of the administered radioactivity was retrieved 20 min after injection. It seems likely that the high level of liver acid soluble radioactivity 2 min after i.p. injection partly depends on a contamination by unabsorbed radionuclide. This is supported by the 20% difference between the ratios of i.p. to s.c. administered radioactivity in the liver acid soluble and RNA-fractions (table). Although the incorporation of labeled orotic acid into the RNA-fraction after s.c. injection lagged behind that after i.p. injection only a minor difference was found between the levels reached at 60 min after injection. This should depend on the high capacity of the mouse liver to accumulate orotic acid[2]. Our result show that in order to avoid contamination of the liver acid soluble fraction and of i.p. sampled blood by unabsorbed radionuclide after short pulse-periods, i.p. injection should be avoided. It is evident that s.c. injection in the neck is preferable as it in addition to an effective incorporation of radioactivity into the liver cells also allows i.p. sampling of blood, a fact that is of great importance when hypoxia must be minimized during liver tissue sampling[10].

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Keto acids and free amino acids during leaf growth in Bauhinia purpurea L.

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Summary. The biosynthesis of keto acids and free amino acids was studied during the growth of Bauhinia purpurea leaves. α-KGA, OAA, pyruvic acid and PEP are the important keto acids observed at various stages. The first 2 metabolites show a progressive increase and PEP → OAA pathway is very active during the process of growth.

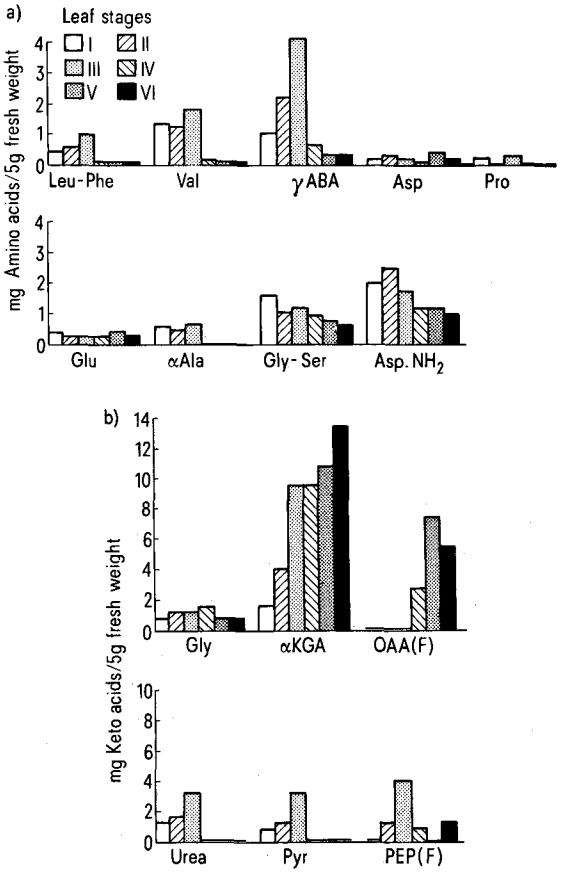
The involvement of amino acids and keto acids in interrelated metabolic pathways has been indicated since long[3]. Changes in the amino acids and proteins with the growth and development of the leaves have been worked out in detail, but little is known about the changes in keto acids in maturing leaves. The present study is aimed at studying changes in keto acids concentrations and correlating with that of amino acids during leaf maturation.

Material and methods. Bauhinia purpurea plant growing in the University campus has been selected for this study. Leaves of different stages (2-, 4-, 6-, 9-, 12- and 15-day-old) were plucked from the tree, brought to the laboratory and analyzed.

The method described by Steward, Wetmore, Thompson and Nitsch[4] has been followed for amino acid extraction. 2-dimensional paper chromatography has been used for their separation. The detailed procedure is same as that described by Pal and Laloraya[5]. Milligrams of different amino acids are calculated in terms of glycine, using a Klett photoelectric colorimeter.

Keto acids have been extracted as 2,4-dinitrophenyl hydrazones (2,4-DNP's) as described by Kaushik[6] and Mukherjee[7,8]. For the separation of keto acids also, 2-dimensional paper chromatography has been employed. Milligrams of different keto acids are calculated in terms of 2,4-DNP of α-ketoglutarate (α-KGA) using a Klett photoelectric colorimeter fitted with blue filter.

Results. The figure shows that with the development of leaves the level of asparagine shows a gradual decline after the stage II, while glutamine is present only in trace



B. purpurea. Showing levels of amino acids and keto acids in different stages of leaves. *a* Amino acids: Leu & Phe, leucine and phenylalanine; Val, valine; γ-ABA, γ-amino butyric acid; Pro, proline; Ala, α-alanine; Glu, glutamic acid; Asp, aspartic acid; Gly & Ser, glycine and serine; Asp·NH_2, asparagine. *b* Keto acids: Gly, glyoxylic acid; OAA (F), oxaloacetic acid (fast-moving isomer); α-KGA, α-ketoglutaric acid, urea; Pyr, pyruvic acid; PEP (F), phosphoenolypyruvic acid (fast-moving isomer).

