mannose or galactose, susceptibility of the culture to ethidium bromide mutagenesis was influenced by the degree of respiratory repression imposed by the sugar in question. Thus the more repressive the sugar, the greater the rate of mutation. We have now tested the effect of alternative, non-utilizable catabolite-repressing sugars, such as mannitol, sorbose and xylose, used at high concentrations, but insufficient to cause growth lag or a considerable decrease in growth rate. Cells were previously grown in 0.5% glucose-containing medium, to minimize catabolite repression, and transferred to ethidium bromide-containing media of 0.5% glucose plus a non-utilizable sugar (Fig. 2b). The results indicate that such substances, to a lesser or greater degree, interfere with ethidium bromide-induced petite mutation; xylose (3%, w/v) was particularly effective although less so than glucose itself (total concentration 10%, w/v). It seems likely that this behaviour is associated with the degree of catabolite repression imposed. Hence it is concluded that one component at least, of the protection of the p-factor afforded by glucose against mutagenesis, is explicable in terms of repression of respiratory activity and that other non-utilizable catabolite repressors can mimic the effect of glucose in this regard. Further, it is interesting to consider that, although pre-conditioning of a culture to 5% (w/v) glucose raises susceptibility to mutagenesis in repressing regimes (Hammond et al., 1974), the converse is true for cells pre-conditioned with 0.5% glucose. Finally, whereas the degree of glucose repression of cells before drug treatment had some effect as far as susceptibility to ethidium bromide petite mutagenesis was concerned (Fig. 1a), the presence or absence of the unmetabolizable sugar in the pre-growth medium was of no consequence in the time-course of mutagenesis.

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Autorepression of Ethidium Bromide Mutagenesis in Kluyveromyces lactis, a petite-Negative Yeast

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The petite mutation was first described by Ephrussi et al. (1949a,b), who was studying the effects of acriflavine on the yeast Saccharomyces cerevisiae. The mutation, which is cytoplasmically inherited, gives rise to colonies of decreased size and is characterized by a respiratory deficiency. Slonimski et al. (1968) first used ethidium bromide as a petite mutagen. Bulder (1963) found that not all yeasts are capable of producing petite mutants and referred to those that were incapable as petite-negative yeasts. Bulder argued that the mutation occurs, but it is lethal. In an attempt to establish this, he demonstrated the existence of microcolonies, formed by abortive petite mutants. Luha (1972) has described the use of low concentrations of ethidium bromide to produce microcolonies in Kluyveromyces lactis.

Figs. 1(a) and 1(b) show the growth curves for K. lactis grown in four different
Fig. 1. Growth kinetics of *K. lactis* grown in ethidium bromide

Growth conditions were as described by Luha (1972). Cultures were sampled for haemacytometer counting, and plating in duplicate on 2% (w/v) glucose agar at the times shown. Plates were incubated for 3 days at 30°C. (a) Growth curves of cultures; (b) Change in viability of cultures; (c) Induction of petite cells (cells giving rise to microcolonies). Ethidium bromide concentrations (μM): ○, 62.5; △, 125; ▲, 187.5; ■, 250; ▽, 375; ○, 500.
concentrations of ethidium bromide over 24h. Fig. 1(a) shows total cell count, Fig. 1(b) shows the percentage of viable cells and Fig. 1(c) shows a much shorter time-course using two higher concentrations as well. It is apparent that during the short time-course microcolony cells (cells giving rise to microcolonies on plating) form quickly, but that there is a recovery and these revert to wild-type cells, provided sufficiently high concentrations of ethidium bromide are used. There then follows an irreversible petite mutation. A similar recovery has been observed with the petite-positive yeast *Saccharomyces cerevisiae* (Hall et al., 1976; Criddle et al., 1976) except that higher concentrations of ethidium bromide were required to bring about recovery in *K. lactis* than for *S. cerevisiae*. After 24h treatment with ethidium bromide.
it was seen that, the higher the concentration of the drug used, the greater the proportion of viable cells in the culture.

Fig. 2 shows that when growth is prevented, by using a variety of methods, there is a decrease in the mutagenicity of ethidium bromide, and the increase in viability with increased ethidium bromide concentration [as seen in Fig. 1(b)] is lost. (When cultures are not shaken, there is decreased aeration of the growth medium, which inhibits the growth of *K. lactis*, an obligate aerobe.)

Fig. 1(a) shows that increasing the concentration of ethidium bromide increases the mean generation time of *K. lactis*. Micro-colony cells are shown to be capable of at least limited division after mutagenesis, because the total cell count increases in an exponential manner throughout the experiment. If micro-colony cells were completely inviable, the rate of increase of total cell count would fall off, and microcolonies would not be produced.

Our results suggest that, in contrast with the situation in *Saccharomyces cerevisiae* where ethidium bromide induces petite mutants in non-dividing cells, cell division is essential for mutation in *K. lactis*. The faster the rate of division, the greater will be the percentage of mutants formed in a given time (assuming the mutation rate is similar in each case). Hence we can explain the paradoxical increase in viability with increased mutagen concentrations. This is supported by the data shown in Fig. 2 where growth was prevented by various means and where there is little or no increase in viability with increasing ethidium bromide concentrations.

This viability effect is quite different, however, from the recovery from the effects of high concentrations of ethidium bromide shown in Fig. 1(b). This has been explained (Cridde et al., 1976; Hall et al., 1976) as fragmentation of mitochondrial DNA followed by stimulation of a repair system. This effect seems to be occurring in *K. lactis* also, but only when extremely high drug concentration is used.

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**A Cadmium-Binding Glycoprotein from the Liver of the Plaice** *(Pleuronectes platessa)*

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Concern for heavy-metal pollution has stimulated great interest in the structure and function of horse kidney metallothionein, a cadmium-binding protein of low molecular weight containing 6% Cd and 2% Zn and >30% cysteine residues, with a thiol group: Cd ratio of 3:1, and no aromatic residues. Binding of Cd to thiol groups results in the appearance of a characteristic 250nm absorption (Kagi & Vallee, 1961). Similar proteins have been found in rat, rabbit and man (Bremner, 1974). Preliminary studies on Cd metabolism in fish have suggested a similar protein is produced when plaice are exposed to Cd in seawater (Coombs, 1974). The isolation and characterization of this piscine metallothionein is the subject of this communication.