BIOCHEMICAL AND PHYSIOLOGICAL STUDIES OF THE PURIFIED TOXIN OF WALTERINNESIA AEGYPTEA ‘THE EGYPTIAN BLACK SNAKE’

BY A. H. MOHAMED AND O. ZAKY
The Department of Physiology, Faculty of Medicine, Abbassia, Cairo

(Received 9 January 1954)

INTRODUCTION

Waltermnesia aegyptea or ‘The Black Snake’ which lives in the Sinai Desert near Suez is extremely poisonous. The snake was named after Walter Innes (1923), who was the first to discover the presence of the snake in the Eastern Egyptian Desert. Many fatal attacks of this snake upon camping soldiers and local inhabitants have been reported. Anderson (1925) made a valuable zoological study of the snake. Curckill (1929) described two cases of death occurring as a result of the bite of this snake. Death occurred from 6 to 24 hr. after the bite. Biochemical and physiological studies of venoms from different poisonous animals living in Egypt have been reported by many investigators, e.g. Wilson (1921), Shousha (1928), Hassan and Mohammed (1940) and Karimi (1955). Physiological effects of the different snake venoms were reviewed by Essex, (1945).

In this work a technique for preparing the toxin of W. aegyptea in a crystalline form is described. The minimum lethal dose of the toxin has been determined. Biochemical and physiological studies are reported.

PREPARATION

(1) First method. The snake is allowed to bite a rubber membrane covering a small beaker. The volume of the venom thus collected is about 0.7 ml. It is a viscous yellowish liquid. It is diluted 100 times with distilled water, then clarified by addition of about 1 g. aluminium sulphate crystals followed by 5 ml. of lime water. Pure acetone is added gradually until a heavy flocculent precipitate is obtained. This is then centrifuged and is washed three times with acetone, then with ether. Finally the precipitate obtained is dried under vacuum. The residue thus obtained is a white amorphous substance. The venom can be obtained in a crystalline form if rewashed with ether, centrifuged, then dried under reduced pressure over phosphorous pentoxide at 30°C.

(2) Second method. The venom collected after one bite is diluted 100 times with 0.1 N-HCl, then neutralized to pH 7 by addition of N-NaOH. The solution is saturated with picric acid crystals, and then allowed to stand for 24 hr. The toxin thus precipitated is centrifuged, then repeatedly extracted with 80% acetone in water (v/v) until no further picrate is recovered. A few drops of conc. HCl are
Studies of the purified toxin of W. aegyptea

added to the toxin, followed by excess of pure acetone. The precipitated toxin hydrochloride thus obtained is centrifuged, then washed successively with acetone and ether. Finally, the crystalline salt of the toxin is obtained by drying the precipitate in vacuum over phosphorous pentoxide.

The yield of one bite (about 0.7 ml.) for either method is 35–50 mg. of fine crystalline toxin.

BIOCHEMICAL STUDY

The purified crystalline toxin dissolves readily in distilled water to give a colourless but frothy solution. It is found to be slightly alkaline (pH = 7.4). The toxin can be precipitated from the aqueous solution by alcohol, acetone or ether. The dried toxin leaves no residue after burning.

Subcutaneous injections of the aqueous solution of toxin into albino rats showed that the minimum lethal dose is 0.03 mg./100 g. body weight (average of twenty-four observations). A period of about 50–80 min. elapses between injection and death of animal. Boiling of the solution of toxin in water destroys its toxicity. One mg. of toxin when injected after boiling is harmless to rats. Incubation of the toxin solution at 37°C for 24 hr. is sufficient to destroy its toxicity.

A solution containing 1 mg. toxin/ml. water gives positive reactions with Millon, xanthoproteic and biuret tests. Half-saturation with ammonium sulphate does not precipitate the toxin but it is precipitated by complete saturation.

An aqueous solution of the toxin, when treated with picric acid, gives an immediate yellow precipitate which dissolves on heating and reappears on cooling, suggesting that the toxin is a secondary proteose.

PHYSIOLOGICAL STUDY

(a) Effect on the isolated uterus of the guinea pig. The uterus of a virgin guinea-pig of about 250 g. body weight was excised and suspended in oxygenated Tyrode solution at a constant temperature of 37°C. The normal activity of the uterus was recorded. 0.2 ml. of a solution containing 1 mg. toxin/ml. Tyrode solution was added to the organ bath. An immediate contraction was obtained. The effect was persistent, but after washing the normal activity returned. The addition of 1 ml. of a solution containing 2 mg. of atropine sulphate in Tyrode solution abolished the excitatory effect of the toxin (see Fig. 1).

(b) Effect on the isolated intestine of the rabbit. The jejunum of a freshly killed rabbit of 1 kg. body weight was excised and suspended in oxygenated Tyrode solution kept at a constant temperature of 37°C. The normal activity was recorded. On addition of 0.2 mg. of toxin in Tyrode solution an immediate slight relaxation occurred followed by vigorous and persistent contraction. Several washings were necessary to abolish the effect. The addition of 1 ml. of atropine sulphate abolished the effect of the toxin, whether added before or after the toxin solution. The effect of bellafoline was similar to that of atropine.

(c) Effect on the perfused frog’s heart. Isolated hearts of medium-sized frogs were perfused with Ringer–Locke solution. The normal activity of the heart was recorded.
The introduction of varying doses of the toxin into the perfusing fluid had the following effects:

1. The heart rate was decreased.
2. The height of contraction was increased.
3. Partial block followed by complete block (Fig. 2) occurred. The heart stopped in diastole.
4. In some experiments extrasystoles followed by compensatory pauses were observed.
5. Washing abolished all the above effects and revived the heart (Fig. 2).

