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Synthesis of total RNA, DNA, and protein was followed in a haploid yeast auxotroph during starvation for required amino acids and uracil, and during shift-up and shift-down conditions.

During amino acid starvation, synthesis of macromolecular constituents was not immediately affected, reflecting the presence of large intracellular amino acid pools. Under conditions in which there was no net RNA and protein synthesis (amino acid and uracil starvation), total DNA continued to be synthesized for a total increase of 10-15%. The results suggested that cells engaged in DNA synthesis at the introduction of starvation conditions complete replication of the genome but do not initiate new periods of DNA synthesis. Upon return of a starved culture to complete medium, a period of DNA synthesis was required before the culture began to divide at the rate characteristic for complete medium. Compounds which are known

to inhibit DNA synthesis in bacteria had no effect upon starved yeast cultures returned to complete medium.

Under shift-down conditions, cultures exhibited a lag in response to that observed during amino acid starvation. During shift-up conditions, DNA and protein increased in a stepwise fashion before assuming a continuous rate of increase characteristic of the new medium. During shift-up RNA was synthesized initially at a high rate; this was found not to be due to exclusive synthesis of a particular class of RNA.

Control of Macromolecular Synthesis in *Saccharomyces cerevisiae*

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To my parents, who made this possible.

In memory of Karl Seethaler

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INTRODUCTION

In bacteria, understanding of the synthesis of catalytic, structural, and informational macromolecules has been greatly aided by the knowledge of how these processes behave during transitions between different growth conditions. Under conditions of steady-state growth, the cellular contents of the different classes of macromolecules exhibit strictly quantitative interrelationships which are functions of the growth rate supported by the medium. During transitions between different steady-state conditions, the syntheses of various macromolecular species are uncoupled until quantitative relationships characteristic of growth in the new medium are established. From studies of the individual patterns of RNA, DNA, and protein synthesis in the transition period, models have been proposed for the *in vivo* regulation of these components. Furthermore, investigation of the synthesis of macromolecules during starvation of bacterial cultures for required amino acids has provided evidence for relationships between protein synthesis and initiation of chromosome replication. This has led to the postulation of regulatory mechanisms controlling synthesis of messenger and stable RNA fractions. Discovery of the phenomenon of chromosome alignment in amino acid starved

bacterial cultures has provided a valuable tool for the study of the origin and direction of chromosome replication. Clearly, many of the concepts of the molecular biology of bacterial systems originated from relatively basic consideration of the behavior of a bacterial cell during transitions between different environmental conditions.

Yeast offer a unique vehicle for the study of cellular regulatory mechanisms. Saccharomyces cerevisiae is a eukaryotic organism possessing a defined nucleus with 18 genetic linkage groups thus far identified. In addition, the growth rate can be varied by the selection of different growth media. Thus, yeast provide an easily manipulated system with a high order of complexity which can be used to test predictions made from bacterial systems and could serve as a basis of comparison for other eukaryotes.

LITERATURE REVIEW

Before reviewing macromolecular synthesis in yeast, it is pertinent to consider the regulation of macromolecule synthesis in procaryotes. Bacteria provide well defined systems in which the interrelationships among various cellular components have been closely examined. For cells in balanced growth (defined by Campbell, 1957, as an increase in every extensive property of the whole system by the same factor over any time interval), the content of different cellular constituents and their relationships to each other vary as a function of the steady state rate of growth; the cell exhibits a continuum of physiological states dependent upon the growth rate supported by the environment. Schaechter, Maaløe, and Kjeldgaard (1958) showed that in Salmonella typhimurium cell mass, the average number of nuclei per cell and the content of RNA and DNA could be described as exponential functions of the growth rate at a given temperature. From the values obtained for mass, RNA, and DNA per cell, they calculated that the amount of protein synthesized per minute per unit of RNA was almost independent of growth rate. Thus the increase in protein synthesized per minute per nucleus at increasing growth rates reflected an increase in ribonucleoprotein particles per nucleus. However, at low growth rates, the proportionality between the rate of protein synthesis and RNA content was not observed.

Kjeldgaard and Kurland (1963) found that the various RNA fractions in the cell are not regulated coordinately at all growth rates. By separating the soluble and ribosomal RNA fractions at various growth rates, they determined that the ratio of soluble RNA to DNA is a constant at all growth rates, and that the increase of RNA content per nucleus with increasing growth rates results from an increase in the ratio of ribosomal RNA to DNA. Their data support the assumption that the rate of protein synthesis per unit weight of ribosomal RNA is a constant at all growth rates. At steady state growth conditions, the syntheses of ribosomal and soluble RNA are regulated independently. Ecker and Schaechter (1963) showed that in Salmonella typhimurium the relative ribosome concentration (ribosome content/soluble protein) was a linear function of the growth rate under steady state conditions; their results are consistent with the hypothesis that the rate of soluble protein synthesis in balanced growth is directly proportional to the ribosome content. Recently Forschhammer and Kjeldgaard (1968) showed that the amount of message activity in RNA isolated from cultures of Escherichia coli growing at different rates was nearly proportional to the amount of ribosomal RNA.

During a transition between different states of balanced growth, the close relationship maintained among the syntheses of various macromolecular constituents of the cell is temporarily disrupted. In this period, the rates of synthesis of the different classes of macromolecules are dissociated until the quantitative relationships

are re-established at a ratio characteristic of balanced growth in the new environment. Kjeldgaard, Maaløe, and Schaechter (1958) found in Salmonella typhimurium that, during a shift-up from a medium supporting a low growth rate to a medium supporting a higher rate, the rate of RNA synthesis was immediately affected and quickly assumed a rate characteristic of the new medium. DNA synthesis and cell division continued at the pre-shift rate for definite periods, then quickly assumed the new rates. The times at which the latter two rate changes occurred were 20 min. and 70 min., respectively, regardless of the magnitude of the shift in steady-state growth rates. Increase in cell mass showed a short lag before reaching the new rate. The number of nuclei per cell remained constant for the first 35 min. after the shift, and then doubled. By 70 min. after the shift, the cells had reached the size and composition characteristic of cells in balanced growth in the new medium. Similar patterns of response were observed in shift-up experiments using two strains of Escherichia coli. During the period immediately following transfer in a shift-up experiment, RNA was synthesized at a rate greater than that characteristic of cells in balanced growth in the new medium (Kjeldgaard, 1961). Analysis of the various cellular RNA fractions during shift-up showed that the initial high rate of total RNA increase could be accounted for by a preferential synthesis of ribosomal RNA at a rate greater than normal. Similar results have been reported

for Aerobacter aerogenes undergoing shift-up conditions (Neidhardt and Magasanik, 1960). Kjeldgaard (1961) concluded that both ribosome formation and the synthesis of ribosomal protein were accelerated during the first 30 min. of the shift-up in response to an increased synthesis of ribosomal RNA. Data from incorporation of labeled amino acids into protein during this period agreed with the hypothesis that the rate of total protein synthesis increased in response to an expanding protein synthetic system (i. e., to ribosome formation). The earlier conclusion that the ratio between ribosome content and rate of protein synthesis is constant seemed to hold for shift-up conditions. This was supported by the work of Schleif (1967), who demonstrated that the ratio between the rate of synthesis of ribosomal protein and the rate of synthesis of total protein (α) in Escherichia coli B/r increased from a value characteristic of balanced growth in succinate minimal medium to a value characteristic of balanced growth in glucose minimal medium within 2 to 5 minutes after a shift-up. Furthermore, Schleif computed the average growth rate of a polypeptide per ribosome to be 13 ± 2 amino acids per second in succinate medium ($\alpha = 0.08$), glucose medium ($\alpha = 0.15$) and during a shift-up from succinate to glucose medium. The fact that ribosomal RNA and the information for ribosomal protein were formed at increasing rates during a period of DNA synthesis at the preshift rate suggested that DNA synthesis is independent of its template activity (Kjeldgaard, 1961).

During a shift-down, the patterns of macromolecular synthesis were quite different from those observed during shifts-up. In Salmonella typhimurium (Kjeldgaard, Maaløe, and Schaechter, 1958), net synthesis of mass and RNA immediately ceased after transfer to the new medium; these quantities did not begin to increase for 40 min. following transfer, then gradually assumed the steady-state rate of increase. Cell number and DNA, on the other hand, continued to increase at the preshift rate for a period before assuming a lower rate. Similar effects have been noted for cultures in which a required component of the medium had become limiting (Neidhardt and Magasanik, 1960). Maintenance of the preshift rate of cell division during shift-down resulted in a reduction of the number of nuclei per cell. It can be assumed that during a shift-up, the cell will be entering a higher state of repression; this assumption was supported by experiments in which cultures subjected to shifts-up were transferred back to minimal medium after varying periods of incubation in rich medium (Kjeldgaard, Maaløe, and Schaechter, 1958). The shorter the period of incubation in rich medium, the less time was required by the culture for adaptation to the minimal medium upon subsequent shift-down. Conversely, a cell undergoing a shift-down can be considered to be entering a higher state of induction. This was confirmed by Hayashi and Spiegelman (1961), who showed that messenger RNA was synthesized during the period of shift-down

when there was no apparent net RNA synthesis.

It has been firmly established that the bacterial chromosome is replicated semi-conservatively in an ordered, sequential fashion and that, at generation rates less than 1.5 doublings/hour, replication of the entire chromosome must be completed before a second round of replication can be initiated (Meselson and Stahl, 1958; Cairns, 1963; Lark, Repko, and Hoffman, 1963). Not all of the events in the normal replication of DNA are dependent upon the synthesis of other macromolecules in the cell; as stated above (Kjeldgaard, Maaløe, and Schaechter, 1958), DNA synthesis will continue during shift-down conditions which inhibit the net synthesis of RNA and protein. Maaløe and Hanawalt (1961) showed that during starvation for one or more required amino acids, which immediately inhibited further net increase of RNA and protein, cultures of E. coli continued to synthesize DNA to an increase of about 40%. On the basis of theoretical calculations, this is the increase expected if, at the time of amino acid removal, all cells continued rounds of replication in progress to completion but did not reinitiate new rounds. The authors proposed that the DNA replication cycle, once initiated, continued to completion without a requirement for RNA or protein synthesis. However, a new round of replication required the synthesis of other macromolecules and could not be initiated under conditions of amino acid starvation. When thymine-requiring cells actively synthesizing DNA

were starved for thymine, a rapid loss of viability (thymineless death) set in within 40 minutes. It was found that when a thymineless amino acid auxotroph was subjected to conditions of amino acid starvation, the cell population gradually became immune to thymineless death; upon readdition of amino acids, this immunity was lost over a period of 40 to 50 minutes. Using autoradiographic techniques, Hanawalt et al. (1961) showed that during inhibition of protein and RNA synthesis a gradually decreasing fraction of cells participated in DNA synthesis. This suggested that, at the time of inhibition, cells that had almost finished replication continued DNA synthesis for a short time while cells which had just begun replication continued for a longer period. Further experiments showed that cultures containing a fraction of cells immune to thymineless death contained a similar fraction unable to synthesize DNA. Lark, Repko, and Hoffman (1963) predicted that under conditions of amino acid starvation, bacterial chromosomes became aligned at a common terminus and that upon readdition of amino acids replication proceeded from a common origin. Using density-labeling techniques, they were able to verify this prediction and to show that a pulse label near the chromosome origin could be recognized after several generations of random growth followed by chromosome alignment by amino acid starvation. However, they observed that DNA made during amino acid starvation appeared to replicate more slowly than that synthesized

prior to or after starvation. The phenomenon of chromosome alignment by amino acid starvation has also been observed in Bacillus subtilis (Anraku and Landman, 1968) and Salmonella typhimurium (Lark and Chan, 1969). Chromosome-aligned cultures prepared by this technique have been used to study the origin and direction of replication of chromosomal markers by transduction (Abe and Tomizawa, 1967; Wolf, Newman, and Glaser, 1968) and by mutagenesis of the replication point (Cerda-Olmedo, Hanawalt, and Guerola, 1968; Ward and Glaser, 1969).

The inhibition of net synthesis of RNA during amino acid starvation involves only the stable RNA fractions. Forschhammer and Kjeldgaard (1968) showed that messenger synthesis occurred at identical rates in the presence or absence of required amino acids in Escherichia coli. Other workers (Edlin et al., 1968; Stubbs and Hall, 1968) showed that a specific message (tryptophan mRNA) was made under conditions of tryptophan starvation. However, Lavellé and De Hauwer (1968) reported that the level of tryptophan mRNA during arginine starvation was greatly decreased in both stringent and relaxed strains of Escherichia coli.

Amino acid starvation and shifting experiments have demonstrated that only certain steps in the chromosome replication cycle are dependent upon concomitant RNA and protein synthesis. Several reports have shown that the events in cell division are, in turn,

dependent upon one or more of the events in DNA replication. Clark (1968) and Helmstetter and Pierucci (1968) used synchronously dividing cultures of Escherichia coli B/r to show that completion of a round of DNA replication was a necessary and sufficient condition for cell division. According to their results, cell division did not occur if replication was inhibited prior to completion of a round of replication, but it was independent of the initiation of new rounds. Thus the steps leading to cell division in Escherichia coli B/r took place in the absence of DNA synthesis. In an extensive study, Helmstetter and Cooper (1968) and Cooper and Helmstetter (1968) presented evidence for a model describing the relationship between chromosome replication and cell division in Escherichia coli B/r. The model predicted that the time for a replication point to traverse the chromosome and the time between the arrival of a replication point at the chromosome terminus and subsequent cell division were constant. In order that these values remain constant at generation times less than the time required for a round of replication, the model predicted the initiation of new replication points on the chromosome prior to the completion of DNA replication at pre-existing points. Helmstetter et al. (1969) proposed that both the frequency of cell division and initiation of new replication points were governed by the rate of synthesis of a hypothetical initiator protein.

In contrast to bacterial systems, comparatively little is known

about the regulation of macromolecular biosynthesis in yeast. Investigations with these organisms have been concerned in large part with the timing of synthesis of different macromolecules during the cell cycle. Ogur, Minckler, and McClary (1953) showed that under conditions in which there was a fair degree of synchrony in initiation of budding (65% of the cells carrying buds), the DNA content per cell approximately doubled toward the end of the first budding cycle. Beam et al. (1954) observed that a portion of a population of dividing yeast cells exhibited increased resistance to X-irradiation. This portion increased under conditions which increased the frequency of budding cells, and disappeared under conditions (nitrogen starvation) in which division ceased. They concluded that the radio-resistant portion of the culture was accounted for by increased resistance of budding cells to X-irradiation, and suggested that this resistance may have been associated with gene multiplicity during bud formation. Mitchison and Walker (1959) measured the increase in total RNA during the cell cycle of the fission yeast Schizosaccharomyces pombe by comparing cell length in a randomly dividing population with absorption at 263 nm. Although their method was subject to considerable experimental error, their results showed that total RNA increased continuously throughout the cell cycle. Williamson and Scopes (1960) used synchronized cultures of Saccharomyces cerevisiae prepared by a method based on alternate feeding and

starvation periods combined with temperature changes to study the synthesis of nucleic acids during the cell cycle. They found that during three consecutive cell cycles, the appearance of buds was closely followed by a rapid doubling in the amount of DNA. Later experiments with improved techniques showed that DNA synthesis started just after completion of division and was coincident with emergence of buds (Williamson, 1964). Since the pattern of DNA synthesis observed during growth of synchronous cultures may have been an artifact resulting from the moderately harsh procedures used to induce synchrony, Williamson (1965) investigated the timing of DNA synthesis in randomly dividing cells of S. cerevisiae. His procedure involved pulse-labeling cultures with radioactive adenine, followed by removal of radioactivity from RNA. Comparison of grain count and relative bud length (a measure of cell age) from autoradiograms of the treated cells indicated that DNA was synthesized almost exclusively during the first quarter of the cell cycle. These results were in complete agreement with the rates of adenine incorporation in control experiments using synchronous cultures. Kleinkauf (1967) observed an oligonucleotide fraction in S. cerevisiae which increased at the onset of budding; increase of this fraction was inhibited by conditions which also inhibited bud formation. Recently, Smith, Tauro, and Halvorson (1968) showed that under conditions which derepressed mitochondria formation, replication of mitochondrial DNA was

periodic in synchronous cultures of Saccharomyces lactis. Mitochondrial and nuclear DNA taken at different times in the cell cycle were separated by density gradient centrifugation; comparison of the ratio of mitochondrial to nuclear DNA showed that the synthesis of mitochondrial DNA was discontinuous and occurred at a different interval of the cell cycle than synthesis of nuclear DNA. As in S. cerevisiae, DNA synthesis occurred during the early part of the cell cycle; this was followed by a period during which neither mitochondrial nor nuclear DNA was synthesized. The authors suggested that mitochondrial DNA replication may require the periodic synthesis of a protein coded by nuclear DNA. In contrast with the results reported for S. cerevisiae and S. lactis, Halvorson et al. (1964) observed that DNA synthesis occupied the first three-fourths of the cell cycle in synchronous cultures of the hybrid yeast S. dobzhanskii x S. fragilis.

Williamson and Scopes (1960) found that total RNA increased linearly and continuously throughout the cell cycle of synchronous cultures of S. cerevisiae, with a period of no net synthesis extending from the time of cleavage through the early stages of bud formation. Neither Mitchison and Walker (1959) nor Williamson and Scopes (1960) found clear evidence for exponential increase in RNA during the cell cycle of yeast. Mitchison and Lark (1962) investigated the synthesis of RNA in randomly dividing cells of Schizosaccharomyces

pombe by autoradiography. Cultures of yeast were pulse-labeled with radioactive adenine and autoradiograms were prepared. Comparison of grain count with cell length (a measure of cell age) showed that the rate of RNA synthesis increased through the cell cycle, with no detectable drop in rate at the time of nuclear division. Continuous synthesis of RNA throughout the cell cycle of S. cerevisiae has been reported by Gorman et al. (1964). Total protein has been shown to increase continuously through the cell cycle (Williamson and Scopes, 1961; Gorman et al., 1964), although Sylven et al. (1959) and Williamson and Scopes (1961) reported that total nitrogen content increased in a stepwise fashion, with one period of increase per generation. Mitchison and Wilbur (1962) prepared autoradiograms of Schizosaccharomyces pombe cultures pulse-labelled with radioactive amino acids or glucose. Comparison of cell length with grain count indicated that the labelled precursors were incorporated into protein and carbohydrate at continuously increasing rates during the cell cycle.

It has been shown in bacteria that the maximal rate of enzyme synthesis is dependent upon the number of gene copies coding for the particular enzyme, and that the observed rate of enzyme synthesis in synchronous cultures will reflect this parameter as well as repressor and effector levels (Masters and Pardee, 1965). Therefore, the potential rate of enzyme synthesis will double at a discrete time in the bacterial cell cycle depending upon the map position of the

corresponding gene. It has also been shown that transcription and chromosome replication are not functionally linked, that is, the portion of the template being replicated is not available to RNA polymerase; the template is accessible to transcription at all other times in the cell cycle. In contrast to the results obtained with bacteria, Halvorson et al. (1964) and Gorman et al. (1964) presented evidence that, in yeast, the genome is available for transcription only at limited periods during the cell cycle. Using synchronous cultures of Saccharomyces cerevisiae, they showed that α-glucosidase was synthesized in a stepwise fashion with a single rise in enzyme content near the beginning of the division cycle. Failure of addition of inducer to alter the timing of enzyme synthesis suggested that the phenomenon was not due to an unstable regulatory system. To support this conclusion, a similar experiment was performed with the hybrid yeast S. dobzhanskii x S. fragilis which contained multiple non-allelic structural genes for β-glucosidase, all under similar regulatory control. Two distinct periods of enzyme synthesis were observed during each cell cycle, one characteristic of S. dobzhanskii and the other of S. fragilis. Similar results were obtained with synchronous cultures of S. cerevisiae carrying multiple non-allelic genes for invertase and alkaline phosphatase; two periods of synthesis for each enzyme were observed during the cell cycle. The authors concluded that in all three species and in the hybrid, transcription was ordered

during the cell cycle and that RNA polymerase does not have access to specific areas of the genome at all times. Expression of a particular gene was limited to a certain period in the cell cycle, and modification of expression by induction or repression could function only during this period. The authors suggested that the observed ordered transcription paralleled gene replication; to test this hypothesis, the timing of expression of two closely linked genes (β-galactosidase and β-glucosidase) was examined. It was found that the times of initiation of synthesis for the two enzymes differed only by 3% of a generation.

Bostock et al. (1966) investigated the timing of enzyme synthesis in synchronous cultures of Schizosaccharomyces pombe and obtained results similar to those found in bacteria. Aspartate transcarbamylase and ornithine transcarbamylase activities were seen to increase periodically through the cell cycle, with a single burst of enzyme activity midway through the cycle. Measurement of sucrase, maltase, and alkaline phosphatase activities in synchronous cultures showed that there was a continuous increase of all three enzyme activities through the cell cycle. In the growth medium used, maltase and sucrase were at basal levels and alkaline phosphatase was constitutive. Thus, in this organism, there was both periodic and continuous synthesis of enzymes. In the case of continuous enzyme synthesis, the authors were unable to distinguish between

continuous transcription modified by feedback control, or periodic transcription of a number of genes in different positions on the genome. In the case of periodic synthesis of aspartate transcarbamylase and ornithine transcarbamylase, the authors noted that initiation of enzyme steps occurred during periods of no net DNA synthesis. Since continuous synthesis of some enzymes was observed, and since the periodic synthesis of other enzymes did not occur during the time of gene replication, it was concluded that the mode of enzyme synthesis in Schizosaccharomyces pombe more closely resembled that of bacteria than that of S. cerevisiae.

