Solute transport and plasma-membrane lipid composition in Saccharomyces cerevisiae NCYC 366.

Keenan, Michael Hugh John

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SOLUTE TRANSPORT AND PLASMA-MEMBRANE LIPID COMPOSITION IN SACCHAROMYCES CEREVISIAENCYC 366

Submitted by
MICHAEL HUGH JOHN KEENAN
For the degree of Ph.D.
of the University of Bath
1981

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SUMMARY

The effects of alterations in fatty-acyl unsaturation in the plasma membrane of Saccharomyces cerevisiae NCYC 366 on the kinetics of accumulation of eight solutes were examined. The $K_T$ and $V_{\text{max}}$ values obtained for L-arginine and L-lysine accumulation were different for cells enriched with oleyl residues to those obtained for cells enriched with linoleyl residues, whilst for D-glucose only the $V_{\text{max}}$ value was altered. The $K_T$ and $V_{\text{max}}$ values obtained for accumulation of L-asparagine, and L-glutamine, and dihydrogen phosphate, sulphate and calcium ions were identical for cells enriched with either residue. Inclusion of palmitoleyl residues in the plasma membrane resulted in $K_T$ and $V_{\text{max}}$ values for L-lysine and L-asparagine accumulation which were different to those obtained with cells enriched with either oleyl or linoleyl residues. The effects of temperature on accumulation of L-lysine and L-asparagine by cells with plasma membranes enriched with oleyl or linoleyl residues were also examined. The results are discussed in terms of specific lipid environments of proteins involved in the transport of those compounds.

Many experiments yielded results which were independent of plasma-membrane compositional change but were interesting in themselves and therefore were worthy of comment. These were included.
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PLASMA-MEMBRANE LIPID COMPOSITION AND TRANSPORT KINETICS IN SACCHAROMYCES CEREVISIAENCYC 366

DATA WHICH ARE INDEPENDENT OF PLASM-MEMBRANE LIPID COMPOSITION:

Effect of Storage Time on Solute-Accumulating Ability of Organisms

Effect of Cold-Osmotic Shock on Arginine-Accumulating Ability of Organisms

Effect of Arginine Accumulation on the Size of Organisms

Some Aspects of Accumulation and Retention of Solutes by Saccharomyces cerevisiae

Expression of the General Amino-Acid Permease

Accumulation of L-Asparagine and L-Glutamine

Plasma-membrane Lipid Unsaturation can Affect
the Kinetics of Solute Accumulation by
Saccharomyces cerevisiae

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INTRODUCTION
GENERAL INTRODUCTION

Organisms which survive do so because they have some selective advantage. In the microbial world these advantages can be readily split into two categories, namely selection for high growth rates (r-selection) and selection for large size (K-selection). Eukaryotes cannot compete with the faster growing prokaryotes in the rapid colonization of transient resources, but their attributes permit their success in more stable environments (Carlile, 1980). Such stable environments are found in brewer's wort and wine must, where yeasts of various types have become the dominant organisms, although one might reasonably argue that human intervention has played its part. Implicit in the concept of competition is success gained at the expense of another. For example, an ability rapidly to utilize available nutrients will deplete the source for would-be competitors. Central to nutrient utilization is the ability to transport these nutrients. An interesting example of the result of uptake selection can be found amongst yeasts with regard to sugar utilization.

Brewer's yeast (Saccharomyces cerevisiae) will ferment D-glucose more rapidly than D-fructose when the two are provided in an equimolar mixture (Dubrunfaut, 1847; Bourquelot, 1886; Peynaud and Domercq, 1955). This is not unexpected as wort, in which D-glucose is the predominant monosaccharide, has been the medium in which the organism has been propagated for many millions of generations. Just such an equimolar mixture is to be found in sound and mature wine grapes (Peynaud and Ribéreau-Gaynon, 1971).
Kloeckera apiculata, the yeast which has been shown to initiate the fermentation of most clarets (Domercq, 1956) utilises both hexoses with equal efficiency.

The great, sweet white wines of Sauternes, however, are required to undergo "le pourriture noble", (noble rot), caused by the mould Botrytis cinerea, which preferentially utilizes D-glucose (Ribereau-Gaynon and Peynaud, 1960) thereby enriching the Sauternes must with D-fructose. A yeast which is frequently responsible for the initiation of fermentation for these sweet white wines, Torulopsis stellata, (bacillaris) (Domercq, 1956) can take advantage of this situation as it will selectively ferment D-fructose, an opportunity shared with another "Sauternes" yeast, Saccharomyces bailii (elegans) (Dubourg, 1897).

