Studies on the mechanism of the lethal toxic action of streptolysin "O" and the protection by certain antiserotonin drugs

S. P. HALBERT, R. BIRCHER, and E. DAHLE *New York, N. Y.*

Intraperitoneal pretreatment of mice with several antiserotonin drugs of different chemical families has been shown to afford protection against the acute intravenous lethal toxicity of streptolysin "O." The protection was manifested by indefinite survival of a small percentage of mice receiving a streptolysin dose which killed all of the control animals, as well as by a significant prolongation of the survival time in those which eventually died. Some of the antiserotonin agents had no streptolysin protective effects. No correlation could be observed between the antiserotonin potency of these drugs, as measured with rat tissues by in vitro and in vivo techniques and the streptolysin protective capacity. Other drugs revealed no significant protection against acute streptolysin toxicity. These included parasympathomimetics and inhibitors, sympathomimetics and inhibitors, antihistamines, an anesthetic (barbiturate), an analgesic (salicylate), a calcium chelator (EDTA), and a corticosteroid (prednisolone). Calcium ions appeared to afford a very slight protection against the lethal toxicity of streptolysin "O." An antiserotonin agent (UML-491) with significant streptolysin protective potency did not protect mice against the lethal or convulsive effects of saponin.

Although it is firmly established that streptococcal infections initiate rheumatic fever, the mechanisms involved are rather obscure. Hypersensitivity factors,[1] autoimmune processes,[2] or an unusual intoxication[3, 4] have been suggested; and cogent arguments can be mustered to support each viewpoint. With regard to the latter, there is abundant circumstantial evidence compatible with the idea that streptolysin "O" may be the streptococcal product involved in the development of cardiac lesions. This evidence has been summarized in a previous publication.[4]

From the Departments of Ophthalmology Research and Pharmacology, Columbia University, College of Physicians and Surgeons.

This report represents paper VII of this series and was supported by research grants from the Office of Naval Research, New York Heart Association, and the Life Insurance Medical Research Fund.

Received for publication Oct. 3, 1962.

Accepted for publication Nov. 12, 1962.

In earlier reports from this laboratory, the value of immunodiffusion techniques for the analysis of human infections was pointed out.[5-11] When such an approach was applied to streptococcal disease, it was found that numerous extracellular products were released in vivo by these microorganisms, but very few cellular components reached antibody-forming sites in significant amounts. Considerable effort was devoted to separating the extracellular substances, and a number of them were purified to a considerable degree, among them streptolysin "O."[7, 8] Because of an element of uncertainty regarding the identity of the active principle in various cardiotoxic effects obtained by other authors with crude preparations,[12-16] in vivo pharmacologic studies with these purified streptolysin fractions were carried out electrocardiographically.[4] These experiments demonstrated that the cardiotoxic effects in rabbits were extraordinarily rapid. Animals given 3 to 5 times an LD_{50} dose of either a Group A or Group C streptolysin preparation revealed striking electrocardiographic changes, often ending in ventricular fibrillation within 4 seconds after completion of the rapid intravenous injection.

The extreme rapidity of death caused by this toxin in acute rabbit experiments prompted the speculation that the mechanism of death under these circumstances might be due to the release of small molecular weight pharmacologically active substances in vivo. The present study was undertaken in order to explore this possibility. This article deals with the effects of various drugs of known pharmacologic properties on the acute lethal toxicity of streptolysin "O." It was assumed that protective or enhancing effects by some of these agents might shed light on the mechanisms involved in the lethal activity.

Materials and methods

The Group A and Group C streptolysin "O" preparations were obtained by continuous flow electrophoresis and hydroxyl apatite column chromatography as reported previously.[7, 8] The Group A material was more potent than the Group C, the hemolytic titers being 160,000 and 60,000 units per milligram protein, respectively. The crude concentrates from which they had been derived contained at least 20 and 9 antigens for the Group A and C organism, respectively. In both instances, quantitative immunodiffusion assays of the fractions used here demonstrated that after purification, streptolysin "O" had been isolated from almost all the other components of the original complex mixtures. Traces of diphosphopyridine nucleotidase were present in both, but an earlier study had shown that this enzyme was not responsible for the cardiotoxic effects observed.[4] In addition, the group A streptolysin contained minute traces of a second, unidentified contaminant. Activation of the streptolysin "O" solutions was carried out with cysteine, in 0.1M phosphate buffer at pH 7.2, as in an earlier report.[6]

The albino mice used were strain CF1* female, 20 to 22 grams in weight. The rabbits were female albinos, 2.8 to 3.2 kilograms, and were all obtained from one dealer.

The mouse electrocardiograms were obtained with a Grass polygraph model 5.† The rabbit electrocardiograms were taken with a Sanborn Twin-Viso Cardiette‡ and the amplitude and paper speed were used as previously.[4]

The drugs employed in the mouse experiments included the following:

Parasympathomimetics

physostigmine salicylate§

*Carworth Farms, New City, N. Y.
†Grass Company, Quincy, Mass.
‡Sanborn Company, Cambridge, Mass.
§Merck & Co., Inc., Rahway, N. Y.

acetylcholine chloride*
Parasympathetic inhibitors
atropine sulfate*
n-ethyl-nortropine-benzhydryl ether-HBr (UK-738)†
Sympathomimetic
epinephrine‡
Sympathetic inhibitor (beta receptors)
dichloroisoproterenol (DCI)§
Antiserotonin agents†
d-lysergic acid diethylamide tartrate (LSD-25)
1-methyl-d-lysergic acid-butanolamide bimaleate (UML-491)
methylergonovine maleate
(d-lysergic acid-butanolamide bimaleate) (Methergine)
1-methyl-dihydro-d-lysergic acid-butanolamide tartrate (DH-UML)
d-brom-lysergic acid diethylamide (BOL-148)
the pyrazole derivative, 1(N-methyl-piperidyl-4') 3-phenyl-benzyl pyrazolone-5 (KB-95)
Antihistamines
Tripelennamine – HCL (Pyribenzamine)¶
9-(N-methyl-piperidyliden-4') thioxanthene maleate (BP-400)†
Tranquilizers
Reserpine phosphate,¶ a serotonin and catechol amine liberator
Thioridazine hydrochloride
(Mellaril)† a phenothiazine derivative; 2 methyl-mercapto-10-(2[n-methyl-2-piperidyl]ethyl) phenothiazine HCl.
This agent also possesses moderate antiserotonin activity.
Histamine liberator
48/80‖
Calcium ions
calcium glucono-galacto-gluconate†
Calcium chelating agent
ethylenediaminetetraacetate (EDTA)
Corticosteroid
prednisolone phosphate#
Analgesic, antipyretic
sodium salicylate
Anesthetic
sodium pentobarbital (Nembutal)**
Serotonin creatinine complex††