To support the earlier conclusion of Halvorson et al. (1964) that the genome was transcribed in an ordered manner during the cell cycle of Saccharomyces cerevisiae, further experiments were performed by Tauro and Halvorson (1966). The timing of increase in α-glucosidase activity in synchronous cultures was determined in different strains of S. cerevisiae carrying one or more non-allelic structural genes for α-glucosidase. The various α-glucosidase (M) genes were under similar regulation but functioned independently; the induced level of α-glucosidase was a strict function of the gene dosage, and the time of expression of a particular gene was independent of the level of induction. Since the various M genes were scattered over the genome, it was possible to determine from the pattern of α-glucosidase synthesis in strains carrying multiple

M genes whether the timing of gene expression was related to gene position. It was found that the number of periods of enzyme synthesis corresponded to the number of unlinked structural genes and that each of the structural genes had a unique time of expression in the cell cycle which depended upon its chromosomal location. Tauro, Halvorson, and Epstein (1967) investigated the order of expression of a number of genes on the same chromosome and genes equidistant from the centromere on separate chromosomes in diploid strains of S. cerevisiae to determine the mode and direction of chromosome transcription. Increase in activity of ten different enzymes was followed in synchronous cultures, and the times of expression were compared with map positions. Their data were inconsistent with a model in which transcription was initiated at the centromere and proceeded outward in both directions, but were consistent with unidirectional transcription starting at the chromosome end. Models predicting regional transcription or bidirectional transcription with origins at chromosome ends could not be ruled out.

Other workers have reported discontinuous enzyme synthesis in synchronous cultures of yeast. Sylven et al. (1959) observed cyclic variations in dipeptidase and proteinase A activity, with maximum activity occurring in the stages preparatory to cell division and minimum activity during bud formation; however, it was not known whether the oscillations in activity were due to de novo synthesis and

degredation or to conversion of inactive enzyme precursors. Eckstein, Paduch and Hilz (1967) reported that DNA polymerase showed a cyclic appearance in the cell cycle of synchronous cultures of S. cerevisiae. Polymerase activity reached a maximum at the onset of DNA synthesis and declined to a minimum during DNA replication. Significantly, the cyclic appearance of DNA polymerase activity continued under conditions of inhibition of DNA replication and cell division (i.e., X-irradiation). The frequency of appearance remained the same, and the maximal activity remained constant but increased when DNA replication resumed. The authors did not determine whether or not the cyclic appearance of enzyme was due to de novo synthesis. Scopes and Williamson (1964) found that the rate of oxygen uptake in synchronous cultures of S. cerevisiae increased in a series of abrupt steps, with one step per generation. They suggested that this might reflect periodic de novo synthesis of respiratory enzymes or mitochondria. Esposito (1967) observed that X-ray or ultraviolet-induced intragenic and intergenic recombination exhibited a characteristic pattern in synchronous cultures of S. cerevisiae. Recombination rose to a high level prior to DNA synthesis, reaching a maximum at the start of replication, then declined during replication, reaching a minimum just after completion. She suggested that the periodic timing of recombinational events in yeast may be due to periodic synthesis or activation of enzymes associated with recombination, e.g. dark repair enzymes. In contrast to results reported

for other gene products, Tauro et al. (1968) found that ribosomal and transfer RNA were produced continuously over the cell cycle in S. cerevisiae. In order to determine whether this phenomenon was due to a continuous availability of these genes for transcription or to periodic transcription of a large number of ribosomal and transfer RNA genes, Schweizer, McKechnie and Halvorson (1969) estimated the number of cistrons for ribosomal and transfer RNA by DNA-RNA hybridization. Their results indicated that yeast nuclear DNA contains 140 cistrons for both 18 and 26 s ribosomal RNA and 320 to 400 cistrons for total transfer RNA (or an average number of 5 to 7 identical cistrons for each transfer RNA species). Continuous synthesis of both RNA species throughout the cell cycle could result either from the ordered transcription of RNA cistrons scattered over the genome or from ordered transcription over a full cell cycle of RNA cistrons clustered in one complete linkage group.

Little is known about the behavior of macromolecular synthesis in yeast during a transition between different growth conditions. Kudo and Imahori (1965) investigated the effect of shift-up following nitrogen starvation upon RNA synthesis in S. cerevisiae. They found that when stepped-up cells were pulse-labelled for five minutes with radioactive phosphate, the label was incorporated to a large extent into the polysome fraction. Treatment of the pulsed extracts with dilute ribonuclease removed radioactivity from the polysome

and ribosome fractions, which suggested that the labeled RNA was present in strands holding the polysome together rather than the ribosomes themselves. However, the base composition of the labeled RNA was similar to that of ribosomal RNA; if a radioactive uracil pulse was followed by a chase with cold uracil, the label moved from the polysome fraction to the 80 s ribosomal fraction. These results suggested that the RNA labeled during shift-up following nitrogen starvation was ribosomal RNA found first in the polysomes and later in the 80 s ribosomes. The authors interpreted these results to indicate that nascent ribosomal RNA serves as a template for protein synthesis (possibly ribosomal protein) before it is incorporated into mature ribosomes. Mitchison and Gross (1964) looked at the synthesis of RNA in cultures of Schizosaccharomyces pombe during shift-down from a rich medium to a minimal medium containing ethanol. They found that after step-down there was no increase in optical density for 25 minutes and no increase in total RNA for one hour. However, incorporation of radioactive precursors indicated that RNA was being synthesized during this period. On the basis of pulse-chase experiments, nucleotide analysis of labelled RNA, and sedimentation properties of labelled RNA, the authors concluded that unstable messenger RNA was synthesized in the period following shift-down.

MATERIALS AND METHODS

Cultures

Saccharomyces cerevisiae strain 5015-D, a haploid yeast requiring methionine, tryptophan, adenine, and uracil was used in all experiments except one. This strain was originally obtained from Dr. H. L. Roman, Department of Genetics, University of Washington, and has the genotype: a, me-2, ad-2, ur, tr-1. In one experiment, Saccharomyces cerevisiae strain 3701-B was used. This strain is a haploid uracil auxotroph, and was obtained from the same source as strain 5015-D. Cultures were maintained on TCA agar slants at 4° and transferred periodically.

Growth Conditions

Cells were grown aerobically in Wickerham's complete defined medium (Wickerham, 1946), Wickerham's minimal defined medium (Wickerham, 1946) supplemented with the various requirements, or in TCA medium. The composition of the different media is shown in Table 1. Unless noted otherwise, supplements were added to Wickerham's minimal medium in the same concentrations used in Wickerham's complete medium. For measurement of radioactive adenine incorporation, the medium was supplemented with 5 μg adenine-8-^{14}C/ml (specific activity 0.0125-0.0250 μc/μg; New England Nuclear Corp., Boston, Mass.) Cells were grown from a

Table 1. Composition of growth media.

Component	Concentration in Grams/Liter		
	TCA Medium	Wickerham's Complete Medium	Wickerham's Minimal Medium
glucose	20	20	20
yeast extract	10		
tryptone	5		
H_3BO_3		1×10^{-5}	1×10^{-5}
$CuSO_4 \cdot 5H_2O$		1×10^{-5}	1×10^{-5}
KI		1×10^{-5}	1×10^{-5}
$FeCl_3 \cdot 6H_2O$		5×10^{-5}	5×10^{-5}
$ZnSO_4 \cdot 7H_2O$		7×10^{-5}	7×10^{-5}
$(NH_4)_2 SO_4$		1.0	1.0
biotin		2×10^{-6}	2×10^{-6}
KH_2PO_4		0.875	0.875
K_2HPO_4		0.125	0.125
$MgSO_4 \cdot 7H_2O$		0.5	0.5
NaCl		0.1	0.1
$CaCl_2 \cdot 2H_2O$		0.1	0.1
thiamin HCl		4×10^{-4}	
pyridoxine HCl		4×10^{-4}	
uracil		0.01	
l-histidine		0.005	
l-methionine		0.005	
l-tryptophan		0.005	
inositol		0.002	
Ca pantothenate		4×10^{-4}	
adenine sulfate		0.01	

loop inoculum in 100 ml medium in 250 ml or 500 ml flasks for 18 hours on a reciprocal shaker at 30°, then harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 6.6). For growth studies, cells were resuspended in 50-300 ml fresh medium in 250 ml, 500 ml, or 1000 ml flasks and incubated at 30° on a Dubnoff metabolic shaking incubator or, in later experiments, on a New Brunswick gyratory water bath shaker. Rapid transfer of cultures to different media was accomplished by collecting the cells on 90 mm type B-6 membrane filters (Carl Schleicher and Schuell Co., Keene, N. H.) followed by washing with 1 liter of prewarmed medium and resuspension in new medium.

Measurement of Macromolecular Constituents

For determination of total protein, 5 ml cell samples were washed with 0.1 M phosphate buffer (pH 6.6), hot 5% perchloric acid, and finally with 95% ethanol. Protein was extracted from the residue with 1N NaOH for 10 minutes at 100°. Protein in the extract was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

For determination of total DNA, 5 ml cell samples were washed with phosphate buffer (0.1 M, pH 6.6), followed by cold 5% trichloroacetic acid. Lipid was extracted twice with hot 95% ethanol, and the residue was evaporated to dryness under vacuum. DNA was

determined in the residue using a modified version of Kissane and Robins fluorometric procedure (Kissane and Robins, 1958). To each sample, 200 μl of 3, 5-diaminobenzoic acid reagent was added; samples were incubated at 60° for 30 minutes, then 5 ml 0.6 N perchloric acid was added to each tube. Insoluble material was removed by centrifugation and fluorescence of the supernatant was determined using an Aminco fluoro-microphotometer with a primary wavelength of 405 nm and a secondary wavelength of 520 nm. Calf thymus DNA was used as standard.

Total RNA was determined either colorimetrically or by incorporation of adenine-8-^{14}C. For colorimetric determinations, 5 ml cell samples were washed twice with cold 0.1 M phosphate buffer (pH 6.6), three times with cold 5% trichloroacetic acid, and twice with 95% ethanol. Nucleic acids were extracted from the residue with 5% perchloric acid at 70° for 15 minutes; RNA in the extract was determined by the orcinol method (Mejbaum, 1949) using yeast RNA as standard. For measurement of incorporation of adenine-8-^{14}C into RNA, 0.5 ml cell samples were suspended in 0.5 ml cold trichloroacetic acid to a final concentration of 5%; cells were collected on type B-6 membrane filters and washed with approximately 15 ml cold 5% trichloroacetic acid. Filters were placed in scintillation vials and dried; 5 ml scintillation fluid (3.0 g 2, 5 diphenyloxazole + 0.1 g 1, 4-bis-[2-(5-phenyloxazolyl)]-benzene per liter toluene) was

added and radioactivity was determined using a Model 3000 Packard Tri-Carb Liquid Scintillation Spectrometer.

Growth of cultures was followed by determination of total cell number using a Model F Coulter Counter equipped with a 100 μ aperture. Cell samples were diluted 1:100 or 1:200 with filtered 0.9% saline solution containing 0.2% formalin. Since cells of strain 5015-D do not dissociate after division but tend to form aggregates, diluted cell samples were subjected to a 30 second period of sonication prior to counting, using a Model W-140-C Branson cell disruptor at 100 watts output. This treatment disrupted cell clumps without cell breakage or separation of buds from parent cells. In some experiments, growth was followed by the increase in optical density of the culture at 520 nm using a Beckman Model DU spectrophotometer.

Isolation and Analysis of Pulse-Labeled RNA

For analysis of RNA pulse-labeled during logarithmic growth or under shift-up conditions, cell growth and label incorporation were terminated by pouring the culture over crushed ice. Cells were harvested by centrifugation at 3000 r.p.m. for 5 minutes at 0°, then washed three times with cold 10 mM tris buffer (pH 7.0) containing 5 mM $MgCl_2$. Cells were resuspended in 2 ml cold tris-$MgCl_2$ buffer, and disrupted with an Eaton press (Eaton, 1962) at 10,000 lbs. pressure. Sodium dodecylsulfate (2%) was added to

the extract to a final concentration of 0.2%, followed by an equal volume of phenol saturated with tris-$MgCl_2$ buffer. The emulsion was centrifuged at 10,000 r.p.m. for 10 minutes at 0°, and RNA was precipitated from the aqueous (top) layer by the addition of 2 volumes of cold 95% ethanol. The precipitate was washed with 5 ml cold 95% ethanol and dissolved in 2 ml cold tris-$MgCl_2$ buffer. For density gradient centrifugation, 0.3 ml of the RNA solution was layered on a 3.7 ml linear sucrose gradient (5% to 20% in 5 mM $MgCl_2$). Gradients were centrifuged at 37,000 r.p.m. for 6 hours at 4° using a Beckman Model L2 preparative ultracentrifuge with an SW-56 titanium rotor. Five-drop fractions were collected by puncturing the bottom of the tube. To each fraction, 100 μg of bovine serum albumin was added, and RNA was precipitated by the addition of 1 ml cold 5% trichloroacetic acid containing 5 μg adenine/ml. Fractions were collected on type B-6 membrane filters and washed with about 15 ml cold 5% trichloroacetic acid containing 5 μg adenine/ml. Filters were dried and radioactivity was determined as described above.

Materials

3,5-diaminobenzoic acid was obtained from commercial sources. For DNA determinations, the reagent was prepared by dissolving 0.3 g of diaminobenzoic acid per 1.0 ml 4N HCl; this solution was decolorized with Norit A decolorizing carbon in three to

five steps until the reagent reached a pale yellow color. In later experiments, a purified grade of 3, 5-diaminobenzoic acid dihydrochloride was used; 0. 44 g of the dihydrochloride was dissolved per milliliter of distilled water and used without further purification.

For inhibitor studies, 5-fluorodeoxyuridine (FUDR) was obtained as a gift from Hoffman-La Roche, Inc., Nutley, New Jersey.

All chemicals unless noted otherwise were of the highest purity available and were used as obtained from commercial sources.

RESULTS

Total Adenine-^{14}C Incorporation as a Measure of Total RNA Synthesis

Since some difficulty was encountered in achieving reproducible results using the orcinol method to determine total RNA content in growth studies, it was decided to use incorporation of adenine-8-^{14}C as a measure of total RNA synthesis. An overnight culture of strain 5015-D grown in Wickerham's minimal medium supplemented with methionine, tryptophan, uracil, and adenine was transferred to medium of the same composition but containing 5 μg adenine-^{14}C/ml. After 3 hours and 6 hours of aerobic incubation, samples were taken to determine total trichloroacetic acid-precipitable radioactivity and radioactivity released by 12-hour incubation in 1N NaOH. The results showed that 2.9% of the trichloroacetic acid-precipitable radioactivity was resistant to 1N NaOH. The ratio of NaOH resistant radioactivity to acid-precipitable radioactivity is in good agreement with the DNA/RNA ratio of cells grown in supplemented Wickerham's minimal medium as determined by colorimetric determinations of RNA and DNA. In a second experiment, an overnight culture of 5015-D grown in Wickerham's complete medium was transferred to Wickerham's complete medium with adenine-^{14}C and incubated at 30 C. After 4 hours incubation, samples were taken

to determine total trichloroacetic acid-precipitable radioactivity and the radioactivity released by incubation in 5% perchloric acid at 90 C for 20 min. Results showed that 0.9% of the total cold acid-precipitable counts were resistant to hot perchloric acid. These experiments suggest that about 98% of the total cold acid-precipitable radioactivity can be accounted for as RNA, and in the remaining experiments, incorporation of adenine-^{14}C into the cold acid-precipitable fraction was considered to reflect total RNA synthesis. Measurement of DNA synthesis by NaOH-resistant incorporation of labeled adenine gave ambiguous results either by acidification of the NaOH digestion or by neutralization followed by adsorption of DNA to membrane filters in the presence of 6x saline-sodium citrate. The values obtained using the fluorimetric procedure of Kissane and Robins were more consistent, and this method was used in succeeding experiments.

Macromolecular Constituents of Strain 5015-D at Different Growth Rates

In order to determine the RNA, DNA, and protein content of strain 5015-D at different growth rates, overnight cultures of yeast grown in Wickerham's minimal medium supplemented with methionine, tryptophan, adenine, and uracil, Wickerham's complete medium or broth medium were washed and resuspended in fresh media. The cultures were monitored until well into logarithmic growth, and

Table 2. Macromolecular constituents of strain 5015-D at different growth rates.

growth medium	generation time	protein		RNA[a]		DNA	
		μg/cell	% dry weight[b]	μg/cell	% dry weight[b]	μg/cell	% dry weight[b]
broth	95 min	5.12×10^{-6}	32.8	56.02×10^{-7}	35.8	1.17×10^{-8}	0.75
Wickerham's complete	200 min	3.78×10^{-6}	24.2	30.89×10^{-7}	19.8	0.88×10^{-7}	0.56
Wickerham's minimal + methionine, tryptophan, adenine, and uracil	296 min	3.83×10^{-6}	24.5	23.19×10^{-7}	14.9	0.61×10^{-7}	0.39

[a] corrected for DNA

[b] based on 1 mg dry weight cells = 6.41×10^{7} cells

samples were taken for measurement of the various constituents and for total cell counts. Results are shown in Table 2. The total amount of RNA, DNA, and protein per cell as measured by the procedures described above increased with increasing growth rate (except for protein content of cells grown in the synthetic media). The values reported here are higher than those of Ogur et al. (1962) perhas due to strain differences and different methods of analysis.

Amino Acid and Pyrimidine Starvation of Strain 5015-D

To determine the effects of amino acid and pyrimidine starvation on various cellular constituents of cultures in logarithmic growth in complete medium, a series of experiments was carried out in which increases in absorbancy, cell numbers, and RNA, DNA, and protein were measured during starvation for methionine, tryptophan, and uracil. Figure 1 shows the effect on increase in optical density at 520 nm of starvation for one or more of the required amino acids and uracil. Overnight cultures of 5015-D grown in Wickerham's complete medium containing 0.1% casamino acids were washed and resuspended in fresh medium. When cultures were in logarithmic growth, the cells were rapidly transferred to flasks of prewarmed Wickerham's minimal medium supplemented with: adenine, adenine + tryptophan, adenine + uracil, or adenine + uracil + tryptophan. In

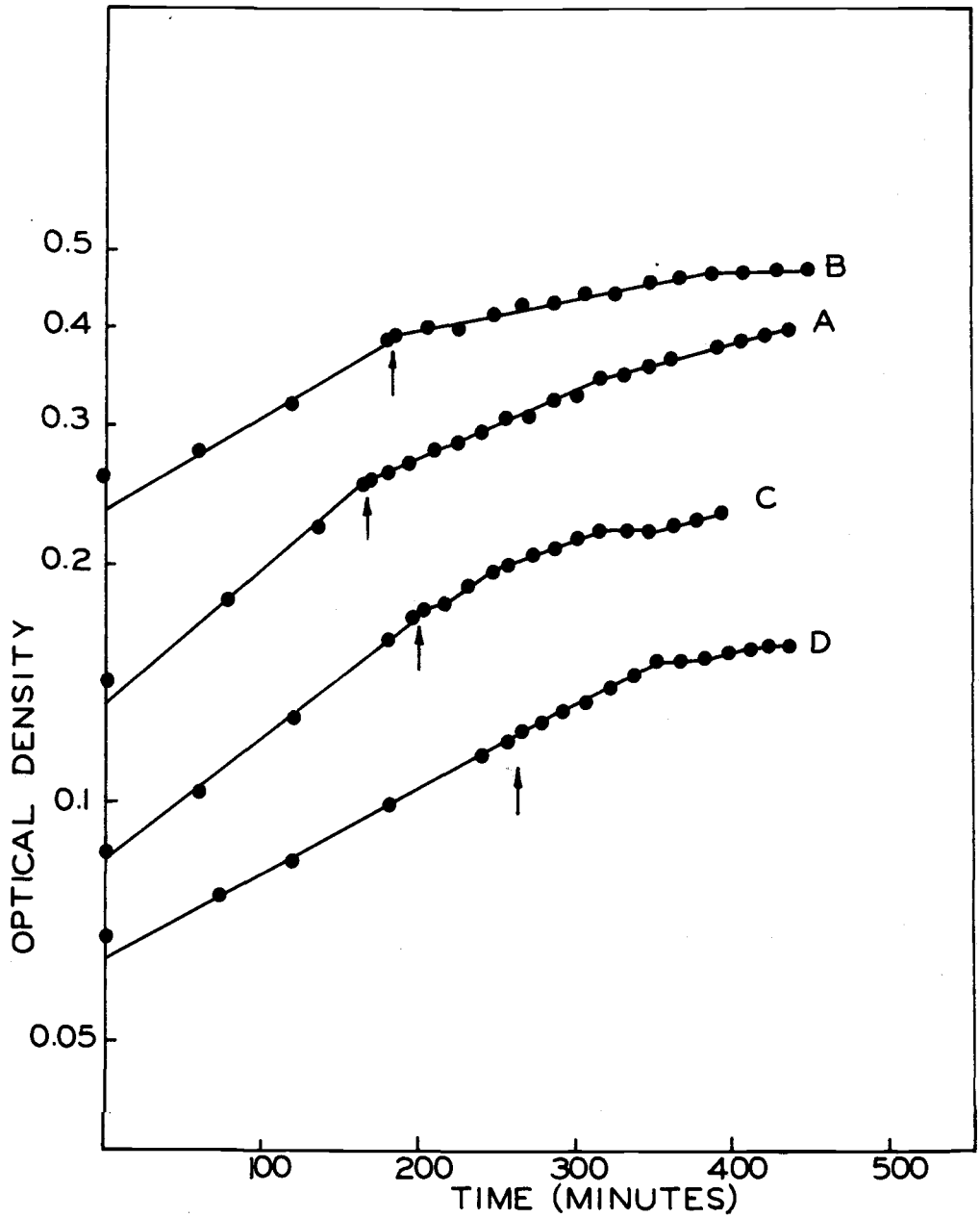

Figure 1. Effect of amino acid and pyrimidine starvation on the growth of strain 5015-D. (A) Transfer to Wickerham's minimal medium + adenine + tryptophan (starvation for methionine and uracil). (B) Transfer to Wickerham's minimal medium + adenine (starvation for methionine, tryptophan, and uracil). (C) Transfer to Wickerham's minimal medium + adenine + uracil (starvation for methionine and tryptophan). (D) Transfer to Wickerham's minimal medium + uracil + adenine + tryptophan (starvation for methionine). Arrow indicates time of transfer from Wickerham's complete medium + 0.1% casamino acids to supplemented Wickerham's minimal medium.

all cases, cell mass continued to increase at the same or reduced rate for a considerable period after transfer. After varying periods of incubation (1.5-3 hours) in the deficient media, increase in optical density assumed a lower rate or ceased entirely.

