JOURNAL OF BACTERIOLOGY, Jan. 1973, p. 263-270
Vol. 113, No. 1

Effect of Reversible Inhibition of Deoxyribonucleic Acid Synthesis on the Yeast Cell Cycle

MARTIN L. SLATER

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111

Received for publication 20 July 1972

Hydroxyurea (HU) preferentially inhibited deoxyribonucleic acid (DNA) replication and division in *Saccharomyces cerevisiae*. Growth, ribonucleic acid synthesis, and protein synthesis were less sensitive to this drug. Upon addition of HU, cells underwent one cycle of budding and the nuclei migrated into the necks between the mother cells and buds. Neither the nucleus nor the cells divided. Removal of HU allowed immediate resumption of DNA synthesis. Nuclear division, budding, and cell division occurred 1.5, 2, and 4 hr, respectively, after HU was removed. If protein synthesis was blocked at the time HU was removed, budding and cell division did not occur. These results were interpreted to indicate that HU prevents accumulation of the potential to initiate a new cell cycle.

The timing of deoxyribonucleic acid (DNA) synthesis, nuclear and cell division (18), as well as the discontinuous synthesis of various enzymes during the yeast cell cycle were described (16). Thus, markers in the "DNA-division cycle" and cell "growth cycle" are known (8). Temperature-sensitive mutants which define the causal connection between DNA synthesis, budding, nuclear division, and cell division have been characterized. These mutations are lethal at the restrictive temperature (1, 4-6), which prevents the study of what happens when the inhibition is removed.

This paper reports the effects of reversible inhibition of yeast DNA synthesis by hydroxyurea (HU). HU has been used as a specific reversible inhibitor of DNA synthesis in eukaryotic and prokaryotic cells (13, 14). In vitro studies with *Escherichia coli* and a variety of mammalian cell lines demonstrated a direct effect of HU on ribonucleotide reductase. This was interpreted to support the proposition, suggested by earlier in vivo studies, that HU selectively inhibits DNA synthesis by inhibiting ribonucleotide reduction (2). This inhibitor was used here on synchronized and random populations of yeast to study the effects of temporary inhibition in the cell cycle. Anisomycin, a specific inhibitor of protein synthesis in yeast (19), was used to study the accumulation of division and budding potential during inhibition by HU.

MATERIALS AND METHODS

Growth of cells and labeling procedures. The diploid strain used throughout this study was National Yeast Culture Collection no. 239, supplied by D. H. Williamson, Medical Research Council, Mill Hill, London, England. The semidefined growth medium, preparation of synchronized cultures, and convention used in measuring the "per cent bud bearing cells" were those described by Williamson (17). In some experiments, large and small cells were separated after the third starvation cycle by the method of Mitchinson and Vincent (9), except that a cell suspension in starvation medium was layered on a 2 to 12% Ficoll gradient.

Total cell counts were determined by a modification of the method of Williamson (17). Formalin (0.4%) was included in the diluent. Cell suspensions (5 ml) were sonically treated for 15 sec by using the needle probe at the minimum setting of a Biosonic III sonic oscillator. The model B Coulter Counter, with a 100-μm aperture tube, was used for counting cells. Preliminary experiments showed a 30% increase in cell counts after 10 sec of sonic treatment and no further increase after 30 sec.

Viable count was performed by using the pour plate technique with 0.7% soft agar and plates containing 2% agar. Dilutions were made in starvation medium (17). The same viable counts were obtained with the spreading and pour plate techniques.

Macromolecular synthesis was measured by a modification of the procedure of Hartwell (4). Growth medium containing 0.1 μCi of ^{14}C-adenine, ^{14}C-cytosine, and ^{14}C-uracil per ml, or 10 μCi of ^{3}H-leucine per ml, and twice the concentration of unlabeled bases or amino acid was inoculated to 8×10^3 cells/ml with a log-phase culture and incubated overnight to mid-log phase. The culture was then adjusted to 5 to 10×10^5 cells/ml. Synthesis of ribonucleic acid (RNA) and protein was measured by suspending duplicate 0.5-ml samples in 5 ml of ice-cold 10% trichloroacetic acid with 4 μg of bovine serum albumin per ml for at least 30 min. The precipitates were collected on glass fiber filters (Whatman, type GF/A), washed with three volumes of cold 5% trichloroacetic acid, two volumes of cold 1% acetic acid, and dried in scintillation vials. The radioactivity was counted in toluene containing Liquifluor (New England Nuclear Corp., Boston, Mass.). The synthesis of DNA was measured by adding 2-ml samples to 2 ml of 2 N NaOH, incubating at room temperature overnight, adding 4 ml of ice-cold 25% trichloroacetic acid and bovine serum albumin to 4 μg/ml, and collecting and treating the precipitates as described above, except that membrane filters (Millipore Corp., Type HA) were used.

