*Proc. Indian natn. Sci. Acad.* **B 46** No. 5 pp 659-666 (1980)

# Hepatopancreatic Trehalase of *Macrobrachium lamarrei* (Crustacea: Decapoda)

RAMESH CHANDRA MURTHY and PADMA SAXENA*
*Department of Zoology, University of Lucknow, Lucknow*

*( Received 3 May 1980; after revision 23 October 1980 )*

The effect of nine factors was investigated on the hepatopancreatic trehalase of *Macrobrachium lamarrei*. Its optimum *p*H is 5.5 and optimum temperature 60°C. Glucose, the end product, suppressed its activity above 0.01 M. When the incubation time was prolonged trehalase activity remained constant upto 160 min. but thereafter the rate of activity decreased, finally becoming zero. Increasing enzyme concentration produced a similar effect. Its Km value is $1.56 \times 10^{-3}$ M. Dialysis produced no effect on enzymic activity. Out of 17 chlorides tested, while six produced no effect, eleven suppressed its activity. Stronger solution(s) of $MnCl_2$, $SnCl_2$, $PtCl_4$ and $HgCl_2$ caused complete inhibition. Of the 19 amino acids tested, seven exerted no effect, four enhanced and seven inhibited its activity; one (isoleucine) produced both effects. Serine caused the maximum and threonine the minimum activation; conversely, norleucine caused the maximum and glutamine the least inhibition.

**Key Words: Crustacea. Decapoda,** *Macrobrachium lamarrei,* **Hepatopancreatic trehalase**

## Introduction

The enzyme trehalase, first detected in insects (Fraenkel 1940), hydrolyses one molecule of trehalose into two molecules of glucose. Among crustaceans, its presence has been reported in the digestive system of *Porcellio* and *Oniscus* (Lockwood 1968) but no work seems to have been done on its properties. Since the midgut gland of *Macrobrachium lamarrei* showed strong trehalase activity (Murthy 1978a) the effect of nine factors, including metallic chlorides and amino acids was studied on it. The two latter factors were included to ascertain whether or not trehalase required ions for its activation.

## Material and Methods

The midgut gland of *M. lamarrei* Milne Edward was removed from 100 individuals and pooled in ice cold distilled water, weighed and homogenised. The homogenate was centrifuged at 3000 × g for 15 mins. at 4°C and the supernatant diluted with water to yield a concentration of 4 mg/ml of hepatopancreas.

*Request for reprint is to be made to P Saxena

The assay system consisted of : 0.3 ml buffer solution, 0.2 ml of 0.1 M trehalose (both solutions pre-incubated at 37°C) and 0.1 ml of enzyme extract. In controls heat denatured enzyme was added. After 4 hrs incubation at 37°C the reaction was stopped by adding 0.5 ml each of 5% $ZnSO_4$ and 5% $Ba(OH)_2$ solutions and the released glucose estimated by Nelson-Somogyi's method as elaborated by Halliwal (1965). Of the nine factors—*p*H, temperature, end product, incubation period, substrate and enzyme concentration, dialysis, metallic chlorides and amino acids—*p*H was the first factor to be studied, in order to ascertain its optimum value at which the effect of the eight remaining factors was investigated.