In a second experiment, the effect of amino acid starvation on increase in total cell number and total adenine incorporation was investigated. Overnight cultures of 5015-D grown in Wickerham's complete medium were washed and resuspended in fresh medium; when cultures were in logarithmic growth, they were rapidly transferred to Wickerham's minimal medium supplemented with all requirements except methionine, tryptophan, or methionine and tryptophan. Results are shown in Figures 2, 3, and 4, respectively. During methionine starvation, the rate of increase of total cell number dropped about 20% immediately after transfer to the deficient medium; after 1.5 hours of incubation the rate again decreased about 80%. Total adenine incorporation continued at a high rate for about 45 min, then gradually leveled off until, after 2.5 hours incubation, adenine incorporation ceased. Total counts incorporated during 4 hours of methionine starvation increased by 140%. During tryptophan starvation, increase in total cell number dropped to a low rate after one hour of starvation. Similarly, adenine incorporation continued at a high rate for about 30 minutes, then assumed a low rate for the balance of the experiment. During 4 hours of tryptophan

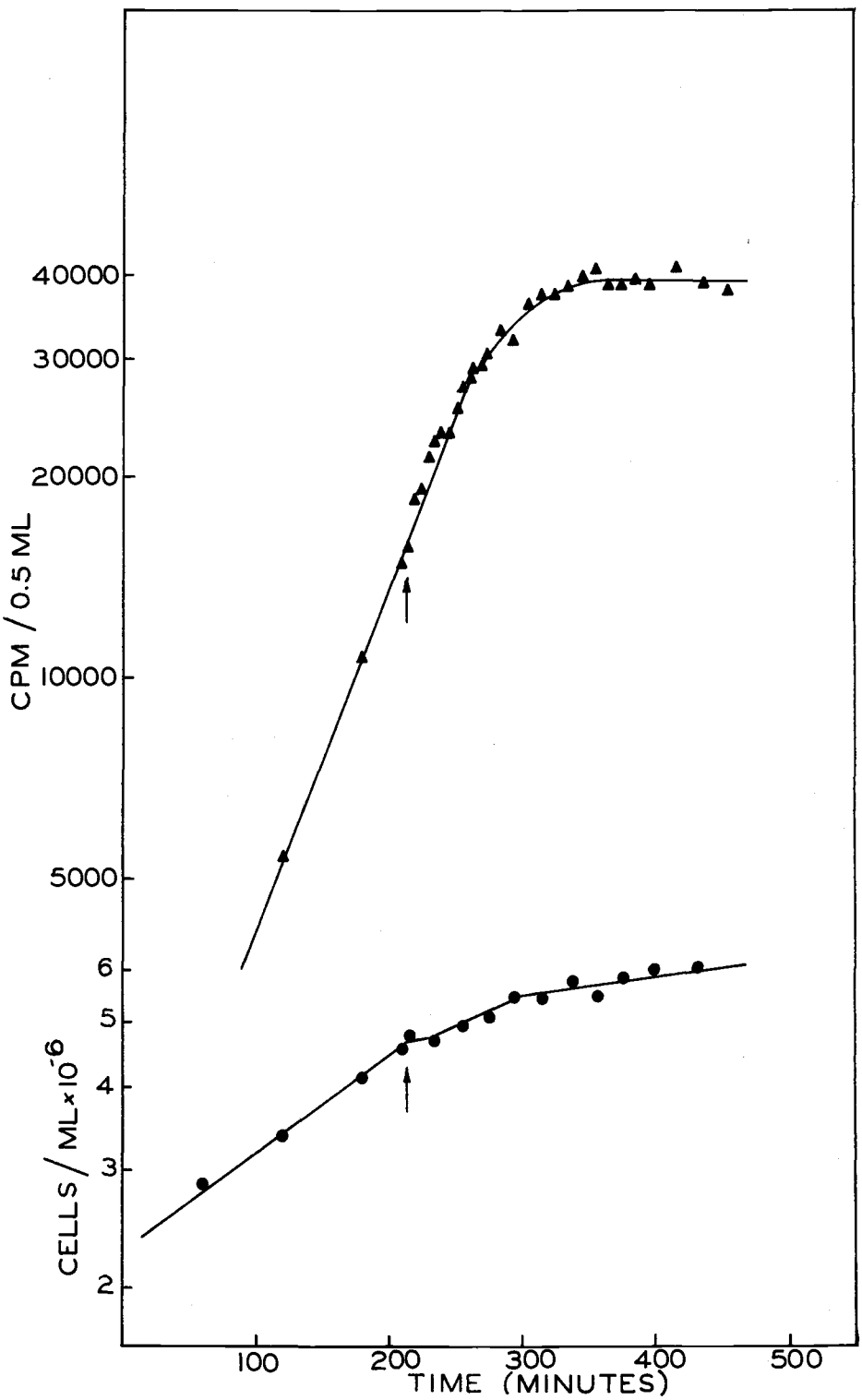

Figure 2. Effect of methionine starvation on cell number and RNA synthesis. Symbols: (●) cell count; (▲) RNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + adenine + uracil + tryptophan.

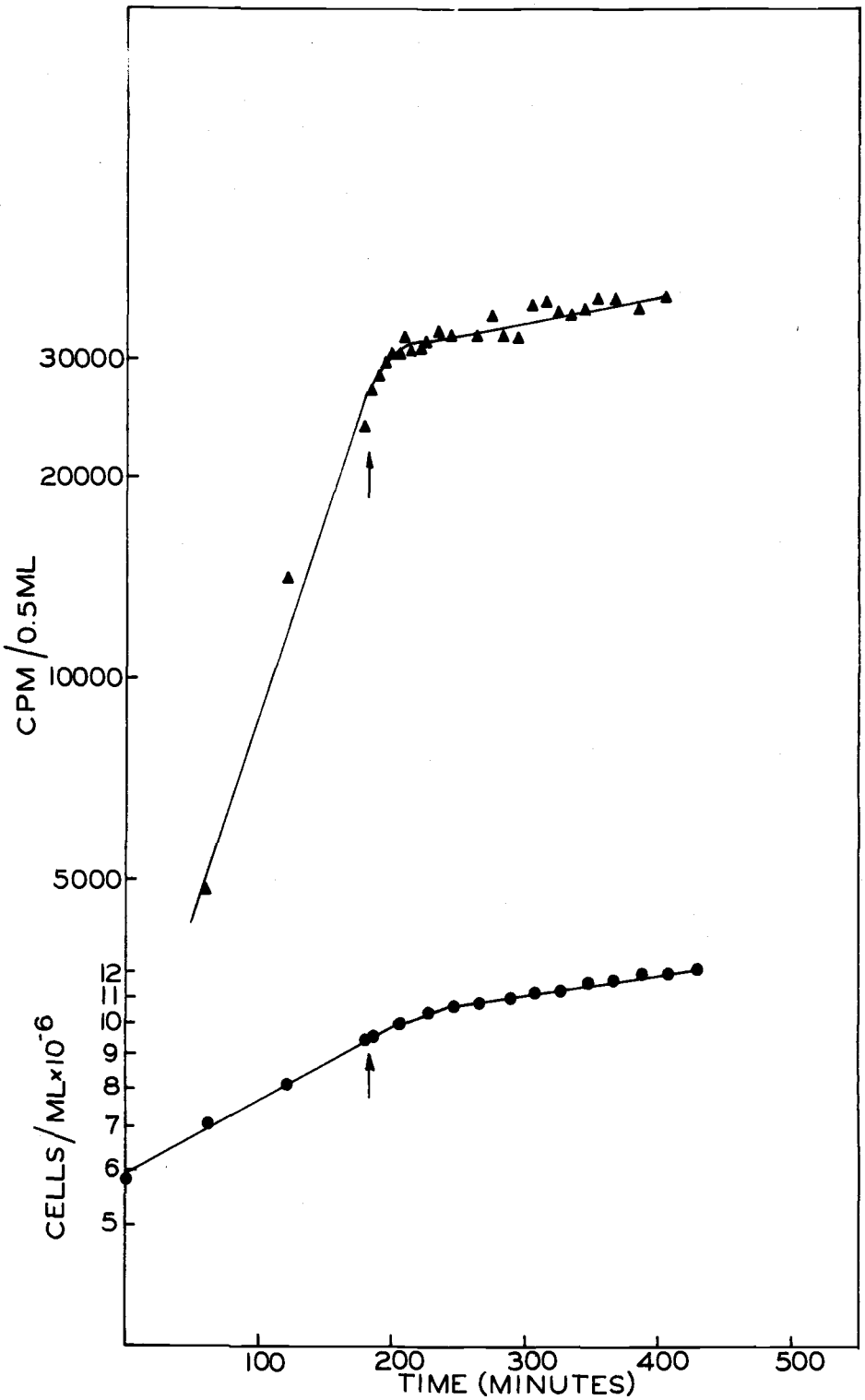

Figure 3. Effect of tryptophan starvation on cell number and RNA synthesis. Symbols: (●) cell count; (▲) RNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + adenine + uracil + methionine.

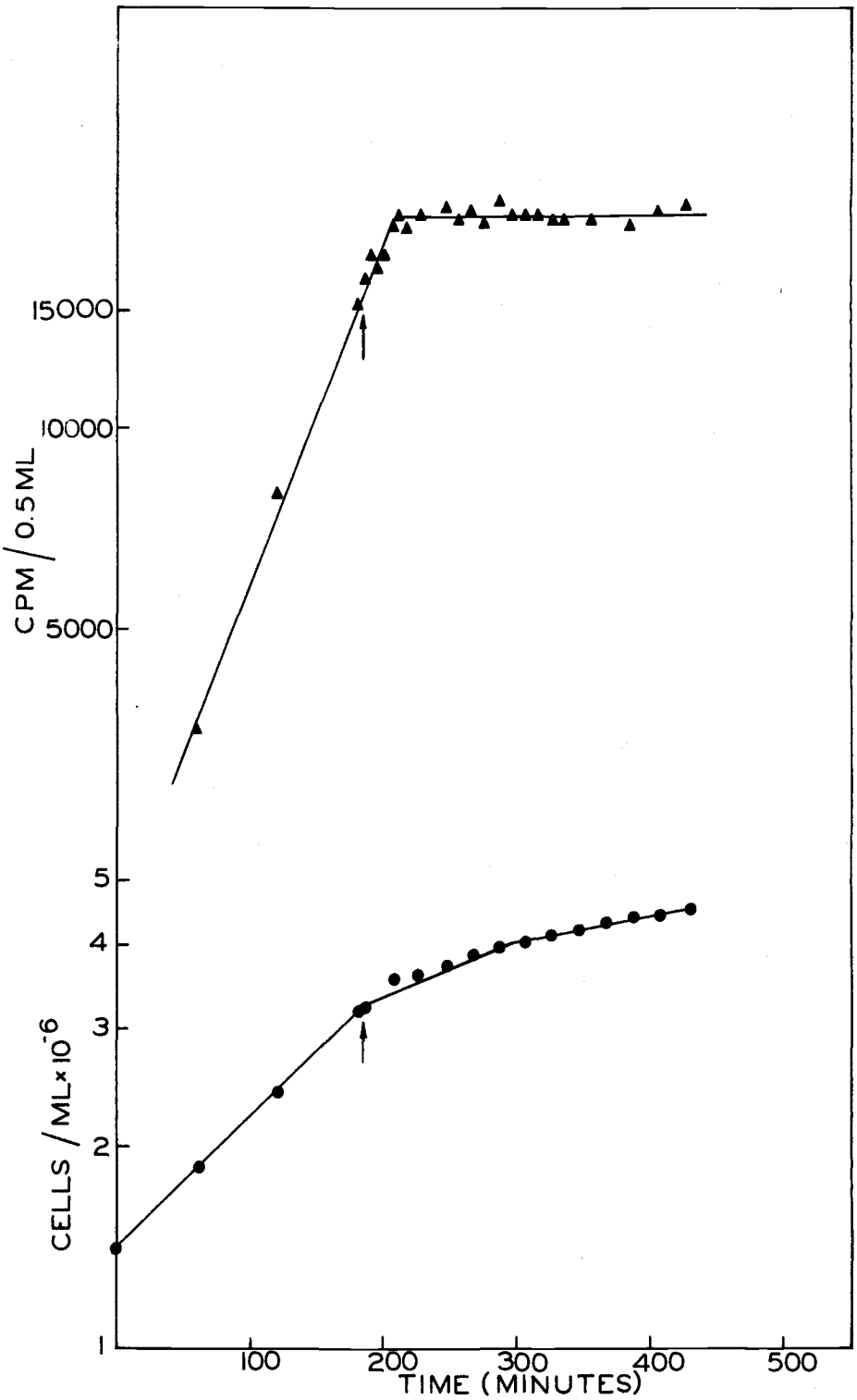

Figure 4. Effect of starvation for methionine and tryptophan on cell number and RNA synthesis. Symbols: (●) cell count; (▲) RNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + adenine + uracil.

starvation total counts incorporated increased by about 30%. During starvation for both methionine and tryptophan, increase in cell number assumed a low rate immediately after transfer; the rate was again reduced after 2 hours of starvation. The rate of adenine incorporation continued at a high rate initially; after 30 minutes of incubation, adenine incorporation ceased.

Figure 5 shows the effect of tryptophan, adenine, and uracil starvation of a culture of 5015-D grown on broth medium. An overnight culture of yeast grown in broth medium was washed and resuspended in fresh medium; when the culture was in logarithmic growth, cells were rapidly transferred to Wickerham's minimal medium with methionine. As seen from the results, increases in optical density and total RNA immediately ceased after transfer. However, DNA continued to increase at approximately the same rate during the first 30 min of starvation, then showed no further increase for the remainder of the incubation period. Total increase in DNA during starvation for tryptophan, adenine, and uracil was about 11%.

The effect of starvation of 5015-D for methionine, tryptophan, and uracil on RNA, DNA, and protein synthesis is shown in Figure 6. An overnight culture of yeast grown in Wickerham's complete medium was washed and resuspended in fresh medium; when the culture was in logarithmic growth it was rapidly transferred to Wickerham's minimal medium containing adenine. After transfer

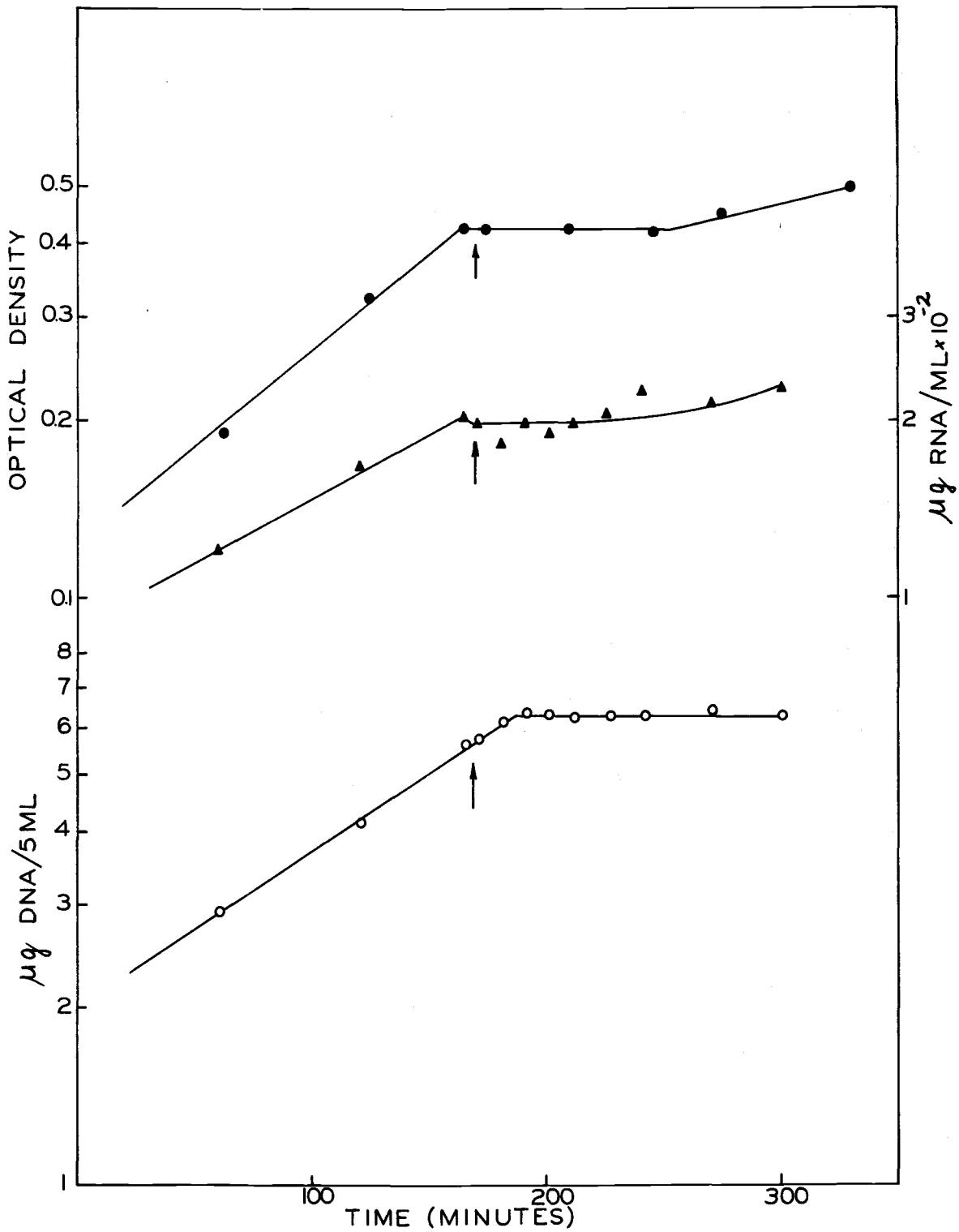

Figure 5. Synthesis of macromolecular constituents during starvation for tryptophan, adenine, and uracil. Symbols: (●) optical density; (▲) RNA synthesis; (○) DNA synthesis. Arrow indicates time of transfer from broth medium to Wickerham's minimal medium + methionine.

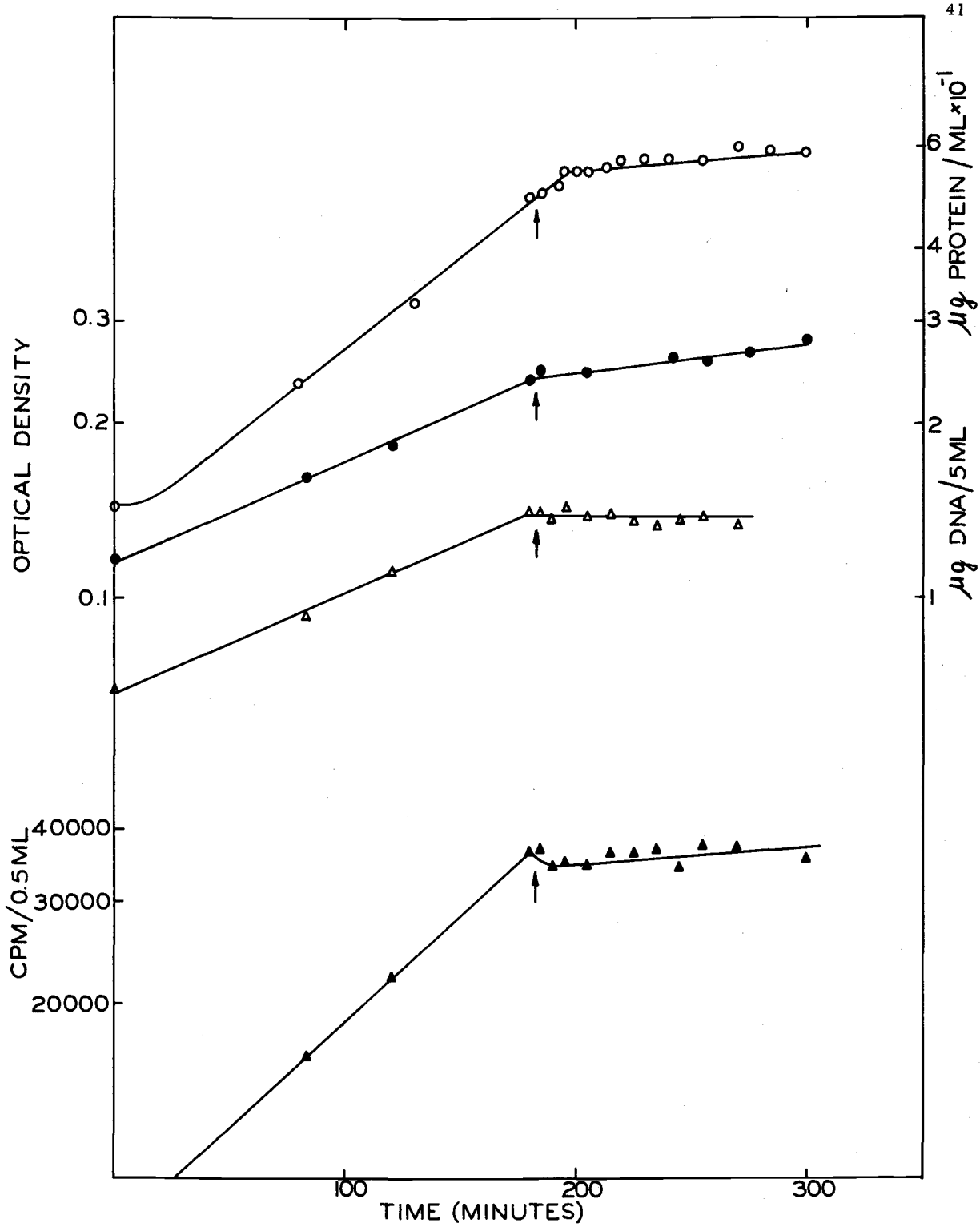

Figure 6. Synthesis of macromolecular constituents during starvation for methionine, tryptophan, and uracil. Symbols: (●) optical density; (△) protein synthesis; (▲) RNA synthesis; (○) DNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + adenine.

to the deficient medium, increase in RNA and protein ceased immediately; cell mass increased at a lower rate (as seen above). For the first 10-15 min after transfer, DNA increase seemed to continue at about the same rate, then dropped to a lower rate for the rest of the incubation period. Total increase in DNA during the incubation period was about 17%. The above experiment involved starvation for uracil as well as methionine and tryptophan. To make sure that the results did not reflect merely the exhaustion of uracil pools, a similar experiment was carried out in which cells growing in Wickerham's complete medium were harvested and rapidly transferred to Wickerham's minimal medium supplemented with adenine and uracil; in this case, the culture was starved only for required amino acids. Results are shown in Figure 7. Although the culture exhibited a lag period and was not in balanced growth at the time of transfer to the deficient medium, the behavior of the culture during amino acid starvation was similar to that in previous experiments. Total DNA increased about 10% during the first 50 minutes after transfer and showed no further increase during the remainder of the incubation period, while cell number continued to increase at a reduced rate. These results support the assumption that the effects noted in previous experiments were due to the absence of required amino acids in the medium.

