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# Hepatopancreatic sucrase of *Macrobrachium lamarrei* (Crustacea, Caridea, Palaemonidae)

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**Abstract.** The effect of seven factors was studied on the activity of hepatopancreatic sucrase of *Macrobrachium lamarrei*. Its optimum pH is 6·0 and optimum temperature 50° C and its activity was suppressed by the two end products, glucose and fructose. On prolonging incubation period sucrase activity remained constant up to 8 hr, declined thereafter, finally becoming zero. Increasing enzyme concentration produced a similar effect. Its $K_m$ value is $4{\cdot}5 \times 10^{-2}$ M. Dialysis suppressed its activity by 17·9%.

**Keywords.** Crustacea ; caridea ; palaemonidae ; *Macrobrachium lamarrei* ; sucrase ; hepatopancreatic sucrase.

## 1. Introduction

The enzyme sucrase ($\beta$-fructofuranosidase, EC 3.2.1.26) is known to occur in the digestive system of a wide variety of crustaceans (Mansour-Bek 1954; van Weel 1970; Vonk 1960). Yet information on its kinetic properties is scanty, being confined to its response to pH (Agarwal 1963, 1964; Martin 1966; Newcomer 1956; Nicholls 1931; Wiersma and van Ween 1928). The present work was conducted to determine the properties of this enzyme by studying the effect of seven factors on hepatopancreatic sucrase of *Macrobrachium lamarrei*, a freshwater shrimp, reported to be rich in this enzyme (Murthy 1978).

## 2. Materials and methods

Hepatopancreatic glands from 100 *Macrobrachium lamarrei* Milne Edward were pooled in ice cold distilled water, dried between filter-paper sheets, weighed and homogenized in distilled water. The homogenate was centrifuged at 3000 xg for 15 min at 4° C and the supernatant diluted to a concentration of 10 mg (wet weight)/ml (or 0·1 ml ≡ 1 mg) of hepatopancreas. The assay system consisted of: appropriate buffer 0·3 ml, 0·3 M sucrose 0·2 ml and enzyme extract 0·1 ml; in controls a heat denatured enzyme was added. After incubating the mixtures at 37° C for 4 hr, following Bernfield's (1955) colorimetric method for estimating hexose sugars, the reaction was stopped by adding 0·5 ml of 3,5-dinitrosalicylic reagent ; thereafter the mixtures were warmed for 15 min, the volume raised to

6 ml with distilled water and readings taken at 540 nm. Under conditions similar to those for the enzyme assay, direct reaction of glucose with dinitrosalicylic reagent gives 1 extinction unit = 0·6 mg glucose. Of the seven factors—pH, temperature, end products, incubation period, enzyme and substrate concentration and dialysis—pH was the first factor to be studied, in order to ascertain its optimum value, at which the effect of the six remaining factors was investigated. Some experimental details are given in §3. The presented values of each factor represent the mean of five replicates.

## 3. Observations

### 3.1. *Determination of optimum pH*

According to the results using two buffer series (0·1 M sodium citrate buffer from pH 3·5–6·5 and 0·1 M Sorenson's phosphate buffer from pH 5·5–8·0), sucrase remained quite active from pH 4·5-7·0 and its optimum activity occurred at pH 6·0 (figure 1). While its optimum pH with both buffers coincided, enzymic activity at this pH (6·0) with phosphate buffer was 9·7% greater than that using citrate buffer.

