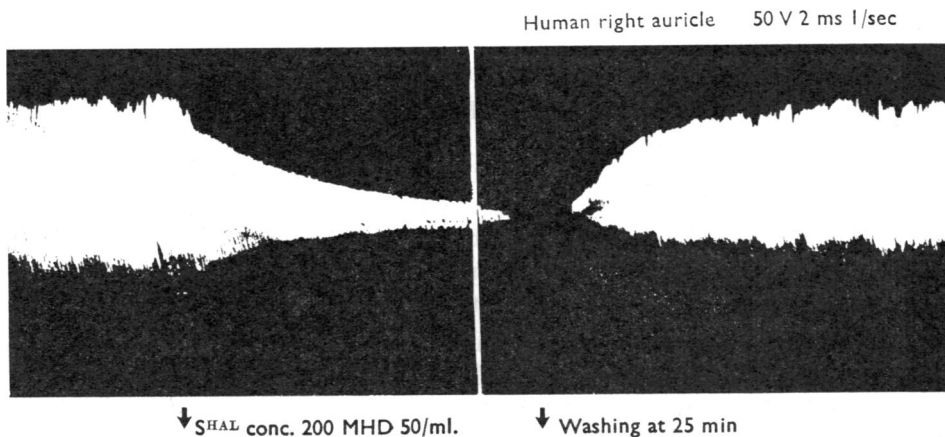

Human right auricle   50 V 2 ms 1/sec

↓ S^HAL conc. 200 MHD 50/ml.   ↓ Washing at 25 min

Fig. 2. Effect of streptolysin Halbert (S^HAL) on the contractions of human auricular myocardium driven electrically.

auricular myocardium, electrically stimulated, showed a similar inhibition of contractile force when subjected to the action of active streptolysin (Fig. 2). The degree of inhibition varies greatly, however, with different preparations cf streptolysin. In most cases the effect is slow and progressive, reaching a maximum in 15–20 min (Fig. 2).

These streptolysins were all activated chemically before use, being almost entirely in the oxidized state. On the other hand, with at least one preparation of streptolysin (S^{G1}) massive inhibition was obtained within a few seconds, being maximal in 5 min. This preparation contained streptolysin in the naturally reduced state and so required no activation. The action potential of the isolated rabbit auricle was also found to be rapidly reduced and distorted by S^{G1}. Total protection was obtained by prior neutralization of S^{G1} by antibody.

The variability of the *in vitro* effects of different preparations of streptolysin O requires explanation. We have some evidence that streptolysin O may exist in biologically more or less active forms. Survival time with a naturally reduced streptolysin S^L was five times shorter than with S^M (Table 1). Reduced streptolysin O, oxygen labile by definition, is very rapidly destroyed when added to the highly oxygenated medium required by the beating auricle. Studies of its rate of destruction showed that the naturally reduced form is more resistant to oxidation. Another possibility is the presence of some substance which stabilizes the reduced form or enhances its action in some preparations of streptolysin.

TABLE 1

TOXICITY OF INTRAVENOUS STREPTOLYSIN IN Balb MICE

	Dose of streptolysin in $MHD50/20$ g body weight	No. of survivors	Average survival time (sec)
S^M	250	50/50	Indefinite
	500	0/21	153
	1,000	0/31	124
	2,000	0/45	72
	5,000	0/19	45
S^L	500	0/9	103
	1,000	0/34	25

Effect of streptolysin on the perfused isolated rat heart

The perfusion of as little as 50 $MHD50$ of activated streptolysin O was seen to bring about rapid arrest of the beating rat heart. In all cases the ventricles stopped beating before the auricles. With increasing doses (50–300 $MHD50$) the mean time of ventricular arrest shortened (Table 2). With doses of more than 300 $MHD50$, the ventricles stopped beating in about 30 sec, whatever the dose. If the time taken by the streptolysin to reach the heart—that is, 14 sec—is subtracted from this period, it seems that an overwhelming dose of streptolysin caused ventricular arrest within a minimal delay of 16 sec.