In all previous amino acid starvation experiments, cultures

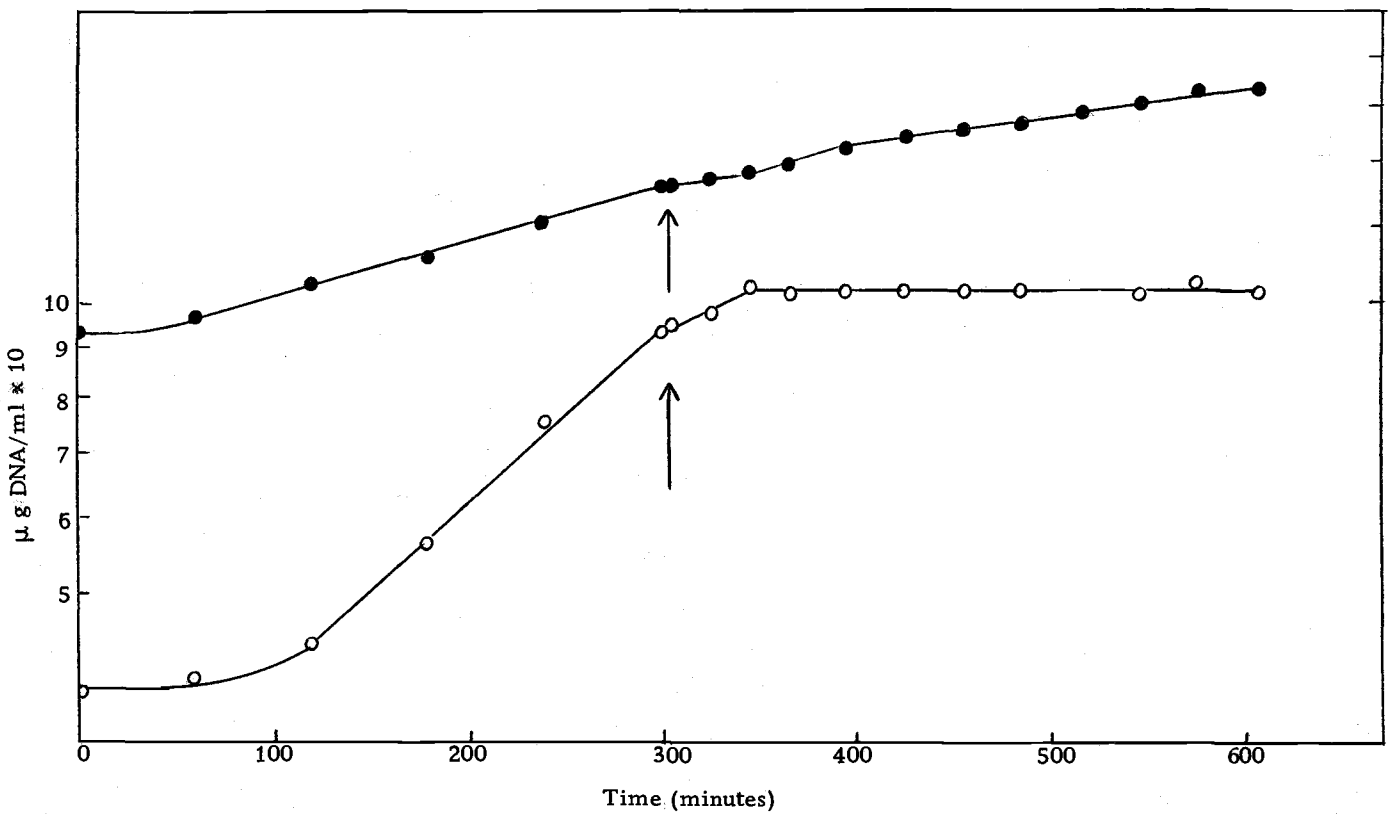

Figure 7. DNA synthesis during amino acid starvation. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + adenine + uracil.

growing on broth medium or Wickerham's complete medium were transferred to Wickerham's minimal medium lacking one or more of the required amino acids and bases. Thus the cells were subjected to a shift-down situation as well as starvation for required nutrients. Consequently, an additional set of experiments was undertaken to determine if the results observed in previous experiments were caused by conditions other than amino acid starvation. Cultures growing on Wickerham's complete medium were rapidly harvested and resuspended in Wickerham's complete medium less methionine and tryptophan. In the first experiment, increase in total DNA was followed, and radioactive adenine incorporation was followed in the second experiment. Results are shown in Figures 8 and 9, respectively. As expected, total DNA increased in a brief period following transfer to the deficient medium, and showed no further increase during the remainder of the experiment. Adenine incorporation continued during amino acid starvation in a manner similar to that shown in Figure 4, probably for the same reason (see Discussion). Continued synthesis of RNA under these conditions prevents a clear interpretation of the increase in DNA during amino acid starvation; however, the results are those expected on the basis of earlier experiments in which cultures grown on Wickerham's complete medium were subjected to amino acid starvation in Wickerham's minimal medium. The results shown in Figures 8 and 9 suggest that effects

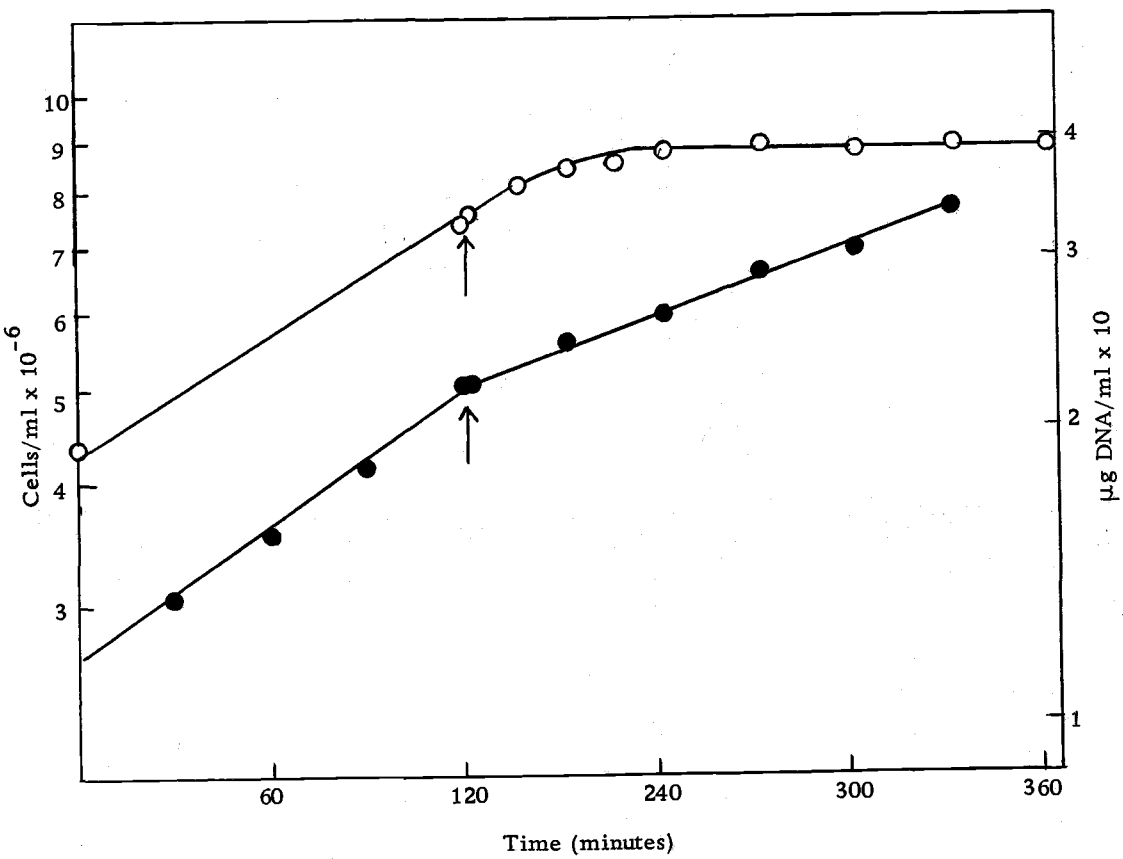

Figure 8. DNA synthesis during amino acid starvation. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's complete medium less methionine and tryptophan.

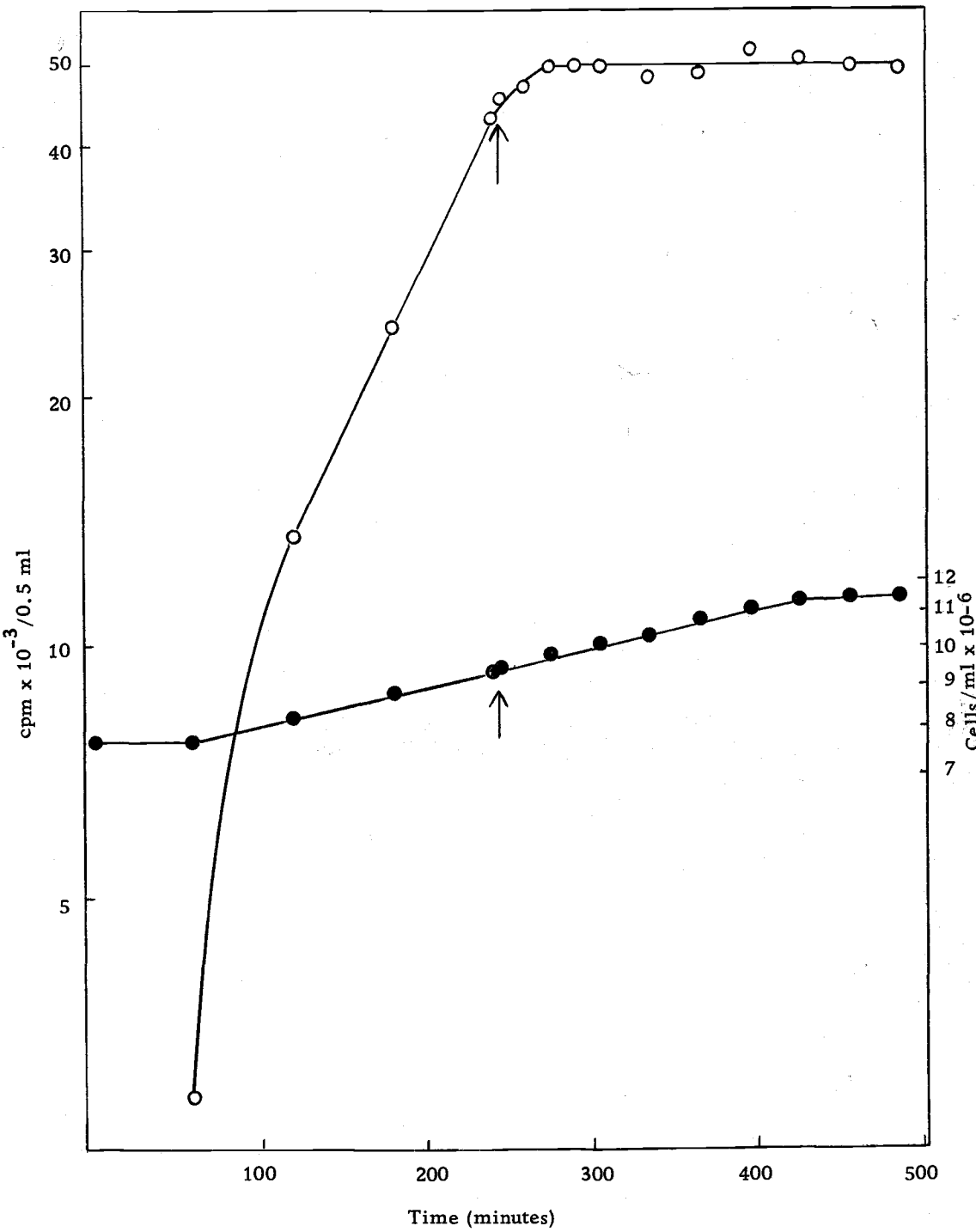

Figure 9. RNA synthesis during amino acid starvation. Symbols: (●) cell number; (○) RNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's complete medium less methionine and tryptophan.

on RNA and DNA synthesis noted earlier are indeed due to the removal of required nutrients rather than the introduction of shift-down conditions.

Figure 10 shows the effect of starvation for a carbon source on DNA synthesis. In this experiment, Saccharomyces cerevisiae strain 3701-B, a uracil auxotroph, was employed. Starvation of strain 5015-D for glucose would leave the required amino acids as alternate carbon sources, while removal of methionine and tryptophan might induce other effects; use of strain 3701-B circumvents this problem. A culture of strain 3701-B growing on Wickerham's complete medium was rapidly harvested and resuspended in Wickerham's minimal medium plus uracil and lacking glucose. Upon transfer to the deficient medium, there was an immediate cessation of cell division. In contrast to the results of amino acid starvation experiments, there is also an immediate inhibition of DNA synthesis, and no increase in DNA was observed over four hours of incubation in the absence of glucose.

The significant feature of cultures starved for methonine, tryptophan and uracil was the continued synthesis of DNA in the absence of net protein and RNA synthesis during the initial period of starvation. To obtain some information concerning the nature of this small increase in DNA, the behavior of starved cultures upon readdition of required nutrients was investigated. In the first experiment, an overnight culture of yeast growing in TCA medium was

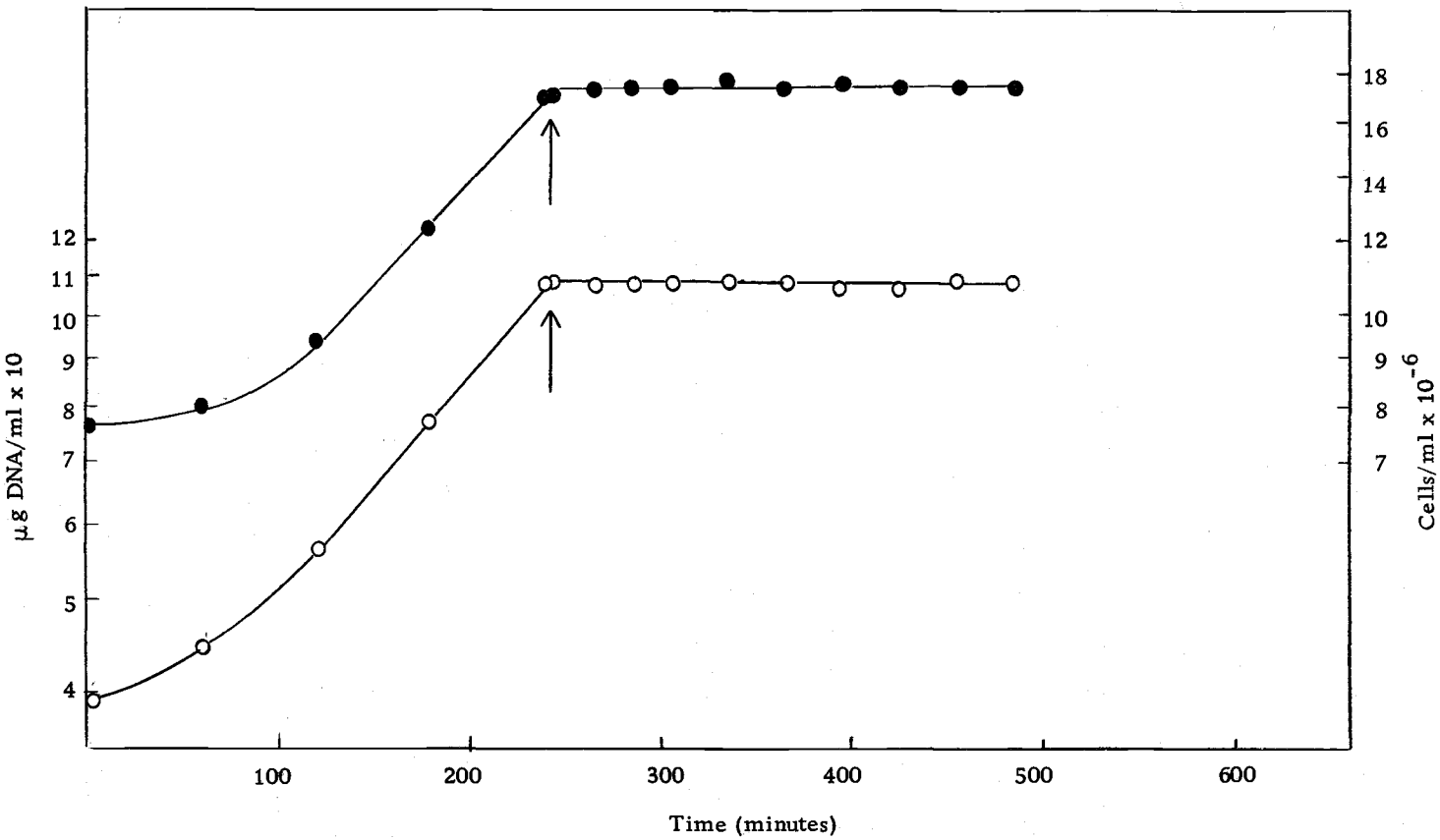

Figure 10. DNA synthesis during starvation for carbon source. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer from Wickerham's complete medium to Wickerham's minimal medium + uracil less glucose.

subjected to a twelve hour period of starvation in Wickerham's minimal medium plus adenine. The culture was then filtered and cells were resuspended in broth medium. Figure 11 shows the increase in total DNA and cell number during incubation in broth medium. There was no net increase in DNA for the first 90 minutes of incubation; at this time DNA synthesis began and rapidly assumed a rate characteristic of cells in balanced growth on broth medium. In contrast to the pattern of DNA synthesis, cell number increased slowly over the first four hours of incubation, then the rate of increase quickly rose to that characteristic of cells in balanced growth. Comparison of the time of increase in the rate of cell division with the DNA curve (dashed vertical line in Figure 11) shows that this event coincided with almost an exact doubling of the total DNA. A similar experiment is shown in Figure 12. In this case, a culture growing in Wickerham's complete medium was subjected to a twelve hour starvation period in Wickerham's minimal medium plus adenine, then returned to Wickerham's complete medium. In this experiment, there was approximately a three hour lag before DNA synthesis began at a rate characteristic of cells growing in Wickerham's complete medium. As in the previous experiment, increase in cell number remained at a low rate for the first six hours of incubation, then assumed a higher rate. As shown by the vertical dashed line, this point also coincided with an exact doubling of total DNA. In both

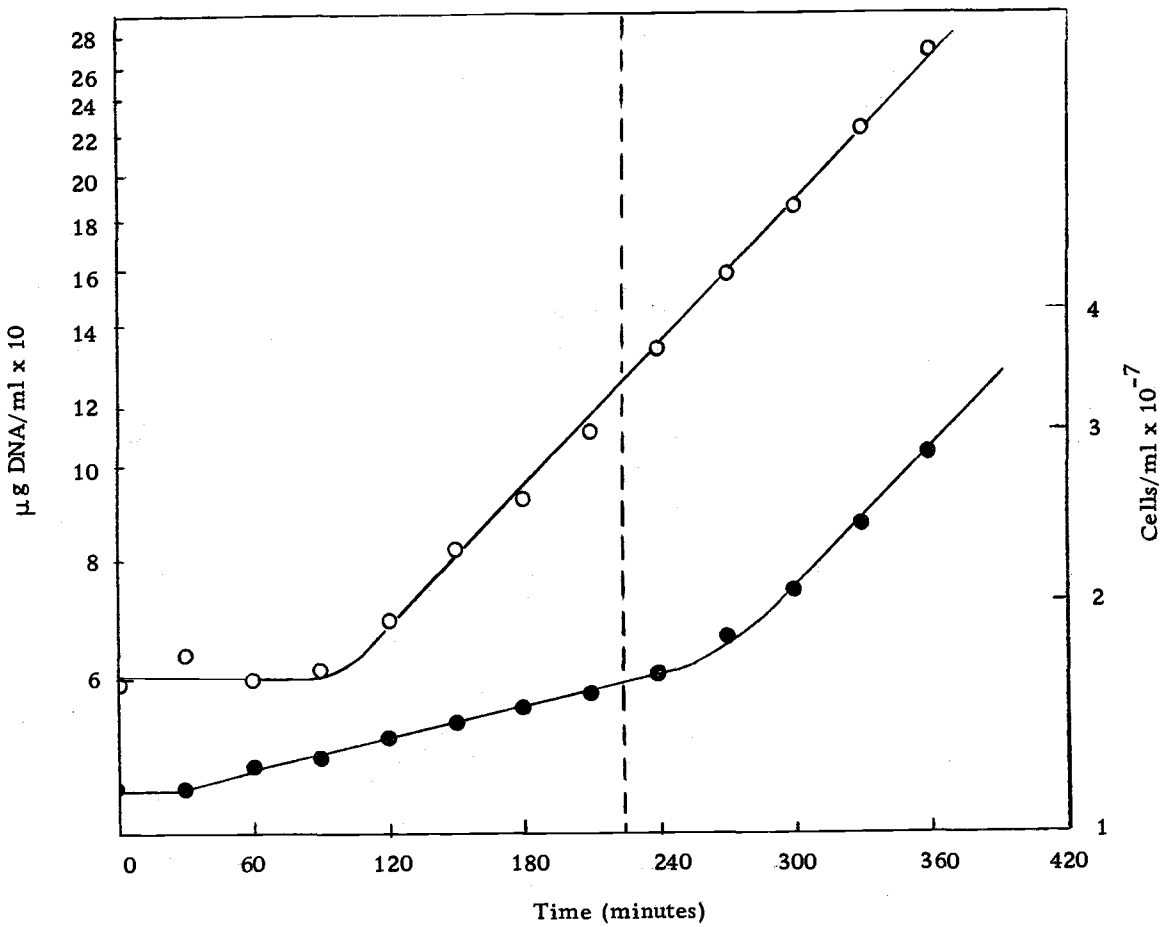

Figure 11. DNA synthesis during growth of amino acid-starved cells in broth medium. Symbols: (●) cell number; (○) DNA synthesis. The abscissa indicates time after transfer from Wickerham's minimal medium + adenine.

