COLCHICINE STIMULATION OF YEAST GROWTH FAILS TO REVEAL MITOSIS

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Received for publication February 28, 1938

The formation of the yeast bud has generally been believed to be a partitioning of the materials between the mother cell and the bud, rather than involving a mitotic division of the nuclear material. Three investigators have presented evidence for a mitotic division, but the evidence has not been entirely convincing. Colchicine delays mitotic division at the metaphase and has been helpful in the study of cell division in both animal and plant cells. A proper concentration of the drug should delay any mitotic division of yeast, change the rate of growth and permit the observation of division figures. Instead, colchicine stimulates yeast growth and fails to show mitosis.

I

The yeast used is a pure strain of *Saccharomyces cerevisiae* Hansen from a single cell isolated by the writer in 1928 (A. T. C. No. 4360) and was grown at 28°C. in 10 ml. Williams' original medium consisting of sugar 20 grams, $(NH_4)_2SO_4$ 3 grams, KH_2PO_4 2 grams, asparagin 1.5 grams, $CaCl_2$ and $MgSO_4$ each 0.25 gram aq. dest. 1000 ml. The medium was tubed, sterilized and inoculated as described by Richards (1932). Mallinckrodt's U. S. P. colchicine was used in the experiment in preference to Merck's colchicine as the latter was not chloroform free. Two check experiments with the Merck drug gave very nearly the same results so that with yeast either might have been used and without significant differences.

[1] Experiments series 162 and 163 were grown at New Haven, 164 at Woods Hole and 155 and 156 at Buffalo.

The growth of the yeast was measured by counting the number of cells present in 1/250th cu. mm. with an hemocytometer; by means of a photoelectric nephelometer (Richards and Jahn, 1933), improved by using a General Electric Blocking Layer Photoelectric Cell and a Weston Model 600 microammeter with knife edge pointer and mirror; and by centrifuging the cells into calibrated, capillary bottom, centrifuge tubes. More than one criterion of growth is necessary for the analysis of the conditions determining growth (Richards, 1934) and the interrelations of the several criteria will be discussed elsewhere. The metal surface of the Bright Line Hemocytometer permits more rapid filling of the cell by capillarity, thereby lessening the error due to unequal distribution of the yeast. The growth measured by the nephelometer is a function of the number of cells present and their size distribution and is expressed as

$$E = \log I - \log I_0$$

when I is the density of the suspension at the time of measurement and I_0 the density of the suspension at the time of seeding the population.

II

Concentrations of colchicine weighed out and added in the proportion of one to one million parts of medium gave very little less growth than the control populations and concentrations greater than ten times this amount stimulated the yeast growth progressively until a concentration of 1 per cent was reached. Four and one-half per cent concentration, which is near the solubility limit of the drug, gave less stimulation (fig. 1, *A*). In terms of percentage of the control the amounts of stimulation of cell number and nephelometric density were about the same and the agreement of the several determinations is shown by the figure. The average size of the yeast cells grown in the medium and in the medium plus 1 per cent colchicine and the distribution of the sizes of the cells showed no significant differences; as determined by photographing the yeast, projecting the images of the photographs, tracing them and measuring the area of the

