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EFFECT OF EXCESS L-METHIONINE ON THE UTILIZATION OF C^{14}- LABELED GLUCOSE BY *SACCHAROMYCES CEREVISIAE*

by

Wynanda M. O'Malley

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In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Wynanda M. O'Malley

entitled "Effect of Excess L-methionine on the Utilization of C^{14}-labeled Glucose by Saccharomyces cerevisiae."

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

Dissertation Director	Date 4/7/65

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

	7 April 1965
Kenneth Ventcam	7 April 1965
A R Kaufman	19 April 1965
James W. Berry	19 April 1965

*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

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SIGNED: Lynanda M. O'Malley

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ABSTRACT

A wild type strain of Saccharomyces cerevisiae (SC-10-2) was grown with normal L-methionine (6.7 μMoles per 100 ml of medium) and excess L-methionine (400 μMoles per 100 ml of medium). Excess L-methionine resulted in early retardation of growth which was followed by stimulation at the later phases.

Glucose-1-C^{14} and glucose-6-C^{14} were used as substrate additions. The $C^{14}O_2$ evolved, RNA produced, and the S-adenosyl-methionine (S-AM) produced were studied.

Specific activities of the $C^{14}O_2$ evolved indicated operation of the hexose monophosphate pathway particularly during the early phases. Excess L-methionine was found to result in lowered ratios of the specific activity of 1-$C^{14}O_2$ to that of 6-$C^{14}O_2$.

Excess L-methionine also resulted in increased RNA production. The specific activities of the RNA's were found to be greater when glucose-6-C^{14} was used as the substrate addition than when glucose-1-C^{14} was used.

Induction of the methionine activating enzyme achieved by growing the inoculum in a medium containing excess L-methionine, resulted in yields of S-adenosylmethionine (S-AM) about four times as large as those obtained when no attempt at induction was made.

The yields of S-AM gradually increased from 20 hours to 44 hours when a peak was reached which was followed by a rapid decline.

All data obtained suggested a crucial period in the growth of the organism occurring at about 30 hours. S-Adenosylmethionine appears to play an active role in the growth of the organism.

INTRODUCTION

Since the first biochemical application of isotope techniques by Hevesy (1923) in an investigation of the uptake of lead by plants, we find an ever increasing number of references involving these techniques in the literature.

The approaches to this type of investigation have several aims, all of them directed toward discovering facts concerning the biochemistry of plants and animals. Facets of such studies include discovering how a given chemical compound is assimilated, how it is dissimilated, and how the breakdown products are used for the biosynthesis of new compounds. All are aspects of metabolism.

Many investigations involved the use of radioactive glucose as a tracer substance. Impetus was given to investigations of glucose metabolism when it was discovered that this sugar was assimilated in cells by routes other than the classical glycolytic pathway of Embden-Meyerhof-Parnas (EMP). At least two other pathways are now known to occur. One of these has been variously called the "Warburg-Dickens pathway" (Dickens, 1953), the "hexose-monophosphate oxidation shunt" (HMP), and the "pentose phosphate pathway". The other is the Entner-Doudoroff pathway (Entner and Doudoroff, 1952).

Bloom et al. (1953a, 1953b) initiated the first use of specifically C^{14} labeled glucose to measure the relative amount of glucose metabolized by the pentose pathway and the EMP pathway. They measured the yield of C^{14} in CO_2 produced from glucose-1-C^{14} and glucose-6-C^{14} in tissue slices of various rat organs. The following year Blumenthal et al. (1954) performed a similar experiment employing yeast as the test organism. Since this time, studies of this nature have been reported in the literature, each one more elegant than the preceding one in its approach to the mathematical treatment of the experimental data. An excellent review of these studies has been published by Wood, Katz and Landau (1963).

The origins of the ribose and deoxyribose of the nucleic acids constitute a problem directly related to the pathways of glucose dissimilation.

Early tracer studies on the origin of ribose in glucose-grown Escherichia coli by Lanning and Cohen (1954, 1955) and Bernstein (1956) indicated that ribose arises mainly from the oxidative decarboxylation of 6-phosphogluconate to ribulose-5-phosphate. Sowden et al. (1954) and David and Renaut (1955) reached the same conclusion for ribose synthesis in Torula utilis. The latter were able to show that RNA-ribose obtained from T. utilis grown on glucose-2-C^{14} as its sole source of carbon possessed a specific activity similar to that of the glucose. They concluded that the D-ribose skeleton was formed by

the elimination of the aldehyde carbon of D-glucose.

Bernstein and Sweet (1957, 1958) studied the biosynthesis of deoxyribose in E. coli grown on specifically labeled glucose and concluded that a considerable portion of the sugar might have been derived from glucose through the loss of carbon 1.

Bagatell, Wright and Sable (1958, 1959) studied the biosynthesis of ribose and deoxyribose in E. coli grown for varying lengths of time in a synthetic medium. Sodium acetate was used as the sole source of carbon and either acetate-1-C^{14} or glucose-6-C^{14} as the tracer. The distribution of C^{14} in the deoxyribose was very similar to that of the ribose, giving rise to the concept of reduction of ribose to deoxyribose. This research supported the findings of Grossman and Hawkins (1957) that ribose derivatives are reduced enzymatically to deoxyribose derivatives. David and Jaymond (1958) employed Candida utilis and concluded that deoxyribose was formed through the elimination of carbon 1 of glucose with ribose as an intermediate.

Barker et al. (1961), while attempting to improve the biosynthetic preparation of C^{14} labeled nucleotides, found that the addition of unlabeled adenine to the medium reduced the specific activities of the ribosyl residues of the RNA of Candida utilis growing on glucose-C^{14}. These findings were subsequently confirmed on six different occasions. Barker et al. (1962) then grew C. utilis on glucose-1-C^{14} and glucose-6-C^{14} as the sole source of carbon in the absence of

adenine and in the presence of 50 mg of adenine per liter of medium. The ratio of the specific activity of CO_2 from glucose-1-C^{14} to that of glucose-6-C^{14} was reduced from 3.26 to 2.12 in the presence of adenine. The possible explanation offered was that the glucose metabolized via 6-phosphogluconic acid was reduced in proportion by the addition of adenine. However, adenine seemed to have little effect on the relative labeling of the purine nucleotides by glucose-1-C^{14} and glucose-6-C^{14}. There was some indication, based on the relative specific activities of the ribose of the purine nucleotides, that C-6 was retained to a greater extent than C-1. In the ribose of the pyrimidine nucleotides, C-1 was retained to a greater extent than C-6.

Exogenous adenine has been shown to participate in the biosynthesis of nucleic acids of rats (Brown et al., 1948). It has been shown that other organisms can also incorporate exogenous adenine into the nucleic acids. Among these are Torulopsis utilis (Kerr, Seraiderian and Brown, 1951) and Saccharomyces cerevisiae (DiCarlo, Schultz and McManus, 1951). Adenine can also serve as the sole source of purine for RNA. This has been demonstrated to be the case for T. utilis (Kerr et al., 1951) as well as S. cerevisiae (Abrams, 1952). DiCarlo et al. (1951) reported that adenine could serve as the sole source of nitrogen in cultures of S. cerevisiae.

Adenine was proven to be involved in the formation of S-adenosylmethionine (S-AM) which is produced by some microorganisms.

This compound was first discovered by Cantoni (1951), who found that the condensation product of methionine and adenosinetriphosphate led to the formation of "active methionine." Cantoni (1953) chemically characterized the compound as S-AM and gave it a structural formula which later was corroborated by Baddiley and Jamieson (1954).

As early as 1952, Smith and Schlenk reported the accumulation of S-AM in yeast when excess amounts of methionine were present in the growth medium. Various investigations on S-AM production have singled out the yeasts as the most important organisms in S-AM studies. Yeasts are not only capable of producing the compound but can also accumulate it (Schlenk and DePalma, 1957a, 1957b; Schlenk, Dainko and Stanford, 1959). S-Adenosylmethionine is stored in the vacuoles of yeast cells (Svihla and Schlenk, 1960) and can be transferred to daughter cells of budding yeasts. Gawel, Turner and Parks (1962) cultured a series of microorganisms representing a variety of microbial types under conditions designed to promote the formation of S-AM. Only S. cerevisiae was found to produce a measurable amount of S-AM.

O'Malley (unpublished data) found that the amount of S-AM produced in S. cerevisiae in the presence of excess L-methionine (400 μmoles per 100 ml of medium) was definitely influenced by the age of the culture. The greatest accumulation of S-AM occurred at 44 hours after which there was a rapid decline in concentration. Moore and Yall (1963) reported that in the early phases of growth of a strain of yeast

(SC-10-4) a large amount of methionine inhibits growth, but that growth is stimulated in the later phase and that the final cell yield is greater in the presence of excess methionine than in normal amounts of methionine (6.7 μMoles per 100 ml of medium). However, this particular strain did not accumulate S-AM.

The enzymatic synthesis of S-AM has been investigated with partially purified enzymes derived from rabbit liver, baker's yeast, and Escherichia coli (Cantoni and Durrell, 1957; Mudd and Cantoni, 1958; Tabor and Tabor, 1960). In all three instances, it appeared that there was a condensation of the adenosyl moiety of ATP with methionine to form the sulfonium nucleoside. Mudd (1962) has elucidated the details involved in the role of the "methionine activating" enzyme.

Pigg, Sorsoli and Parks (1963) employed S. cerevisiae and determined that the methionine activating enzyme was induced in the presence of excess L-methionine. The same workers (1964) reported the accumulation of S-AM in the cells until a high level was attained which occurred after three hours. The appearance of S-AM was accompanied by the disappearance of free methionine which had been incorporated from the medium by the cells.

