Hepatopancreatic Amylase Activity as a Function of Warm-Adaptation in a Fresh-Water Field Crab

Thermal adaptation of crabs was studied with reference to the animal oxygen consumption[1], carbohydrate metabolism[2] and blood ionic composition[3]. All these changes were studied in the organism; but cellular or molecular changes in thermal adaptation have not been reported so far. In this note the hepatopancreatic amylase activity with reference to the warm-adaptation of a crab is reported.

Paratelphusa hydrodromus were collected from fields and stocked in the laboratory aquaria for at least 1 week before being used for experimentation. 2 batches of crabs were used for experimentation – 1 at room temperature (26 °C) and the other warm-adapted for 14–16 days at 36 °C according to RAO and VENKATA REDDY[4]. Both the batches were in a normal feeding state, fed daily on earthworms and frog muscle slices. Aqueous extracts of the hepatopancreatic tissue of the crab was found to give a positive response to the test for amylase[5]. The enzyme was extracted into 80% glycerol from the acetone powder of hepatopancreatic tissue as obtained according to the method of PROSSER and VAN WEEL[6]. The tissue from 5–6 animals was pooled to extract the enzyme. Amylase activity of the 1% glycerol extracts (1 g acetone powder in 100 ml glycerol) was determined according to the method described by OSER[7].

The Figure illustrates that the enzyme from warm-adapted animals is thermolabile, i.e. it shows lower activity with increasing temperatures, in contrast to that of normal animals. The rate function curves (Figure) differ in their slopes and establish a 'counter-clockwise rotatory compensation', described by PROSSER[8]. He suggests the occurrence of change in Q10, on counter-clockwise rotatory compensation. Consequently, the enzymatic rate, as measured by the Q10 approximation (Table), suggests a significant decrease on adaptation in the 25–35 °C range. The diminution of the Q10 may be attributed to the changes in activation energy of the enzyme[8]. The lowering of Q10 at these temperature ranges may not be due to thermal damage since at the higher temperature following this range, no such lowering is seen. The thermolabile amylase (Figure) of warm-adapted crabs may be the result of a compensatory mechanism to warm-adaptation. Since the enzyme is concerned with the hydrolysis of starch and glycogen[9], warm-adaption may result in changes in carbohydrate metabolism of these crabs. The change in the enzyme activity might be the result of a change in the qualitative property of the enzyme molecule and could be attributed to the macromolecular change on warm-adaptation. Such changes during thermal adaptation are known to be associated with the net synthesis of functional proteins[10–13]. The rotatory compensation as such of amylase during thermal acclimation has been reported only in a few animals[14].

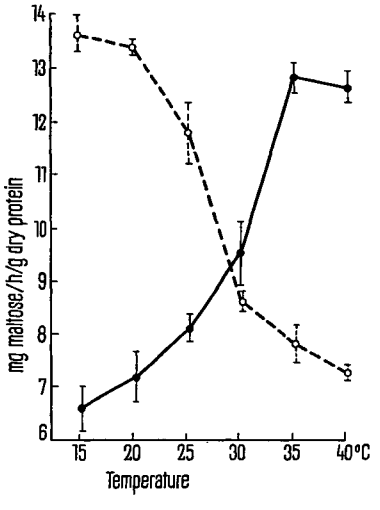
It is of considerable interest to note that the activity of amylase of normal crabs at room temperature (26 °C) and the same in the warm-adapted crabs at 36 °C tends to be equal. The adaptation thus results in the restoration of the rate process of amylase to its normal level.

The real significance of the change in the activity of amylase during adaptation, however, must be sought in the carbohydrate metabolism. It is well known that the Krebs cycle enzymes increase on cold- and decrease on warm-adaptation[15,16]. Further, it was suggested[16] that

Q10 of hepatopancreatic amylase activity in *Paratelphusa hydrodromus*

Temperature range	Normal crabs at 26 °C	Crabs adapted to 36 °C	Change in Q10 on warm-adaptation (Students *t*-test)
(1) 15–20 °C	0.81 ± 0.41[a]	0.73 ± 0.06	26 °C = 36 °C at *P* 0.01
(2) 20–25 °C	1.22 ± 0.44	1.36 ± 0.14	26 °C = 36 °C at *P* 0.01
(3) 25–30 °C	1.73 ± 0.52	0.41 ± 0.03	26 °C > 36 °C at *P* 0.01
(4) 30–35 °C	2.10 ± 0.28	0.84 ± 0.07	26 °C > 36 °C at *P* 0.01
(5) 35–40 °C	1.00 ± 0.24	1.07 ± 0.14	26 °C = 36 °C at *P* 0.01

[a] Each value is the mean of 6 observations ± standard deviation.



