hyaluronidase, and the fact that all capsulated streptococci undergo spontaneous decapsulation even though they cannot be shown to produce hyaluronidase, would indicate that hyaluronidase is not responsible for this process.

In attempting to characterize the fluoride effect, it was found that the addition of magnesium or calcium to the test system tended to block the ability of fluoride to delay decapsulation. This may mean that fluoride acted in the system by combining with metallic ions necessary for enzyme action. However, MgF_2 and CaF_2 are highly insoluble compounds and it is felt that the observed blocking effect could also be due to the removal of available fluoride from the system.

Summary. (1) Attempts to demonstrate an agent in beta hemolytic streptococci which would accelerate the normal rate of capsular disintegration were unsuccessful. (2) Of various chemical agents tested, the following ions were ineffective in inhibiting this disintegration: Lead, cadmium, barium, manganese, lithium, silver, mercury, arsenite, iodide, bromide, periodate, cyanide and oxalate. (3) The following agents were found to retard decapsulation: Fluoride, azide, arsenate, citrate, and formaldehyde. The effect of fluoride was lost upon the addition of magnesium or calcium ions. (4) Fluoride did not inhibit the decapsulating action of streptococcal hyaluronidase. (5) The effect of temperature was found to be compatible with the assumption of a hypothetical enzyme system causing decapsulation.

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Action Mechanism of Cobra Venom, Cardiotoxin and Allied Substances on Muscle Contraction. (19106)

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The excised toad heart stops beating if perfused with a solution of cobra venom*, cardiotoxin†(1,2) or cardiac glucosides. The similarity of cobra venom to saponin was often noted by earlier workers, but later it has been shown, more closely, to resemble digitalis and digitalis-like substances(3,4). Now that a number of active principles of cobra venom have been isolated—some in a highly purified state—it was considered worthwhile to study the mode of action and to compare them with known cardiac drugs. Cardiotoxin, one of the many active constituents of cobra venom, has been isolated from it by fractional precipitation method(1). It is a protein and on weight basis it is 90-100 times more potent than crude venom. It contains less than 2% of neurotoxin and haemolysin.

Method. An isolated frog or toad heart (average weight of 250 g and 150 g respectively) was perfused with Ringer's solution. The minimum concentration of a drug required to stop the heart was taken as that amount which when perfused in the 5 cc of the test solution caused the systolic contracture. Test solutions were made up in salt mixture; the composition of which was as follows: NaCl, 6.5 g; $CaCl_2$, 0.12 g; $NaHCO_3$, 0.2 g; the total volume made up to 1000 cc. The pH was 7.2-7.4.

Results. Table I shows the concentration of each drug required for cardiac arrest. The

* Cobra venom was extracted from the poison glands of cobra (*Naja tripudians*) and dried in a vacuum desiccator over fused calcium chloride.

† Cardiotoxin brings about the stoppage of perfused toad heart or heart beat when administered intravenously into an anaesthetized cat(2).

1. Sarkar, N. K., *Ann. Biochem. and Exp. Med.*, 1948, v8, 11.

2. Sarkar, N. K., *J. Ind. Chem. Soc.*, 1947, v24, 227.

3. Epstein, D., *J. Pharmacol.*, 1921, v43, 697.

4. Gunn, J. A. and Epstein, D., *J. Pharm. and Pharmacol.*, 1933, v6, 182.

TABLE I. Actions of Saponin, Digitoxin, Strophanthin, Cobra Venom and Cardiotoxin on Isolated Amphibian Heart.

Substance	Min. conc. producing systolic arrest	Condition of heart: (a) On perfusing with test solution	Condition of heart: (b) After washing with Ringer's	Washing time, min
Cobra venom	1/500 for toad 1/1200 frog	Stopped in systole	Remained unchanged	40-60
Cardiotoxin	1/1050 toad 1/2500 frog	"	"	40-60
Saponin	1/1000 toad 1/1000 frog	"	Revived	20-30
Digitoxin	1/400 toad 1/1500 frog	"	"	20-25
Strophanthin	1/450 toad 1/2250 frog	"	"	50-60

last column of the table shows the time required to wash out the effect of each substance. From the table it will be also seen that the toad heart as a rule is more resistant to all drugs, except saponin, than is the frog heart.

It will be noted that the characteristic systolic contracture is caused by solutions of cobra venom, cardiotoxin, saponin, digitoxin and strophanthin. The cardiac arrest brought about by saponin and the drugs of the digitalis group can be completely reversed by washing; while the action of cobra venom and cardiotoxin cannot, even by repeated washing with Ringer. Among the other differences between these drugs, saponin has effects on red blood cells, the nervous system and the respiratory system, while cardiotoxin and the digitalis drugs lack such actions. Cobra venom too, has these properties of saponin, because it contains other active principles besides cardiotoxin.