Svihla and Schlenk (1959, 1960) were of the opinion that the S-AM accumulated by the cells is not used during the growth of the culture. Degradation of S-AM was observed to occur at a very slow rate,

if at all, in the yeast S. cerevisiae by Shapiro and Mather (1958) and Mudd (1959).

S-Adenosylmethionine has been linked to the methylation of nucleic acids. The existence of methylated compounds in DNA was reported by Wyatt (1951) and Wyatt and Cohen (1953). The earliest evidence of a methylated component of RNA was obtained by Cohn (1957) and Davis and Allen (1957) who isolated a naturally occurring unidentified nucleotide from yeast. Littlefield and Dunn (1958) were the first to observe the presence of small amounts of three methylated adenines and of thymine in RNA's from various sources. Adler et al. (1958) reported the occurrence of two methylated guanines in yeast RNA. Soon after the publication of these reports, the origin of the methyl group of these compounds came under investigation (Mandel and Borek, 1961a, 1961b, 1963). Fleissner and Borek (1962) reported the existence of RNA methylase which was found to be involved in the methylation of the base components of soluble RNA when the latter are already in polynucleotide form. These same authors reported (1963) that in addition to the methyl receptor being macromolecular RNA, the direct methylating agent is S-adenosylmethionine.

STATEMENT OF PROBLEM

The purpose of this investigation was to study the effect of L-methionine concentration in the presence or absence of exogenous adenine on the utilization of specifically labeled radioactive glucose by the yeast Saccharomyces cerevisiae with reference to (1) the age of the culture, (2) the CO_2 evolved, (3) the production of ribonucleic acid, (4) the production and accumulation of S-adenosylmethionine, and (5) the possible relationship between ribonucleic acid and S-adenosylmethionine.

MATERIALS AND METHODS

Organism

A strain of Saccharomyces cerevisiae (SC-10) originally obtained from F. P. Hungate of the General Electric Company, Richland, Washington was used for all experiments. This strain produces and accumulates S-AM in the presence of L-methionine (Yall, 1962). Prior to this investigation, this property of the yeast strain was verified on three separate occasions. The patterns of accumulation exhibited were identical for each trial experiment.

Media

The stock culture was maintained on agar slants of glucose yeast extract peptone (GYEP) of the following composition: glucose (2.0%), Bacto yeast extract (1.0%), Bacto peptone (2.0%), and Bacto agar (1.5%). The yeast was grown in the "complete" synthetic medium of Roman (1956) for the experiments.

"Normal" L-methionine refers to 10 mg/l or 6.7 μMoles/100 ml as designated by Roman (1956). "Excess" L-methionine refers to 597 mg/l or 400 μMoles/100 ml (Yall, 1962). The synthetic medium was prepared with either normal or excess L-methionine and dispensed in 100 ml quantities in 500 ml wide-mouth Erlenmeyer flasks. These

flasks were plugged with cotton and autoclaved at 15 pounds pressure for 15 minutes.

The synthetic medium containing normal or excess L-methionine was supplemented with glucose-1-C^{14} or glucose-6-C^{14} which had been sterilized separately by autoclaving and added aseptically to the flasks of medium. The radioactivity of the glucose was assayed in counts per minute (CPM/ml) in a Packard Tricarb Liquid Scintillation Spectrometer System, model 314 EX before addition to the medium.

Radioactive glucose was obtained from the California Corporation for Biochemical Research, Los Angeles, California.

Preparation of the Inoculum

The inoculum of Saccharomyces cerevisiae (designated SC-10-2) was prepared from 24-hour cultures grown on GYEP agar slants at 30° C. The growth from these slants was used to prepare a cell suspension in sterile saline which gave a 20% transmittance at 650 mμ on a Lumetron photoelectric colorimeter. Five ml of this suspension were used to inoculate the flasks of Roman's medium. For the glucose-1-C^{14} experiment, the medium contained "normal" L-methionine; for the glucose-6-C^{14} experiment the medium contained "excess" L-methionine. In all other respects, the preparation of the inoculum was the same. Duplicate determinations of the dry weight of cells in 5 ml cell suspension proved to be 10.4 mg for the 1-label and 10.6 mg for

the 6-label. Inoculated flasks were incubated on a Brunswick shaker for 24 hours at 30° C. Cells were harvested aseptically and suspended in sufficient sterile saline to give the same percent transmittance in the experiments. One ml of the prepared cell suspensions was employed to inoculate each of the flasks used in the experiments.

Experimental Protocol

For the glucose-1-C^{14} and the glucose-6-C^{14} experiments, the yeast was cultured with continuous agitation by a Brunswick shaker at 22° C. in parallel flasks containing either normal or excess L-methionine. Cells were harvested after 20, 26, 32, 44, 48, and 56 hours of growth for the glucose-6-C^{14} experiments and at 20, 32, and 44 hours for the glucose-1-C^{14} experiments. The protocol for the experiments was not identical in all respects.

In some instances, the CO_2 being evolved was trapped in 10 ml of a 20% NaOH solution. The NaOH solution was dispensed in 35 mm x 75 mm centrifuge tubes of 30 ml capacity. The tubes were suspended several centimeters above the level of the medium contained in the flasks by means of glass tubing fused to the tube. The other end of the glass tubing was inserted into a #10 black rubber stopper which fitted into the mouth of the flasks. The rubber stoppers and CO_2 collection tubes were sterilized as a unit by autoclaving before being inserted into the flasks.

In those instances in which CO_2 was being recirculated, a sterile #10 black rubber stopper was securely taped inside the neck of the flasks.

In order to obtain significantly large numbers of cells, duplicate or triplicate flasks of medium were inoculated and incubated. In the glucose-1-C^{14} experiment, CO_2 was trapped for 20, 32, and 44 hour growth periods with normal as well as excess L-methionine. In the glucose-6-C^{14} experiment, CO_2 was trapped for the entire period of growth (i.e. 20, 26, 32, 44, 48, and 54 hours) or CO_2 was not permitted to escape for the entire growth period, or free exchange of gases took place.

Measurement of Growth

After the flasks of synthetic medium were seeded with standardized inoculum, they were cultured with continuous agitation by a Brunswick shaker at 22° C. The cells were harvested after 20, 26, 32, 44, 48, and 54 hours. The amount of growth was measured at these intervals. Dry weight determinations were made by filtering appropriate samples of the various cultures through tared Whatman #542 filter paper and drying to a constant weight.

Extraction Procedures

The nucleic acids were extracted and hydrolyzed by a modification of the method of Ogur et al. (1952). This method consisted of

extraction with 0.2 N perchloric acid, acidified alcohol, and alcohol-ether to remove all non-nucleic acid ultraviolet absorbing material. The ribonucleic acid (RNA) was extracted by treating with 1 N perchloric acid at 4° C. for 18-24 hours followed by three washings in the cold with the same reagent. The deoxyribonucleic acid (DNA) was extracted and hydrolyzed by treatment for 50 minutes with 1 N perchloric acid at 70° C. The RNA was hydrolyzed by similar treatment. As a result of this treatment, the nucleic acids were split to purine bases and pyrimidine nucleotides (Loring, 1955).

S-Adenosylmethionine was extracted by the method of Schlenk, Dainko and Stanford (1959). This method consists of suspending the cells in 1.5 N perchloric acid (volume:volume, 1:4). The suspension was held at 4° C. overnight with constant stirring. Extracted cells were collected by centrifugation and washed twice with cold 1.5 N perchloric acid. The supernatant fluid was then assayed by column chromatography on a Dowex 50 x 8 ion exchange resin of 100-200 mesh with 1 N, 2 N, and 4 N HCl (Schlenk and DePalma, 1957a). The eluate was collected in 5 ml portions using a fraction collector. The eluted portions were monitored continuously for ultraviolet absorption at 255 mu by an LKB Uvicord Absorptionmeter. The S-AM was eluted by 4 N HCl.

In some instances, the above method was used to get RNA as well as S-AM, since the former is eluted by the 1 N HCl. DNA could

then be obtained by extraction of the cell residue using 1 N perchloric acid at 70° C. for 50 minutes. This process also hydrolyzed the DNA.

Radioactivity Determinations

The radioactivity of the various fractions was determined in a Packard Tricarb Liquid Scintillation Spectrometer System, model 314 EX. Duplicate aliquots of each sample were dispensed in low potassium glass counting vials and the dioxane-methanol scintillator of Bray (1960) was employed. Corrections for quenching caused by acid or color in the system were made using an internal benzoic acid-C^{14} standard and applying the correction factor of Kinnory et al. (1958).

All radioactivity determinations were made at the 95% confidence level.

The μMoles of S-AM were computed according to the method of Schlenk and DePalma (1957a).

The quantity of RNA formed was estimated according to the method of Sueoka and Cheng (1962). In this method the amount of nucleic acid was estimated from ultraviolet absorption at 260 mμ. For convenience, a conversion factor of 0.02 OD_{260} for 1 μg/ml of nucleic acid was used.

RESULTS

The Effect of L-Methionine on Cell Growth

Table 1 illustrates the effect of normal and excess L-methionine on the growth of Saccharomyces cerevisiae SC-10-2. In the presence of excess L-methionine the growth of the culture was retarded during the first 24 hours in contrast to the culture grown in the presence of normal L-methionine. Between 24 and 32 hours the effect of excess L-methionine became negated and all cell yields expressed as mg dry weight were approximately the same after 32 hours of growth. Beyond this period, excess L-methionine appeared to have a slight effect in promoting growth which was most apparent at 44 hours.

