

bin develops. It is then likely that the formation of this multichain network favours the stabilization of the hemoprotein in the R conformation (R, 'relaxed', in the presence of oxygen). In the same manner, the Hill coefficient diminishes when the molecular weight of the conjugates is increased (respectively 2.70, 2.33 and 1.40 for 6 h, 32 h and 5 days reaction times) and this later observation reflects a lack of cooperativity in O<sub>2</sub>-binding of the high molecular weight compounds.

In conclusion, it has been found that the low molecular weight hemoglobin-dextran conjugates exhibit good Barcroft's curves, that the cooperativity in O<sub>2</sub>-binding of linked hemoglobin is retained, and that solutions of conjugates do not present any hemagglutination phenomena nor toxicity when injected into rabbits. Therefore the possibility of their use as oxygen-carriers can be reasonably expected and some in vivo experiments are now being carried out to investigate this.

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### Effect of acrylonitrile on trehalase, phosphorylase and acetylcholinesterase activities in *Tribolium castaneum* Herbst and *Trogoderma granarium* Everts

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**Summary.** Acrylonitrile inhibited trehalase and phosphorylases in larvae and adults of *Tribolium castaneum*. In *Trogoderma granarium* larvae phosphorylases alone were inhibited. Acetylcholinesterase was not affected.

Literature survey indicates that hitherto very little work has been done on the effect of acrylonitrile, a highly toxic fumigant, on enzyme systems of storage pests. Because of its extensive industrial usage, most of the biochemical studies of acrylonitrile are limited to mammals only. Trehalase is an important enzyme in the carbohydrate metabolism of insects as it regulates the level of trehalose – a circulating source of energy<sup>2</sup>.

Phosphorylases (active and inactive) are another group of enzymes closely associated with the energy supply in insects<sup>3</sup>. The effect of fumigants on trehalase and phosphorylases has not been investigated so far. Acrylonitrile has been reported to increase acetylcholinesterase (AChE) ac-

tivity in the brains of exposed rats<sup>4</sup>. In fact, the level of AChE (and catalase) activity has been used as a criterion for occupational chronic poisoning by acrylonitrile in industry<sup>5</sup>. In the present investigation, the effect of fumigation with acrylonitrile on the 3 enzymes mentioned was studied in *Tribolium castaneum* (larvae and adults) and *Trogoderma granarium* (larvae).

**Materials and methods.** 14-day-old larvae of *T. castaneum* and *T. granarium* and 2–3-week-old *T. castaneum* adults obtained from laboratory cultures were weighed into 300-mg batches. In the petri dishes containing *T. castaneum* adults, 3 folded filter paper-strips of 5 × 1 cm were placed to absorb their defensive secretions. The insects were

Effect of acrylonitrile fumigation at sublethal and LD50 levels on certain enzyme activities in *T. castaneum* and *T. granarium*

Species	Stage	Acrylonitrile dosage (mg/l)	Trehalase <sup>a</sup>	Phosphorylase <sup>b</sup>		AChE <sup>c</sup>
				Active	Total	
<i>T. castaneum</i>	Adult	Nil	955.62 ± 43.89	0.28 ± 0.01	1.05 ± 0.05	0.36 ± 0.02
		0.53	714.61 ± 52.66 <sup>d</sup>	0.09 ± 0.01 <sup>d</sup>	0.61 ± 0.05 <sup>d</sup>	0.35 ± 0.02
		1.05	553.44 ± 71.13 <sup>d</sup>	N.D.	0.40 ± 0.03 <sup>d</sup>	0.34 ± 0.02
	Larva	Nil	698.73 ± 24.24	1.54 ± 0.04	2.74 ± 0.26	0.69 ± 0.02
		0.40	618.56 ± 39.32	1.14 ± 0.08 <sup>d</sup>	2.08 ± 0.02 <sup>d</sup>	0.71 ± 0.01
		0.79	534.75 ± 17.51 <sup>d</sup>	0.79 ± 0.05 <sup>d</sup>	1.43 ± 0.12 <sup>d</sup>	0.69 ± 0.02
<i>T. granarium</i>	Larva	Nil	470.12 ± 67.69	1.88 ± 0.23	3.04 ± 0.24	0.23 ± 0.01
		0.47	433.22 ± 49.36	1.32 ± 0.03 <sup>d</sup>	2.29 ± 0.09	0.24 ± 0.01
		0.93	461.06 ± 67.60	0.49 ± 0.01 <sup>d</sup>	2.24 ± 0.15 <sup>d</sup>	0.22 ± 0.01

<sup>a</sup> µg glucose produced/mg protein/h. <sup>b</sup> µmoles inorganic phosphate released/mg protein/h. <sup>c</sup> µg acetylcholine (chloride) hydrolyzed/mg insect/h. All values are mean ± SE of 6 estimations. <sup>d</sup> Significantly inhibited (p < 0.05). N.D.: Not detected, below limits of detectability.

fumigated at the predetermined sublethal and LD50 doses for 24 h in 2.5 l desiccators at 25–30 °C and 40–90% RH. In each experiment, there were 6 replicates each for control and sublethal dose, and 12 for LD50. At the end of the exposure period, mortality was found to be nil or negligible (<1%) in control and sublethal treated batches. Survivors from each of 2 desiccators fumigated at LD50 were pooled, and from this pool about 300 mg were weighed and utilized for the enzyme assays.

