THE EFFECT OF L-METHIONINE ON THE UPTAKE AND
UTILIZATION OF GUANINE-8-C\textsuperscript{14} BY

SACCHAROMYCES CEREVISIAE

by

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STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

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Irving Yall
Associate Professor of Microbiology
In sincere appreciation to Dr. Irving Yall for his guidance and encouragement throughout this investigation.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>STATEMENT OF PROBLEM</td>
<td>15</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>16</td>
</tr>
<tr>
<td>A. Organisms</td>
<td>16</td>
</tr>
<tr>
<td>B. The Media</td>
<td>16</td>
</tr>
<tr>
<td>C. Growth of the Organism</td>
<td>17</td>
</tr>
<tr>
<td>D. Extraction Procedures</td>
<td>18</td>
</tr>
<tr>
<td>E. Identification of Isolated Compounds</td>
<td>19</td>
</tr>
<tr>
<td>F. Radioactivity Determinations</td>
<td>20</td>
</tr>
<tr>
<td>RESULTS</td>
<td>21</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>38</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>49</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>51</td>
</tr>
</tbody>
</table>
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine</td>
<td>22</td>
</tr>
<tr>
<td>II.</td>
<td>The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine and adenine</td>
<td>23</td>
</tr>
<tr>
<td>III.</td>
<td>The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine and aminopterin</td>
<td>24</td>
</tr>
<tr>
<td>IV.</td>
<td>The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine, adenine and aminopterin</td>
<td>25</td>
</tr>
<tr>
<td>V.</td>
<td>The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine</td>
<td>28</td>
</tr>
<tr>
<td>VI.</td>
<td>The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine and adenine</td>
<td>29</td>
</tr>
<tr>
<td>VII.</td>
<td>The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine and aminopterin</td>
<td>30</td>
</tr>
<tr>
<td>VIII.</td>
<td>The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C(^{14}) in the presence of excess and normal amounts of L-methionine, adenine and aminopterin</td>
<td>31</td>
</tr>
<tr>
<td>Table</td>
<td>The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>IX.</td>
<td>The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine and adenine</td>
<td>33</td>
</tr>
<tr>
<td>X.</td>
<td>The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine, and aminopterin</td>
<td>34</td>
</tr>
<tr>
<td>XI.</td>
<td>The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine, adenine and aminopterin</td>
<td>35</td>
</tr>
<tr>
<td>XII.</td>
<td>The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine, adenine and aminopterin</td>
<td>36</td>
</tr>
<tr>
<td>XIII.</td>
<td>Total cell mass harvested per flask</td>
<td>37</td>
</tr>
</tbody>
</table>
ABSTRACT

Methionine, an essential amino acid in many organisms, has been interconnected with purine metabolism by its condensation with Adenosinetriphosphate to form S-adenosylmethionine (AM). In the yeast, Saccharomyces cerevisiae, the presence of excess L-methionine is known to stimulate the de novo production of adenine in order to overcompensate for the trapping of adenine groups in the formation of AM. Adenine is readily converted to guanine in these yeasts.

The present experiment is devised to investigate the effect of excess L-methionine on guanine utilization in the yeast. In some experiments, aminopterin, an antifolic acid compound, was employed to block endogenous purine synthesis.

Guanine-8-C\textsubscript{14} was taken up by the cells during a 44 hour exposure. However, adenine in the media reduced the amount of guanine-8-C\textsubscript{14} incorporated into nucleic acids. The specific activity of isolated guanine components indicated that adenine was readily interconverted to guanine.

When aminopterin was present, guanine was readily incorporated into the cells but only a slight amount was utilized for nucleic acid synthesis. The remaining guanine was concentrated in the cell in
nucleotide pools. Adenine still supplied a good proportion of nucleotide guanine.

No guanine interconversion to adenine was observed in any of the growth conditions.
INTRODUCTION

Methionine was isolated from casein by Müller in 1923 and in the same year was shown by Jackson and Block (Stekol, 1955) to be one of the essential amino acids in the rat diet. Methionine is required by many microorganisms but it can be replaced by cysteine or homocysteine provided that methyl group donors such as choline, betaine or dimethyl propriothetin are present (Buess, 1961). Borsook and Dubnoff (1947) and Dubnoff and Borsook (1948) have shown that the methylation of cysteine or homocysteine can be one of the general pathways of methionine biosynthesis.

Methionine is involved in many reactions. It can be deaminated to form alpha-ketobutyric acid or it can be reversibly demethylated to form homocysteine. Homocysteine then reacts with serine to form the intermediate cystathionine which can be cleaved to form cysteine and homoserine (du Vigneaud et al., 1942; Anslow et al., 1946). Homoserine, deriving its carbons 2, 3, and 4 from methionine, can be directed on to the biosynthesis of glucose with propionic acid as an intermediate (Kisliuk, Sakami and Patwardhan, 1956). In higher animals the conversion of methionine sulfur to cysteine sulfur is essentially irreversible but some microorganisms such as Neurospora crassa can catalyze the reverse of these reactions (Horowitz, 1947; Fling and
Methionine can be incorporated into the structure of protein as methionine or as cysteine, following the reactions described above.

Methionine is also involved in another series of reactions broadly classified as transmethylation reactions, first recorded by Hofmeister in 1894. He observed that inorganic tellurite could be converted to dimethyl tellurite by the dog (Cantoni, 1952). Keller et al. (1949) proved that the CH₃ group is transferred intact inasmuch as there is no loss of hydrogen from the unit. Methionine itself can be involved in the biological transfer of the labile methyl group but Cantoni (1951) found that S-adenosylmethionine (AM), a condensation product of methionine and the high energy purine compound, adenosinetriphosphate (ATP), functions more efficiently in transmethylation reactions.

Pfeffer and Shapiro (1963) found AM to be involved in several enzymatic reactions that are essential to the biosynthesis of the amino acid methionine in various microorganisms, mammalian tissues, and plant tissues. *Aerobacter aerogenes*, for example, uses AM as a methyl group donor as shown by the following pathway (Shapiro, 1962):

\[
\text{L-homocysteine} + \text{AM} \rightarrow \text{L-methionine} + \text{S-adenosyl-L-homocysteine} + H^+.
\]

S-Methylmethionine can also serve as the methyl donor in the above reaction (Shapiro, 1956). S-Adenosylmethionine can be regenerated from S-adenosyl-L-homocysteine through the participation of
N-5-methytetrahydrofolic acid (Duerre and Schlenk, 1962). Shapiro et al. (1963) indicated that the thionium compound had not been shown to be a source of any other part of the methionine molecule. The enzyme S-adenosylmethionine homocysteine transferase participated in the methylation of homocysteine. In the yeast, Saccharomyces cerevisiae, this pathway is one of the major methods of methionine biosynthesis (Shapiro and Almenas, 1963).

In studying the metabolic activity of A. aerogenes and various strains of Escherichia coli, it was discovered that the adenine moiety of AM could be transferred to L-homocysteine forming S-adenosyl-L-homocysteine and methionine. In this case the entire methionine molecule was derived from AM. Pfeffer and Shapiro (1962) indicated that this transadenosylation with the concomitant cleavage of the sulfur-ribose linkage had not been reported before. S-Adenosylmethionine homocysteine adenosyl-transferase was found to be the enzyme involved in E. coli K-12, K113-3 and in the Texas strain by Pfeffer and Morris (1963).