In all instances, with the exception of reserpine, the drugs were dissolved in 0.1M phosphate pH 7.2 at the appropriate concentrations. Reserpine was dissolved in water. In most cases, the dosages of the drugs used represented about one fifth of the reported intravenous LD_{50} values. In the mouse experiments, they were administered intraperitoneally 15 to 30 minutes prior to the challenge intravenous dose of streptolysin. Two drugs, reserpine and 48/80, were given 24 hours prior to the challenge. Two others (adrenaline and acetylcholine), because of the rapidity of their effect in vivo, were given 2 minutes prior to the challenge dose.

*Merck & Co., Rahway, N. J.
†Sandoz Pharmaceuticals, Hanover, N. J.
‡Parke, Davis & Company, Detroit, Mich.
§Eli Lilly & Co., Indianapolis, Ind.
¶Ciba Pharmaceuticals, Summit, N. J.
‖Burroughs Wellcome & Co., Tuckahoe, N. Y.
#E. R. Squibb and Sons, New York, N. Y.
**Abbott Laboratories, Inc., North Chicago, Ill.
††California Biochemical Corporation, Los Angeles, Calif.

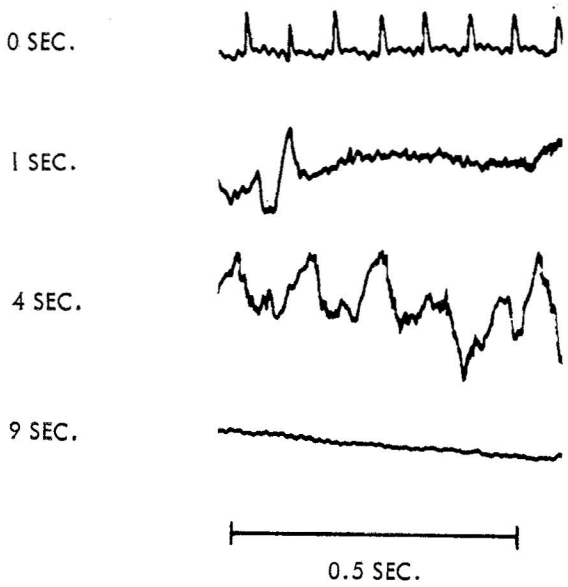
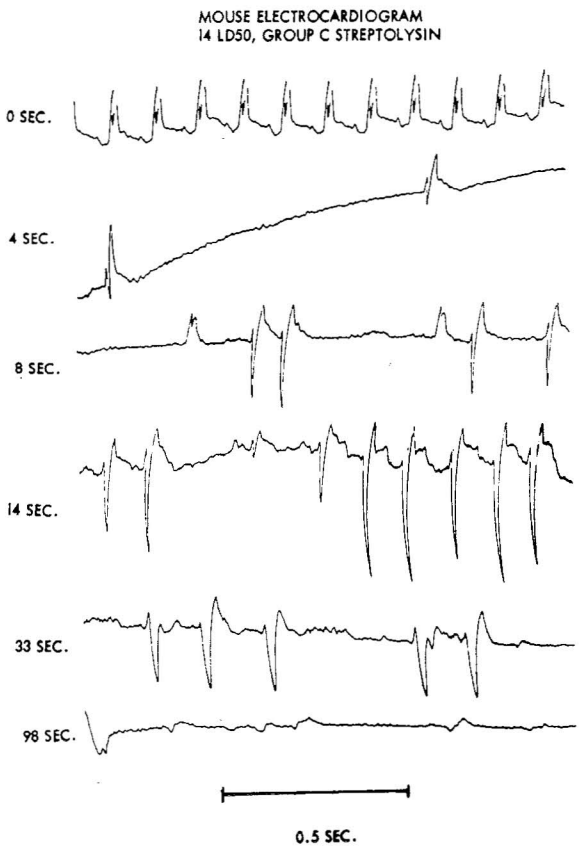
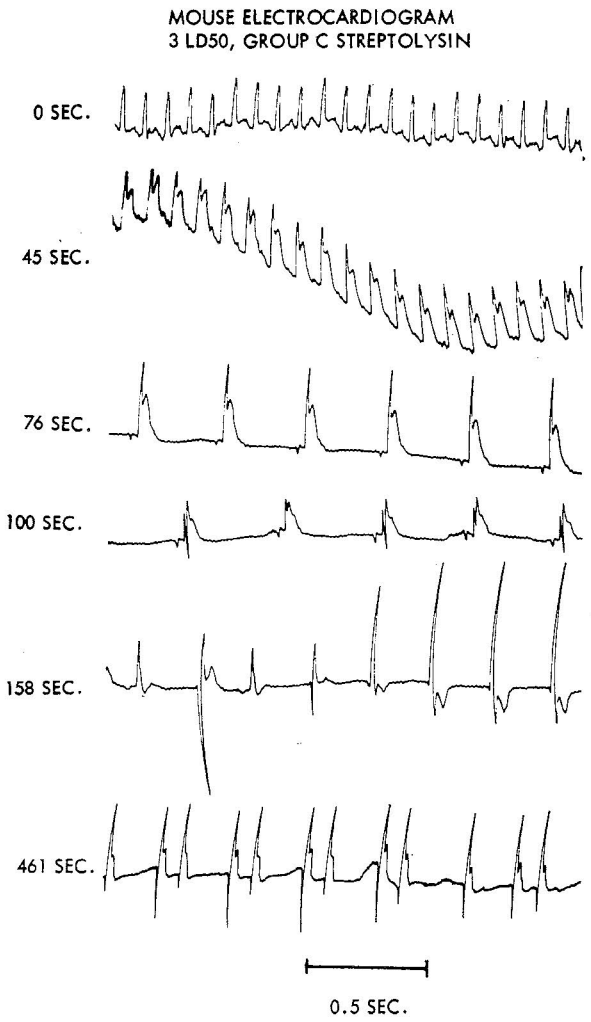


