TEMPERATURE AND pH ABNORMALCY ON THE ACTIVITIES OF SUCCINATE, GLUTAMATE AND GLYCEROPHOSPHATE DEHYDROGENASES IN CELL FREE EXTTRACTS OF SHEEP LIVER

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Summary: (1) Some kinetic properties of succinate, glutamate and glycerophosphate dehydrogenases were compared using cell free extracts of sheep liver as source of enzyme. (2) The enzyme-substrate complex of succinate dehydrogenase was found to be dependent on pH and temperature. (3) The pH was found to exert an uncompetitive type of inhibitory regulation on the enzyme activity (4). The ionizing groups essential for succinate dehydrogenase activity at 32°C were found to be imidazolium (histidine) and ammonium (α, cystine), at 37°C the ionizing groups are mostly ammonium (α, cystine) whereas at 45°C the ionizing groups are both imidazoluim (histidine) and ammonium (α, cystine).

Key words: sheep liver dehydrogenases effect of temperature and pH

Body temperature is the resultant of the heat produced and heat lost to the environment (15). During temperature compensation increase in heat production is known to be contributed by the metabolism of the liver, (15) which in turn is mediated by enzymes. The maximum velocity (Vmax) state of the enzyme activities are subjected to inhibitory regulation at physiological pH, so that the economy of the cell is last disturbed (11). Similarly the temperature and pH dependent kinetics are mediated and regulated by cytoplasmic proteins within the cell (1). In pathological states such as fever and other ailments, the pH and thermal states will be altered (15), which may induce significant changes in the structural and functional aspects of enzymes thereby affecting the general enzyme activity and cell metabolism. Under these circumstances it is felt highly desirable to study the properties of selected enzymes in the physiological states and see how the pH and temperature abnormalcy will alter the catalytic properties of the enzymes and the possible role of cytosol proteins in providing biochemical basis for protection of enzymic disturbance within the healthy cells. The succinate, glutamate and glycerophosphate dehydrogenases were selected in the present investigation to represent central oxidative metabolism, neutralisation of ammonia toxicity and fatty infiltration respectively in the liver.

MATERIALS AND METHODS

Sheep were killed by decapitation and the livers transferred quickly to a clean dry beaker jacketed with ice in a Thermos flask, thus maintaining a temperature at about 0°. The tissue was homogenized in cold 0.025 M buffer (pH 7.7) for 3 minutes with a motor driven ground glass homogenizer. The homogenate was made at a concentration of 100 *mg* per *ml* (Wet weight)

and centrifuged at 2,000 r.p.m. for about 10 min to sediment the cellular debris. Appropriately diluted supernantant was used as the source of enzyme.

Succinate dehydrogenases activity (SDH) was assayed by the colorimetric method described by Nachlas *et al.*, (9). The method was modified as follows; the reaction mixture contained in a final volume of 2 *ml;* 75 μ moles sodium succinate (pH 7.7), 100 μ moles KH_2PO_4--K_2HPO_4 buffer (pH 7.7), 4 μ moles INT, 0.5 μ moles PMS and 0.1 *ml* of appropriately diluted homogenate. After incubation for 30 min at 37°, the reaction was stopped by the addition of 6 *ml* glacial acetic acid and the formazan formed due to reduction of the dye was extracted into 6 *ml* toluene. After overnight extraction in cold, the colour was read in UV spectrophotometer (Hilger and Watts, England) at 500 *mμ*.

The cytochrome linked glycerophosphate dehydrogenase (GPDH) and glutamate dehydrogenase (GDH) were determined by the colorimetric method described by Lee and Lardy (6). The reaction mixture contained in 2 *ml* volume; 50 μ moles sodium glutamate of sodium glycerophosphate (pH 7.7), 100 μ moles KH_2PO_4 - K_2HPO_4 buffer-(pH 7.7), 4 μ moles INT, 0.5 μ moles PMS, 0.1 μ moles KCN and 0.1 *ml* of appropriately diluted homogenate. 0.01 μ moles of NAD was added to the reaction mixture when glutamate dehydrogenase activity was measured. After 30 minutes incubation at 37°C, the reaction was stopped and the colour was measured as given before. Individual zerotime controls were maintained for all the samples by the addition of glacial acetic acid to the reaction mixture plus homogenate before incubation and the enzyme activities are expressed in μ moles formazan formed/*mg* protein/*hr*. The standard calibration curve of formazan was prepared by taking INT in different concentrations and allowed for reduction in the presence of NADH and the enzyme assay components. The formazan formed was extracted into toluene as described before. The optical densities were found to be proportional to the formazan concentrations.

