To study the effects of hormones, vegetative buds from seedlings grown for 120 h were excized and dipped in the hormone solution. The control set was dipped in hormone solution at 0 °C. After soaking for the required time, buds were washed thoroughly with distilled water and employed for enzyme assay. Of all the different hormones tested, kinetin was found to be effective in enhancing the enzyme activity. As shown in the figure, the optimal concentration was 10^{-7} *M*, where about 70–80% increase was noticed, higher concentrations were found to be inhibitory. The kinetics of the hormone treatment showed that the enzyme activity started increasing after 4 h and reached a maximum at 24 h. Other hormones did not have much effect. IAA at 10^{-6} *M* could enhance by only 11–16% and GA_3 at 10^{-6} *M* enhanced enzyme activity by 22–29% (table). Another cytokinin, benzyladenine was totally ineffective. Cyclic AMP, tested at 10^{-6} *M*, 10^{-5} *M* and 10^{-4} *M*, also did not show any effect.

Effect of different hormones on Leucine aminotransferase activity. Here kinetin is tested at 10^{-6} *M* for the sake of comparison

Hours of treatment	Percentage increase in specific activity of enzyme		
	GA_3 (10^{-6} *M*)	IAA (10^{-6} *M*)	Kinetin (10^{-6} *M*)
4	0	0	20
8	2.8	11.9	26
12	11.2	16.5	30
16	16.5	7.8	32
24	29.9	6.08	54

To find out the mechanism of action of kinetin, effect of different inhibitors was tested. RNA synthesis inhibitors, such as cordycepin (20 µg/ml) and α-amanitin (5 µg/ml) had no effect. Actinomycin-D sometimes increased the enzyme level by 10–15%. However, cycloheximide, a potent inhibitor of protein synthesis, stopped the increase of LAT activity of kinetin by almost 100%. We have confirmed that all these inhibitors do act at the concentrations tested, in pea, by radioactive precursor incorporation study (Sihag, Sopory and Guha-Mukherjee, unpublished) and also they do not have any significant effect on total protein content of the tissue.

Effect of Various Factors on the Activity of Trehalase from the Larvae of *Sesamia inferens* Walker (Insecta)

A. K. AGARWAL[1]

Department of Zoology, University of Lucknow, Lucknow–226007 (India), 23 February 1976.

Summary. Trehalase from the salivary glands and the midgut of *Sesamia inferens* showed optimum activity at pH 5.8, and at temperatures of 50 and 60 °C respectively. The increase in the incubation period, enzyme concentration, and substrate concentration respectively increased the end-product, the hydrolysis, and the rate of hydrolysis of the substrate. Dialysis did not affect, tryptophan accelerated, and other amino acids and end-product inhibited the enzyme activity.

FRAENKEL[2] first detected trehalase (α-α-glucoside-1-glucohydrolase, E.C. 3.2.1.28) in insects and then FRÈREJACQUE[3] found its optimum activity at pH 5.8. GILMOUR[4] did not include it among the digestive enzymes, although it was detected in the gut or/and salivary glands of *Chilo simplex*[5], aphids[6], *Lucilia serricata*[7] and *Leucophaea maderae*[8]. The trehalase activity seems generally to be greatest in the salivary glands and gut[9], although it is distributed in most of the insect tissues. An excellent review on trehalase has been published by WYATT[9]. Properties of trehalase from the salivary glands and midgut of *S. inferens* have been studied separately.

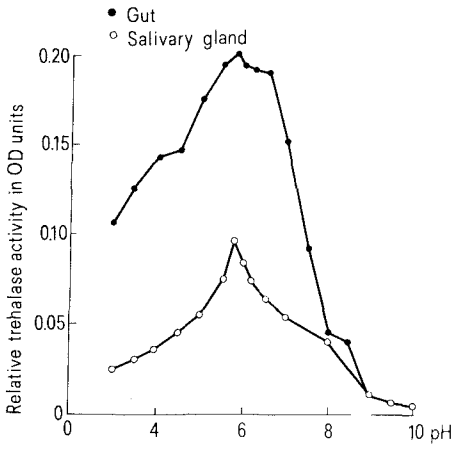

Fig. 1. The pH-trehalase activity curves of the larvae of *S. inferens*.

Materials and methods. The reaction mixture containing 0.2 ml of 1.0% trehalose solution, 0.2 ml of suitable buffer and 0.2 ml of enzyme extract[10] was incubated at 37 °C (except in case of temperature experiments) for the appropriate time periods. The reaction was stopped and the amount of reducing sugar formed was estimated[10]. The pH for the optimum activity was found by experiments at different pH values ranging from 3.0 to 10.0 using

[1] The author wishes to express his thanks to Professor R. RAKSHPAL for his constructive criticism, and to University Grants Commission, India, for awarding a Junior Research Fellowship.

