Transverse Septa in *Geotrichum lactis*

A number of problems associated with the structural differentiation of yeast cell walls remain unresolved. The fine structure of isolated and purified walls of the fungus *Geotrichum lactis* has been studied by the standard techniques of electron microscopy. This report describes structures corresponding to the septa formed during the growth of the pseudomycelium.

The yeast, *G. lactis* (Colección Española de Cultivos Tipo No. 1280), was cultivated on Hansen's medium at 27 °C for 16 h. Cells were recovered by repeated washing and centrifugation, and exposed for 5 min to ultrasonic disintegration. The yeast, which before treatment appears as a pseudomycelium, disintegrates and separates into pieces corresponding to cell compartments delimited by 2 septa. After centrifugation, the sedimented material was shaken with small glass beads in a Braun disintegrator, recentrifuged and thoroughly washed. The residue consisted of empty cell walls, fragments thereof, and some intact cells. Differential centrifugation (García Mendoza and Villanueva[1]) was used to obtain pure preparations of cell walls. These walls were freed of lipids by extraction with ether, treated with 1 *N* KOH according to Kessler and Nickerson[2], and thoroughly washed and lyophilized. The preparations were shadowed by the usual techniques, and electron micrographs taken with a Siemens Elmiskope I microscope.

Recent studies indicate that the cell walls of fungi consist of microfibrils embedded within an amorphous matrix. Cross walls have been studied after isolation from mycelium and from conidia[3,4] and special attention has been devoted to the role of pores in the septa. The classical septum of *Ascomycetes* and *Deuteromycetes* is a simple disc with a central pore, and is formed as an ingrowth of the lateral wall. Multiperforated septa with pores of different sizes have also been described in moulds, in yeast[3,4], and in the fungal components of lichens[5,6]. In *Fusarium culmorum*, septations with multiple perforations have been demonstrated in large hyphae, and probably in conidiophores, where large amounts of cellular substance must be translocated in order to manufacture large conidial masses. Macroconidia also show multiperforated septa. The exact nature, function and ontogeny of septa are uncertain.

The cross walls of *G. candidum* have been reported[3] to contain 20–50 micropores. We have found no pores in septa of *G. lactis*. Because *G. lactis* grows in the form of a pseudomycelium, each cell probably remains independent, so that there is no need for perforations in the septa.

In electron micrographs of the chemically treated cell walls of *G. lactis*, the outside of the wall appears quite smooth with few fibrils. Figure 1 represents a side view of one septum partially separated from the hypha, and Figures 2 and 3 isolated septa. They show intermeshing of fibrils without definite orientation, embedded within an amorphous matrix. Some fibrils projected beyond the edges of the ruptured septum. Many more such fibrils are seen in specimens which have been boiled in alkali for several hours, but again no micropores or central pores

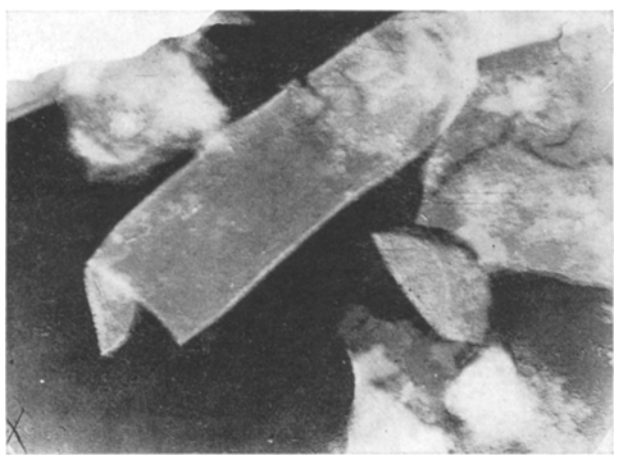

Fig. 1. Electron micrograph of cell compartments of *Geotrichum lactis* showing at one end a bent septum.

