Choline Esterase in Cobra Venom.

THE venom of the Indian Cobra (*Naia Naia Vel tripudians*) is a substance of very complicated composition and possesses a remarkable pharmacological activity. A large amount of literature has collected on the subject and many investigators have attempted to explain the cause or the causes which are responsible for the peculiar toxic manifestations produced by the venom. Most of the investigations reported in the literature are centred around the question of the identification and separation of the various constituents, to each of which can be attributed a particular toxic property. Earlier workers like Calmitte[1] believed that the protein fraction (toxalbuminoids) was the active constituent in the venom but later experiments tended to show that certain principles of a non-protein nature might also be involved. Faust[2] reported the isolation of a non-nitrogenous, non-glucosidal principle from the cobra venom (ophiotoxin) which was very closely related in its physical, chemical and pharmacological characters to the saponins. This principle produced all the effects of whole snake venom, except agglutination. That the enzymes present in the venom might have some part to play in bringing about the toxic manifestations of venom poisoning was first brought out by Mathews[3] who showed that the destruction of fibrinogen by the protease present in the *crotalus* (*crotalus adamenteus*) venom was the chief cause of the failure of the blood to clot in *crotalus* poisoning. This finding was later confirmed by Billing.[4] These observations naturally drew the attention of a number of workers (Ganguly,[5] Ghosh *et al*[6]) to the study of the nature and physical and biochemical characteristics of the various enzymes or enzyme complexes present in the cobra venom. Recently, Iyengar, Sehra and Mukerji[7] investigated in detail the nature of the protease in the cobra venom and attempted to explain the biochemical significance of the presence of this enzyme with special reference to the use of the venom in therapeutics. Roy and Chopra[8] studied the comparative biochemical characteristics of the cobra and Russell's viper venom with a view to identify, if possible, the constituents of the venom primarily responsible for the poisoning symptoms associated with snake bites.

The action of the cobra venom in experimental animals was investigated by Chopra and Iswariah.[9] It was shown that the main action of this venom in lethal and sub-lethal doses is on the respiratory system, the effect being one of initial stimulation and final paralysis. The respiratory centre is probably primarily involved but the motor end-plates in the diaphragm and other respiratory muscles are also affected almost simultaneously. There is considerable divergence of opinion with regard to the exact mechanism involved in bringing about the respiratory paralysis. Chopra and Iswariah (*loc. cit.*) produced evidence to indicate that the paralysis is chiefly central in origin. Cushny and Yagi,[10] Houssay[11] and Kellaway and Holden,[12] on the other hand, thought that the seat of motor paralysis was peripheral resembling very closely 'curare' action. Of late, evidence has been advanced that most, if not all, motor nerves on stimulation liberate acetylcholine at their ends and that this substance (and not the nerve itself) carries the impulse across the synapse to the end-organs. It has also been shown that this liberated acetylcholine is quickly destroyed in the blood due to the presence of a specific choline esterase in the plasma. Therefore, if acetylcholine is prevented from reaching the receptor end-organs by its destruction through choline esterase, apparently no

contraction of the muscles can take place, or in other words, a paralysis of the respiratory and other musculature will result. The paralysis resulting from cobra venom is generally considered to be due to a neurotoxin with specific action on the respiratory centre and nerve endings. In view of the recent ideas with regard to the chemical transmission of nerve impulses, it was considered of interest to study the effect of cobra venom on acetylcholine-choline esterase system of blood. It was expected that some light on the possible mechanism of action of the venom might be obtained from this angle.

The blood serum of the cat was used as the source of choline esterase. The continuous titration method of Stedman, Stedman and White[13] was employed for the determination of enzyme activity using acetylcholine chloride as the substrate. The original activity of the serum expressed as c.c. of 0·032 N. NaOH has been compared with the activity of the serum under the influence of cobra venom in various concentrations. A very striking activating effect of the cobra venom, on the choline esterase content of the serum was noticed, the activation increasing with the concentration of the venom. The Russell's viper venom tested similarly failed to show any measurable effect. This marked activation led us to suspect the possible existence of choline esterase itself in the cobra venom. The two venoms (Cobra and Russell's viper venom) were therefore tested for their choline esterase activity without the addition of the serum. Three different specimens of the cobra venom which were stored in the laboratory for periods varying from six months to two years (Table I) were tested for their choline esterase content and in all three, a very powerful choline esterase activity could be demonstrated, the most recent sample (six months' old) showing the highest activity.

The specific nature of the activity of choline esterase was studied by Stedman, Stedman and Eason[14] and by Stedman, Stedman and White (*loc. cit.*). They have shown that liver esterase is without any appreciable action on esters of choline and that choline esterase exerts little, if any, action on simple esters such as methyl butyrate. Roy[15] studied the rate of hydrolysis of ethyl

TABLE I.

Substrate : acetylcholine, 0·25 gm., in 100 c.c. ; Enzyme : dried venom 10 mg. Period of reaction 1 hour.

Experiment ..	1	2	3
Age of venom (in months) ..	> 24	12	6
Activity (in c.c. of 0·0231 N. NaOH)	4·70	21·80	41·10

butyrate in the presence of cobra venom and showed that in high concentration (5 per cent.) and under prolonged period of incubation, cobra venom had a fairly strong esterase activity while this activity was almost negligible in low concentrations (0·2 to 0·02 per cent.). In our experiment with 0·005 per cent. venom, no hydrolysis of ethyl butyrate occurred in 20 minutes incubation, while under identical conditions, a marked splitting up of acetylcholine chloride could be demonstrated. This goes to show that the esterase present in the venom is predominantly of the type of choline esterase. The well-known inhibitory effect of eserine on choline-esterase was also tested. Cobra venom when mixed with eserin (physostigmine) in a concentration of 1/50,000, lost its cholin esterase activity considerably, indicating that probably the choline esterase in the venom is of an identical nature with the esterase in the blood serum. Russell's viper venom, on the other hand, did not show any measurable choline esterase activity.

The biochemical and pharmacological significance of this finding is interesting. The presence of choline esterase in cobra venom offers a new and fairly satisfactory explanation of the peculiar toxic manifestations (paralysis) produced by cobra venom on biological tissues. Cobra venom, as is well known, is characterised by an action on the nervous mechanism. The choline esterase in the venom will, when introduced into the system, naturally supplement the activity of the choline esterase already present in the blood serum, and will help in bringing about a complete destruction of acetylcholine. Without the mediation of acetylcholine, nervous impulses will not be transmitted to the respiratory muscles and a paralysis will

supervene. This view-point, however, does not satisfactorily explain the specific paralytic effect of the cobra venom on the respiratory centre. It has not yet been conclusively demonstrated that impulses in the central nervous system are carried through the mediation of acetylcholine, though Dikshit[16] (1934) has demonstrated the presence of acetylcholine in the ventricles of the brain and suggested that humoral transmission may also occur in the central nervous system. Further work on the different aspects of this interesting problem is in progress.

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