YEAST

A Source of Biochemical Intermediates

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Modern medical science has focused attention on the chemistry of living cells. This report describes methods for recovering from yeast, compounds essential to all living tissue. Nucleic acid is readily extracted from yeast in yields of 6% on a dry basis. From it, nucleotides and nucleosides are prepared by hydrolysis. Yeast enzyme systems are used to convert nucleosides to phosphorylated esters, and to prepare sugar phosphates. This organism is also a good source for glutathione and cozymase. The yeast derivatives described are being used in research on fundamental biochemical mechanisms. Prepared in radioactive form by biosynthesis, they are powerful investigative tools. Adenosine nucleotides, glutathione, and nucleic acid, among others, are being studied as possible therapeutic agents in metabolic diseases. Economical recovery methods from available raw materials for these hitherto rare chemicals are of considerable potential value.

THE USE OF YEAST as a primary source for compounds which are assuming growing importance to biochemical and medical science has increased as attention is focused on the chemistry of living cells. Although yeast is classed as a plant, the similarity of its metabolic patterns to those of mammalian tissue makes possible the recovery of many complex molecules which also occur in the cells of higher animals. The efficiency with which yeast is able to synthesize these substances and the ease with which yeast can be grown in large quantities make it a more advantageous source than animal tissue for some of the most essential cell intermediates.

Here are presented brief summaries of methods used for preparing nucleic acids, nucleotides, coenzymes, and other cell metabolites from yeast, and some potential applications in medicine and biochemistry are mentioned. This is not a review in the usually accepted sense, and no attempt has been made to cover all of the literature pertaining to the compounds discussed. Only those references have been cited which seem most pertinent to the discussion, and, because methods are the primary concern, reports published during the past 25 years have been emphasized. The pioneer work of such men as P. A. Levine, F. G. Hopkins, C. A. Neuberg, and numerous others during the 50 years from 1875 to 1925 is still the nucleus of knowledge about yeast derivatives from which modern preparative techniques are developing.

In this discussion, the term "yeast" is used rather nonspecifically. Because nearly all yeasts contain most of the compounds described, choice of a strain as a raw material is frequently a matter of commercial availability rather than genetic selection. For maximum yield, there is little doubt, however, that special strains unusually rich in one or more components could be developed by the application of modern yeast breeding and screening techniques.

The amounts of five important biochemicals found in yeast and rat liver are shown in Table I. With the exception of coenzyme II, the content of these compounds in yeast compares favorably with liver, which is generally considered a good source for such substances.

The presentation is divided into three parts, which are shown in Table II: (1) direct extraction of cell substances, under which the preparation of nucleic acid and its degradation products, coenzymes, glutathione, and ergosterol is discussed; (2) use of yeast enzymes as catalysts for phosphorylation of nucleotides and sugars; and (3) biosynthesis of isotopically labeled metabolites.

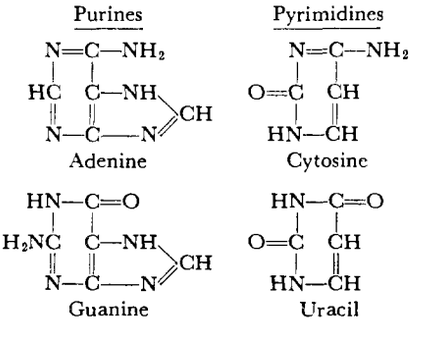
Table I. Occurrence of Some Compounds in Yeast and Liver Tissue

	Pressed Lager Yeast	*Rat Liver*
	Mg. per 100 grams	
Ribonucleic acid	1500	1000
Coenzyme I	50	30
Coenzyme II	5	30
Coenzyme A	25	30
Glutathione	150	250

Nucleic Acid

Ribonucleic acid (RNA) occurs in all living matter. Yeast is one of the richest known sources, the concentration ranging from 4.5% to as high as 10% on a dry basis (*8*, *10*, *29*). In the native state, ribonucleic acid occurs as a nucleoprotein, primarily in the cytoplasm. Efficient recovery requires rupture of the cell wall, followed by some treatment to split the nucleic acid from the protein. Thereafter the problem is one of concentration and purification with an attempt to preserve the polymeric structure of the compound which is, unfortunately, sensitive to extremes of pH and to elevated temperature.

