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Esterase activity in the hepatopancreas of *Macrobrachium lamarrei* (Crustacea: Decapoda)[1]

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Summary. The activity of the hepatopancreatic esterase of the fresh water prawn *Macrobrachium lamarrei* was optimal at pH 7.4 and temperature 40 °C. The activity increased with the increase in incubation period and enzyme concentration. The Michaelis constant (K_m) of the enzyme was 2.1×10^{-3} M.

Very little work has been done on the properties of the digestive esterases of crustaceans, although almost every crustacean investigated so far has been shown to possess these enzymes[4]. As strong esterolytic activity was recorded in the hepatopancreatic extracts of *Macrobrachium lamarrei*[5], a study of the properties of the enzyme concerned (arylesterase; E.C. 3.2.1.2) was undertaken.

Material and methods. Animals were collected from the local river Gomati. The hepatopancreas was dissected out, weighed immediately and homogenized in cold distilled water using an all-glass homogenizer, and the homogenate was centrifuged at $3000 \times g$ for 15 min at 4 °C. The supernatant was used as the enzyme source. For enzyme assay the reaction mixture containing 0.2 ml of homogenate supernatant, 0.5 ml of buffer, 0.5 ml of substrate solution (β-naphthyl acetate, 5×10^{-4} M in distilled water) and 0.6 ml of distilled water was incubated at 37 °C (except for temperature experiments). After 30 min incubation, 0.5 ml of cold solution of fast blue B (0.45%) was added and the reaction was stopped by adding 0.5 ml of 40% trichloroactic acid. The esterase activity was estimated by the colorimetric method of Seligman et al.[6]. 1 unit of esterase activity was defined as the amount of enzyme liberating 1 µmole of β-naphthol at 37 °C in 1 h.

The enzyme activity was estimated at several pH-values ranging from 4.5 to 9.5 using different buffer systems (0.1 M sodium citrate-HCl buffer for pH 4.5–5.5; 0.1 M Sorensen's phosphate buffer for pH 5.5–7.0; 0.1 M veronal sodium-HCl buffer for pH 7.0–9.5). Effects of all other factors were studied at the pH at which optimal

pH and temperature optima of esterases from various crustaceans

Name of the animal	Substrate used	Optimum pH	Optimum temperature
Astacus fluviatilis[10]	Tributyrin	5.2–6.5	–
Thalamita crenata[11]	Tributyrin	6.97	–
Panulirus japonicus[9]	β-naphthyl acetate	6.9–8.0	30 °C
	β-naphthyl laurate	6.9–8.6	35 °C
	β-naphthyl stearate	5.3	35 °C
A. astacus and *Cambarus affinis*[12]	Phenyl acetate and Phenyl butyrate	8.5	–
Diogenes bicristimanus[8]	Amyl acetate and Olive oil	7.4	–
Streptocephalus dichotomus[13]	Milk fat	5.2–6.5	–
Paratelphusa masoniana[14]	Ethyl butyrate	6.2	–

activity was observed. The effects of temperature on the enzyme activity were studied by incubating the reaction and control mixtures at various temperatures ranging between 10 and 60 °C. To study the effect of incubation period, samples were withdrawn at different time intervals from a pool of reaction mixture incubated at 37 °C, and assayed for the endproducts liberated. For the effect of enzyme concentration, the concentration of the homogenate ranged from 0.5–5.0 mg hepatopancreas/ml. For substrate concentration experiments, different concentrations of substrate, ranging from 10×10^{-5} M to 12×10^{-4} M, were used in the reaction mixture.

Results and discussion. The optimum activity of esterase from the hepatopancreas of *M. lamarrei* lies around pH 7.4 (figure 1). As the stomach is the chief site of digestion in *M. lamarrei* and the pH of this region is 6.4–6.7[7], the activity of esterase in this region would be around 86.8–90.9% of the optimum. The pH optima of crustacean esterases appear to be dependent upon the natyre of substrate used and it ranged from pH 5.2 to 8.5 (table). The optimum pH for the hepatopancreatic esterase of *M. lamarrei* also falls in the above range and closely resembles that of *Diogenes bicristimanus* (pH 7.4)[8].