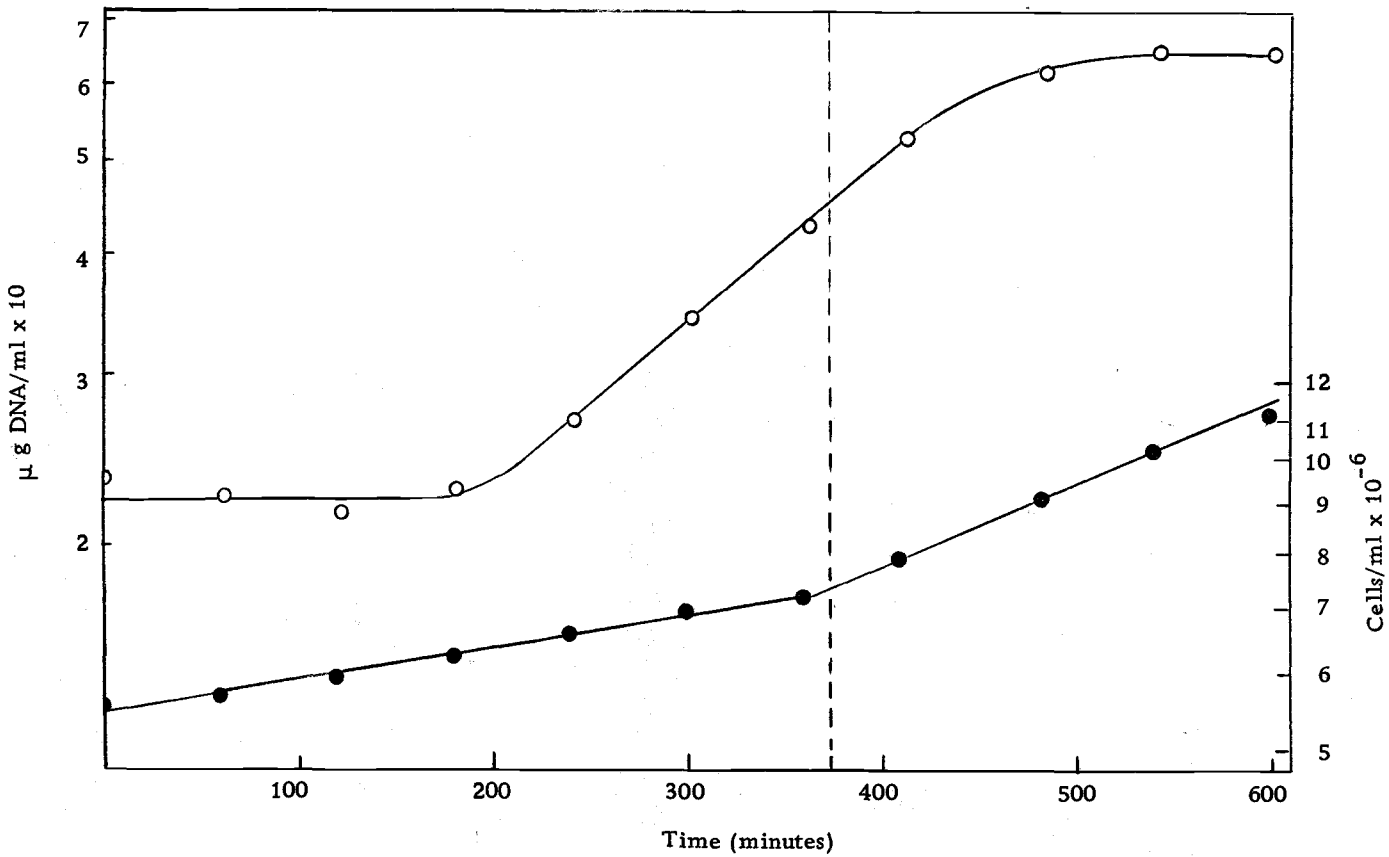

Figure 12. DNA synthesis during growth of amino acid-starved cells in Wickerham's complete medium. Symbols: (●) cell number; (○) DNA synthesis. The abscissa indicates time after transfer from Wickerham's minimal medium + adenine.

experiments, the total increase in cells per milliliter at the time of rate increase was about 30%. In contrast to the TCA-grown cells, the culture returned to Wickerham's complete medium exhibited a lag in DNA synthesis after approximately eight hours of incubation.

To determine whether the lag in DNA synthesis after readdition of amino acids and uracil was peculiar to this cellular constituent, or reflected a more general lag in cell metabolism, RNA synthesis was measured during incubation of a starved culture in Wickerham's complete medium. As shown in Figure 13, incorporation of radioactive adenine began soon after return of the culture to complete medium and continued at a high rate during the period corresponding to the lag in DNA synthesis.

To gain a better understanding of the effect of amino acid and uracil starvation on the cell, the effect of inhibition of DNA synthesis on cultures returned to complete medium after a period of amino acid and uracil starvation was investigated. Two compounds, nalidixic acid and 5-fluorodeoxyuridine (FUDR) are known to be specific inhibitors of DNA synthesis in Escherichia coli (Goss et al., 1965; Cohen et al., 1958). Although these inhibitors have been used in studies with yeast (Esposito, 1967; Mounolou and Perrodin, 1968), it has not been shown that they specifically inhibit the synthesis of

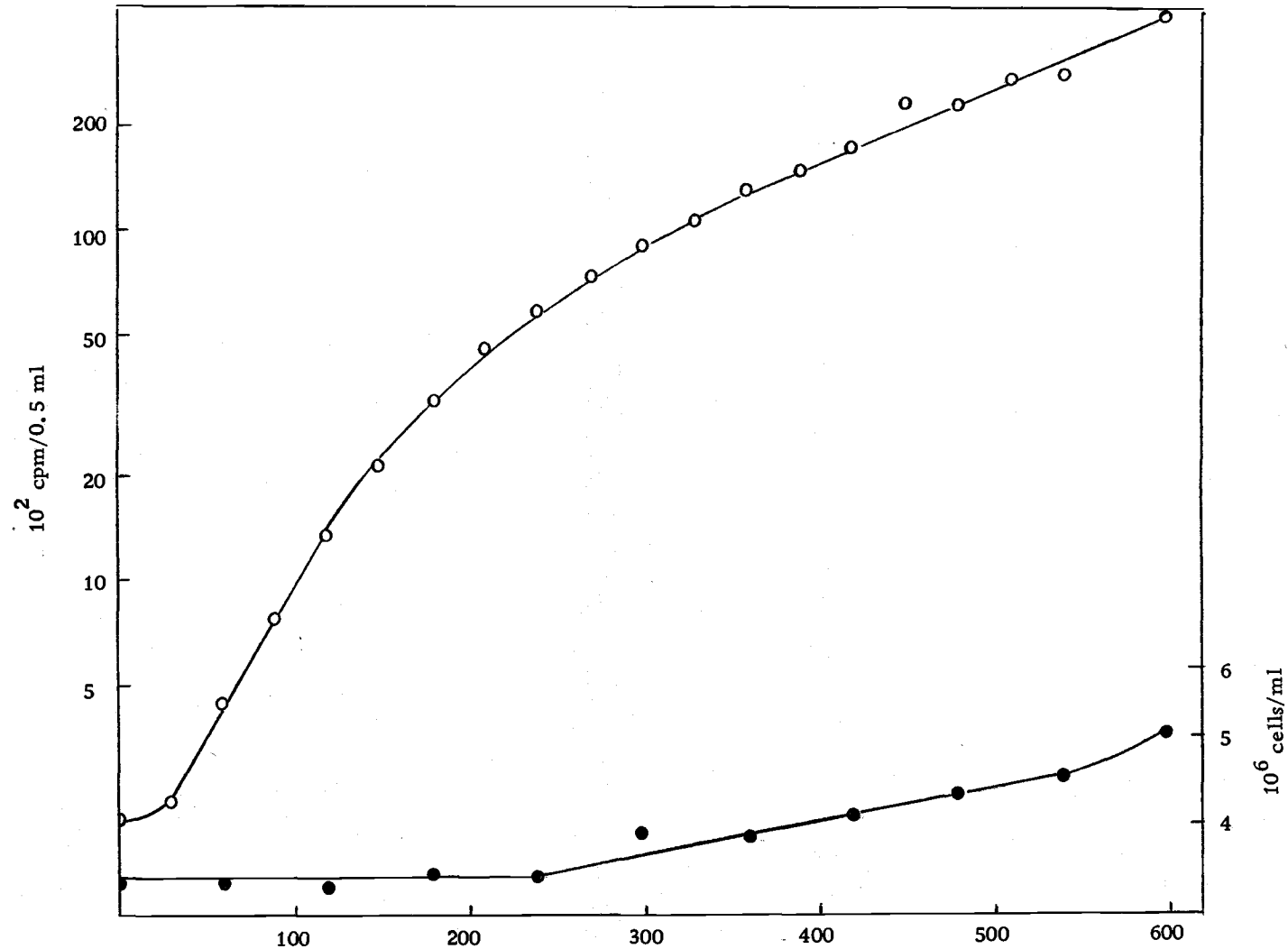

Figure 13. RNA synthesis during growth of amino acid-starved cells in Wickerham's complete medium. Symbols: (●) cell number; (○) RNA synthesis. The abscissa indicates time after transfer from Wickerham's minimal medium + adenine.

DNA. Consequently, a series of experiments was carried out to investigate the effect of these two compounds on DNA and RNA synthesis in strain 5015-D. Nalidixic acid or FUDR was added to cultures growing in Wickerham's complete medium and increases in total DNA, adenine-^{14}C incorporation, and cell number were followed. The effects of addition of FUDR to cultures growing in Wickerham's complete medium on DNA and RNA synthesis are shown in Figures 14 and 15, respectively. The rate of cell division dropped during the first 30 minutes after addition of the inhibitor, then returned to a normal value. The rate of adenine incorporation seemed to drop after addition of FUDR, but resumed at the previous rate after 3 hours. Similarly, the rate of DNA synthesis underwent a drop, then returned to a normal value. The effects of nalidixic acid on DNA and RNA synthesis are shown in Figures 16 and 17, respectively. Both DNA and RNA synthesis were only temporarily affected. Increase in total DNA stopped shortly after addition of nalidixic acid but resumed at the previous rate after 60 minutes. Adenine incorporation was affected more strongly; incorporation stopped about 30 minutes after addition of the inhibitor and did not resume for 3 hours. The rate of cell division was only slightly inhibited during the first hour after addition of nalidixic acid. Neither FUDR nor nalidixic acid brought about a permanent

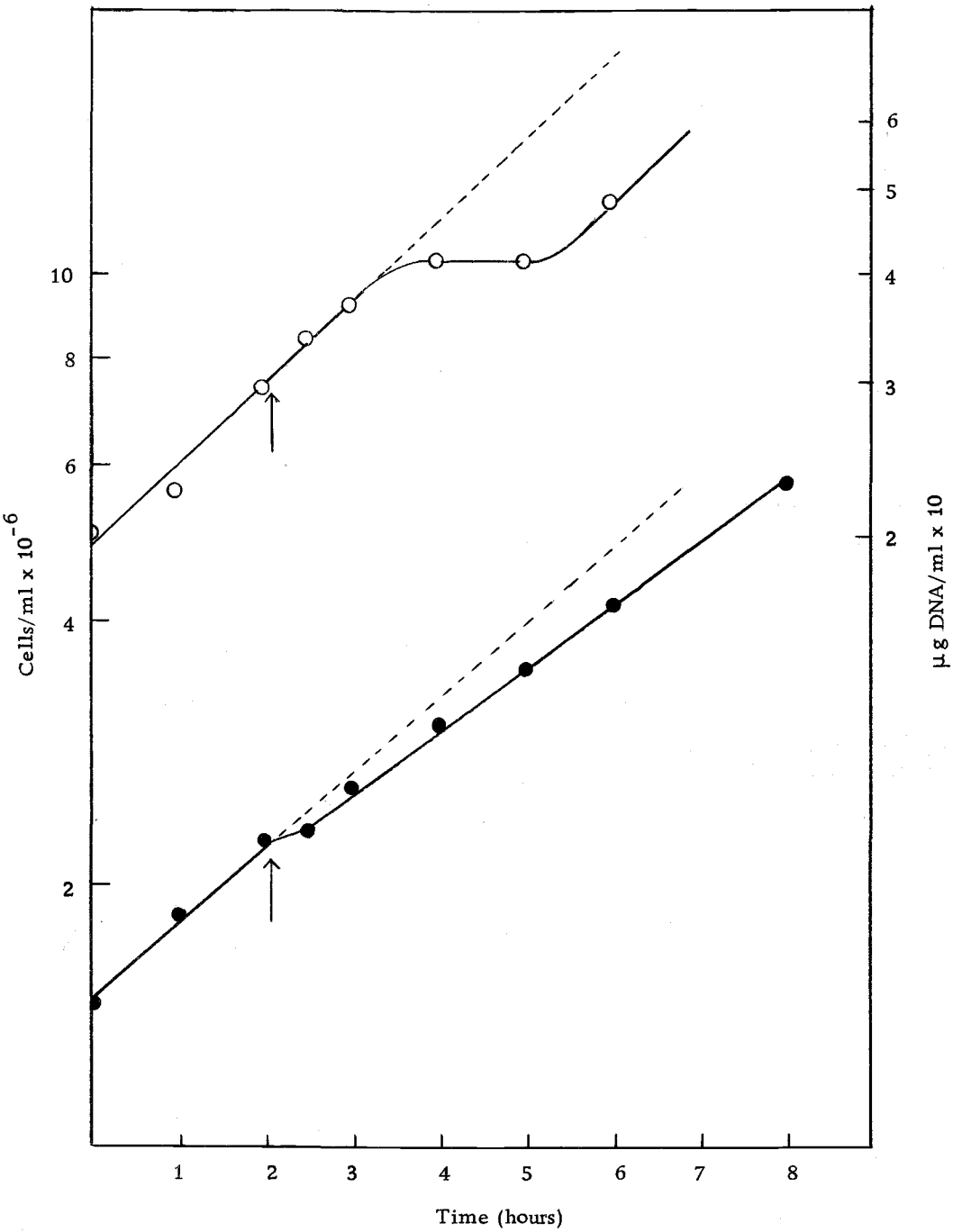

Figure 14. Effect of FUDR on DNA synthesis during growth in Wickerham's complete medium. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of addition of FUDR (1 mg/ml). Dotted line indicates control culture without inhibitor.

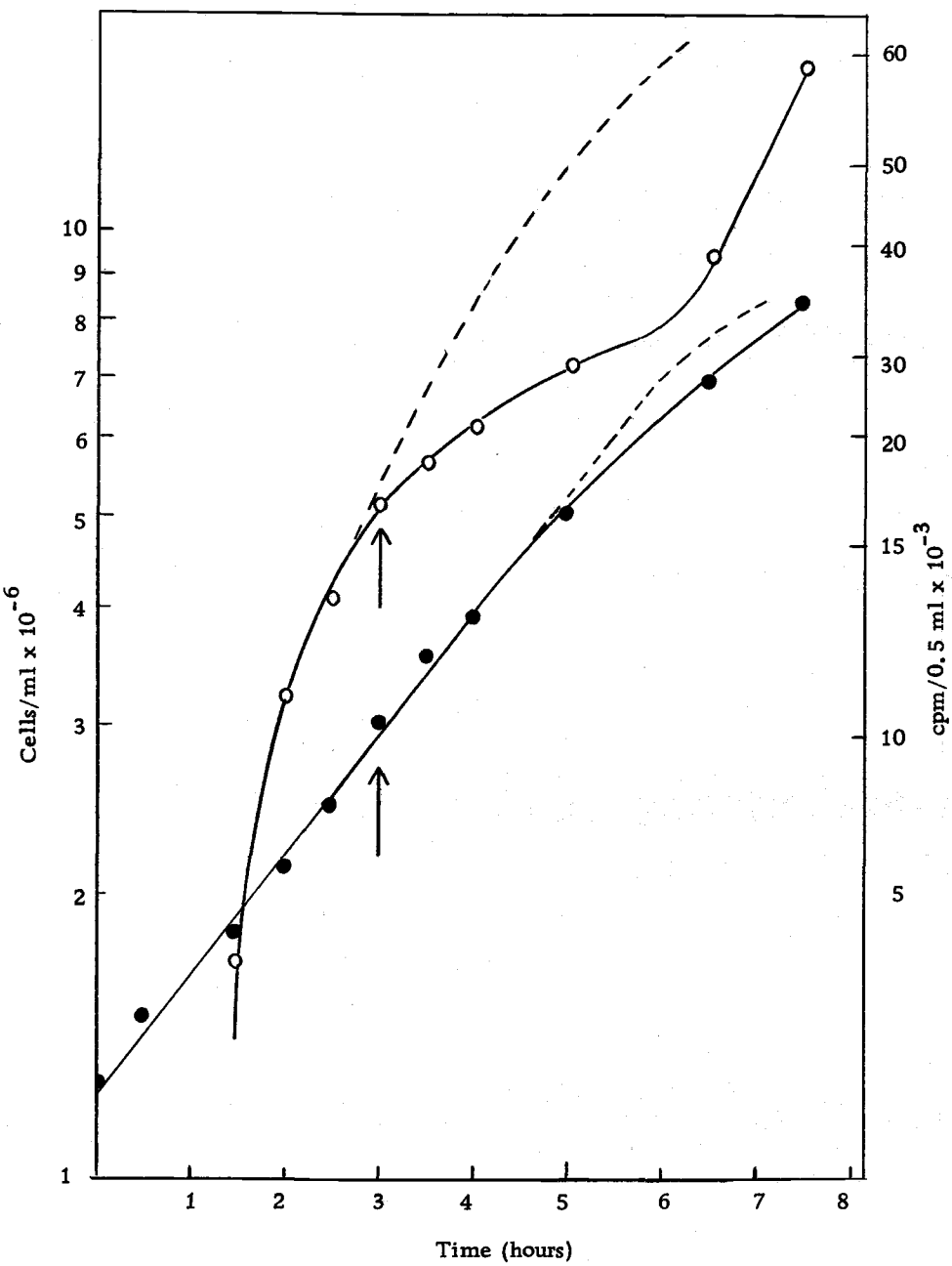

Figure 15. Effect of FUDR on RNA synthesis during growth in Wickerham's complete medium. Symbols: (●) cell number; (○) RNA synthesis. Arrow indicates time of addition of FUDR (1 mg/ml). Dotted line indicates control culture without inhibitor.