Rate-temperature curves of hepatopancreatic amylase of normal (solid line) and warm acclimated (broken line) *Paratelphusa hydrodromus*. Plots are the mean of 9 observations and their standard deviation. R. V. K. is grateful to the Council of Scientific and Industrial Research of India for placing him in the Scientists' Pool.

[1] J. L. ROBERTS, Physiol. Zoöl. *30*, 232 (1957).
[2] J. M. DEAN and F. J. VERNBERG, Biol. Bull. *129*, 87 (1965).
[3] K. PAMPAPATHI RAO and R. RAMACHANDRA, J. exp. Biol. *38*, 29 (1961).
[4] K. PAMPAPATHI RAO and V. VENKATA REDDY, Comp. Biochem. Physiol. *5*, 65 (1962).
[5] J. H. WELSH and R. I. SMITH, *Laboratory Exercises* in *Invertebrate Physiology* (Burgess Publ. Co., Minneapolis 1960).
[6] C. L. PROSSER and P. B. VAN WEEL, Physiol. Zoöl. *31*, 171 (1958).
[7] B. L. OSER, in *Hawk's Physiological Chemistry*, 14th edn (McGraw-Hill Book Co., New York 1965), p. 518.
[8] C. L. PROSSER, in *Comparative Animal Physiology* (W. B. Saunders, 1961), chapt. 9, p. 246.
[9] J. S. FRUTON and S. SIMMONDS, in *General Biochemistry* (Asia Publishing House, Madras 1960), p. 433.
[10] H. KOFFLER, Proc. 9th Int. Congr. Bot. *2*, 196 (1959).
[11] D. R. EKBERG, Comp. Biochem. Physiol. *5*, 123 (1962).
[12] A. B. DAS and C. L. PROSSER, Comp. Biochem. Physiol. *21*, 449 (1967).
[13] A. B. DAS, Comp. Biochem. Physiol. *21*, 469 (1967).
[14] C. L. SMITH, J. exp. Biol. *28*, 141 (1951).
[15] H. J. PRECHT, in *Physiological Adaptation* (Ed. C. L. PROSSER; The American Physiological Society, Inc., Washington, D.C. 1958), p. 50.
[16] K. SAROJA and K. PAMPAPATHI RAO, Z. vergl. Physiol. *50*, 35 (1965).

both the glycolytic and HMP pathways in earthworm may be more active in cold than in normal ones, whereas in warm worms glycolytic pathway is less active than in the normal worms. This in its turn may suggest the breakdown of more sugars in cold- and less sugars in warm-adaptation. It has been pointed out[2] that the temperature adaptation results in quantitative changes in carbohydrate metabolism in crabs, rather than qualitative shifts. The increase in the activity of amylase in warm-adapted crabs may ensure the availability of more sugars in cold[17].

Zusammenfassung. Im Hepatopankreas der Krabben sind Fermentsysteme, die eine Wärmeadaptation besitzen. Diese lässt sich mit ähnlichen physiologischen Erscheinungen bei kaltblütigen Vertebraten vergleichen.

R. V. KRISHNAMOORTHY and V. VENKATA REDDY

Department of Zoology, Sri Venkateswara University, Tirupati and Department of Zoology, Government Arts College, Anantapur (Andhra Pradesh, India), 22 February 1968.

[17] The authors wish to thank Dr. K. S. SWAMI, Head of the Department of Zoology, Sri Venkateswara University for offering facilities. They are also grateful to Prof. K. PAMPAPATHI RAO for helpful criticism.

Proximal Tubular Reabsorptive Capacity in Rats with Chronic Experimental Pyelonephritis

The classical concept of chronic pyelonephritis[1,2] assumes that the inflammatory process proceeds from the pelvis to the medullary interstitium, resulting in various types of tubular damage. Thus, normal glomeruli empty in severely damaged or disrupted tubules, or, on the contrary, aglomerular nephrons with almost intact tubuli are formed. Contrary to this concept, BRICKER[3] proposed an 'intact nephron hypothesis' suggesting that the seriously damaged nephrons participate only to a small extent in urine formation. Consequently, most of the urine is produced by intact or slightly damaged nephrons. In the course of the disease, the number of these nephrons decreases gradually. Recent micropuncture studies in rats with experimental pyelonephritis have consistently shown[4,5] that both proximal and distal reabsorption in nephrons, in which the tubular fluid movement is still demonstrable, do not differ essentially from those found in healthy rats. In these experiments, reabsorption was estimated using tubular fluid/plasma inulin concentration ratio. This method, however, fails to give accurate information about the intrinsic reabsorptive capacity of the tubular wall which can actually be decreased; this decrease may be overcome by tubular dilatation resulting in an increased reabsorptive surface, as described for example by WIEDERHOLT et al.[6] in epinephrectomized rats.