Fig. 1. Electrocardiograms of mice receiving group C streptolysin "O" intravenously. *A*, 28 LD_{50}.



B, 14 LD_{50}.



C, 3 LD_{50}.

Results

The clinical manifestations of a lethal intravenous dose of reduced purified streptolysin "O" in mice were quite similar to those seen in rabbits.[4] Generalized tonic and clonic convulsions followed the injection within as short a time as 15 seconds or less, up to 10 to 15 minutes. In rare instances with smaller doses, convulsions developed 1 to 3 hours afterward, but most animals which survived 3 minutes survived indefinitely. Following the convulsions, cessation of respiration and micturition rapidly occurred. Similar patterns were obtained earlier by other workers,[17-19] in spite of the fact that comparatively crude preparations had been used.

The relative toxic potencies of the two streptolysin fractions for mice are shown in Table 1. The Group C material revealed an LD_{50} of 10.3 μg dry weight (6.1 μg protein) while the Group A showed an LD_{50} of 2.1 μg dry weight or

protein. The similar steepness of the toxicity curves in both instances may be pointed out, as further evidence that the lethal agents were the same in both preparations. In addition to the similar clinical pattern of death in mice and rabbits, electrocardiograms performed on the mice revealed that the electrical activity of the heart becomes as rapidly disorganized in this species as in rabbits[4] following streptolysin injection. Several examples of these effects are seen in Fig. 1A, B, and C, with approximately 28, 14, and 3 LD_{50} of the Group C streptolysin. As in rabbits, it appeared that the convulsions always followed the electrocardiographic disturbances.

Of the various drugs tested, only some of those possessing antiserotonin activity revealed a clear protective action against the lethal toxicity of streptolysin. One of the most effective of these agents was the lysergic acid derivative, UML-491 (1-methyl-d-lysergic acid-butanolamide bimaleate). When given at a dose of 1 mg. (48 mg. per kilogram) intraperitoneally 15 to 30 minutes prior to an intravenous challenge dose of approximately 2 × LD_{50} of streptolysin, the protection was manifested by a significant prolongation of life in most of the animals, and an indefinite survival of a small, variable percentage. The animals which survived the streptolysin challenge were dazed for several hours, but by the next morning were almost as active as normal mice. They usually survived for at least 1 week with no further gross evidence of ill effects. All of the controls succumbed to this quantity of streptolysin, the vast majority within a period of 1 or 2 minutes. The results are summarized in Fig. 2. The related antiserotonin agent, BOL-148 intraperitoneally (24 mg. per kilogram) also was significantly protective, while DH-UML intraperitoneally (24 mg. per kilogram) showed only a suggestion of increased survival time. Methylergonovine maleate intraperitoneally (24 mg. per kilogram) was essentially nonactive, the animals dying in the same short intervals as the controls. It may be recalled that the latter is the nonmethylated derivative of UML-491, while DH-UML is hydrogenated at the 9–10 position. Both of these alterations apparently decrease the streptolysin protective activity. The related hallucinogenic compound, LSD-

Table I. Lethal toxicity of purified Group A and Group C streptolysin "O" for mice

Streptolysin	*Dose (μg dry weight)*	*Results*										*LD_{50} (μg)*
Group C	19.0	D 1'*	D 1'	D 1½'	D 1½'	D 1½'	D 2'	D 2'	D 2'	D 2'	D 2'	
	12.5	D 1'	D 1½'	D 2'	D 2'	D 4'	D 5'	D 9'	S	S	S	10.3
	6.3	S	S	S	S	S	S	S	S	S	S	
Group A	10.0	D ¼'	D ¼'	D ¼'	D ½'	D ½'	D ½'	D ¾'	D ¾'	D 1'	D 1'	
	5.0	D ½'	D 1'	D 1'	D 1½'	D 1½'	D 2'	D 2'	D 2½'	D 3'	D 3'	2.1
	2.5	D 3'	D 7'	D 8'	D 13'	D 180'	D 180'	D 180'	S	S	S	
	1.3	S	S	S	S	S	S	S	S	S	S	

All of the injections were given in 0.1 ml., and the streptolysin was reduced to the activated state by cysteine.

*D 1' died in 1 minute, D 1½' - died in 1½ minutes, etc., S survived for 3 days.

