SULPHUR UTILIZATION BY YEAST

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INTRODUCTION

This communication is restricted to a consideration of *Saccharomyces* yeasts, and deals with the requirements for sulphur and with the uptake, turnover and distribution of sulphur in these organisms.

Of the sulphur compounds which occur in yeast, quantitatively the most important are the amino-acids cysteine, cystine and methionine. Cystine and methionine are located almost entirely in the proteins, and the glutathione of the cytoplasm accounts for the bulk of the cysteine. Other sulphur-containing constituents of importance are present, including derivatives of biotin and thiamine, traces of sulphate in inorganic and ester form, as well as in nucleotide combination as "active sulphate", derivatives of cysteine such as cysteamine, a part of the coenzyme A molecule, and derivatives of methionine such as *S*-adenosylmethionine. However, these compounds collectively represent only a small fraction of the total sulphur of the cell.

SULPHUR CONTENT OF YEASTS

A survey of the available literature on the total sulphur content of brewer's and baker's yeasts (see *Table 1*) reveals a fairly wide range of values. Much of the variation undoubtedly arises from the variety of analytical procedures used and from the fact that many of the analyses were carried out on commercial preparations of dried yeasts grown on industrial or vegetable wastes, and for which the exact conditions of culture, harvesting, separation from residual medium, *etc.*, were not specified. From the standpoint of sulphur utilization the nutritional history of a yeast culture is important since the

Table 1. Total sulphur content of Saccharomyces cerevisiae

<i>Authors</i>	<i>Yeast strains</i>	<i>Total sulphur</i> (% of dry wt. of yeast)
Fink and Just ¹	Brewer's strain	0.19, 0.20
Block and Bolling ²	2 Baker's strains	0.34-0.44
	5 Brewer's strains	
Dent ³	Baker's strain	0.53
Czyörgy ⁴	Brewer's strain	0.65
	Baker's strain	0.86
McManus, Schultz and Maynard ⁵	4 Baker's strains	0.35-0.45
Lindan and Work ⁶	Baker's strain	0.36
	Brewer's strain	0.55
Suomalainen and Keränen ⁷	3 Baker's strains	0.28-0.34
	Brewer's strain	0.44-0.49

sulphur content, and more particularly the methionine and glutathione contents, can vary with the nature and amount of assimilable sulphur in the growth medium⁸⁻¹⁰. According to some authors⁷ aeration is a further factor and can lead to lower total sulphur values. Significant differences between the sulphur content of brewer's and baker's yeasts have been reported^{6, 7}, although this is not a general finding². On the basis of the more reliable data an average value for the sulphur content of these yeasts appears to be about 0.4 per cent of the dry weight.

Reported values for cystine, methionine and glutathione in yeasts are equally varied. Thorne¹¹ quotes the following range of figures (expressed as a percentage of the dry weight): glutathione-S, 0.05-0.10; cystine-S, 0.13-0.27; methionine-S, 0.15-0.28. Glutathione-S appears to represent some 20-30 per cent of the total sulphur, about 90 per cent being present as reduced glutathione^{12, 13}. In a sulphur-deficient medium the glutathione content can be reduced to as little as one-tenth of normal values¹⁰.

Some data for cystine and methionine are given in Table 2. The methionine content can be increased considerably (up to three-fold) by inclusion

Table 2. Cystine and methionine content of *Saccharomyces cerevisiae* (expressed as g S/100 g dry wt. yeast)

Authors	Yeast strains	Cystine-S	Methionine-S
Stokes <i>et al.</i> ¹⁴	Brewer's strain	—	0.17, 0.18
Stokes and Gunness ¹⁵	1 Strain	—	0.14
Block and Bolling ²	2 Baker's strains	0.11-0.13	0.25-0.32
	5 Brewer's strains	—	—
Horn, Jones and Blum ¹⁶	Brewer's strain	—	0.12
Barton-Wright ⁹	Brewer's strain	—	0.12-0.19
Brunner ¹⁷	6 Brewer's strains	0.12-0.19	—
Lindan and Work ⁶	Baker's strain	0.21	—
	Brewer's strain	0.29	—
Tamura <i>et al.</i> ¹⁸	Brewer's strain	—	0.34
Chiao and Peterson ¹⁹	6 Strains	0.07-0.10	0.15-0.18
Baumgarten, Mather and Stone ²⁰	Yeast preparations	—	0.15, 0.32
Nelson <i>et al.</i> ²¹	53 Strains	—	0.11 (av.)

of the amino-acid in the growth medium⁹. Methionine supplementation also leads to a marked increase in the content of *S*-adenosylmethionine²². The variations among the published values make it difficult to obtain a reliable estimate of the ratio of cystine-S to methionine-S in yeast, especially as few workers have attempted both analyses on the same yeast sample.