Synchronized cultures could not be prelabeled, so radioactive precursors were added to the growth medium (at the same concentration used for steady-state labeling) at the beginning of the experiments.

The usual thymidine label could not be used because yeast do not take it up and have no thymidine kinase (3). Evidence that the procedure used accurately measured DNA and RNA synthesis depends on the known composition and pattern of synthesis of DNA and RNA in yeast. Chemical measurements demonstrate that 2% of yeast nucleic acid is DNA (5) and that DNA replication ends at an age of 0.28 generation, whereas RNA synthesis is continuous through the cell cycle. By using the procedure described above, 1.7% of the trichloroacetic acid-precipitable ^{14}C counts were NaOH resistant in steady-state labeling experiments. During synchrony experiments, bursts of increase of alkali-resistant counts followed by plateaus (remaining constant for six to seven samples taken at 7-min intervals, in preliminary experiments) were measured while total trichloroacetic acid-precipitable ^{14}C counts increased continuously throughout the cell cycles. When NaOH-resistant material was centrifuged and washed three times with water, about one-third of the counts were lost. Of the remaining counts, 97% were deoxyribonuclease sensitive.

Medium changes. Cells were rapidly collected on membrane filters (Millipore Corp., type GS), washed with 3 volumes of prewarmed medium without HU, and resuspended in new medium. When macromolecular synthesis was measured, the medium used for washing and resuspension contained the same concentrations of labeled precursors and carriers as the original growth medium.

Nuclear staining and phase-contrast microscopy. Samples (5 ml) were removed from growing cultures, 0.5 ml of formalin was added, and the samples were rapidly centrifuged. The concentrated suspensions were immediately spread in a thin layer of 2% agar on a slide for phase-contrast microscopy or placed on 10- by 15-mm blocks of 2% agar for nuclear staining. After 5 to 10 min at room temperature, the blocks of agar were pressed against albumin-coated cover slips and the nuclei were stained by the Giemsa stain procedure of Robinow and Marak (12). Cells were photographed with Kodak HC136 film using a Wild Photomicrographic Camera II and M20 microscope.

Inhibitors. HU was added to cultures as a powder. Unless otherwise stated, the final concentration was 0.075 M. Solutions of anisomycin were made shortly before use. Preliminary experiments showed that 3×10^{-5} M was the minimal concentration which completely inhibited protein synthesis. The concentration used in the experiments cited in the text was 10^{-4} M.

Several inhibitors of DNA synthesis were examined in addition to HU. Inhibition by fluorodeoxyuridine was not reversible but could be prevented by uridine (but not by deoxyuridine, uracil, or thymidine). Cytosine, arabinoside, and deoxyadenosine did not inhibit growth. Methotrexate did inhibit growth, but cells did not appear to accumulate at any particular phase of the cell cycle.

Chemicals. HU was obtained from K & K Laboratories, Plainview, N.Y. Anisomycin was supplied by K. Butler of Pfizer Pharmaceuticals, Groton, Conn. Gurr Giemsa stain (R66) and Gurr buffer were obtained from Biomedical Specialties, Los Angeles, Calif. Ficoll (molecular weight, 400,000) was obtained from Pharmacia Fine Chemicals, Piscataway, N.J. The radioactive compounds adenine-8-^{14}C (30–50 mCi/mmole), uracil-2-^{14}C (25–50 mCi/mmole), cytosine-2-^{14}C (20–30 mCi/mmole), and L-leucine-4,5-^{3}H (30–50 Ci/mmole) were products of New England Nuclear Corp., Boston, Mass.

RESULTS

Effects of varying concentrations of HU on cell division and macromolecular synthesis. Portions of a steady-state culture were added to flasks containing various amounts of HU. The increase in cell number (Fig. 1) and the incorporation of radioactive precursors into DNA, RNA, and protein (Fig. 2) were measured. The minimal concentration of HU which completely inhibited DNA synthesis and cell division was 0.075 M. At this concentration, RNA and protein synthesis were inhibited by 15 and 10%, respectively, and cell number increased by 66%. Higher concentrations caused greater inhibition of RNA and protein synthesis, but the extent of residual division was not altered. HU at 0.05 M slowed division but did not stop it for at least 4 hr.

