Compound TX: RF 0.95 (Silica gel G and chloroform:ethanol 9:1 developer) it was obtained as a brown powder from methanol. m.p. 169–170° (Uncorrected). Mol. wt. by Rasts' method 659.2 $[\alpha]_D^{24.5°}$ chloroform + 33.17° m.p. of chloroplatinate 138° m.p. of picrate 215° m.p. of dinitro-benzoate 138° UV Ethanol λ max. nm 225, 281, 292.

IR CH_2Cl_2 at 3450 (NH) 2860 (Broad) 1720, 1680 cm^{-1} (CO). Mass spectra—same type of fragmentation as TY. Higher fragments could not be obtained.

Three other components were also obtained in a homogeneous condition, one from benzene eluate and two more from chloroform:ethanol (9:1) eluates but they could not be further characterised. It appears that the plant material contains at least 9 components as shown by the TLC study of the partially purified samples.

Pharmacological Studies

The following pharmacological studies have been made on the two extracts A and B. (a) Preliminary screening in mice and rats. (b) Analgesic study (Gujral, M. L. and G. Khanna (1956) in Wistar Albino rats. (c) Anti-convulsant activity with Wistar Albino rats. (d) Effect on skeletal muscle frog rectus abdominus muscle. (e) Effect on smooth muscle—Guineapig ileum—as per the method described by the Staff, Department of Pharmacology, Edinburg University (1968). (f) Effect on Cardiovascular system of Dog (Jackson, D. E., 1939).

Both fractions have no significant effect on (i) autonomic and behavioural changes; (ii) analgesic studies and (iii) anti-convulsant action. Extract A possesses nicotine-like effect producing skeletal muscle contraction ganglion stimulation in small doses and ganglion blockade in large doses. Extract B possesses skeletal muscle relaxing property, minimal muscarinic blocking effect and negligible ganglion blocking effect and histamine releasing property.

Acute toxicity studies have given LD_{50} of the extract A as 89.15 mg/kg and in the case of Extract B there is no mortality of the experimental animals even upto a dose of 500 mg/kg.

Acknowledgement

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1. Kartha, A. R. S. and Menon, K. N., *Curr. Sci.*, 1952, **21**, p. 315.
2. Chopra, R. N., *Indigenous Drugs of India*, 1958.
3. Nadkarni, A. K., *Indian Materia Medica*, (3rd ed.), Vol. I.

PHENOLIC ACIDS AS POTENTIAL INHIBITORS OF PLANT AMYLASE

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Abstract

Plant amylase derived from two sources (*Lens culinaris* and *Phaseolus aureus*) was inhibited *in vitro* by salicylic acid, caffeic acid and gallic acid. There was total inhibition at 50 mM concentration of the inhibitors. The inhibition was of non-competitive type with an apparent K_i values as 14, 8 and 8.5 mM for salicylic acid, caffeic acid and gallic acid respectively.

Introduction

PHENOLIC acids are known to act as analogues of growth hormones[1,2] and influence many enzyme systems[3,4]. Amylases occur widely in germinating seeds and play an important role during germination. The interaction of phenolic compounds with amylases was, therefore, considered to be of physiological significance. This paper deals with the *in vitro* interaction of caffeic acid, gallic acid and salicylic acid with mungbean and lentil seed amylase.

Materials and Methods

The enzyme preparation was carried out as described previously[5]. The seeds of local cultivars of

mungbean (*Phaseolus aureus* Roxb.) and lentil (*Lens culinaris* Medik) were sterilized in 1% sodium hypochlorite solution, washed thoroughly with distilled water and then germinated in dark at 25 ± 2° C. Six-day old seedlings (40 g) were homogenized in chilled 0.1 M acetate buffer (pH 5.2). The homogenate was filtered and then centrifuged at 4000 rpm for 10 min. The supernatant was subjected to ammonium sulphate precipitation between 20 and 80% saturation and the precipitates, collected by centrifugation at 16000 rpm for 15 min were suspended in acetate buffer (20 ml). The enzyme activity was measured at 540 nm with 3,5-dinitrosalicylate reagent[6]. The reaction mixture in a total volume of 2 ml contained 0.5 ml of 1% buffered starch as substrate, 0.5 ml phenolic acids (1–50 mM) added to the starch solution, 0.5 ml enzyme extract and acetate buffer. It was incubated for 10 min at 30° C. One amylase unit corresponds to the release of 1 mg maltose in 30 min under assay conditions. Specific activity of the enzyme is the number of enzyme units per mg protein. Protein was measured by the method of Lowry *et al.*[7] using BSA as standard.