SULPHUR DISTRIBUTION STUDIED WITH THE AID OF SULPHUR-35

Tracer experiments using sulphur-35 have been carried out with a brewer's strain of *Saccharomyces cerevisiae* which have provided data on the sulphur content of the yeast and on the relative amounts of cystine and methionine present. Yeasts utilize inorganic sulphate for growth, and in these experiments the growth medium for the brewer's yeast contained

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$\text{Na}_2^{35}\text{SO}_4$ (0.78 mM, equiv. to 25 mg S/l.) as the sole sulphur source. Incorporation of sulphate-S into the yeast was determined from both the percentage loss of sulphur-35 from the medium and the appearance of sulphur-35 in the cells. The course of sulphate incorporation closely paralleled that of yeast growth (Figure 1) and during the phase of active growth, sulphate-S incorporation into whole cells or into the protein fraction was proportional to the amount of cells formed (Figure 2).

Less than 5 per cent of the sulphur-35 taken up was released back into the medium in organic form and since labelled sulphate was the only source

Figure 1. Growth of yeast and incorporation of ^{35}S -sulphate-sulphur into yeast as a function of time: $\circ-\circ$, yeast growth; $\bullet-\bullet$, sulphate uptake from medium; $\circ-\circ$, sulphate incorporation into yeast; $\text{Na}_2^{35}\text{SO}_4$ in medium 0.78 mM; temperature 30°

Figure 2. Relation between ^{35}S -sulphate incorporation and yeast growth: $\circ-\circ$, whole cells; $\bullet-\bullet$, TCA-insoluble fraction; $\text{Na}_2^{35}\text{SO}_4$ in medium 0.78 mM; temperature 30°

of sulphur, its loss from the medium provided a reliable measure of the total sulphur content of the yeast. A group of 26 experiments gave a value of $0.35 (\pm 0.08)$ g S/100 g dry wt. yeast. This value agreed with sulphur determinations made on samples of the yeast after ashing with cupric nitrate²³, or combustion by the Schöniger flask method, the mean of 24 such determinations being $0.36 (\pm 0.03)$ g S/100 g dry wt. yeast. Under the conditions chosen and with sulphate as the sulphur source, the sulphur content showed little variation over the growth period (see *Figure 1*) and was independent of the sulphate concentration of the medium over a wide range (10–100 mg sulphate-S/l.). The value of 0.35 per cent obtained from these experiments is in accord with the earlier data of Block and Bolling² and of McManus, Schultz and Maynard⁵.

The ratio of cystine to methionine was determined on yeast grown in the presence of $\text{Na}_2^{35}\text{SO}_4$ as sole sulphur source by a procedure which will be described in detail elsewhere²⁴. In outline it was as follows: samples of yeast and also of the trichloroacetic acid-insoluble fraction of yeast, amounting to 6 mg dry wt., were first oxidized with performic acid at 0° , following the method described recently by Schram, Moore and Bigwood²⁵ and Moore²⁶, the cystine and methionine residues present being converted to cysteic acid and methionine sulphone respectively. The oxidized yeast was then hydrolysed with 6N hydrochloric acid. Hydrolysis after rather than before oxidation circumvents losses of sulphur which can occur due to the prolonged action of hydrochloric acid on cystine and methionine^{5, 19}. The hydrolysate was subjected to one-dimensional chromatography on Whatman 3MM paper using phenol:acetic acid:water (160:1:40) as solvent, and the chromatogram then scanned for sulphur-35. Two clearly defined radioactive bands were obtained corresponding to cysteic acid and methionine sulphone, with well separated R_f values of 0.10 and 0.40 respectively. The radioactive bands were cut from the chromatogram, combusted separately by the Schöniger flask technique and the labelled sulphate obtained converted to benzidine sulphate which was then assayed with a thin end-window Geiger-Muller tube as described previously²⁷. The ratios of the counts for the cysteic acid and methionine sulphone bands on each chromatogram gave the required cystine/methionine ratio. Close agreement was obtained among successive determinations for this ratio in an extensive series of experiments. The summarized data for three *Saccharomyces* yeasts is shown in *Table 3*.

