AN, decreased significantly the NCCR activity of liver mitochondria. The solvent D had no effect on the mitochondrial enzymes.

Discussion. Evidence is now accumulating that there is an increase in the rate of energy metabolism of the liver cell upon stimulation by androgen steroids, and that this is reflected in concomitant increase in the biosynthetic activities of liver[6,7]. About the action of androgens upon the mitochondrial electron transport systems, we know very little. Our early experiments revealed a testosterone-induced increase of oxygen consumption and of succinate oxidase activity of rat liver cells, which is evidence of an increased rate of energy metabolism[8].

ENGEL and SCOTT[9], studying the effects of steroid hormones upon the rates of reactions mediated by NADH, showed that testosterone added to a reaction system containing corticosterone blocked the corticosterone-inhibitory effect on glutamate dehydrogenase in beef liver. JENSEN and NEUHARD[10–12] obtained evident inhibitory effect of corticosteroids upon the NADH-cytochrome *c* reductase systems of heart sarcosomes.

We have obtained a stimulatory action of testosterone, in low concentrations, upon the activity of isolated liver mitochondrial oxidative enzyme systems. This action depends on the structure of androgen used, because testosterone and 5α-androstane-17β-ol-3-one showed stimulatory effects, but their nitrate esters had an inverse effect. It can be mentioned that in Enzyme Nomenclature[13] we did not found enzymes with the capacity to hydrolase a steroid nitrate group. We believe that the steroid nitrate esters acted by a mechanism similar to the action of corticosteroids. For the stimulatory action of steroid molecules a 17β-OH and a 3-ketone group is necessary. A double bond at 4-5 position did not influence it. By introducing a nitrate-group into 17β-position, the stimulatory effect of steroid molecule decreased significantly.

Résumé. Le testostérone et la 5α-androstane-17β-ol-3-one stimulent l'activité de la succinoxydase des mitochondries intactes et l'activité de la NADH-cytochrome-*c*-réductase des mitochondries à membranes lésées, extraites du foie du rat blanc. Les nitrates-esters des mêmes stéroïdes réduisent l'activité des deux enzymes.

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Choline Esterase Activity in the Nervous System and the Innervated Organs of the Scorpion, *Heterometrus fulvipes*

Various types of synaptic transmitters occur in the nervous system of arthropods, especially the crustaceans and insects, but too little is known about them in arachnids[1,2]. Acetylcholine was detected in the nervous system of *Limulus polyphemus*[1,3] and the ganglia of the spider, *Heteropoda regia* and 2 species of scorpions, *Buthus europaeus* and *Heterometrus maurus*[4]. Acetylcholine esterase activity was determined in the nervous system and the innervated organs of *Limulus polyphemus*[5]. Small amounts of 5-hydroxytryptamine were also found in the nervous tissue of *Limulus*[6] and the venom apparatus of arachnids[7,8]. Except for these few studies, not much is known about the transmitters in arachnids.

Earlier investigations on the central nervous system of the scorpion[9] showed the occurrence of choline esterase (ChE) in the ventral nerve cord and a diurnal rhythm in its activity. This prompted us to believe that ACh–AChE system might be prevalent in the scorpion as a transmitter system. The present investigation was undertaken to test the validity of the statement.

Material and methods. The commonly available South Indian scorpion, *Heterometrus fulvipes*, was used during the experimentation. The choline esterase activity was determined using the method of Metcalf[10] with due modifications to suit the present material. The incubation mixture contained 0.1 ml of 1% homogenate (W/V) in 0.25 *M* sucrose solution and 1.0 ml of buffer-substrate solution (9 vol. of buffer + 1 vol. of 0.04 *M* acetylcholine chloride solution). 0.008 *M* acetylcholine chloride solution was prepared by mixing 0.04 *M* acetylcholine chloride and the buffer in 1:4 ratio. The mixture was incubated at 37 °C for $^1/_2$ h and the reaction was stopped by adding

Table I. Acetylcholine esterase activity in the nervous system of the scorpion

No.	Tissue	Molar concentration of the substrate 0.004
1	Brain	992.00 – 1292.00 (1143.00 ± 121.80)
2	Suboesophageal mass	1072.00 – 1332.00 (1222.00 ± 131.90)
3	Anterior part of the cord	872.00 – 972.00 (914.5 ± 38.00)
4	Posterior part of the cord	532.00 – 852.00 (759.5 ± 129.00)
5	Segmental nerve	752.00 – 972.00 (889.5 ± 65.20)

Activity expressed as μg of ACh hydrolysed/mg wet wt./h. Values in parentheses are the average of 8 values ± S.D.

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2.0 ml of alkaline hydroxylamine hydrochloride solution and 1.0 ml of 1:1 hydrochloric acid. The colour developed by the addition of 1.0 ml of 10% ferric chloride solution was read at 540 nm in Hilger and Watts (England) UV-Spectrometer. The amount of unreacted acetylcholine was determined from the standard graph and the enzyme activity was calculated. Inhibition by eserine was studied by adding 0.05 ml of the drug to the incubation mixture besides the other reagents.