In this paper, the intrinsic reabsorptive capacity was measured in proximal tubuli of pyelonephritic rats using the shrinking-drop technique as described by GERTZ[7]; in this method, the half-time of the intratubular shrinkage of an isotonic saline drop injected between 2 oil drops is measured. The original method of GERTZ based on photographic recording of drop-shrinkage was replaced by direct measurements with a filar ocular micrometer. Transit time of fluid in the proximal tubule was measured with lissamine green according to STEINHAUSEN's method[8] modified by GERTZ et al.[9]. Fractional reabsorption in proximal convolution was calculated according to the equation of BRUNNER, RECTOR and SELDIN[10] originally introduced by GERTZ et al.:

$$\% \text{ reabsorption} = \left(1 - \frac{1}{\text{antilog } (0.301\ T/t_{1/2})}\right) 100$$

where T is transit time of lissamine green in proximal convolution, $t_{1/2}$ is half-time of the drop-shrinkage.

Our data (see Table) were obtained from 12 rats in which unilateral pyelonephritis according to PRÁT et al.[11] was produced by injecting a culture of *Escherichia coli* i.v. during temporary ligation of the (left) ureter. Within 5–6 weeks, the pathological picture of a granulated contracted pyelonephritic kidney developed. In another group of animals with experimental bilateral pyelonephritis, chronic fistula of the urinary bladder[12] had been prepared

[1] J. OLIVER, J. Urol. *63*, 373 (1950).
[2] S. E. FRANKLIN and J. P. MERRILL, Am. J. Med. *28*, 1 (1960).
[3] N. S. BRICKER, P. A. F. MORRIN and S. W. KIME JR., Am. J. Med. *28*, 77 (1960).
[4] N. BANK and H. S. AYNEDJIAN, J. Lab. clin. Med. *68*, 728 (1966).
[5] H. LUBOWITZ, M. L. PURKERSON and N. S. BRICKER, Nephron *3*, 73 (1966).
[6] K. HIERHOLZER, M. WIEDERHOLT and H. STOLTE, Pflüger's Arch. ges. Physiol. *291*, 43 (1966).
[7] K. H. GERTZ, Pflüger's Arch. ges. Physiol. *276*, 336 (1963).
[8] M. STEINHAUSEN, Pflüger's Arch. ges. Physiol. *277*, 23 (1963).
[9] K. H. GERTZ, J. A. MANGOS, G. BRAUN and H. D. PAGEL, Pflüger's Arch. ges. Physiol. *285*, 360 (1965).
[10] F. P. BRUNNER, F. C. RECTOR JR. and D. W. SELDIN, J. clin. Invest. *45*, 603 (1966).
[11] V. PRÁT, D. BENEŠOVÁ, L. PÁVKOVÁ and F. ČERVINKA, Acta med. scand. *165*, 305 (1959).
[12] J. HELLER, Physiologia bohemoslov. *9*, 150 (1960).

	No.	$t_{1/2}$	TT	FR	BUN
Controls (healthy rats)	16	8.87 ± 0.93	9.10 ± 0.67	50.9	11.45 ± 1.37
Controls (temporary ureteral ligation)	9	9.02 ± 1.08	8.75 ± 0.85	49.8	13.00 ± 1.57
Unilateral pyelonephritis (temporary ureteral ligation + *Escherichia coli* i.v.)	12	8.98 ± 1.40	9.27 ± 1.07	51.2	12.86 ± 0.93
Bilateral pyelonephritis (chronic fistula of the urinary bladder)	8	9.11 ± 1.27	9.19 ± 0.97	50.3	28.03 ± 2.76

No., number of animals; $t_{1/2}$, half-time of oil shrinkage; TT, transit time of lissamine green in proximal convolution (both values in sec); FR, calculated % reabsorption in proximal convolution; BUN, blood plasma urea nitrogen concentration, in mg%. Values presented as mean + residual standard error.