It will be seen that the ratios are virtually the same in the three yeasts. In the protein methionine predominates and, taking cystine and methionine as the major sulphur constituents, a ratio of 0.63 corresponds to a cystine

Table 3. Cystine-methionine ratios of some Saccharomyces yeasts

Yeast	Cystine-S/Methionine-S	
	Whole yeast	Yeast protein
<i>S. cerevisiae</i> (Brewer's strain)	1.16 (± 0.05)	0.63 (± 0.02)
<i>S. cerevisiae</i> (Baker's strain)	1.21	0.64
<i>S. carlsbergensis</i>	1.16	0.65

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content of 38.7 per cent and a methionine content of 61.3 per cent of the protein-S. In whole cells there is slightly more cystine than methionine, this being accounted for by the appreciable amount of cysteine present as glutathione in the non-protein fraction of the cells. It is of interest that in detailed studies of the distribution of sulphur in *Escherichia coli*, Roberts *et al.*²⁸ obtained very similar cystine/methionine relationships, namely, equimolar amounts in whole cells and a ratio of 1:2 for cystine-S to methionine-S in the protein.

SULPHUR AND YEAST GROWTH

A source of available sulphur is essential for yeast growth. Almost all species of yeasts are able to utilize inorganic sulphate and methionine as sulphur sources, but considerable differences have been found in their ability to assimilate the sulphur of cysteine, cystine and glutathione^{29, 30}, as well as that of other organic sulphur compounds. Among *Saccharomyces* yeasts sulphate has been reported as the best source and methionine as slightly less effective, although in some strains it is equivalent to sulphate²⁹. Glutathione utilization is generally equivalent to, or slightly less effective than, that of methionine, although the inability of a baker's yeast to grow on this compound has been reported³⁰. Cystine and cysteine are at best poorly utilized or are ineffective as sulphur sources^{10, 29-31}.

The response of a brewer's yeast to sulphate and to some sulphur-containing amino-acids is shown³¹ in Figure 3. Optimum growth was

Figure 3. Growth of a brewer's strain of *S. cerevisiae* on various sulphur sources; growth determined after 2 days at 30°

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obtained with concentrations of sulphate above 10 mg S/L, the growth response being practically linear up to a level of 6 mg S/l. The response to methionine and to glutathione showed a similar pattern, but less yeast was produced. However, when added to the sulphate-containing medium both

compounds increased the rate of growth (*Table 4*), although the final yeast yield was unaltered. These compounds probably stimulate growth by supplying the intact molecules of methionine and cysteine respectively for protein synthesis, and, as will be described below, the role of methionine in this connection is accentuated by its very rapid rate of entry into the yeast²⁷.

Table 4. Effects of some sulphur compounds on the growth of a brewer's yeast in a medium containing sulphate

Na_2SO_4 and sulphur compounds in medium 0.78 mM (25 mg S/l.); period of growth 24 h; temperature 30°

<i>Compound added</i>	<i>Growth of yeast as a % of controls</i>
None	100
L-Methionine	132.6
L-Cysteine	65.5
L-Cystine	104.7
Glutathione (reduced)	117.9
S-Methyl-L-cysteine	85.2
Taurine	103.3

Cystine was completely ineffective as a sulphur source. At low concentrations cysteine supported limited growth but at concentrations above about 25 mg S/l. cysteine was toxic and produced diminished growth (*Figure 4*), no growth occurring in the presence of 200 mg cysteine-S/l.¹⁰ Cysteine in low concentrations acted as a supplementary sulphur source in the presence of sub-optimum concentrations of sulphate, methionine or glutathione, but at concentrations above 25 mg S/l. it depressed growth, and this effect could

Figure 4. Growth of a brewer's strain of *S. cerevisiae* with various sources of sulphur in the medium; growth determined after 2 days at 30°

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not be overcome by raising the sulphate level of the medium. Other thiols have been tested for possible growth-inhibitory properties in the presence of sulphate, as shown in *Table 5*. Mercaptoacetate was as effective as cysteine, homocysteine showed a slight effect, while cysteamine inhibited growth

Table 5. Effects of some thiols on the growth of a brewer's yeast

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Growth measured after 2 days at 30° and expressed as mg dry wt./10 ml medium; sulphate in medium 10 mg S/l.; compounds added at a level of 100 mg S/l.