Production of $C^{14}O_2$

Tables 2 and 3 record the specific activities of $C^{14}O_2$ obtained from glucose-1-C^{14} and glucose-6-C^{14} respectively. The specific activities which have been recorded cumulatively are higher for the $C^{14}O_2$ obtained from glucose-1-C^{14} than for those obtained from the glucose-6-C^{14}. In Table 2 a slight lowering effect can be noted at 32 hours and at 44 hours in the presence of adenine and normal L-methionine; no such effect was noted in the presence of adenine and excess L-methionine. Excess L-methionine both in the absence and

Table 1. The effect of L-methionine concentration on the cell yield of Saccharomyces cerevisiae SC-10-2.

Culture Age	L-Methionine	
	6.7 μMoles/100 ml medium	400 μMoles/100 ml medium
Hours	mg*/100 ml medium	mg*/100 ml medium
20	124	78
26	163	90
32	171	179
44	232	237
54	218	245

*Recorded as mg dry weight of cells
Cells grown at 22° C. in 100 ml of medium
Results representative of six experiments

Table 2. Effect of L-methionine and adenine concentration on the specific activity of $C^{14}O_2$ obtained from glucose-1-C^{14} by Saccharomyces cerevisiae SC-10-2.

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mMole	No adenine Counts/min/mMole
6.7	20	38×10^3	26×10^3
	32	48×10^3	51×10^3
	44	65×10^3	68×10^3
400	20	26×10^3	22×10^3
	32	42×10^3	37×10^3
	44	63×10^3	57×10^3

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 3. Effect of L-methionine and adenine concentration on the specific activity of $C^{14}O_2$ obtained from glucose-6-C^{14} by Saccharomyces cerevisiae SC-10-2.

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mMole	No adenine Counts/min/mMole
6.7	20	9×10^3	8×10^3
	32	11×10^3	17×10^3
	44	16×10^3	18×10^3
400	20	11×10^3	15×10^3
	32	12×10^3	19×10^3
	44	14×10^3	24×10^3

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 4. Comparison of the ratios of the specific activities of $C^{14}O_2$ obtained from glucose-1-C^{14} to that from glucose-6-C^{14} by Saccharomyces cerevisiae SC-10-2.

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mMole	No adenine Counts/min/mMole
6.7	20	4.27	3.03
	32	4.16	3.05
	44	3.99	3.71
400	20	2.40	1.40
	32	3.43	1.98
	44	3.39	2.19

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

presence of adenine resulted in a lowering of the specific activities.

A definite effect of the presence of adenine can be noted in Table 3 which records the specific activities of $C^{14}O_2$ obtained from glucose-6-C^{14}. In the presence of adenine the specific activities are lowered, the extent of this reduction is greater when excess L-methionine is present.

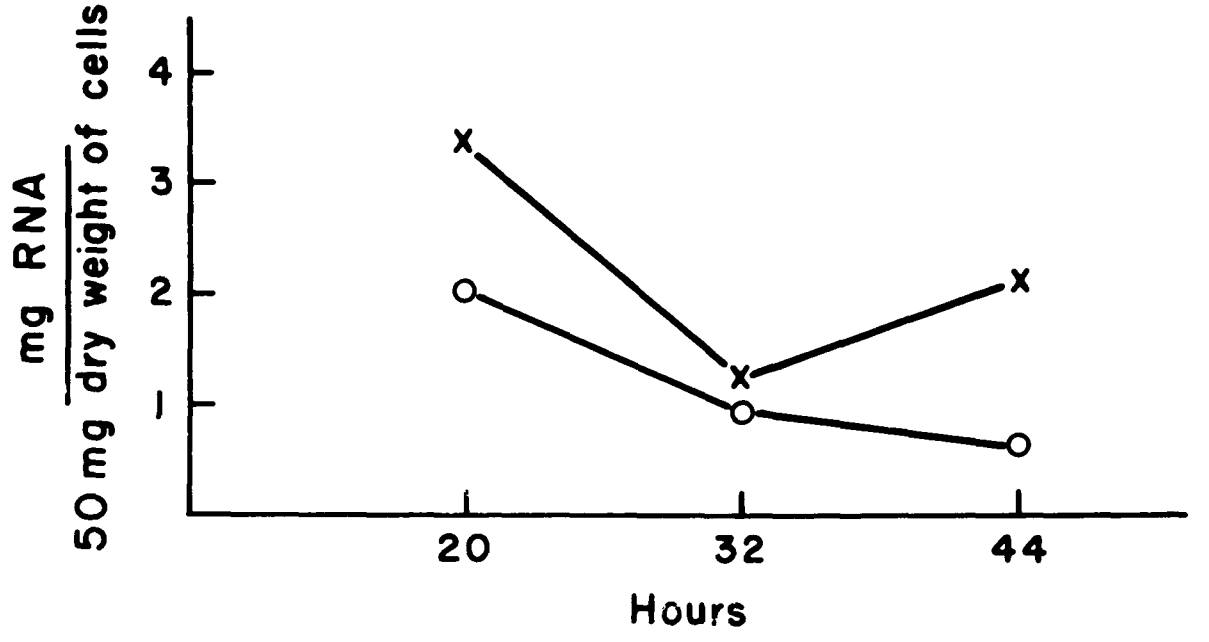
Table 4 gives the ratios of the specific activities. These ratios are higher in the presence of adenine. This is true regardless of the concentration of L-methionine used. The effect of excess L-methionine is greater because it reduces the ratios to a greater extent than normal L-methionine.

Yield of RNA and Specific Activities of RNA

Figures 1 through 6 record the yield of RNA in mg per 50 mg dry weight of cells under the various experimental conditions employed with glucose-1-C^{14}. In each instance the yield was higher with excess L-methionine at 20, 32, and 44 hours. The greatest differences were observed when the flasks were plugged with cotton and the smallest when CO_2 was being collected. When cultures were grown with rubber stoppers taped into the neck of the flasks, differences between normal and excess L-methionine were also observed but to a lesser extent. The presence or absence of adenine affected the RNA yield only slightly. In those instances where very slight differences were observed, adenine

Figure 1.--Yield of RNA in 100 ml of medium with 4.0 uMoles of adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with cotton plugs.

Figure 2.--Yield of RNA in 100 ml of medium without adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with cotton plugs.


mg RNA
50 mg dry weight of cells
4
3
2
1
20
32
44
Hours

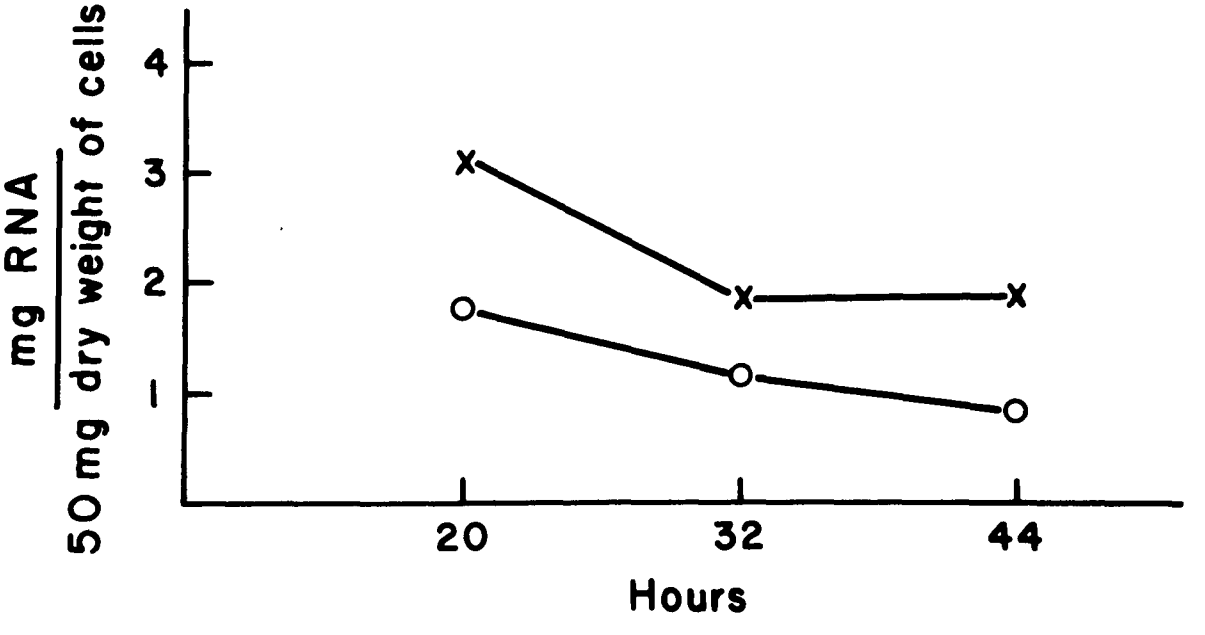
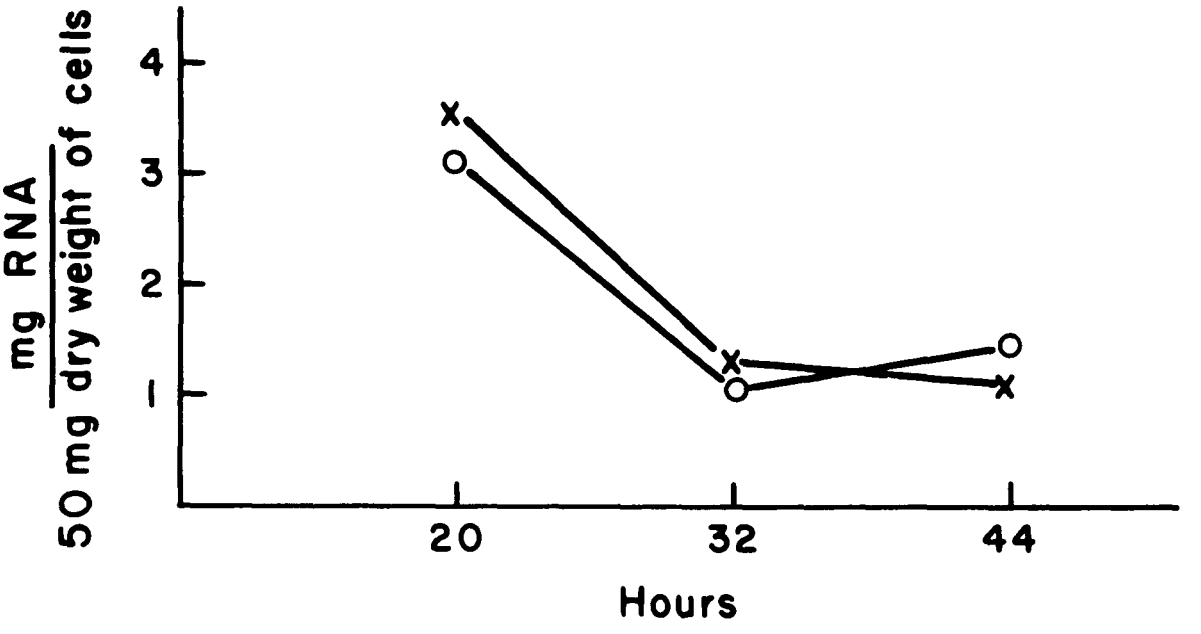