The e.c.g. of the perfused beating rat heart recorded by surface electrodes showed a small, clear-cut P wave and QRS spike. In the electrode position adopted, the two waves were in opposite directions, the P being a small upward deflection about 1 mm high, the QRS a downward one generally about 10 mm (1 mV = 0.5 cm). Ventricular arrest after administration of streptolysin was preceded by progressive diminution of the height of the QRS wave, the latter disappearing with the last ventricular contraction (Fig. 3). In

some cases inversion of the spike was noted before its disappearance. The *P* wave was found to persist much longer than the *QRS* wave. Disappearance of the *P* wave was taken to mean auricular arrest.

TABLE 2

INTERVAL BETWEEN THE INFUSION OF STREPTOLYSIN O AND THE VENTRICULAR ARREST OF THE ISOLATED PERFUSED RAT HEART

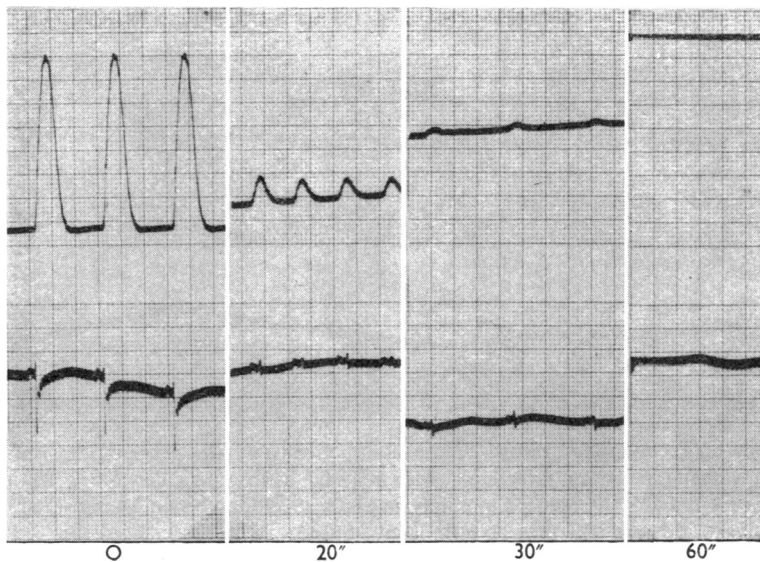| Dose of streptolysin	No. of hearts perfused	Mean time of ventricular arrest (sec)
<50 *MHD*50	5	Not obtained in four cases; 246 for the fifth
50–150 *MHD*50	8	186 (not obtained in one case)
150–300 *MHD*50	8	106 (not obtained in one case)
300–600 *MHD*50	3	32
600–900 *MHD*50	2	30
1,750 *MHD*50	1	29

Fig. 3. Effect of 81 *MHD*50 of reduced streptolysin O on the contractions (upper trace) and e.c.g. (lower trace) of the isolated perfused rat heart. Sections of a continuous record taken before and at 20, 30 and 60 sec after the perfusion of streptolysin. Speed: 25 mm/sec.

Auricular arrest was followed by the e.c.g. as well as by direct observation of the auricles themselves. Auricles were found to be much more resistant to streptolysin than ventricles, and auricular arrest was obtained in only half the cases followed (nine out of eighteen). It could take place as long as 45 min after the ventricular arrest. In other cases the *P* waves persisted indefinitely, the auricles beating, however, at a much slower rhythm than normally—30–60 beats/min, instead of 200–240. In some cases, after a long interval of complete ventricular arrest, varying from 10 min to as much as 1.5 hr, ventricular contractions reappeared. Slow and irregular at first, they subsequently became more regular. Their height, however, always remained very much less than initially—that is, before the administration of streptolysin—although they became quite normal in other respects in two experiments.

It was observed that the effects of streptolysin on the rat heart were of three different types, roughly corresponding to the dose.