The nature of enzyme inhibition by phenolic acids was evaluated according to Lineweaver-Burk method[8]. The values of apparent inhibition constant (K_i) for individual phenolic acids were determined from Dixon plots drawn between 1/v and inhibitor concentration[9]. For these plots, the enzyme was assayed by using 1 and 2% substrate concentrations and 5 different phenolic acid concentrations in the range of 0-40 mM. Other experimental conditions were the same as described above for enzyme assay. For the determination of K_i values with different phenolic acids, the linear lines in Dixon plots were extended backwards as indicated in figure 2.

Results and Discussion

Table 1 summarizes the results of the percentage inhibition of amylase derived from two pulse sources by phenolic acids. This shows that the three phenolic acids used are potent amylase inhibitors. The inhibition of enzyme activity increased with increase in the concentration of phenolic acids to the extent that there was total inhibition at 50 mM concentration. The Lineweaver-Burk plots with and without inhibitors (figure 1) indicated that the inhibition was of non-competitive type, *i.e.* the inhibitors had no effect on K_m. The Dixon plots drawn between 1/v and salicylic acid, caffeic acid and gallic acid (figures 2A–C) indicated the value of K_i as 14, 8 and 8.5 mM respectively, suggesting that salicylic acid is comparatively a less potent inhibitor than the other two phenolic acids.