<i>Compound added</i>	<i>Growth without compound</i>	<i>Growth with compound</i>	<i>% Inhibition of growth</i>
L-Cysteine	83.9	35.9	57
Cysteamine	87.0	82.6	5
Glutathione (reduced)	90.4	86.1	5
DL-Homocysteine	91.2	80.8	11
DL-Penicillamine	82.0	80.8	2
Sodium mercaptoacetate	90.4	39.6	56
Sodium mercaptosuccinate	90.4	86.4	4

only when the concentration of sulphate was low. Glutathione, mercaptosuccinate and penicillamine were without effect, and the first two compounds, like homocysteine, enhanced growth at sub-optimum sulphate concentrations. Recently β -mercaptoethanol has also been reported as a growth inhibitor for yeast³². Cysteine affects yeast growth by prolonging the lag phase and slowing down the rate of cell division. It is also an effective inhibitor of respiration^{10, 33}.

A number of amino-acids in equimolar concentration were found to annul the growth inhibition. These included glutamic and aspartic acids, serine, homoserine and *S*-methylcysteine. Glucose-1-phosphate, glucose-6-phosphate, fructose-1,6-diphosphate and zinc salts were among other compounds which produced the same effect. These compounds were unable to reverse the prolongation of the lag phase, but they promoted faster growth in the logarithmic phase.

Cysteine is known to depress the growth of other micro-organisms^{28, 34-36}. Its precise mode of action is not clear. It has long been known to be an inhibitor of yeast aldolase³⁷ and it may act by complexing with metal ions essential for the activity of respiratory and other enzymes.

SULPHUR TRANSPORT IN YEAST

The differences in effectiveness of various sulphur compounds as sulphur sources for yeasts may be due to the presence or absence in the cells of enzymes capable of metabolizing these compounds, to the production of toxic effects by certain of these compounds, as appears the case for cysteine, or simply to differences in the ease with which they penetrate the cell membrane. This last factor prompted a study of the transport of sulphur compounds in yeasts. The uptake of inorganic sulphate and of six sulphur amino-acids has so far been studied with the brewer's yeast used in earlier experiments^{27, 38}. The yeast suspension (final concentration 2 per cent wet wt. pressed yeast/vol.) was added to a medium containing each sulphur

compound labelled with sulphur-35 and present at a level of 10 mg S/l. The loss of labelled sulphur from the medium and its corresponding appearance in the yeast were determined at intervals over a 4-hour period. *Figures 5 and 6* show the typical patterns of uptake obtained. They fall into four distinct groups.

Figure 5. Uptake of sulphur compounds by a brewer's yeast as a function of time; compounds in medium 0.312 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°

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Figure 6. Uptake of sulphur compounds by a brewer's yeast as a function of time; compounds in medium 0.312 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°

(Reproduced by courtesy of the *Journal of General Microbiology*)

(i) Sulphate and methionine uptake were similar in that they increased steadily with time, resulting in virtually complete removal of each compound from the medium. Methionine uptake was more rapid than that of sulphate and also of all the other sulphur compounds under study.

(ii) Cystine was not taken up by the yeast to any significant extent, and

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cysteine was accumulated at only a very slow rate. This explains in part their ineffectiveness as sulphur sources.

(iii) Ethionine uptake took place to a limited extent, the labelled sulphur then being retained in the yeast and incorporated partially into protein.

(iv) *S*-Methylcysteine and *S*-ethylcysteine were also taken up only to a limited extent, but unlike ethionine part of their sulphur was released back into the medium (75 per cent in the case of *S*-ethylcysteine and 48 per cent in the case of *S*-methylcysteine). The nature of the released sulphur has not so far been established, but chromatographic studies on the medium from *S*-ethylcysteine uptake experiments indicate that at least one compound distinct from the original amino-acid is present.