More detailed experiments have shown that unlike intact "Sauternes" yeast, broken cells will ferment D-glucose preferentially, a feature in common with brewer's yeast, (Gottshalk, 1946). The plasma membrane is therefore implicated as the point of selectivity, and indeed Sols (1956) has shown that the carrier which is responsible for transport of both of these hexoses in "Sauternes" yeast has a greater affinity for D-fructose than it does for D-glucose which is a situation precisely opposite to that in brewer's yeast (Kotyk, 1967), where the carrier has a greater affinity for D-glucose. The origins of these phenomena will never be known.

Yeast have been used for the production of alcoholic beverages for thousands of years, and references can be traced back as far
as the Babylonian empire (Rose, 1977). The leavening of bread has an equally long history. However, throughout most of that time, yeast was considered merely as an ingredient.

Yeasts were not observed as single cells until Antonie van Leeuwenhoek viewed them down his microscope in 1680, and presented his drawings to the Royal Society in London (Rose and Harrison, 1969). However, some 150 years later, they were still only referred to as "yeast globules" by Cagniard-Latour (1838). Yeast gained respectability when Louis Pasteur finally reported that fermentation was due to living cells in his Etudes sur le Vin (1866) and Etudes sur la Bière (1876). Since that time, increased understanding of the underlying biochemical processes has enabled man to utilize yeasts in a greater variety of ways. For example, yeast are now involved in production of carbon dioxide and ethanol for industrial usage, whilst whole cells, and extractable compounds such as vitamins, coenzymes and amino acids may be used as food supplements, as well as maintaining a traditional role in bread and beverage manufacture.

Yeast has many advantages for use in the laboratory. They are unicellular; they grow readily in rather acid media and therefore are not very susceptible to bacterial contamination; with certain reservations they can withstand any practicable osmolarity of the medium, from 5 M sodium chloride to distilled water; unlike bacteria they are clearly visible under the light microscope, they are eukaryotic, yet unlike animal cells they may be readily separated from their medium when necessary. Yeast can therefore be described as an ideal model organism.
PLASMA MEMBRANE COMPOSITION

Plasma membranes can be prepared from *Saccharomyces cerevisiae* by two methods. One requires lysis of protoplasts (or sphaero-plasts) formed after enzymic digestion of whole cells by preparations containing a β-glucanase. In early studies, snail gut juice was used (Boulton and Eddy, 1962), but most workers now use Zymolyase, either 5,000 or 60,000. The plasma membrane may then be separated from the rest of the lysate by differential or isopycnic centrifugation (Boulton and Eddy, 1962; Boulton, 1965). More recently, this technique gained an extra step, namely lactoperoxidase-catalysed iodination of the outer surface of sphaeroplasts, which aids the isolation of the plasma membrane (Longley et al., 1968; Schibeci et al., 1973). The second method involves mechanical disruption of whole cells and subsequent separation of plasma membranes or membrane-rich fractions by centrifugation (Suomalainen et al., 1967; Matile et al., 1967). This second method generates preparations which contain more carbohydrate than does the first (Rose, 1976). Most authors would agree that the main constituents of the yeast plasma membrane are protein and lipid with smaller quantities of nucleic acid and carbohydrate.

**Protein**

Majority opinion puts the protein content of the yeast plasma membrane between 35% and 49% dry weight (Boulton, 1965; Suomalainen et al., 1967; Longley et al., 1968; Schibeci et al., 1973).
1973), but variation as wide as 26% dry weight (Matile et al., 1967), and 65% dry weight (Christensen and Cirillo, 1972) have been reported. These variations are almost certainly due to one or more of three factors: differences in (i) cultural conditions, (ii) growth phase of the culture and (iii) techniques used to determine the protein content.

Unlike lipids, the proteins of the plasma membrane have not been divided into chemical groupings. The only classification is functional. The traditional concept of transport proteins, structural proteins and enzymes as a basis for classification is interesting. For example, there is evidence for the presence of numerous transport proteins in the plasma membrane, but as yet none have been isolated and identified. Also as far as is known, no yeast plasma membrane proteins have been identified as purely structural. It is likely, however, that transport proteins and enzymes effect a structural role in addition to their demonstrable role. Enzymes within the plasma membrane are primarily involved with cell envelope growth (Suomalainen and Nurminen, 1972), an important exception to this is ATPase, although this could well be included as a transport protein.