(1) With less than 50 MHD50, four out of five hearts did not stop beating. Some diminution of the force of contraction occurred with slowing. The most remarkable feature in this group, however, was arrythmia. This could appear as early as 4 min after the perfusion of streptolysin, and was seen to persist for as long as 1 or 2 hr after a very small dose of streptolysin (10 *MHD*50 or less). Momentary arrest of the heart would be followed by a series of contractions of diminishing height, the first being the highest, followed by another pause. From tracings taken at greater speed, and from electrocardiography, it became apparent that the slowing and irregularities were caused, in some cases at least, by atrio-ventricular block (Fig. 4). Similar arrythmia was observed in the two cases where ventricular arrest was not obtained with larger doses of streptolysin.
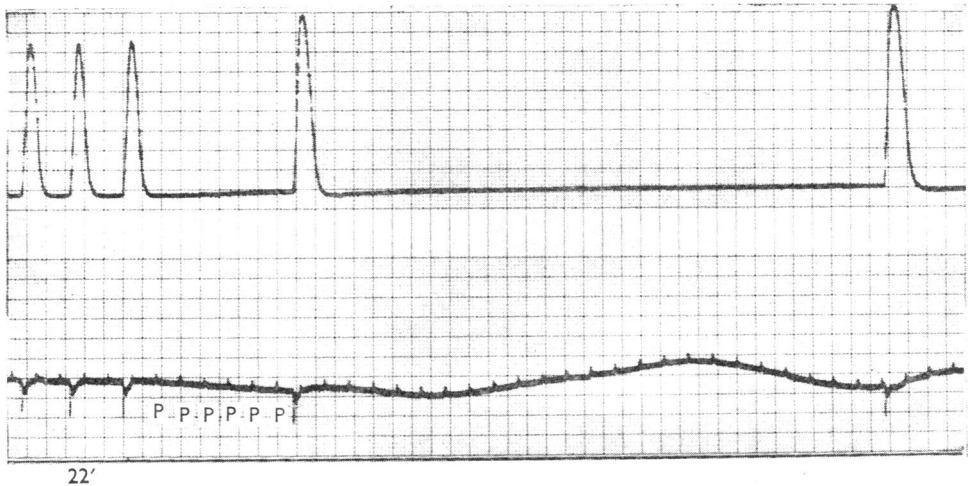

Fig. 4. Section of a continuous record of the contractions and the e.c.g. of the isolated perfused rat heart showing persistent arrythmia with marked atrio-ventricular block 22 min after the perfusion of 7 *MHD*50 of reduced streptolysin Halbert. Block began 14 min after the perfusion of streptolysin and was still present 1 hr later. Speed: 25 mm/sec.

(2) With doses of 50–300 MDH50, ventricular arrest was obtained in nearly all cases, preceded by a progressive decrease in the force of the contractions. Auricular arrest occurred in half the cases. Diverse types of arrythmia were observed in two-thirds of the experiments. Apart from a few extra-systoles and some bouts of fibrillation, the most frequent anomaly was progressive atrio-ventricular block. This could be 2 : 1, 3 : 1, 4 : 1 and finally became complete.

(3) With large doses of 300–600 MHD50 or more, the whole heart, auricles as well as ventricles, was seen to stop beating within 16 sec after the toxin reached the heart ; the main feature was a rapid diminution of the height of the contractions as well as that of the *QRS* spike, both finally disappearing almost simultaneously with the *P* wave.

An interesting phenomenon, particularly visible in this and the preceding dose range, was a marked increase of tonus as indicated by a rising base line, which continued after the ventricles had ceased to beat and seemed to indicate a kind of contracture of the ventricles (Fig. 3).

It should be emphasized that all these toxic effects were observed only with reduced streptolysin O and were absent when the toxin was in the oxidized state.

Effect of antibody

The toxic effects of streptolysin O on the perfused rat heart were completely prevented by prior neutralization of the toxin with antibody. Control experiments were carried out with antibody alone, as well as reducing agent alone.

Effect of streptolysin O on the respiration of cardiac muscle

The most evident effect of streptolysin O on auricular myocardium *in vitro* is a diminution of its contractile force. Muscular contraction is obviously dependent on energy, which is liberated by mechanisms of cellular oxidation. It seemed worth while, therefore, to investigate the oxygen consumption of heart muscle.