Sulphate uptake was not greatly increased over a 20-fold concentration range and methionine uptake was approximately doubled. The transport mechanisms for these two compounds appear to be largely saturated at low concentrations. This is in contrast to the findings with cysteine, *S*-ethylcysteine and ethionine, the uptakes of which were greatly increased by raising their concentration in the medium. Calculations have been made of the ability of the yeast to concentrate sulphate and sulphur amino-acids, the comparative data being shown in *Table 6*. Methionine was the most

Table 6. Ability of a brewer's yeast to concentrate sulphur compounds

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[³⁵S]-Sulphur amino-acids and Na₂³⁵SO₄ 0.312 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°; uptake period 0.5 h

<i>Compound</i>	<i>Ratio of internal concentration of ³⁵S in yeast to concentration in medium</i>
L-Methionine	56.2
DL-Ethionine	11.5
L-Cysteine	17.2
<i>S</i> -Methyl-L-cysteine	30.5
<i>S</i> -Ethyl-L-cysteine	21.1
Na ₂ SO ₄	4.9

readily accumulated compound, being taken up 11 times as rapidly as sulphate and over 3 times as rapidly as cysteine. Further experiments have shown that after 1 h the yeast was able to accumulate 0.46 mg sulphate-S/g dry wt. yeast. Of this 59.7 per cent became associated with the protein fraction, 5.1 per cent was present as free sulphate and 35.2 per cent was present as other forms of non-protein-S. The incorporation of sulphate-S into protein amounted to 0.24 mg S/g dry wt. yeast/h, while the corresponding figures for the incorporation of methionine-S and cysteine-S, estimated from other data, were 0.28 and 0.08 mg S/g dry wt. yeast respectively.

From the relatively low concentration of free sulphate in yeast, it appears that sulphate entering the yeast cell is readily converted to organic-S, the rate-limiting step probably being the rate of transport across the cell membrane. Methionine on the other hand is much more rapidly taken up

by the yeast but is incorporated into protein at a comparable rate to sulphate, so that it also accumulates in the non-protein-S fraction. Cysteine is taken up somewhat faster than sulphate and enters protein at a much slower rate, but a further pathway in its utilization is conversion to glutathione, and to what extent this occurs under these conditions has not yet been determined.

FACTORS AFFECTING SULPHATE UPTAKE

Sulphate uptake was previously studied by Kleinzeller, Kotyk and Kováč³⁹ and by Kotyk⁴⁰ in a baker's yeast. The process was shown to require a source of energy, such as glucose, and a supply of nitrogen, such as ammonium ions, and uptake was suppressed by certain metabolic inhibitors. Crocomo and Menard⁴¹ obtained similar results with *Torulopsis utilis*.

In experiments with a brewer's yeast the endergonic nature of sulphate uptake has been confirmed. Metabolic inhibitors such as azide and 2,4-dinitrophenol which uncouple oxidative phosphorylation, markedly reduce sulphate uptake (*Table 7*). During an examination of the action of various

Table 7. Effects of some metabolic inhibitors and added sulphate on sulphate uptake by yeast

Na₂³⁵SO₄ 0.156 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°; uptake period 1 h; effects of inhibitors expressed as the % decreases in sulphate uptake compared with controls in the absence of added compounds

Compound added	% Decrease in sulphate uptake when molar ratio compound/sulphate is			
	1	2	5	10
Sodium azide	57.9	70.6	85.0	98.0
2,4-Dinitrophenol	47.5	67.4	100	100
Iodoacetic acid	47.8	78.8	100	—
Mercuric chloride	100†	—	—	—
Sodium selenate	48.3	63.2	79.1	90.7
(Sodium sulphate	48.6	66.2	78.5	90.9)

† 60.4% inhibition at molar ratio 0.1.

inhibitors it was found that selenate strongly depressed sulphate accumulation and that its effect resembled that of added unlabelled sulphate which competes with the uptake of the labelled compound by dilution. Further experiments have shown that selenate is a competitive inhibitor of sulphate transport and that it has an affinity for the absorption sites of the cell comparable with that of sulphate itself. This is in accord with the earlier work of Fels and Cheldelin⁴² who found selenate to be a competitive inhibitor of sulphate in yeast growth. The sulphate uptake mechanism is a fairly specific one. Sulphonic acids, despite their close structural relationship to sulphate, were without effect on uptake. Certain of the sulphur amino-acids, including methionine, ethionine, cysteine and homocysteine were slightly inhibitory, but the reason for this is not clear.

