DIVISION SYNCHRONIZATION IN A RESPIRATORY DEFICIENT YEAST[1]

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Synchronization of cell division in a variety of microorganisms has been achieved by temperature shifts or by starvation. Two reports exist which mention synchronization of the budding process in yeast by starvation (Beam *et al.*, 1954; Ogur *et al.*, 1953). In both cases, the demonstration of synchronization was incidental to the primary purpose of the experiments performed, and was therefore not quite so complete as one might like.

In the present study, starvation was for glucose. Starvation for glucose alone on a complete medium was achieved by the use of a respiratory deficient strain which will grow on yeast extract and peptone only with the addition of a fermentable substrate. It was felt that, just as periodic temperature shifts have been useful in some synchronization procedures (Lark and Maaloe, 1954; Zeuthen and Scherbaum, 1954), that periodic starvation might likewise enhance the degree of synchronization. It will be seen that this expectation was not confirmed.

MATERIALS AND METHODS

The yeast strain 19.1, a haploid segregant from the Winge strain 55 (*Saccharomyces chevalieri* × *S. cerevisiae* hybrid) has been described previously (Campbell and Spiegelman, 1956.) The yeast extract peptone medium was made by adding the following to 1 L of water: $(NH_4)_2SO_4$, 6 g; KH_2PO_4, 2 g; $CaCl_2 \cdot 2H_2O$, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; 60 per cent sodium lactate, 6 ml; agar (where added), 20 g; carbohydrate as specified. Synthetic medium was made according to the formula of Burkholder (1943).

Direct microscopic counts of cell suspensions were generally made in a Spencer bright line counting chamber and occasionally in a Petroff Hauser chamber. Cells were classified by visual inspection into the following categories: (1) single, nonbudding cells; (1B) single, budding cells; (2U) unequal pairs of cells; (2E) equal pairs of cells; (3), (4), (5), etc. The borderlines of these categories are arbitrary, and are shown diagrammatically in figure 1. When one or more cells in a pair or larger group was budding, no separate category was used. In subsequent counts, we considered a structure such as 2U or 2E to comprise two cells, but one group of cells.

Viable counts were made by plating of suitably diluted aliquots on complete medium containing 2 per cent glucose. The diluted aliquot was suspended in about 2.5 ml of 0.7 per cent agar which was then poured onto the surface of the plate and allowed to solidify. Plates were incubated at 29 C for 2 days.

Unless otherwise specified, cultures were grown in 250 ml flasks containing from 10 to 40 ml liquid on a horizontal shaker (240 oscillations per min) at 29 C. For certain studies the cells were obtained from a chemostat which had been modified so as to dilute the culture periodically by a factor 2 rather than to add liquid continually dropwise. This was accomplished by inserting an intermittent siphon into a Rotman chemostat (Rotman, 1955), between the growth tube and the feed supply. The volume of the siphon was sufficient to effect, upon discharge, a dilution of the growing culture by a factor 2.

pH Adjustments, when necessary, were accomplished by the addition of a suitable amount of 4 N HCl or 1 N NaOH. They were checked with a Beckmann pH meter.

RESULTS

Effects of glucose concentration on growth of strain 19.1. Figure 2 shows the effect of glucose concentration on growth of strain 19.1 on complete medium. Within the range 0.05 per cent to 1 per cent the maximum cell yield varied linearly with the input glucose concentration.[2]

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[2] The K_S value for glucose fermentation by this strain is about 0.02 per cent.

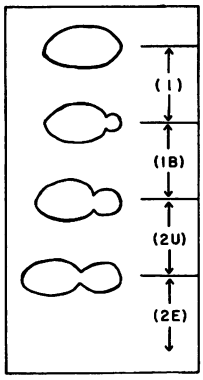

Figure 1. Diagrammatic representation of stages in the normal division cycle of Saccharomyces. (1) Single cell. (1B) Budding cell. (2U) Unequal pair. (2E) Equal pair.

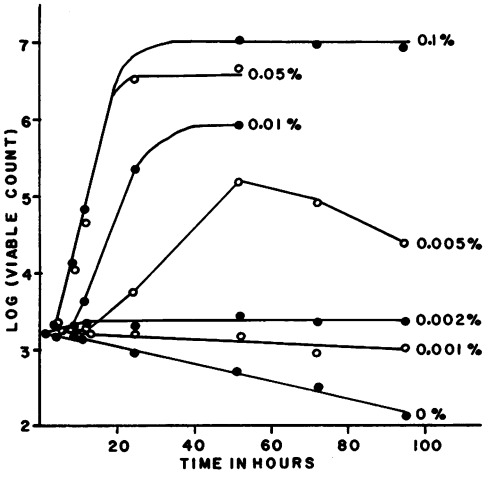

Figure 2. Growth of strain 19.1 at subsaturation levels of glucose. Curves are labeled with the concentration of glucose used. Inoculum from stationary phase culture in 2 per cent glucose broth.