Previously it was thought that calcium was involved in the development of systolic contracture of the perfused frog's heart caused by cobra venom(5). Without it the heart stopped in diastole. Similar results with digitalis drugs were obtained by Loewi(6). Our findings (Table II) indicate that calcium is not essential. When perfused with isotonic solution of NaCl, KCl or both together, the heart stops in diastole. When cobra venom or cardiotoxin is added to such a solution, the heart gradually revives and finally stops in systole and remains so even after prolonged washing with Ringer.

These results and the results obtained from the study of the action of cobra venom and cardiotoxin on the excitability and the contractility of nerves and muscles(7) led to the conclusion that they both act, perhaps, directly on muscle. To understand why the muscle contracts under the influence of cobra venom, the following experiments were performed.

An isolated muscle fibre from frog sartorius was placed in isotonic saline and 0.05 cc of cobra venom solution (1/2000-1/5000) was dropped on it. An extremely rapid contraction followed; amounting to 40-45% of the original length. Experiments using rabbit psoas muscle fibres were carried out in Petri-dishes containing 0.0015 M $MgCl_2$, 0.04 M veronal-acetate buffer of pH 7.0 and 0.1 M KCl. Cobra venom sufficient to make the final concentration to 1/1000-1/2000 when added, caused a contraction similar to that seen with frog muscle.

Muscle fibres in which the cell membrane has been removed by treatment with 50% glycerine solution no longer contract when cobra venom is added. These fibers were made according to the method of Szent-Györgyi(8). Thin strips of the musculus psoas of rabbit were treated with 50% glycerol, at 0°C for 24 hours and were kept for

5. Gottdenker, F. and Wachstein, M., *J. Pharmacol.*, 1940, v69, 117.

6. Loewi, O., *Arch. f. Exp. Path.*, 1917, v82, 131; 1918, v83, 366.

7. Sarkar, N. K., and Maitra, S. R., *J. Am. Physiol*, 1950, v163, 209.

8. Szent-Gyorgyi, A., *Biol. Bull.*, 1949, v96, 140.

TABLE II. Influence of Different Ions on the Action of Cobra Venom and Cardiotoxin on Isolated Toad's Heart.

Substances in the perfusion fluid	Condition of heart on perfusion after washing—with the solution	with Ringer
a. Perfused with KCl	Stops in diastole	—
b. The same heart is then perfused with KCl and cobra venom	Heart revives and then stops in systole	Remained so
a. Perfused with NaCl, KCl	Heart stops in diastole	
b. The same heart is then perfused with NaCl, KCl and cobra venom	Heart revives and then stops in systole	,, ,,
a. Perfused with KCl	Stops in diastole	
b. The same heart is then perfused with KCl and cardiotoxin	Heart revives and then stops in systole	,, ,,
Cobra venom (alone)	Stops in systole	,, ,,
Cardiotoxin (alone)	,,	,, ,,

4-5 days at −20°C in the same solution. It seemed of interest to investigate the possible effect of cobra venom on the recombination of actin and myosin in pyrophosphate treated glycerol-fibres(9); such fibres after treatment with cobra venom did not show any contraction on the addition of ATP over 0.26 M KCl. If association has been effected by cobra venom, one should expect contraction up to the concentration of .45 M KCl like glycerinated muscle fibres do.

Threads made of actomyosin prepared according to Weber(10) show no contraction on addition of cobra venom or cardiotoxin. Actin and myosin prepared according to the method of Straub(11) and Szent-Györgyi(12) still show superprecipitation on the addition of ATP and respective co-partner after treatment with cobra venom or cardiotoxin, indicating that these two muscle proteins are not affected directly by these compounds.

These results suggest that the pharmacological action of snake venoms requires the presence of the cell membrane, without which there will be hardly any contraction.

Summary. Cobra venom, cardiotoxin, saponin and drugs of the digitalis series bring about the stoppage of the movements of the excised heart. The cardiac arrest caused by cobra venom, unlike that by saponin and digitalis, is irreversible, *i.e.*, cannot be washed out. Unlike living muscle fibres, glycerinated muscle fibres and actomyosin threads show no contraction under the influence of cobra venom or cardiac drugs. These drugs have no effect on myosin and actin either.

9. Szent-Gyorgyi, A. E., *Enzymologia.*, 1950, v14, 246.

10. Weber, H. H., *Arch. ges. Physiol.*, (*Pflügers*) 1934, v36, 109.

11. Straub, F. B., *Studies*, 1942, v2, 3.

12. Szent-Gyorgyi, A., *Muscular contraction* (second edition) Academic Press p146.

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Specific Determination of Serum Creatinine.* (19107)

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The estimation of serum creatinine by Jaffe's alkaline picrate reaction does not differentiate between creatinine and non-creatinine chromogen. This has led many workers

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