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## SOLUBILITY OF PROLINE AND ITS BIOLOGICAL SIGNIFICANCE

K. S. KRISHNA SASTRY, M. UDAYAKUMAR, R. DEVENDRA AND A. A. MEHKRI

Division of Crop Physiology, The University of Agricultural Sciences, GKVK, Bangalore 560 065, India

PROLINE is the amino acid which accumulates in large quantities (upto 15,000 to 20,000  $\mu\text{g g}^{-1}$  dry weight) under moisture stress in leaves<sup>1-3</sup>. Steward and Lee<sup>4</sup> have shown that 90% of the free amino acid that accumulates under saline conditions is proline. Proline shows unusual properties which are characteristics of hydrophilic colloids<sup>5</sup>. Recently, Paleg *et al.*<sup>7</sup> have shown that proline, betaine and several other compounds like hydroxyproline, glycine, valine and glycerol protect enzymes against a wide range of thermal sensitivity. Proline could also increase solute potential and this is relevant in view of its large accumulation and high solubility<sup>6</sup>. The solubility of proline in solutions of other compounds that are known to exist in the cell sap was investigated. The solubility of proline, glycine, glucose, sucrose, mannitol and KCl in saturated solutions of these compounds was determined.

The data in table 1 shows that in 100 ml each of the saturated solutions, the solubility of proline varies from 85 to 185 g while in these saturated solutions the solubility of other substances ranged from 14.9 to 64.8 g. The unique variation of the solubility of proline is perhaps due to the ability of proline to form micelles<sup>6</sup>.

The variation of the solubility of proline could be of great biological significance. For a given molar con-

TABLE 2

Per cent increase in weight of groundnut seeds after soaking in equimolar (0.5 M) solutions of different solutes in water

Solutes	Per cent imbibed after different periods of soaking		
	1 hr	2 hr	4 hr
Proline	15.0	19.2	30.4
Glycine	13.6	21.4	26.0
Sucrose	12.6	17.2	20.0
Mannitol	13.4	20.0	27.0
KCl	12.9	17.6	23.4
Water only	19.0	33.4	44.4

CD at 5%, Time 0.94; Treatment 1.32; Time  $\times$  Treatment 2.29.

centration while the solute potential created by proline is equivalent to that of any other organic substance, the available free water to sustain metabolic functions could be higher. This was tested by studying the imbibition by seeds of groundnut in equimolar concentrations of several solutions and water. It can be seen from table 2 that the per cent imbibition at the end of the 4 hr was highest in water followed by solutions of proline and other solutes. This order was, however, slightly different at lower intervals of soaking.

Cucumber cotyledons grown in a medium containing benzyladenine and potassium<sup>8</sup> for 72 hr were kept in open petridishes at 28°C until 50% reduction in weight was noticed. Such cotyledons were transferred to water and 0.1 M solutions of proline, glycine and KCl. The per cent increase in fresh weight of such cotyledons in 15 min was determined (table 3). The data showed that increase in fresh weight was more in

TABLE 1

Solubility of different solutes (g/100 ml) in saturated solutions at 25°  $\pm$  1° C

Solutes	Saturated solution of				
	KCl	Glycine	Mannitol	Glucose	Proline
Proline	137.8	85.7	185.6	141.3	—
Glycine	24.2	—	19.5	14.9	16.2
Mannitol	23.6	20.5	—	20.2	18.4
KCl	—	33.3	53.3	30.7	32.2
Glucose	64.0	64.8	36.4	—	63.7

TABLE 3

Per cent increase in fresh weight (uptake of water) in 15 min by cucumber cotyledons, stressed to lose 50% moisture and placed in 0.1 M concentrations of different substances

Solutions	Percent increase in fresh weight	
	Experiment I	Experiment II
Proline	59.45	63.41
Glycine	41.23	48.61
KCl	33.12	31.57
Water	60.79	62.86

proline compared to other solutions and was nearly as good as in water.

In all cases along with water, entry of the solutes into the cotyledons was also observed. Studies on the property of diffusibility of proline compared to other commonly occurring solutes across the membrane systems are in progress.

These properties show that proline can exist in solution along with other solutes, even though the concentration of the other solutes is high. When equimolar solutions are compared, water potential of proline solution appears to be higher. This is an important property which could be of biological significance.