Figure 1 shows the classic structure of what might be referred to as the polyribonucleotide monomer. This shows four bases attached through *n*-glucosidic linkages to the 5-carbon sugar, D-ribose, with the ribosides so formed cross-linked through phosphate esters attached to the 2′ and 3′ positions. Recent evidence supports the conclusion that a 5′ ester linkage also occurs and that the concept



of simple linear polymerization requires *modification* (*4*, *7*).

The bases which occur in varying molar ratios, depending on the source of nucleic acid, are adenine, guanine, cytosine, and uracil as shown at the bottom of the preceding column.

Table II. Yeast as a Source of Some Biochemical Intermediates

1. Direct Extraction
   - A. Nucleic acid and degradation products
   - B. Coenzymes
   - C. Glutathione
   - D. Ergosterol
2. Use of Yeast Enzymes
   - A. Adenosine triphosphate and degradation products
   - B. Hexose phosphates
3. Synthesis of Isotopically Labeled Metabolites

The method for the recovery of ribonucleic acid from yeast most widely used commercially in the United States has been described (*24*).

Briefly, it consists of the plasmolysis of the cell and release of the nucleic acid by alkaline treatment. The protein and cell residue are then coagulated by boiling at pH 5.0 and the solids are removed by filtration. A crude iron complex is recovered from the filtrate by treatment with ferric chloride. The washed iron complex is reslurried and decomposed by adjustment to a pH of 8.0 to 9.0 and heating to about 60° C., causing the flocculation of iron hydroxide, which is then removed by filtration. The adsorptive properties of the hydroxide floc help to remove impurities at this stage. The filtrate is adjusted to pH 2.0 and added to 2 volumes of alcohol. Nucleic acid precipitates out. It is centrifuged, washed with alcohol, and dried. The yield ranges from 1 to 2% of pressed yeast weight.

The product is primarily ribonucleic acid, although a few per cent (traces to as much as 5%) of desoxyribonucleic acid unavoidably remains. Yeast nucleic acid so prepared is free of protein (biuret and Millon's tests) and gums. It is partially depolymerized, having a molecular weight of under 10,000. The nitrogen and phosphorus content and their ratio are 95% of those calculated for the classically postulated tetranucleotide structure.

Yeast nucleic acid and its salts have for many years been used pharmaceutically in tonics, particularly in Europe and South America. The mixed nucleotides, a hydrolysis product, has been used in the treatment of leukopenia. Polynucleotides stimulate the proliferation of leukocytes and lymphocytes (*14*).

Reports by Robertson (*36*) and Gardner (*13*) indicate that ribonucleic acid may be effective, in conjunction with growth factors of the B vitamin group, in prolonging life. The ability of ribonucleic acid to complex metals, including those such as copper, manganese, iron, and magnesium which are known to catalyze certain enzyme reactions, is well established (*30*) and merits further consideration of possible clinical application. Neutral soluble metal salts of nucleic acid have been prepared and are commercially available.

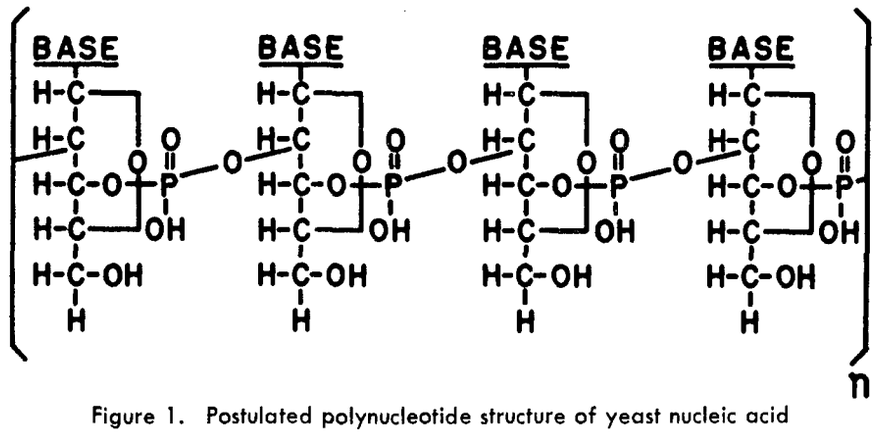


Figure 1. Postulated polynucleotide structure of yeast nucleic acid

The biological functions of ribonucleic acid are still largely a matter of speculation. It is believed to play a fundamental role in growth processes, possibly including the mechanism of protein synthesis, and may be involved in the formation of antibodies.

