copied on transparent graph paper. The size of the bud was obtained by cutting and weighing the paper images on an analytical balance. Cell size is expressed in arbitrary units related to the weight of the paper image.

Initial experiments showed that treatment of the yeast with isoamyl alcohol in standard buffer caused a rapid loss of β-D-galactosidase activity. High concentrations of Mn^{2+} [(1 to 5) × $10^{3}M$], which were about 100 times higher than those used for the measurement of enzyme activity in extracts (*5*), stabilized the activity for at least 3 hours. However, serum albumin (3 mg/ml) and $5 \times 10^{-4}M$ dithiothreitol were required in addition to $10^{-3}M$ Mn^{2+} for enzyme stabilization in single cells.

Because of the high β-D-galactosidase activity in single yeast cells and the lapse of 3 to 5 minutes between mixing with substrate and the first measurement of enzyme activity, it was imperative to reduce the rate of the enzyme reaction and to shorten the time required for the assay. We found that addition of glycerol reduced the rate of β-D-galactosidase reaction; 5 percent glycerol gave a rate suitable for the time schedule of the assay. At this glycerol concentration the enzyme reaction was linear with time for at least 5 minutes (Fig. 1A). Under these conditions the assay procedure was shortened by consecutive assay of single cells in drops on the same slide. In each case, the rate of formation of fluorescent product in these cells could be extrapolated to zero time of the reaction.

Figure 1B shows the effect of *p*H on β-D-galactosidase activity in single cells. In the presence of 5 percent glycerol no activity was observed at *p*H 7.2, whereas there was substantial activity in its absence. This activity increased as the *p*H was raised to 7.6 and reached a plateau in the range of 7.6 to 8.0.

To verify that the permeability barrier of most cells was broken by the isoamyl alcohol treatment, individual cells from a treated culture were allowed to accumulate fluorescein by a process known as fluorochromasia (*6*). Cell suspensions previously incubated with fluorescein diacetate (Nortok Associates) were examined under a fluorescence microscope. No cells with intact membranes were found after treatment of about 10^{7} cells with isoamyl alcohol. A similar test with lyophilized yeast cells revealed less than ten fluorescent cells per 10^{7} cells in each slide.

To follow the pattern of β-D-galactosidase synthesis during the cell cycle, a random population of yeast cells was dispersed into the microchamber, examined for enzymatic activity, and photographed. Droplets of similar size (about 100 μm diameter) were selected for the assay; relatively small variations in droplet size did not affect our results since the rate of the increase of fluoresence of the whole droplet was measured. Cells without buds and cells with intermediate-size buds had similar levels of enzyme (Fig. 2). In contrast, cells with full-size or nearly full-size buds contained about twice this level of enzyme. No intermediate levels of enzyme were found. The doubling of the level of enzyme is periodic and occurs near the end of the cell cycle period. These results, which are in agreement with those obtained previously by the use of synchronous cultures (*7*, *8*), show that it is possible to study directly the changes of enzyme level in yeast on individual cells taken from a random population. This procedure also avoids artifacts resulting from induced synchrony.

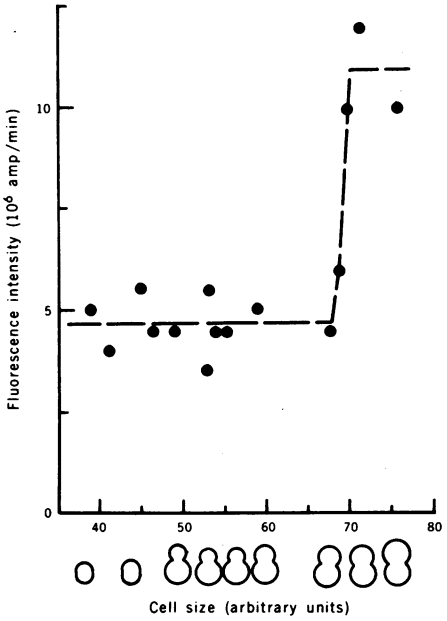

Fig. 2. Levels of β-D-galactosidase activity in single *S. lactis* cells during the cell cycle. The shape of the cells is drawn diagrammatically. The arbitrary units are described in the text.

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Duffy Blood Group and Malaria

Implications from the study of Miller *et al.* (*1*) that Fy(a-b-) erythrocytes are resistant to *Plasmodium vivax* raises some questions concerning selection of the *FyFy* genotype in zones endemic for malaria.

In testing Saudi Arabs for the sickle cell trait and Duffy antigens, we found 31 out of 37 sickle cell trait carriers (AS) to be *FyFy*. Of 106 normal homozygotes (AA), 46 were *FyFy*. The significant excess of *FyFy* among sickle trait carriers ($P < .01$) could be due to continuous African gene flow into Saudi Arabia, assortative mating (*AS FyFy* × *AA FyFy*), linkage between the hemoglobin beta-chain and Fy structural loci, or additive resistance against *P. falciparum* and *P. vivax* malaria in the presence of hemoglobin S and Fy(a-b-).

P. falciparum apparently maintains the AA,AS polymorphism in endemic areas; *P. falciparum* malaria was holoendemic, and *P. vivax* mesoendemic in the oases of eastern Saudi Arabia until 1948 (*2*), and sickle trait carriers average 25 percent in these oases. Accordingly, we favor the proposal that at least among Saudi Arabs, the *AS FyFy* genotype may have been selected by concurrence of endemic *falciparum* and *vivax* malaria, and that this genotype provides a greater survival advantage than either AS or FyFy alone in mixed *falciparum-vivax* infection. Alternatively, we propose that Fy(a-b-) erythrocytes may have partial resistance to *P. falciparum*, comparable to that conferred by hemoglobin S.

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