At subsaturation levels of glucose the yield per g of sugar was less and the lag phase was longer; with less than 0.002 per cent glucose there was no increase in viable count even over a period of several days. It is known, furthermore, that the cells are able to grow to full yield in a chemostat (glucose limiting) at immeasurably low working concentrations of glucose (Campbell, 1953).

These facts suggest that the medium as prepared is not suitable for optimal growth; but that its composition is so modified during growth that it becomes more suitable. Such situations are not uncommon (Hinshelwood, 1946). The

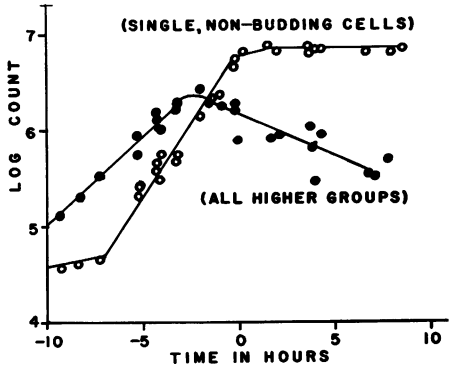

Figure 3. Change in composition of population undergoing starvation for glucose.

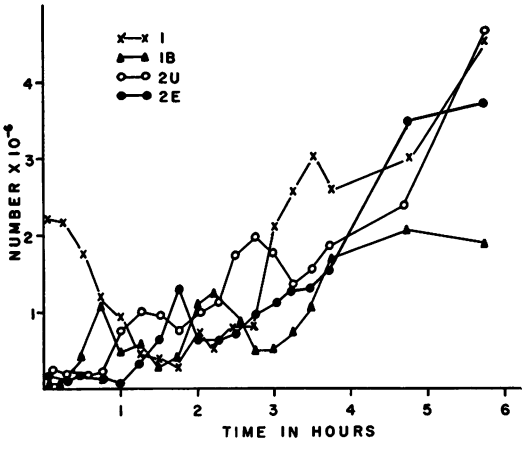

Figure 4. Changes in composition of population upon dilution into 1 per cent glucose broth after starvation. Symbols as in figure 1.

unique feature of the present data is that the medium is unsuitable only for a cell with a low rate of energy supply.

Starvation for glucose. The best synchronization was obtained with starvation for 0.1 per cent glucose. The pattern of growth as a population approached such starvation is shown in figure 3. The last major change was the separation of the pairs of cells (which comprised the majority component of a log phase population) into single cells. To make data from different experiments comparable, the zero of the time scale has always been placed at the point where the ratio equalled 1.2. The value of this ratio varied from 1.7 in the logarithmic phase to 1.1 in the stationary phase. It is not plotted in figure 3, but is shown for a similar situation in figure 5.

As the stationary phase approached, budding

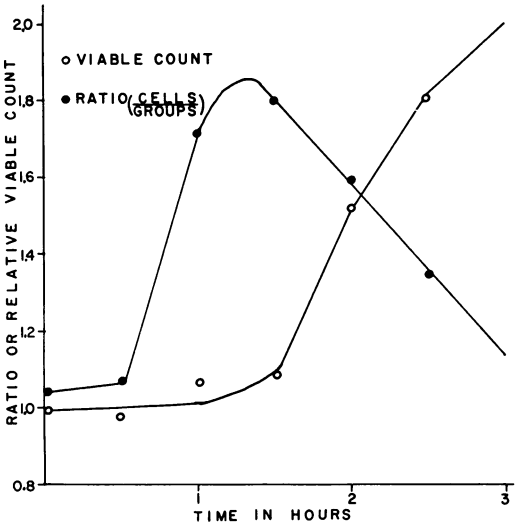

Figure 5. Changes in composition and in viable count during a single starvation cycle.

ceased, but cells which had already budded continued their development into cell pairs and then separated. The result was a culture consisting predominantly of single nonbudding cells.

Synchronization by starvation and renewal. The result of adding an aliquot from such a starved population to 2 per cent glucose broth is shown in figure 4. Such curves were run with cultures which had starved for various lengths of time. As the time of starvation was increased, the time to reach maximum budding increased. This was expected; old stationary phase cells show a longer lag than do young stationary phase cells. The percentage of budding cells at the maximum, however, did not vary significantly within the time interval studied (10 hr from time 0 of figure 3).