### 3.2. *Effect of temperature*

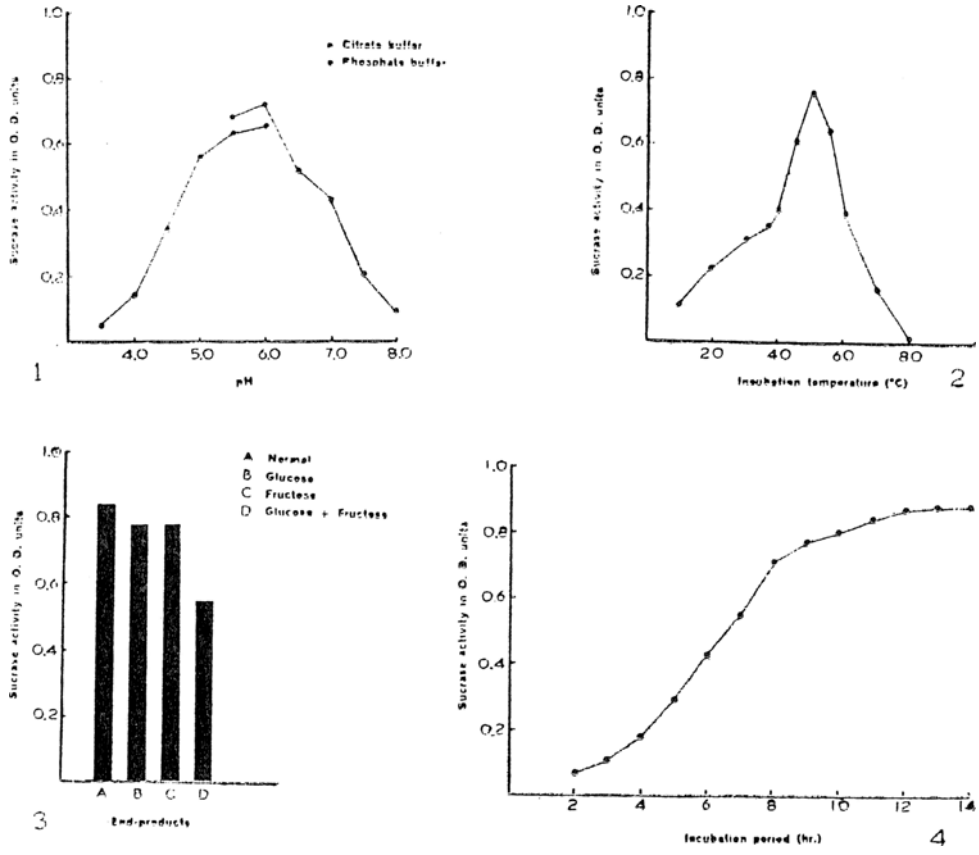
Enzymic activity was tested at eleven temperatures ranging from 10°-80° C (figure 2). Sucrase showed optimum activity at 50° C, although remaining quite active from 20°-6° C. The activity increased slowly from 10°–40° C but steeply from 40°–50° C; above 50° C it fell sharply becoming almost zero at 80° C.

### 3.3. *Effect of end products*

Four tubes, *A* to *D*, of the assay system were prepared and to three, *B* to *D*, the end product(s) were added before their incubation. No end product was added to tube *A*, being meant to serve as the blank for calculating normal enzymic activity. In the parallel controls of *B* to *D*, the end product(s) were added after their incubation. The details are tabulated below :

| | Tube *A* | Tube *B* | Tube *C* | Tube *D* |
|---|---|---|---|---|
| 1% Glucose, in ml | 0·0 | 0·1 | 0·0 | 0·05 |
| 1% Fructose, in ml | 0·0 | 0·0 | 0·1 | 0·05 |
| Distilled water, in ml | 0·2 | 0·1 | 0·1 | 0·1 |

The solutions of glucose and fructose when added separately caused inhibition of sucrase activity to the same extent, by 7·2%; whereas a mixture of both caused 34% inhibition (figure 3).