In 1963, Rosenthal and Buchanan reported an alternate pathway for the biosynthesis of the methyl group of methionine which depended on the presence of 5-methytenahydrofolic acid, homocysteine, reduced pyridine dinucleotide, ATP, FAD, Mg$^{++}$ and an enzyme containing a derivative of Vitamin $\text{B}_{12}$ as a prosthetic group (Guest et al., 1962). Earlier, Rosenthal and Buchanan (1962) noted that ATP was only
required in catalytic amounts and that it could be replaced by a fraction isolated from the $B_{12}$ enzyme. This fraction contained both a cofactor for the methionine synthesis and an enzyme that forms the cofactor from ATP. Magnum and Scrimgeon (1962) using pig liver and Larrabee et al. (1963) using *E. coli* K-113-3 substantiated that this latter enzyme was S-adenosylmethionine synthetase. It was shown that AM could replace S-adenosylmethionine synthetase, ATP and $Mg^{++}$. Kisliuk (1963) reported that AM did not function by the direct transfer of its labile methyl group.

It has been postulated that the AM operates as an activator of the vitamin $B_{12}$ in its conversion to coenzyme $B_{12}$, the prosthetic group of the enzyme involved in the synthesis of methionine from homocysteine and 5-methyltetrahydrofolic acid. Weissbach, Redfield and Peterkafsky (1961) and Peterkafsky and Weissbach (1962) reported that in cell free extracts of *Clostridium tetanomorphum*, vitamin $B_{12}$ is converted to the coenzyme form by the reduction of its cobalt valence and the replacement of the cyanide moiety of the vitamin by an adenine nucleotide derived from ATP. The same activation of vitamin $B_{12}$ has been reported in *Propionibacterium shermanii* by Brady, Castanera and Barker (1962). Vitamin $B_{12}$ deficiencies have been found by Lanzer and Kratzer (1962) to decrease the synthesis of methionine in turkeys. Its importance in methionine biosynthesis has been substantiated by Fox and Ludwig (1962) and Morningstar and Kisliuk (1963).
For additional information on the nature of the thionium compound, S-adenosylmethionine, and its importance in the transfer of labile methyl groups, the following papers are of interest: Shapiro, 1955; Cantoni and Durrell, 1957; Schlenk and Shapiro, 1959; Svihla and Schlenk, 1959; Shapiro and Schlenk, 1960; Fruton and Simmonds, 1961; Gawel, Turner and Parks, 1962; Yall and Henney, 1961; Henney, 1961; and Yall, 1962.

The unusual relationship between an amino acid and the high energy purine compound, ATP, has stimulated investigations into the other possible interrelations of methionine with purine metabolism.

Observing the effect of methionine on the growth of Bakers' yeast, Schmidt, Seraidarian and Greenbaum in 1954 indicated that a net increase in de novo purine synthesis overcompensated for the "trapping" of adenine groups by methionine in the biosynthesis of AM. Reddi (1955) discovered that methionine caused an increase in growth and synthesis of protein and RNA in Pseudomonas hydrophila as well as an increase in phosphorus turnover in RNA. Schmidt et al. (1956) determined that this increase in RNA synthesis in Bakers' yeast was not due to the direct transfer of the labile methyl group of methionine or methionine derivatives such as AM.

Excess L-methionine was shown by Yall and Henney (1961) to alter the purine and nucleic acid metabolism of both the wild and adenine requiring mutant of Saccharomyces cerevisiae. Yall (1962)
in further investigations with the adenine requiring mutant of the same yeast found that in the presence of excess L-methionine there was a proportionate increase in cell growth as the adenine concentration of the media was increased.

Using compounds containing radioactive C\(^{14}\), the carbon atoms of the synthesized purine nucleotides were found to be derived from CO\(_2\), glycine, 1-carbon residues from formate, formaldehyde, serine or glycine and from the preformed purine bases themselves (Roberts et al., 1957). Applying this information, Buchanan (1960) set up a schematic representation of de novo purine synthesis:

![Diagram of purine synthesis](attachment:image.png)
Brown et al. (1948) and Brown (1953) indicated that once the purine ring was formed it remained intact and its integrity was
maintained through reactions such as those involving the interconversion of the bases.

Brown and Roll (1955), investigating the effect of the incorporation of exogenous purines into the cell on de novo purine synthesis, discovered that the preformed purines were readily utilized for nucleotide synthesis. An example of this is Lactobacillus casei which uses preformed adenine and guanine to furnish 80% of the RNA purines in the cell. RNA renewal is much more rapid than DNA synthesis which is limited by the cell's potential to reproduce itself (Bennett and Karlsson, 1957).

Smith (1962) indicated that in high concentrations, the exogenous purines have a strong negative feedback inhibition on endogenous synthesis. The exogenous metabolite, normally the end product of the biosynthetic pathway, will exert an inhibitory effect on the biosynthetic step by raising the level of the intracellular pool. The enzymes responsible for the synthesis are in themselves controlled by the amount of purines in the system which are, in many experimental cases, supplied preformed in the media (Levin and Magasanik, 1961).

The uptake of purines by the yeasts has been observed in 18 species and is considered a general phenomenon (Roush and Shieh, 1962). Roush, Questiaux and Domas (1959) described the accumulation of purines in Candida utilis as being controlled by active transport. They implied that a separate transport system existed for each purine
with both respiratory and fermentative processes as the source of energy. In addition, they reported the formation of a purine pool in the yeast due to a rapid uptake that was not compensated for by an equally rapid metabolism. Potter (1960) also described the presence of metabolic pools which were made up of the nucleotides of the purines and pyrimidine bases. Conversion to RNA and DNA took place after the bases were changed to nucleotides. Only the 5' monophosphates were contributed to the pool by the biosynthetic pathways. Henderson (1962a) indicated that this general metabolic pool as described above was compartmentalized in ascites tumor cells. The preformed exogenous purines made up one pool while the purine nucleotides derived from the de novo pathway comprised the second. In order for the exogenous nucleotides to be utilized by the cell, they must first pass into the de novo pool.

Henderson (1962b) reported that the purine bases do not remain concentrated for any length of time in the cell. Saffron and Scarano (1953) demonstrated the presence of a nucleotide phosphorylase in pigeon liver cells that catalyzed the condensation of ribose 1, 5 diphosphate with adenine to form adenylic acid. They discovered a similar phosphorylase in yeast cells that catalyzed the condensation of adenine and 5' phosphoribosyl pyrophosphate to form adenylic acid. Lieberman, Kornberg and Sims (1954) proved that the 5' phosphoribosyl pyrophosphate was derived from ATP and ribose 5' phosphate. Balis et al. (1956) and Bennett and Krueckel (1955) believed that the purines
exerted a growth promoting effect on the cell only after being converted to this active state. Potter (1960) indicated that with the exception of cytosine, each nucleoside is in reversible equilibrium with the corresponding base due to the activity of the nucleoside phosphorylase.

Derivatives of folic acid were found to be the transmethylating agents involved in two of the steps in the de novo pathway of purine synthesis. These reactions are the methylation of glycinamide ribotide to formylglycinamide ribotide and the methylation of 5-amino-4-imidazolecarboxamide ribotide to 5-formamido-4-imidazolecarboxamide ribotide (see pages 6 and 7 above).