Nucleotides, Nucleosides, And Derivatives

One important use of yeast nucleic acid is as a starting material for a variety of purine and pyrimidine nucleotides and nucleosides. Efforts in this direction are, to some extent, reflected in the patent literature (*25–28*). Both chemical and enzymic processes have been described for breaking down nucleic acid to its component nucleotides. Alkaline hydrolysis (3% caustic at 60° C. for 2 hours) is commonly used for this purpose. The purine nucleotides are readily separated from those containing pyrimidine bases by precipitation as cuprous salts, the pyrimidines being recoverable from the filtrate by means of heavy metals. For relatively small scale operations, nucleotides of exceptionally high purity can be obtained directly through ion exchange chromatography on Dowex-1 as described by Cohn (*5*). Nucleotides are readily dephosphorylated by the enzyme preparation, emulsin. This can also be accomplished by chemical means. The purine ribosides are easily hydrolyzed to the pentose, D-ribose, and the purine bases—adenine or guanine—and from these hypoxanthine and xanthine are prepared by deamination.

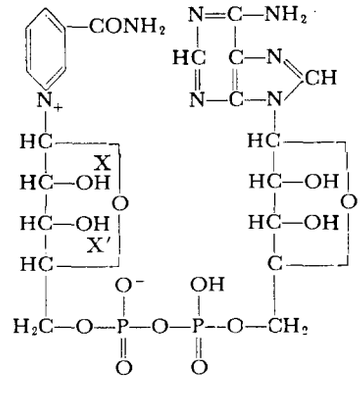
Figure 2 shows the multiplicity of products obtainable from the nucleotide, yeast adenylic acid.

Work in recent years has established that yeast adenylic acid, prepared according to classical methods, is a mixture of two isomers separable by ion exchange chromatography and designated temporarily as A and B. The nature of the isomerism has not been settled. Present evidence favors phosphate esterification on the 2′ or 3′ carbons with no definite assignment of which compound represents the 2′- and which the 3′-phosphate. Doherty (*12*), Kornberg and Pricer (*22*), and more recently Kaplan and coworkers (*21*) have shown that the isomers differ in enzyme specificity. For example, the A isomer phosphate linkage appears identical to the nonpyrophosphate ester group which occurs in triphosphopyridine nucleotide (TPN).

Again referring to Figure 2, it is shown that by a series of phosphate exchanges, the series, adenosine, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) can be formed. Each of these can be deaminated by use of sodium nitrite and acid to form inosine compounds. Adenosine and adenosine-5-phosphate (AMP) are readily split by acid hydrolysis to D-ribose and D-ribose-5-phosphate, respectively, plus the adenine base. As is well known, adenosine triphosphate hydrolyzes more or less spontaneously in aqueous solution to adenosine diphosphate and adenosine monophosphate, so that the formation of these is relatively simple. In the formation of adenosine monophosphate alkaline hydrolysis is also employed, since the 5′-phosphate ester is stable to alkaline hydrolysis.

Coenzymes

Among the coenzymes known to contain adenine nucleotide is coenzyme I, diphosphopyridine nucleotide (DPN) or cozymase.



Closely related to diphosphopyridine nucleotide is coenzyme II or triphosphopyridine nucleotide, which differs from it by having an additional phosphate in the 2′ or 3′ position, shown as X and X′. Both diphosphopyridine nucleotide and triphosphopyridine nucleotide function as hydrogen acceptors in oxidative transformation of intermediary metabolism, such as glycolysis, fermentation, and the tricarboxylic acid cycle. Yeast is an excellent source of diphosphopyridine nucleotide, comparing favorably to liver in this respect, but the amount of triphosphopyridine nucleotide normally present is not sufficient to make its recovery from yeast economical.