mg RNA
50 mg dry weight of cells
4
3
2
1
20
32
44
Hours

Figure 3. --Yield of RNA in 100 ml of medium with 4.0 μMoles of adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with CO_2 collectors.

Figure 4. --Yield of RNA in 100 ml of medium without adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with CO_2 collectors.


mg RNA / 50 mg dry weight of cells
4
3
2
1
20
32
44
Hours

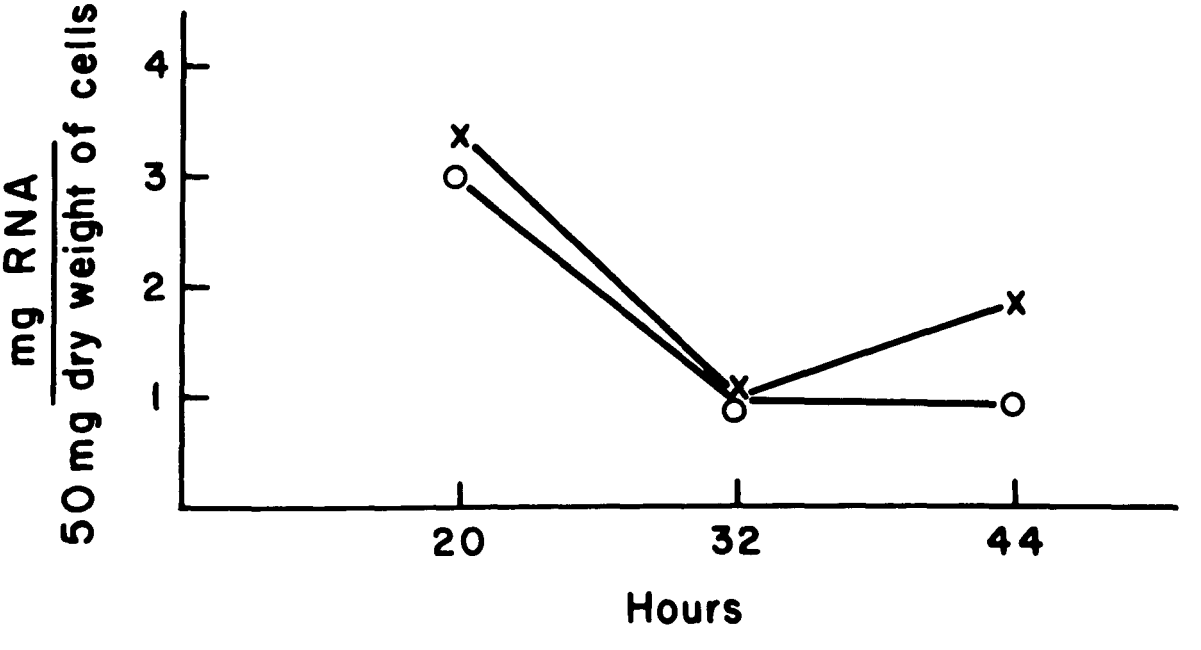
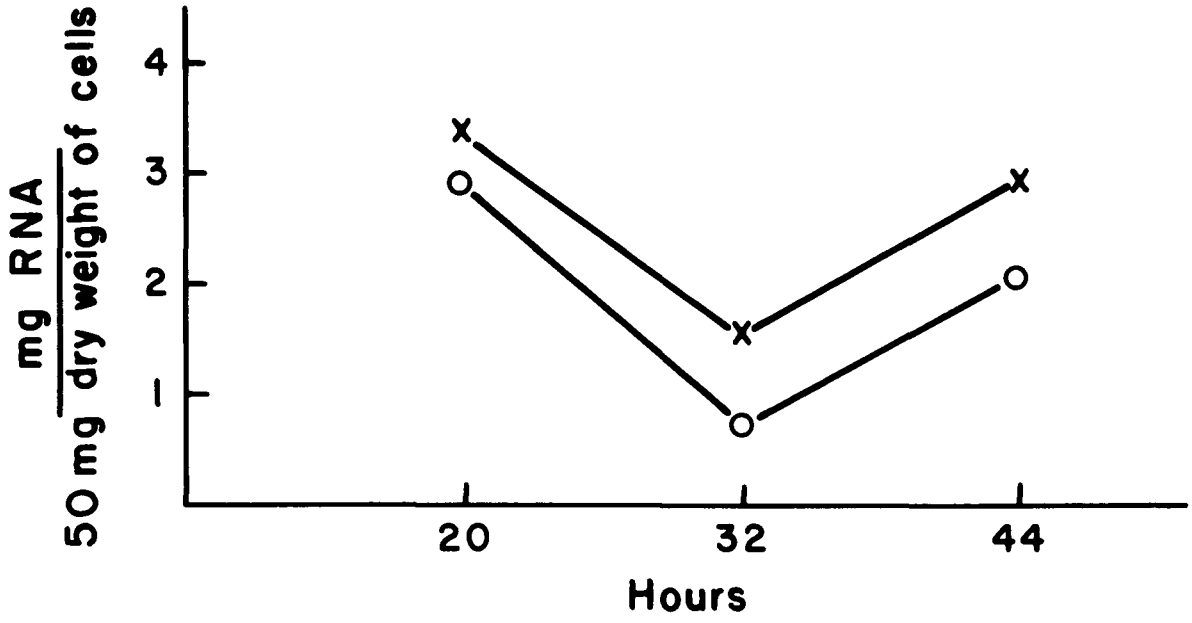
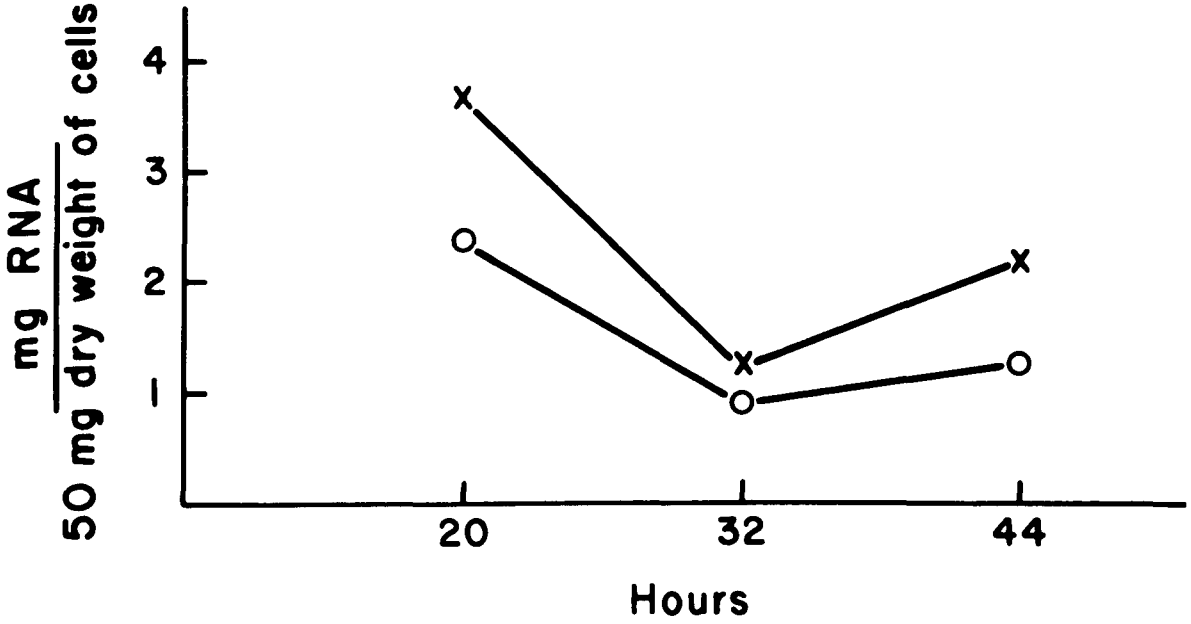

mg RNA / 50 mg dry weight of cells
4
3
2
1
20
32
44
Hours

Figure 5. --Yield of RNA in 100 ml of medium with 4.0 μMoles of adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with rubber stoppers.