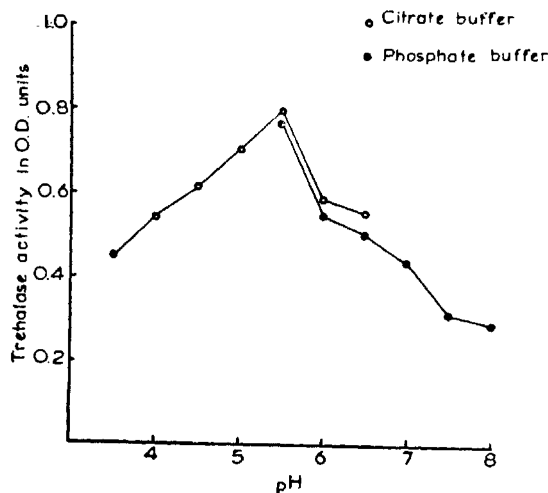


**Figure 1** Effect of *p*H on hepatopancreatic trehalase

## Results

*Determination of optimum pH*: The results using two buffer systems (0.1M sodium citrate buffer for *p*H range 3.5-6.5 and 0.1M Sorenson's phosphate buffer for *p*H range 5.5-8 0) show that the enzyme remained quite active from *p*H 3.5-7.0 and that its optimum activity occurred at *p*H 5.5 (figure 1). While the optimum *p*H with both buffers coincided, the enzyme preparation using phosphate buffer showed an activity of 91.6% as compared to that using citrate buffer.

*Effect of temperature*: Trehalase activity was tested at various temperatures, ranging from 10° to 80°C. The enzyme showed optimum activity at 60°C (figure 2), although it remained quite active from 37° to 70°C.

*Effect of end product (glucose)* : To the assay system, 0.2 ml of glucose solution of ten strengths, from 0.0025-0.25 M was added. At weaker concentrations up to 0.01M, no effect, but at higher ones an inhibitory effect was produced (figure 3).