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amount upto the stage III followed by complete disappearance. The quantity of γ-aminobutyric acid gradually increases up to stage III, followed by a sharp decline. Proline is observed only in the stage I and III, while α-alanine shows its presence from the stage I to stage III. However, the concentrations of these compounds are much lower compared with asparagine.

The major keto acid spots detected in different stages of leaves are α-KGA, OAA, pyruvic acid and PEP; the first 2 comprise the bulk of keto acids. α-KGA increases markedly with the growth of leaves; an almost linear increase is observed. A 7fold increase is recorded between the first stage and the sixth leaf stage where it shows a high value of 13.6 mg/5 g fresh weight of leaves.

Oxaloacetate is untraceable during the first 3 stages of leaf growth; it appears at the IVth stage and increases markedly at the Vth stage, whereafter it decreases slightly at the VIth stage. Another important metabolite PEP, which is not present in stage I leaves, increases up to the stage III, followed by a low value during later period of growth. The amount of pyruvic acid and urea (which also forms its hydrazone derivative) also increases with the age of the leaves up to the IIIrd stage, followed by their disappearance in later stages. Glyoxylic acid, however, does not exhibit any marked changes.

Discussion. Changes in keto acids and amino acids with the growth of leaves clearly reveal that there is a progressive accumulation of α-KGA, and at late stages of OAA. Disappearance of pyruvic acid and lower value of PEP after IIIrd stage of leaf growth would indicate that PEP → OAA pathway is very active during the early periods of leaf growth. Absence of OAA during this period is therefore due to its rapid utilization for growth reactions, while its later accumulation is due to sluggish rate of metabolism and synthesis of protein. Webb and Fowden[9], working on the keto acid changes in the leaves of Arachis hypogaea, have suggested that as leaves develop they pass from a state of rapid net protein synthesis during the period of their active growth to one where the rate of protein synthesis is much reduced and only counterbalances protein breakdown in the mature leaf. Present findings agree with this view, and it appears that, with the increasing age of the leaves, the transamination reactions utilizing the keto acids for the synthesis of amino acids is affected and becomes sluggish. Since α-KGA and OAA involving transaminations are the most common in plants, they tend to show the greatest accumulation. These keto acids being key metabolites of oxidative citric acid cycle, their accumulation envisages sluggish operation of this cycle particularly at these points. Indeed it has been shown during recent years that the protein synthesis per se is not affected during ageing but the breakdown processes are activated[10].

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Colchicine inhibits stimulated release of gastric histamine but not activation of histidine decarboxylase

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Summary. In the rat, gastric mucosal histamine is mobilized and histidine decarboxylase activated by treatment with insulin or pentagastrin. Colchicine pretreatment prevented the histamine release without preventing the enzyme activation. The results suggest a) that histamine release and histidine decarboxylase activation are independent events, and b) that microtubules are involved in the release of histamine.

Gastrin mobilizes histamine from endocrine cells in the rat oxyntic mucosa, at the same time activating the histamine-forming enzyme in these cells[1,2]. It has been argued that the reduction of gastric histamine triggers off the activation of histidine decarboxylase by 'lessening of end-product repression'[1]. Agents, such as colchicine and vinblastine, which disaggregate microtubules, are known to interfere with release processes, such as secretion of insulin from the B-cell[3,4] and secretion of catecholamines from the adrenal medulla[5]. The purpose of the present study was to examine the effects of colchicine on histamine content and histidine decarboxylase activity in the rat stomach.

Adult male rats (weighing 150–250 g) of the Sprague-Dawley strain were used. They were fasted for 48 h (tap water ad libitum) before the experiments. In 1 experiment the rats received 5 mg/kg colchicine i. p. and were then given pentagastrin s. c. $3^1/_2$ h later and killed after 1 h. In another experiment the rats received first colchicine and after 30 min 2.5 U/kg insulin s. c.. They were killed $4^1/_2$ h later. Controls were given 0.9% saline. At sacrifice,

Effect of pentagastrin on gastric histamine content and histidine decarboxylase activity after pretreatment with colchicine

Treatment	A. Mucosal histamine conc. μg/g, mean ± SEM (n)	B. Histidine decarboxylase activity pmoles CO_2/mg/h, mean ± SEM (n)
1. Saline	59 ± 2.4 (8)	5.7 ± 0.7 (8)
2. Colchicine	63 ± 1.8 (5)	8.2 ± 1.3 (5)
3. Pentagastrin	41 ± 2.5 (27)	15.1 ± 1.0 (36)
4. Colchicine and pentagastrin	56 ± 3.4 (23)	17.1 ± 1.0 (23)

1A–2A: N.S.; 1A–3A: $p < 0.001$; 1A–4A: N.S.; 2A–4A: N.S.; 1B–2B: N.S.; 1B–3B: $p < 0.001$; 1B–4B: $p < 0.001$; 2B–4B: $p < 0.001$.

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