The enzyme showed optimum activity at 40 °C (figure 2) although it was quite active between 10 and 45 °C. At temperatures higher than 40 °C the enzyme activity decreased sharply, and ultimately at 60 °C no activity was observed. The temperature optimum has been studied only in *Panulirus japonicus*[9] where it ranged from 30 to 35 °C depending on the nature of substrate used (table). These values are lower, when compared to that from *M. lamarrei*. Enzyme from *M. lamarrei* was destroyed at 60 °C while that of *D. bicristimanus* was destroyed at a slightly higher temperature (65 °C)[8] showing that the enzyme from the former is less heat resistant than that of the latter.

The hydrolysis of the substrate increased with the increase in incubation period (figure 3) and enzyme concentration (figure 4) for some time, after which the rate was slowed down, showing probably an inhibition effect by the accumulating hydrolytic products of the substrate or a partial thermal enzyme-denaturation with the incubation time.

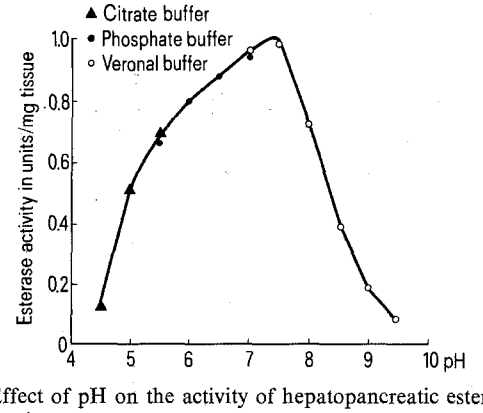

Fig. 1. Effect of pH on the activity of hepatopancreatic esterase of *M. lamarrei*.

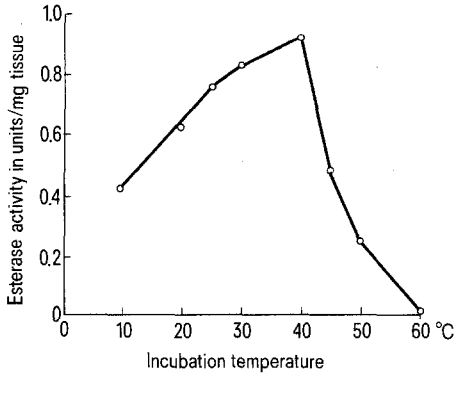

Fig. 2. Effect of temperature on the activity of hepatopancreatic esterase of *M. lamarrei*.

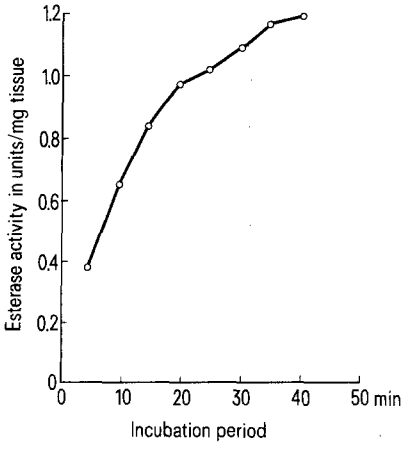

Fig. 3. Effect of incubation period on the activity of hepatopancreatic esterase of *M. lamarrei*.

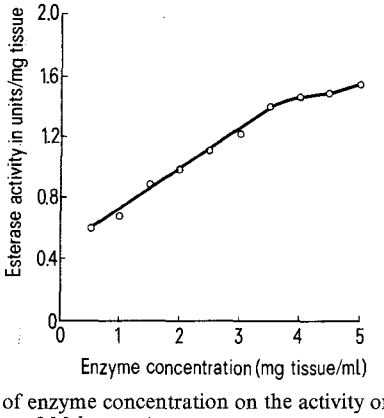

Fig. 4. Effect of enzyme concentration on the activity of hepatopancreatic esterase of *M. lamarrei*.

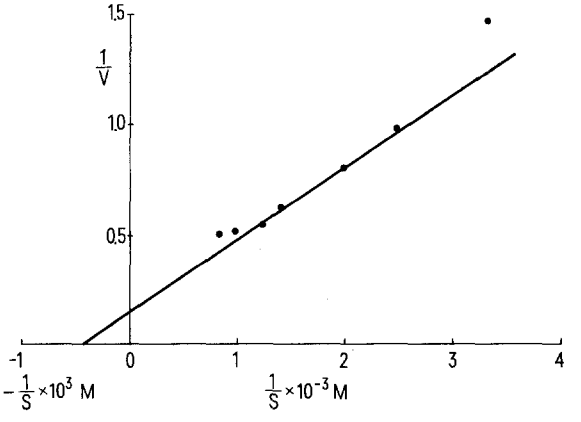

Fig. 5. Lineweaver-Burk plot for Michaelis constant (K_m) of hepatopancreatic esterase of *M. lamarrei*. (V = Enzyme activity in units/mg tissue; S = substrate concentration, M).