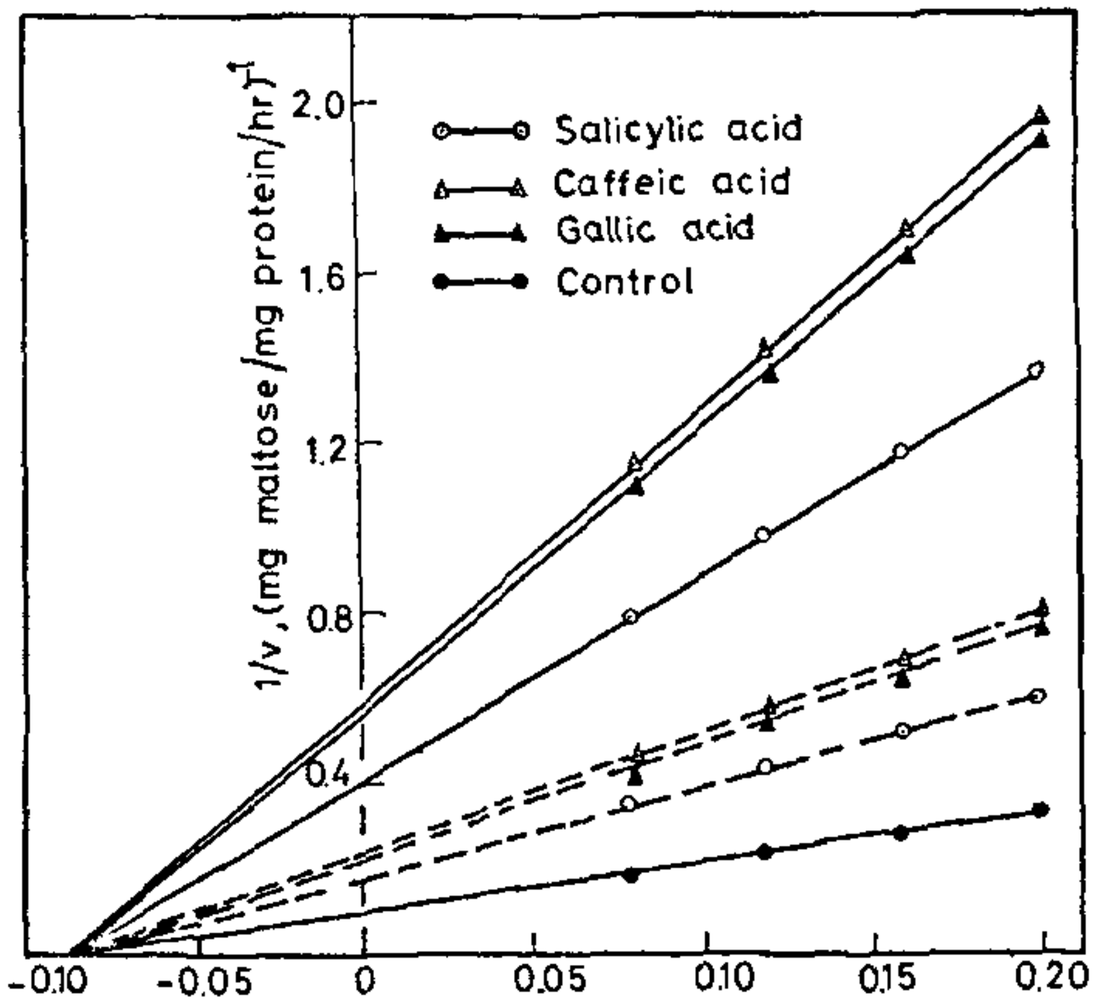
The inhibition of amylase activity by phenolic acids observed here is supported by many examples showing the nature of phenolics inhibiting various enzymes during extraction from plant tissues[10,11]. Demos *et al.*[12] and Koch[13] reported that phenolic acids, when added to suspensions of mungbean hypocotyl mitochondria, reduced the respiration rates as well as induced a loss of coupling suggesting the phenolic implications in mitochondrial energy metabolism. In addition, there are other reports where phenols have been shown as cofactors or inhibitors of enzymes[14,15]. The phenolic compounds are thought to affect enzymes in two ways: (a) they reduce the solubility of enzyme proteins by forming insoluble protein-phenolic complexes[16] and (b) they inhibit the enzymes activity by forming a soluble but inactive enzyme-inhibitor complex[11,17]. The inhibitory activity of phenolic acids used in this paper appear to be of the second type as the