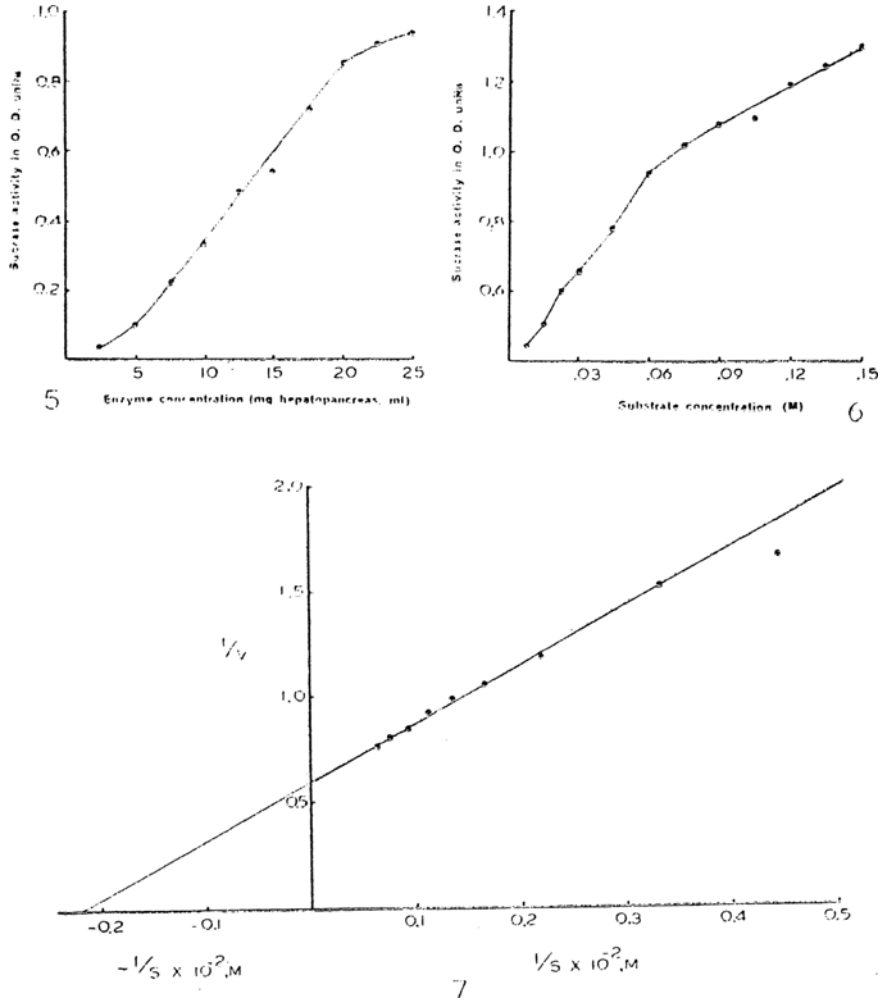

**Figures 1-4.** 1. Effect of pH on hepatopancreatic sucrase. 2. Effect of temperature on hepatopancreatic sucrase. 3. Effect of end products on hepatopancreatic sucrase. 4. Effect of incubation period on hepatopancreatic sucrase.

### 3.4. *Effect of incubation period*

Results of incubating 13 tubes for progressively longer duration by one hour show that from 2-8 hr the rate of increase of formation of end products was more or less linear (figure 4). Thereafter, the build-up of hexose sugars decreased becoming constant after 14 hr of incubation. This pattern indicates a somewhat constant rate of enzymic activity up to 8 hr, followed by decreased activity, reaching finally close to zero at 14 hr.

### 3.5. *Effect of enzyme concentration*

Enzyme extracts of ten concentrations, ranging from 2·5 mg/ml to 25 mg/ml of hepatopancreas, were tested (figure 5). The liberation of hexose sugars increased more or less linearly up to an enzyme concentration of 20 mg/ml. Above it the increase was considerably slower, becoming almost nil at 25 mg/ml.




**Figures 5-7.** 5. Effect of sucrase concentration on its activity. 6. Effect of substrate concentration on hepatopancreatic sucrase. 7 Lineweaver-Burk plot for Michaelis constant ($K_m$ value) of hepatopancreatic sucrase.