The initial measurements of counts per min per ml of culture referred to in Fig. 2 were: DNA, 2.24×10^3; RNA, 119×10^3; and protein,

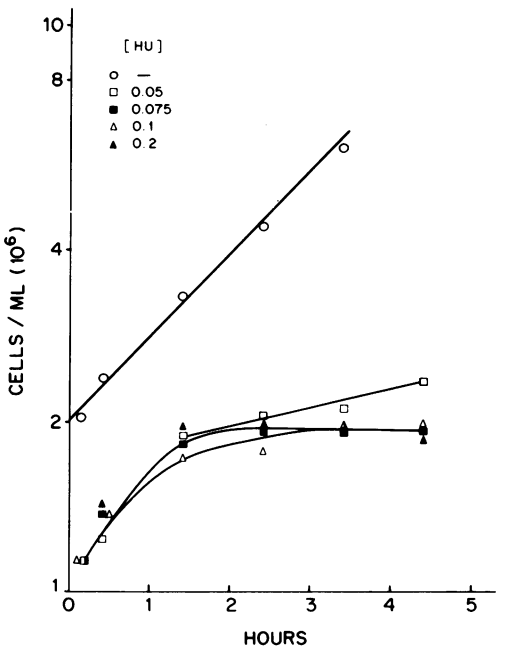

FIG. 1. *Concentration dependence of the effect of HU on cell division. Portions of an exponentially growing culture were distributed into flask containing graded amounts of HU. Samples were removed at various times, and total cell number was measured. The control culture received no HU. Symbols: O, control; □, 0.05 M HU; ■, 0.075 M HU; △, 0.1 M HU; ▲, 0.2 M HU.*

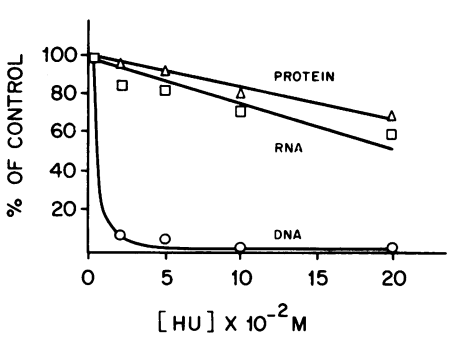

FIG. 2. *Concentration dependence of the effect of HU on macromolecular synthesis. Portions of a prelabeled culture were distributed into flasks containing graded amounts of HU. At the time of addition of HU and 2 hr later, samples were removed. The incorporation of radioactivity into DNA, RNA, and protein was measured. The results are expressed as percent incorporation into the control which received no HU. Symbols: O, DNA; □, RNA; △, protein.*

49.7×10^3.

Reversibility of inhibition by HU. To determine if HU was fungistatic or fungicidal, part of a log-phase culture was exposed to the inhibitor, and viable and total cell counts were measured. After increasing to a plateau value, viable count remained constant for at least five generations (Fig. 3).

The effect of reversible inhibition on macromolecular synthesis was determined by adding HU to part of a steady-state culture growing in the presence of radioactive precursors. After 2 hr, the inhibitor was removed from a portion by filtering, washing, and resuspending the cells in inhibitor-free medium (Fig. 4). Upon addition of HU, DNA replication stopped immediately. Cell number increased by 66% and then stopped. RNA and protein synthesis continued at nearly the control rate for about 2 hr, but gradually decreased. In the subculture from which the inhibitor was removed, DNA synthesis resumed abruptly and cell division resumed 2 hr (one generation time) later.

Chain formation in reversibly inhibited asynchronous cultures. Microscope examination during the experiment referred to in Fig. 4 showed chain formation in the reversibly inhibited culture. The effect of temporary inhibition on chaining was studied further by performing the following experiment. HU was added to part of a log-phase culture and removed after 2, 3, or 4 hr. The percentage of the populations consisting of chains of three or more cells or of five or more cells was determined under the microscope after squirting 0.5-ml samples through a Pasteur pipette 30 times to bre p clumps. In this experiment, buds of any size were scored as cells (e.g., two large cells with

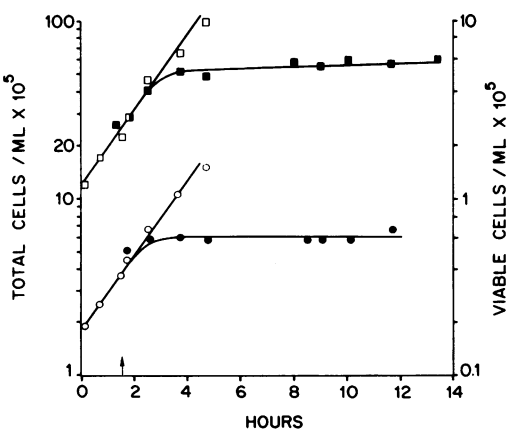

FIG. 3. *Effects of HU on viability. HU to a final concentration of 0.075 M was added to part of a steady-state culture at the time indicated by the arrow. Viable and total cell numbers during subsequent incubation were measured. Symbols: O, total cell number in control; ●, total cell number with HU; □, viable count in control; ■, viable count with HU.*

one small bud was scored as a chain of three). Chaining was also monitored in the control culture which was never exposed to HU and in a culture from which the inhibitor was never removed (Fig. 5). In the control and permanently inhibited cultures, no chains of five were seen. Chains of three never accounted for more than 20% of the permanently inhibited culture. In this culture, eventually all of the cells accumulated as doublets whose parent cell and bud increased in volume at the same rate. Chain formation was extensive in the reversibly inhibited cultures. Chains of five or more increased to over 30% of the culture inhibited for 4 hr (two normal generation times).