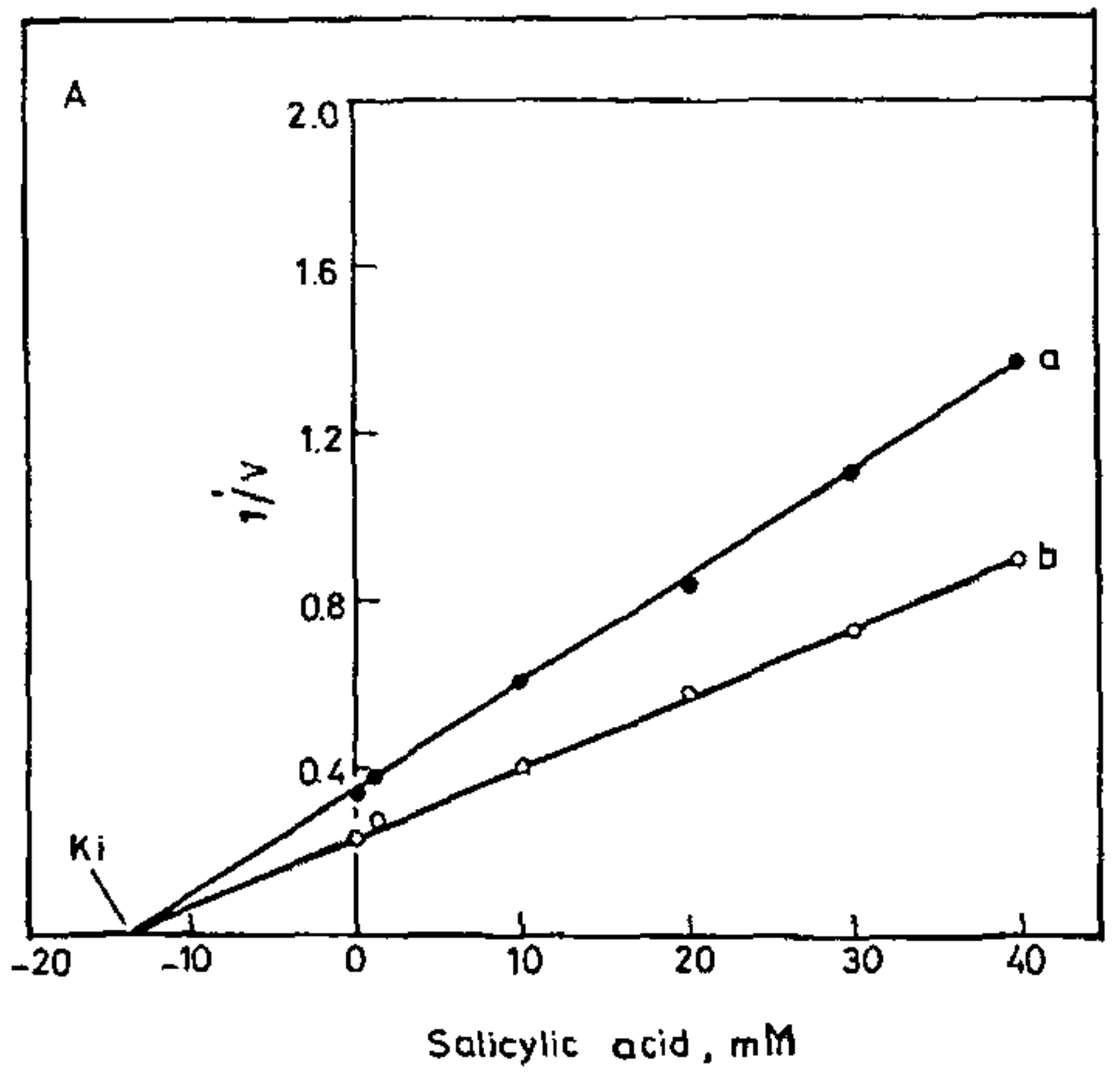
Table 1

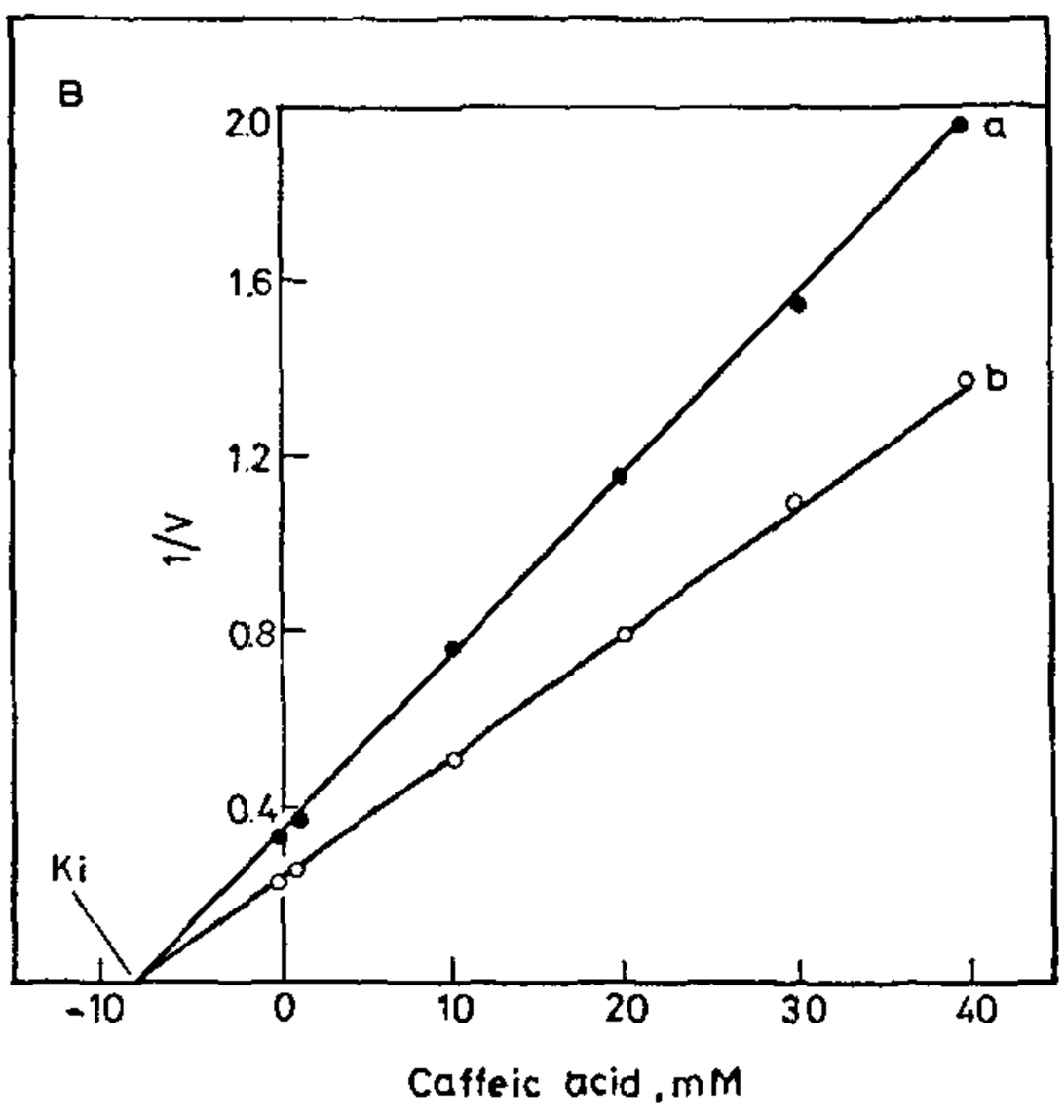
Inhibition of amylase by phenolic acids

Inhibitor concentration (mM)	Enzyme source, inhibitors, inhibition (%)					
	Lens culinaris			*Phaseolus aureus*		
	Salicylic acid	Caffeic acid	Gallic acid	Salicylic acid	Caffeic acid	Gallic acid
1	10.5	8.2*	17.0	7.8*	8.0*	12.4
10	43.2	56.5	56.5	41.5	52.3	56.6
20	59.5	70.4	70.1	58.3	66.7	65.2
30	69.0	77.9	77.5	66.3	77.2	77.8
40	75.2	82.6	82.3	75.4	80.9	81.6
50	100.0	100.0	100.0	100.0	100.0	100.0

* Values are not significantly different from control at $P = 0.01$ level.