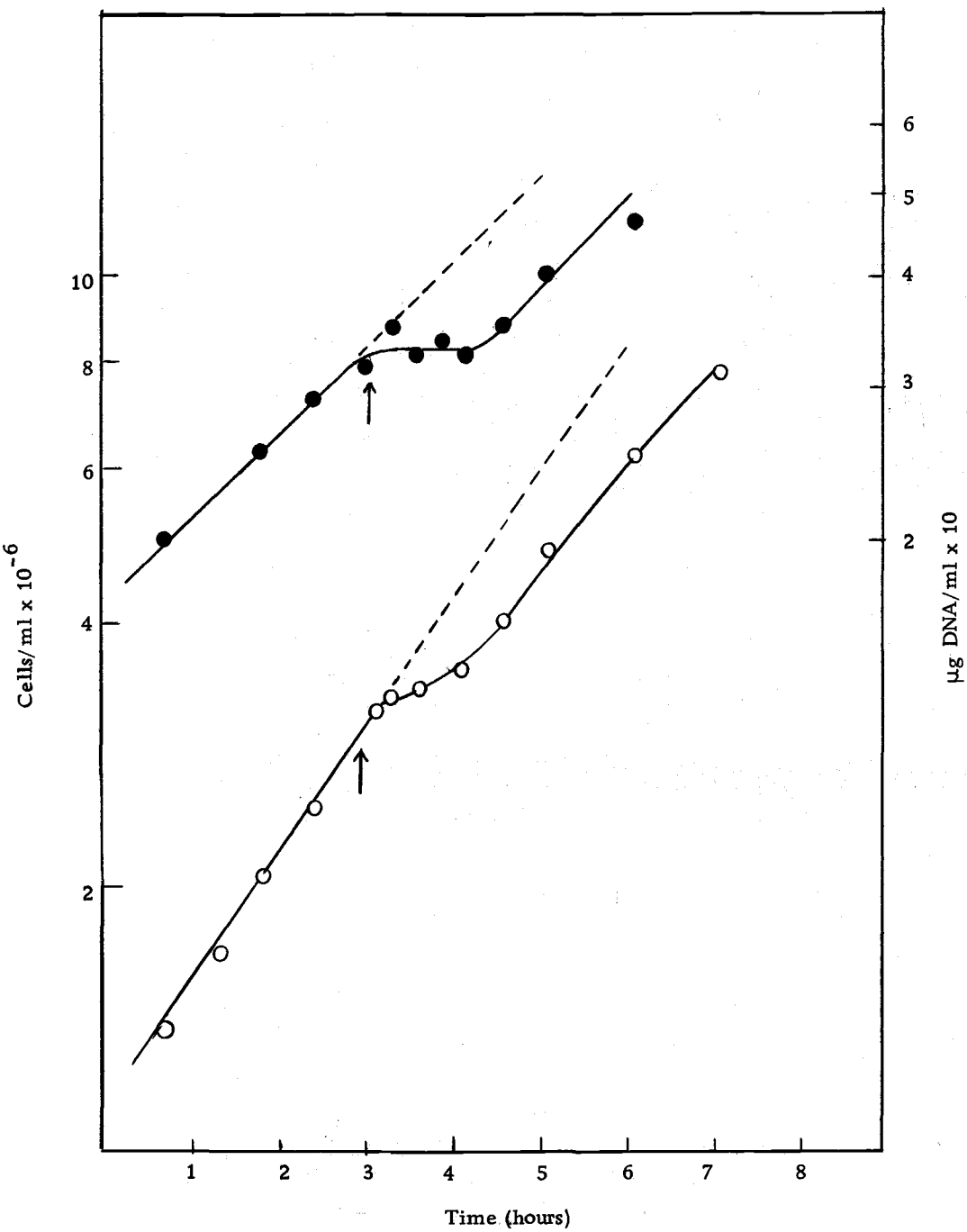

Figure 16. Effect of nalidixic acid on DNA synthesis during growth in Wickerham's complete medium. Symbols: (○) cell number; (●) DNA synthesis. Arrow indicates time of addition of nalidixic acid (50 µg ml). Dotted line indicates control culture without inhibitor.

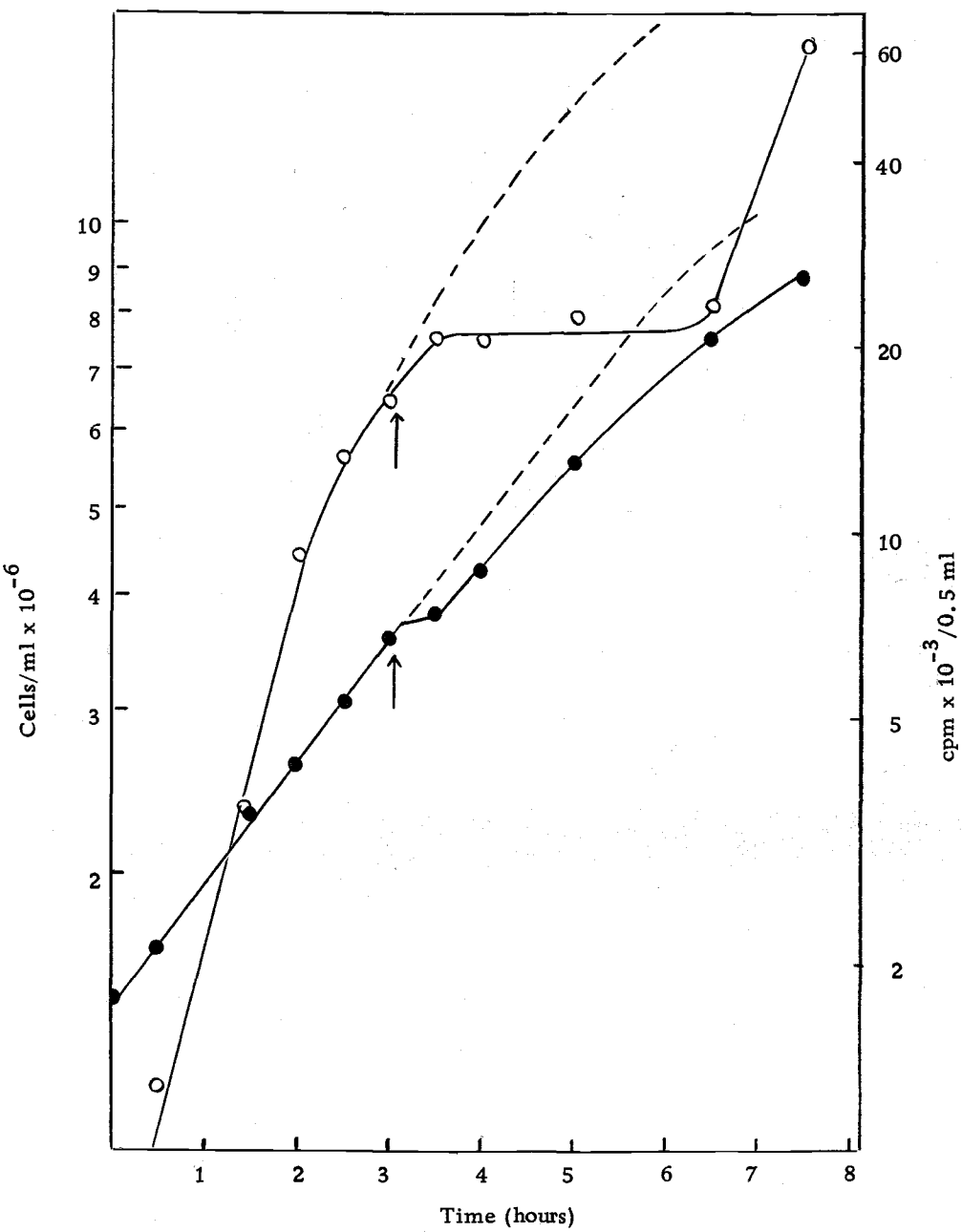

Figure 17. Effect of nalidixic acid on RNA synthesis during growth in Wickerham's complete medium. Symbols: (●) cell number; (○) RNA synthesis. Arrow indicates time of addition of nalidixic acid (50 μg/ml). Dotted line indicates control culture without inhibitor.

inhibition of DNA and RNA synthesis or cell growth, suggesting that yeast cells in logarithmic growth are only temporarily sensitive to these compounds.

The effects of transfer of amino acid and uracil-starved cultures to Wickerham's complete medium + FUDR and Wickerham's complete medium + nalidixic acid are shown in Figures 18 and 19, respectively. In these experiments, the presence of the inhibitors had little or no effect on the behavior of the cultures after return to Wickerham's complete medium. DNA synthesis resumed within 3 hours of incubation in complete medium, and underwent about 2 doublings before ceasing. Cell number increased at a low rate for the first 5-6 hours, then doubled at a higher rate before tapering off. Comparison of these results with those shown in Figure 12 shows that the presence of FUDR or nalidixic acid had no effect on DNA synthesis or increase in cell number. Final cessation of DNA synthesis and cell division occurred in both the control culture and in the cultures growing in the presence of inhibitors; this probably reflects exhaustion of an essential nutrient from the medium.

Macromolecular Synthesis during Shift-Down Conditions

The results reported in Table 2 show that the amount of DNA, RNA, and protein per cell varies as a function of the growth rate.

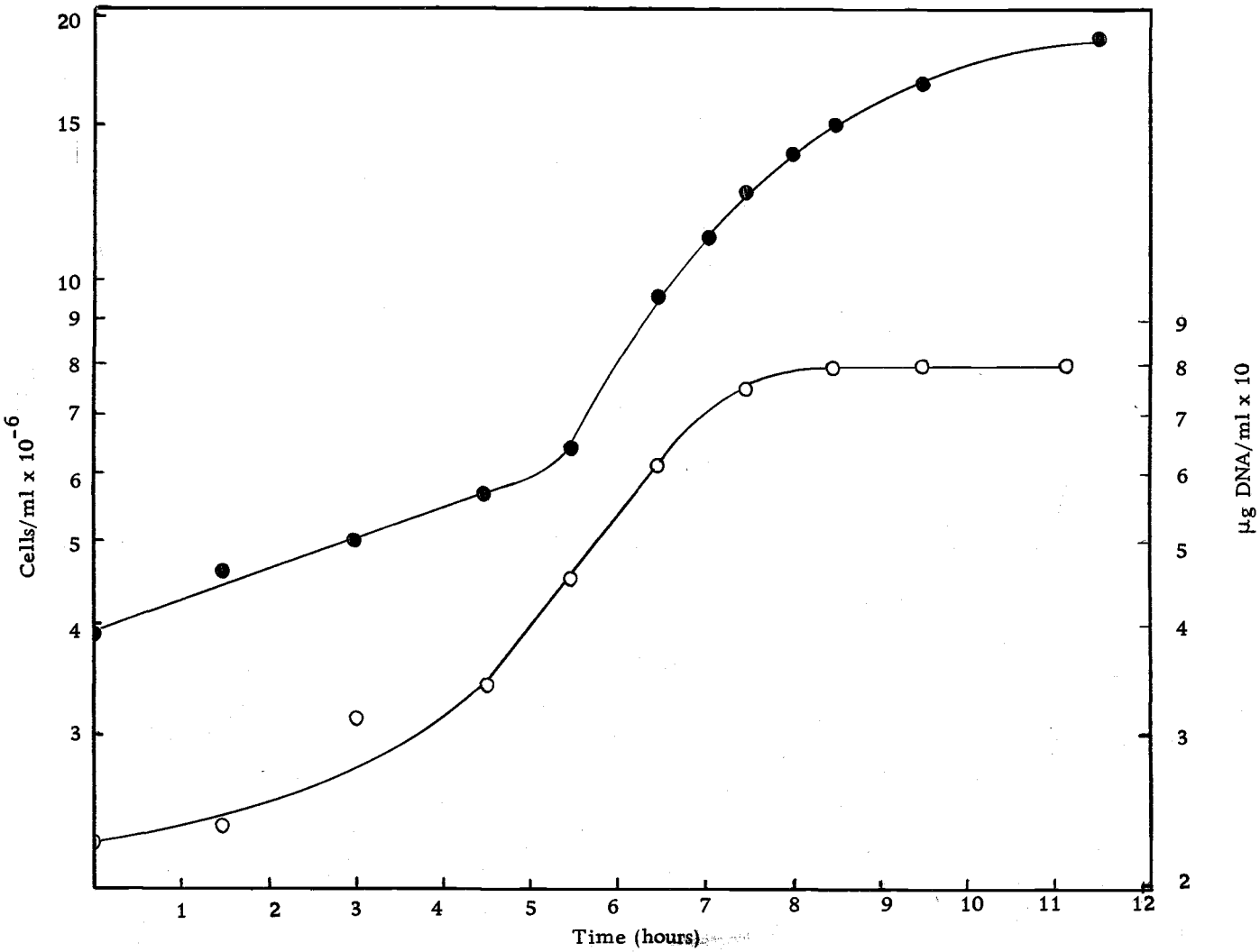

Figure 18. Effect of FUDR on amino acid-starved cells growing in Wickerham's complete medium + 1 mg FUDR/ml. The abscissa indicates time after transfer from Wickerham's minimal medium + adenine. Symbols: (●) cell number: (○) DNA synthesis.

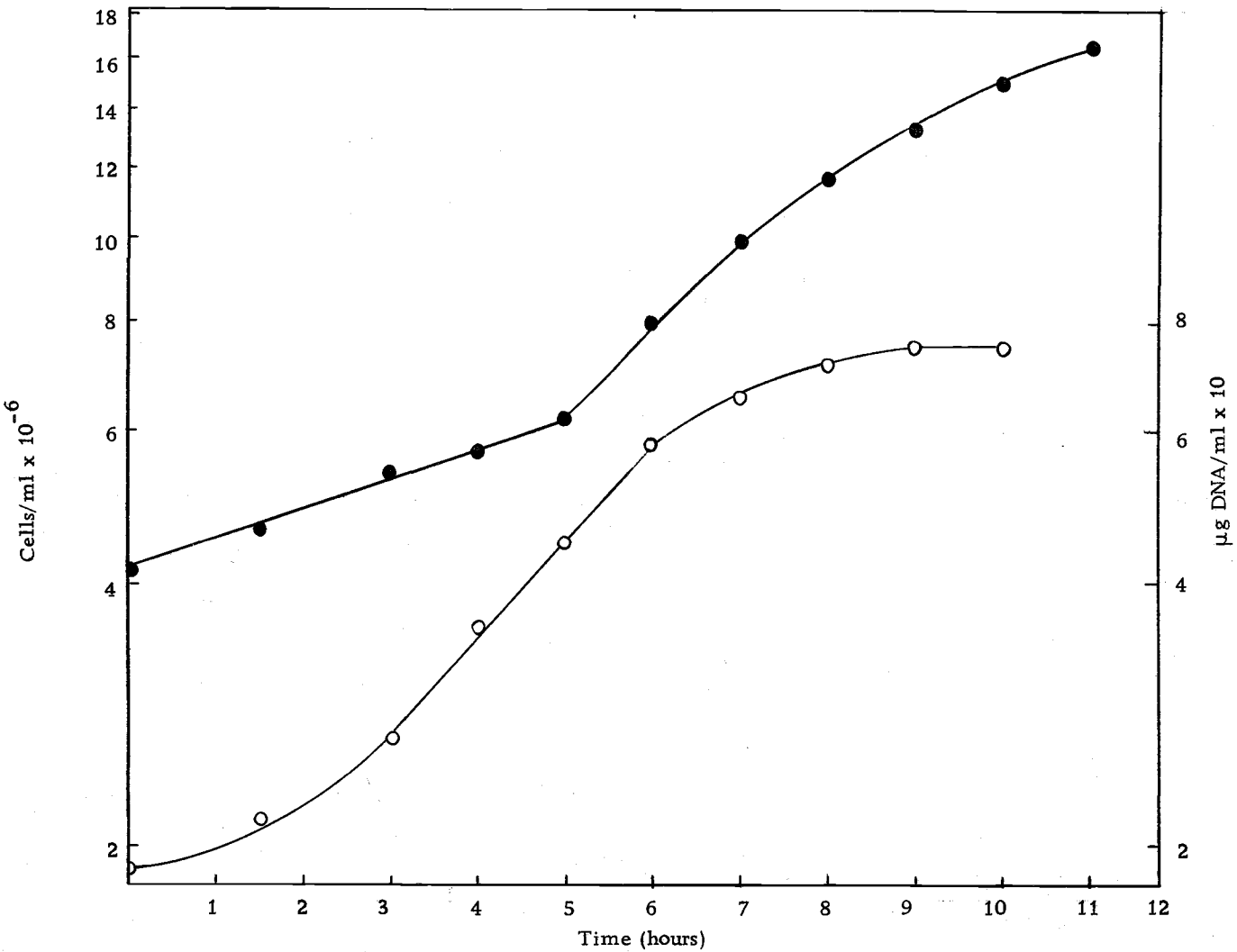

Figure 19. Effect of nalidixic acid on amino acid-starved cells growing in Wickerham's complete medium + 50 µg nalidixic acid/ml. The abscissa indicates time after transfer from Wickerham's minimal medium + adenine. Symbols: (●) cell number; (○) DNA synthesis.

On the basis of these results, the effect on macromolecular synthesis of shifting a culture growing logarithmically at one rate to a medium supporting a different growth rate was investigated. In the first case, the effect on RNA, DNA, and protein synthesis was observed during a shift-down from broth medium to Wickerham's minimal medium supplemented with methionine, adenine, tryptophan, and uracil. An overnight culture of 5015-D grown in broth medium was washed and resuspended in fresh medium. When the culture was in logarithmic growth, cells were rapidly transferred to Wickerham's minimal + methionine, tryptophan, adenine, and uracil. Results are shown in Figures 20 and 21. Increase in total cell counts showed a consistent pattern similar to that observed in the amino acid starvation experiments; the rate of increase of cell number dropped 60% and remained constant for the first 120 min after shift-down, then assumed a lower rate. The rate of adenine incorporation remained close to the pre-shift rate for the first 30 min after transfer, then gradually decreased over the next 60 min of incubation in minimal medium. During the remainder of the incubation time, adenine incorporation was roughly linear at a low rate. After transfer to minimal medium, the rate of increase in total DNA immediately dropped to about 14% of the rate in broth medium; after 60 min incubation, increase in DNA exhibited a brief lag, then continued at a reduced rate during the rest of the incubation period. Increase

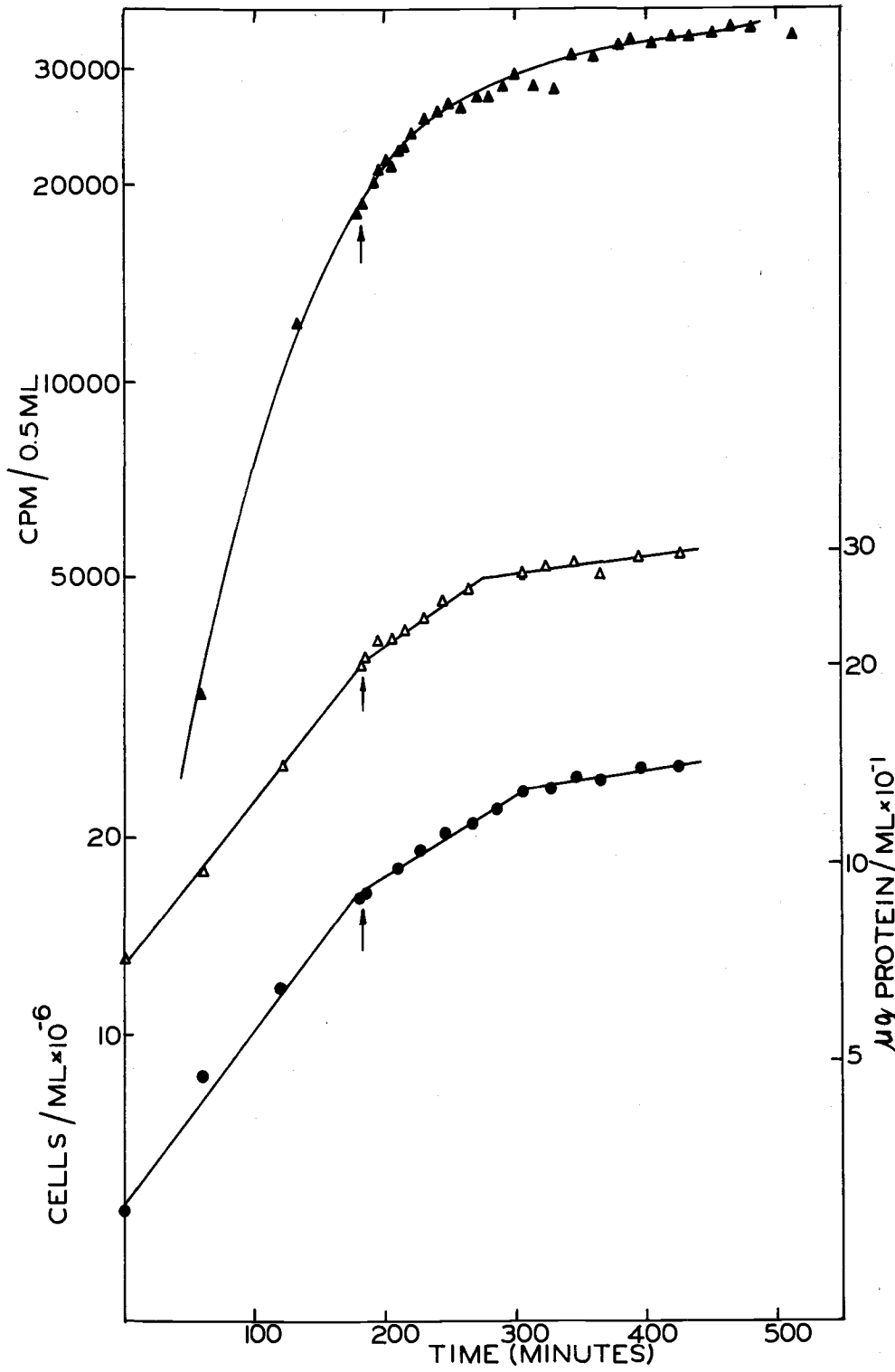

Figure 20. Shift-down from broth medium to Wickerham's minimal medium + adenine + uracil + methionine + tryptophan. Symbols: (●) cell number; (△) protein synthesis; (▲) RNA synthesis. Arrow indicates time of transfer.