**Lipid**

Since techniques for lipid extraction and analysis vary considerably, the comparison of data is not ideal. The lipid composition of the plasma membrane is very sensitive to influences
such as growth temperature (Hunter and Rose, 1972), growth phase
(Watson and Rose, 1980) and degree of aeration (Jollow et al.,
1968). This must always be borne in mind when compositions
are quoted. Reviews of yeast lipids, membranes and phospho-
lipids (Hunter and Rose, 1971; Rattray et al., 1975; Magnall
and Getz, 1973) provide a detailed insight into this complex
field, far more so than within this limited report. Even so
a relatively simple and generalized view may be given.

The main lipid classes of the yeast plasma membrane are
glycerophospholipids and sterols, although small quantities of
sterol esters and mono-, di-, and tri-acylglycerols have been
reported (Erwin, 1973).

Glycerophospholipids are substituted fatty-acyl diesters
of sn-glycerol-3-phosphoric acid. In yeast the substitution is
almost entirely by choline, ethanolamine, inositol, serine
or glycerol, producing phosphatidylcholine (PC), phosphatidyl-
ethanolamine (PE), phosphatidylinositol (PI), phosphatidyl-
serine (PS) and phosphatidylglycerol (PG), respectively. On
a moles percent basis, the relative proportions of each of
the phospholipids have been variously reported as: PE, 35%;
PC, 23%; PS + PI, 28% (Longley et al., 1968); PE, 20%;
PC, 34%; PS + PI, 28% (Kramer et al., 1978); PE, 25% and PC 30%
(J.A. Hossack, unpublished data). Individual lipids within
these groups differ in fatty-acyl content but, in all lipids,
unsaturated residues are more prevalent at position 2 on the
glyceryl moiety. The most prevalent fatty-acyl residues are
C\textsubscript{16:1} (cis-9-hexadecenoic acid) and C\textsubscript{18:1} (cis-9-octadecenoic acid), which account for 40 - 50% and 25% respectively of the residues present. The remainder comprises C\textsubscript{16:0} (hexadecanoic acid), 15%; C\textsubscript{18:0} (octadecanoic acid), 5%; and various shorter chain acids which are either mono-unsaturated (C\textsubscript{14:1}) or saturated, down to as short as six or eight carbon atoms. Manipulation of the relative quantities of these and other fatty-acyl residues is an important technique discussed in the section on altering membrane composition.

The structural feature which links all sterols is the cyclopentanoperhydrophenanthrene ring. The sterol content of Saccharomyces cerevisiae, including free and esterified forms, is usually between 0.1 and 2.0% of the cell dry weight (Shaw and Jefferies, 1953), although values as great as 10% have been reported (Dulaney et al., 1954), depending on growth conditions. On a dry weight basis, 6% of the yeast plasma membrane was found to be sterol (Longley et al., 1968; Schibeci et al., 1973). It naturally follows that not all sterols and related compounds found in Saccharomyces cerevisiae are found in plasma membranes; indeed sterol esters may be preferentially concentrated in certain intracellular structures (Hunter and Rose, 1972; Hossack et al., 1973).

The principal sterol found in most yeasts is ergosterol (Dulaney et al., 1954; Hunter and Rose, 1971), with 24,(28)-dehydroergosterol second, although these were reported in equal amounts in Saccharomyces cerevisiae NCYC 366 by Longley et al.,
(1968). Dulaney et al. (1954) also found zymosterol in many yeasts which was proposed as an intermediate in ergosterol biosynthesis by Fryberg et al. in 1973.

Proudlock et al. (1968) thought that the major function of sterols in yeast is their structural influence upon the dynamic state of the membrane, and only sterols with the required conformation can be expected to produce a functional membrane. Two basic requirements are that the A and B rings are planar and that the molecule has a long alkyl side chain at C-17. Chemical characteristics of sterols associated with functional membranes have been thoroughly reviewed by Nes (1974).
STRUCTURE OF THE PLASMA MEMBRANE

In 1835, Carl Nageli examined pigment penetration into damaged and undamaged plant cells. He concluded that the undamaged cells had a continuous boundary, which he named the "plasma membrane". It was to this membrane that he attributed the osmotic properties of cells which he observed some time later when working with Cramer. In experiments which examined the rate of entry of molecules into cells, Overton, (1899) inferred that biomembranes contain lipids, on the evidence that the more polar a molecule was, the less rapidly it traversed the membrane. He did not, however, speculate on the organization of the lipid in the membrane.