Figure 6. --Yield of RNA in 100 ml of medium without adenine and 6.7 μMoles (o-o) or 400 μMoles (x-x) of L-methionine by Saccharomyces cerevisiae SC-10-2 in flasks with rubber stoppers.


mg RNA / 50 mg dry weight of cells
4
3
2
1
20
32
44
Hours


mg RNA / 50 mg dry weight of cells
4
3
2
1
20
32
44
Hours

appeared to result in a slight lowering of the amount of RNA produced.

It was also observed that the RNA yield was in all instances greatest at 20 hours, then dropped sharply at 32 hours, and in most instances recovered to a greater or lesser extent by 44 hours. It was noted that the gain was less in the flasks provided with CO_2 collectors.

Comparable data for glucose-6-C^{14} were obtained but are not represented here. The same pattern of RNA production was developed.

Tables 5 through 7 illustrate the effect of L-methionine concentration on the specific activities of the RNA's obtained with glucose-1-C^{14} under the various experimental conditions employed. On the whole, the specific activities were relatively low, a reflection of the lack of incorporation of the labeled material since in only one instance was more than one percent of the radioactivity originally introduced recovered from the RNA fraction. The specific activities are recorded as counts per minute per mg of RNA. If the counts were less than 15 above background, the specific activities were recorded as a trace. In all instances, the specific activities were lowest at 32 hours. There was no consistent pattern to indicate that the specific activities were higher at 20 hours than at 44 hours. This seemed to be the case only when cotton plugs were used. The specific activities obtained in the presence of adenine appeared to be somewhat lower than those obtained in the absence of adenine.

Table 5. Effect of L-methionine and adenine concentration on the specific activity of RNA obtained from Saccharomyces cerevisiae SC-10-2 in flasks with cotton plugs. Glucose-1-C^{14}

L-Methionine µMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mg RNA	No adenine Counts/min/mg RNA
6.7	20	trace	trace
	32	trace	573
	44	682	1193
400	20	445	454
	32	trace	trace
	44	697	957

*4.0 µMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 6. Effect of L-methionine and adenine concentration on the specific activity of RNA obtained from Saccharomyces cerevisiae SC-10-2 in flasks with CO_2 collectors. Glucose-1-C^{14}

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mg RNA	No adenine Counts/min/mg RNA
6.7	20	442	431
	32	trace	585
	44	trace	586
400	20	300	963
	32	trace	438
	44	529	762

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 7. Effect of L-methionine and adenine concentration on the specific activity of RNA obtained from Saccharomyces cerevisiae SC-10-2 in flasks with rubber stoppers. Glucose-1-C^{14}

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/mg RNA	No adenine Counts/min/mg RNA
6.7	20	trace	523
	32	trace	447
	44	383	793
400	20	608	707
	32	288	328
	44	494	431

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Incorporation of the 1-C^{14} label was greatest when cotton plugs were used. The effect of excess L-methionine occurred only when adenine was absent from the medium and tended to result in a lowering of the specific activities especially at the 44 hour level except when CO_2 was collected.

Table 8 represents the comparable data for the 6-C^{14} label. These cultures were not grown without adenine, therefore, no specific activities are available for this experimental condition. Again it was noted that there was very little effect on the specific activities due to the presence of excess L-methionine. The specific activities at 32 hours were lowest. A greater incorporation of the 6-C^{14} label was observed at 44 hours than at 20 hours. This was most obvious when the cotton plugs or rubber stoppers were employed.

The extent of the incorporation of the 6-C^{14} label was greater than that of the 1-C^{14} label as was reflected in the specific activities of the RNA's obtained.

Production and Specific Activity of S-Adenosylmethionine

Tables 9, 10, and 11 report the yield of S-AM per 100 ml of medium for glucose-1-C^{14} under the various experimental conditions. Tables 12, 13, and 14 show the comparable data for the glucose-6-C^{14} experiment. The most striking difference between the data from the two experiments was the much higher yields of S-AM with the 6-C^{14} label.

Table 8. Effect of L-methionine concentration on the specific activity of RNA from Saccharomyces cerevisiae SC-10-2 in the presence of adenine.* Glucose-6-C^{14}.

Culture Age	6.7 μMoles L-methionine/100 ml medium		
	Cotton plugs	CO_2 collectors	Rubber stoppers
Hours	Counts per min/μMole	Counts per min/μMole	Counts per min/μMole
20	702	983	605
32	trace	trace	494
44	1240	1362	1286
	400 μMoles L-methionine/100 ml medium		
20	934	1148	693
32	710	487	trace
44	1292	1172	1103

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 9. Effect of L-methionine and adenine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in flasks with cotton plugs. Glucose-1-C^{14}

L-Methionine µMoles per 100 ml medium	Culture Age Hours	Adenine* µMoles/100 ml medium	No adenine µMoles/100 ml medium
6.7	20	0.13	0.23
	32	0.34	0.35
	44	0.24	0.48
400	20	0.33	0.34
	32	0.35	0.54
	44	0.48	1.50

*4.0 µMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 10. Effect of L-methionine and adenine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in flasks with CO_2 collectors. Glucose-1-C^{14}

L-Methionine μMoles per 100 ml medium	Culture Age -- Hours	Adenine* μMoles/100 ml medium	No adenine μMoles/100 ml medium
6.7	20	0.27	0.21
	32	0.41	0.12
	44	0.68	0.39
400	20	0.52	0.63
	32	0.62	0.20
	44	1.42	1.41

*4.0 μMoles per 100 ml medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 11. Effect of L-methionine and adenine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in flasks with rubber stoppers. Glucose-1-C^{14}

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* μMoles/100 ml medium	No adenine μMoles/100 ml medium
6.7	20	0.43	0.33
	32	0.15	0.13
	44	0.32	0.26
400	20	0.76	0.64
	32	0.33	0.27
	44	0.20	0.76

*4.0 μMoles per 100 ml medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 12. Effect of L-methionine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with cotton plugs. Glucose-6-C^{14}

	Total S-AM production in μMoles/100 ml medium	
Culture Age Hours	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
20	1.39	1.88
26	trace	3.21
32	1.25	4.64
44	1.87	5.76
48	0.62	3.99
54	0.56	3.02

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 13. Effect of L-methionine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with CO_2 collectors. Glucose-6-C^{14}

	Total S-AM production in μMoles/100 ml medium	
Culture Age Hours	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
20	1.0	2.37
26	trace	3.81
32	0.78	5.49
44	1.41	6.74
48	1.63	5.25
54	0.85	2.53

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 14. Effect of L-methionine concentration on the production of S-adenosylmethionine by Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with rubber stoppers. Glucose-6-C^{14}

	Total S-AM production in μMoles/100 ml medium	
Culture Age Hours	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
20	0.97	2.72
26	1.00	3.52
32	1.37	4.37
44	2.22	7.23
48	1.68	6.21
54	0.63	5.01

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

All yields involving the 1-C^{14} label were low, but it is also noted that the yields at 44 hours were greatest in the presence of excess L-methionine. The amounts of S-AM obtained when flasks had cotton plugs or CO_2 collectors were approximately the same but yields were considerably lower when the flasks had rubber stoppers. When excess L-methionine was used, the yield was consistently higher than with normal L-methionine. The presence of adenine in the medium did not appear to have resulted in increased S-AM production.

The data for the 6-C^{14} label are more complete in that assays for S-AM were made at several additional periods during the growth of the yeast. It was evident excess L-methionine in the medium had a dramatic effect on S-AM production. The various culture conditions also appeared to have had an effect for when the culture flasks were provided with rubber stoppers, the S-AM yield was higher in the later hours than when cotton plugs were used. The yields when CO_2 was being collected appear to be intermediate. These observations probably are the result of the adaptation to changed environmental conditions which the yeast produced.

The 6-C^{14} data also show the pattern of S-AM production and accumulation. There appears to be a steady rise from 20 hours to 44 hours when the accumulation reaches its peak. After 44 hours there was a drop. This pattern was more clearly illustrated for flasks with cotton plugs and either normal or excess L-methionine (Figs. 7 and 8).

Figure 7. --Relationship of S-AM production to growth and RNA production in the presence of 4.0 μMoles of adenine and 6.7 μMoles of L-methionine. Flasks with cotton plugs.