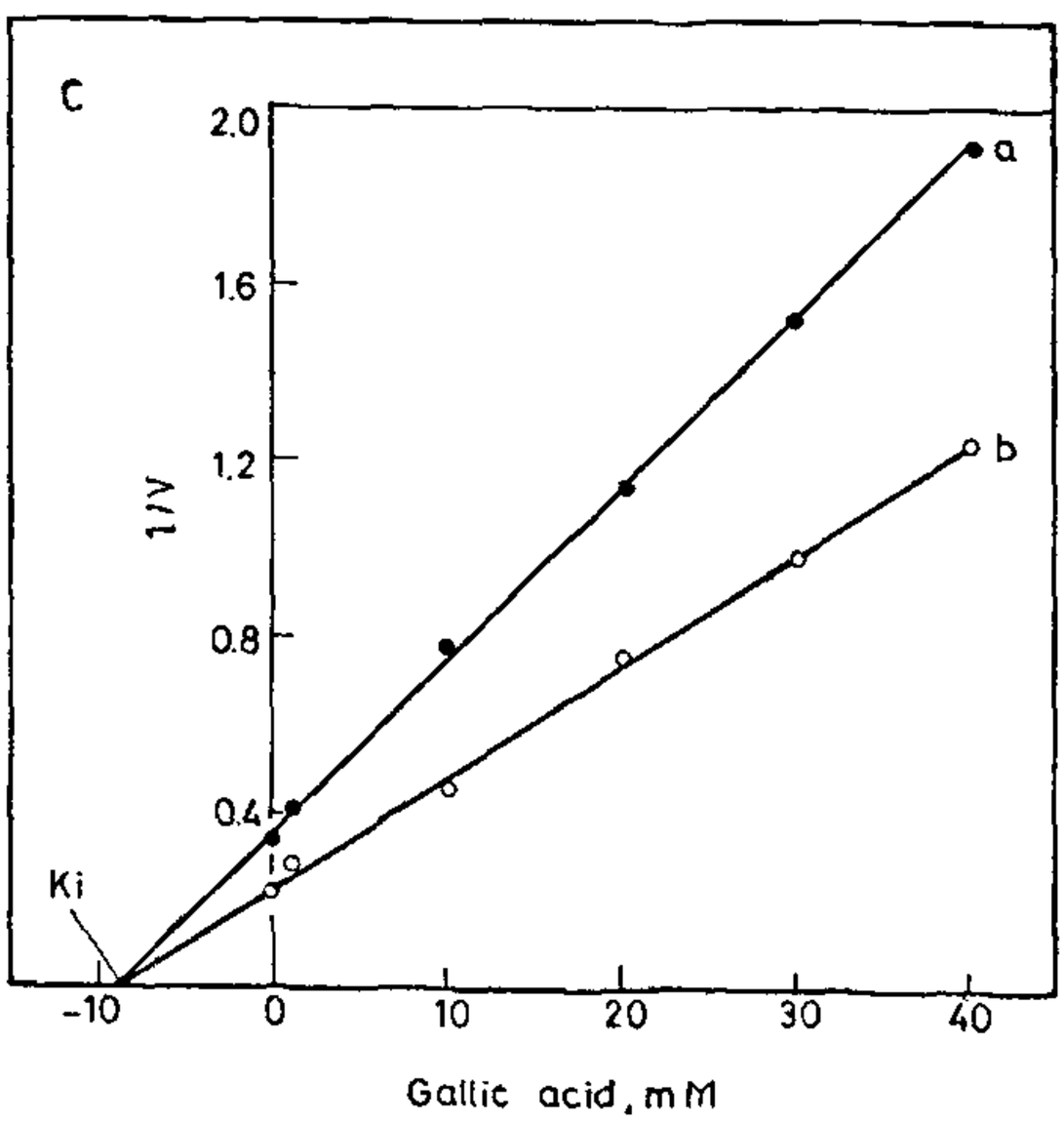


Figure 1. Lineweaver-Burk plots showing the non-competitive type of inhibition of lentil amylase by phenolic acids. Broken and continuous lines indicate 10 and 40 mM of inhibitors, respectively except for control.

Figure 2. Plots of 1/v *vs.* salicylic acid (2A), caffeic acid (2B) or gallic acid (2C) concentrations at fixed substrate concentrations. Assays were carried out in the presence of varying inhibitor concentrations (0–40 mM) at 1 and 2% (a and b) substrate concentrations. The lines of the plots were extended backwards to obtain the value of K_i.

enzyme protein was not precipitated by the addition of any of the three phenolic acids used. The amylase inhibition studies may partly reflect a mechanism of action for widely occurring phenolics in plant growth and metabolism.

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1. Vendrig, J. C. and Buffel, K., *Nature (London)*, 1961, **192**, 276.
2. Wain, R. L. and Taylor, H. F., *Nature (London)*, 1965, **207**, 167.
3. Blank, G. E. and Sondheimer, E., *Phytochemistry*, 1969, **8**, 823.