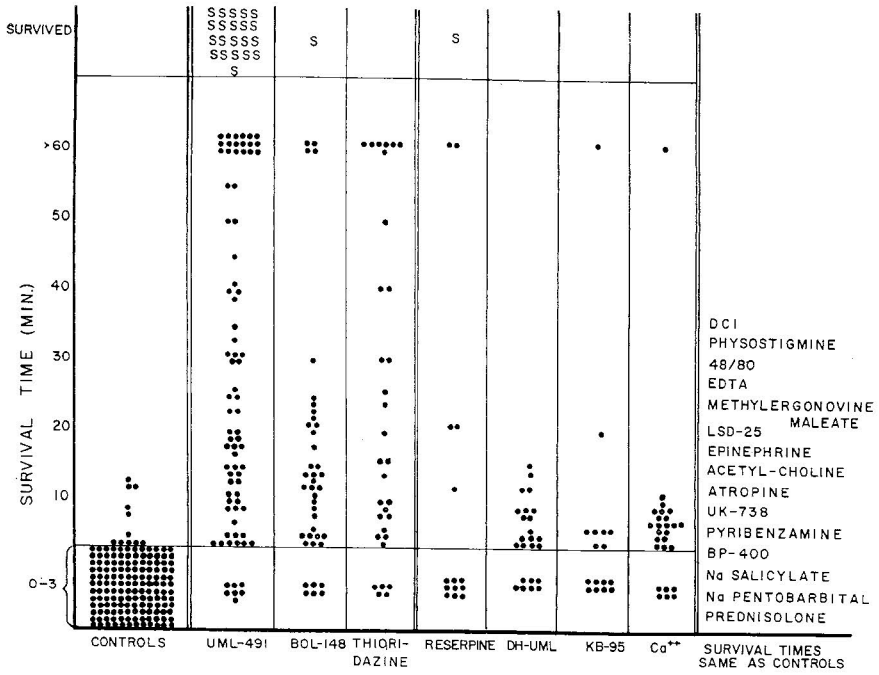


Fig. 2. Effects of various drugs on the acute lethal toxicity of streptolysin "O." In all instances, the streptolysin challenge (2 × LD_{50}) was administered intravenously at an appropriate interval following the intraperitoneal injection of the drug. Each point represents one mouse. s = survival time for at least 3 days.

25 intraperitoneally (24 mg. per kilogram) also had no effect on the streptolysin toxicity.

The phenothiazine tranquilizer, thioridazine hydrochloride (24 mg. per kilogram) possessed appreciable protective properties. In addition, calcium ions, furnished as calcium glucono-galacto-gluconate (110 mg. Ca^{++} per kilogram) showed slight protection, as did reserpine intraperitoneally (3.8 mg. per kilogram). The latter is known to deplete tissue stores of serotonin and catecholamines.

As may be seen in Fig. 2, sodium pentobarbital, the calcium chelator (EDTA), parasympathetic and sympathetic inhibitors, parasympatho- and sympathomimetic agents, antihistamines, the histamine liberator 48/80, sodium salicylate, and prednisolone, all had no effects whatever on the survival times of streptolysin challenged animals. In some instances, death may have been accelerated by these agents, but the short intervals involved made meaningful data difficult to obtain.

In the case of sodium pentobarbital anesthesia, where no protection was afforded, the sequence of clinical events was somewhat different from that seen in unanesthetized mice receiving the same challenge dose of streptolysin. Under this anesthesia, all mice showed a rapid cessation of respiration within 3 to 10 seconds following the streptolysin injections. Within 1 to 2 minutes, they re-

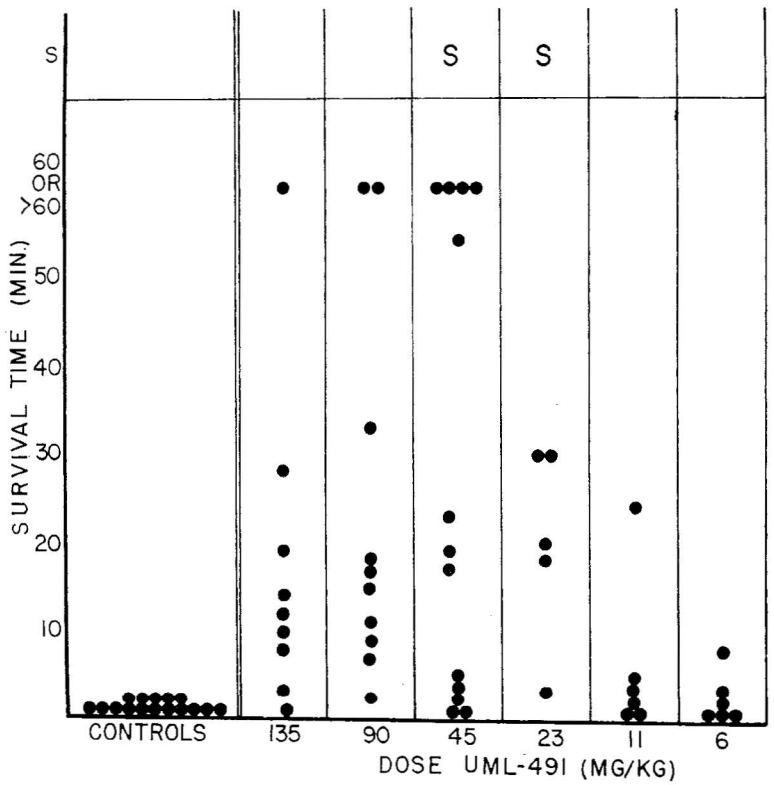


Fig. 3. Dose assay of the antiserotonin drug, UML-491, required for a protective effect against 2 × LD_{50} of streptolysin "O" in mice.

vealed weak, generalized muscular contractions, followed by micturition. Normal mice without anesthesia, and those receiving all the other drugs tested, revealed convulsions first, which were then followed by cessation of respiratory movements. The reason for this different sequence is not clear.

An assay of the antiserotonin drug, UML-491, needed to produce a significant streptolysin protective response was carried out with the results shown in Fig. 3. It may be seen that between 11 and 23 mg. per kilogram given intraperitoneally was required to reveal a detectably increased survival time upon intravenous streptolysin challenge. The smaller vaue is one seventeenth of the intravenous LD_{50} of UML-491 for mice.