The activity of the enzyme increased with the increase in substrate concentration. The data regarding the effect of substrate concentration on esterase activity, when plotted in a Lineweaver-Burk plot for the Michaelis constant (K_m), gave a value of 2.1×10^{-3} M (figure 5).

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Estimation of pore passage time of red blood cells in normal subjects and patients with renal failure

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Summary. The average transit time of single red blood cells through a nuclepore membrane filter (pore diameter and length, 5 µm and 10 µm, respectively) was measured using an improved method and was shown to be an index of deformability. An increased passage time, indicating reduced deformability, was observed in renal failure.

Because of the importance of the deformability of red cells in relation to blood flow through the microcirculation, a number of methods to measure or quantify this property of red cells have been proposed and developed. In our previous study[2] a filtration method using a nuclepore membrane filter[3, 4] was applied to measure the deformability of red cells in fresh human blood exposed to hypercapnia. Although the method is open to ambiguity in the interpretation of the results, due to many factors affecting blood flow through the filter, its simplicity and speed made it possible to detect a rapid decrease of the deformability of red cells after blood sampling and its reduction by hypercapnia. In the present study, we tried to obtain a better index of red cell deformability than the originally proposed passage time of the whole blood[4], since the rheological property of blood in the filtration method is affected by the deformability of red cells and hematocrit as well. It seemed possible that a simple filtration model would permit an analysis of the hematocrit dependence of the filtration characteristics, and an evaluation of the average time required for a red cell to pass through a pore of the filter. Thus, a comparison of red cell deformability between normal subjects and patients suffering from renal failure accompanied with anemia could be made.

20 ml of venous blood from each healthy subject was drawn into a syringe containing 1 ml of heparin solution (1000 units). To obtain red cell suspensions with reduced hematocrits, the blood was divided into 5 parts, each of which was diluted by an appropriate amount of plasma from the fresh venous blood of the same subject. The time required for 0.5 ml of each sample to pass through a filter under a pressure difference of 10 cmH_2O was measured at 37 °C. The sample preparation and measurement were made within 30 min after blood sampling. Then the hematocrit of each sample was determined by a microhematocrit method. 2 ml of venous blood from patients was anticoagulated with

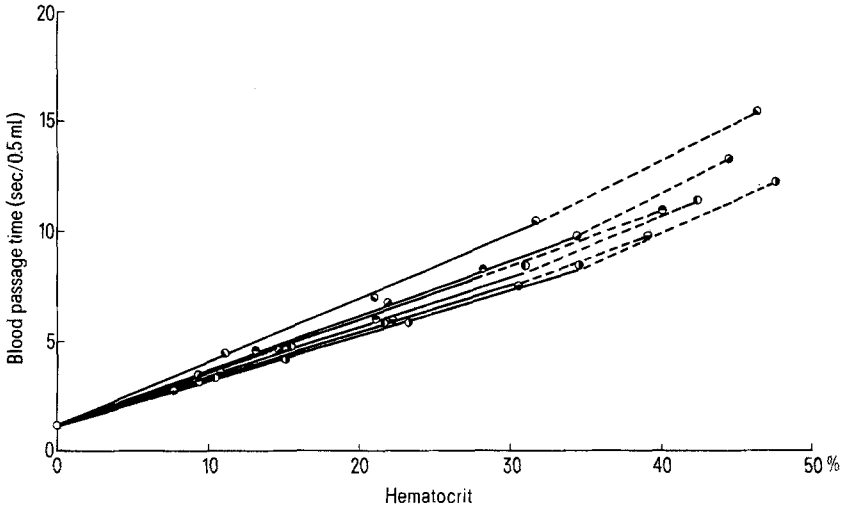

Fig. 1. Relation between blood passage time and hematocrit in 6 normal subjects.