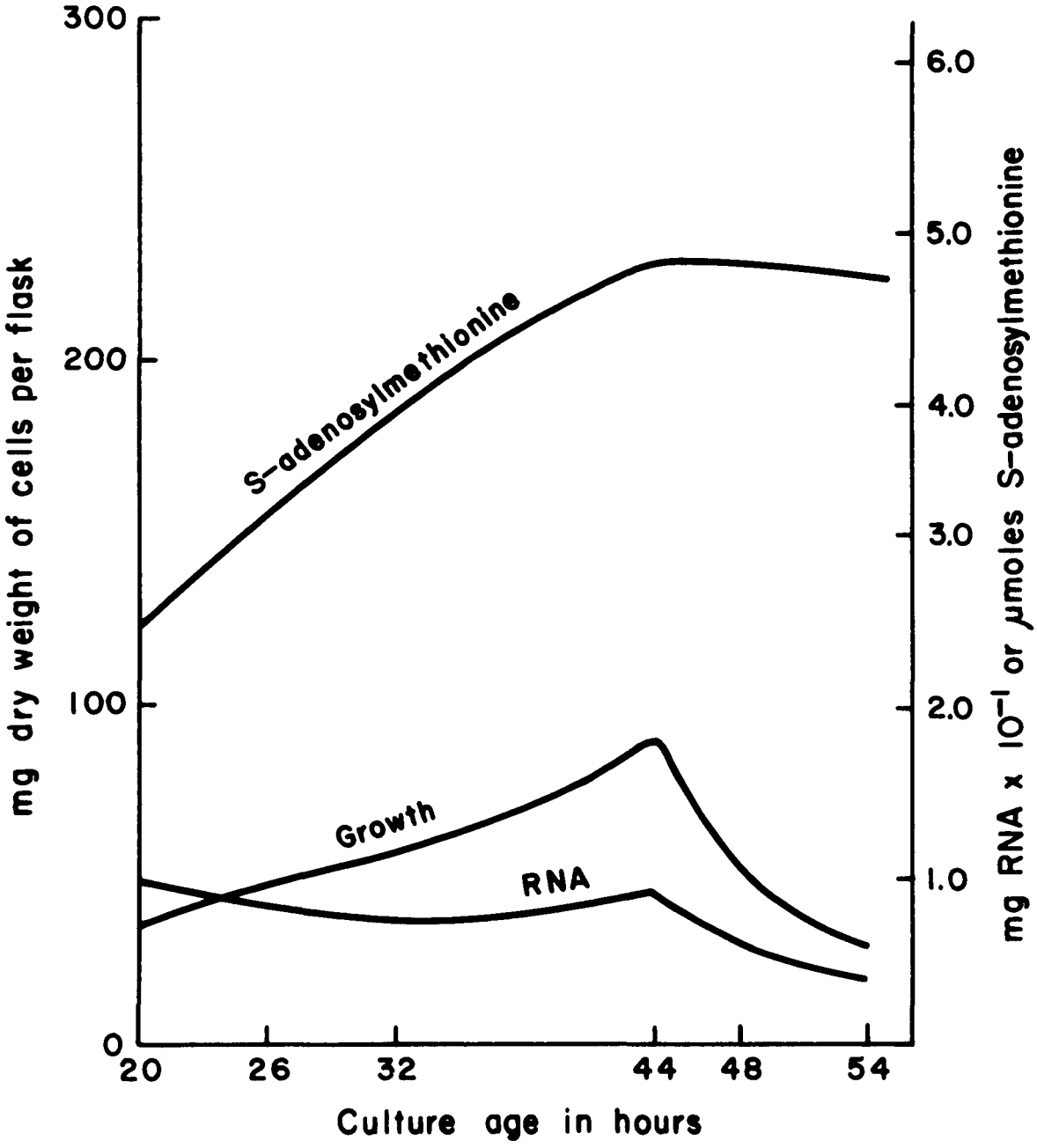
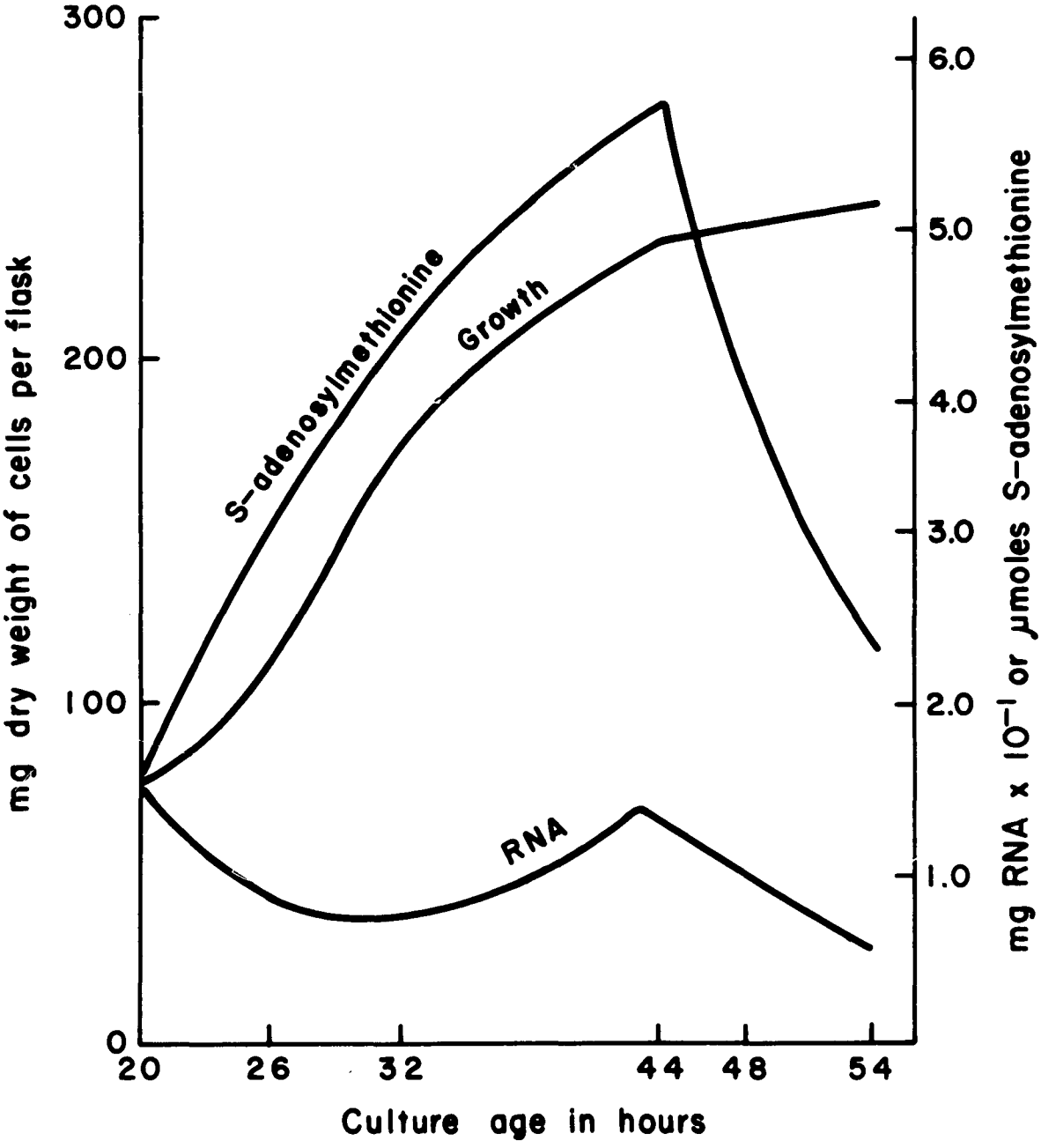

300
200
100
0
20
26
32
44
48
54
6.0
5.0
4.0
3.0
2.0
1.0
S-adenosylmethionine
Growth
RNA
mg dry weight of cells per flask
mg RNA x 10^{-1} or μmoles S-adenosylmethionine
Culture age in hours

Figure 8. --Relationship of S-AM production to growth and RNA production in the presence of 4.0 μMoles of adenine and 400 μMoles of L-methionine. Flasks with cotton plugs.


300
200
100
0
20
26
32
44
48
54
6.0
5.0
4.0
3.0
2.0
1.0
S-adenosylmethionine
Growth
RNA
mg dry weight of cells per flask
mg RNA x 10^{-1} or μmoles S-adenosylmethionine
Culture age in hours

Tables 15, 16, and 17 record the specific activities of S-AM when glucose-1-C^{14} was used as the substrate addition. It is noted that, on the whole, under all experimental conditions the specific activities are lowest at 32 hours and highest at 44 hours. The specific activities appear to be of the same magnitude with both normal and excess L-methionine.

The presence of adenine in the medium appeared to have exerted some influence on the incorporation of the label since the specific activities particularly at 44 hours were higher in the presence of adenine. This influence was most noticeable when CO_2 was collected or the flasks had rubber stoppers.

Tables 18, 19, and 20 show the comparable 6-C^{14} data. When cotton plugs were used the specific activity of the S-AM produced in the presence of excess L-methionine was higher at all but the 44 hour level than when the cells were grown with normal L-methionine.

In viewing Tables 19 and 20 the high specific activities of S-AM obtained from cells grown in normal L-methionine are immediately noted. This is true only for the 20 and 26 hour cultures. After 26 hours the label seems to be no longer detectable when CO_2 collectors were used in the flasks.

When cells were grown in the presence of excess L-methionine, the label appeared to have been incorporated to about the same extent under all experimental conditions.

Table 15. Effect of L-methionine and adenine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in flasks with cotton plugs. Glucose-1-C^{14}.

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/μMole	No adenine Counts/min/μMole
6.7	20	1700	600
	32	2550	2000
	44	trace	1600
400	20	trace	trace
	32	1160	1200
	44	2500	1950

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 16. Effect of L-methionine and adenine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in flasks with CO_2 collectors. Glucose-1-C^{14}

L-Methionine μMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/μMole	No adenine Counts/min/μMole
6.7	20	trace	trace
	32	1100	trace
	44	4700	2400
400	20	trace	1200
	32	1300	2450
	44	4600	2800

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 17. Effect of L-methionine and adenine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in flasks with rubber stoppers. Glucose-1-C^{14}

L-Methionine µMoles per 100 ml medium	Culture Age Hours	Adenine* Counts/min/µMole	No adenine Counts/min/µMole
6.7	20	1050	800
	32	trace	trace
	44	2900	2000
400	20	1000	750
	32	trace	trace
	44	3000	1800

*4.0 µMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 18. Effect of L-methionine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with cotton plugs. Glucose-6-C^{14}

Culture Age	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
Hours	Counts/min/μMole	Counts/min/μMole
20	8000	2150
26	8700	3250
32	trace	trace
44	2640	trace
48	trace	2430
54	trace	3720

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 19. Effect of L-methionine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with CO_2 collectors. Glucose-6-C^{14}

Culture Age	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
Hours	Counts/min/μMole	Counts/min/μMole
20	10,300	2,500
26	12,500	trace
32	trace	trace
44	trace	1,040
48	trace	trace
54	trace	3,320

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

Table 20. Effect of L-methionine concentration on the specific activity of S-adenosylmethionine obtained from Saccharomyces cerevisiae SC-10-2 in the presence of adenine* in flasks with rubber stoppers. Glucose-6-C^{14}

Culture Age	6.7 μMoles L-methionine per 100 ml medium	400 μMoles L-methionine per 100 ml medium
Hours	Counts/min/μMole	Counts/min/μMole
20	10,740	2,500
26	11,880	2,800
32	trace	1,600
44	8,800	1,440
48	trace	trace
54	trace	1,260

*4.0 μMoles per 100 ml of medium
Cells grown at 22° C. in 100 ml of medium
Results representative of two experiments

DISCUSSION

Factors Affecting Growth

The increased yield of cells of Saccharomyces cerevisiae SC-10-2 in the presence of excess L-methionine was reported by Yall (1962) and Moore and Yall (1963). The inhibition of growth in the early phase was also reported by the same authors. Their observations were made on cultures started from a small inoculum rather than a large one as was employed in this study. Nevertheless, a clearly discernible inhibitory effect due to the presence of excess L-methionine was noted.