The protein content was determined by the method of Lowry *et al.*, (7), using bovine serum albumin as standard. The mean values of enzyme activity levels of either duplicates or triplicates were used for calculation. The maximum velocities (Vmax) and the Michaelis constants (*Km*) were calculated by the method of least squares.

Abbreviations:

INT = 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium Chloride
PMS = Phenazine methosulphate.

Enzymes:

SDH = Succinate dehydrogenase
(Succinate : INT — Oxidioreductase E.N. 1.3.99.1)

GPDH = Glycerophosphate dehydrogenase
(Glycerophosphate : Cytochrome *C* Oxidoreductase, E.N. 1.1.99.5).

GDH = Glutamate dehydrogenase (L-glutamate : NAD Oxdoreductase, E.N.1.4.1.2)

All the assays were made under the conditions measuring initial velocities after due standardisation.

RESULTS AND DISCUSSION

The pH optimum in KH_2PO_4 - K_2HPO_4 buffer is about 8.0 for SDH and 8.5 for GDH and GPDH (Fig 1). The pH optimum without PMS and with triphenyl tetrazolium chloride as electron acceptor is about 9.0 for SDH and GDH (11) indicating that pH optimum is dependent upon the dye used as electron acceptor and this is in accordance with McShan (8).

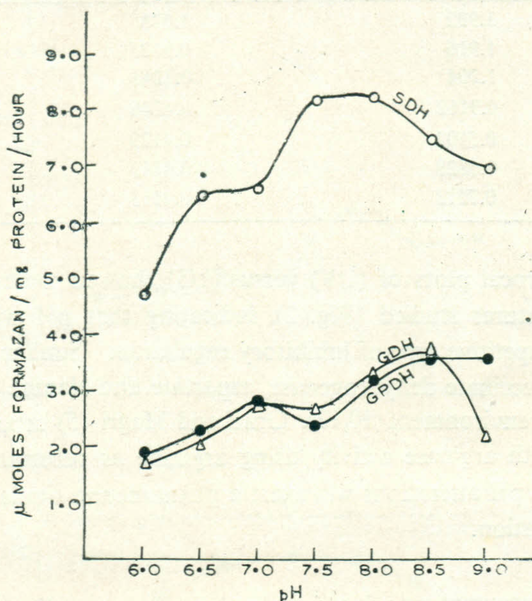

Fig. 1: Effect of pH on dehydrogenases.

Enzyme activities are known to undergo reversible transitions between active and inactive forms in response to shifts in pH and temperature (2, 13, 14, 16) Ghosh and Fishman (4) have reported that the Michaelis constant of rat intestinal alkaline phosphatase was pH dependent and similar relationship could be envisaged for the succinate dehydrogenase.

In the present study, the Michaelis constant of succinate dehydrogenase was found to be dependent on pH and also temperature (Table I). At 32° and 37°C the Km values were found to decrease with increase in pH value up to 8.0 pH and started increasing at pH values 8.5 and 9.0. Thus the lowest Km values were obtained at pH 8.0 while at 45°C the lowest Km value was at pH 7.0, suggesting that the enzyme efficiency and the pH dependency vary with the temperature. A general effect of temperature increase reflects a decrease in Km values in general and at each temperature, further, the variations in Km values are pH dependent. Thus the Km values are dependent upon pH and temperature. These variations in Km value are

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having a metabolic significance, since, the pathological states such as fever and other ailments may induce changes in the pH and thermal states and these alterations in physiological stage may alter the Km values for the substrate which reflects the efficiency of the enzymes in the hydrolysis of endogenous substrates.

