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BIOCHEMICAL CHARACTERISTICS OF THE CHOLINESTERASE OF THE TURNIP MOTH *Agrotis segetum*

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Gnawing moths are widespread in all cotton-planting regions, and, among them, the turnip moth *Agrotis segetum* Schiff does considerable damage to the cotton harvest. The localization and dynamics of the activity of the cholinesterase (CE) of this pest have been studied to a comparatively small extent [1]. We have investigated the substrate specificity and sensitivity to inhibitors of different chemical natures of the CE of turnip moth caterpillars present in various phases of development, since such a type of investigation may lead to the creation of effective means of protecting cotton plants.

In the experiments we used turnip moth caterpillars of the 2nd-3rd, 4th-5th, and prepupal instars. Cholinesterase activity was investigated in homogenates prepared from the heads of the insects in 0.05 M phosphate buffer at a ratio of 1 g of tissue to 9 ml of buffer. The homogenate was centrifuged at +4°C on a Beckman centrifuge (USA) at 10,000 rpm for 20 min. The supernatant part of the homogenate was used for determining CE activity by Ellman's method [2] from the rates of hydrolysis of acetylthiocholine (ATC), propionylthiocholine (PTC), and butyrylthiocholine (BTC).

The interaction of the CE with the inhibitors was evaluated from the values of K_2 [3] at an inhibitor concentration causing causing 50% inhibition of the initial activity of the enzyme (25°C, pH 7. 4) on incubation for five minutes.

The experiments showed that the caterpillars of the 2nd-3rd and 4th-5th instars contained an enzyme capable of cleaving ATC, BTC, and PTC; ATC was hydrolyzed at the highest rate, PTC somewhat more slowly, and BTC most feebly. The enzyme activity of the caterpillars of the 4th-th instars was twice as great as that of the 2nd-3rd instars.

Homogenates of the heads of caterpillars in the prepupal phase did not hydrolyze ATC, PTC, and BTC. Apparently the functioning of the cholinergic system of the moths ceases in this stage of development.

It is interesting to note that Charyeva et al. [4], on investigating the CE activity of whole caterpillars (5th instar) of the cotton bollworm moth *Helicoverpa armigera* Hbn., showed the presence of an analogous enzyme which, like the turnip moth CE, hydrolyzed ATC, PTC, and BTC.

To answer the question of whether the cholinesterase activity was due to the action of a single enzyme, we determined the sensitivity of the enzyme from the moth heads to inhibitors of different structures while using ATC, BTC, and PTC as substrates (Table 1). The values of K_2 depended little on the chemical natures of these substrates. Consequently, the

TABLE 1. Sensitivity of the CE of Turnip Moth Heads to Inhibitors of Various Chemical Natures

Inhibitor	$K_2 \cdot M^{-1} \cdot min^{-1} \times 10^5$		
	ATC	BTC	PTC
Eserine	2.82	3.34	2.92
O-Pentyl S-(anabasinoethyl) phenylphosphonothioate	0.243	0.275	0.205
O-Isopentyl S-(lupininomethyl) phenylphosphonothioate	2.97	3.11	3.34
Di-O-isopropyl S-(piperidinobut-2-ynyl) phosphorothioate	26.4	23.1	20.1
Diisopropyl phosphorofluoridate	527	491	501

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homogenate of the caterpillar heads contained a single CE that hydrolyzed ATC at a high rate and was sensitive to the action of eserine, which gives grounds for assigning this enzyme to the class of acetylcholine acetyl hydrolyses — EC 3. 1.1.7.

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