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# ACETYLCHOLINESTERASE ACTIVITY OF *SCHISTOSOMA MANSONI**

BY

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Bülbring, Lourie, and Pardoe (1949) demonstrated the presence of acetylcholine in *Trypanosoma rhodesiense* and its absence in the erythrocytic forms of malaria parasites. They suggested that acetylcholine might play a role connected with the motility of parasites. Should this hypothesis be correct, mechanisms for the hydrolysis of acetylcholine should be present not only in protozoan, but also in metazoan parasites which have a well-developed muscular apparatus. Several helminths contain esterase activity since fatty acid esters of glycerol as well as acetylcholine are hydrolysed by homogenates and extracts of these organisms (Bacq and Oury, 1937; Penoit-De Cooman, 1940; Artemov and Lurje, 1941; Penoit-De Cooman and Van Grembergen, 1942). However, whether helminths contain a specific acetylcholinesterase has not been determined previously. Therefore, this problem was investigated in the trematode, *Schistosoma mansoni*. It was found that this parasite contains an acetylcholinesterase which, in many respects, is similar to that occurring in the nervous tissue of vertebrates.

## Methods and Materials

Adult schistosomes were obtained as in previous studies (Bueding, 1950; Bueding and Peters, 1951). Cholinesterase activity of worm homogenates was determined according to Nachmansohn and Rothenberg (1945) in an atmosphere of 5 per cent $CO_2$ in nitrogen. After temperature equilibration (38° C.) the substrate was tipped from the sidearm into the main compartment. Enzymatic activity which remained constant for 20 minutes was expressed in $\mu$l. $CO_2$ liberated per mg. of worms (dry weight) during the first 15 minutes. $CO_2$ production by the reaction mixture without homogenate, and by the homogenate without substrate, was measured simultaneously and appropriate corrections for these " blank " values were made in the calculation of the esterase activity of the worms. Unless specified otherwise, the concentration of the substrate in the reaction mixture was $4 \times 10^{-2}$M. In order to save material and in order to increase the sensitivity of the assay procedure, Warburg flasks were used whose total volume did not exceed 5 ml.

## Results

It has been demonstrated that acetylcholine esterase does not split butyrylcholine in concentrations at which a high rate of hydrolysis of acetylcholine is observed,

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whereas other choline-splitting esterases, e.g., serum choline esterase, hydrolyse butyrylcholine at a more rapid rate than acetylcholine (Nachmansohn and Rothenberg, 1945; Augustinsson and Nachmansohn, 1949). As illustrated in Table I, the rate of hydrolysis of acetylcholine by schistosome homogenates was three to four and one-half times greater than that of butyrylcholine. This observation suggested that the schistosomes contain acetylcholinesterase and, in addition, a weaker non-specific cholinesterase. Acetylcholinesterase activity was the same in male and in female schistosomes (Table I) and remained constant for at least several months when the intact organisms were stored in the frozen state at −30° C. Acetylthiocholine and butyrylthiocholine were hydrolysed by schistosome homogenates at a somewhat faster rate than acetylcholine and butyrylcholine respectively.

Homogenates of the muscle of *Ascaris lumbricoides* and of the filarial worm, *Litomosoides carinii*, hydrolysed acetylcholine at a more rapid rate than butyrylcholine (Table I). These observations indicate the presence of acetylcholinesterase in two other helminths. However, it should be noted that the acetylcholinesterase activity of *Ascaris* muscle was considerably lower than that of schistosomes.

TABLE I

HYDROLYSIS OF ACETYLCHOLINE AND OF BUTYRYLCHOLINE BY HOMOGENATES OF HELMINTHS

Substrate concentration, 0.04M

| Homogenate of | $\mu$l. $CO_2$/mg. during first 15 min. | | | | | |
|---|---|---|---|---|---|---|
| | Acetylcholine Exp. No. | | | Butyrylcholine Exp. No. | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Male schistosomes .. .. .. | 5.8 | 6.45 | 6.1 | 1.2 | 2.0 | 1.6 |
| Female schistosomes .. .. .. | 5.7 | 6.7 | 6.1 | 1.3 | 1.9 | 1.75 |
| Mixture of male and female schistosomes | 5.8 | 6.6 | 6.0 | 1.3 | 2.2 | 1.7 |
| Filariae .. .. .. .. .. | 15.1 | 15.5 | 16.3 | 6.7 | 6.2 | 6.5 |
| *Ascaris* muscle .. .. .. .. | 0.37 | 0.52 | 0.36 | 0 | 0.08 | 0 |

That the hydrolysis of acetylcholine by *Schistosoma mansoni* was catalysed by a specific acetylcholinesterase, which could be separated from the enzyme responsible for the hydrolysis of butyrylcholine, was shown by centrifuging the homogenate at 18,000 r.p.m. for 60 minutes (0°–2° C.) and homogenizing the residue in a solution containing 0.1 M-NaCl and 0.025 M-$NaHCO_3$. Acetylcholinesterase activity was present in this preparation, but butyrylcholine was not hydrolysed by it (Table II). When this preparation was centrifuged at 3,500 r.p.m. for 15 minutes (0°–2° C.), little or no hydrolysis of acetylcholine occurred in the supernatant. The solubility of the schistosome acetylcholinesterase could be increased considerably by ultrasonic treatment of the homogenized residue (5 ml.) in a 9 KC Raython sonic oscillator for $2\frac{1}{2}$ minutes at 1° C. (Table II).