Little serious thought was given to structure of the plasma membrane until 1925 when Görter and Grendel, in a now classic experiment, calculated that there was sufficient lipid in erythrocyte ghosts to form a bilayer. Using this information and the misconception that biological membranes had a surface tension too low for an exposed lipid bilayer, Danielli and Davson (1935) proposed that a membrane comprised a protein-lipid-lipid-protein sandwich 7.5 nm wide. It is now known that not only had Görter and Grendel miscalculated, but also that phospholipid headgroups can account for the low surface tension in biomembranes. However, support for the Danielli-Davson model was found in electron micrographs of sectioned membranes which showed two electron-dense layers (protein) about an electron transparent layer (lipid). This model was christened the unit membrane by
Robertson in 1959. However, pretenders to the throne existed. For example Lucy (1964) proposed a unicellular membrane structure. Two other models by Hybl and Dorset (1970) and Pettiplace et al. (1971) incorporated the fact that there does not seem to be enough lipid available in the membrane to be arranged in two continuous layers with the fatty-acyl chains oriented transversely in the membrane. Both models assumed a lengthwise orientation of the lipid molecules with a sheath of protein on the outward facing surfaces. Nevertheless, the unit membrane remained the dominant theory for thirty seven years, when Singer and Nicholson (1972) presented the fluid mosaic model.

In the fluid mosaic model, globular proteins are interpolated to varying degrees in a phospholipid bilayer. Amphipathic phospholipids are orientated transversely with their polar headgroups towards the outside of the membrane and hydrophobic fatty-acyl chains towards the centre of the membrane. In the original model, sterols were not included but these may be readily accommodated. Viewing the sterol as a club-shaped molecule, the thick end, which contains the functionally significant polar hydroxyl group (position 3) locates itself with the phospholipid headgroups and the narrow end, formed by the acyl side chain (position 17), rests alongside the fatty-acyl side chains of the phospholipids.
TRANSPORT AS A FUNCTION OF THE YEAST PLASMA MEMBRANE

The contents of living cells are different from their surroundings, a situation which is maintained by a limiting membrane. To sustain life it is necessary for extracellular substrates to enter the cell, while provision must be made for egress of metabolic waste products which would otherwise accumulate in toxic amounts to the detriment of the cell. The lipid nature of the plasma membrane poses real problems when attempting to understand penetration of many biologically important molecules across it.

Collander et al. (1931) showed that, in general, the more lipid-soluble compounds were, the faster they penetrated cells. In a classic series of experiments, the rate of diffusion of some thirty organic non-electrolytes across the plasma membrane of the plant cell, Chara ceratophylla, were found to be correlated with the olive oil-water partition coefficients (Collander and Bärlund, 1933). These data were subsequently refined by Stein in 1967 (Stein, 1967), who showed that the rate of diffusion was inversely proportional to the putative number of hydrogen bonds that must be broken for the solute to partition into a hydrophobic environment.

Inorganic ions and other charged molecules are highly hydrated. For example, they may be associated with up to twenty water molecules. The energy required for release from this hydration shell into the hydrophobic region of the plasma membrane
is prohibitively large to be useful in biological systems (Quinn, 1976). Many inorganic ions are, however, accumulated by cells, and accumulated very rapidly. In *Saccharomyces cerevisiae*, glucose was found to penetrate more rapidly than could be predicted from its size and lipid solubility (Ørskov, 1945). To explain such exceptions, a carrier concept was invoked (Cirillo, 1961). Other important considerations for this carrier were: (i) finite rates of transport can be achieved if enough solute is added to one side or other of the membrane, so making Fick's first law of diffusion no longer applicable; (ii) a number of enzyme poisons inhibit transport when added in concentrations low enough to exclude any non-specific effects on membrane permeability; (iii) 'transport sites' possess a remarkable chemical specificity and, in many processes, can even discriminate between optical isomers. If one adds to these considerations the need for a carrier to be located in a partly hydrophobic lipid layer, then virtually only proteins could be considered as candidates for carrier molecules.

The 'carrier' view of transport is now generally accepted. In yeasts, and *Saccharomyces cerevisiae* in particular, many different transport systems have been shown to exist, and as time passes more are being found.