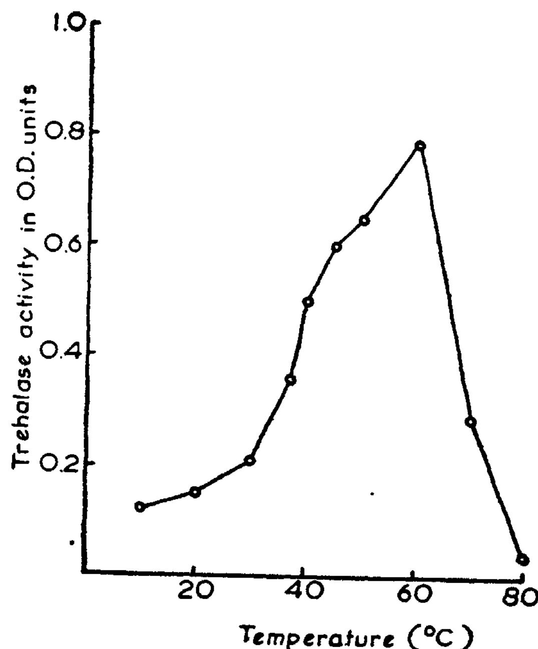


**Figure 2** Effect of temperature on hepatopancreatic trehalase

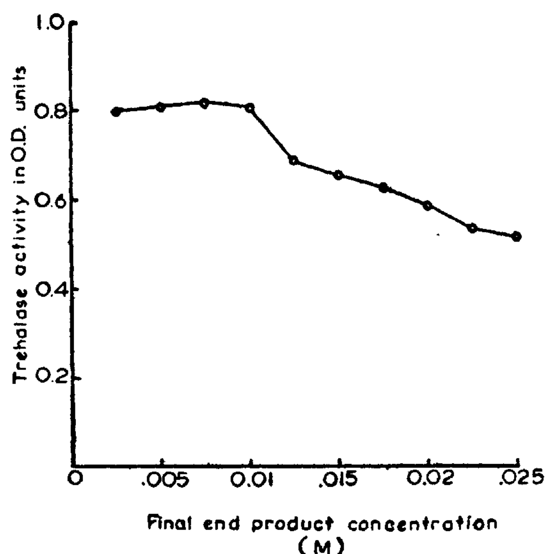


**Figure 3** Effect of end product (glucose) on hepatopancreatic trehalase

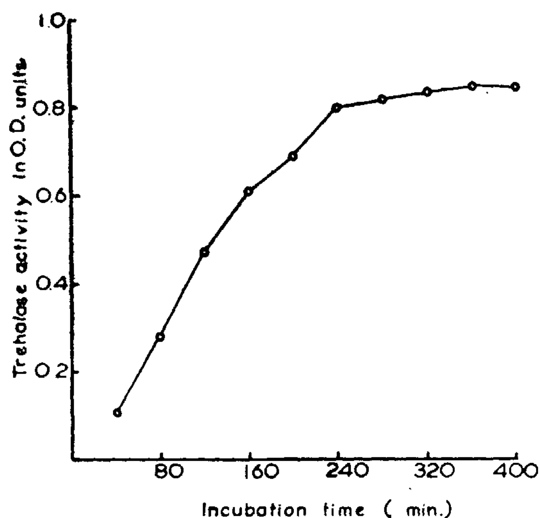


**Figure 4** Effect of incubation period on hepatopancreatic trehalase

*Effect of incubation period*: For determining the effect of incubation period, ten tubes were incubated for progressively longer duration by 40 mins. Glucose formation increased upto 360 mins., becoming constant at 400 mins. (figure 4). The rate of increase was almost linear up to 160 mins., somewhat slower between 160 and 240 mins. and negligible after 240 mins. This pattern shows that trehalase activity remained upto 160 mins., thereafter it decreased reaching finally close to zero at 400 mins.

*Effect of substrate concentration*: Trehalose solutions of fourteen concentrations, from 0.005-0.04 M, were tested. Glucose formation increased up to 0.03 M, becoming constant at 0.04 M. Consequently, a 0.04 M solution of trehalose is essential for converting virtually all trehalase into ES-complex. The Michaelis constant (*Km* value), derived from this data, is $1.56 \times 10^{-3}$M (figure 5).

*Effect of enzyme concentration*: Enzyme extracts of ten strengths increasing by 1 mg hepatopancreas/ml were tested. Glucose formation increased with increasing concentration of the enzyme at a more or less constant rate up to 6 mg hepatopancreas/ml (figure 6); above this strength it declined progressively. Thus, the rate of increase of trehalase activity shows a decrease above 6 mg hepatopancreas/ml.

*Effect of dialysis*: The activity of an enzyme preparation dialysed for 24 hrs against double distilled water at 4°C did not differ from that of undialysed enzyme.

In the study of factors 8 and 9 dialysed enzyme preparation, as given under factor 7, was used.

*Effect of metallic chlorides*: Seventeen metallic chlorides were tested, each in six concentrations ranging from $5 \times 10^{-4}$M to $1 \times 10^{-1}$M, Since 0.2 ml of the metallic