Before folic acid (FA) participates in these reactions, it first is reduced to the tetrahydrofolic acid state (THFA). Folate reductase or tetrahydrofolate dehydrogenase was found to be the enzyme involved (Zakrzewski, 1963). There are conflicting viewpoints as to the nature of this reduction. Futterman (1957) postulated that this process took place in two steps: Folic acid $\xrightarrow{\text{DPNH}}$ Dihydrofolic acid

$$\text{Dihydrofolic acid} \xrightarrow{\text{TPNH}} \text{Tetrahydrofolic acid}$$

Zakrzewski and Nichol (1958 and 1960) believed that this reduction took place in one step with two moles of TPNH being oxidized for every mole of folic acid reduced.

Sakami (1955), in his study of the role of folic acid in glycine and serine metabolism, found that THFA could accept a methyl group of serine, formic acid and formaldehyde and that it was involved in the
transmethylation of thymine, purines and methionine. Berg (1953) indicated that the conversions of formate into the methyl group of methionine, beta carbon of serine, cystathionine, the 2 and 8 carbons of the purines and choline occurred under the same conditions.

Jaenicke (1955) found that the N-10-formyl THFA derivative was the first major product formed in the transfer of the beta carbon of serine to methyl group acceptors. He also discovered that the formyl group of this compound was in equilibrium with other one carbon donors and acceptors in the system. Thus the concept of the methyl group pool was substantiated. Greenberg (1953) and Jaenicke (1955) showed that ATP was needed to fix formate to THFA in the formation of the active molecule. N-10-formyl THFA was later implicated by Greenberg et al. (1955) as the methyl group donor in purine synthesis. It formylates 5-amino-4-imidazolecarboxamide 5' phosphoribotidate which then loses water to form inosinic acid. The actual transmethylation proceeds in the absence of ATP.

Jaenicke (1956) studied pigeon liver systems and postulated the following: (1) Serine + THFA \text{pyridoxal PO}_4^{---} \text{Mn}^{++} \rightarrow \text{Hydroxymethyl THFA} + \text{glycine}

(2) \text{Hydroxymethyl THFA} + \text{TPN} \rightarrow \text{N-10-formyl THFA} + \text{TPNH}.

Eighty per cent of the hydroxymethyl THFA was converted to the N-10-formyl THFA.
Flaks, Erwin and Buchanan (1957) have implicated both N-10-formylTHFA and N-5-N-10-anhydroformylTHFA as the agents transformylating the glycinamide ribotide and supplying carbon 2 of the purine ring.

The structure of these transmethylating agents are as follows:

**FOLIC ACID**

(pteroylglutamic acid)

\[
\begin{align*}
\text{COOH} & \\
(CH_2)_2 & \\
\text{CH--NH--C--C=CH--NH--CH_2--C} & \\
\text{COOH} & \\
\end{align*}
\]

\[\text{R}\]

**THFA**

\[
\begin{align*}
\text{R--NH--CH_2--C} & \\
\text{H} & \\
\text{N} & \\
\text{H} & \\
\text{NH_2} & \\
\end{align*}
\]

**N-10-FORMYLTHFA**

\[
\begin{align*}
\text{R--N--CH_2--C} & \\
\text{CHO} & \\
\text{H} & \\
\text{N} & \\
\text{H} & \\
\end{align*}
\]

There are two antimetabolites of the folic acid derived coenzymes that inhibit purine synthesis indirectly. These are aminopterin and amethopterin. Aminopterin binds very tightly to the enzyme tetrahydrofolic dehydrogenase and it can be displaced only at excess natural substrate levels. It is bound 100,000 times more strongly to the enzyme than folate (Zakrzewski, 1963). Peters and Greenberg (1959) found that aminopterin can completely inhibit folic acid
reductase (folic acid dehydrogenase) at very low concentrations. The degree of inhibition is a function of enzyme concentration.

**AMINOPTERIN**

(4-amino-pteroylglutamic acid)

\[
\text{COOH} \quad (\text{CH}_2)_2 \quad \text{H} = \text{H} \quad \text{C} = \text{C} \quad \text{C} = \text{NH} - \text{CH}_2 - \text{C} \quad \text{N} = \text{N} \quad \text{N} = \text{G} - \text{NH}_2 \\
\text{CH} - \text{NH} - \text{C} = \text{C} \quad \text{C} = \text{NH} - \text{C} - \text{NH} \quad \text{COOH} \quad \text{C} \quad \text{C} \quad \text{NH}_2
\]

The strong affinity of the drug to the substrate is due to the stronger basicity of the NH₂ group on C₂ and the N₁ on the antimetabolite as compared to the natural substrate (Perault and Pullman, 1961).

Aminopterin does not inactivate the enzyme but, as a result of its binding, prevents the formation and regeneration of the folic acid derived coenzymes (Zakrzewski, 1963). However, Blakley and McDougall (1961) question this interpretation by calling it non-competitive inhibition. The enzyme and inhibitor in their opinion undergo a slow irreversible change to an inactive product. Zakrzewski concluded that the substrate and the inhibitor actively competed for the enzyme.

Aminopterin has been used effectively in the treatment of leukemic cells which have a high requirement for folic acid. Before treatment, formate C¹⁴ was demonstrated to be incorporated into both purine components of RNA and DNA and into protein. After the addition
of the antimetabolite, the incorporation was inhibited almost entirely
(Handschumacher and Welch, 1960).

Aminopterin was effective in inhibiting purine synthesis in E.
coli (Wolley and Pringle, 1950). In this organism there was found to
be an accumulation of the 5-amino-4-imidazolecarboxamide ribotide
after introduction of aminopterin.

Stekol (1955) reported many experiments in which folic acid
deficiencies in higher animals decreased the utilization of labiled C$^{14}$
of formate, glycine, serine, and methionine for choline and creatine
synthesis, indicating that not only purine synthesis, but also other
pathways important to cell growth were affected by folic acid antag-
onists. In 1958, Johnson, Corte and Jasmin noted that aminopterin
inhibited the activation of acetate to form acetyl CoA suggesting that
the inhibitor is functioning in a different manner than had been noted
previously.
STATEMENT OF PROBLEM

The purpose of this study is to determine the effect of excess L-methionine on the uptake and utilization of guanine-8-C\textsuperscript{14} by the yeast \textit{Saccharomyces cerevisiae} with special interest directed to:

(1) nucleic acid biosynthesis as affected by a preformed purine, and

(2) the effect of the antifolic acid antagonist (aminopterin) on the cell's ability to carry out interconversions of the purine bases.
MATERIALS AND METHODS

A. The Organism

Saccharomyces cerevisiae strain SC-10-2 was the test organism in this experiment. It showed no requirement for either adenine or guanine. Excellent growth was obtained by growing the yeast in the presence and absence of each purine. Inhibition of growth was obtained with aminopterin.