A small series of tests was carried out in rabbits to determine whether the serotonin antagonist, UML-491, could also prolong survival in this species following a small lethal challenge dose of streptolysin "O." For this purpose, in most instances, 1.7 LD_{50} (1.25 mg. dry weight) of the reduced streptolysin "O" (group C) was given intravenously 10 to 15 minutes following an intravenous injection of this drug at 2 mg. per kilogram. This dosage represents one fourteenth of the intravenous LD_{50} of UML-491 for rabbits. Electrocardiograms were taken simultaneously. All 10 controls died within 1½ to 3 minutes, as shown in

Fig. 4, while 10 of the 16 treated rabbits survived from 37 to 240 minutes. The significance of these results is quite clear, but it is possible that the drug may not be as effective in this species as in mice. In the rabbits which were protected against streptolysin toxicity, the electrocardiograms often showed variable and transitory disturbances during the prolonged survival period. An example of this is shown in Fig. 5, which also shows the typical electrocardiographic pattern[4] of the untreated control rabbit which received the same streptolysin solution. The UML-treated rabbit, on the other hand, revealed a variety of transitory electrocardiographic disturbances, including bigeminal rhythm, ST segment depression, and ST segment notching, alternating with apparently normal electrocardiographic tracings (18 minutes and 74 minutes). Unfortunately, terminal electrocardiograms were not obtained, but death occurred in this animal after 120 minutes.

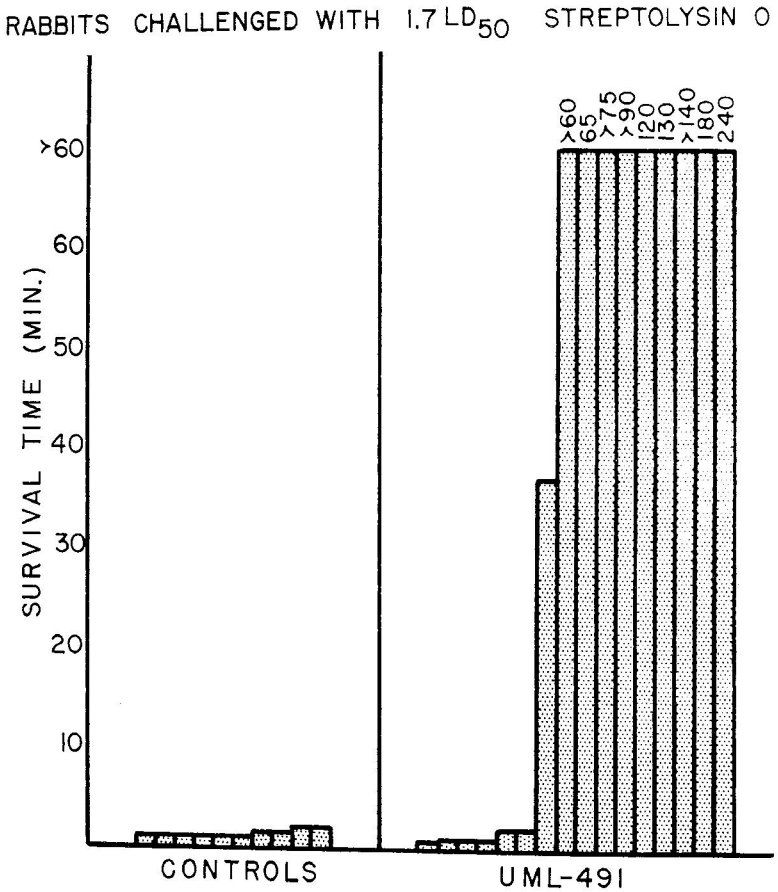


Fig. 4. Protective effect of the antiserotonin drug, UML-491, against the lethal property of streptolysin "O" for rabbits. The drug (2 mg. per kilogram) was administered intravenously 10 minutes prior to the intravenous challenge with streptolysin "O." The numbers above the columns represent the survival time in minutes.

When the various drugs which were tested for streptolysin protective properties were also examined for their antiserotonin potencies, it could be seen that no correlation between the two was clearly evident (Table II). It is of some interest, however, that the lysergic acid derivatives, devoid of streptolysin protection in mice, were those that seemed to show some central nervous system excitatory effects (LSD-25, DH-UML, and methylergonovine maleate) as judged by gross visual impression. On the other hand, those with streptolysin protective properties appeared to cause some sedation in the mice (UML-491, BOL-148). Thioridazine HCl, the phenothiazine tranquilizer, also produced an apparent inhibitory motor effect as seen visually. The dose of reserpine chosen caused such an extreme tranquilization that the animals appeared almost motionless when they were challenged with streptolysin. The protective activity of this agent was rather limited, however, compared to those mentioned above. It thus appears that central nervous depression by itself was not an important factor in this response. Perhaps even more convincing evidence of this was the lack

Table II. Antiserotonin potency and streptolysin protective properties of various drugs

Drug	*Mouse LD_{50} intravenously mg./Kg.*	*Antiserotonin activity*		*Streptolysin protective potency*	*Chemical family*
		In vitro (rat uterus)*	*In vivo* (rat paw edema)*		
UML-491	185	400	400	++	Lysergic acid derivative
BOL-148	20	103	29	+	Lysergic acid derivative
Thioridazine HCl	53	7	—	+	Phenothiazine derivative
KB-95	145	140	20	± ?	Pyrazole derivative
LSD-25	46	100	100	0	Lysergic acid derivative
DH-UML	41	184	194	± ?	Lysergic acid derivative
Methyl ergonovine maleate	85	61	152	0	Lysergic acid derivative
BP-400	23	121	3	0	Thioxanthene derivative
UK-738	14	3	—	0	Nortropine derivative
Reserpine	8.3 (i.p.)	Depletes serotonin stores		±	Yohimbine-related alkaloid

*In relation to LSD-25 = 100

Table III.

Saponin dose (mg.)	*Group*	*Results*									
1.5*	Control	D 10″	D 10″	D 10″	D 15″	D 15″	D 20″	D 20″	D 20″	D 25″	D 30″
	UML-491	D 5″	D 5″	D 5″	D 5″	D 5″	D 10″	D 10″	D 10″	D 10″	D 10″
1.0	Control	D 10″	D 15″	D 15″	D 15″	D 20″	D 40″	DOV	DOV	DOV	DOV
	UML-491	D 10″	D 10″	D 10″	D 10″	D 10″	DOV	DOV	DOV	DOV	DOV
0.25	Control	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV
	UML-491	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV
0.125	Control	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	S	S
	UML-491	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV	DOV

Lack of effect of the antiserotonin drug UML-491 on saponin toxicity in mice. All animals received 1 mg. of drug intraperitoneally 15 to 25 minutes prior to saponin challenge intravenously. D10″ = died in 10 seconds; DOV = died overnight; S = survived 3 days, etc.

