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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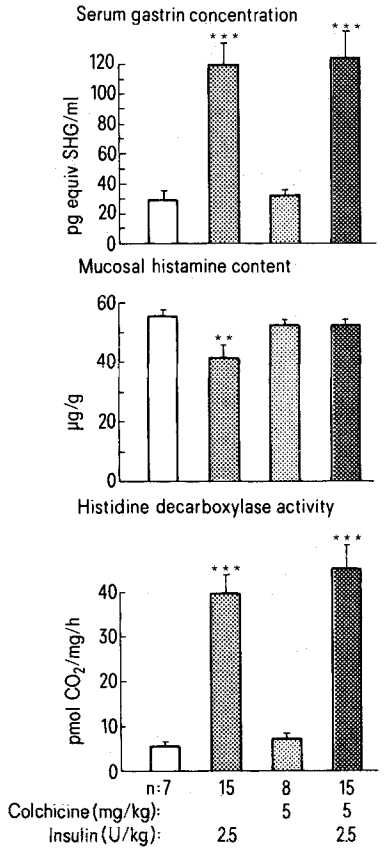

Effect of insulin on serum gastrin concentration, gastric mucosal histamine content and histidine decarboxylase activity after pretreatment with colchicine. Statistical analysis was made according to Student's t-test: Significant difference between untreated controls and drug-treated animals are indicated by **for $0.001 < p < 0.01$ and ***for $p < 0.001$. Colchicine alone or in combination with insulin had no significant effect on the mucosal histamine content, whereas insulin alone caused reduction of the histamine content ($0.001 < p < 0.01$). The difference in serum gastrin concentration and histidine decarboxylase activity between colchicine-treated and colchicine + insulin-treated rats was significant ($p < 0.001$).

blood was drawn from the aorta. Serum was lyophilized and stored at −25 °C until analysis. Gastrin was determined by radioimmunoassay, using antibodies raised in rabbits against synthetic human gastrin I. The assay technique, its accuracy and reliability on rat serum has been described elsewhere[6]. The oxyntic mucosa was scraped off the stomach wall and homogenized in 0.1 M phosphate buffer, pH 7.0, to a final concentration of 100 mg (wet weight) per ml. The histamine content of these extracts was measured fluorometrically[2] and the histidine decarboxylase activity was determined radiometrically as described in detail elsewhere[6].

Colchicine had no effect on serum gastrin concentration, gastric histamine content or histidine decarboxylase activity in fasted rats. However, colchicine prevented the reduction of gastric histamine following injection of insulin (figure) or pentagastrin (table), but did not prevent the activation of histidine decarboxylase. Insulin-stimulated gastrin release was not inhibited by colchicine (figure).

Microtubules are thought to be involved in the process of peptide hormone secretion, e.g., by promoting margination of cytoplasmic granules prior to exocytosis. Colchicine is known to prevent the assembly of subunits into microtubules[7] and has therefore been widely used in studies on the role of this organelle in hormone secretion. Our results seem to suggest that release of histamine from the endocrine cells in rat oxyntic mucosa is dependent upon an intact microtubular arrangement. Further, the results argue strongly against the contention that histidine decarboxylase is activated as a result of a reduced histamine content. On the contrary, it appears that gastrin-stimulated histamine release and gastrin-stimulated activation of histidine decarboxylase are 2 independent processes. Finally, the results give no indication that colchicine blocks gastrin release. This will be the subject of a separate study.

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Chromosome aberrations in mice by the antifungal antibiotic, nystatin

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Summary. Nystatin, a fungicide of current medical use, was tasted in mice for its effect on chromosomes of bone marrow cells. A significant increase of aberrations, mostly of chromatid type, was observed over a period of from 15 min to 15 days following the application of the drug. Our data indicate a non-random distribution of the breaks.

The antifungal antibiotic nystatin, isolated as an intracellular product of Streptomyces nourscei[1] was found effective also in human monilial infections. The chromosome-breaking property of nystatin appeared not to have been studied before, which initiated the present investigation.

Material and method. 6-week-old random bred Swiss strain mice, Mus musculus, were injected with a 25% aqueous solution of nystatin[2] at a dose of 50 mg/kg b.w. (equivalent to a current human therapeutic dose), and another set with distilled water as controls. Their bone marrow cells were fixed at 8 different intervals (table 1) for the assessment of chromosome aberrations from slides prepared according to the colchicine-citrate-acetic alcohol-Giemsa-air-drying technique[3,4].

Results. As found with the use of other antibiotics[4–9], the aberrations induced by nystatin were mainly chromatid constrictions and gaps, subchromatid and chromatid breaks, and to a minor extent fragments of unknown origin and translocations. In the control series, the last 2 types were not encountered, while the frequencies of other types were very low (table 1). The average was