chlordene epoxide or HCE *in vivo* in the pig, but if the *in vitro* processes observed in pig liver microsomes reflect events *in vivo*, then for each racemic epoxide this animal possesses a hydrolytic detoxication mechanism for one enantiomer and an oxidative detoxication mechanism for the other. The housefly, on the other hand, appears to detoxify these compounds chiefly by oxidative mechanisms which are qualitatively similar for both species and are susceptible to inhibition by insecticide synergists of the 1,3-benzodioxole type (inhibitors of microsomal oxidation); the toxic action of the epoxides in the housefly is consequently synergized in combination with certain of these inhibitors[5,6]. The pig, however, might be afforded some protection against synergist combinations involving the racemic epoxides by the apparent ability of its liver microsomes to detoxify half the toxic material by a hydrolytic process unaffected by synergists of this type. Furthermore, synergist combinations with those enantiomers, not at present obtainable, that are rapidly hydrolysed by pig liver microsomes should be toxic to the housefly and innocuous to the pig.

These observations illustrate the way in which comparative metabolic studies with different species can indicate possible pathways to selective toxicity.

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Chitin in the New Wall of Regenerating Protoplasts of *Candida utilis*

Although many results have been published in the past decade on the formation and physiology of yeast protoplasts, little information has been presented so far concerning the *de novo* synthesis of cell walls by these protoplasts[1,2] in defined conditions. Knowledge of the components of new cell walls which appear during the reversion of yeast protoplasts constitutes the first stage in the study of cell wall biosynthesis.

Candida utilis CECT 1061 cells were grown in Winge medium consisting of 2 per cent glucose (w/v) and 0·3 per cent yeast extract (w/v). Protoplasts have been obtained by helicase attack of cells in the exponential phase of growth as described elsewhere[3]. Preliminary experiments have shown that protoplasts prepared by treatment with "strepzyme" and helicase behave similarly in immunological tests[3] and in their regenerating properties.

The protoplasts were incubated at 30° C in the growth medium to which was added 0·55 M $MgSO_4$. Samples were taken at intervals and their development was followed in a phase contrast microscope. Light and electron micrographs of this phenomenon have recently been published by Uruburu *et al.*[4], and these show that all the initially spherical protoplasts changed to tubular forms (after about 18 h), from which new yeast cells later originated.

Normal and regenerated cell walls were freed from cytoplasm by disruption with glass beads in a Braun disintegrator, followed by differential centrifugations. Parallel studies on the chemical composition of both wall preparations were carried out and colorimetric determinations of protein[5], carbohydrate[6] and hexosamine[7] were performed. Characterization and evaluation of sugars were performed by chromatographic techniques after hydrolysis in determined conditions. The results are shown in Table 1.

Table 1. CHEMICAL COMPOSITION OF CELL WALLS OF *Candida utilis*

	Normal cell (per cent)	18 h regenerated cell (per cent)
Total carbohydrate (anthrone)	78	50
Glucose	49	49
Mannose	28	0·5–1
Total hexosamine[7]	1	20
Total *N*-acetylglucosamine (chitinase)	0·5–1	22–27
Protein[5]	9·5	15
Free and bound lipids (gravimetrically)	1	0·5

The considerable content of glucosamine in regenerated walls suggests the presence of chitin. To verify this hypothesis these walls were incubated in the presence of commercial fungal chitinase (Koch Light) and the products liberated by the action of the enzyme were studied by chromatography. A high content of *N*-acetylglucosamine and chitobiose, characteristic components of chitin, were detected with a diphenylamine reagent. This last result is in agreement with the solubility in acid and alkali of regenerated cell walls.

The fact that the amount of chitin (22–27 per cent) in regenerated walls is similar to that of mannan in normal cells suggests that this glucosamine polymer substitutes for the mannose complex when, in certain conditions, a new wall is synthesized by protoplasts. Necas and Svoboda[8] have reported that the synthesis of mannan in the regenerating wall needs very particular physical conditions in the surrounding medium. A very small amount of chitin occurs in normal yeast cell walls, which means that the mechanism of synthesis of this component is always present in the yeast. We have no evidence, however, that during the preparation of protoplasts the mechanism of mannan synthesis can be damaged, nor that the protoplast builds this wall with a more resistant complex, that is, with chitin, as a protection in an adverse environment.

In view of the differences in composition between normal and regenerated cell walls, another question which arises is whether the mannan is a typical component of the ellipsoidal form of the yeast, and chitin of the tubular form. Further studies on the chitin content after different periods of regeneration should throw light on whether this chitin constitutes a component intermediate, or a definite structure in the "new" yeast as a result of some derangement of the membrane-bound cell wall synthesizing machinery. Work is now in progress on the structure of these regenerated walls.

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