The oxygen consumption of the auricular myocardium of mice (and rats) was studied in the Warburg apparatus. The normal consumption under different physiological conditions was first established. For whole mouse auricles dissected at 4° C, it was found to be 3 μl./mg of fresh tissue/hr. This consumption was quite stable for at least 4 hr (Table 3).

TABLE 3

EFFECT OF ACTIVATED STREPTOLYSIN MERIÉUX ON THE OXYGEN CONSUMPTION OF MOUSE AURICLES
Dissection at 4° C.

Treatment of mice	Oxygen consumption (μl./mg fresh tissue/hr)			
	First hr	Second hr	Third hr	Fourth hr
Killed by i.v. injection of air (controls)	2·87	3·12	3·15	3·08
Killed by i.v. S^M 1,000 $MHD50/20$ g	2·07	2·63	2·64	2·63
Effect	−27·8%	−15·7%	−16·2%	−14·6%
Killed by i.v. S^M 2,000 $MHD50/20$ g	1·97	2·34	2·32	2·28
Effect	−31·3%	−25%	−26·3%	−25·9%
Killed by i.v. S^M 5,000 $MHD50/20$ g	1·48	1·94	2·08	2·18
Effect	−48·4%	−37·8%	−33·9%	−29·2%

Auricles from mice killed by an intravenous injection of streptolysin O showed a markedly reduced consumption of oxygen (Table 3). This reduction was shown to be proportional to the dose of streptolysin, but not in a linear way. Moreover, prior neutralization of streptolysin O by antibody prevented the death of the injected animal. The auricles from these protected animals, killed by an intravenous injection of air, showed a normal oxygen consumption (Fig. 5).

We mentioned before that S^M produced intravascular and intracardiac thrombosis. It was therefore of great importance to determine the possible interference of the thrombosis phenomenon with the oxygen consumption of myocardium. Three different anticoagulants—heparin, calcium heparinate and coumadin—were used in doses sufficient to prevent blood clotting after an injection of streptolysin. In the animals thus treated death nevertheless occurred with the same doses of streptolysin. The oxygen consumption of the auricles taken from the animals pretreated with anticoagulants, although less depressed than in animals which had not received anticoagulant, was much lower than normal. That death in itself was not the cause of this decrease was proved by the study

of animals killed by an intravenous injection of air. The auricles of these animals showed an oxygen consumption similar to that of auricles from hearts removed under ether anaesthesia.
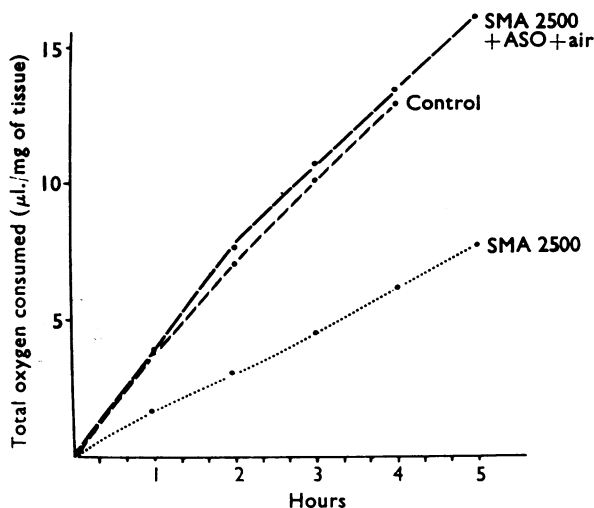

Fig. 5. Inhibitory effect of reduced streptolysin Mérieux (SMA) on the oxygen consumption of mouse auricles and absence of this effect with streptolysin previously neutralized by antistreptolysin O (ASO). Dissection at 37° C under oxygen.

Control experiments were carried out with animals given the reducing agent (mercaptoethanol) alone, and the anticoagulant alone. The oxygen consumption was found to be normal in both cases. A final control was carried out with animals given oxydized inactive streptolysin. They were subsequently killed by an injection of air, and the oxygen consumption of their auricles was shown to be normal or, in some cases, slightly higher.