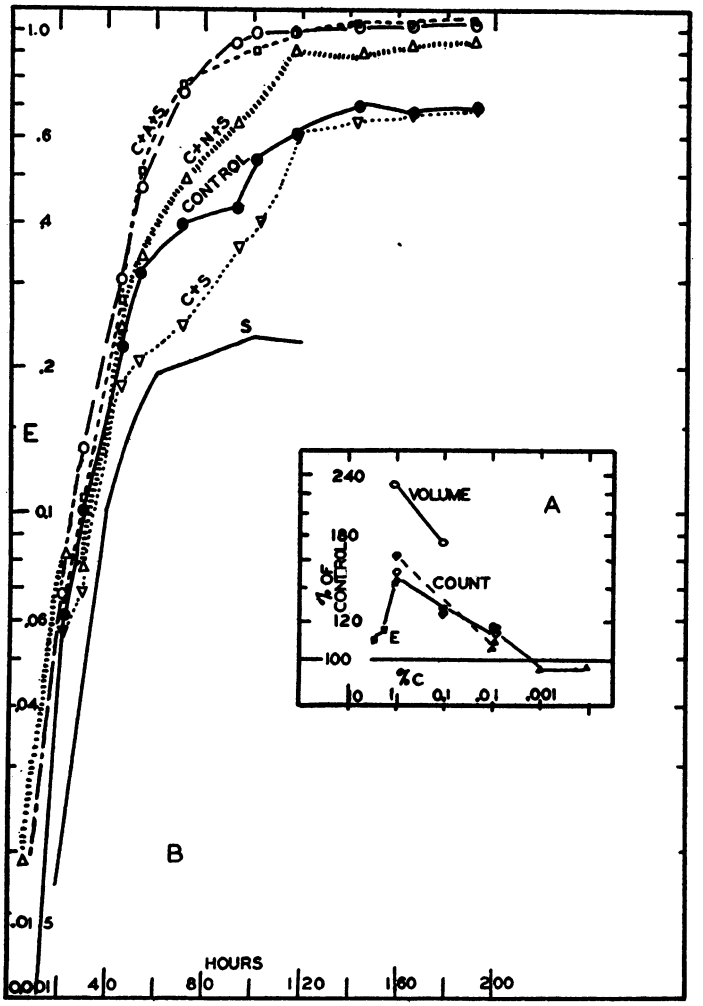

FIG. 1. *A*, THE INCREASED YIELD OF YEAST WITH ADDED COLCHICINE. *B*, THE GROWTH OF YEAST POPULATIONS IN DIFFERENT COMBINATIONS OF NUTRIENTS (*cf.* TEXT)

Control in Williams' medium. *C* = colchicine. *S* = sugar. *A* = asparagin. *N* = $(NH_4)_2SO_4$.

enlarged photographs with a planimeter. Consequently, the proportionally greater volume must be due to inefficient packing of the cells into the capillary tubes by the centrifuge.

The increased yield of yeast, while marked, is not of the order of magnitude to be expected from the presence of a bios, because far greater concentrations of the drug are required for a comparable stimulation. To determine the nature of the stimulation, series 164 was analysed to learn the changes in the sugar, alcohol, and hydrogen ion concentrations at frequent intervals during the growth cycle. Previous experience had shown that the sugar concentration is a measure of the food in the medium used by the cells and that the unfavorableness of the medium from the accumulation of waste products was proportional to the fermentation which could be measured in terms of the alcohol concentration (Richards, 1932).

The growth of the cells in the medium without the drug produced two cycles (fig. 2), which are characteristic for this yeast in this medium. The growth curve of the yeast grown in 1 per cent colchicine in addition to the medium grew directly to a maximum crop in a single cycle of growth. The concentration of sugar in the medium is about the same during the first 60 hours and then decreases more rapidly than in the control cultures. The hydrogen ion concentration is diminished when the drug is added, but that of the colchicine cultures then increases faster than in the control cultures. The alcohol concentration in the colchicine cultures increases to a maximum some six times greater than that of the control cultures and does not show a period of equilibrium associated with the end of the first cycle of growth such as is observed in the alcohol concentration of the control series.

The equilibrium marking the end of the first growth cycle of the control cultures results from a selective killing of the larger buds by the toxic waste products in the medium and a decrease in the available food supply. The addition of food just before the end of the first cycle prevents the retarded growth and the population continues directly to a maximum crop limited by the medium becoming too unfavorable for further increase (Richards, 1934).

As only one cycle occurs when the colchicine is present, the addition of the drug must act either as a food or by making the

medium less unfavorable even though the alcohol and other products of fermentation are proportionally greater. To evaluate the extent of these two possibilities an experimental series was grown in the complete medium, the medium with 1 per cent

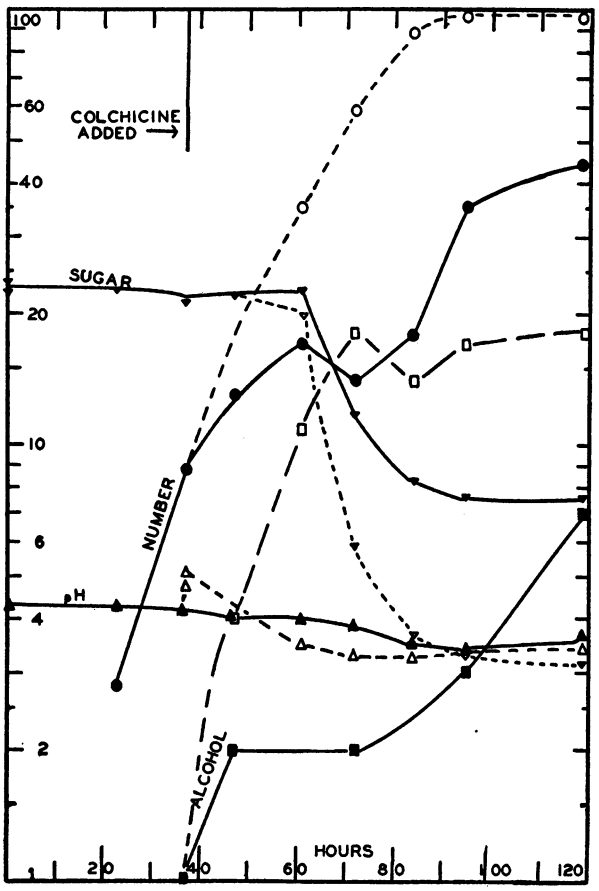

FIG. 2. INCREASED NUMBER OF YEAST PRODUCED BY THE ADDITION OF COLCHICINE AND THE CHANGES WITH TIME OF THE SUGAR, HYDROGEN ION AND ALCOHOL CONCENTRATIONS OF THE MEDIUM WITH AND WITHOUT THE DRUG

colchicine, and in 1 per cent colchicine with the mineral salts and the following combinations of nutrients: sugar, ammonium sulfate, asparagin, asparagin and ammonium sulfate, asparagin and sugar, and ammonium sulfate and sugar, the concentrations being

exactly the same as in the control medium except for the nutrients intentionally omitted.