Effects of reversible inhibition on synchronized cultures. Synchronized cultures were used to study more closely the effects of reversible inhibition on budding and cell division.

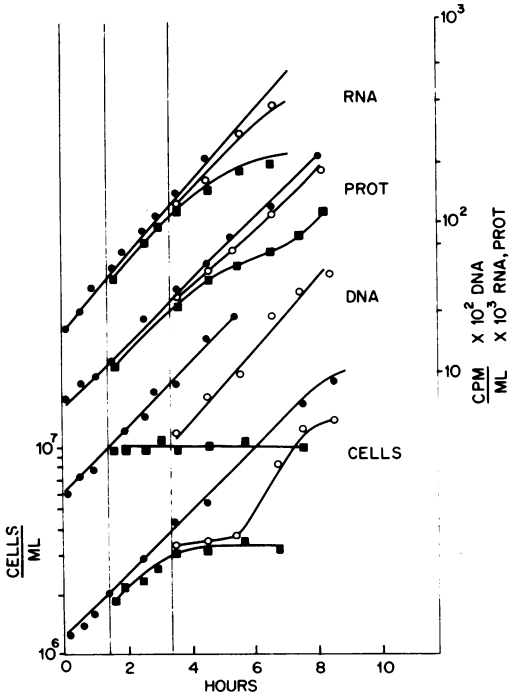

FIG. 4. *Time course of the effects of permanent and temporary inhibition by HU on cell division and macromolecular synthesis in asynchronous cultures. HU was added to part of a steady-state culture growing in the presence of radioactive precursors at the time indicated by the first line. At the time indicated by the second line, the inhibitor was removed from a subculture. Total cell number and the incorporation of radioactivity into DNA, RNA, and protein were measured. Symbols: ●, control; ■, permanently inhibited culture; ○, reversibly inhibited culture.*

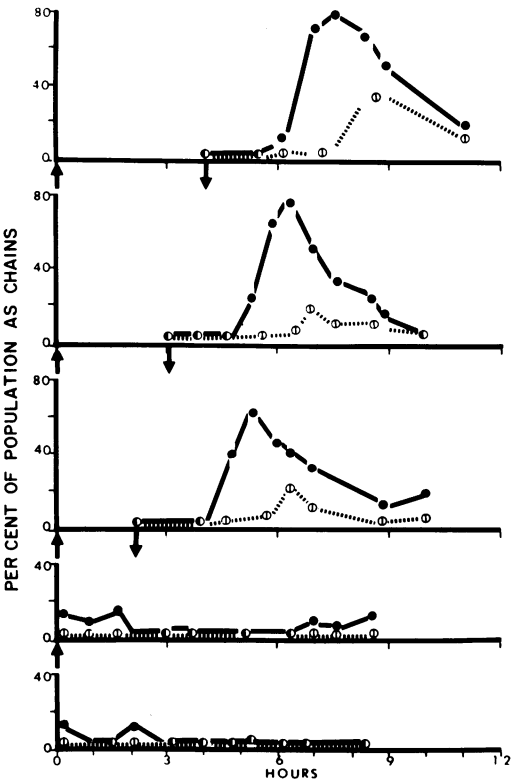

FIG. 5. *Chain formation in reversibly inhibited asynchronous cultures. HU was added to part of an exponentially growing culture at zero time. At the times indicated by the arrows, parts of the culture were washed free of the inhibitor and placed in inhibitor-free medium. Samples were squirted through a Pasteur pipette 30 times and examined under the microscope. Buds of any size were counted as cells. Symbols: Ⓓ, chains of five or more; ●, chains of three or more.*

HU was added to part of a synchronized population growing in the presence of radioactive precursors. After 2 hr (one generation time), the inhibitor was removed (Fig. 6). The addition of HU prevented DNA replication but allowed one (and only one) generation of buds to appear and grow. When the inhibitor was removed there was a burst of DNA synthesis, but no new buds appeared until a generation time after reversal. There was still no cell division so that as the new buds grew, the culture consisted of chains of four cells. After another generation time, there was another rather poorly synchronized cycle of budding. At this time, cell numbers began to increase. During the time HU was present, RNA increased by 80% and protein by 88% of the control values.