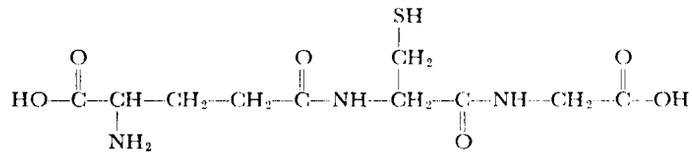
One method for recovering diphosphopyridine nucleotide from yeast, illustrated in Figure 3, combines the extraction procedures of Williamson and Green (*11*) with the chromatographic purification worked out by Horecker and Kornberg (*19*).

The yeast is extracted and washed with hot water. The combined filtrate and wash is defecated with basic lead acetate and a crude diphosphopyridine nucleotide is recovered from the filtrate of this precipitation, by formation of the insoluble silver salt. The silver salt is decomposed with hydrogen sulfide, the silver sulfide removed, and a diphosphopyridine nucleotide of about 65% purity recovered by adding the filtrate to 10 volumes of alcohol. The 65% diphosphopyridine nucleotide is then further purified by chromatography on a Dowex-1 column buffered with formate.

Coenzyme A, the composition of which has only recently been determined (*15*), also contains adenine nucleotide, in this case coupled, it is believed, to a thioethanolamine derivative of pantothenic acid. The compound plays a key role in transacetylation reactions by which fatty acids are synthesized in the body. Yeast contains coenzyme A in about the same concentrations as diphosphopyridine nucleotide. Beinert and coworkers (*1*) have published a note on the recovery of coenzyme A from yeast which utilizes another yeast derivative, glutathione, as a coprecipitating and reducing agent.

Glutathione

Glutathione, which occurs in pressed yeast in a concentration of from 100 to 300 mg. %, was first discovered in 1921 by Hopkins (*17*), who confirmed its structure in 1929 (*18*) as γ-glutamylcysteinylglycine



This molecule has several obvious points of instability—for example, the SH group which is readily oxidized, forming the disulfide in alkaline solution or in the presence of traces of iron, copper, and certain other metals and an oxygen source (air, peroxides). The γ-glutamylcysteine peptide bond is rather labile, being completely hydrolyzed in 1 *N* hydrochloric acid at 90° C. in 1 hour (*3*). Most methods for recovering glutathione from yeast are based on the work of Hopkins and of Pirie (*34*).

Pressed yeast is plasmolyzed and extracted with an acidified organic solvent. The cell residue is filtered and washed with dilute acid. The highly insoluble cuprous glutathione complex is precipitated from the filtrate. The cuprous salt is then recovered, washed, slurried with water, and decomposed with hydrogen sulfide. The filtrate after cuprous sulfide removal is concentrated under vacuum, mixed with alcohol, and chilled, causing glutathione of high purity to crystallize out.

The product melts with decomposition at 190–192° C. and assays better than 99.5% reduced glutathione by iodine titration. The tripeptide contains 13.7% nitrogen and 10.4% sulfur.

The increased interest in the nature and function of sulfhydryl enzymes and sulfur-containing hormones has resulted in renewed study of the biochemical role of glutathione. Patt and coworkers (*33*) and Cronkite *et al.* (*9*) have demonstrated the relationship of sulfhydryl to radiation damage. There is a definite prophylactic effect when glutathione is administered prior to radiation. Binkley (*2*) has isolated from kidney a specific glutathionase which splits the glutamyl peptide linkage. Hanes, Hird, and Isherwood (*16*) have shown that the tripeptide plays a role in enzymatic peptide synthesis in kidney homogenates.

Zwemer, Vollmer, and Carey (*42*) have shown that glutathione has protective action against lethal doses of potassium in rats, mice, and guinea pigs.