B. The Media

The synthetic complete media of Roman (1956) was used to ascertain the effect of excess (400 umoles/flask) and normal (3.35 umoles/flask) L-methionine on the metabolism of guanine-8-C\textsuperscript{14} in Saccharomyces cerevisiae. One hundred ml quantities of the media were dispensed into 500 ml Erlenmeyer flasks and sterilized under 15 pounds pressure for 15 minutes. Four different growth conditions were set up as follows: (1) guanine-8-C\textsuperscript{14} was the sole purine source in both excess and normal amounts of L-methionine; (2) adenine supplemented guanine-8-C\textsuperscript{14} in excess and normal amounts of L-methionine; (3) guanine-8-C\textsuperscript{14} and the antifolic acid antagonist aminopterin were added to excess and normal L-methionine; (4) adenine supplemented the guanine-8-C\textsuperscript{14} and aminopterin in the excess and normal amounts of L-methionine.
Adenine, guanine-8-C\textsuperscript{14}, aminopterin, and the two concentrations of methionine were sterilized separately under the same conditions and aliquots were analyzed in a Beckman D. U. spectrophotometer (Beavin et al., 1955). Four moles of adenine, 3.35 moles of guanine-8-C\textsuperscript{14}, 7.5 moles of aminopterin, 3.35 moles of normal methionine, and 400 moles of excess methionine were added aseptically, where required, to the Erlenmeyer flasks of the media.

The radioactivity of the guanine-8-C\textsuperscript{14} was determined in a Packard Tri-Carb Liquid Scintillation Spectrometer (see page 20). The specific activity of the purine is shown in an accompanying table. Flasks to which no adenine or guanine-8-C\textsuperscript{14} had been added were used as controls.

C. Growth of the Organism

An inoculum of the organism was taken from a glucose yeast extract slant on which it had been maintained and transferred to a flask of Roman's synthetic media containing excess amounts of L-methionine. This culture was grown for 24 hours at 30\textdegree C with continuous aeration on a Brunswick shaker (Henney, 1961).

The cells were harvested by centrifugation and adjusted to 4.0% transmission in a Coleman spectrophotometer with sterile distilled water. Aliquots of two ml were added aseptically to the flasks containing the appropriate purines. These flasks were incubated with
shaking for 44 hours at 22° C and harvested by centrifugation and then washed three times with distilled water. The washings were added to the supernatant and the cells were resuspended to volume in distilled water. Duplicate one ml aliquots of the cells were plated on Whatman no. 542 filter disks (previously washed in alcohol, dried and weighed) and then dried at 70° C to a constant weight. The amount of cells harvested per flask was then computed by the dry weight measurements.

The supernatants and cells were stored at -20° C until needed.

D. Extraction Procedures

The yeasts were extracted for ribonucleic acid (RNA) by the following procedure. The cells were collected by centrifugation and the supernatant was saved for later analysis. The packed cells were suspended in 1.5 N perchloric acid (volume: volume, 1: 2.5). This suspension was held at 4° C for 24 hours with constant stirring. The extracted cells were washed twice with 1.5 N perchloric acid and collected by centrifugation (Schlenk, Dainko, and Stanford, 1959). The 1.5 N fraction was then assayed by chromatography on a Dowex 50 resin with 0.5 N, 2 N, 3 N, and 4 N hydrochloric acid (Schlenk and DePalma, 1957). The fractions were monitored for absorption at 255 mu by a LKB Uvicord Absorptionmeter. Fractions showing absorption were assayed for radioactivity.
Deoxyribonucleic acid (DNA) components were extracted and hydrolyzed from the cell residue by the use of 1 N perchloric acid at 70° C for 50 minutes. The hydrolysis was sufficient to split the nucleic acids to purine bases and pyrimidine nucleotides (Loring, 1955).

E. Identification of Isolated Compounds

The material developed from the Dowex column was tentatively identified by the u.v. absorption spectrum on the Beckman D. U. spectrophotometer. Those fractions that showed significant amounts of radioactivity were collected and condensed by evaporation under vacuum and the radioactive nucleic acid derivatives were separated by thin layer chromatographic techniques (Hagdahl, 1961). The adsorbant and binding layer used on the chromatoplates was diethylaminoethyl cellulose. One dimensional chromatography was employed using tertiary butanol, hydrochloric acid and water (v:v:v, 70:6.7:23.3) as the solvent system (Smith and Markam, 1950). Ultraviolet absorbing spots were removed from the glass plates, eluted with 0.1 N hydrochloric acid (Carter, 1950) and quantitated on the Beckman D. U. spectrophotometer.

The 1 N perchloric acid fraction was developed on Whatman no. 1 chromatographic paper using the same solvent system. The ultraviolet absorbing spots were cut from the paper (Wyatt, 1955) eluted with 0.1 N hydrochloric acid and quantitated with the Beckman
D. U. spectrophotometer. Specific activities of all isolated compounds were determined.

F. Radioactivity Determinations

The radioactivity of the various fractions was determined in a Packard Tri-Carb Liquid Scintillation Spectrometer. Duplicate aliquots of the sample were dispensed in low potassium glass vials and a dioxane-methanol scintillator was employed (Bray, 1960). Correction of color and acid quenching of the system was accomplished by the use of an internal benzoic acid C\textsuperscript{14} standard (Kinnory et al., 1958).

Whole cells and cell debris were solubilized in 1 ml of formamide at room temperature for 24 hours before the scintillator was added. It was found that this was sufficient to realize an approximate count from the suspended cells (Mitchison and Wilbur, 1962).
RESULTS

Before the addition of replicate aliquots of guanine-8-C\textsuperscript{14} into the test flasks, an aliquot of the material was assayed quantitatively on the Beckman D. U. spectrophotometer and its radioactivity was measured on the Tri-Carb Liquid Scintillation Spectrometer. Guanine-8-C\textsuperscript{14} with a total concentration of 3.35 umoles and specific activity of 700, 372 counts per minute per umole was added to each flask.

After the 44 hour exposure to the radioactive guanine-8-C\textsuperscript{14}, the cells and supernatants collected were measured for radioactivity by liquid scintillation procedures. The presence of excess amounts of L-methionine did not affect the extent of guanine-8-C\textsuperscript{14} incorporation into the cells after the incubation period as compared to the incorporation into the cells grown in normal L-methionine. Tables I to IV indicate the amount of radioactivity detectable and the percentage of guanine-8-C\textsuperscript{14} that was taken up by the cells.

The cells were extracted for the nucleic acids as described. That the 0.5 N HCl eluate from the Dowex 50 column contained the RNA nucleotides was determined by qualitative measurements on the Beckman D. U. spectrophotometer and by later analysis by thin layer chromatography. In the cells grown with adenine, 67% of the radioactivity incorporated was found in the RNA nucleotides in the normal
TABLE I

The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C\textsuperscript{14}\* in the presence of excess and normal amounts of L-methionine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>cpmp/Flask Detected</th>
<th>Radioactivity Detected (cpm)</th>
<th>Per Cent Activity in Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal L-methionine#</td>
<td>774,000</td>
<td>1,530,000</td>
<td>2,304,000</td>
</tr>
<tr>
<td>Excess L-methionine#</td>
<td>901,000</td>
<td>1,407,000</td>
<td>2,308,000</td>
</tr>
</tbody>
</table>

*original radioactivity introduced = 2,317,000 cpmp/flask

#normal L-methionine = 3.35 umole/flask
excess L-methionine = 400 umole/flask
TABLE II

The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-$^{14}$C in the presence of excess and normal amounts of L-methionine and adenine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>cpm/Flask Detected</th>
<th>Radioactivity Detected (cpm)</th>
<th>Per Cent Activity in Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal L-methionine and adenine</td>
<td>1,273,000</td>
<td>2,288,000</td>
<td>45%</td>
</tr>
<tr>
<td>Excess L-methionine and adenine</td>
<td>1,015,000</td>
<td>2,060,000</td>
<td>50%</td>
</tr>
</tbody>
</table>