The effect of L-methionine on growth or the accumulation of a product has been reported by several workers. Giri and Krishnaswamy (1954) reported the stimulatory effect on growth and riboflavin production by large amounts of methionine in a strain of S. cerevisiae which accumulated riboflavin.

From studies with mutant strains of Neurospora crassa (Emerson, 1949) it was recognized that homoserine is an important precursor of threonine. Aspartic acid is phosphorylated by adenosine triphosphate (ATP) involving the enzyme aspartokinase, the aspartyl phosphate is reduced and the reduced product in turn is converted to homoserine in the presence of homoserine dehydroganase. These two enzymes were first obtained in partially purified form from yeast by

Black and Wright (1955a, 1955b, 1955c). The conversion of homoserine to threonine was demonstrated with extracts of E. coli and yeast (Watanabe, 1955). Threonine in turn is a precursor of the alpha-ketobutyric acid used in the microbial biosynthesis of isoleucine (Strassman et al., 1955, 1956; Umbarger and Brown, 1958).

The repression of aspartokinase synthesis by methionine in S. cerevisiae was reported by Robichon-Szulmajster and Corrivaux (1963). Karrasevitch and Robichon-Szulmajster (1963) reported that methionine specifically inhibits the synthesis of homoserine dehydrogenase and that it was susceptible to feedback inhibition by methionine as well as by lysine and threonine.

It appears that we have two enzymes which control not only the intracellular levels of the amino acids methionine and threonine but also indirectly the level of isoleucine. It is conceivable, therefore, that excess L-methionine interferes with the biosynthesis of these amino acids which are essential for the growth of the organisms.

Reversal of the biosynthetic pathway of methionine, trans-thiomethylation, or degradation of the S-adenosylmethionine formed by the organism may overcome the retardation in growth. These processes would result in the formation of homoserine, alpha-aminobutyric, and alpha-ketobutyric acid respectively. All of these can be metabolized to threonine (Black and Wright, 1955d). Reversal of the biosynthetic pathway has been shown to occur in Neurospora (Teas et al., 1948).

Schlenk and Smith (1953) believe transthiomethylation to be of doubtful validity and the degradation of S-adenosylmethione occurs at a very slow rate if at all in S. cerevisiae according to Shapiro and Mather (1958) as well as Mudd (1959). There is some evidence that at least in a mutant of Aerobacter aerogenes the labeled carbon of S-adenosylmethionine-C^{14} shows up in the cellular proteins (Shapiro et al., 1963). The possible degradation of S-adenosylmethionine will be more fully discussed later.

It seems that in the culture of S. cerevisiae the early retardation of growth in the presence of excess L-methionine can be overcome by the organism.

Production of Radioactive CO_2

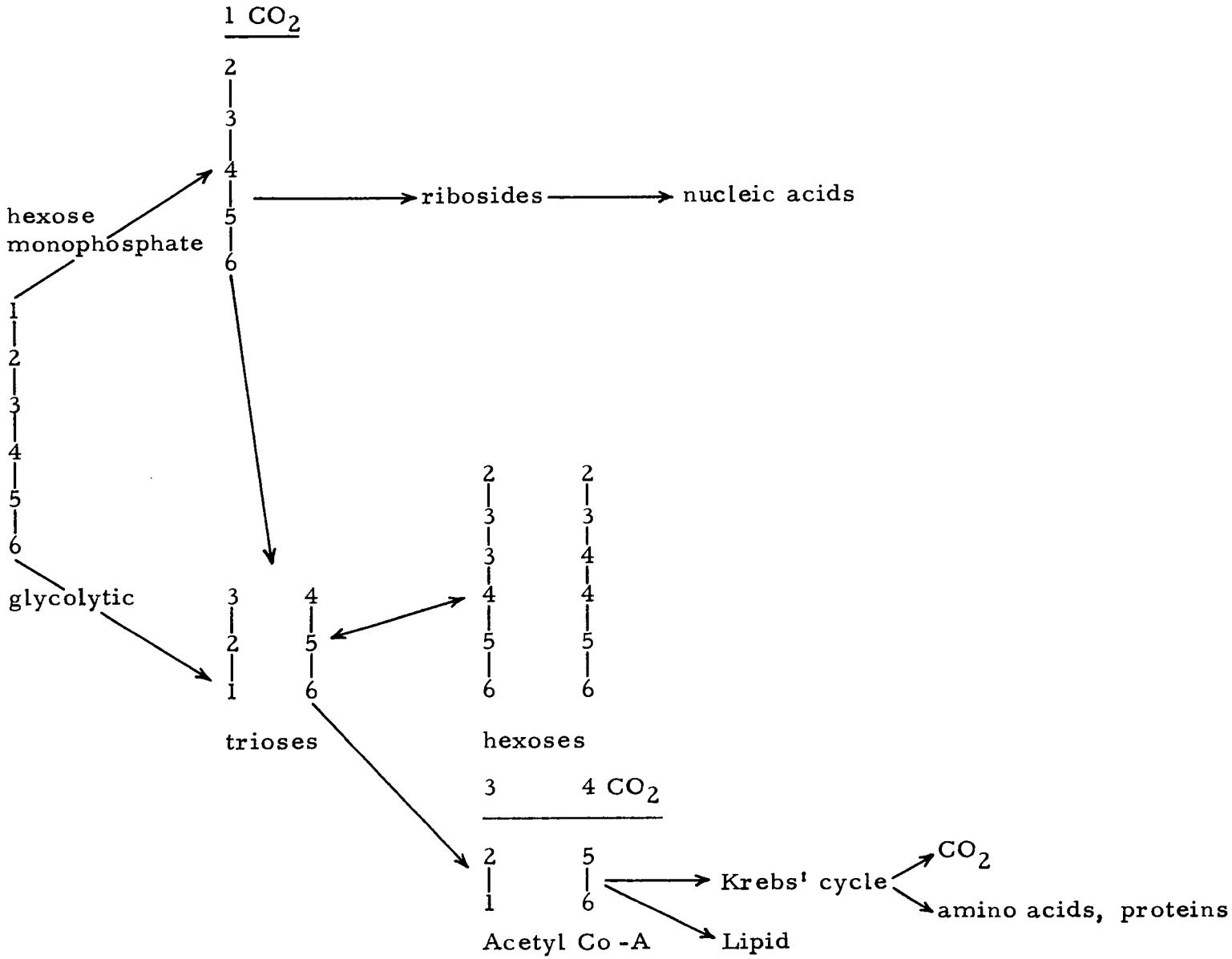
In the breakdown of glucose through the Embden-Meyerhof-Parnas or glycolytic pathway, carbon atoms 1 and 6 are equivalent metabolically, assuming that both triose phosphates are in complete equilibrium. In degradation through the hexose monophosphate pathway (HMP), on the other hand, C-1 is lost primarily as CO_2, while C-6 will not be lost until the second recycling of the tricarboxylic acid cycle. The preferential oxidation of C-1 to CO_2 over that of C-6 has often been cited as evidence for the operation of the glycolytic pathway. If the ratio of CO_2 formation from glucose-C-1 to CO_2 formation from glucose-C-6 is more than 1, this is considered as evidence for the

operation of the HMP pathway. Wang et al. (1956, 1958) have reported that in S. cerevisiae the glycolytic pathway operates to the extent of about 88 percent and the hexose monophosphate pathway to about 13 percent. In yeast the contribution of glycolytic degradation is increased to 95 percent by anaerobic conditions (Blumenthal et al., 1954). Wood and Katz (1958) and Katz and Wood (1963) reported the effect of recycling in the HMP pathway on the yields of radioactive CO_2 from glucose-1-C^{14} and glucose-6-C^{14} as influenced by the metabolism of the glycolytic pathway, the hexose monophosphate pathway, and the utilization of glucose-6-C^{14} in other pathways. Figure 9 illustrates the relationship between these two major pathways.

The data presented in Table 4 give evidence of the operation of the HMP pathway, but no attempt was made to interpret the extent. The lower ratios when excess L-methionine was used support the evidence for retardation of growth in the earlier stages. One would expect the HMP pathway to be operative during the earlier stages of growth when RNA formation is at its peak--requiring ribose for ribonucleotides derived from glucose via the hexose monophosphate pathway.

The addition of adenine to the medium resulted in increased specific activity ratios. This effect was in sharp contrast to that observed by Barker et al. (1962) who reported that in Candida utilis the addition of 50 mg adenine per liter of medium lowered this ratio.

Figure 9. --Relationship of the glycolytic pathway to the hexose monophosphate pathway.