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different buffer systems. All other experiments were performed at pH for the optimum activity. The enzyme activity was studied at temperatures from 10 to 90 °C. To see the effect of incubation periods, a pool of reaction mixture was incubated at 37 °C and the samples were taken out at different intervals of time for the assay of glucose formed. The effect of substrate concentration was observed at 1.0 to 7.0% trehalose concentrations. The enzyme concentration effect was studied at enzyme concentrations ranging from 1 pair of salivary glands or 1 midgut to 8 pairs of salivary glands or 8 midguts per ml. The effects of dialysis and different amino acids were observed as described elsewere[10]. To study the effect of end-product, glucose was mixed prior to the addition of the enzyme to the reaction mixture, while in the controls after stopping the reaction.

Results and discussion. The salivary gland and midgut trehalase from *S. inferens* showed optimum activity at pH 5.8 (Figure 1); but more than half of the optimal activity at the pH 4.5–8.0 and 3.0–7.5, respectively. As the pH range of the midgut is 7.9–8.2, at this pH range the trehalase activity will be very low, i.e. only 20–30% of the optimum. The pH for the optimum activity of gut and whole body trehalase of insects ranged from 5.0 to 5.8 and 5.2 to 6.5, respectively (Table I).

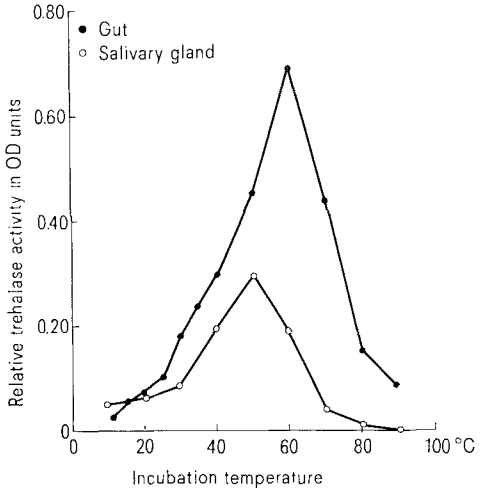

Fig. 2. The temperature-trehalase activity curves of the larvae of *S. inferens*.

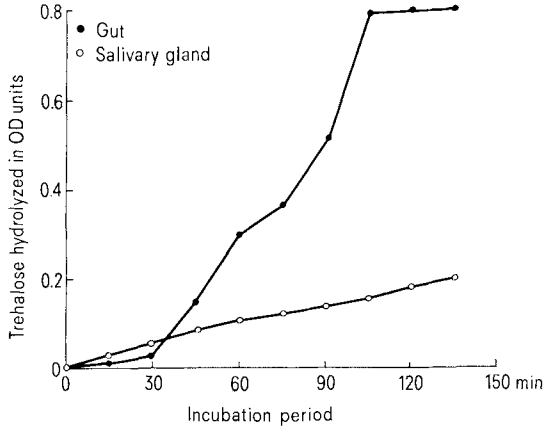

Fig. 3. The incubation period-trehalase activity curves of the larvae of *S. inferens*.

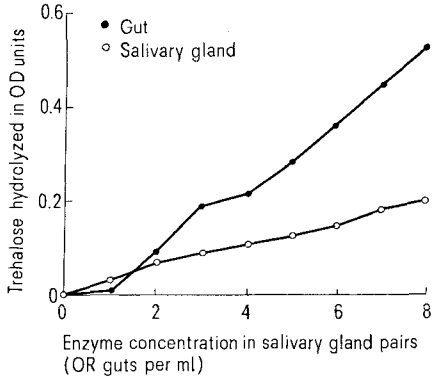

Fig. 4. The trehalase concentration-trehalase activity curves of the larvae of *S. inferens*.

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Table I. pH for the optimum activity of trehalase from various insects

Insects	Source	pH
Blaberus discoidalis[11]	Gut	5.0
Bombyx mori[12]	Gut	5.4
Hyalophora cecropia[13]	Gut	5.7
Chilotraea infuscatellus[14]	Gut	5.8
Melanoplus differentialis[15]	Whole body	5.5
Phormia regina[16]	Whole body	6.5
Melolontha vulgaris[17]	Whole body	6.5
Trinervitermes trinervoides[18]	Whole body	6.5
Bombyx mori[19]	Whole pupa	5.2
Galleria mellonella[20]	Whole body	5.5

Table II. Effect of amino acids on gut trehalase activity of the larvae of *S. inferens*

Amino acids used	Final strength (*M*)	Inhibition (%)
DL-α-alanine	0.025	44.87
DL-arginine	0.025	38.46
L-cystine	0.0125	6.41
L-glutamine	0.025	20.51
Glycine	0.025	28.20
L-hydroxyproline	0.025	38.46
L-leucine	0.025	7.69
DL-methionine	0.025	17.94
DL-phenylalanine	0.025	48.71
L-proline	0.025	38.46
DL-serine	0.025	10.26
L-tryptophan	0.0125	–34.61
L-tyrosine	0.0125	7.69
DL-valine	0.025	38.46

Trehalase from the salivary glands and midgut of *S. inferens* showed optimum activity at 50 and 60 °C, respectively (Figure 2) and more than half activity at temperature 35–60 and 45–70 °C, respectively. As the larvae were reared at 32 °C at this temperature, the activity of gut trehalase will be only about 40% of the optimum. Trehalase from *Trinervitermes trinervoides*[18] and *Hodotermes mossambicus*[21] showed optimum activity at 52 °C.