### 3.6. *Effect of substrate concentration*

Sucrose solutions of 12 concentrations, ranging from 0·0075 to 0·15 M, were tested. The rate of liberation of hexose sugars was almost linear up to 0·06 M concentration; after which it gradually slowed down (figure 6). The Michaelis constant ($K_m$ value) of hepatopancreatic sucrase as calculated from the collected data is $4{\cdot}5 \times 10^{-2}$ M (figure 7).

### 3.7. *Effect of dialysis*

An enzyme preparation dialysed for 24 hr at 4° C against double distilled water suffered a 17·9% loss in activity.

## 4. Discussion

In *Astacus fluviatilis* the optimum activity of gastric juice sucrase was reported to occur at pH 6·0 (Wiersma and van Ween 1928), as well as over a small pH range 4·5–5·0 (Krüger and Graetz 1928). The optimum activity of hepatopancreatic sucrase of *Marinogammarus obtusatus* (4·2–6·4; Martin 1966), *Porcellio laevis* (5·5–6·5; Newcomer 1956) and of *Thalamita crenata* (7·74–7·87; van Weel 1960) takes place over a narrow pH range. In contrast, that of *Corophium volutator* and *Orchestia gammarella* (5·8, 6·0 respectively; Agarwal 1963, 1964), *Ligia oceanica* (5·8; Nicholls, 1931) occurs at a sharp pH. The optimum pH of hepatopancreatic sucrase of *M. lamarrei* being 6·0 is a sharp peak. Being lower than the pH of the stomach contents (6·4–6·7; Murthy 1978), sucrase activity *in vivo* would therefore be about 66·6–72·25% of its full activity *in vitro*.

The effect of the six remaining factors apparently remains uninvestigated on crustacean sucrase. However, as the effect of three of them, temperature, end products and dialysis has been studied on insectan sucrase, the findings on *M. lamarrei* have been compared with the available insectan data. The optimum temperature of hepatopancreatic sucrase of *M. lamarrei* corresponds to that of *Sarcophaga ruficornis* and *Musca domestica* (50° C; Sinha 1976), but is higher than that of *Blatella germanica* (25° C; Day and Powning 1949), *Sesamia inferens* (30°C for gut, 35° C for salivary glands; Agarwal 1976), *Acyrthosiphon pisum* (35° C; Srivastava and Auclair 1962), *Chrysomphalus aonidum* and *Aonidiella aurantii* (37° C; Ishaaya and Swirski 1970) and *Lygus disponsi* (37° C; Hori 1971).

Inhibition by the end products of sucrase activity, as occurs in *M. lamarrei*, has been recorded in insects like *Bombyx mori* (Horie 1959), *Dysdercus fasciatus* (Khan and Ford 1967), *A. pisum* (Srivastava and Auclair 1962) and *S. inferens* (Agarwal 1976); no effect was recorded in *Aedes aegypti* (Yang and Davies 1968) and *L. disponsi* (Hori 1971) by their accumulation.

The results in the case of three factors, (i) prolonged incubation, (ii) increasing concentration of enzyme and (iii) substrate are similar, as an initial fast hydrolysis of the substrate undergoes slowing down. The slowing down after prolonged incubation can be due either to the inhibitory effect of glucose and fructose formed or to a depletion of sucrose or to a combination of both. The retardation by relatively higher concentrations of the substrate can be due to the conversion of the total enzyme into ES-complex, as postulated by Karlson (1969). However, accumulation of formed hexose sugars can be a contributory factor. That by stronger enzyme extracts can be attributed either to rapid exhaustion of the substrate due to excessive enzyme or to the inhibitory effect of the formed end products or to a combination of both.

At present, reduced activity of hepatopancreatic sucrase of *M. lamarrei* after dialysis can neither be explained nor compared. However, dialysed sucrase from the gut and salivary glands of lepidopterous larvae showed 8% activation and 37·5% inhibition respectively after dialysis (Agarwal 1976).

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