*original radioactivity added = 2,317,000 cpm/flask

#normal L-methionine = 3.35 umole/flask
excess L-methionine = 400 umole/flask
TABLE III

The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C\(^{14}\) in the presence of excess and normal amounts of L-methionine and aminopterin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>cpm/Flask Detected</th>
<th>Radioactivity Detected (cpm)</th>
<th>Per Cent Activity in Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supernatant</td>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>Normal L-methionine# and aminopterin</td>
<td>330,000</td>
<td>1,355,000</td>
<td>1,685,000</td>
</tr>
<tr>
<td>Excess L-methionine# and aminopterin</td>
<td>618,000</td>
<td>1,820,000</td>
<td>2,440,000</td>
</tr>
</tbody>
</table>

*original radioactivity added = 2,317,000 cpm/flask

#normal L-methionine = 3.35 umole/flask

excess L-methionine = 400 umole/flask
TABLE IV

The distribution of radioactivity after a 44 hour exposure of the yeast to guanine-8-C\textsuperscript{14} in the presence of excess and normal amounts of L-methionine, adenine and aminopterin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>cpm/Flask Detected</th>
<th>Radioactivity Detected (cpm)</th>
<th>Per Cent Activity in Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supernatant</td>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>Normal L-methionine# adenine and aminopterin</td>
<td>528,000</td>
<td>1,435,000</td>
<td>1,963,000</td>
</tr>
<tr>
<td>Excess L-methionine# adenine and aminopterin</td>
<td>519,000</td>
<td>1,445,000</td>
<td>1,965,000</td>
</tr>
</tbody>
</table>

*original radioactivity added = 2,317,000 cpm/flask

#normal L-methionine = 3.35 umole/flask

excess L-methionine = 400 umole/flask
L-methionine while the RNA nucleotides from the excess L-methionine contained 72%. In the cells that were grown in normal and excess L-methionine alone, the RNA nucleotides contained 72% and 85% of the radioactivity incorporated respectively. Aminopterin decreased the amount of radioactive guanine-8-C\textsuperscript{14} used for RNA synthesis. In the cells grown with normal and excess L-methionine and aminopterin, 6.3% and 9.7% of the radioactivity incorporated was found in the RNA nucleotides respectively. The RNA nucleotides of the cells grown with normal and excess L-methionine, adenine and aminopterin accounted for 6.9% and 11% of the total.

Free bases that were present in the nucleotide pools accounted for the remainder of the activity extracted from the cells with 1.5 N perchloric acid. Guanine was the major component of these pools as proven by separation by thin layer chromatography and by quantitative and qualitative measurements on the Beckman D. U. spectrophotometer. The Rf value of .21 and maximum and minimum u. v. absorption at 248 mu and 235 mu corresponded with those of a known standard. The cells grown without aminopterin in normal L-methionine contained about 16% of the activity of the intact cell in these nucleotide pools. Cells grown with aminopterin contained 75% to 85% of the activity in this fraction.
The DNA of the cells in all cases showed a very small percentage of the total activity incorporated. Only 1% to 5% of the radioactivity was found in this fraction.

The cells, in preparation for extraction, were collected by centrifugation after being stored at freezing temperatures. The resulting supernatant (water fraction), containing both pooled guanine-8-C\textsuperscript{14} and aminopterin accounted for 5% to 13% of the radioactivity. Tables V to VIII list the results of the extraction procedures.

The nucleic acids were hydrolyzed by the methods discussed above and the resulting purine bases when isolated by chromatography were tested for radioactivity and the specific activities in counts per minute per umole measured. The specific activity of the guanine components isolated from the cells grown in excess L-methionine was much higher in all cases than the specific activities of the guanine obtained from the cells grown in normal amounts of L-methionine. The specific activity of the guanine-8-C\textsuperscript{14} introduced was much higher than that found in the isolated compounds. There was no activity found in any of the adenine compounds isolated.

When the specific activities of the guanine components isolated from the nucleotide pool were measured, it was found that those extracted from the cells grown under natural conditions had activities of 92,000 cpm/umole and 99,000 cpm/umole. When aminopterin was employed, the specific activities of the isolated guanine components
### TABLE V

The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-$\text{C}^{14}$ in the presence of excess and normal amounts of L-methionine.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine*</th>
<th>Excess L-methionine*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activity cpm/flask</td>
<td>% of whole cell</td>
</tr>
<tr>
<td>Whole cell</td>
<td>1,530,000</td>
<td>1,407,000</td>
</tr>
<tr>
<td>1.5 N HClO$_4$ fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 N HCl (RNA)</td>
<td>1,200,000</td>
<td>72.0%</td>
</tr>
<tr>
<td>3.0 N HCl</td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>4.0 N HCl</td>
<td>238,000</td>
<td>16.0%</td>
</tr>
<tr>
<td>1.0 N HClO$_4$ (DNA)</td>
<td>22,000</td>
<td>1.4%</td>
</tr>
<tr>
<td>Water</td>
<td>68,000</td>
<td>4.4%</td>
</tr>
<tr>
<td>Cell debris</td>
<td>600</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

*normal L-methionine = 3.35 µmole/flask
excess L-methionine = 400 µmole/flask
**TABLE VI**

The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C\(^{14}\) in the presence of excess and normal amounts of L-methionine and adenine.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine*</th>
<th>% of whole cell</th>
<th>Excess L-methionine*</th>
<th>% of whole cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activity cpm/flask</td>
<td></td>
<td>activity cpm/flask</td>
<td></td>
</tr>
<tr>
<td>Whole cell</td>
<td>1,015,000</td>
<td></td>
<td>1,045,000</td>
<td></td>
</tr>
<tr>
<td>1.5 N HCl(_4) fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 N HCl (RNA)</td>
<td>687,000</td>
<td>67.0%</td>
<td>758,000</td>
<td>72.0%</td>
</tr>
<tr>
<td>3.0 N HCl</td>
<td>trace</td>
<td></td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>4.0 N HCl</td>
<td>173,000</td>
<td>16.0%</td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>1.0 N HCl(_4) (DNA)</td>
<td>21,000</td>
<td>2.0%</td>
<td>29,000</td>
<td>2.8%</td>
</tr>
<tr>
<td>Water</td>
<td>130,000</td>
<td>12.0%</td>
<td>134,000</td>
<td>12.8%</td>
</tr>
<tr>
<td>Cell debris</td>
<td>4,000</td>
<td>&lt;1.0%</td>
<td>2,000</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

*normal L-methionine = 3.35 umole/flask
excess L-methionine = 400 umole/flask
### TABLE VII

The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-C\(^{14}\) in the presence of excess and normal amounts of L-methionine and aminopterin.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine*</th>
<th>Excess L-methionine*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activity cpm/flask</td>
<td>% of whole cell</td>
</tr>
<tr>
<td>1. Whole cell</td>
<td>1,355,000</td>
<td>1,821,000</td>
</tr>
<tr>
<td>2. 1.5 N HCl(_4) fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 N HCl (RNA)</td>
<td>86,000</td>
<td>6.3%</td>
</tr>
<tr>
<td>3.0 N HCl</td>
<td>trace</td>
<td>1,417,000</td>
</tr>
<tr>
<td>4.0 N HCl</td>
<td>1,070,000</td>
<td>78.0%</td>
</tr>
<tr>
<td>3. 1 N HCl(_4) (DNA)</td>
<td>18,000</td>
<td>1.3%</td>
</tr>
<tr>
<td>4. Water</td>
<td>180,000</td>
<td>13.0%</td>
</tr>
<tr>
<td>5. Cell debris</td>
<td>&lt;1.0%</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