Two distinct maxima are visible in the curves for budding cells and for pairs (figure 4). The

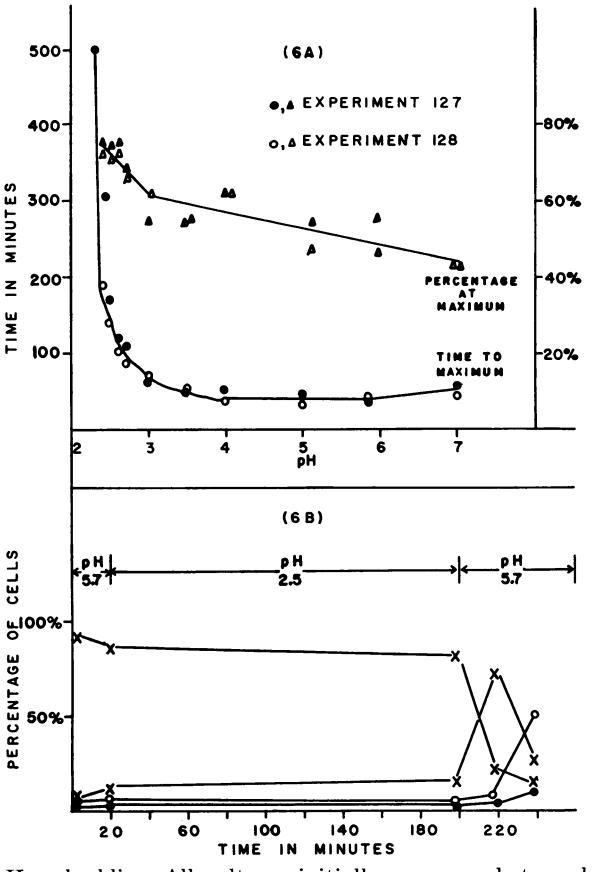

Figure 6. Effects of pH on budding. All cultures initially grown and starved for glucose at pH 5.7. Then diluted into fresh glucose broth at various pH values. 6*B* shows the effect of a sudden shift from sublethal low pH back to 5.7.

TABLE 1

Effect of temperature on budding

Temperature	Time to Obtain Maximum Budding	Budding Cells at Maximum
C	*min*	%
30	53	43
25	65	51
20	111	51
15	188	56
10	352	51
4	*	

All cultures originally grown and starved for glucose at 30 C. Then diluted into glucose broth at various temperatures.

* No budding in 48 hr.

distance between the maxima is equal, within experimental error, to the normal division time on this medium.

There was thus clearly some synchronization, but it was not good enough to serve as more than a curiosity. It seemed therefore more practical to use the method described above of periodically diluting the culture by a factor 2 with fresh medium. The behavior of the culture during such a cycle is shown in figure 5. The maximum rate of increase of viable count was more rapid than the logarithmic growth rate characteristic of the medium. The viable count corresponded closely to the total number of groups of cells seen under the microscope, which implies that the observed pairs of cells generally were not separated during the process of diluting and plating.

As mentioned above, one might expect such a periodic process to increase the degree of synchronization because the cells should arrive at the starved condition already synchronized from the previous starvation. To test this point, populations of freshly starved cells (within 1 hr of time 0) were divided into two portions. Portion (a) was inoculated into 1 per cent glucose broth. Portion (b) was put through three starvation cycles on 0.1 per cent glucose broth and then inoculated into 1 per cent glucose broth. The percentages of budding cells at the maximum point were 40.1 $\pm$ 5.4 and 44.4 $\pm$ 7.6 ($2\sigma_m$), respectively. The difference is not significant, and therefore no such cumulative synchronizing effect has been demonstrated.

Many attempts were made to improve the degree of synchronization within such cycles. The period, the input glucose concentration, and the

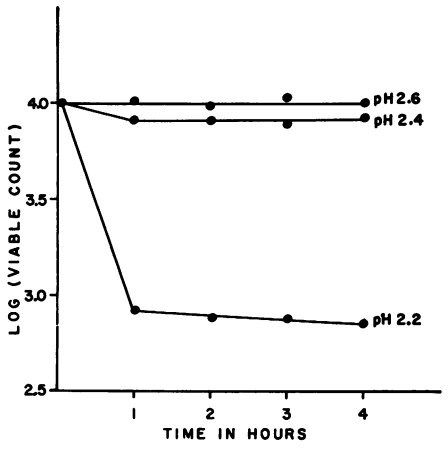

Figure 7. Viability of cells placed at low pH values. Conditions as in figure 6.

temperature were all varied, but no significant improvement was observed. Accordingly, a survey was undertaken of the effects on the budding process of physical and chemical agents, using fully starved cells as the starting material.