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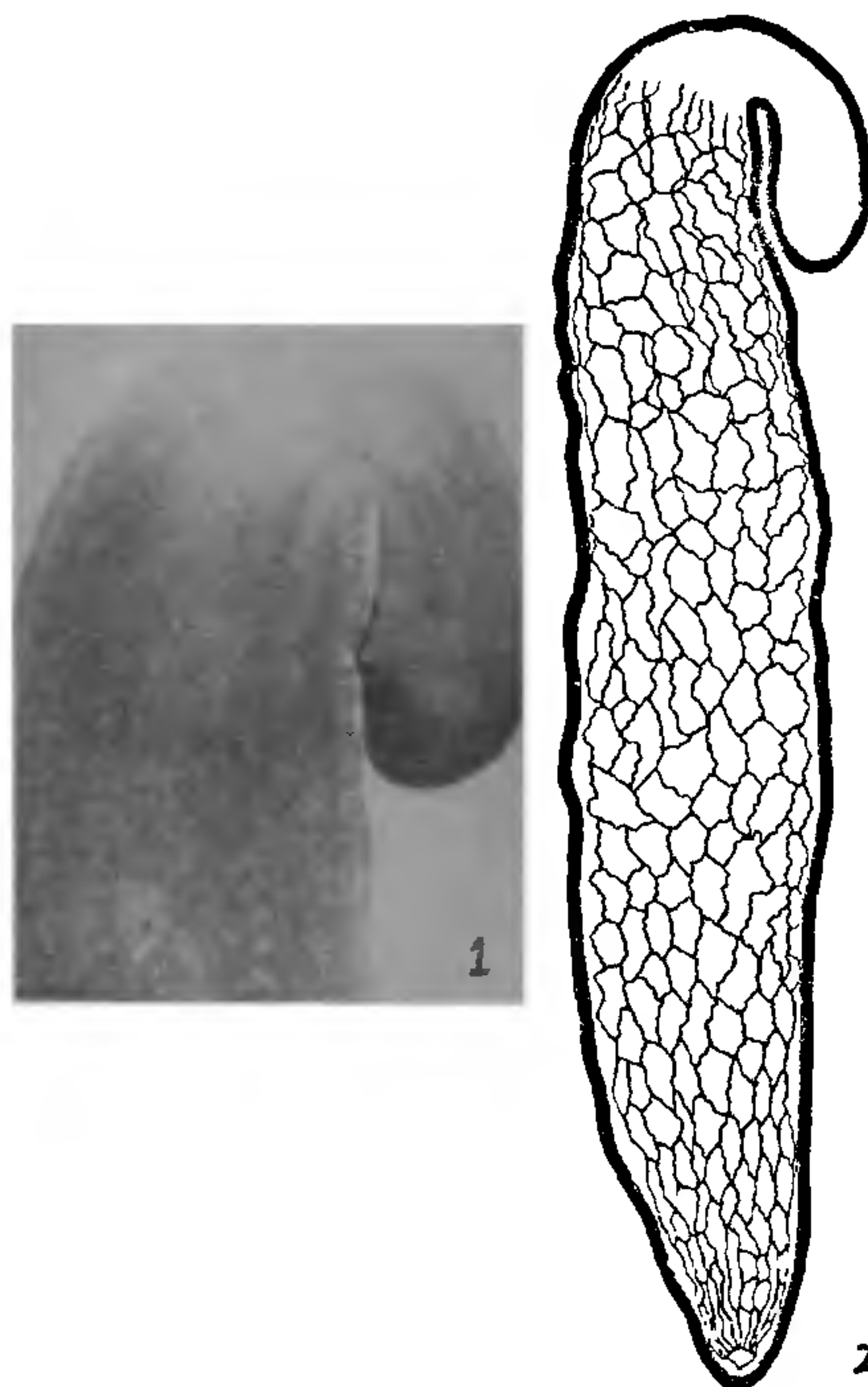
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## ESTERASE ACTIVITY IN THE OSMOREGULATORS

H. FERNANDEZ, U. RASHEED, S. S. SIMHA,  
Department of Zoology, Osmania University,  
Hyderabad 500 007, India.

ORIGINAL observations on the osmoregulatory system of caryophyllaeids date from the mid 19th century and are largely confined to *Caryophyllaeus mutabilis* and *Archigetes* species. A few notable references in this regard are from Hunter<sup>1</sup>, Blanchard<sup>2</sup>, and Mackiewicz<sup>3</sup>. Little attention has been given on the osmoregulatory system in other species. The present communication deals with the osmoregulatory system of two species of caryophyllaeids *Lytocestus indicus* and *Pseudocaryophyllaeus indica*.

Fresh specimens were collected in cold physiological saline from freshwater siluroid fish, *Clarius batrachus* and immediately fixed in cold 4% formalin for one hour. The parasites were washed in cold distilled water three times and transferred into incubation medium of Holt & Withers<sup>4</sup> maintained at 37°C.



Figures 1, 2. 1. *Lytocestus indicus* showing the esterase activity in the excretory canals. 2. Diagrammatic representation of excretory system in *L. indicus*.

The authors found that *L. indicus* and *P. indica* were selectively stained and the number and the arrangement of canals in both the types of parasites were identical. Five pairs of longitudinal canals originate