*normal L-methionine = 3.35 umole/flask  
excess L-methionine = 400 umole/flask
TABLE VIII

The distribution of radioactivity in cellular components after a 44 hour exposure to guanine-8-\(^{14}\)C in the presence of excess and normal amounts of L-methionine, adenine and aminopterin.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine*</th>
<th>Excess L-methionine*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activity (cpm/flask)</td>
<td>% of whole cell</td>
</tr>
<tr>
<td>Whole cell</td>
<td>1,435,000</td>
<td>1,445,000</td>
</tr>
<tr>
<td>2. 1.5 N HClO(_4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 N HCl (RNA)</td>
<td>100,000</td>
<td>6.9%</td>
</tr>
<tr>
<td>3.0 N HCl</td>
<td>trace</td>
<td>1,108,000</td>
</tr>
<tr>
<td>4.0 N HCl</td>
<td>1,218,000</td>
<td>80.0%</td>
</tr>
<tr>
<td>3. 1.0 N HClO(_4)</td>
<td>45,000</td>
<td>3.1%</td>
</tr>
<tr>
<td>4. Water</td>
<td>70,000</td>
<td>4.8%</td>
</tr>
<tr>
<td>5. Cell debris</td>
<td>1,000</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

*normal L-methionine = 3.35 umole/flask  
excess L-methionine = 400 umole/flask
were approximately 3.5 to 5 times as high. Some dilution was apparent however, and the specific activities of those from the excess L-methionine were higher in all cases.

The specific activities of the guanine components isolated from DNA approximated those of the RNA nucleotides. Tables IX to XII summarize these results.

Dry weight measurements of the total cell growth indicate that the presence of excess L-methionine stimulated growth up to twice that found with normal L-methionine. Table XIII indicates these figures.
TABLE IX

The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14}* in the presence of excess and normal amounts of L-methionine.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine#</th>
<th>Excess L-methionine#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cpm/umole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>compound</td>
<td>compound</td>
</tr>
<tr>
<td>RNA nucleotides</td>
<td>guanine 289,100</td>
<td>guanine 364,912</td>
</tr>
<tr>
<td>nucleotide pool</td>
<td>guanine 92,000</td>
<td>guanine</td>
</tr>
<tr>
<td>DNA nucleotides</td>
<td>guanine 278,000</td>
<td>guanine 350,000</td>
</tr>
<tr>
<td>Water</td>
<td>guanine 116,332</td>
<td>guanine 76,248</td>
</tr>
</tbody>
</table>

*specific activity of guanine-8-C\textsuperscript{14} introduced = 700,372 cpm/umole

#normal L-methionine = 3.35 umole/flask
excess L-methionine = 400 umole/flask
TABLE X

The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\textsuperscript{14}\textsuperscript{*} in the presence of excess and normal amounts of L-methionine and adenine.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine#</th>
<th>Excess L-methionine#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>compound</td>
<td>cpm/umole</td>
</tr>
<tr>
<td>RNA nucleotides</td>
<td>guanine</td>
<td>202,732</td>
</tr>
<tr>
<td>nucleotide pool</td>
<td>guanine</td>
<td>99,000</td>
</tr>
<tr>
<td>DNA nucleotides</td>
<td>guanine</td>
<td>190,000</td>
</tr>
<tr>
<td>Water</td>
<td>guanine</td>
<td>196,614</td>
</tr>
<tr>
<td></td>
<td>guanine</td>
<td>211,110</td>
</tr>
<tr>
<td></td>
<td>guanine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>guanine</td>
<td>200,000</td>
</tr>
<tr>
<td></td>
<td>guanine</td>
<td>138,820</td>
</tr>
</tbody>
</table>

\*specific activity of guanine-8-C\textsuperscript{14} introduced = 700,372 cpm/umole

\#normal L-methionine = 3.35 umole/flask

excess L-methionine = 400 umoles/flask
TABLE XI

The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-\textsuperscript{14}C in the presence of excess and normal amounts of L-methionine and aminopterin.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine#</th>
<th>Excess L-methionine#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cpm/umole</td>
<td>cpm/umole</td>
</tr>
<tr>
<td>RNA nucleotides</td>
<td>guanine 250,000</td>
<td>guanine 284,900</td>
</tr>
<tr>
<td>nucleotide pool</td>
<td>guanine 442,000</td>
<td>guanine 489,000</td>
</tr>
<tr>
<td>DNA nucleotides</td>
<td>guanine 200,000</td>
<td>guanine 260,000</td>
</tr>
<tr>
<td>Water</td>
<td>guanine</td>
<td>guanine</td>
</tr>
</tbody>
</table>

*specific activity of guanine-8-\textsuperscript{14}C introduced = 700,372 cpm/umole

#normal L-methionine = 3.35 umole/flask

excess L-methionine = 400 umole/flask
The distribution of radioactivity in isolated compounds after a 44 hour exposure to guanine-8-C\(^{14}\) in the presence of excess and normal amounts of L-methionine, adenine and aminopterin.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal L-methionine#</th>
<th>Specific activity</th>
<th>Excess L-methionine#</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>compound</td>
<td>cpm/umole</td>
<td>compound</td>
<td>cpm/umole</td>
</tr>
<tr>
<td>RNA nucleotides</td>
<td>guanine</td>
<td>67,369</td>
<td>guanine</td>
<td>89,802</td>
</tr>
<tr>
<td>nucleotide pool</td>
<td>guanine</td>
<td>368,000</td>
<td>guanine</td>
<td>450,000</td>
</tr>
<tr>
<td>DNA nucleotides</td>
<td>guanine</td>
<td>45,300</td>
<td>guanine</td>
<td>77,000</td>
</tr>
<tr>
<td>Water</td>
<td>guanine</td>
<td></td>
<td>guanine</td>
<td></td>
</tr>
</tbody>
</table>

*specific activity of guanine-8-C\(^{14}\) introduced = 700,372 cpm/umole

#normal L-methionine = 3.35 umole/flask
excess L-methionine = 400 umole/flask
### TABLE XIII
Total cell mass harvested per flask.

<table>
<thead>
<tr>
<th>Content</th>
<th>Weight in mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Guanine-8-C(^{14}) and normal methionine</td>
<td>47.8</td>
</tr>
<tr>
<td>2. Guanine-8-C(^{14}) and excess L-methionine</td>
<td>84.0</td>
</tr>
<tr>
<td>3. Guanine-8-C(^{14}), adenine and normal L-methionine</td>
<td>60.2</td>
</tr>
<tr>
<td>4. Guanine-8-C(^{14}), adenine and excess L-methionine</td>
<td>97.2</td>
</tr>
<tr>
<td>5. Aminopterin and normal L-methionine</td>
<td>14.0</td>
</tr>
<tr>
<td>6. Aminopterin and excess L-methionine</td>
<td>9.0</td>
</tr>
<tr>
<td>7. Guanine-8-C(^{14}), aminopterin and normal L-methionine</td>
<td>8.1</td>
</tr>
<tr>
<td>8. Guanine-8-C(^{14}), aminopterin and excess L-methionine</td>
<td>14.0</td>
</tr>
<tr>
<td>9. Guanine-8-C(^{14}), aminopterin, adenine, and normal L-methionine</td>
<td>23.3</td>
</tr>
<tr>
<td>10. Guanine-8-C(^{14}), aminopterin, adenine, and excess L-methionine</td>
<td>12.6</td>
</tr>
</tbody>
</table>
DISCUSSION

After exposure of Saccharomyces cerevisiae SC-10-2 to guanine-8-C\(^{14}\) in the presence of normal and excess amounts of L-methionine (adenine and aminopterin added where needed), it was noted that the cells grown in the excess L-methionine had a higher cell yield than those grown in normal L-methionine. These results are consistent with the findings of Yali (1962).