Results and discussion. Table I shows the levels of enzyme activity in various regions of the central nervous system. The activity was very high in the integrating centres like the brain and the suboesophageal mass where there is a complex network of synapses, while it was very low in the segmental nerves where there are no synapses. The activity in the cord was intermediate. Here again the activity in the anterior mesosomatic part of the cord was higher than in the posterior metasomatic part. The high choline esterase activity in the nervous system, especially the neuropilar regions, suggests the possibility that ACh is involved in conduction and transmission of impulses and this may be a potential transmitter in the scorpion.

The fact that ACh may be a synaptic transmitter led to the study of AChE in the different innervated organs. The activity levels in these tissues are shown in Table II. The activity was very high in the pedipalp and tail muscles which control the movements of the pedipalp and the tail respectively. The activity was low in blood and intermediate in the heart tissue. In general, the activity in these tissues was low compared to the nervous tissue. The presence of choline esterase in the innervated organs suggests its importance in the transmission of impulses. AChE may be present in blood to hydrolyse excess ACh present in the different regions of the animal.

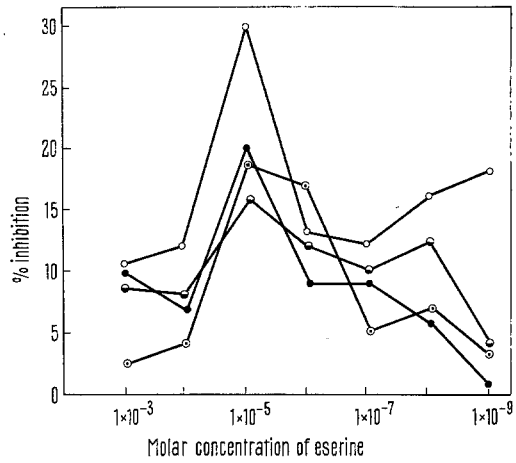
It is of interest to note that the level of enzyme activity depended upon the molar concentration of the substrate used. There was a two-fold increase in the enzyme activity in all the tissues studied when the substrate concentration was raised from 0.004–0.008 *M*. The accumulation or liberation of ACh in greater amounts might be responsible for the activation of the enzyme activity.

When compared with the choline esterase activity in the nervous system and the innervated organs of *Limulus*[5], the activity found in the scorpion is many times higher.

Table II. Acetylcholine esterase activity in different innervated organs of the scorpion in comparison with that in the ventral nerve cord

No.	Tissue	Molar concentration of Acetylcholine 0.004
1	Ventral nerve cord	872.00 – 1152.00 (957.00 ± 109.10)
2	Pedipalp muscle	452.00 – 872.00 (732.00 ± 135.60)
3	Tail muscle	612.00 – 992.00 (749.50 ± 115.30)
4	Heart	332.00 – 792.00 (559.50 ± 159.20)
5	Blood	272.00 – 550.00 (364.00 ± 109.60)

Activity expressed as µg of ACh hydrolysed/mg wet wt./h. In case of blood the activity is per 0.1 ml. Values in parentheses are the average of 8 values ± S.D.



Percentage inhibition of ChE in different tissues of the scorpion at various molar concentrations of the drug. ○—○, heart; ●—●, ventral nerve cord; ⊙- -⊙, pedipalp muscle; ◒—◒, tail muscle.

Inhibition of choline esterase by eserine was also tested and the results are presented in the accompanying Figure. Eserine inhibited the enzyme activity in all the tissues and at all concentrations. The choline esterase of the heart was inhibited to the greatest extent (30%) and its inhibition was also higher than in other tissues at all concentrations of the drug. The maximum inhibition of ChE in the ventral nerve cord was only 20% while in the muscle tissue it varied from 15–18%. It is of interest to note that eserine inhibition was the highest in all the tissues when its molar concentration was 1×10^{-5}, which is a typical characteristic of true choline esterases[10].

To summarize, the presence of choline esterase in the nervous system and the innervated organs of scorpion showing very high activity suggests the possibility that it might act as a transmitter system. The action of eserine shows the specific nature of the enzyme. High eserine sensitivity of this enzyme in the heart muscle shows that ACh may play a prominent role in the normal heart function. The presence of cardiac ganglion on the heart of the scorpion and the available pharmacological evidence[11] suggest the neurogenic nature of the heart and hence the presence of ChE in the heart is of great importance. In view of this it is assumed that the ACh–AChE system might be operating in the scorpion as an efficient transmitter mechanism[12].

Zusammenfassung. Die Cholinesteraseaktivität im Zentralnervensystem und in anderen Geweben des Skorpions *Heterometrus fulvipes* wurde gemessen, und ein verschieden hoher Gehalt wurde gefunden. Eserin hemmte die Enzymaktivität um 15–30%.

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