*All mice dying rapidly convulsed after 5 to 15 seconds shortly after which death occurred. Many of those which died overnight after receiving 1.0 mg., convulsed briefly within 1 minute, and then recovered temporarily.

of detectable effects of deep barbiturate anesthesia on the streptolysin toxicity.

Studies with saponin, a streptolysin mimicking agent. Saponin, a plant glycoside (or mixture) from the soapwort plant, is known to be rather similar to streptolysin "O" in many of its toxic properties, as has been shown by the studies of Cantoni and Bernheimer.[27] For this reason, tests were carried out on the effects of UML-491 on intravenous saponin toxicity in mice. The clinical pattern of death in mice receiving large lethal doses of saponin was similar to that seen with streptolysin "O." Convulsions usually occurred sooner with saponin, and sometimes respirations did not cease for some while after the convulsions. In addition, smaller lethal doses of saponin caused death overnight with few immediate gross symptoms. As can be seen in Table III, pretreatment of the mice with UML-491, at a dose of 1 mg. (48 mg. per kilogram) intraperitoneally 15 to 25 minutes prior to the challenge intravenous dose of saponin, had no detectable effect on the toxicity. Neither the convulsions nor the lethal outcome was ameliorated by this drug.

Serotonin toxicity. Since many of the active drugs possessed appreciable antiserotonin properties, it was felt of importance to test the toxicity of serotonin itself in the mice and rabbits used in these studies. Four mice given an intravenous dose of 5 mg. of serotonin creatinine sulfate complex per 0.5 ml. (240 mg. per kilogram) showed immediate convulsions. One died in 20 seconds, while the other 3 recovered after a temporary period of prostration. Four mice receiving 2 mg. of this serotonin solution showed no ill effects.

Four rabbits were given intravenous injections of this serotonin preparation, and were followed electrocardiographically. The doses administered were 40, 50, 60, and 100 mg. in a solution of 20 mg. per milliliter (13, 17, 20, and 33 mg. per kilogram, respectively). All rabbits survived without gross ill effects but revealed very temporary sinus bradycardia lasting from 5 to 30 seconds. In the rabbit receiving 50 mg., one extra systole was seen during this period.

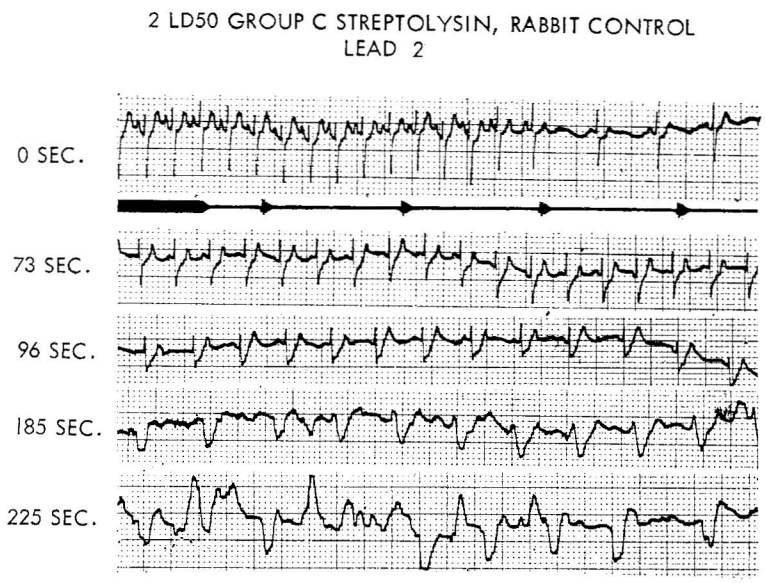


Fig. 5, A, Electrocardiogram of control rabbit receiving 1.7 × LD_{50} streptolysin "O."

Discussion

The data presented in this report make it clear that certain drugs are capable of interfering with the acute lethal toxicity of streptolysin "O" in mice and rabbits. The agents active in this regard also possessed varying degrees of antiserotonin properties, but there appeared to be little correlation between the potency of this activity and the streptolysin protective capacity. However, the antiserotonin potencies were measured with rat tissues, a species not examined in the above studies. One assay was in vitro (rat uterus), and the other in vivo (rat paw edema).[20, 21] Since striking discrepancies were even found between these two determinations (e.g., KB-95 and BP-400), it is conceivable that the antiserotonin properties of these drugs might still account for some of their streptolysin protective effects. In addition, Woolley, Shaw, and Campbell[22-24] have pointed out that certain serotonin antimetabolites have serotonin mimetic properties under some circumstances. It is possible that such effects also contributed to the apparent lack of correlation between the antiserotonin properties and the interference with streptolysin toxicity.

On the other hand, the relative lack of lethal toxicity of serotonin for the rabbit and mouse makes it appear somewhat unlikely that release of this naturally occurring pharmacologically active substance could entirely account for the acute streptolysin toxicity. As will be shown in a subsequent report, the role of liberated potassium ions is strongly implicated in these processes, but the protection afforded by the antiserotonin drugs cannot be accounted for on this basis. A visual impression was gained that serotonin antagonists tending to have a sedating effect were protective, while those which were excitatory were not.