Cell permeability to aminopterin was noted by observation of the brilliant aminopterin-yellow color taken up by the cells after exposure to the drug. The effect of aminopterin was measured in part by the decreased cell growth of the yeast as compared to controls. Belogersky and Spirin (1960) and Reddi (1955) have equated the growth rate of cells to their nucleic acid concentration. By this relationship it can be assumed that aminopterin was effective in blocking the synthesis of purines from small precursors (specifically formate carbon) and thus inhibiting the eventual production of nucleic acids. Handschumacher and Welch (1960) described the influence of aminopterin on nucleic acid metabolism of both mammalian and microbial cells.

Aminopterin, in creating a folic acid deficiency in these cells, has essentially transformed them into purineless "mutants" of the wild type.
Guanine-8-C$^{14}$ was readily taken into the cells in all growth conditions. The fact that it was incorporated into *Saccharomyces cerevisiae* under normal growth circumstances (no aminopterin) supports the findings of Kerr et al. (1951), DiCarlo et al. (1951) and Roush et al. (1959).

In normal growth conditions the addition of adenine showed a slight increase in the growth rate as compared to that obtained when guanine was the sole source of purine. However, more guanine-8-C$^{14}$ was incorporated into the cells and into RNA nucleotides in the latter case. This indicates that adenine spared the requirement of guanine for RNA synthesis by the yeast. This can be explained more effectively when the specific activities are noted. In both cases, the specific activities of the nucleic acid guanine extracted from the cells grown with adenine in the media were much lower than with guanine as the purine source. This indicates that adenine was utilized as a source of guanine in the yeast. The fact that adenine can be converted readily to guanine was supported by Brown (1953), Henney (1961), Yall (1962), Cerwin (1963) and Moore (1964). However, that this interconversion continued in the presence of the preformed guanine in the media contradicted the results found by Kerr et al. (1951) and Balis et al. (1951). The conversion of purine adenine to nucleotide guanine has been noted in other organisms (Fairley and Loring, 1949; Hamilton, Brown and
The specific activity of the RNA guanine isolated from cells grown with guanine as the sole purine source was 40% to 50% lower than that added originally to the media. This would indicate that endogenous purine synthesis was progressing at a significant rate and that exogenous guanine was not directed efficiently to RNA synthesis. Feedback or end product inhibition did not curtail the endogenous synthesis of the purine to the extent that might be expected from reports by Kerr et al. (1951) and Cowie and Bolton (1957). They indicated that there is little if no dilution of the preformed purine when the amount added to the media is sufficient to meet the requirements of the organism.

The presence of excess L-methionine however did have an effect on the amount of guanine-8-C\textsuperscript{14} directed on to nucleic acid production. In both normal and aminopterin treated cells, more guanine-8-C\textsuperscript{14} was utilized for RNA synthesis in excess L-methionine. Excess L-methionine is known to stimulate the production and utilization of adenine groups in Saccharomyces cerevisiae (Yall, 1962) and RNA purine synthesis in P. hydrophila (Reddi, 1955). Under the conditions of this experiment, a greater utilization of guanine groups was seen. However, excess L-methionine did not stimulate a greater de novo synthesis of guanine groups. The specific activity of the isolated
compounds was much higher than that grown in the normal L-methionine. This confirmed the findings of Schmidt et al. (1956). DNA synthesis was not affected to any extent by the two different concentrations of L-methionine, corroborating the results of Reddi (1955).

Aminopterin, the 4-amino derivative of folic acid was employed in this experiment to exclude the use of small purine precursors in the synthesis of purine nucleotides and force the cells to utilize preformed guanine-8-C\textsuperscript{14} for nucleic acid production. The ability of guanine to furnish nucleotide adenine was investigated. In the presence of the inhibitor, there was a basic level of growth over the amount of cells inoculated. This threshold level of endogenous synthesis has been described in purine mutants (Abrams, 1951 and Balis et al., 1956) and in incomplete aminopterin inhibition in leukemic cells (Handschumacher and Welch, 1960).

Guanine did not appear to be an efficient growth factor in aminopterin treated cells, for the growth when this purine was present barely surpassed that of the control. When adenine was present in the media, the total cell yield was doubled over that of the controls. Again, slightly more exogenous guanine-8-C\textsuperscript{14} was taken into the cells grown in the absence of adenine, but approximately the same amount was utilized for RNA synthesis.

Ten times less guanine was directed to RNA nucleotides than in the normal growth conditions. This is consistent with the fact that
in folic acid deficient monkeys the RNA content of their liver cells is lower and the P$^{32}$ incorporation is less efficient than in normal cells (Brown and Roll, 1955). When the specific activities of the guanine components were appraised, it could be concluded that de novo synthesis was continuing to a significant extent. When adenine was present, the specific activity of the isolated guanine nucleotides was three times lower than when guanine was the sole purine source. This indicates that adenine was efficiently interconverted to guanine and was used in preference to the exogenous guanine. Brown and Roll (1955) indicated that with folic acid antagonists in the rat the relative amounts of guanine derived from exogenous adenine was increased. The results of this experiment supported this finding.

Between 75% and 80% of the exogenous guanine incorporated into the aminopterin treated cells was not utilized for RNA or DNA nucleotide synthesis. That purines can be stored in living cells before use has been proven. Siminovitch and Graham (1955) described the metabolic pools in _E. coli_ composed of the nucleosides, nucleotides, and free bases. Cowie and Bolton (1957) described the two pools in _Candida utilis_ as taking up adenine and guanine into the cell until the external supply was nearly exhausted. After being altered to the nucleotide level (2nd pool) the components were utilized for nucleic acid synthesis. Roush and Shieh (1962) reported that even in the absence of an active transport system purines could diffuse into and
out of living cells. The preceding observations partly explain the high concentrations of guanine found in the yeast.

The specific activities of the guanine components isolated from these pools were much higher than those obtained from the RNA and DNA nucleotides. Guanine directed on to nucleic acid synthesis must be taken from these pooled bases. However, there was some dilution that indicated that endogenous synthesis was still competing with the exogenous purines for utilization by the cell.

In the absence of aminopterin, only the cells grown in normal L-methionine exhibited a small nucleotide pool. Those cells grown in excess L-methionine depleted the nucleotide pool. They utilized the purines to a greater extent because of the stimulus to growth and RNA synthesis by excess L-methionine. The specific activity of the guanine isolated from the normal L-methionine pools was 14% of the guanine inoculated originally indicating the de novo synthesis was very active in supplying purines to the nucleotide pool.