The effect of substrate concentrations on the acetylcholinesterase activity of *S. mansoni* is recorded in Fig. 1. The optimal concentration was found to be 0.04 M. Higher concentrations of acetylcholine had an inhibitory effect. By contrast, the rate of hydrolysis of triacetin increased with the substrate concentration, but even

TABLE II

ESTERASE ACTIVITY OF FRACTIONATED HOMOGENATES OF *Schistosoma mansoni*

Substrate concentration, 0.04M

| Fraction | $\mu$l. $CO_2$/mg. during first 15 min. | | | |
|---|---|---|---|---|
| | Acetylcholine Exp. No. | | Butyrylcholine Exp. No. | |
| | 1 | 2 | 1 | 2 |
| Untreated homogenate | 6.7 | 5.9 | 1.8 | 1.4 |
| Supernatant obtained by centrifugation of homogenate at 18,000 r.p.m. (1 hr.) | 2.1 | 2.2 | 1.4 | 1.2 |
| Residue obtained by centrifugation of homogenate at 18,000 r.p.m. (1 hr.) | 4.5 | 3.6 | 0.4 | 0 |
| Supernatant obtained by centrifugation at 3,500 r.p.m. (15 min.) of suspended residue | 0.7 | 0 | 0 | — |
| Supernatant obtained by centrifugation at 3,500 r.p.m. (15 min.) of suspended residue subjected to *ultrasonic vibration* | 4.35 | 3.3 | 0 | — |

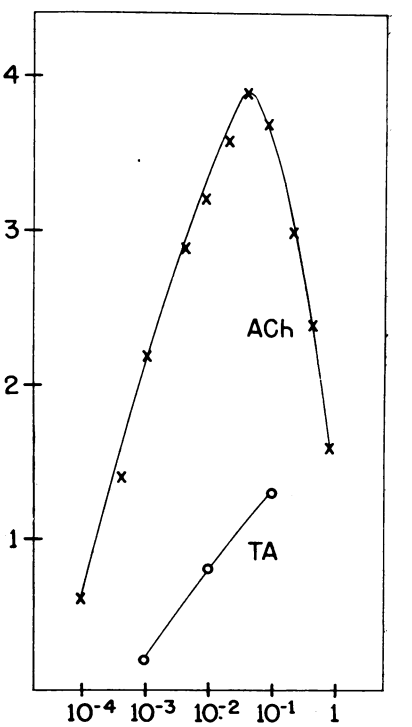


FIG. 1.—Effect of substrate concentration on acetylcholinesterase activity of *Schistosoma mansoni*. A suspension of the residue obtained by centrifugation of a schistosome homogenate at 18,000 r.p.m. (1 hr., 1° C.) was used as a source of enzyme. Abscissae: log of molar substrate concentration. Ordinates: esterase activity ($\mu$l. $CO_2$/mg.). ACh = Acetylcholine. TA = Triacetin.

at the highest concentration which was tested (0.1 M), triacetin was hydrolysed at a rate which was considerably lower than that observed with optimal concentrations of acetylcholine.

As with vertebrate tissues, the activity of acetylcholinesterase of schistosomes was inhibited by physostigmine and neostigmine. Complete inhibition was observed at molar concentrations of $1 \times 10^{-5}$ with either of these two compounds.

## Discussion

Acetylcholinesterase of *Schistosoma mansoni* is similar to the acetylcholinesterases of other species in the following respects: (1) In concentrations at which a high rate of hydrolysis of acetylcholine is observed butyrylcholine is not hydrolysed. (2) Reduction below as well as an increase above the optimal concentration of acetylcholine results in a decrease of the enzymatic activity. (3) Triacetin is hydrolysed at a slow rate. These properties distinguish acetylcholinesterase from other choline ester-splitting enzymes (Augustinsson and Nachmansohn, 1949). The only observed difference between the acetylcholinesterase of *S. mansoni* and that obtained from other tissues consists in the fact that the optimal substrate concentration for the schistosome enzyme is higher. The presence in schistosomes of a specific acetylcholinesterase, similar in properties and in concentration to that of central nervous tissue, suggests that acetylcholine may play a functional role in this parasite. This hypothesis is supported by an unpublished finding of Nachmansohn (personal communication) that acetone powders of schistosomes contain choline acetylase, i.e., an enzyme which catalyses the synthesis of acetylcholine from choline and acetate in the presence of adenosinetriphosphate and coenzyme A (Nachmansohn, Hestrin, and Varipaieff, 1949), at a rate of 135 to 150 $\mu$g. acetylcholine per g. per hour. Bülbring *et al.* (1949) have correlated the motor activity of parasitic protozoa with the presence and the production of acetylcholine. The presence of acetylcholinesterase and of choline acetylase in *Schistosoma mansoni* suggests that this applies also to parasitic metazoa, particularly because acetylcholinesterase activity has been detected in two other helminths, the filarial nematode, *L. carinii*, and in *Ascaris lumbricoides*. Baldwin and Moyle (1949) have shown that eserine does not sensitize *Ascaris* muscle to the effect of low concentrations of acetylcholine. It should be noted that the acetylcholinesterase activity of this tissue is considerably lower than in the schistosomes and the filariae.

## Summary

The trematode, *Schistosoma mansoni*, contains a specific acetylcholinesterase. Its concentration in the parasite is of the same order of magnitude as that of mammalian brain. Some properties of this enzyme are described and compared with acetylcholinesterases from other species.

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