The budding cycles were delayed for one

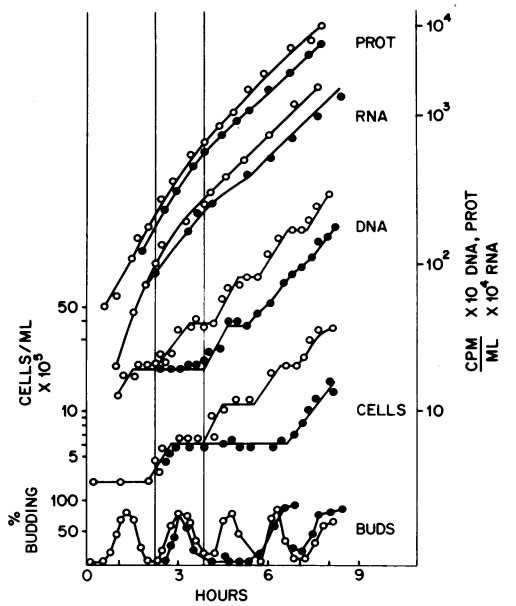

FIG. 6. *Effects of reversible inhibition by HU on budding, division, and macromolecular synthesis in synchronized cultures. Cells of a synchronized population were added to labeled medium. At the time indicated by the first line, HU was added to a portion of the culture. At the time indicated by the second line, the inhibitor was removed. The portion never exposed to HU served as the control. Symbols:* O, *control;* ●, *reversibly inhibited culture.*

generation time and division by two generation times after reversal of inhibition, regardless of the length of time the culture was exposed to the inhibitor (Fig. 7, 8). HU was added to part of a synchronized culture and removed from portions after 1, 1.5, and 2 hr. In each case, 2 hr after inhibition was relieved there was a cycle of budding, and 2 hr later there was a second cycle of budding accompanied now by the first division.

Effects of HU on nuclear migration and division. In the normal cell cycle, the nucleus begins to migrate into the bud at an age of about 0.5 generation. Chromosome separation followed by nuclear division occurs at an age of about 0.75 generation (18). If DNA synthesis is prevented, the nucleus migrates into the bud but does not divide (6).

When HU was added to a random population, the cells accumulated as doublets, with the nucleus in the isthmus between the parent cell and bud (Fig. 9). To study the effects of permanent and temporary inhibition by HU on nuclear migration and division, synchronized cultures were used (Fig. 10). HU did not delay migration of the nucleus into the neck between the parent cells and buds, but did not allow nuclear division. When the inhibitor was removed from a subculture one generation time after its addition, the normal 1.5 hr (0.75 generation time) between DNA synthesis and nuclear division elapsed before the nuclei divided.

Effects of inhibition of protein synthesis after reversible inhibition by HU. The observed lags in budding, nuclear division, and cell division, after reversible inhibition, indicated that the potential to initiate a new cell cycle did not accumulate in the presence of HU, in spite of continued RNA and protein synthesis.

To confirm this interpretation, anisomycin was used to block protein synthesis after reversible inhibition by HU.

Anisomycin was added to subcultures of a random population at the time HU was removed after 2.5, 3.5, and 4.5 hr of growth in the presence of HU (Fig. 11). In no case did division occur in the presence of anisomycin.

To determine if continued protein synthesis is needed throughout the period between reversal of HU inhibition and the onset of division, a more detailed experiment was done. Part of a

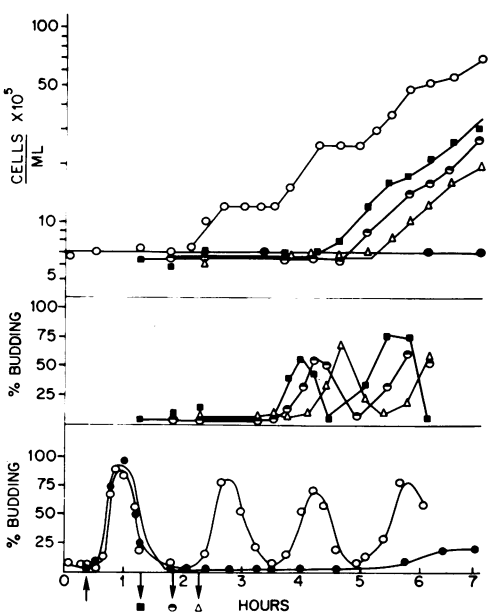

FIG. 7. *Effect of inhibition with HU for different times on budding and division. HU was added to part of a synchronized population at the time indicated by the first arrow. At the times indicated by the arrows, subcultures in inhibitor-free media were made. Symbols:* O, *control;* ●, *permanently inhibited culture;* ■, ⊖, *and* Δ, *cultures exposed to HU for 1, 1.5, and 2 hr, respectively.*