TABLE I: Apparent michaelis constants (mM) of succinate dehydrogenase at different temperature and pH.

pH	32°C	37°C	45°C
6.0	1.932	1.678	0.5610
6.5	1.916	0.9625	0.5106
7.0	1.204	0.8243	0.1032
7.5	0.9162	0.6246	0.1391
8.0	0.5102	0.4129	—
8.5	0.5828	0.4483	—
9.0	0.7013	0.4513	0.3009

The double reciprocal plots of (1/V) versus (1/S) showed somewhat parallel relationship at all the three temperatures studied (Fig. 2), indicating that pH will exert a phenomenon similar to that of uncompetitive type of inhibitory regulation. Similar type of inhibitory regulation was observed for succinate dehydrogenase, aspartate and alanine amonotransferases by the alterations in the protein environment (10,12). Grazi and Magri (5) reported similar type of somewhat parallel curves with arginase activity using arginine as substrate at different pH values. Thus it appears that the pH alterations will exert a phanomenon similar to that of uncompetitive type of inhibitory regulation.

If the Michaelis constant was found to be dependent on pH and temperature, it is likely that Vmax may also be dependent on these factors and it was found to be the case in the present study (Fig. 3). At temperature of 32°C, the optimal Vmax values were found between pH 7.0 and 8.0 giving a broad curve covering these points. At 37°C, the curve shows a relatively narrow optimal range at pH 8.0 whereas at 45°, the narrow optimal range is at pH 8.0 (Fig. 3). The double reciprocal plots of (1/S) versus (1/V) indicate a similar trend (Fig. 2). At temperatures of 32°, the plots from pH values 7.0 to 8.0, at 37°, the plot at pH 8.0 and at 45° the plot at pH 7.0 represents the tendency of shift towards the "x" axis, thereby suggesting the hyper activity at these pH values in the respective temperature environment. This is in consonance with the pH-Log Vmax relationship as evinced in the Fig.3.

Dixon and Webb (3) have shown that a plot of log Vmax against pH should exibit a horizontal segment of the curve over the pH range in which the enzyme exists in the optimum active form and that the ionization of groups to form inactive enzyme species should appear on such plots as lines of integral slope running down from the horizontal. From the positions