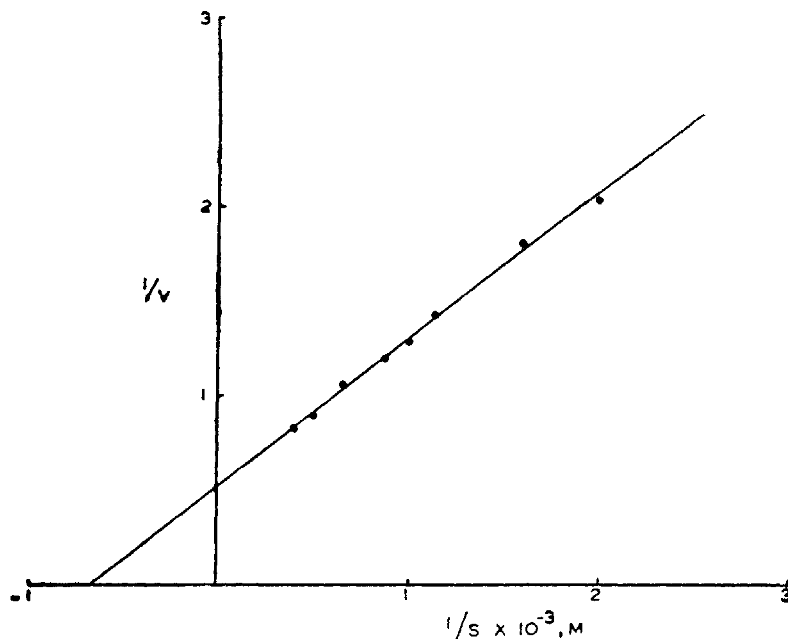


**Figure 5** Lineweaver-Burk plot for Michaelis constant (Km value) of hepatopancreatic trehalase

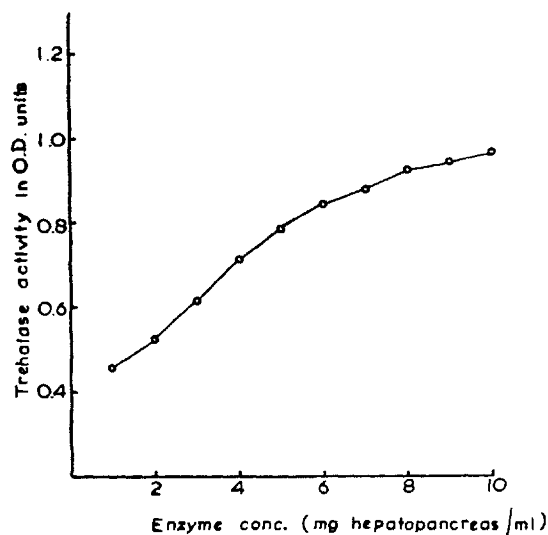


**Figure 6** Effect of enzyme concentration on its activity

chloride solution was added to the assay system, its final concentration was one-fourth of the original.

Trehalase activity remained unaffected by chlorides of Li, Na, K, Ca, Rb and Sr (table 1). It remained unaffected by chlorides of Mg, Co, Cu and Zn also but only at weaker concentrations; their stronger solutions inhibited its activity. The remaining six chlorides, of Al, Mn, Ba, Pt, Hg and Sn, inhibited the activity at all concentrations, the inhibition being total with stronger solutions of Mn, Pt, Hg and Sn.

*Effect of amino acids :* Nineteen amino acids were tested, each in six concentrations ranging from $5 \times 10^{-4}$ M to $1 \times 10^{-1}$ M. Since 0.2 ml of the amino-acid solution was added to the assay system, its final

**Table 1** *Effect of metallic chlorides on the activity of hapatopancreatic trehalase of* M. lamarrei

| Chloride | | Effective molar concentration(s) | Relative trehalase activity | % Activation | % Inhibition |
|---|---|---|---|---|---|
| Nil | | — | 100 | Nil | Nil |
| Lithium | $Li^{+}$ | All concentrations | 100 | Nil | Nil |
| Sodium | $Na^{+}$ | All concentrations | 100 | Nil | Nil |
| Magnesium | $Mg^{++}$ | $2.5 \times 10^{-2}$ | 81 | — | 19 |
| Aluminium | $Al^{+++}$ | $2.5 \times 10^{-2}$ | 26 | — | 74 |
| Potassium | $K^{+}$ | All concentrations | 100 | Nil | Nil |
| Calcium | $Ca^{++}$ | All concentrations | 100 | Nil | Nil |
| Manganese | $Mn^{++}$ | $2.5 \times 10^{-3}$ | 00 | — | 100 |
| Cobalt | $Co^{++}$ | $2.5 \times 10^{-3}$ | 74 | — | 26 |
| Cupric | $Cu^{++}$ | $2.5 \times 10^{-3}$ | 64 | — | 36 |
| Zinc | $Zn^{++}$ | $2.5 \times 10^{-3}$ | 72 | — | 28 |
| Rubidium | $Rb^{+}$ | All concent·ations | 100 | Nil | Nil |
| Strontium | $Sr^{++}$ | All concentrations | 100 | Nil | Nil |
| Cadmium | $Cd^{++}$ | $2.5 \times 10^{-3}$ | 67 | — | 33 |
| Stannous | $Sn^{++}$ | $2.5 \times 10^{-3}$ | 00 | — | 100 |
| Barium | $Ba^{++}$ | $2.5 \times 10^{-2}$ | 66 | — | 34 |
| Platinum | $Pt^{++++}$ | $1.25 \times 10^{-2}$ | 00 | — | 100 |
| Mercuric | $Hg^{++}$ | $1.25 \times 10^{-2}$ | 00 | — | 100 |