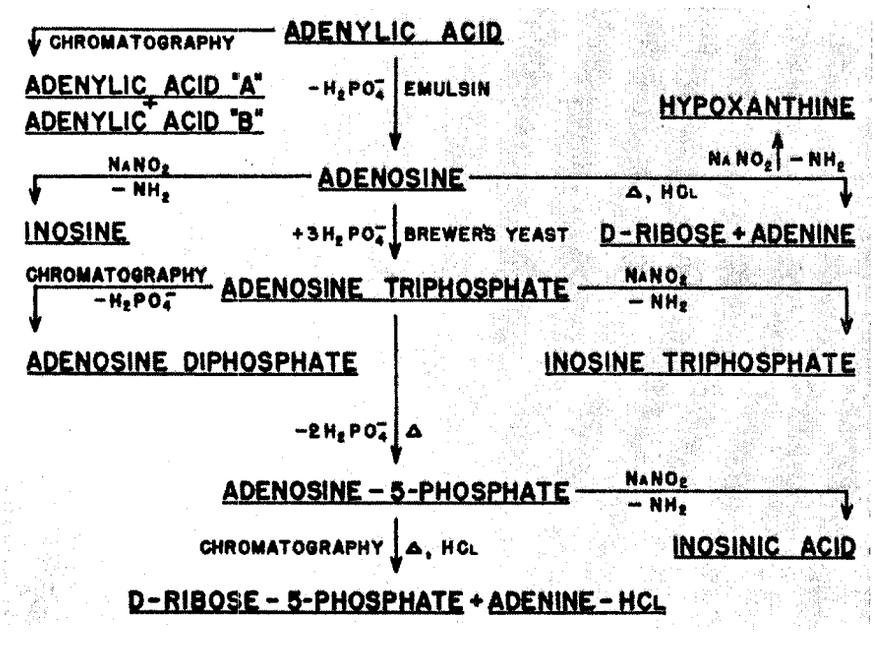
Glutathione is clearly an important tool for investigators in physiology and biology. Methods have been developed to prepare it in stable, neutral, sterile form as the lyophylized monosodium salt. Similar preparations of oxidized glutathione have also been made.

It seems appropriate at this point to mention another yeast derivative, ergosterol, which has recently come to renewed prominence. This vitamin D precursor is also a suitable starting material for the preparation of adrenal cortical hormones and could be used for this purpose in tonnage quantities. Such a large scale use for yeast substance could be expected to have a fundamental effect on the economics of yeast production and the preparation of yeast derivatives.

Use of Yeast Enzymes

In the brewing and fermentation industries yeast acts as a catalyst, converting available carbohydrate to alcohol by a series of intracellular enzyme reactions. The use of yeast enzymes for biochemical synthesis offers attractive possibilities for the preparation of many valuable intermediates. In these instances, however, the need for greater control and specificity leads to the use of extracellular enzymes or extract fractions rather than the intact cell. A well-known example of this class of yeast derivative is invert-

Figure 2. Compounds derived from yeast adenylic acid



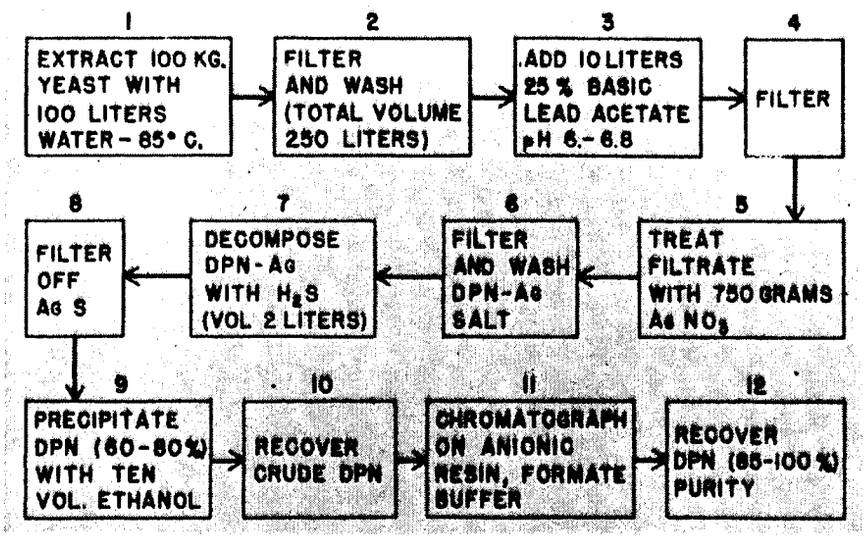


Figure 3. Preparation of coenzyme I from yeast

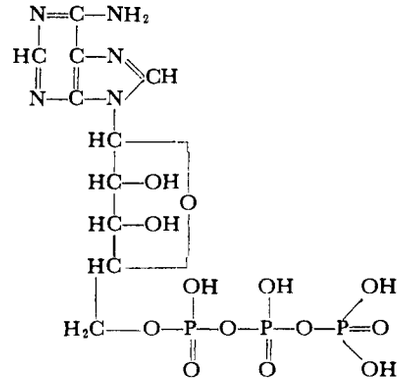
ase, which has numerous commercial uses.