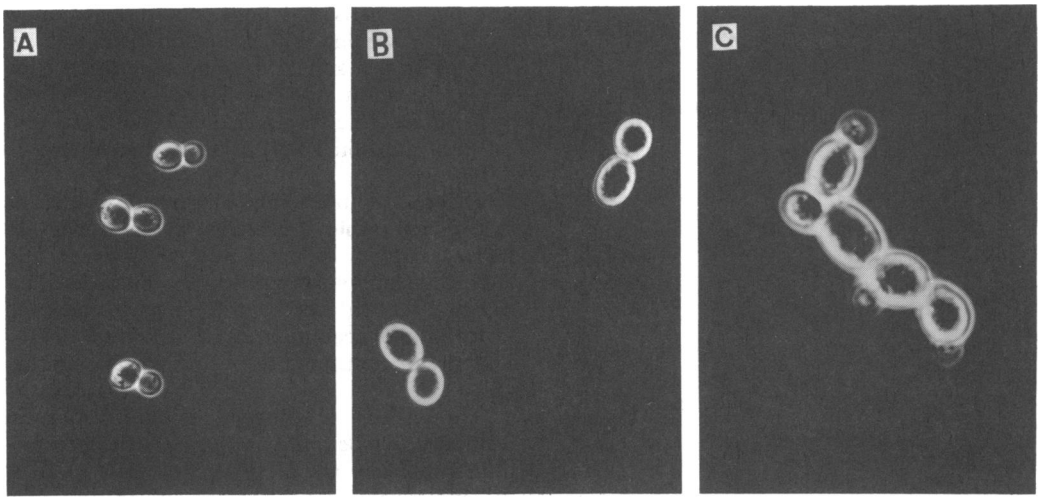

FIG. 8. *Cells from control, permanently inhibited, and reversibly inhibited cultures. Samples were removed at time 5.5 hr in the experiment referred to in Fig. 7. A, control; B, permanently inhibited culture; C, reversibly inhibited culture.*

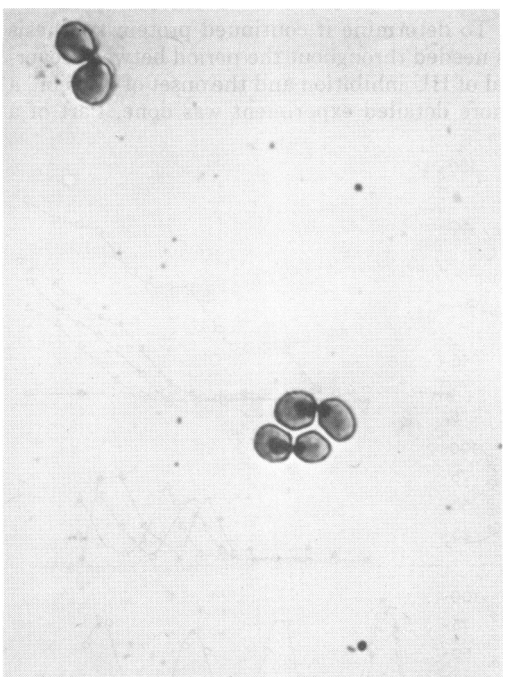

FIG. 9. *Nuclear staining of asynchronous culture exposed to HU. HU was added to an asynchronous culture. A sample was removed for nuclear staining 4 hr after the inhibitor was added.*

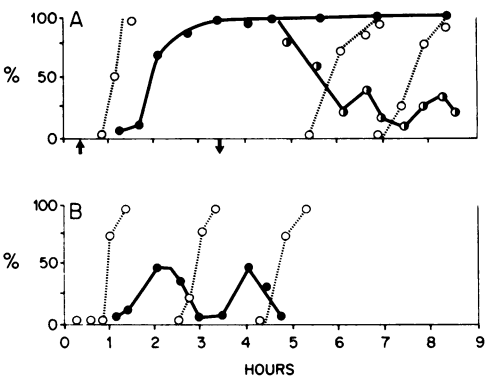

FIG. 10. *Effect of permanent and temporary inhibition by HU on nuclear division and migration. At the time indicated by the first arrow, HU was added to part of a synchronized population. Duplicate 5-ml samples were removed for nuclear staining as described in Materials and Methods. Part A, culture exposed to HU. Symbols: O, percentage of budding; ● and ◑, percentage of populations with nuclei in the necks between parent cell and bud of permanently and reversibly inhibited cultures, respectively. Part B, control. Symbols: O, percentage of budding; ●, percentage of population with nuclei in the necks between parent cell and bud.*

log-phase culture was exposed to HU for 2.5 hr. At the time the inhibitor was removed and at 20-min intervals thereafter up to 3 hr, anisomycin was added to various subcultures. Cell number in the subcultures was measured at the time of addition of anisomycin and again after 5 hr (Fig. 12). No cell division occurred in the presence of anisomycin, regardless of the time of recovery from HU.