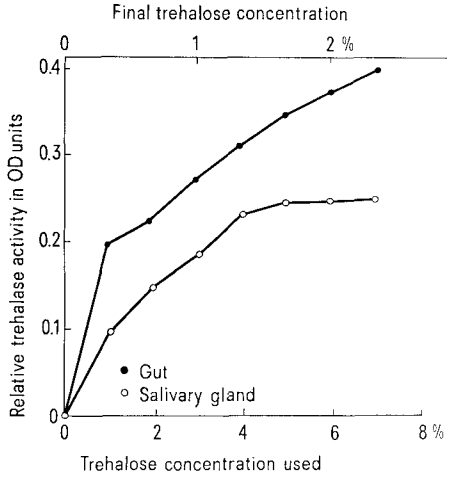

Fig 5. The trehalose concentration-trehalase activity curves of the larvae of *S. inferens*.

With the increase of incubation period, the quantity of end-product continued increasing in case of salivary gland trehalase, but in case of midgut trehalase there was almost no increase after about 100 min (Figure 3). Rate of hydrolysis of trehalose increased with the increase of concentration of trehalase from the gut and the salivary glands of *S. inferens* (Figure 4).

The activity of the salivary glands trehalase increased as the concentration of trehalose increased, but after 5.0% trehalose concentration its activity became almost constant; however, in case of midgut trehalase the activity continued increasing even after 5.0% trehalose concentration (Figure 5).

Dialysis did not affect the activity of the midgut trehalase. Tryptophan accelerated while all other amino acids used inhibited the activity of trehalase (Table II). The activity of trehalase was inhibited even when the concentration of glucose was as low as 0.5%.

It may be concluded that the activity of trehalase was very low at the temperature at which the larvae of *S. inferens* were reared and at the pH of their gut, and its activity was lowered even by as low concentration of glucose as 0.5%. It may be added that presence of an enzyme in abundance does not mean that it functions effectively.

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Changes of the Phosphatidylcholine Content and the Number of Synaptic Vesicles in Relation to the Neurohumoral Transmission in Sympathetic Ganglia

Á. PÁRDUCZ, Z. KISS and F. JOÓ[1]

Institutes of Biophysics and Biochemistry, Biological Research Centre, H–6701 Szeged (Hungary), 31 May 1976.

Summary. In sympathetic ganglia stimulated in the presence of HC-3, the reduction in number of synaptic vesicles was observed to be accompanied by a significant decrease of the ganglionic phosphatidylcholine content.

The quantal release of transmitter at nerve endings is well established physiologically[2], and there is a great deal of suggestive evidence that its morphological basis involves the packaging of the neurotransmitter in the vesicles seen in the electron micrographs of nerve terminals[3,4]. Concerning the mechanism of the actual release, current hypotheses favour a process of exocytosis and presume some sort of recycling process, whereby the vesicle membrane is transiently incorporated into the plasma membrane and is subsequently made available again for the manufacture of more intracellular vesicles[5,6]. In such a system, the materials composing the vesicle membrane would not be lost from the nerve ending. A useful approach for proving this would therefore be to measure some constituent substance of the vesicle membrane in the nerve endings at time when their number was diminished. Although there is no easily measured membrane component which is specific for vesicles, it is known that the synaptic vesicle wall is especially rich in phosphatidylcholine (PC)[7]. We have therefore designed experiments to measure the PC in sympathetic ganglia, during conditions when there is a dramatic fall in the vesicle content. Our experiments involve the use of hemicholinium (HC-3) to interfere with the choline uptake[8], coupled with repeated stimulation of the nerve; this readily produces a depletion of vesicle number[9] in the superior cervical ganglion (SCG) of the cat.

Cats of both sexes (weighing 1.5–3 kg) were anaesthetized with 40 mg/kg pentobarbital, and ganglia of either side were experimented on in a variety of ways. In the 1st group of animals, the ganglion on one side was simply stimulated through its preganglionic nerve trunk for 5 min; in a 2nd group the stimulation was identical, but the animals had been previously given 10 mg/kg

[1] Acknowledgment: We thank Prof. J. DIAMOND for the helpful suggestions and the critical reading of the manuscript.

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