Those yeast enzymes responsible for phosphorylation and carbohydrate transformations are of special interest. Although phosphate esters of some of the sugars or of the nucleosides can be formed by chemical methods, as shown by Horecker and Seegmiller (*20*) and Todd and Michelson (*38*), enzymic phosphorylations are frequently much simpler and more specific.

Among the sugar phosphates which have been prepared with yeast enzymes are fructose-1,6-diphosphate, fructose-6-phosphate, phosphoglyceric acid, and glucose-6-phosphate. This last is readily prepared from glucose-1-phosphate by use of a yeast extract containing phosphoglucomutase from which the isomerase has been removed by heating at 50° C. Glucose-6-phosphate may be readily converted to 6-phosphogluconic acid by oxidation with bromine, as described by Horecker (*20*).

Although the knowledge of the sugar phosphates has been greatly expanded since their first isolation by Harden and Young, Emden, the Coris, and others, new facts about their chemical properties still come to light. A crystalline form of the barium salt of glucose-6-phosphate not previously reported in the literature has been prepared and characterized. This compound was isolated independently at about the same time, by B. L. Horecker at the National Institutes of Health (*19*). The product differs in several notable respects from the amorphous barium salt generally described in the literature. The salt crystallizes as a heptahydrate and is extremely insoluble in water.

An important use of yeast phosphorylase is the preparation of adenosine triphosphate and its derivatives from adenosine.



Adenosine triphosphate is an essential coenzyme for phosphate transfer in muscle, the bond energy of the pyrophosphate linkages being in the neighborhood of 12,000 calories per mole.

For many years the preferred source of adenosine triphosphate was muscle tissue, but the preparation from yeast is more economical and has the advantage of providing a nearly unlimited source for large scale production. The general procedures in use today owe much to the work of Ostern (*32*). More recently, Kornberg has studied the enzymatic mechanism in detail (*23*). Figure 4 shows one method of commercial preparation.

Adenosine is incubated with plasmolyzed yeast, inorganic phosphate, and dextrose, and its conversion to adenosine triphosphate is measured by disappearance of inorganic phosphate until the reaction is maximized. In this step the yeast strain is of paramount importance. Brewer's lager yeast appears to be especially rich in the desired enzymes.

After the phosphorylation is completed, the reaction is stopped by reduction of the pH. The yeast solids are removed and a crude adenosine triphosphate is precipitated from the filtrate by the addition of barium hydroxide or a soluble barium salt at pH 8.5. This cake is carefully decomposed by addition of sulfuric acid, and a considerably purer adenosine triphosphate, containing some adenosine diphosphate and adenosine monophosphate, is recovered from the filtrate by precipitation with ethyl alcohol. Chromatographic purification of this product is carried out on a Dowex-1 column (*6*) from which the mono-, di-, and triphosphate esters can be recovered in pure form. If adenosine monophosphate is the desired end product the two labile phosphates are first removed by alkaline hydrolysis, heat, or the action of both.

Apart from their general importance in biology, both adenosine triphosphate and adenosine monophosphate are of considerable interest in clinical investigations. Adenosine monophosphate is being used as a pharmaceutical in the treatment of peripheral vascular disease. Good results have been reported when 20 to 100 mg. have been administered daily, intramuscularly or in sublingual tablets (*37*). Its application to other disease where a deficiency of adenine nucleotides may be implicated is being studied. Adenosine triphosphate appears to have a direct action on heart muscle as well as a vasodilating effect.

Synthesis of Isotopically Labeled Nucleotides

All of the foregoing has taken on an added significance since the introduction of isotopic tracer techniques. Biochemical intermediates labeled with isotopic carbon, nitrogen, hydrogen, sulfur, and phosphorus are powerful tools for elucidating biological mechanisms. Incorporation of isotopes into complex molecules by biosynthesis offers an economical route for preparing a wide variety of tagged cell substances.

