Compound fed	Amount fed to 12 plants	% incorporation into alkaloids		Specific activity of pure alkaloids
		unhydrolysed	hydrolysed[a]	
Sodium acetate-1-^{14}C	250 μC	0.007	0.002	9.0×10^{-5} μC/mM
Sodium acetate-2-^{14}C	100 μC	0.006	0.003	2.5×10^{-4} μC/mM
Mevalonic acid-2-^{14}C, DBED salt	50 μC[b]	0.010	0.006	2.0×10^{-4} μC/mM

[a] Hydrolysis removes esterifying acids which, in the case of lycoctonine include methylsuccinic and anthranilic acids[5]. [b] Fed to 6 plants, 6 untreated plants were added prior to extraction of the alkaloids. All counting was performed using the Nuclear-Chicago 'Dynacon', Vibrating-reed electrometer-ionisation chamber assembly.

extracted, hydrolysed and separated by chromatography on alumina. Browniine was isolated as the crystalline perchlorate, lycoctonine as the crystalline hydrate.

From the results given in the Table it can be seen that although the 'incorporations' were poor[6b], they were consistent with the pattern expected from a 'normal' terpene biosynthesis. Within experimental error, the specific activities of the pure browniine and lycoctonine were the same, in all cases, consistent with a common precursor.

HERBERT and KIRBY suggested that the failure to incorporate mevalonic acid into the alkaloids of *D. elatum* was due to unsuccessful competition with the biosynthesis of non-alkaloidal terpenes. (They found 0.02% incorporation of mevalonic acid into β-sitosterol biosynthesized, over 4 days, in *D. elatum*.) However, we suspect that the primary site of alkaloid biosynthesis in *D. brownii* is in the roots and if this is also the case with *D. elatum*, the failure of HERBERT and KIRBY to observe incorporation of mevalonic acid into the alkaloids may be ascribed to their use of detached leaves for their experiments. Their further result, that methionine, methyl-^{14}C was incorporated (0.025%) into delpheline, primarily into the methoxyl groups of the alkaloid, under these conditions, implies either that there is metabolic equilibrium between methylated and demethylated alkaloids in the leaves, or more probably that methylation occurs in the leaves, of alkaloid biosynthesized elsewhere in the plant. There are certainly present in the leaves of *D. brownii* alkaloids which, from their chromatographic behaviour, appear to contain more free hydroxyls than browniine or lycoctonine and which we hope will prove to be the unmethylated precursors of these alkaloids.

Zusammenfassung. Die diterpenoiden Alkaloide Browniin und Lycoctonin wurden *in vivo* aus Mevalonsäure-2-^{14}C hergestellt. Sie zeigten, aus Acetat-1-^{14}C und -2-^{14}C gewonnen, eine geringere Wirksamkeit. Die Biosynthese dieser Alkaloide scheint somit der der anderen Terpene analog.

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[6] (a) Injected in aqueous solution into the stem cavities. (b) The incorporations could probably be improved by using the 'wick method' (E. LEETE, J. Amer. chem. Soc. *84*, 55 (1962)) and feeding earlier in the development of the plant.

A Procedure for the Preparation of Gram-quantities of Bacterial Cell Walls[1]

Many methods are available for the preparation of bacterial cell walls[2]. Most of these, however, suffer from the disadvantage that only small amounts of bacteria (usually to 400 mg dry weight) can be disrupted in a single operation. The use of commercially available blenders or homogenizers for the disruption of large quantities of microorganisms was originally described by LAMANNA and MALLETTE[3] but seems to have been overlooked by students of bacterial cell walls. We have found that the Omni-Mixer Highspeed Homogenizer[4] can be used for the efficient disruption of relatively large quantities of bacterial cells. This homogenizer offers the added advantage over other types of homogenizers in that its stainless steel mixing chamber is mounted on a movable bracket. The chamber can thus be lowered into a temperature-control bath. Using this homogenizer, together with a high-speed centrifuge with large capacity, gram-quantities of cell walls can be readily prepared.