Effect of chemical and physical factors. If the starved cells were washed and placed in the synthetic medium of Burkholder (1943), the budding was much poorer than with the complete medium. The combined data with synthetic medium and with complete medium which had been deionized with Dowex 50 cation exchange resin revealed one definite nutritional requirement for the budding process. Little if any budding occurred in the absence of external Mg^{++}. 10^{-3} M Co^{++} and 10^{-3} M Mn^{++} inhibited budding, and 10^{-2} M Mg^{++} reversed the inhibition.

The effect of temperature is shown in table 1, and that of pH in figure 6*A*. Data for pH values above 7 are not given, because at these pH values the cells tended to clump.

Figure 7 demonstrates that pH 2.3 or 2.4 represented the limiting pH for this strain on this medium, below which death was rapid. At pH values in the range 2.4 to 2.7, it took much longer for the cells to bud, and one might have as many as 80 per cent of the cells budding at one time.[3] The budding cells developed into

[3] In comparing these data with others in the literature, it must be remembered that here we counted as "buds" only those structures which were between the limits indicated in figure 1. There has not been any standard practice in this regard.

cell pairs, but these seldom separated into single cells. Instead, as they continued to grow, they formed chains of cells.

If one took a culture at pH 2.4 at or before the point of maximum budding and suddenly switched the pH to 5.7, the cells soon began to complete the division process to the equal pair stage as shown in figure 6*B*. Thus, part of the division cycle occurred uniformly among the population.

DISCUSSION

The failure of the periodic process significantly to improve the synchronization merits further comment. In the two published cases of synchronization by periodic temperature shifts, some synchronization is obtainable by single shifts (Lark and Maaloe, 1954), or by multiple shifts prior to cell division (Zeuthen and Scherbaum, 1954). The latter case involves the use of sublethal temperatures and is probably not so relevant as the former. In the former, there is a gradual improvement in the degree of synchronization in a series of cycles starting with an unsynchronized logarithmic phase culture.

This result might be taken as showing that the periodic nature of the treatment is instrumental in synchronizing the culture. However, one is really not able to say this with confidence until one has screened a wide variety of nonperiodic treatments and compared them with it.

For example, we can obtain an analogous improvement in our system under certain conditions. If the inoculum is sufficiently old so that the lag is appreciably lengthened, or sufficiently young that it is not completely starved, the first cycle is abnormal. Then it may take two or three cycles before the ultimate cycle characteristic of this periodic treatment is closely approached.

The theory of growth in simple continuous devices such as the chemostat has been well developed (Herbert *et al.*, 1956; Novick, 1955). It can be extended with slight modifications to a periodic dilution process such as we have used here. If the flow rate is above a critical value, growth will be unable to keep pace with dilution, and the culture will be washed out of the apparatus. If the flow rate is below this value, in an ordinary chemostat a constant population density and rate of growth are approached, which depend on the composition of the entering medium and on the flow rate, but not on the initial population density. In our apparatus, the population density and rate of growth do not approach constancy. Instead, an ultimate cycle is approached, whose characteristics are again independent of initial conditions.

Synchronization in such periodic environments has one great advantage and one great disadvantage when compared with synchronization by prior treatments. The advantage is that the ultimate growth cycle must be balanced; i. e., over one cycle, every extensive property of the cell population must double. The average doubling cycle of cells growing unrestrictedly must likewise be balanced, but with synchronization by prior treatments this is frequently not the case. For example, the average cell volume may decrease during the synchronized divisions (Scherbaum, 1956), or two periodic processes may become phased with different periods (Hotchkiss, 1954).

The disadvantage is well illustrated in another paper by the present author (Campbell, 1957). If one wishes to study the correlation of some phenomenon with cell division, one looks for a periodic variation in it occurring at the same time as cell division. Now the fact that the cycles are balanced implies that every periodic change in populational properties is compelled to assume the period imposed by the investigator. Thus a correlation may be fortuitous.

SUMMARY

The effect on a respiratory deficient yeast of starvation for and subsequent renewal of glucose is described. By repeated starvation for glucose one can obtain a balanced growth cycle within which cell division is somewhat synchronized. The effects of Mg^{++}, temperature, and pH were studied. At low, near-lethal pH values, the time required for budding is much prolonged, and as many as 80 per cent of the total cells present can be obtained as single cells with small buds.

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