Azione del G.T.P., dell'α-chetoglutarato e dell'A.T.P. sui sistemi enzimatici ossidasici dei grassi (fegato di cavia). I valori sono espressi in μM di O_2/100 mg di preparato enzimatico secco. Tempo di prova 20 min

A = μM di O_2 consumate globalmente dal sistema enzimatico per ossidare l'acido caprilico.

B = μM di O_2 consumate per la sola formazione di acido acetacetico.

Condizioni sperimentali

Attivatore	Alimentazione normale		Digiuno da 120 h	
	A	*B*	*A*	*B*
G.T.P. (10 μM)	9,80	7,10	8,78	7,95
α-chetoglutarato	10,20	8,00	9,02	7,89
A.T.P. (·10 μM)	2,00	1,60	0	0
A.T.P. (65 μM)	10,80	9,60	6,60	5,98
A.T.P. + succinato (65 μM A.T.P.)*	10,34	7,80	6,00	3,36

* 10 μM di A.T.P. aggiunte direttamente al sistema enzimatico + 55 μM sintetizzate tramite l'ossidazione fosforilante del succinato.

dente a quella che si forma teoricamente durante il processo di ossidazione dell'α-chetoglutarato calcolando P/O = 1) attiva l'ossidazione degli acidi grassi con la stessa intensità dell'α-chetoglutarato. Invece l'A.T.P., aggiunto alla stessa concentrazione di 10 μM, attiva il sistema enzimatico assai meno intensamente.

Si potrebbe prospettare che il G.T.P. abbia agito tramite l'A.T.P. che avrebbe potuto sintetizzarsi, per azione di una transfosforilasi, secondo la seguente reazione:

$$\text{G.T.P.} + \text{A.D.P.} \rightleftharpoons \text{G.D.P.} + \text{A.T.P.} \qquad (3)$$

e che la più intensa azione del G.T.P., in confronto di quella dell'A.T.P., sia dovuta al fatto che l'A.T.P. viene sintetizzato gradatamente dal G.T.P. e quindi potrebbe risentire in minor misura delle attività fosfatasiche. Ci sembra però che quest'ultima ipotesi possa essere esclusa perché lo stesso fenomeno avrebbe dovuto verificarsi anche quando si impiega come attivatore succinato o fumarato i quali, come si è già detto in altra nota[1], agiscono pure sintetizzando gradatamente A.T.P. tramite il processo della fosforilazione ossidativa.

Che il G.T.P. e l'α-chetoglutarato agiscano indipendentemente dall'A.T.P. è confermato anche dal fatto che l'intensità di azione dei sistemi enzimatici preparati da fegati di cavie a digiuno da 120 h rimane praticamente invariata quando l'attivatore è rappresentato dall'α-chetoglutarato o dal G.T.P., invece è intensemente diminuita quando l'attivatore è rappresentato dall'A.T.P. o dal succinato più A.T.P.

In definitiva si può concludere che, impiegando fegato di cavia, il G.T.P. e l'α-chetoglutarato attivano il sistema enzimatico ossidasico dei grassi con le stesse modalità, ed ambedue indipendentemente dall'A.T.P.

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Istituto di Chimica Biologica, Università di Padova, il 16 settembre 1958.

Summary

In guinea-pig liver, G.T.P. and α-ketoglutarate both activate enzyme systems of fatty acid oxidation in the same way, and both act independently of A.T.P.

Amino Acids in *Nopalea cochinellifera*

The plant referred to as *Opuntia* sp. in a previous communication[1] has been identified as *Nopalea cochinellifera*. Besides enzymes, the plant has been examined for carbohydrates, organic acids, and amino acids.

The present communication is a report of the qualitative as well as quantitative amino acid make up of the phylloclade of the plant.

Cactus plants have been analysed for their total nitrogen content by Ducloux[2], for protein content by Benjamin and Old[3], and also by Bonsma and Maré[4]. Piccoli[5] has estimated crude proteins of some varieties of *Opuntia*.

The present study was undertaken to throw some more light on the nitrogenous constituents of a cactus plant, as such detailed information is not available about this group of plants.

Method. Free amino acids were identified in the alcoholic extracts of the fresh phylloclade of the plant by two-dimensional paper chromatographic technique of Consden, Gordon, and Martin[6], using 80% phenol for the first development followed by Partridge's solvent[7] for the second development.

Besides the relative mobilities of the amino acids, appropriate reagents were used to confirm the presence of different amino acids.

For total amino acid composition 50 mg of the dried defatted plant material were hydrolysed with 3 cm³ of 6 *N* hydrochloric acid for 24 h.

After the removal of HCl in the usual manner, the hydrolysate was kept overnight in a dessicator over KOH to remove last traces of HCl. The thin film was then taken up in distilled water, filtered and made up to 5 ml.

Total nitrogen in the hydrolysate was estimated by semimicro Kjeldahl's method. Some of the amino acids were estimated by the circular paper chromatographic technique of Giri[8] *et al.*, and the remaining amino acids were estimated by buffered paper chromatographic technique as described by Krishnamurthy and Swaminathan[9].

Results. The following amino acids were identified in the alcoholic extract of the fresh plant material.

1. Leucines, 2. Phenyl-alanine, 3. Valine, 4. Methionine, 5. τ-amino butyric acid, 6. proline, 7. α-alanine, 8. glutamic acid, 9. threonine, 10. glycine, 11. serine 12. lysine, 13. cystine.