The conclusion which emerges from this group of experiments is that neither the other toxins present in the crude preparation of streptolysin O nor the reducing agent used were responsible for the inhibition of cellular respiration observed. As death only occurred if the streptolysin was reduced and active, and was then accompanied by a diminution of cellular respiration proportional to the dose given, it is likely that both phenomena are attributable to the streptolysin itself.

DISCUSSION

From the data reported, it seems that streptolysin O has manifold toxic properties. It is haemolytic, thrombotic and cardiotoxic. Our studies are chiefly concerned with the last point. The lack of a pure toxin complicated the problem. The available streptolysins contain various quantities of other substances which may possess their own toxicity. Crude streptolysin contains DPNase, which acts by liberating nicotinamide from the molecule of DPN. It has been suggested by Carlson *et al.* (1956) that DPNase may

also have cardiotoxic properties, but these were not evident under our experimental conditions. Oxidized streptolysin Mérieux contains no less than 44,520 units of DPNase/phial; the animal supported the intravenous injection of as much of this DPNase as 20,000 units/20 g without evident effect on the e.c.g., or on the auricular consumption of oxygen. On the other hand, as little streptolysin O as 500 *MHD*50/20 g was sufficient to bring about the death of the animal, preceded by marked alterations in the e.c.g.

The action of streptolysin O *in vivo* is complex. It modifies the coagulation factors favouring intravascular thrombosis. It severely damages the capillary wall, thus producing acute pulmonary oedema, and, as appears from our preliminary work with Dr. Hollmann in electron microscopy (unpublished), massive subendothelial and interstitial oedema of the heart. As we have shown, there is a direct toxic effect on the heart muscle itself, evident even with minute amounts (4×10^{-2} μg). When injected in lethal doses, streptolysin profoundly altered the intra-cardiac conduction processes, leading to atrio-ventricular block and finally to fibrillation. The rapidity of its action is remarkable. The studies on isolated cardiac strips indicated that streptolysin O exerts a potent depressive effect on the contractile myofibrils, which was partially irreversible. Experiments performed on the isolated perfused rat heart reproduced more or less completely the disorders observed *in vivo*, thus confirming a direct toxic effect of streptolysin on the heart. Finally, the profound reduction of the oxygen consumption of cardiac muscle caused by streptolysin suggests that this substance affects enzyme systems which are implicated in the processes of cellular respiration. Further experiments are necessary to determine its precise site of action.

Two other points deserve attention. First, the cardiotoxic effect was a property of reduced streptolysin O and was lacking totally when the toxin was in the oxidized form, at least in the doses used by us. Moreover, prior neutralization by antibody abolished the characteristic cardiotoxic properties of streptolysin *in vitro* as well as *in vivo*, which indicates that the toxic moiety is one of the active antigenic determinants. Second, our experiments do not exclude the possibility that the cardiotoxic effects of streptolysin O are part of a generally cytoxic property of this toxin. In any case, the toxic effects on the heart seemed sufficient in themselves to bring about the rapid death of the animal.

SUMMARY

1. The intravenous injection of reduced streptolysin O brings about rapid death of small laboratory animals. The nature of the toxic effect on the heart has been investigated *in vivo* and *in vitro*.

2. Marked inhibition of the contractile and electrical activity of auricular myocardium of human and animal origin was observed *in vitro* after the addition of reduced streptolysin O to the bathing fluid.

3. The oxygen consumption of auricular myocardium from mice killed with streptolysin was found to be significantly depressed.

4. The isolated perfused rat heart was rapidly brought to a standstill by reduced streptolysin O, with electrocardiographic changes such as *QRS* inhibition, atrio-ventricular block, extrasystoles and fibrillation similar to those seen *in vivo* after an intravenous injection of streptolysin.

5. It is concluded that reduced streptolysin O has a direct toxic effect on the heart, in particular on the junctional tissue and the ventricles.

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