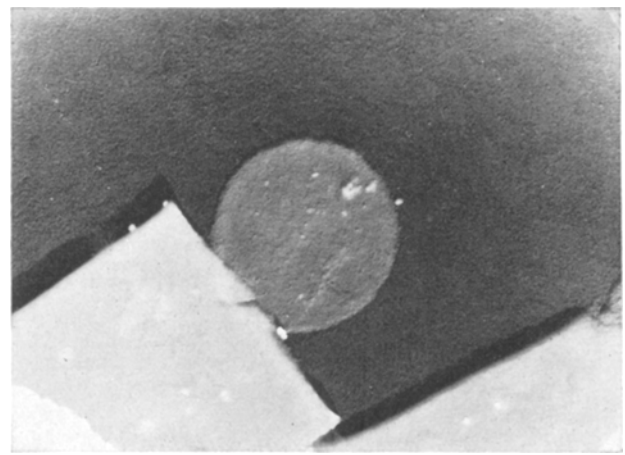

Fig. 2. Electron micrograph of a septum of *Geotrichum lactis* partially separated from the cell wall of the hyphae (× 24,000).

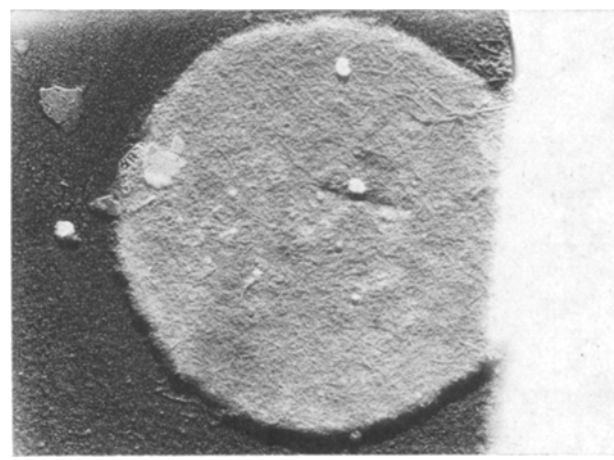

Fig. 3. Higher magnification of a septum of *Geotrichum lactis* showing microfibrils embedded within an amorphous matrix (× 60,000).

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were seen. It was expected that after alkali treatment pores, if present, would be more evident.

SHATKIN and TATUM[7] suggested that septum formation in *Neurospora* occurs by a process of cell wall invagination and fusion. Whether this is the case in *G. lactis* is unknown. However, it is clear that the discs corresponding to septa are readily released from the walls of the pseudomycelium. In the electron microscope, a great number of these discs, more or less free of cell walls, can be seen. The liberation of these septa is probably due to partial chemical digestion of the junction between septum and wall.

These septa correspond to fraction 1 of KESSLER and NICKERSON[2], that is the cell wall after digestion with alkali. Theoretically these should contain only the glucan-protein component, but we have also found mannose in this fraction.

Resumen. Las pareces celulares aisladas de *G. lactis*, después de tratadas con éter y KOH 1 *N*, muestran al examinarles en el electrónico, los tabiques transversales, circulares, de estructura fibrilar no orientada y sin poros, separados del resto de la pared. Su composición es glucano-proteina y manosa.

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Preliminary Studies of the Metabolism of 2-Guanidinomethyl-1:4-benzodioxan Sulphate (Guanoxan)

Guanoxan is a powerful antihypertensive agent in man, and in dogs produces a considerable adrenergic neurone blockade, antagonizing the effects of epinephrine and norepinephrine at α-receptor sites, depleting the norepinephrine content of the heart, spleen and hypothalamus and adrenal glands[1–5].

The metabolism of 2-alkylaminomethyl-1:4-benzodioxan has been studied in man and in animals in detail by MCMAHON et al.[6–8]. Oxidative degradation of the alkylaminomethyl chain and hydroxylation of the aromatic nucleus were the main metabolic pathways found. Although guanidines which exist normally in biological systems undergo many transformations, there is very little evidence, if any, to show that other non-physiological guanidines are metabolized to a great extent. Thus, when taurocyamine[9] is given to dogs, 80% of it is excreted unchanged; sulphaguanidine[10] is acetylated in the amino group of the aromatic ring and streptomycin and dihydrostreptomycin[11] are excreted largely unchanged.

Preliminary recovery experiments with non-radioactive guanoxan showed that it could be extracted with *n*-butanol from salt-saturated urine and assayed colorimetrically by means of the Voges-Proskauer diacetyl reagent for guanidines[12]. When a single dose of guanoxan (88 mg) was administered orally to a beagle dog, the total excretion of the unchanged drug during the 4 days consecutive to dosage was found by the colorimetric assay to be 21–22%.

Guanoxan-(guanidino-^{14}C) was prepared from 2-aminomethyl-1:4-benzodioxan by reaction with S-methyl thiouronium sulphate-(uronium-^{14}C). A Packard Tricarb

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