**Amino Acids**

Uptake of amino acids by *Saccharomyces cerevisiae* is mediated by a wide variety of transport systems with overlapping
specificities (Grenson et al., 1966; Grenson, 1966; Gits and Grenson, 1969; Crabeel and Grenson, 1970; Grenson et al., 1970; Kotyk et al., 1971; Chan and Cossins, 1976). There are at the present time eleven known transport systems and more probably exist (Table 1).

In general, uptake is an active process, unidirectional against a concentration gradient and is trans-inhibited by pre-accumulated amino acid (Kotyk and Řihová, 1972). The energetics of uptake have been well studied by Eddy and his coworkers (Eddy et al., 1970; Eddy and Nowaki, 1971; Seaston et al., 1973; 1976). For example they have established a stoicheometrical relationship between proton influx, potassium ion efflux and glycine accumulation. Other amino acids studied have revealed a proton dependence with various stoicheometries.

The yeast vacuolaes play a central role in compartmentation of soluble amino acids, particularly arginine, glutamine and lysine (Wiemken and Dürr, 1974), with acidic amino acids (aspartate and glutamate) held preferentially in the cytoplasm. The localization of neutral amino acids is not as clear cut, for alanine residues preferentially in the cytoplasm and glycine is taken into the vacuoles, sometimes uncontrollably (Indge et al., 1977).

There are differences between the transport systems for amino acids but there are also many similarities, so it is useful to describe one system in some detail. As previously mentioned arginine is accumulated irreversibly against a
### Table 1. Amino-Acid Transport Systems of *Saccharomyces cerevisiae*

<table>
<thead>
<tr>
<th>Preferred amino acid transported</th>
<th>( K_T ) Value (M)</th>
<th>Reference</th>
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<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>( 10^{-5} )</td>
<td>Grenson <em>et al.</em> (1966)</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>( 2.5 \times 10^{-5} )</td>
<td>Grenson (1966)</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>( 1.2 \times 10^{-5} )</td>
<td>Gits and Grenson (1967)</td>
</tr>
<tr>
<td></td>
<td>( 6 \times 10^{-5} )</td>
<td>Kotyk <em>et al.</em> (1971a)</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>( 7.7 \times 10^{-4} )</td>
<td>Gits and Grenson (1967)</td>
</tr>
<tr>
<td>Threonine</td>
<td>( 1.1 \times 10^{-3} )</td>
<td>Kotyk <em>et al.</em> (1971a)</td>
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<tr>
<td>Serine</td>
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<td></td>
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<tr>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>( 2.8 \times 10^{-4} )</td>
<td>Joiris and Grenson (1969)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td></td>
<td>Kotyk <em>et al.</em> (1971a)</td>
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<tr>
<td>VI</td>
<td></td>
<td></td>
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<tr>
<td>Leucine</td>
<td>( 1.3 \times 10^{-4} )</td>
<td>Gits and Grenson (1969)</td>
</tr>
<tr>
<td></td>
<td>( 6 \times 10^{-5} )</td>
<td>Kotyk <em>et al.</em> (1971a)</td>
</tr>
<tr>
<td></td>
<td>( 1 \times 10^{-3} )</td>
<td>Bussey and Umbarger (1970a, b)</td>
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<tr>
<td>Isoleucine</td>
<td></td>
<td></td>
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<tr>
<td>Valine</td>
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<td>VII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>( 2 \times 10^{-5} )</td>
<td>Crabeel and Grenson (1970)</td>
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Table 1 continued

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<tr>
<th>Preferred amino acid transported</th>
<th>( K_T ) Value (M)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>Histidine</td>
<td>2 \times 10^{-5}</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>Proline(^a)</td>
<td>2.5 \times 10^{-5}</td>
</tr>
<tr>
<td>X</td>
<td>Basic and Neutral (not proline)</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>Arginine</td>
<td>10^{-3}</td>
</tr>
</tbody>
</table>

\(^{a}\) Reported in a strain of *Saccharomyces chevalieri*, a strain considered synonymous with *Saccharomyces cerevisiae* (Lodder, 1970). Proline transport observed, but no \( K_T \) values reported in *Saccharomyces cerevisiae* (Seaston et al., 1973; Woodward and Cirillo, 1977).
concentration gradient and most of this is stored in vacuoles although a small rapidly metabolizable pool is maintained in the cytoplasm (Wiemken and Nurse, 1973; Wiemken and Dürr, 1974).