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NUTRIENT REQUIREMENTS FOR SULPHATE UPTAKE

The medium used in the uptake experiments contained glucose, phosphate, ammonium chloride, citrate buffer, salts and vitamins. Experiments were carried out to determine which components of the medium were necessary for optimum uptake. Omission of glucose, ammonium ions or citrate produced significant decreases in uptake whereas omission of the other constituents was without effect. Uptake values amounting to 95–99 per cent of that in the complete medium were obtained with a simplified medium composed of glucose, citric acid-potassium citrate buffer, ammonium chloride and potassium phosphate. As shown in *Table 8*, omission of glucose

Table 8. Metabolites required for sulphate accumulation by yeast

Glucose 0.28M; ammonium chloride and amino-acids 0.028M; potassium dihydrogen phosphate 4.04 mM; citric acid 5.4 mM; potassium citrate 0.015M; acetic acid-sodium acetate buffer, pH 5.2, 0.02M; Na₂SO₄ 0.156 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°; uptake period 1 h

<i>Composition of medium</i>	<i>Uptake of sulphate</i>
Complete medium	100
Water alone	11.2
Glucose	45.7
Glucose + NH ₄ Cl + citrate + phosphate	97.1
NH ₄ Cl + citrate + phosphate	28.2
Glucose + citrate + phosphate	63.1
Glucose + NH ₄ Cl + phosphate	78.3
Glucose + NH ₄ Cl + citrate	92.6
Glucose + citrate + phosphate + L-aspartic acid	64.5
Glucose + citrate + phosphate + L-glutamic acid	62.3
Glucose + citrate + phosphate + L-glutamine	93.2

drastically reduced sulphate uptake. The requirement for ammonium ions and citrate is also apparent. Phosphate was the least essential component of this medium, its omission producing at the most only a slight decrease in uptake. Ammonium ions could be replaced almost completely by equimolar amounts of L-glutamine but not by L-asparagine, L-glutamic acid or L-aspartic acid. Furthermore, the function of the citrate was not merely that of a buffer, since its replacement by acetate or phosphate buffers of the same strength and pH resulted in a distinct decrease in sulphate uptake. The findings summarized in *Table 8* place the four components of the medium in the following order of importance for sulphate transport: glucose, ammonium ions, citrate, phosphate.

RÔLE OF NITROGEN IN SULPHATE UPTAKE

The necessity for a nitrogen supply has been discussed by Kotyk⁴⁰ in terms of the synthesis of a nitrogenous cell component which combines with sulphate as the first step in its metabolism, on the grounds that labelled sulphate-S when taken up by a baker's yeast appeared rapidly in the nucleotide fraction of the cells. Sulphate is known from the work of Wilson and Bandurski⁴³

to be converted to 3'-phosphoadenosine-5'-phosphosulphate (PAPS) in yeast, and in this form it can undergo reduction to sulphite and sulphide^{44, 45}. It is therefore tempting to suggest that PAPS or adenosine-5'-phosphosulphate (APS) may be the form in which sulphate is transported into yeast³⁹. This would also explain the competitive effect of selenate on sulphate uptake, since ATP-sulphurylase which catalyses the conversion of sulphate to APS can also use selenate as substrate⁴⁶. More recent work by Spencer⁴⁷ has provided evidence that ATP-sulphurylase is directly involved in sulphate uptake and that the ion is transported as APS.

UPTAKE OF SULPHUR AMINO-ACIDS

Some preliminary studies have been made of the accumulation by a brewer's yeast of methionine, ethionine, *S*-methylcysteine, *S*-ethylcysteine and cysteine. In all cases it was endergonic, being negligible at 0° and inhibited by azide and 2,4-dinitrophenol. The uptake of each sulphur amino-acid was blocked by only a small number of closely-related compounds, and then only when these were present in relatively high (ten-fold) concentration, as shown in the case of methionine in *Table 9*. Methionine uptake was depressed to the extent of about 50 per cent or less by ethionine, methionine sulfoxide and sulphone, and homocysteine. Ethionine uptake was reduced by 60 per cent by a ten-fold concentration of methionine, but not by *S*-methylcysteine or *S*-ethylcysteine. *S*-Methylcysteine and *S*-ethylcysteine in ten-fold concentration likewise reduced the uptake of each other