Fumigated and control batches of insects (300 mg each) were separately homogenized at 0–5 °C in 3 ml of 0.1 M, 5.6 pH citrate buffer for trehalase, in 0.1 M NaF for phosphorylases and in 0.9% NaCl for AChE. The homogenate was centrifuged at 10,000 × g for 20 min. Trehalase assay was carried out according to Friedman<sup>6</sup>, and the method of Sutherland and Wosilait<sup>7</sup> was followed for phosphorylases. Glucose was estimated by Nelson's method<sup>8</sup> and inorganic phosphate according to Taussky and Shorr<sup>9</sup>. Protein in the enzyme source was determined<sup>10</sup>. For AChE assay, the homogenate was directly used. About 10 ml of 0.015 M (final concentration) acetylcholine chloride and 1 ml of the insect homogenate were taken for the titrimetric assay of AChE<sup>11</sup>.

**Results and discussion.** Both active and total phosphorylases were significantly inhibited in all the test insects (table). Trehalase was inhibited in *T. castaneum* adults exposed at sublethal and LD50 levels but in the larvae, the inhibition was noted only at LD50 dose. However, in Khapra larvae, one of the most tolerant stages for the majority of fumigants, trehalase activity was found to be least affected by acrylonitrile. The small effect on the AChE activity of the exposed insects is quite interesting. Acrylonitrile either alone or in a mixture with HCN increased AChE activity in nervous tissues of exposed rats<sup>12</sup>. The enhanced AChE activity in the brains of rats exposed to acrylonitrile has been related to the excitatory effect on the central nervous systems<sup>4</sup>.

Trehalase activity, in control batches, was high in *T. castaneum* adults followed by its larvae and *T. granarium*. The reverse was true for phosphorylase activity. The activity of AChE in *T. castaneum* larvae was approximately twice that

of adult stage; the Khapra larvae showed lowest activity. The low phosphorylases and AChE activities in control batches of *T. castaneum* adults may be attributed to inhibition by parabenzoquinones contained in their defensive secretions. These parabenzoquinones are known to be extremely reactive and they readily form addition compounds with proteins<sup>13</sup>. While preparing the homogenates with pestle and mortar at 0–5 °C, the penetrating odor of the defensive secretions of the beetles was observed. In fact, adults of *Tribolium* spp. are known to secrete parabenzoquinones under irritant conditions, i.e. on exposure to fumigants, in abnormal CO<sub>2</sub> or N<sub>2</sub> atmospheres and under cold stress<sup>14,15</sup>. Lord and Potter<sup>16</sup> suspected a material in extracts of *T. castaneum* adults (parabenzoquinones?) which inhibited hydrolysis of AChE.

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## Enkephalins induce asymmetrical effects on posture in the rat

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**Summary.** Enkephalins when applied subarachnoidally induce hind limb postural asymmetry in rats with transected spinal cord. The effect is antagonized by naloxone. Methionine-enkephalin induces predominantly left limb flexions while leucine-enkephalin causes mostly right limb flexions. The data suggest an asymmetry existing in the enkephalinergic system in the rat spinal cord.

Opioid peptides, endorphins and enkephalins have been found recently in brain and pituitary<sup>2</sup>. These substances are known to affect pain perception<sup>3</sup>, behaviour<sup>4</sup> and some endocrine functions<sup>5,6</sup>, and may also play a role in the control of muscular tone<sup>7,8</sup>. In this paper a new property of enkephalins is described; their ability to induce postural asymmetry of the hind limbs in rats with a transected spinal cord. We further report that the side of the asymmetry is dependent on the type of enkephalin used: methionine-enkephalin (Met-enkephalin) predominantly induces flexion of the left limb whereas leucine-enkephalin (Leu-

enkephalin) induces flexion of the right limb. It is suggested that this influence may be due to asymmetric responsiveness of the spinal cord to enkephalins.

**Methods.** Enkephalins were injected into the subarachnoid space of the caudal portion of the spinal cord of 150–180 g male albino rats after laminectomy at the T5–T9 level and cord transection. Operations were carried out over the time interval from 10.00 h to 14.00 h under ether anaesthesia. The spinal cord was doubly ligated at T5–T9 and 20 µl of saline (control) or enkephalin solution in saline (experimental) were administered subarachnoidally, 5 mm