Without sugar, little growth was observed. With the sugar and the mineral salts an appreciable growth occurred, (fig. 1, *B*). This curve was added from a previous series started with a smaller seeding which accounts for the curve being to the right of the others. The similarity of the slopes, which show the relative rate of growth from the plotting on arithlog paper, show that the rate was essentially the same except for less yield. The addition of 1 per cent colchicine to the sugar and mineral salts increases the yield nearly three times and there is a suggestion of two cycles in the growth curve. The crop attained is the same as that of the control series without the drug but in the complete medium. The yeast may obtain nitrogen from the colchicine, although not as readily as from the asparagine and ammonium sulfate.

The addition of ammonium sulfate to the colchicine and sugar gives a still greater yield, but the period of decreasing rate of growth occurs earlier than when the drug is added to the complete medium. The colchicine, asparagin and sugar gives a crop similar to the complete medium, so that the colchicine serves as well without the ammonium sulfate. Asparagin and colchicine without sugar give less yield than with sugar alone and this curve was not plotted.

The experiments show that the colchicine is a food and also buffers the medium, lessening the effect of the increasingly adverse medium on the yeast proliferation so that the population grows directly to the maximum crop without passing through two growth cycles. The period of constant relative rate, or logarithmic growth, lasts longer when the drug is present with an additional source of nitrogen, preferably asparagin, despite the fact that the alcoholic fermentation is greater and the sugar is used up more rapidly.

III

Had the colchicine arrested the division, or bud formation of the yeast as it does with other plants and animals (Nebel, 1937; Allen, 1937) the rate of growth of the populations would have

been less than of the control populations grown without the drug. Even a small retardation would be evident during the period of exponential rate of incrase and would appear as a changed slope of the growth curves. The logarithmic growth period continues beyond that of the control cultures when the colchicine is added to the culture medium and the departure from the straight line in the figures plotted on arithlog paper occurs later and less rapidly. The slightly lesser crops obtained with the lower concentrations of the drug differ from the controls by less than the experimental error (4 per cent) and the differences are not significant (fig. 1, *A*).

Samples of yeast were prepared for microscopic examination by killing in Lavdovsky's or Bouins fluids, mordanted in 2.5 per cent iron alum, stained in 1 per cent aqueous hematoxylin, destained in iron alum or in picric acid and mounted with glycerine jelly. Some of the preparations were made in bulk and the cells separated from the various fluids by gentle centrifuging and the rest as cover glass preparations.

None of the stained preparations showed any grouping of the chromatin that might resemble chromosomes. The chromatin granules were irregularly distributed between the mother cell and the bud depending somewhat on the size of the bud. I failed to note any distinguishing difference between the appearance of the colchicine-treated and the control cells. The yeast were examined at different stages during the growth cycle.

Wager (1898), Wager and Peniston (1910) and Guilliermond (1904–1912), among others, failed to find mitosis in their extensive studies. Fuhrman (1906), Swellengreble (1905) and Kater (1927) describe a mitosis of the yeast nucleus with four chromosomes. The first mentioned paper seems not to be entirely clear. The other two papers publish clear descriptions and figures. Unfortunately the preparations of Kater have faded and are not available for examination.

No yeast cell that I have seen has shown any grouping of chromatin that might be interpreted as chromosomes. Feulgen-stained preparations have been studied (1933). This includes examination of my own preparations and some preparations very

carefully stained by Dr. H. W. Beams for this purpose. The ultra-violet photographs of Ter Louw and of Dr. F. F. Lucas do not show chromosomes (personal communications to the author). The preponderance of negative evidence does not answer the question in a satisfying manner, but it is peculiar that only three observers have been able to see mitotic figures if the budding of yeast is mitotic. It was hoped that the colchicine would make possible the demonstration of mitotic division as it has done with such success for other cells. The drug failed to show any effect on cell division although it does stimulate the growth of populations of yeast.

SUMMARY

The colchicine technique was applied to the study of the growth of yeast populations and failed to show any evidence of other than amitotic cell division. Stained preparations of the cells grown in Williams' medium and in the medium plus from 4.5 per cent to 1 part of colchicine to ten million parts of medium showed no differences in cytological structure. One per cent of the drug gave maximum stimulation of the yeast growth. The stimulation is not of the kind given by a bios, but is due rather to the colchicine serving as a food and as a buffer in lessening the adverse effect of the increasingly unfavorable medium on the growth of the yeast populations.

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