**Table 2** *Effect of amino acids on the activity of hepatopancreatic trehalase of* M. lamarrei

| Amino acid | Effective molar concentration (s) | Relative trehalase activity | % Activation | % Inhibition |
|---|---|---|---|---|
| Nil | — | 100 | Nil | Nil |
| DL-α-Alanine | $2.5 \times 10^{-2}$ | 81 | — | 19 |
| β-Alanine | All concentrations | 100 | Nil | Nil |
| α-Aminobutyric acid | All concentrations | 100 | Nil | Nil |
| γ-Aminobutyric acid | All concentrations | 100 | Nil | Nil |
| L-Asparagine | All concentrations | 100 | Nil | Nil |
| L-Glutamine | $2.5 \times 10^{-2}$ | 79 | — | 21 |
| Glycine | All concentrations | 100 | Nil | Nil |
| L-Hydroxyproline | $2.5 \times 10^{-2}$ | 70 | — | 30 |
| Isoleucine | $1.25 \times 10^{-4}$ | 124 | 24 | — |
| | $2.5 \times 10^{-2}$ | 76 | — | 24 |
| L-Leucine | $2.5 \times 10^{-2}$ | 33 | — | 67 |
| Lysine | $1.25 \times 10^{-2}$ | 181 | 81 | — |
| DL-Methionine | All concentrations | 100 | Nil | Nil |
| Norleucine | $2.5 \times 10^{-2}$ | 24 | — | 76 |
| DL-Phenylalanine | $2.5 \times 10^{-2}$ | 60 | — | 40 |
| L-Proline | $2.5 \times 10^{-2}$ | 74 | — | 26 |
| DL-Serine | $1.25 \times 10^{-2}$ | 197 | 97 | — |
| L-Threonine | $2.5 \times 10^{-2}$ | 151 | 51 | — |
| L-Tryptophan | $2.5 \times 10^{-2}$ | 172 | 72 | — |
| DL-Valine | All concentrations | 100 | Nil | Nil |

concentration in the reaction mixture was one-fourth of the original.

Seven amino acids produced no effect on trehalase activity. These are: β-alanine, α- and γ-aminobutyric acid, asparagine, glycine, methionine and valine (table 2). Serine, lysine, tryptophan and threonine (arranged in descending order of activation), activated the enzyme at almost all concentrations. Isoleucine produced both activatory and inhibitory effects; it activated the enzyme (by 24%) at the weakest but inhibited (by 24%) at the strongest concentration (table 2). The remaining seven amino acids exerted no influence at weaker but inhibited the activity at higher concentrations. These are: norleucine, leucine, phenylalanine, hydroxyproline, proline, glutamine and α-alanine (arranged in descending order of inhibition).

## Discussion

The optimum *p*H of muscle trehalase of *M. lamarrei* being 6.0 (Murthy 1977) is higher than that of its hepatopancreatic enzyme (*p*H 5.5). In those insects also in which the optimum *p*H of muscle and gut trehalase is known, that of muscle trehalase is higher (table 3). The optimum *p*H of hepatopancreatic trehalase falls within the range of optimum *p*H (5.0–5.8) of gut trehalase of insects (table 3). In contrast, it is lower than the *p*H of its own gastric juice, which is 6.4–6.7 (Murthy 1978a); this shows that the activity of hepatopancreatic trehalase *in vivo* would be only 55–65% of its full activity *in vitro*.

The optimum temperature (60°C) of hepatopancreatic trehalase of *M. lamarrei* is higher than that of gut trehalase of *Trinervitermis trinervoides* (55°C; Retief & Hewitt 1973) and that of salivary gland trehalase of larva of *Sesamia inferens* (50°C) but conforms to that of gut trehalase of the latter (60°C; Agarwal 1976). Its being higher than that of the ambient of all these animals while of significance, is hardly fortuitous.