During the past year glutathione labeled with sulfur-35 has been prepared from yeast in these laboratories. The data from a typical experiment of this type are shown in Table III. Between 30 and 50% of the inorganic sulfate in the medium is converted to cellular sulfate. About three fourths of this sulfur appears to be incorporated into organic sulfur compounds of high molecular weight, probably largely proteins, as indicated by the amount of activity remaining in the residue from the plasmolyzed cells. Radioactive glutathione is recovered from the filtrate of the cell debris, representing an over-all yield on a sulfur basis of about 6%. The product may be diluted to any convenient specific activity, the present range being 0.1 to 2.5 μc. per milligram.

Compounds uniformly labeled with carbon-14 may be obtained from yeast by adding to the medium uniformly labeled fermentable carbohydrate. A number of procedures are available for producing these nutrients by photosynthesis (*35*). Labeled acetate and glycine (*39*, *40*) have also been used as a carbon source. Currently, uniformly labeled

Table III. Incorporation of Sulfur-35 into Cell Substance by Yeast

Strain. *S. cerevisiae* (Fleischmann)	
medium (*31*)	
$MgSO_4.7H_2O$	12.5 mg. %
Glucose	1.0 gram %
Total radioactivity added	3.64 μc.
	%
Remaining in medium	61.3
In washed cells	37.1
	98.4
In washed cell residue	29.4
In extract of plasmolyzed cells	8.0
	37.4
S^{35} as GSH in extract	6.5

nucleotides and glutathione are being produced in this laboratory by this method. Radioactive amino acids and yeast carbohydrates can be recovered from the same fermentation.

The entire series of sugar phosphates and most of the adenine nucleotides can also be prepared labeled with phosphorus-32. The incorporation of nitrogen-15 into yeast nucleic acid has been reported (*11*). Yeast utilizes ammonia practically quantitatively and biosynthesis of nitrogen compounds is therefore particularly efficient.

Our modern era of metabolic studies received much of its impetus and inspiration from the revealing work of Büchner on extracellular fermentation by enzymes extracted from yeast. This brief review demonstrates that the simple yeast cell still serves the biochemist in his efforts to unravel the secrets of life.

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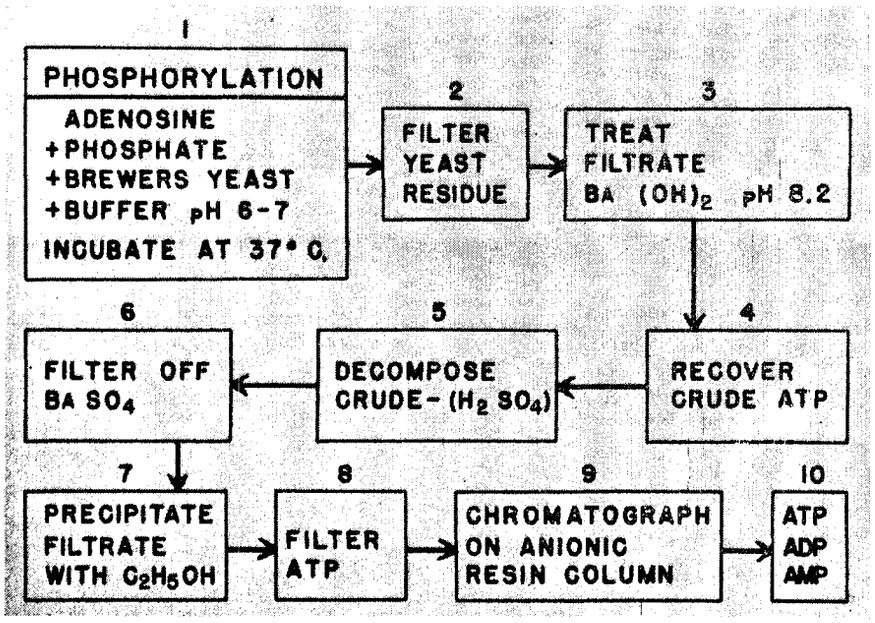
Figure 4. Flow sheet for preparation of adenosine triphosphate from yeast



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