The addition of 0.5 mg. of atropine sulphate to the perfusing fluid abolished these effects.

(d) Effect on the perfused rabbit’s heart. Isolated hearts from freshly killed rabbits of an average weight of 1 kg. were perfused with oxygenated Ringer-Locke solution. After the normal activity had been recorded, 0.1 mg. of the toxin solution was introduced into the inflow rubber tube. After a latent period of 15–60 sec., changes similar to those observed in the case of the frog’s heart occurred: slowing of the heart rate, increase in contractility, appearance of different grades of partial
heart block, complete heart block; and in some experiments extrasystoles followed by compensatory pauses were observed.

These effects were abolished by washing or by injection of 0.5 mg. of atropine sulphate (see Fig. 3).

(e) Effect of salivary secretion. One of the common symptoms observed after injection of toxin intravenously into rats and dogs was increased salivation. Accordingly, it was thought worth while to study the effect of injection of toxin on the rate of salivation in dogs anaesthetized by chloralose and with their salivary ducts dissected and cannulated. The number of drops of saliva collected per minute was taken as an index of the activity of the salivary gland.

Fig. 3

In one experiment one drop was collected per minute. On stimulation of the chorda tympani by faradic current for a period of 30 sec. the rate of secretion rose to five drops per minute. Similar yields were obtainable on repeating stimulation after periods of rest. On the intravenous injection of 2 mg. of the toxin, the flow of saliva increased to seven drops per minute after a latent period of 8 min. The effect persisted for about 35 min. Stimulation of the chorda tympani after injection of toxin increased the salivary flow to twelve drops per minute. Intravenous injection of 1 mg. atropine sulphate abolished the effect of toxin, and no change in rate of salivary flow was observed. Ergotoxin injected intravenously had no effect on the rate of saliva flow after the injection of toxin.

These experiments were repeated with similar results.

DISCUSSION AND CONCLUSIONS

Most of the toxins from different venomous animals when examined chemically have been shown to be protein in nature (Ganguly & Malkana, 1936).

Biochemical study of the black snake toxin has shown that it is probably a proteose since when the aqueous solution of toxin is treated with picric acid it forms a precipitate which dissolves on heating but reappears on cooling.

The effects produced by crude venom are usually difficult to interpret. The possibility that the effect observed may be partially or entirely attributable to foreign contaminants cannot be excluded. Crude venom kept dry or in solution is known to decrease in potency with time (Guena & Calabrese, 1941). It
is therefore preferable to test the action of the venom when it is prepared in a pure crystalline form.

When the toxin is prepared in crystalline form its potency and activity are retained for a very long time. The minimum lethal dose can thus be reliably determined. In this way the toxicities of different venoms can be compared. The toxicity of the black snake venom was found to be ten times that of the Egyptian cobra venom (*Naja haja*).

It can be deduced from the experimental work on isolated organs of amphibia and mammals that this toxin has a parasympathetic effect. Its excitatory action on the isolated uterus of the guinea-pig and on the isolated intestine of the rabbit is abolished by addition of atropine. Again, the increase in the rate of saliva flow as a result of intravenous injection of toxin and the antagonistic effect of atropine are further indications of the parasympathetic effect. In this respect the black snake venom acts similarly to scorpion venom. Scorpion venom, however, has been shown to give an additional sympathetic effect (Mohammed, 1940).

Nevertheless, experiments showed that atropine sulphate when injected after injection of toxin did not save the life of the animal although it prolonged it. This suggests that the venom has another effect, probably a histamine-like action. Feldberg & Kellaway (1937) and Tretchewie & Kellaway (1940) have shown that histamine is liberated by perfused tissues when the venom of various snakes is added to the perfusing fluid. In the study of the Egyptian black snake it has been found that it causes histamine liberation from the skeletal muscles of the rat (Mohammed & Zaky, unpublished). In this respect the black snake venom behaves similarly to Egyptian bee venom (Karimi, 1955).

It is probable that the venom action is dependent on ‘the route of administration’ as has been pointed out by Shottler (1951).

The toxin interferes with the conducting system of the heart. It causes partial and complete block of the perfused amphibian and mammalian hearts. The toxin has also a direct effect on the myocardium since it causes augmentation of the heart beat. The venom may have another excitatory effect on the normally dormant ectopic centres in the walls of the ventricle since in some cases a series of extra systoles occurred.

It seems justifiable to believe that the venom has no damaging or irreversible effects on the different organs since simple washing of the poisoned organ is followed by complete recovery from all the above-mentioned effects.

**SUMMARY**

1. Two methods for preparation of the toxin in a purified crystalline form are described.
2. The toxin is probably a secondary proteose.
3. It has an excitatory parasympathetic effect on the uterus of the guinea-pig and on the intestine of the rabbit. The effect is abolished by atropine.
4. It causes excessive salivary secretion, again abolished by atropine.
5. It causes partial or complete block of perfused isolated amphibian and
Studies of the purified toxin of *W. aegyptea*

mammalian hearts. It also causes extrasystoles. Both effects are abolished by atropine.

6. The minimum lethal dose for rats is 0.035-0.05 mg. toxin/100 g. body weight. Atropine did not save the life of the animal although it prolonged it.

7. The possibility of a histamine-like action of the venom is discussed.

REFERENCES