1 CO2
2
3
4
5
6
ribosides
nucleic acids
hexose
monophosphate
1
2
3
4
5
6
glycolytic
3
2
1
4
5
6
trioses
2
3
3
4
5
6
2
3
4
4
5
6
hexoses
3
4 CO2
2
1
5
6
Krebs' cycle
CO2
amino acids, proteins
Acetyl Co-A
Lipid

However, these workers used 11 times as much adenine as was used in these experiments.

The specific activities of CO_2 reported in Tables 2 and 3 suggest that adenine had very little influence on the metabolism of glucose-1-C^{14} either in the presence of normal or excess L-methionine. On the other hand, the specific activities of CO_2 from glucose-6-C^{14} are lowered in the presence of adenine to a somewhat greater extent with excess than with normal L-methionine. This suggests that the presence of adenine may reduce the proportion of glucose metabolized via the glycolytic pathway.

When under the experimental conditions employed solid rubber stoppers were used there was no exchange of gases with the atmosphere. It might be expected that during the period from 20 hours to 32 hours microaerophilic conditions tended to become established. Changing the CO_2 collectors at 32 hours relieved this condition to some extent.

RNA Synthesis

RNA synthesis is subject to a wide variation. It is dependent on the growth rate and not on the chemical nature of the medium. It does not matter whether the medium is rich in RNA precursors or sources of carbon and nitrogen. It is only the general growth supporting ability of the medium which determines the response of RNA formation. Cells which are rapidly growing are richer in RNA than cells growing more slowly (Neidhart and Magasanik, 1960).

In the observations made in this study the amount of RNA was always greater when excess L-methionine was present in the medium than when normal L-methionine was present. However, this difference was very small when CO_2 was collected (Tables 3 and 4). A possible explanation might lie in the redox state of the glycolytic substrate which is directly related to the phosporylation state of the adenine nucleotide system (Klingenberg, 1964).

There is a direct precursor-product relationship between the purine and pyrimidine nucleosides and the product RNA. If the redox state is altered the adenine nucleotide system is also altered. It would seem that RNA formation is inhibited. An alteration probably also occurred when flasks were stoppered during the entire growth period and conditions went from aerobic to microaerophilic, and then to anaerobic at the later stages. The effect on the redox state apparently is not as great in this instance as when CO_2 was collected in the current experiments.

The specific activities of RNA were greater when glucose-6-C^{14} was used as substrate than when glucose-1-C^{14} was employed. One would expect that, since the operation of the HMP pathway results in retention of the label which is incorporated as ribose into the nucleotides.

Roberts et al. (1957) grew cells of E. coli with various radioactive substrates including glucose-1-C^{14}. Analyses of the nucleic

acids of these cells showed that the specific activities of the purines were about the same but lower than those of the pyrimidines. They concluded that 1-C was used for purine as well as pyrimidine synthesis but the mechanism differed. These same workers also reported that if $C^{14}O_2$ was used, the purines as well as the pyrimidines were labeled. Ribose was not found to be labeled with either substrate.

In this study the presence of excess L-methionine in the medium did not have any clearly defined effect on the specific activities of the RNA's obtained from either the 1-C^{14} or the 6-C^{14} experiment. This one would expect if the chemical nature of the medium was not a critical factor in RNA formation.

However, some effect was noted due to the presence of adenine in the glucose-1-C^{14} experiment. The specific activities tended to be lower under this condition, perhaps evidence of an adenine caused inhibition of the incorporation of 1-C^{14} which was available as CO_2. Cells grown in the presence of adenine were found to grow faster, this effect was most noticeable in the presence of excess L-methionine. The amounts of RNA produced did not differ to any great extent between cultures grown with or without adenine. The lowering of the specific activity of RNA in the presence of adenine may perhaps be considered as being due to the preferential use of exogenous unlabeled purine over that of the labeled purine formed from the liberated 1-$C^{14}O_2$.

S-Adenosylmethionine

In discussing the data on S-adenosylmethionine it is necessary to note that the 24 hour inoculum for the glucose-1-C^{14} experiment was grown in Roman's medium containing 6.7 μMoles L-methionine whereas that for the glucose-6-C^{14} experiment was grown in the same medium to which 400 μMoles L-methionine had been added.

A comparison of the amounts of S-AM produced during the course of the two experiments reveals a striking contrast between the two sets of data obtained. All yields obtained in the 1-C^{14} experiment were very much lower than those obtained in the 6-C^{14} experiment. The cell yields in terms of dry weight were approximately the same for both experiments thus indicating that the presence of excess L-methionine to the growth medium of the inoculum served to induce the methionine-activating enzyme in this strain of yeast. Pigg et al. (1964) reported similar results but with an experiment of only 5 hours duration. They were able to induce the enzyme with a starter concentration of 275 mg L-methionine per liter of medium while in the current experiments 600 mg per liter were used.

Since the quantities of S-AM produced in the presence or absence of adenine did not differ significantly with either normal or excess L-methionine, it was not possible to evaluate the role of adenine in this instance. In addition, since adenine was not omitted in the glucose-6-C^{14} experiment, no evaluation could be made here either.

The specific activities of the S-AM obtained from the 1-C^{14} experiment vary with the experimental conditions employed. However, there was very little if any effect of L-methionine concentration in any one given set of conditions. This would indicate that while the exogenous unlabeled adenine was incorporated into the RNA, the labeled purine formed was incorporated into S-AM. Since the specific activities were about the same with both concentrations of L-methionine, it would appear that the extent of adenine labeling was controlled by the growth rate of the culture since differences in growth rate between cells growing in normal and excess L-methionine were demonstrated to exist.

The specific activities of S-AM obtained in the 6-C^{14} experiment presented essentially the same pattern under all experimental conditions. There was a difference between the specific activities obtained with normal and excess L-methionine in all instances. These differences were particularly evident at the 20 and 26 hour levels when the specific activities obtained with normal L-methionine were four to five times higher than those obtained with excess L-methionine. Inspection of the data reporting the amounts of S-AM produced discloses that up to four times as much S-AM was produced with excess L-methionine. It would appear therefore that the C^{14} label was available to approximately the same extent but was diluted when excess L-methionine increased the production of S-AM. In addition, the retardation of growth in the early stages in the presence of excess

L-methionine could have resulted in a lesser amount of label being available.

The role of S-adenosylmethionine in the growth of the organism remains a moot question at this time. Svihla and Schlenk (1959, 1960) have shown that although high levels of S-AM are accumulated and stored in the vacuole of Candida utilis, the organisms are subsequently unable to use the stored material even under adverse conditions of growth. The findings of Pigg et al. (1964) are in agreement.

On the other hand, since the existence of methylated bases in nucleic acids was first reported, investigations attempting to elucidate their formation, have shown that S-AM is implicated as the direct methylating agent. Svensson et al. (1963) have been able to methylate soluble RNA from log phase cells of E. coli using a partially purified yeast enzyme preparation. These authors do not specifically implicate S-AM but indicate that the methyl group is obtained from methionine. Shapiro et al. (1963) have shown that two mutants of A. aerogenes which utilize sulfonium compounds for growth will use the S and CH_3 groups of S-AM for the synthesis of methionine. Norrell and Yall (1965) using a mutant of S. cerevisiae have found that when S^{35}-AM was used as a growth supplement, a dilution of the label occurs even when there was no methionine present in the medium. This is believed by them to be indicative not only of de novo S-AM production, but also de novo methionine synthesis. The S^{35} label was found in the sulfur

containing amino acids even in the presence of excess unlabeled L-methionine. Schlenk et al. (1958) reported that sulfonium compounds were used in transmethylation. There seems to be little doubt at present that the methyl group as such is being transferred. Tropp et al. (1964) have reported this to be the case in a methionine mutant of E. coli and Sirlin (1963) reported the same for the chironomid Smittia sp.

The data presented in these experiments indicate that at about 30 hours something crucial is happening to the growth of the yeast S. cerevisiae (SC-10-2). This is during the logarithmic phase of the culture, a period during which many reactions occur. The evidence obtained indicates an active role for S-adenosylmethionine. A critical analysis of the fate of S-AM labeled with C^{14}, S^{35}, and H^{3} respectively could shed light on just when and where the label goes during the growth of the cells. Possibly some of the S-AM is involved with transmethylation and biosynthesis of other amino acids, while the remainder is being stored. Although it is believed that methylated bases comprise only two to five percent of the total RNA (Brown, 1963) it is quite conceivable that more detailed and intensive investigations may disclose other transmethylation reactions involving S-adenosylmethionine occurring in cells.

SUMMARY

1. Excess L-methionine (400 μMoles per 100 ml of medium) resulted in a retardation of growth of S. cerevisiae (SC-10-2) during the early phases. However, after 32 hours there was a stimulation of growth.

2. Data obtained from the specific activities of the $C^{14}O_2$ evolved indicated operation of the hexose monophosphate pathway, particularly during the early phases of growth. Excess L-methionine was found to result in lowered ratios of the specific activity of 1-$C^{14}O_2$ to that of 6-$C^{14}O_2$.

3. The presence of excess L-methionine in the medium resulted in increased RNA production. The specific activities of RNA were found to be greater when glucose-6-C^{14} was used as a substrate than when glucose-1-C^{14} was used.

4. Induction of the methionine activating enzyme achieved by growing the inoculum in a medium containing excess L-methionine has resulted in yields of S-adenosylmethionine (S-AM) about four times as large as those obtained when no attempt at induction was made. The yields of S-AM gradually increased from 20 hours to 44 hours when a peak was reached which was followed by a rapid decline.

5. The evidence obtained from the experiments performed suggests that S-AM plays an important role in the growth of the organism.

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