Commercially available cells of *Micrococcus lysodeikticus*[5] were used in these experiments. The glass beads

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were a product of the Minnesota Mining and Manufacturing Company ('Superbrite', Type 130-5005, 0.1 mm diameter) and prior to use, were treated with hot cleaning solution, washed extensively with water and dried. 15 g of dried cells, 250 g of beads and 250 ml of distilled water, all precooled in ice, were put into a 400 ml stainless steel mixing chamber which was also precooled to 0°. The chamber was then transferred into a freezing mixture, which was kept at a temperature of about −5°. It was attached to the homogenizer, and mixing at top speed was carried out for 50 min. This period of time was sufficient for virtually complete breaking of the cells, as judged by the release into solution of non-sedimentable (10,000 × *g*, 20 min) ultraviolet absorbing material (260 mμ). The temperature inside the mixing chamber was 3–4° throughout the operation. At the end of the mixing time, the supernatant was transferred into a 2 l beaker kept in ice. The beads were washed by decantation with 5 × 200 ml of ice-cold water. The supernatant and washings were combined, and centrifuged for 15 min at 3000 RPM in the large (GSA) rotor of the Servall RC-2 refrigerated centrifuge. The precipitate, which contains some unbroken cells and cell membranes, as well as small metal particles (from abrasion of the chamber and stirrer), was discarded. The supernatant was centrifuged at 9000 RPM for 15 min to precipitate the cell walls. The supernatant containing cytoplasmic material was discarded. The top white layer of the precipitate was suspended in 500 ml of water, transferred into clean centrifuge bottles and centrifuged at 9000 RPM. This procedure, resulting in further removal of contaminating cytoplasmic material from the sedimenting cell walls, was repeated twice. Another centrifugation at 3000 RPM was carried out after the last wash to remove additional impurities. This was followed by centrifugation at 9000 RPM for 15 min. The packed cell walls, which at this stage are white, were then suspended in 250 ml of water, and heated for 20 min at 100° to destroy lytic enzymes[6]. They were then treated with trypsin[7] and washed with the aid of the centrifuge three more times with water. Finally, they were lyophilized. The yield from 15 g of cells amounts to 2.0 g of walls.

The preparation obtained is homogeneous as seen in the electron microscope. The walls are rendered completely soluble by lysozyme, are free of ultraviolet absorbing material, and do not contain significant amounts of amino acids other than those usually present in *M. lysodeikticus* walls[8]. The same procedure can also be used for the disruption of small amounts of bacterial cells, using the smaller chambers of the Omni-Mixer, and the same proportions of cells, beads and water. The mixing time can, however, be shortened, since disruption of 1 g of *M. lysodeikticus* cells in the 50 ml chamber is complete within 10 min.

Résumé. Une méthode est décrite, qui permet la rupture de la paroi de bactéries en utilisant des quantités relativement larges à chaque essai, ainsi qu'un équipement de coût modique. La préparation de parois de *M. lysodeikticus* à partir d'une prise de 15 g de bactéries sèches est rapportée en exemple.

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Distribution of Mast Cells in the Mucous Membrane of the Human Nasopharynx

The distribution of mast cells in the various animal and human tissues is well documented in the literature[1–5]. Under normal conditions, certain organs show high density of mast cells while others contain fewer, depending on the amount of connective tissue present[6]. However, a notable increase in the mast cell population is also seen in chronic inflammatory reaction[7], epithelial metaplasia[8], certain hormonal influences[9], precancerous lesions and in tissues to which carcinogenic chemicals have been applied[10–13].

According to HLAVÁČEK and LOJDA[7], the human respiratory tract is rich in mast cells which are increased in chronic inflammatory conditions. Carcinoma of the nasopharynx being the commonest malignant neoplasm amongst the Chinese[14,15], it is intended to study the changes in mast cell population in premalignant lesions of the nasopharyngeal mucosa. The purpose of this preliminary report is to provide data on the normal distribution of mast cells in the mucosa of the human nasopharynx. The multiracial population in the state of Singapore made it possible to compare the findings in specimens obtained from four ethnic groups.

Material and Methods. The tissues for this study were obtained from sixty medico-legal necropsies of apparently healthy male adults who died as a result of accidents, homicide or suicide. The persons necropsied belonged to four racial groups: 20 Chinese, 20 Indians, 10 Malays and 10 others (6 English, 2 Japanese, 1 Javanese and 1

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