The fact that endogenous synthesis of the purines could continue to a certain extent despite the effect of aminopterin could be explained by the fact that the cells inoculated may have had a "pool" of the THFA coenzyme. Welch and Nichol (1952) reported that a supply of THFA is found in yeasts. This is offered as possible confirmation of the above postulation. Until the THFA was depleted, nucleic acid
synthesis could continue. The aminopterin would then prevent the regeneration of folic acid to THFA.

Growth in the presence of aminopterin can also be understood when the many cases of drug resistance are noted. Welch and Nichol (1952) suggested that the resistance to aminopterin could be explained by the development of an alternate pathway in the reduction of FA to THFA. Webb in 1955 found pteridines and p-aminobenzoylglutamic acid in the media when Aerobacter aerogenes was grown in aminopterin. The oxidative cleavage of the drug allowed the bacteria to utilize the components for growth. Candida tropicalis was grown with aminopterin and after an initial inhibitory phase, the cell growth approached 75% to 100% of the normal in 48 to 72 hours. Again a cleavage of the drug was noted. In 1956, Law reported that Streptococcus faecalis and a strain of Tetrahymena could metabolize the antagonists by deaminating the compound to a folic acid derivative. Aerobacter cloacae and E. coli had similar effects on the drug. Saccharomyces cerevisiae was reported to be a naturally resistant organism to this drug. (However, the results of the present experiment do not uphold this claim.)

Welch (1959) called attention to a trend for selecting resistant leukocytes from highly malignant strains of leukemia in mice. A possible pathway for by-passing the drug was suggested. Fischer (1959) indicated that the capacity of the cell to synthesize the THFA analogue could determine the amount of resistance.
The susceptibility of organisms to aminopterin is thus seen to be an individual one. And in *Saccharomyces cerevisiae*, the inhibition has been found to be far from complete. There was enough endogenous synthesis to provide a good proportion of the nucleotide purines and the exogenous supply was not utilized to its full potential.

Adenine was converted readily to nucleotide guanine in normal cells. Because of this it would seem, when aminopterin was present, that adenine would be able to supply the necessary purines for nucleotide synthesis. But in the presence of aminopterin the ability of exogenous adenine was very limited in stimulating growth. Perhaps the inability of the cell to supply DNA thymine was the limiting factor. Hakala in 1957 and Handschumacher and Welch in 1960 indicated that not only must a utilizable purine be added to support growth of aminopterin inhibited cells, but also thymine must be present. The methylation of deoxyuridine 5'phosphate to form thymidylic acid is very susceptible to the antifolic acid antagonists. The uptake of formate, formaldehyde and the methyl group of methionine into thymidylic acid has been shown to be decreased with aminopterin (Kit et al., 1958).

Guanine present in the media also might inhibit the effect of adenine. Pomper (1952) reported that guanine inhibited the utilization of adenine in an adenine purineless mutant of *Saccharomyces cerevisiae*. Webb (1958) found that in aminopterin treated cells of *Aerobacter aerogenes*, even when adenine was effective in reversing the inhibition,
the presence of guanine inhibited the action of adenine. Normal *Aerobacter* could utilize guanine. The same effect was reported in a mutant of *Staphylococcus flavocyanus* (Aaronson, 1955).

In *Saccharomyces cerevisiae* SC-10-2 there was no conversion of guanine to adenine. This finding corroborates the results of Pomper (1952) and Chamberlain and Rainbow (1954) with other strains of this yeast. The possibility of purine interconversion had been studied in many other organisms after the strategic role of inosinic acid (IMP) in the synthesis and general reactions of the purine bases was realized. IMP is the common point in the biosynthesis of the purines after which relatively minor changes lead to the formation of adenylic acid (AMP) and guanylic acid (GMP). In many cases adenine is readily interconverted to guanine. This can be explained by the fact that the amination of IMP in the formation of AMP is a reversible enzymatic process. However, the amination of xanthyllic acid to GMP is known to be irreversible. Buchanan (1960) reported that most amination reactions involving glutamine are irreversible. Other organisms besides *Saccharomyces cerevisiae* have been determined to be unable to convert nucleotide guanine to nucleotide adenine. Some examples are as follows: *Neurospora crassa* 3254 (Pierce and Loring, 1945); 45 independently occurring mutants of *Neurospora crassa* (Mitchell and Houlahan, 1946); *Neurospora* mutant 28610 (Fairley and Loring, 1947 and Loring and Fairley, 1948); *Saccharomyces cerevisiae* (Abrams,
Torulopsis utilis (Kerr et al., 1951); Schizosaccharomyces octosporus (Northam and Norris, 1951); Aerobacter aerogenes (Hamilton, Brown and Stock, 1952); white rat (Brown and Roll, 1955) and a Neurospora mutant (McNutt, 1958).

Other investigators have found that some organisms possess the ability to convert guanine to adenine. Lactobacillus casei has been found by Kidder and Dewey (1949), Balis et al. (1951) and Hamilton, Brown and Stock (1952) to utilize adenine and guanine equally well as precursors of polynucleotide adenine and guanine while Tetrahymena galeii can only use guanine for the interconversion reactions. A strain of Ochromonas has also been shown to convert guanine to adenine (Hamilton, Brown and Stock, 1952). This process was further clarified when it was found that guanine can be deaminated and reduced to the hypoxanthine level in rat liver. The existence of a similar enzyme in bacteria that deaminates and reduces guanosine 5'-phosphate to inosine 5'-phosphate has been substantiated by Guarino and Yureger (1959).

A general scheme for nucleotide interconversion has been set up by Audly and Goodwin (1962) with inosine monophosphate as the central link.
The reductive deamination mechanism by-passes xanthosine monophosphate. The presence or absence of GMP deaminase is the determining factor in this process.
**SUMMARY**

*Saccharomyces cerevisiae* SC-10-2 was inoculated into the synthetic, complete media of Roman to test the utilization of guanine-8-C$^{14}$. Four different growth conditions were set up as follows: (1) guanine-8-C$^{14}$ was the sole purine source in both excess and normal amounts of L-methionine; (2) adenine supplemented guanine-8-C$^{14}$ in excess and normal amounts of L-methionine; (3) guanine-8-C$^{14}$ and the antifolic acid antagonist were added in excess and normal amounts of L-methionine; and (4) adenine supplemented the guanine-8-C$^{14}$ and aminopterin in the excess and normal amounts of L-methionine.

It was found that adenine spared the amount of guanine-8-C$^{14}$ incorporated into the cell. Less guanine-8-C$^{14}$ was utilized for RNA synthesis when adenine was in the media. The fact that adenine decreased the specific activities of the nucleotide guanine compounds isolated indicated that exogenous guanine was not used as the sole source of nucleotide guanine under normal growth conditions in *S. cerevisiae*.

Aminopterin was successful in blocking most of the purine synthesis. The presence of adenine with aminopterin greatly reduced the specific activity of guanine compounds isolated again indicating a preferential utilization of adenine over guanine in purine metabolism.
Excess L-methionine did stimulate an increase utilization of guanine-8-C\textsubscript{14} into RNA purines in every case. However an increase in synthesis of guanine components was not observed to any great extent. DNA synthesis was not affected by the presence of excess L-methionine.

The fact that a threshold level of synthesis was maintained by the cells grown in aminopterin indicated that the yeasts did not have to rely entirely on the exogenous purine for growth. No guanine conversion to adenine was indicated in either the normal or aminopterin treated cells.
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