Inhibition of hepatopancreatic trehalase of *M. lamarrei* by 0.015 M solution of glucose is in agreement with Agarwal's (1976) findings on *S. inferens*. While a solution of this strength caused 36.6% inhibition in trehalase activity of *M. lamarrei*, the degree of inhibition caused

**Table 3** *Optimum pH and Michaelis constant of digestive and muscle trehalase of some insects and of* M. lamarrei

| Animal | *p*H | | Km (M) × $10^{-3}$ | | Reference |
|---|---|---|---|---|---|
| | Digestive trehalase | Muscle trehalase | Digestive trehalase | Muscle trehalase | |
| *Bombyx mori* | 5.4 | — | 2.9 | — | Horie 1959 |
| *Hyalophora cecropia* | 5.7 | 6.5 | 0.4 | 3.6 | Gussin & Wyatt 1965 |
| *Blaberus discoidalis* | 5.0 | 6.0 | 0.5 | 3.3 | Gilby et al., 1967 |
| *Trinervitermis trinervoides* | 5.2 | — | 9.708 | — | Retief & Hewitt 1973 |
| *Heliothis zea* | 5.5 | — | — | — | Burton 1975 |
| *Sesamia inferens* | 5.8 | — | — | — | Agarwal 1976 |
| *M. lamarrei* | 5.5* | 6.0 | 1.56 | 2.85 | Murthy 1977 |

*Present observation

in *S. inferens* has not been given. Surprisingly enough, a glucose solution 20 times stronger than trehalose was found to produce no effect on the activity of trehalase of *Phormia regina* (Friedman 1960).

In the study of prolonged incubation and increasing enzyme concentration, the rate of increase in trehalase activity is reduced after 160 minutes and 6 mg hepatopancreas/ml respectively. The decrease can be due either to the inhibitory effect of the formed glucose or to a depletion of trehalose or to a combination of both.

The *Km* value of hepatopancreatic trehalase ($1.56 \times 10^{-3}$M) of *M. lamarrei* is lower than that of its muscle trehalase ($2.85 \times 10^{-3}$ M, Murthy 1977). This is in agreement with the view of Wyatt (1967) that the *Km* value of gut trehalase of insects (soluble trehalase) is usually lower than that of their body or muscle trehalase (particulate trehalase) (table 3).

Failure of dialysis to affect the activity of hepatopancreatic trehalase is surprising, more so when it reduced the activity of two carbohydrases—sucrase and amylase—from the same source (Murthy 1978b).

Inhibition of trehalase activity by majority of metallic chlorides tested is indicative of inhibition to be due to the chloride ion. Incidentally, this is just the opposite of their effect on amylases in general which are activated by $Cl^-$ (Colowick & Kaplan 1955). The difference in the extent of inhibition would seem to be due to the effect of the metallic cation, which is different for each salt.

The effect of metallic ions has not been widely investigated even on insects. Retief and Hewitt (1973) reported 50% inhibition of gut trehalase of *T. trinervoides* by a $2.2 \times 10^{-4}$M solution of $Mg^{++}$. An inhibitory effect on hepatopancreatic trehalase of *M. lamarrei* was exerted not by this, but by a stronger concentration of $Mg^{++}$. Dahlman's (1971) study of the effect of 10 metallic chlorides on purified trehalase from whole larvae of *Manduca sexta* is worth consideration; while seven divalent cations ($Hg^{++}$, $Mn^{++}$, $Co^{++}$, $Ni^{++}$, $Cu^{++}$, $Zn^{++}$ and $Cd^{++}$) produced an inhibitory effect, the three monovalent cations ($Na^+$, $K^+$ and $Li^+$) produced a slight activatory effect. Of the seven divalent cations tested by Dahlman, with the exception of $Ni^{++}$, which was not tested, all others caused inhibition of hepatopancreatic trehalase; the three monovalent cations were ineffective.

The only information on the effect of amino acids on arthropod trehalase seems to be the study of Agarwal (1976). He studied the effect of one concentration of 14 amino acids on undialysed trehalase from the larval gut of *S. inferens*. On comparison with Agarwal's results the following comments have been considered desirable: (i) Tryptophan activated, whereas α-alanine, glutamine, hydroxyproline, leucine, phenylalanine and proline inhibited the activity of trehalase from both the sources, albeit to different degrees; (ii) Serine produced opposite effects, as it inhibited the insectan but activated the crustacean trehalase; and (iii) Glycine, methionine and valine inhibited insectan but had no effect on hepatopancreatic trehalase.