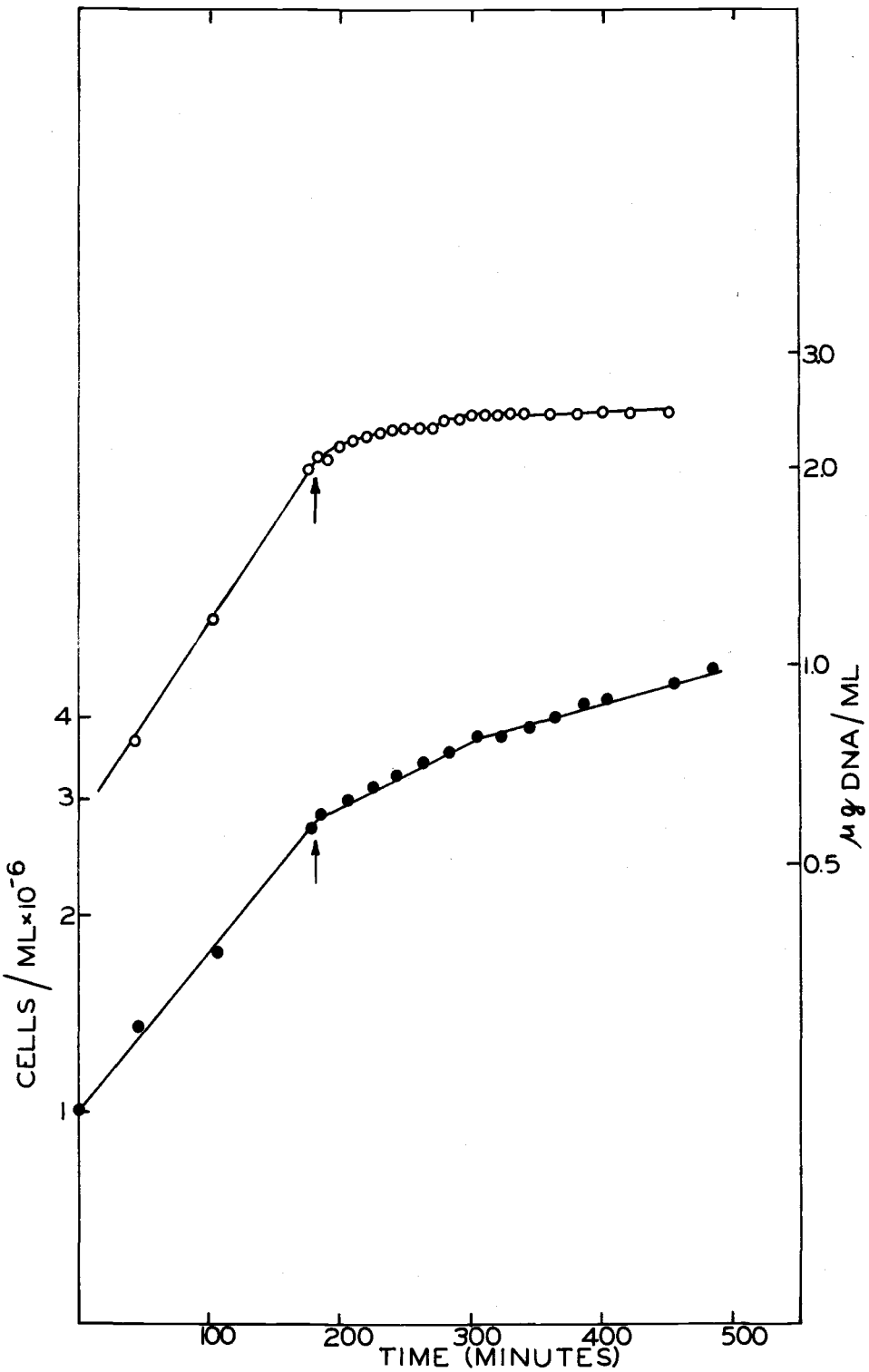

Figure 21. Shift-down from broth medium to Wickerham's minimal medium + adenine + uracil + methionine + tryptophan. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer.

in total protein during shift-down conditions resembled increase in total cell number; the rate of increase dropped immediately upon transfer, and was again reduced after two hours of incubation.

Macromolecular Synthesis during Shift-Up Conditions

In a similar set of experiments, the effect on RNA, DNA, and protein synthesis was investigated during transfer of yeast growing at a low growth rate to media supporting a rapid growth rate. Figures 22 and 23 show the results of a shift-up from Wickerham's minimal medium supplemented with methionine, tryptophan, adenine, and uracil to Wickerham's complete medium. Increase in cell numbers showed, if any, a very brief lag before being re-established at a slightly higher rate. There is some suggestion of a plateau 55 min after transfer, but the results are not unequivocal. In general, the small differences in generation times and rate of increase of cell constituents suggest that the shift in growth conditions is small. Upon transfer to Wickerham's complete, the rate of adenine incorporation immediately shifted to a slightly higher rate, and remained at that rate during the rest of the experiment; no lag in the rate of adenine incorporation during shift-up was observed. There seemed to be a 40 min period after transfer to complete medium during which the rate of protein synthesis lagged, then increased to a higher rate. The rate of DNA synthesis showed a definite 30 min lag after transfer

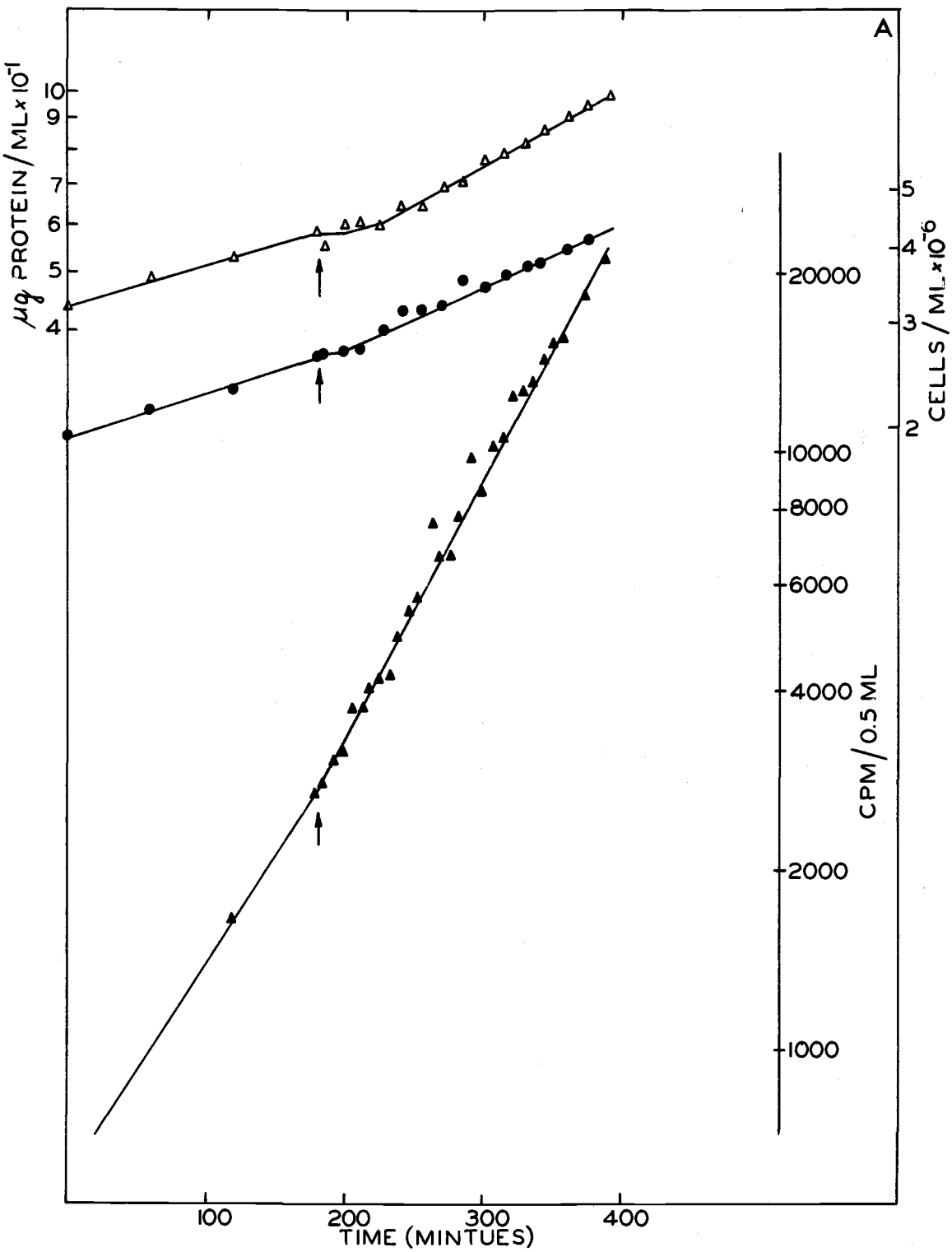

Figure 22. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to Wickerham's complete medium. Symbols: (●) cell number; (▲) RNA synthesis; (△) protein synthesis. Arrow indicates time of transfer.

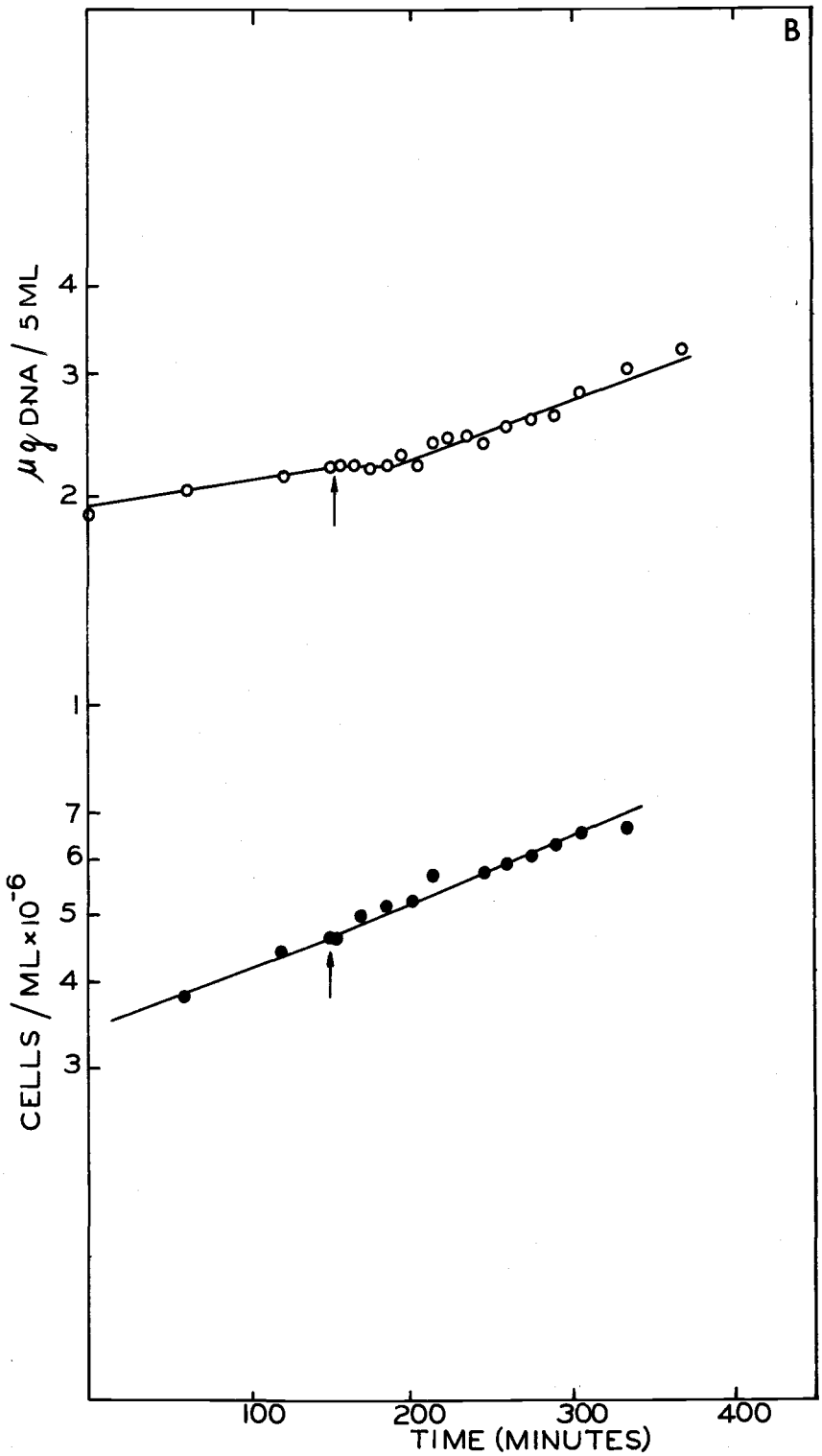

Figure 23. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to Wickerham's complete medium. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer.

to Wickerman's complete medium, then abruptly resumed at a higher rate.

The results of a shift-up from Wickerham's minimal + methionine, tryptophan, adenine, and uracil to broth medium are shown in Figures 24 and 25. After shift-up, increase in protein, DNA, and cell number showed varying lag periods before assuming a higher rate, and all exhibited a step-wise increase during the first 1.5 hours after transfer to broth medium. Increase in total protein gradually increased during the first 40 min after transfer, then became linear. One hour after shift-up there was a 30 min lag in protein synthesis, after which it resumed at the previous rate. Similarly, increase in cell count showed a gradual increase to the post-shift rate; 75 min after transfer, it leveled off for 20 min, then resumed at the previous rate. Increase in DNA exhibited a 50 min lag after shift-up (perhaps remaining at the pre-shift rate) then assumed a higher rate for the next 50 min. After 100 min incubation in broth medium, there was a 30 min lag in the rate of DNA increase, after which it assumed the earlier rate. In contrast to the behavior of other cellular constituents during shift-up, the rate of RNA synthesis increased dramatically and remained at a higher rate for the first hour after shift-up. After this time the rate abruptly dropped about 50% and remained constant for the rest of the experiment. The decrease in the rate of adenine incorporation occurred simultaneously

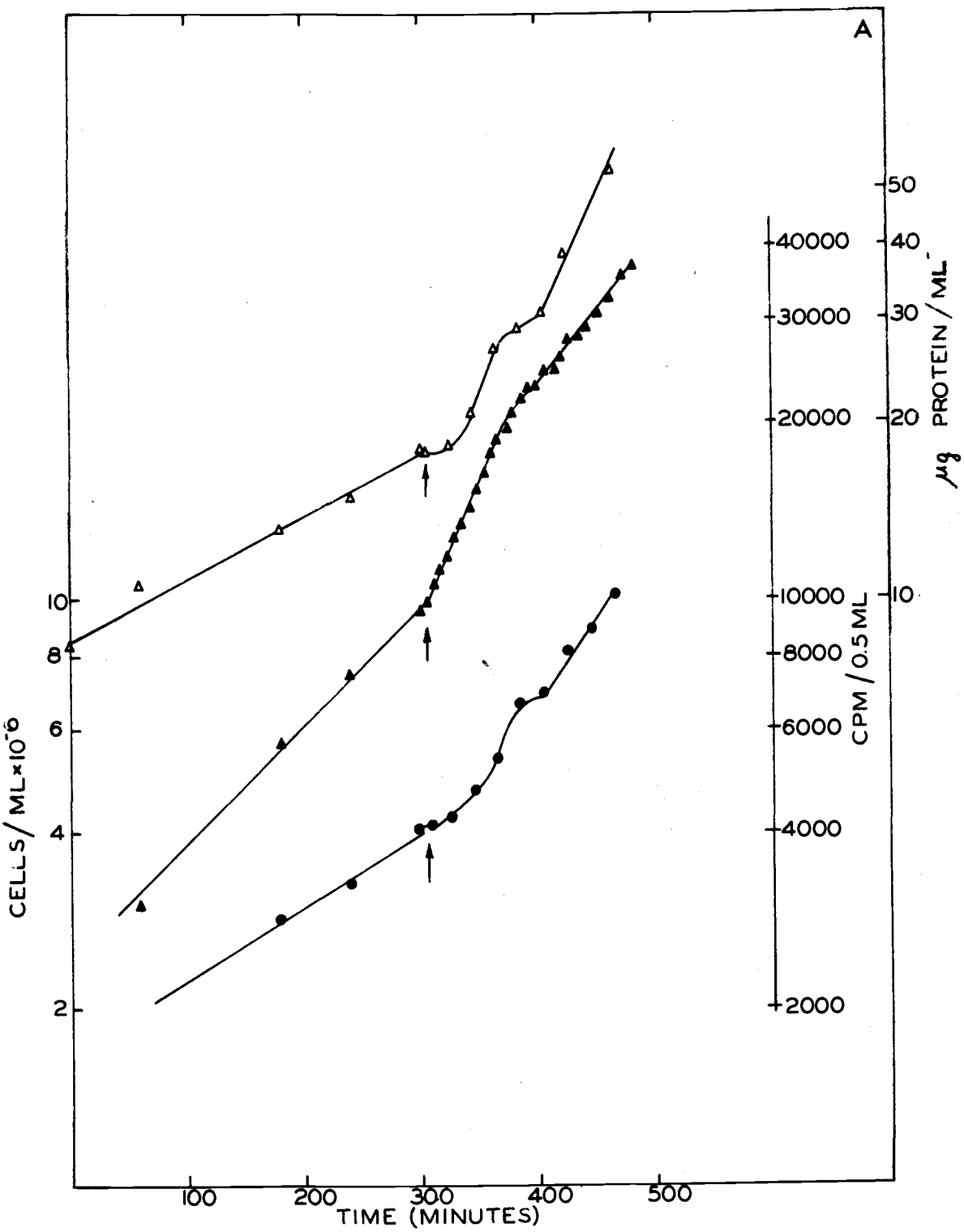

Figure 24. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to broth medium. Symbols: (●) cell number; (△) protein synthesis; (▲) RNA synthesis. Arrow indicates time of transfer.

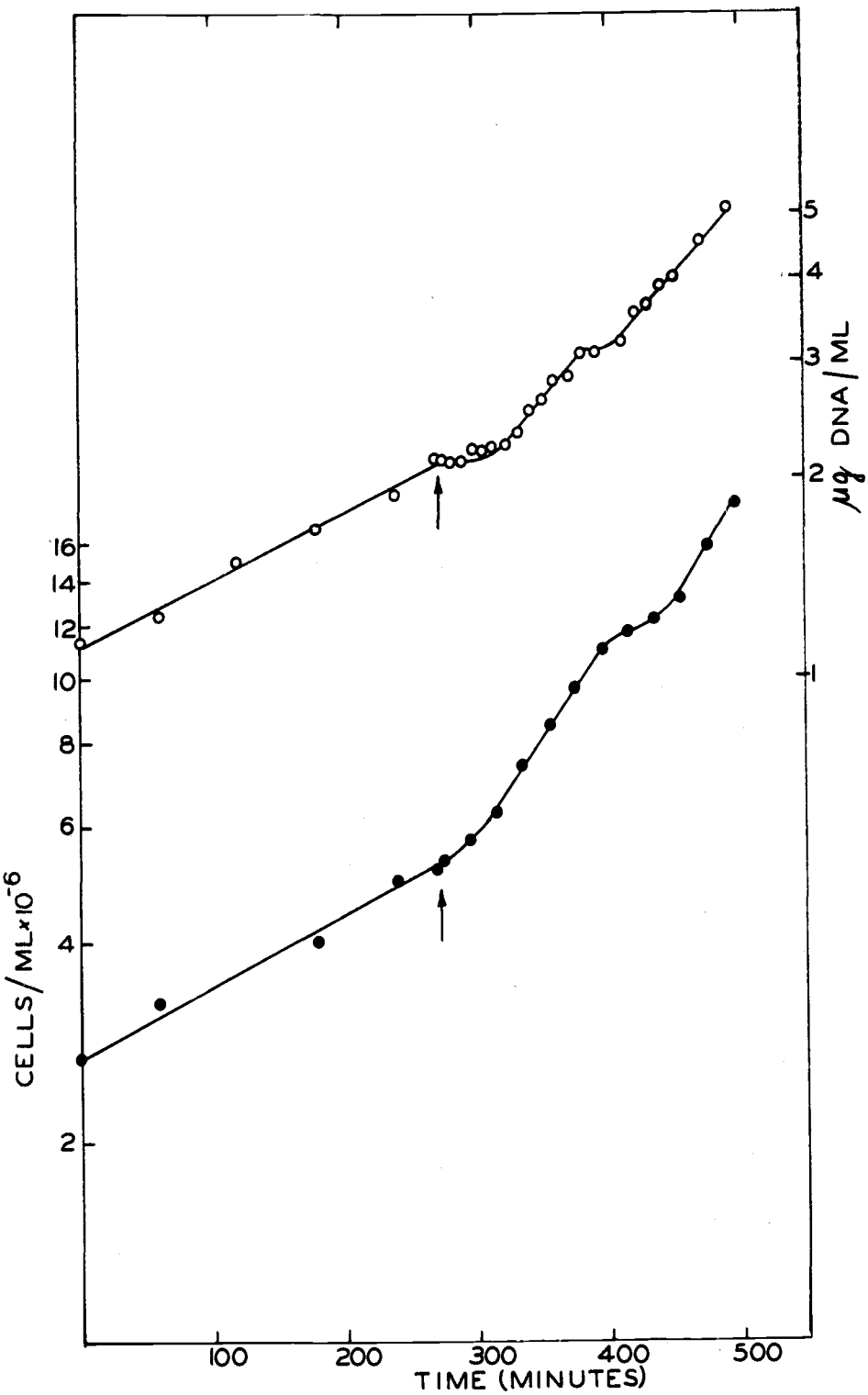

Figure 25. Shift-up from Wickerham's minimal medium + methionine + tryptophan + adenine + uracil to broth medium. Symbols: (●) cell number; (○) DNA synthesis. Arrow indicates time of transfer.

with the step in cell increase. The final rate of adenine incorporation in broth medium was only slightly higher than that in supplemented Wickerham's minimal; this was probably due to dilution of the label in the broth medium.