The susceptibility of mice under barbiturate anesthesia demonstrated that

central nervous system sedation by itself does not increase the resistance to streptolysin toxicity. The different sequence of gross clinical events following streptolysin challenge in sodium pentobarbital–anesthetized mice is curious, but may yield some further clue as to modes of action of this bacterial product. The slight prolongation of life afforded by pretreatment with calcium ions, similarly, may yield clues as to the mechanisms involved in the streptolysin lethal properties. It is of interest in this regard that Woolley has obtained evidence for the role of serotonin in calcium ion transport across cell membrane.[25, 26]

Raskova[13] and her co-workers have carried out extensive studies on the pharmacologic properties of crude Group A streptococcal extracellular concentrates containing streptolysin "O." Perfusion experiments with isolated cat gastrocnemius muscle and cat skin revealed a pronounced vasoconstriction and interference with fluid flow through the vascular tree. It is not clear which of the various toxic factors present in the preparations used were primarily re-

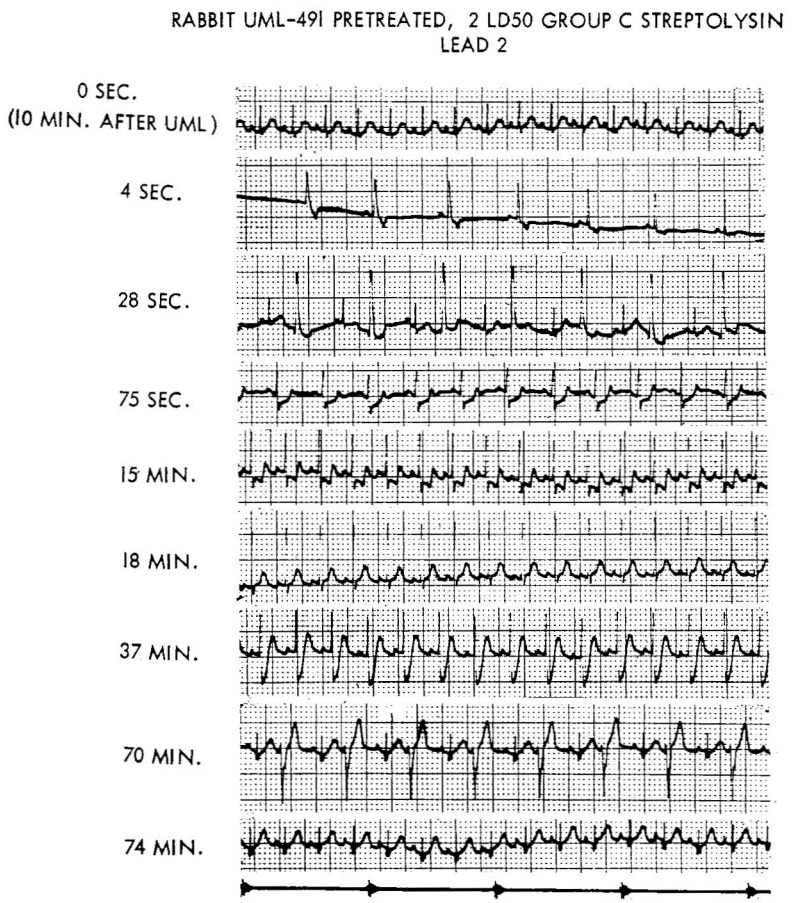


Fig. 5, B, Electrocardiogram of UML-491 pretreated rabbit, which was protected against the rapidly lethal streptolysin effects at the same dose level. This animal died at 120 minutes.

sponsible for these changes. In relation to the present studies, however, it was of interest that an antiserotonin drug, Cepentyl (a cyclopentyl derivative of LSD25), markedly reduced these effects, but an antihistamine drug did not. In similar studies on the muscle contraction of the isolated rat uterus, the streptococcal concentrate used by them caused a slow contraction, but this was not strikingly altered by the serotonin antagonist.

The failure of protection by UML-491 against saponin toxicity may also be pointed out. Earlier work by Cantoni and Bernheimer[27] had indicated many close similarities between streptolysin "O" and saponin, although a few differences were noted. The data recorded here also reveal that there must be distinctions in the mechanisms of toxicity of these two agents.

The ratio of lethal mouse toxicity of the Group A and Group C streptolysin preparations tested is almost exactly the same as the ratio found in rabbits, i.e., about 1 to 5.[4] This value showed a good correlation between the lethal toxicity and hemolytic potencies. Earlier observations by several investigators had revealed that streptolysin "O" Group A and Group C streptococci appeared to be identical.[28] Immunodiffusion studies from this laboratory supported this finding[7, 8] as did the previous studies on the lethal toxicity of the two preparations used here.[4] It thus seems almost certain that the protective effects of serotonin antagonists seen above with the Group C product would apply as well to the Group A material.

It is also of interest that the lethal LD_{50} toxicities of the two streptolysin preparations for mice were about 100 μg per kilogram and 490 μg per kilogram for the Group A and Group C fractions, respectively. The similar values for rabbits previously observed were roughly 50 μg per kilogram and 250 μg per kilogram,[4] indicating that mice were approximately twice as resistant to the lethal toxicity of streptolysin as were rabbits. These data are in rather striking contrast to that found by Howard and Wallace[29] who recorded a difference of susceptibility in these two species of approximately thirtyfold. The reasons for this discrepancy are not clear, but may be related to the relative purity of the streptolysin fractions employed here.

As pointed out in the introduction, circumstantial evidence does exist in the literature linking streptolysin "O" to the pathogenesis of the cardiac lesions of rheumatic fever. A working hypothesis was developed earlier, implicating this toxin in the form of antigen-antibody complexes.[4] Should it be true that streptolysin "O" is actually involved in the etiology of rheumatic fever, it would be of some interest to attempt the treatment of acutely ill rheumatic patients with one of these antiserotonin drugs which possess streptolysin protective properties.

REFERENCES

1. Rich, A. R., and Gregory, J. E.: Further Experimental Cardiac Lesions of the Rheumatic Type Produced by Anaphylactic Hypersensitivity, Bull. Johns Hopkins Hosp. **75**: 115, 1944.
2. Kaplan, M. H.: The Concept of Autoantibodies in Rheumatic Fever and in the Postcommissurotomy State, Ann. New York Acad. Sc. **86**: 974, 1960.
3. McLeod, C. M.: Etiological Considerations and the Prevention of Rheumatic Fever, Maryland State Med. J. **2**: 236, 1953.