Synchronized populations were used to test the effect of anisomycin on budding after reversible inhibition with HU. When protein synthesis was blocked immediately after a 2-hr

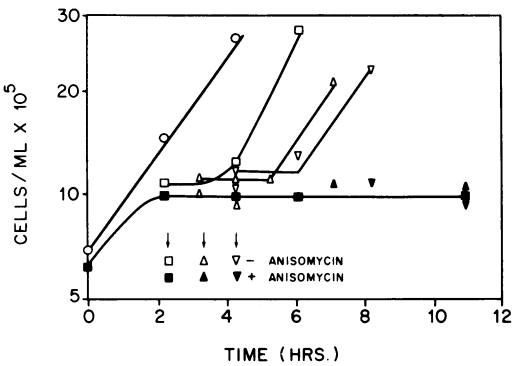

FIG. 11. *Effect on cell division of inhibiting with HU for various time intervals and then adding anisomycin. HU was added to part of log-phase culture at zero time. At the times indicated by the arrows, samples were freed of inhibitor and divided into two flasks, one of which contained anisomycin. Cell number was measured at the time of subculturing and after 2 and 4 hr of further incubation. Symbols:* O, *control;* □ *and* ■, *HU removed after 2.5 hr;* △ *and* ▲, *HU removed after 3.5 hr;* ▽ *and* ▼, *HU removed after 4.5 hr. Closed symbols, anisomycin added to subculture; open symbols anisomycin not added to subcultures.*

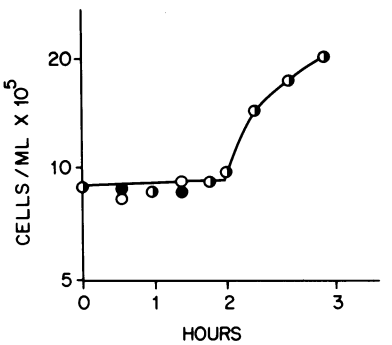

FIG. 12. *HU was added to part of a log-phase culture for 2.5 hr and removed at zero time. Samples were added to flasks with anisomycin at various times. Cell number was measured at the time of sampling* (O) *and after 5 hr of incubation in the presence of anisomycin* (●).

reversible inhibition with HU, no new buds appeared for at least 10 hr (Fig. 13).

DISCUSSION

HU was found to be a fungistatic agent which preferentially inhibits DNA synthesis in yeast. It affects the cell cycle as one would expect of a specific inhibitor of DNA synthesis. That is, 66% of a random population divides, and cells accumulate as doublets with the nucleus in the isthmus between the parent cell and bud (6, 11, 18).

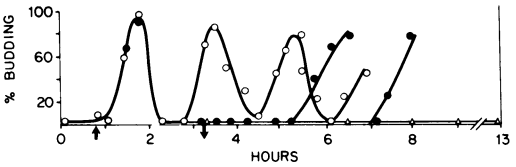

FIG. 13. *Effect on budding of adding anisomycin after reversible inhibition with HU. HU was added to part of a synchronized culture at the time indicated by the first arrow. At the time indicated by the second arrow, two subcultures in HU-free media were prepared. Anisomycin was immediately added to one of subcultures. Symbols:* O, *control;* ●, *HU removed and anisomycin not added;* △, *HU removed and anisomycin added.*

Work done with temperature-sensitive mutants has shown that preventing DNA replication allows one cycle of budding and migration of the nucleus into the neck (6). It is also known from other mutants that these events mark progress toward a new cell cycle, in the sense that if they are prevented, a new cell cycle does not occur (1, 7). The results reported here indicate that bud formation and nuclear migration (in the absence of DNA synthesis) do not mark progress toward a new cell cycle in the sense that their occurrence does not bring the next cycle closer in time. Thus, progress toward nuclear division and a new cell cycle appears to be "frozen" if DNA synthesis is prevented, although events which are temporally and casually related to the new cycle do occur.

The observation that cell division is delayed for two generation times after HU is removed may be explained in two ways. The rate of septum formation may be decreased to the extent that two generation times are required for completion. Alternatively, delay in septum formation in one cycle may cancel its occurrence in that cycle but not interfere with its timing in the next cycle. The first explanation would predict complete septum formation first between the innermost cells (the oldest cells) of the chain. The second explanation would predict septum formation at the necks of the outermost cells first. The precise coincidence of division with the second generation of budding, regardless of the period of inhibition, suggests the latter explanation.