In order to determine whether a particular class of RNA was being preferentially synthesized during the period of increased rate of adenine incorporation in the shift-up experiment, RNA pulse labeled during shift-up conditions was compared with pulse-labeled RNA isolated from cells growing in Wickerham's minimal medium. As a control, cells were grown for 10 hours in 100 ml of Wickerham's minimal medium plus adenine, uracil, tryptophan, and methionine and containing 25.8 μc of $H_3{}^{32}PO_4$. Cells were then rapidly filtered and resuspended in 100 ml of prewarmed Wickerham's minimal medium plus methionine, tryptophan, uracil, and adenine-8-^{14}C (5 μg/ml, specific activity = 0.025 μc/μg). After 7 minutes of incubation with labeled adenine (corresponding to about 2% of the generation time in supplemented Wickerham's minimal medium and about 6% of the generation time in broth medium), cells were poured over crushed ice; RNA was extracted and analyzed on sucrose-$MgCl_2$ gradients. Results are shown in Figure 26. The profile of the pulse-labeled RNA is similar to that of the continuously labeled RNA, but with a relatively larger proportion of the label sedimenting in the region from fraction 39-52. Analysis of RNA pulse-labeled during shift-up is

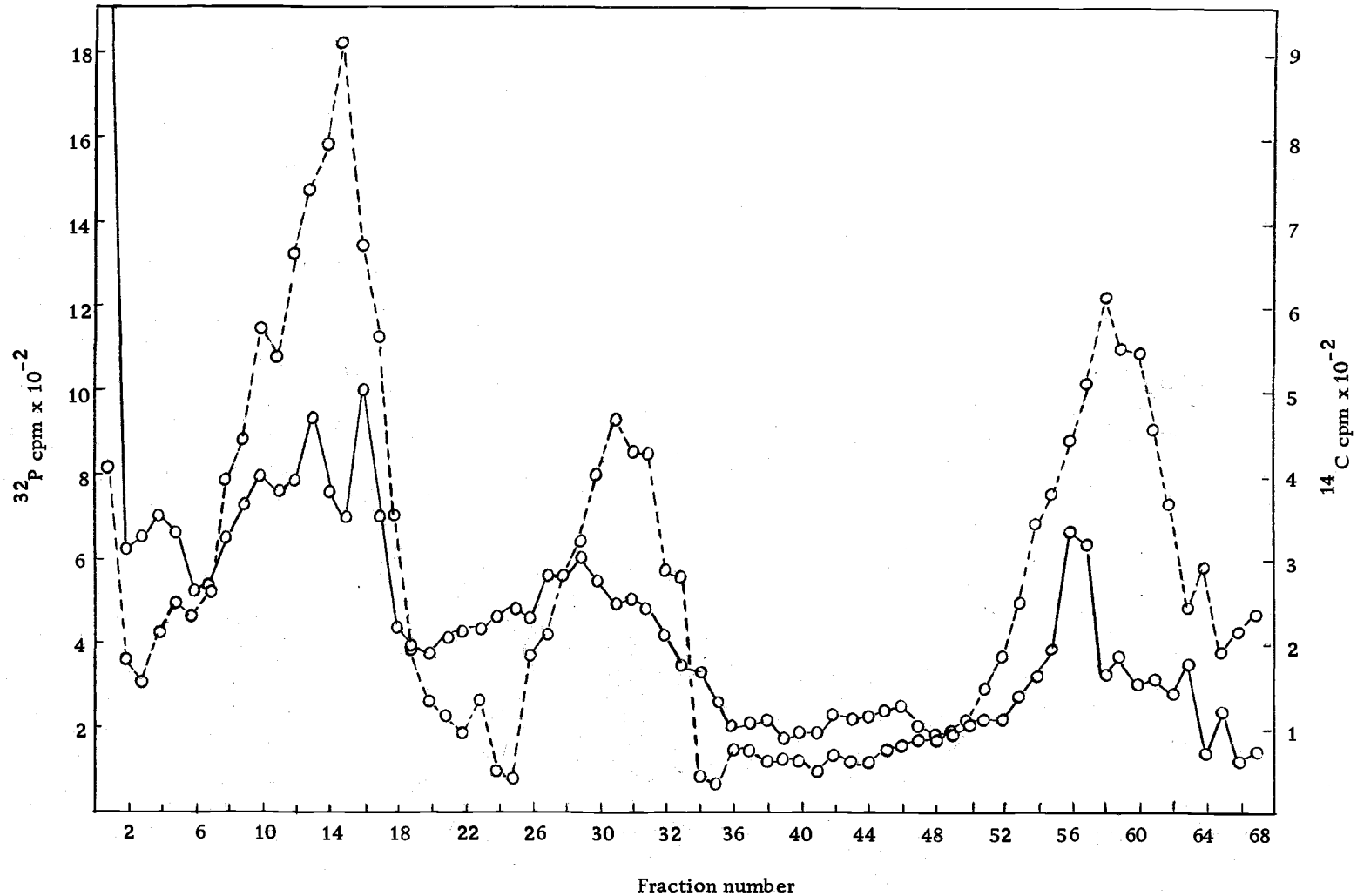

Figure 26. Analysis of RNA pulse-labeled during growth on supplemented Wickerham's minimal medium on a linear 5%-20% sucrose gradient in 5 mM $MgCl_2$. Symbols: ---- ^{32}P; —— ^{14}C.

shown in Figure 27. Cells were grown for 13 hours in 100 ml of Wickerham's minimal medium plus adenine, uracil, methionine, and tryptophan and containing 11.66 μc of $H_3{}^{32}PO_4$, then rapidly transferred to 100 ml of prewarmed TCA medium containing 1 μg adenine/ml. After 5 minutes of incubation, 25 μc of adenine-8-^{14}C was added (final concentration = 3 μg adenine/ml, specific activity = 0.08 μc/μg); the culture was incubated for an additional 7 minutes before the cells were harvested. The RNA was isolated and analyzed on sucrose gradients as before. Two gradients each were analyzed from two experiments; some variability was observed in the amount of the pulsed label sedimenting with the three major fractions. In every case, however, all three major fractions were labeled to a moderate extent during the pulse; in no experiment were the heavier (ribosomal RNA) fractions labeled to the exclusion of the lighter (soluble RNA) fractions. Results of one experiment are shown in Figure 27. In some of the results not shown, the lighter fractions were labeled to a greater degree during the pulse.

It should be noted that in all pulse-labeling experiments, cultures continuously labeled with $H_3{}^{32}PO_4$ were pulse-labeled in the presence of cold phosphate. Thus the ^{32}P profiles in Figures 26 and 27 represent stable RNA species.

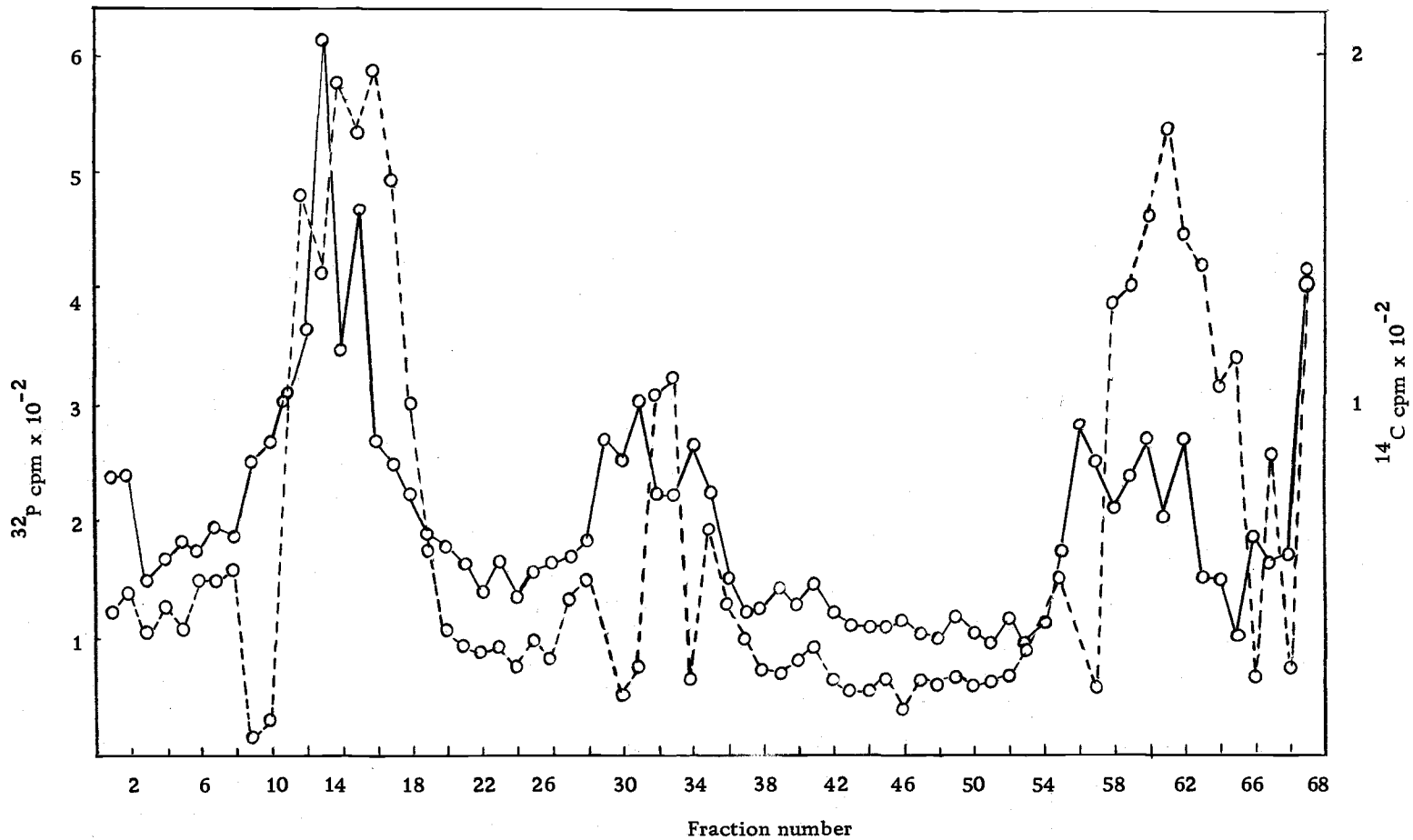

Figure 27. Analysis of RNA pulse-labeled during shift-up on a linear 5%-20% sucrose gradient in 5 mM $MgCl_2$. Symbols: ---- ^{32}P; —— ^{14}C.

DISCUSSION

During the starvation of Saccharomyces cerevisiae for amino acids or amino acids and uracil, cell mass increased for a considerable period (as much as a 50% increase over 4 1/2 hours of incubation). Similarly, striking increases in cell number and adenine incorporation were noted during amino acid starvation. Yeast are known to possess large intracellular amino acid pools and may under some circumstances accumulate exogenous amino acids up to 1000-fold (Surdin et al., 1965); the continued synthesis of RNA during amino acid starvation may represent exhaustion of such intracellular pools. It is of interest that the greatest residual RNA synthesis was observed during methionine starvation. It is known that yeast can accumulate high levels of methionine as S-adenosylmethionine, which is thought to be unavailable to the cell under normal growth conditions (Pigg et al., 1964). The results reported here would suggest that the cell has a relatively larger available pool of methionine than of tryptophan. This is in agreement with measurements of the free amino acid pools of yeast made by Halvorson and Spiegelman (1955). They reported that log phase cultures of Saccharomyces cerevisiae strain K contained 0.027 μM of tryptophan and 0.60 μM of methionine/100 mg dry cells. In contrast to the results above, adenine incorporation immediately ceased during

starvation for methionine, tryptophan and uracil; the amount of total RNA may in fact have dropped slightly (see Figure 6). Also, during starvation for tryptophan, uracil and adenine, cell mass and RNA showed little increase. On the other hand, DNA increased at the original rate for a brief period before leveling off. Total increase in DNA was about 11% during starvation for adenine, uracil, and tryptophan, and about 17% during starvation for uracil, methionine, and tryptophan. Williamson (1965) has shown that in yeast growing at 25°C with a doubling time of about 2 hours, DNA synthesis occupies about the first 27% of the cell cycle. If roughly 30% of the cells in a random population were engaged in DNA synthesis at the time of transfer to the amino acid-deficient media, the observed increase in DNA during amino acid and pyrimidine starvation would be consistent with the idea that all cells engaged in DNA synthesis will continue, while older cells in the population will not initiate a new cycle of DNA synthesis. Furthermore, the small increase occurs during a relatively short period after the cultures were transferred to starvation conditions (less than 60 minutes). If cells engaged in DNA synthesis at the time of transfer continue DNA replication at the same rate, one would expect all rounds in progress at the time of transfer to be completed in less than one generation period. For example, cells growing in TCA medium should complete DNA synthesis within 30 minutes after transfer to the deficient medium. The

experimental results agree quite well with this expectation.

The results reported in Figure 7 show that there is approximately a 10% increase in total DNA during starvation for only methionine and tryptophan, although previous experiments showed that RNA synthesis continued for about 30 minutes under the same conditions (see Figure 4). This suggests that limitations on initiation of DNA synthesis are imposed soon after removal of amino acids from the medium and may be independent of the intracellular amino acid pools. The experiment also suggests that the small increase in DNA noted during starvation for amino acids and uracil was not due solely to uracil limitation.

Figure 8 shows that the same increase in total DNA was seen during incubation in Wickerham's complete less methionine and tryptophan; in this experiment, cells were subjected to amino acid starvation without concurrent shift-down conditions. Therefore, the effect on total DNA increase noted during transfer of cells from Wickerham's complete medium to Wickerham's minimal medium + adenine and uracil was a consequence of removal of required amino acids. Total RNA (Figure 9) continued to increase during incubation in Wickerham's complete medium less methionine and tryptophan; the results are almost identical to those shown in Figure 4 (in which cells were transferred to Wickerham's minimal plus adenine and uracil). This again suggests that the effects noted in Figures 2-4

were consequences of amino acid starvation rather than shift-down conditions.

All data thus far considered show that when required amino acids are removed from a randomly dividing population of yeast, the culture will continue to synthesize DNA for a brief period; this same phenomenon was observed in the absence of net RNA and protein synthesis, i.e., during amino acid and uracil starvation. The time over which this occurs and the net increase in DNA are in agreement with the interpretation that cells synthesizing DNA at the time of amino acid removal will continue DNA replication to completion but will not initiate a new round of replication (although it cannot be determined from the data whether cells continue replication to the completion of a full complement of DNA or complete only a fraction of the genome). This is similar to the effect of amino acid starvation on DNA synthesis in the enteric bacteria (Maaløe and Hanawalt, 1961). If this interpretation is correct, certain predictions can be made concerning the behavior of a starved culture upon readdition of required amino acids. First, if all cells engaged in DNA synthesis have completed rounds of replication in the absence of amino acids, then the culture should be phased with regard to DNA replication, i.e., all cells should begin replication at the same point when returned to complete medium. Second, if cell division is dependent upon some event in the DNA replication cycle, as has

been suggested for bacteria (Clark, 1968; Helmstetter and Pierucci, 1968; Cooper and Helmstetter, 1968), one might expect to see some division synchrony in a starved culture returned to complete medium. Third, if completion of DNA synthesis is a necessary event for cell division and all cells in a starved culture have completed this step, one might expect all or a portion of a starved population to undergo division when returned to complete medium in the presence of a specific inhibitor of DNA synthesis.

A direct test of the first prediction was not considered in this study. Instead, an answer to the second prediction was sought by observing the behavior of DNA and RNA synthesis and cell division in starved cultures returned to complete medium. There was an initial lag in DNA synthesis during which RNA was synthesized at a relatively high rate. After this lag, DNA synthesis commenced and rapidly assumed the rate characteristic of cells in balanced growth in the new medium. In the case of the culture returned to Wickerham's complete medium, DNA synthesis leveled off after 1.5 doublings. In all cases, cell division proceeded at a low rate until the DNA content of the culture had doubled. At this point, cell number had increased by 30%. No evidence of division synchrony or synchronous steps in DNA was observed. Evidently, a period of incubation in complete medium is necessary for reinitiation of DNA synthesis, perhaps reflecting a required period of protein synthesis

prior to DNA replication. The 30% increase in cell number at the time of DNA doubling may be fortuitous, but could represent a connection between DNA replication and cell division. The results are not inconsistent with the hypothesis that starved cells must undergo one complete cycle of DNA replication before cell division is permitted; the gradual increase in cell number would then correspond to division of 30% of the original cell population which had completed DNA replication during the starvation period. This assumes that cells which had completed DNA replication prior to transfer to the starvation medium continued to divide at a low rate during the starvation period; in fact, increases in cell number at reduced rates during starvation conditions were observed. Failure to observe any synchrony in DNA synthesis or cell division could be related to the same lack of synchrony in amino acid starved bacterial cultures upon readdition of amino acids. Lark, Repko, and Hoffman (1963) observed that DNA made during amino acid starvation of Escherichia coli appeared to replicate more slowly that synthesized prior to or after starvation.

It was hoped that the pattern of cell division of a starved culture returned to Wickerham's complete medium in the presence of FUDR or nalidixic acid would provide some evidence for a relationship between cell division and DNA synthesis. Preliminary experiments with cultures growing logarithmically in Wickerham's complete

medium indicated that both of these compounds led to only a transitory inhibition of DNA synthesis and adenine incorporation. Also, when starved cultures were returned to Wickerham's complete medium supplemented with either FUDR or nalidixic acid, increases in cell number and total DNA were quite similar to those of control cultures without inhibitors. Apparently, S. cerevisiae is only temporarily sensitive to these inhibitors; an explanation of this must await a more thorough investigation of the mode of action of FUDR and nalidixic acid in yeast cells in different physiological states.

In a shift-down from broth medium to supplemented Wickerham's minimal medium, adenine incorporation and increase in total protein remained at the pre-shift rate but gradually leveled off to a rate lower than that characteristic for cells in balanced growth in minimal medium. The initial high rate may, again, represent a large intracellular amino acid pool so that, within the cell, actual shift-down conditions are not reached until the pools have been depleted by incubation in minimal medium. Upon transfer to minimal medium, the rate of increase in cell number showed an immediate drop to a rate comparable to that of cells in balanced growth in minimal, but after 2 hours incubation abruptly underwent a second drop to a much lower rate. In general, there seems to be a lag in response among all three quantities, after which the rates of increase

drop to a low value; the condition of balanced growth in minimal medium was not reestablished during the course of the experiments. In contrast to the results of the amino acid starvation experiments, the rate of DNA synthesis during shift-down from broth medium to minimal medium immediately dropped to a low value. When total DNA increased about 10%, there was a brief lag before DNA synthesis resumed at a lower rate. Here again, the rate characteristic of balanced growth in minimal medium was not attained. However, the increase in total DNA prior to assumption of final low rate was the same as that observed during tryptophan, adenine, and uracil starvation shown in Figure 5.

Although there was little difference between the pre- and post-shift rates of increase in RNA, DNA, protein, and cell number during a shift-up from supplemented Wickerham's minimal medium to Wickerham's complete medium, it is of interest that all quantities except RNA exhibited a slight lag period after shift-up. The rate of RNA synthesis immediately assumed a higher rate which was maintained over the balance of the experiment. This phenomenon was much more evident in a shift-up from supplemented Wickerham's minimal to broth medium. Here RNA synthesis assumed a high rate which was maintained for the first 70 min after the shift. Kudo and Imahori (1965) have shown that upon a shift-up of nitrogen-starved yeast cells to a rich medium, newly synthesized RNA appears

in the polysome fraction and later moves to the 80S fraction, and that the base composition of this RNA is similar to that of ribosomal RNA. Thus it was thought that the increased rate of RNA synthesis during a shift-up from minimal to rich medium might represent a synthesis of ribosomal RNA. Analysis of RNA pulse-labeled with adenine-^{14}C during shift-up from supplemented Wickerham's minimal medium to broth medium indicated that all three major fractions (identified by continuous labeling with $H_3{}^{32}PO_4$ prior to shift-up) were labeled during the pulse. Although some variation in the relative distribution of label in the three fractions was observed, it was clear that the heavier (ribosomal RNA) fractions were not being preferentially labeled to the exclusion of the lighter fractions. These results suggest that all RNA species were pulse-labeled during shift-up conditions, and that the increased rate of total adenine incorporation observed during the initial period after shift-up reflects an increased rate of synthesis of all RNA species. This is in contrast to the situation in the enteric bacteria, in which ribosomal RNA is synthesized preferentially during shift-up (Kjeldgaard, 1961; Schleif, 1967). It should be noted that these experiments are not conclusive enough to rule out the possibility that ribosomal RNA may be synthesized at a higher rate than other species; they demonstrate only that the RNA synthesized immediately after shift-up is not exclusively ribosomal RNA.

The stepwise increases in total DNA and cell count following shift-up to broth medium resemble a partially synchronous division, and suggest that shift-up conditions may bring a portion of the cell population into phase with regard to DNA synthesis and cell division. However, a stepwise increase in total protein was also observed; in a synchronous culture of yeast protein increases continuously throughout the cell cycle (Williamson, 1964).

SUMMARY

Synthesis of total RNA, DNA, and protein was followed in a haploid yeast auxotroph during starvation for required amino acids and uracil, and during shift-up and shift-down conditions.

During amino acid starvation, synthesis of macromolecular constituents was not immediately affected, reflecting the presence of large intracellular amino acid pools. Under conditions in which there was no net RNA and protein synthesis (amino acid and uracil starvation), total DNA continued to be synthesized for a total increase of 10-15%. The results suggested that cells engaged in DNA synthesis at the introduction of starvation conditions complete replication of the genome but do not initiate new periods of DNA synthesis. Upon return of a starved culture to complete medium, a period of DNA synthesis was required before the culture began to divide at the rate characteristic for complete medium. Compounds which are known to inhibit DNA synthesis in bacteria had no effect upon starved yeast cultures returned to complete medium.

Under shift-down conditions, cultures exhibited a lag in response similar to that observed during amino acid starvation. During shift-up conditions, DNA and protein increased in a stepwise fashion before assuming a continuous rate of increase characteristic of the new medium. During shift-up RNA was synthesized

initially at a high rate; this was found not to be due to exclusive synthesis of a particular class of RNA.

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