Table 9. Effect of various compounds on the uptake of L-[³⁵S]-methionine by a brewer's yeast

(Reproduced by courtesy of the *Journal of General Microbiology*)

L-[³⁵S]-Methionine 0.156 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°; uptake period 20 min; effects of compounds expressed as the % decrease in methionine uptake compared with controls in the absence of added compounds

Compound added	% Decrease in methionine uptake when molar ratio compound/methionine is			
	1	2	5	10
Na ₂ SO ₄	1.1	1.7	1.6	1.8
L-Cysteine·HCl	2.3	5.5	9.0	13.2
DL-Homocysteine	8.5	14.9	27.0	37.7
<i>S</i> -Methyl-L-cysteine	6.6	9.6	13.3	16.6
<i>S</i> -Ethyl-L-cysteine	—	—	15.2	21.4
DL-Methionine sulfoxide	11.8	20.9	36.3	46.5
DL-Methionine sulphone	16.1	26.4	40.4	45.9
L-Ethionine	12.5	20.9	32.5	46.0
Glutathione (reduced)	5.7	5.6	7.6	2.5
DL-Norleucine	—	—	16.9	23.3
DL- α -Aminobutyric acid	—	—	—	8.9
L- α -Alanine	—	—	—	8.9
Amino-acid mixture A†	7.5			
Amino-acid mixture B‡	7.9			
Amino-acid mixture A + B	14.1			

† Mixture *A* contained the following amino-acids, each 0.156 mM: L- α -alanine, L-arginine·HCl, L-aspartic acid, L-citrulline, L-glutamic acid, glycine, L-proline, L-serine, L-threonine.

‡ Mixture *B* contained the following amino-acids, each 0.156 mM: L-cystine·HCl, L-histidine·HCl, DL-isoleucine, L-leucine, L-lysine·HCl, L-phenylalanine, DL-tryptophan, L-tyrosine, DL-valine.

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by about 60 per cent. Cysteine uptake was partly suppressed (32 per cent) by *S*-methylcysteine, but not by homocysteine, and an interesting finding was that it was enhanced by glutathione (30 per cent increase at equimolar concentration, 65–80 per cent increase at ten-fold concentration). These rather restricted competitive effects on the uptake of the sulphur amino-acids suggest that a number of fairly specific transport mechanisms are involved. This contrasts with the findings of other workers^{48–50} on the uptake of such amino-acids as glutamic acid, valine and phenylalanine by yeasts.

The competitive effects were shown to operate primarily on the transport of the amino-acids into the cell. This is illustrated in *Table 10* by the effects

Table 10. Effects of L-ethionine and DL-methionine sulphone on the partition of L-methionine-sulphur in a brewer's yeast

(Reproduced by courtesy of the *Journal of General Microbiology*)

L-[³⁵S]-Methionine 0.156 mM; L-ethionine and DL-methionine sulphone 1.56 mM; yeast 2% (wet wt. pressed yeast/vol.); temperature 30°; uptake period 20 min; figures in parentheses show the % decreases over the corresponding controls

Compound added	% Uptake of methionine into yeast	
	TCA-soluble fraction	TCA-insoluble fraction
None	58.5	11.4
L-Ethionine	26.1 (55.4)	10.8 (5.3)
None	51.3	10.4
DL-Methionine sulphone	25.4 (50.5)	9.0 (13.5)

of ethionine and methionine sulphone on methionine uptake, which depressed the appearance of methionine in the non-protein-S fraction of the cells. The increase in cysteine uptake produced by glutathione was also primarily an effect on the uptake process (*Table 11*). This was associated with an increase in the incorporation of cysteine-S into protein which was most probably due to the increased availability of cysteine within the cell.

Table 11. Effect of reduced glutathione on the partition of cysteine-sulphur in a brewer's yeast

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L-[³⁵S]-Cysteine·HCl, 0.312 mM; reduced glutathione 3.12 mM; uptake period 2 h; figures in parentheses show the % increases over the corresponding controls; other details as in *Table 10*

Compound added	% Uptake of cysteine into yeast	
	TCA-soluble fraction	TCA-insoluble fraction
None	14.9	9.7
Reduced glutathione	30.2 (104.1)	13.9 (43.3)

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