Owing to the inhibitory effect of the majority of the tested amino acids, the alfa amino ($-NH_2$) and carboxylic (-COOH) groups, being common to all, may be inferred to be responsible for the inhibition. The

third, acyl (R–), group which is characteristic for each amino acid, could likewise be held responsible for difference in the degree of inhibition or activation caused by the amino acids. On this basis the strong inhibition caused by norleucine and leucine can be attributed to the inhibitory effect of their respective R-groups ($\beta$-methyl valeryl and isocaproic groups) in conjunction with the same effect of their $-NH_2$ and –COOH groups. Likewise the strong activation caused by serine and lysine can be attributed to the activatory effect of their respective R-groups (hydroxy propionyl and amino caproic groups) which more than completely neutralize the inhibitory effect of $-NH_2$ and – COOH groups.

**Acknowledgements**

We thank the Head, Department of Zoology, University of Lucknow for making available all necessary facilities; RCM is also thankful to the University Grants Commission for the award of a Junior Research Fellowship.

**References**

Agarwal A K 1976 Effect of various factors on the activity of trehalase from the larvae of *Sesamia inferens* Walker (Insecta); *Experientia* **32** 1518-1519

Burton R L 1975 Carbohydrate digestion in the adult moth *Heliothis zea*; *J. Insect Physiol* **21** 1855-1857

Colowick S P and Kaplan N O 1955 *Methods in Enzymology*. Vols. I and II (New York: Academic Press)

Dahlman D L 1971 Purification and properties of trehalase from tobacco worm larvae; *J. Insect Physiol.* **17** 1677-1687

Fraenkel G 1940 Utilization and digestion of carbohydrates by the adult blowfly; *J. exp. Biol.* **17** 18-29

Friedman S 1960 The purification and properties of trehalase isolated from *Phormia regina* Meig; *Arch. Biochem. Biophys.* **87** 252-258

Gilby A R, Wyatt S S and Wyatt G R 1967 Trehalases from the cockroach *Blaberus discoidalis:* Activation, solubilization and properties of the muscles enzyme and some properties of the intestinal enzyme; *Acta. Biochim. Polo.* **14** 83-100

Gussin A F S and Wyatt G R 1965 Membrane-bound trehalase from cecropia silkmoth muscle; *Arch. Biochem. Biophys.* **112** 626-634

Halliwal G 1965 Hemicelluloses in *Methods of Enzymatic Analysis* ed Bergmeyer H V pp. 77-78 (New York: Academic Press)

Horie Y 1959 Physiological studies on the alimentary canal of the silkworm *Bombyx mori*. II. Carbohydrases in the digestive fluid and in the midgut tissue; *Bull. Sericul. Exp. Sta. Japan* **15** 365-381

Lockwood A P M 1968 Feeding and digestion in *Aspects of the physiology of Crustacea*, pp. 254-264 (Edinburgh : Oliver and Boyd)

Murthy R C 1977 Properties of trehalase from the muscles of fresh water prawn *Macrobrachium lamarrei; Experientia* **33** 480-481

—— 1978a Study of carbohydrases in the digestive system of *Macrobrachium lamarrei* Edwards (Crustacea : Decapoda); *Comp. Physiol. Ecol.* **3** 13-16

—— 1978b Studies on the digestive physiology of *Macrobrachium lamarrei* Milne Edwards (Crustacea : Dacapoda); Ph. D. thesis, University of Lucknow

Retief L W and Hewitt P H 1973 Purification and properties of trehalase from the harvester termite, *Trinervitermis trinervoides; J. Insect Biochem.* **3** 345-351

Wyatt G R 1967 The biochemistry of sugars and polysaccharides in insects; in *Advances in Insect Physiology* eds. J W L Beament, J E Treherne and V B Wigglesworth Vol. 4, pp. 309-325 (New York: Academic Press)