4. Van-Lelyveld, L. J. and Brodrick, H. T., *Agroplantae*, 1975, **7**, 13.
5. Tayal, M. S., Kumar, S. and Goel, A. K., *Curr. Sci.*, 1981, **50**, 785.
6. Silano, V., Pocchiari, F. and Kasarda, D. D., *Biochim. Biophys. Acta.*, 1973, **317**, 139.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
8. Lineweaver, H. and Burk, D. J., *J. Am. Chem. Soc.*, 1934, **56**, 658.
9. Dixon, M., *Biochem. J.*, 1953, **55**, 170.
10. Anderson, J. W., *Phytochemistry*, 1966, **7**, 1973.
11. Loomis, W. D. and Battaile, J., *Phytochemistry*, 1966, **5**, 429.
12. Demos, E. K., Woolwine, M., Wilson, R. H. and McMillan, C., *Am. J. Bot.*, 1975, **62**, 97.
13. Koch, S. J. and Wilson, R. H., *Ann. Bot.*, 1977, **41**, 1091.
14. Lee, T. T., *Plant Physiol.*, 1977, **59**, 372.
15. Van-Sumere, C. F., Cottenie, J., DeGreef, J. and Kint, J., *Recent Adv. Phytochem.*, 1972, **4**, 165.
16. Williams, A. H., in *Enzyme chemistry of phenolic compounds*, (ed.), J. B. Pridham, Pergamon Press, London, 1963, p. 87.
17. Zanobini, A., Vanni, P. and Firenzuoli, A. M., *Experientia*, 1967, **23**, 1015.

MYOGLOBIN LEVELS IN THE MYOCARDIA OF SOME REPRESENTATIVE VERTEBRATES

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ABSTRACT

Studies on the myocardial myoglobin levels of some representative vertebrates were carried out. The lowest myoglobin level has been observed in the myocardia of *Rana*. The ventricular myocardia of *Cybium*, *Calotes* and *Geomyda* exhibit higher myoglobin levels than their respective atria. The myocardia of flying forms *Columba* and *Pteropus* have higher levels than the less active forms. *Pteropus* myocardia show a uniquely high myoglobin level. The active flying forms exhibited a preponderance of myoglobin in the right ventricle than the left.

INTRODUCTION

MYOGLOBIN is concerned with the storage and transport of oxygen at the cellular level in the muscular tissue. Functionally it is an oxygen carrier facilitating oxygen diffusion into the cell[1]. Myoglobin plays an important role in the adaptation of animals to diverse physiological conditions. Grote[2] suggested that in tissues with critical oxygen supply, there is the possibility of an increased myoglobin concentration. The role of myoglobin in adaptation to high altitude had been reported by various investigators[3,4]. Similarly, programmed treadmill running was also found to induce a higher concentration of myoglobin in skeletal muscles of rats[5]. According to Catlett *et al.*[6] there exists a higher myocardial myoglobin level in the flying forms of *Columba* than the non-flying forms. Differential distribution pattern of myoglobin in the various myocardial chambers of some vertebrates has also been reported[7,8]. However information regarding the distribution pattern of myoglobin in the various chambers of vertebrates belonging to diverse habitats and activity levels is lacking. The present study has therefore been undertaken to elaborate the distribution pattern of myoglobin and its possible significance in the myocardia of some representative vertebrates belonging to diverse habitats and activity levels.

Abreviations: RA—Right atrium; LA—Left atrium; RHV—Right half of ventricle; LHV—Left half of ventricle; RV—Right ventricle; LV—Left ventricle.

MATERIALS AND METHODS

The list of nine vertebrates investigated and their respective habitats are given in table 1. The myocardial tissue from different chambers were carefully excised and the myoglobin content was estimated employing the method of Tappan and Reynafarjee[3].

RESULTS

Myoglobin levels in the various chambers of the myocardia of the vertebrates investigated are given in table 2. The data are represented as histograms (figure 1), showing the distribution pattern of myoglobin among the various animals and in the different chambers.