Caution must be observed in accepting these interpretations, since the inhibitor was not completely specific for DNA synthesis, and the synchronization involved starvation and replenishment. The 66% increase in cell number with 0.075 M to 0.2 M HU, in spite of increasing effects on RNA and protein synthesis, argue for DNA synthesis as the primary event affected,

as does the stage of the cell cycle at which development stops. Williamson (17) showed that DNA replication ends at a cell age of 0.28 generation. According to the age distribution of random cultures (11), 66% of the cells had completed DNA synthesis at the time HU was added. Recently, HU was used with another lower eukaryote, *Chlorella*, in a study which provided biochemical evidence that the primary effect was on DNA synthesis (10). The pattern of gradually increasing effect on RNA synthesis and the kinetics of recovery from temporary inhibition were similar to those reported here. Williamson (17) found that the timing of DNA synthesis in the cell cycle is not affected by the synchronization procedure. Tauro et al. (15) found that the timing of bursts of enzyme synthesis was the same in cells synchronized by selection and by starvation. Therefore, in yeast, markers in the "DNA-division cycle" and the cell "growth cycle" appear unaltered by the starvation procedure.

Bearing these notes of caution in mind, HU appears to "freeze" cell cycle progress and "cancel" septum formation. The primary effects of the inhibitor appear to be on DNA replication.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI O 9465 from the National Institute of Allergy and Infectious Diseases, to Moselio Schaechter, and by a postdoctoral fellowship grant 1-F02-CA-49773-01 from the National Cancer Institute, to M. L. Slater.

LITERATURE CITED

1. Culotti, J., and L. H. Hartwell. 1971. Genetic control of the cell division cycle in yeast. III. Seven genes controlling nuclear division. Exp. Cell Res. **67**:389-401.
2. Elford, H. L. 1968. Effect of hydroxyurea on ribonucleotide reductase. Biochem. Biophys. Res. Commun. **33**:129-135.
3. Grivell, A. R., and J. F. Jackson. 1968. Thymidine kinase: evidence for its absence from *Neurospera crassa* and some other micro-organisms, and the relevance of this to the specific labeling of deoxyribonucleic acid. J. Gen. Microbiol. **54**:307-317.
4. Hartwell, L. H. 1967. Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93**:1662-1670.
5. Hartwell, L. H. 1970. Biochemical genetics of yeast. Annu. Rev. Genet. **4**:373-396.
6. Hartwell, L. H. 1971. Genetic control of the cell division cycle in yeast. II. Genes controlling DNA replication and its initiation. J. Mol. Biol. **59**:183-194.
7. Hartwell, L. H., J. Culotti, and B. Reid. 1970. Genetic control of the cell division cycle in yeast. I. Detection of mutants. Proc. Nat. Acad. Sci. U.S.A. **66**:352-359.
8. Mitchison, J. M. 1971. The biology of the cell cycle. Cambridge University Press, New York.
9. Mitchison, J. M., and W. S. Vincent. 1965. Preparation of synchronous cultures by sedimentation. Nature (London) **205**:987-989.
10. Moors, W. J., and F. N. C. M. Kryzer. 1972. Dissociation of nuclear DNA replication from concomitant protein synthesis of *Chlorella*. Biochim. Biophys. Acta **269**:153-161.
11. Puck, T., and J. Steffen. 1963. Life cycle analysis of mammalian cells. Biophys. J. **3**:379-397.
12. Robinow, C. F., and J. Marak. 1966. A fiber apparatus in the nucleus of the yeast cell. J. Cell Biol. **29**:129-151.
13. Rosenkranz, H. S., and J. A. Levy. 1965. Hydroxyurea: a specific inhibitor of deoxyribonucleic acid synthesis. Biochim. Biophys. Acta **95**:181-183.
14. Sinclair, W. K. 1965. Hydroxyurea: differential lethal effects on cultured mammalian cells during the cell cycle. Science **150**:1729-1731.
15. Tauro, P., and H. O. Halvorson. 1966. Effect of gene position on the timing of enzyme synthesis in synchronous cultures of yeast. J. Bacteriol. **92**:652-661.
16. Tauro, P., H. O. Halvorson, and R. L. Epstein. 1968. Time of gene expression in relation to centromere distance during the cell cycle of *Saccharomyces cerevi-seae*. Proc. Nat. Acad. Sci. U.S.A. **59**:277-284.
17. Williamson, D. H. 1965. The timing of deoxyribonucleic acid synthesis in the cell cycle of *Saccharomyces cerevisiae*. J. Cell Biol. **25**:517-528.
18. Williamson, D. H. 1966. Nuclear events in synchronously dividing yeast cultures, p. 81-101. *In* I. L. Cameron and G. M. Padilla (ed.), Cell synchrony. Academic Press Inc., New York.
19. Vazquez, D., E. Battaner, R. Neth, and R. E. Monro. 1969. The function of 80S ribosomal subunits and effects of some antibiotics. Cold Spring Harbor Symp. Quant. Biol. **34**:369-375.