4. Halbert, S. P., Bircher, R., and Dahle, E.: The Analysis of Streptococcal Infections. V. Cardiotoxicity of Streptolysin "O" for Rabbits in Vivo, J. Exper. Med. **113**: 759, 1961.
5. Halbert, S. P., Swick, L., and Sonn, C.: The Use of Precipitin Analysis in Agar for the Study of Human Streptococcal Infections. I. Oudin Technic, J. Exper. Med. **101**: 539, 1955.
6. Halbert, S. P., Swick, L., and Sonn, C.: The Use of Precipitin Analysis in Agar for the Study of Human Streptococcal Infection. II. Ouchterlony and Oakley Technics, J. Exper. Med. **101**: 557, 1955.
7. Halbert, S. P.: The Use of Precipitin Analysis in Agar for the Study of Human Streptococcal Infections. III. The Purification of Some of the Antigens Detected by These Methods, J. Exper. Med. **108**: 385, 1958.
8. Halbert, S. P., and Auerbach, T.: The Use of Precipitin Analysis in Agar for the Study of Human Streptococcal Infections. IV. Further Observations on the Purification of Group A Extracellular Antigens, J. Exper. Med. **113**: 131, 1961.
9. Halbert, S. P., Di Sant'Agnese, P., and Rotman Kotek, F.: Staphylococcal Antibodies in Cystic Fibrosis of the Pancreas, Pediatrics **26**: 792, 1960.
10. Halbert, S. P., and Keatinge, S. L.: The Analysis of Streptococcal Infections. VI. Immunoelectrophoretic Observations on Extracellular Antigens Detectable With Human Antibodies, J. Exper. Med. **113**: 1013, 1961.
11. Halbert, S. P., and Keatinge, S. L.: Purification of Group C Streptococcal Extracellular Antigens Detectable With Naturally Occurring Human Antibodies, Fed. Proc. **21**: 24, 1962.
12. Kellner, A., Bernheimer, A. W., Carlson, A. S., and Freeman, E. B.: Loss of Myocardial Contractility Induced in the Isolated Mammalian Heart by Streptolysin "O," J. Exper. Med. **104**: 361, 1956.
13. Raskova, H.: Pharmacology of Some Toxins, Prague, 1958, Czechoslovak Academy of Sciences.
14. Bernheimer, A. W., and Cantoni, G. L.: The Cardiotoxic Action of Preparations Containing the Oxygen-Labile Hemolysin of Streptococcus Pyogenes. I. Increased Sensitivity of the Isolated Frog's Heart to Repeated Application of the Toxin, J. Exper. Med. **81**: 295, 1945.
15. Schwab, J. H., Watson, D. W., and Cromartie, W. J.: Production of Generalized Shwartzman Reaction With Group A Streptococcal Factors, Proc. Soc. Exper. Biol. & Med. **82**: 754, 1953.
16. Schwab, J. H., Watson, D. W., and Cromartie, W. J.: Further Studies of Group A Streptococcal Factors With Lethal and Cardiotoxic Properties, J. Infect. Dis. **96**: 14, 1955.
17. Todd, E. W.: Lethal Toxins of Hemolytic Streptococci and Their Antibodies, Brit. J. Exper. Path. **19**: 367, 1938.
18. Herbert, D., and Todd, E. W.: Purification and Properties of a Hemolysin Produced by Group A Hemolytic Streptococci (Streptolysin "O"), Biochem. J. **35**: 1124, 1941.
19. Bernheimer, A. W., and Cantoni, G. L.: The Toxic Action of Preparations Containing the Oxygen-Labile Hemolysin of Streptococcus Pyogenes. III. Induction in Mice of Temporary Resistance to the Lethal Effect of the Toxin, J. Exper. Med. **86**: 193, 1947.
20. Erspamer, V.: Pharmacology of Indole Alkylamines, Pharmacol. Rev. **6**: 425, 1954.
21. Doepferner, W., and Cerletti, A.: Comparison of Lysergic Acid Derivatives and Antihistamines as Inhibitors of the Edema Provoked in the Rat's Paw by Serotonin, Internat. Arch. Allergy **12**: 89, 1958.
22. Shaw, E., and Woolley, D. W.: Some Serotonin-Like Activities of Lysergic Acid Diethylamide, Science **124**: 121, 1956.
23. Woolley, D. W., and Shaw, E. N.: Evidence for the Participation of Serotonin in Mental Processes, Ann. New York Acad. Sc. **66**: 649, 1957.
24. Woolley, D. W., and Campbell, N. K.: Serotonin-Like and Anti-Serotonin Properties of Psilocybin and Psilocin, Science **136**: 777, 1962.

25. Woolley, D. W.: A Probable Mechanism of Action of Serotonin, Proc. Nat. Acad. Sc. U. S. A., 44: 197, 1958.
26. Woolley, D. W.: Serotonin Receptors. I. Extraction and Assay of a Substance Which Renders Serotonin Fat-Soluble, Proc. Nat. Acad. Sc. U. S. A., 44: 1202, 1958.
27. Cantoni, G. L., and Bernheimer, A. W.: The Toxic Action of Preparations Containing the Oxygen-Labile Hemolysin of Streptococcus Pyogenes. IV. Comparison of Cardiotoxic Action With That of Saponin, J. Pharmacol. and Exper. Therap., 91: 31, 1947.
28. Todd, E. W.: The Streptolysins of Various Groups and Types of Hemolytic Streptococci; a Serological Investigation, J. Hygeine 39: 1, 1939.
29. Howard, J. G., and Wallace, K. R.: The Comparative Resistances of Rabbits, Guinea Pigs, and Mice to the Lethal Actions of Streptolysin "O" and Saponin, Brit. J. Exper. Path. 34: 185, 1953.