Total amino acids. 1 cm³ of the hydrolysate contained 0·143 mg of total nitrogen.

The following Table gives the quantities of individual amino acids in 100 g of the dried plant material.

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[4] H. C. Bonsma and G. S. Maré, Union S. Africa, Dept. agr. Forestry Bull. *236*, 71 (1942).

[5] G. Piccoli, Agr. Coloniale (Italy) *37*, 267 (1943).

[6] R. Consden, A. H. Gordon, and A. J. P. Martin, Biochem. J. *37*, XIII (1944).

[7] S. M. Partridge and R. G. Westall, Biochem. J. *42*, 238 (1948).

[8] K. V. Giri, A. N. Radhakrishnan, and C. S. Vaidyanathan, J. ind. Inst. Sci. *35*, 145 (1953).

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Amino acid	Per 100 g of dried material	Expressed as g of N_2/100 g of dried material
Leucines	1·23	0·130
Phenylalanine	0·41	0·035
Tyrosine	traces	—
Alanine	0·67	0·105
Arginine	1·09	0·350
Threonine	0·220	0·026
Glycine	0·680	0·127
Serine	0·230	0·031
Glutamic acid	1·010	0·096
Aspartic acid	0·670	0·070
Methionine	0·068	0·006
Valine	traces	—

The nitrogen metabolism in the plant seems to be low as it contains 1·56% total nitrogen on dry weight basis. 0·98% nitrogen has been accounted for by estimation of amino acids. Histidine, lysine, and cysine were found to be only in traces and hence were not estimated. The number of free amino acids in the plant was practically the same as the total amino acids except for the τ-amino butyric acid which was detected in the free state.

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Résumé

L'examen de la *Nopalea cochinellifera* pour en déterminer la composition amino-acide a révélé que le métabolisme de l'azote est bas. Sur 1,56% d'azote total du tissu sec, la présence de 0,98% a pu être expliquée par la détermination, au moyen de la chromatographie sur papier, de la quantité d'amino-acides qui y est contenue.

Organic Acid and Carbohydrate Metabolism in *Nopalea cochinellifera*

The occurrence of organic acids and carbohydrates in chlorophyllous tissues of plants and their interconvertibility is a well established fact. BENNET-CLARK[1], in his review of literature on this subject, emphasized that succulent plants exhibit diurnal fluctuations in the acid content of their chlorophyllous tissues and thereby exhibit a special type of metabolism. He included plants belonging to *Crassulaceae, Cactaceae, Liliaceae,* as examples of this type of metabolism. Plants belonging to these families accumulate organic acids in their chlorophyllous tissues in dark and lose them on illumination. WARBURG[2] indicated the possibility of this type of metabolism in plants belonging to other families also, possessing strong cuticle.

WOLF[3] observed a reciprocal relationship between organic acids and carbohydrates in crassulacean plants.

KREBS[4] proposed a new theory to correlate the variation in organic acids and carbohydrates in plants.

The plant in the present investigation is a spineless cactus and has a close relationship with *Opuntia*. It has been fairly worked out in detail for its organic acid content, carbohydrate content, amino acid content, and enzyme systems and the present communication is a report of results regarding its carbohydrates and organic acids. and their variation over a period of 24 h.

Materials and Methods. Qualitative examination of the phylloclade of the plant for organic acids was done in the alcohol extracts of the plant. Circular paper chromatographic technique developed by GIRI and RAO[5] was used to identify organic acids; pentanol-formic acid as the developing solvent, and bromophenol blue, ammonical silver nitrate, and ammonium-vanadate as the spray reagents[6]. Sugars were identified in alcoholic extracts by the same technique using butanol; acetic acid; water = 4:1:5[7] for development of chromatograms, and aniline-hydrogen phthalate[8], aniline-diphenylamine phosphoric acid, α-naphthylaminephosphoric acid, and triphenyl tetrazolium chloride as spray reagents[9]. Titratable acidity of the plant was determined by the method of THOMAS and BEEVERS[10]. 5·0 g of the fresh phylloclade tissue was sliced before plunging it into 100 ml of boiling water. Boiling was continued for 20 min and after cooling it was titrated against 0·1 *N* NaOH using a few drops of phenolphthalein as indicator. The titratable acid number (T.A.N.) was expressed as the number of ml of 0·1 *N* NaOH required to neutralize the acid content in the boiled residue and liquid originating from 100 g of the fresh tissue.

Reducing and total sugars were estimated in the alcoholic extracts of the plant by the method of SOMOGYI[11].

These studies were carried out over a period of 24 h in January 1957 in Bombay.

Results. The following organic acids were found to be present in the plant; malic acid, citric acid, and traces of oxalic acid. Sugars-fructose, glucose, sucrose, maltose, and traces of raffinose.

The following Table gives the variation of organic acids during 24 h of a day.

Table I

Winter January Bombay	Time	Temperature °C	Malic Acid	Citric Acid	Oxalic Acid
	8·0 A.M.	21	+ + + +	+ +	—
	12·0 noon	28	+ + +	+	—
	4·0 P.M.	27·5	+	+	—
	8·0 P.M.	26·0	+	+	—
	12·0 midnight	22·0	+ +	+	—
	4·0 A.M.	22·0	+ + +	+ +	—

+ + + + = very prominent; + + + = prominent; + + = moderate; + = present; – = traces.

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