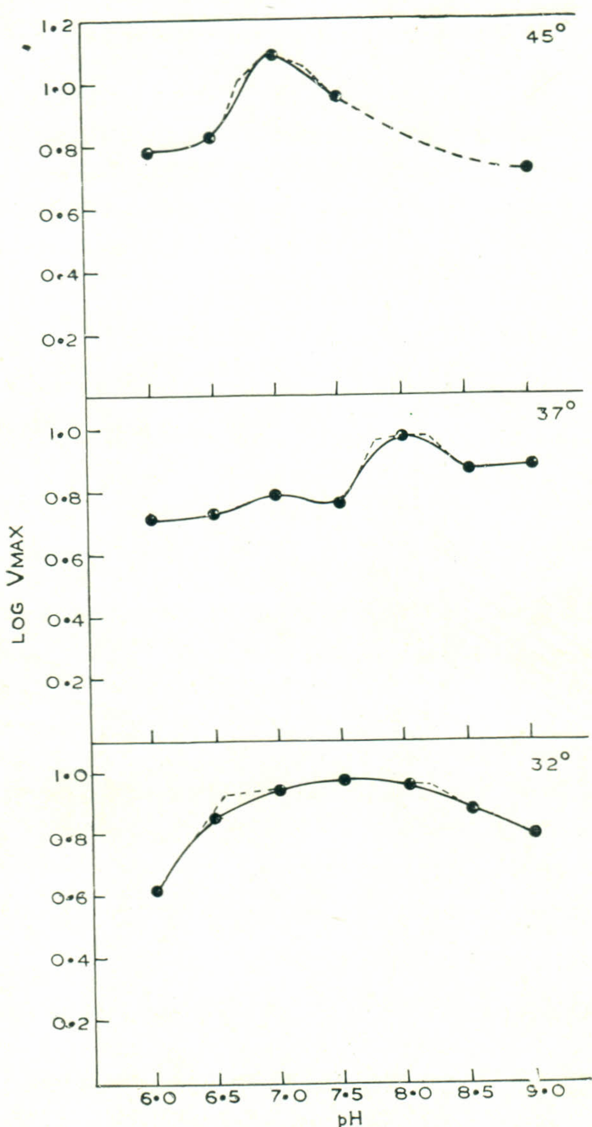

Fig. 2: Double reciprocal plots of initial velocity against succinate concentration at a series of different pH values and temperatures.

of the bends in the graph, the pKs of the ionizing groups in the ES complex which affect the activity of the groups which either form part of the active centre of closely associated with it can be obtained (3). Since a change of the temperature will itself produce a change in the stage of ionization of each of these groups, by determining the curves at different temperatures the change in each pK with temperature can be measured by the displacement of the bends (3). In the present

experiment, the integral slopes running down were observed at pH 6.6 and 8.2 at 32°C at 7.8 and 8.2 at 37°C and at 6.75 and 7.25 at 45°C indicating that at 32° the ionizing groups essential for SDH are imidazolium (histidine) and ammonium (α, cystine), at 37° the ionizing groups are mostly ammonium (α, cystine) whereas at 45° the ionizing groups are both imidazolium (histidins) and ammonium (α, cystine) as suggested by Dixon and Webb (3). Thus at physiological temperature the emphasis of the active site is towards the ionization of the ammonium (α, cyctine) whereas as under hypothermic and hyperthermic states the ionization of active centre is shifted also to imidazolium (histidine), indicating the existence of a regulative factor which is highly operative at the physiological state, which interferes with the contribution of ionization potential of imidazolium (histidine) in the catalytic centre of succinate dehydrogenase.

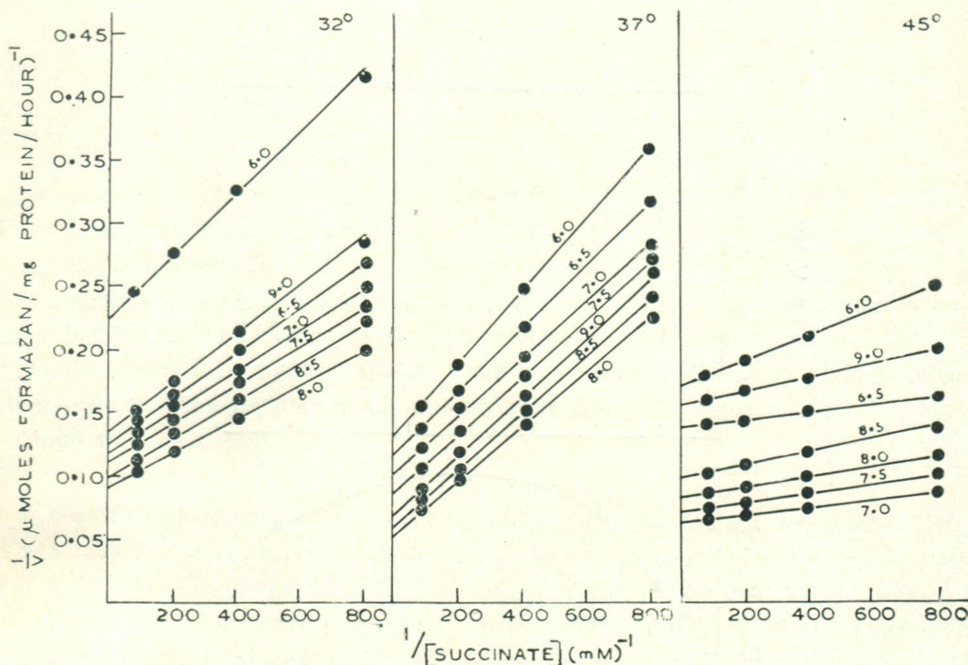

Fig. 3: Effect of pH and temperature on the Vmax of succinate dehydrogenase activity at different temperatures.

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