In 1966, Grenson demonstrated the presence of an arginine-specific transport system in ammonia-grown cells with a $K_T$ value of about $10^{-5}$ M, other workers have calculated values of $3 \times 10^{-5}$ M (Boller et al., 1975) and $10^{-5}$ M (Chan and Cossins, 1976).

Subsequent work (Chan and Cossins, 1976), showed a further arginine-specific transport system, with saturation kinetics giving a $K_T$ value of $10^{-3}$ M. On the other hand, the basic and neutral-amino acid transport system (GAP) (Grenson et al., 1970) transports arginine with a $K_T$ value of $10^{-6}$ M. This activity is not expressed in ammonia-grown cells. Proton dependence of arginine transport has been demonstrated (Seaston et al., 1973).

The number of protons cotransported with arginine is probably one with the high-affinity arginine transport system and two with the GAP. The low-affinity arginine transport system was not investigated.

In sphaeroplasts of Saccharomyces cerevisiae, both specific systems for arginine still operate (Boller et al., 1975), but the $K_T$ value for the high-affinity system is decreased some twenty-fold from 30 to $1.5 \times 10^{-6}$ M, showing that its affinity for arginine has increased. The lower affinity system was not investigated in so much detail.

In whole cells and sphaeroplasts, the inhibition patterns of the high-affinity system are the same. Only basic amino acids
can inhibit L-arginine uptake, and the order of effectiveness is:
L-canavanine > L-lysine > L-histidine > L-ornithine > D-arginine
> D-histidine. Of these, L-canavanine and D-arginine are known to
inhibit competitively, and L-histidine inhibits in a non-competitive
manner.

Opekarová et al. (1975) found that the rate of arginine
transport was lowered by 40 to 80% when exponentially growing yeast
cells were transferred from growth medium to dilute salt solutions
or distilled water. This was accompanied by loss of several
proteins from the membrane surface or periplasmic space.
Protein synthesis was required for resumption of activity. One
of these proteins (mol. wt. 5000, about 6 x 10^6 per cell) showed
a high binding affinity for arginine (and lysine), so it was
inferred that this protein played a necessary scavenging role in
transport of arginine.

In 1975, Boller et al. characterized a specific transport
system for arginine in isolated yeast vacuoles with a K_m value
the same as that for whole cells, namely 3 x 10^{-5} M. This system
was unlike the whole-cell transport systems in several respects.
Although in the absence of external arginine the vacuoles did
not leak arginine, when arginine was present both inside and
outside the vacuoles stoicheometrical exchange occurred. One
arginine molecule came out as one went in. 2,4-Dinitrophenol
and azide, both of which are very potent inhibitors of whole-
cell arginine transport, were ineffective on the vacuolar system.
Furthermore, it was shown that not only L-canavanine and D-arginine
inhibited vacuolar arginine transport competitively but that L-histidine did too. From this and other competition studies (less inhibition of vacuolar transport by L-lysine, L-ornithine, L-canavanine and L-N\(^\text{5}\)-l-iminoethyl)-ornithine and less inhibition of sphaeroplast transport by L-arginin acid, 5-guanidino-n-valeric and D-arginine), it would seem that the whole cell-sphaeroplast transport system most readily recognizes the L-\(\alpha\)-amino group whereas the vacuolar system most readily recognizes the region of the guanidino group. A physiological role for the vacuolar molecule for molecule exchange is, however difficult to visualize.

In 1979, (Dürr et al., 1979), the role of the vacuole was further elucidated by linking high polyphosphate contents in yeast vacuoles to accumulated arginine, and it was proposed that polyphosphate acts as an ion-exchange surface with particular affinity for arginine. This would explain why vacuoles do not leak but only give up their arginine in exchange for another, i.e. the vacuoles are impermeable to the polyphosphate-arginine complex. The system has the added advantage of being able to store large quantities of nitrogen (arginine) and phosphate in a relatively osmotically inactive form. It is possible that polyphosphate can be replaced by other anionic substances though these have yet to be identified.

Cowie and McClure (1959) and Halvorsen and Cowie (1961) suggested the existence of metabolic non-expandable and expandable pools for amino acids in yeast. This vacuolar involvement may
be the realization of that concept. The presence of two consecutive transport systems may lead to confusion in interpreting transport data and, although no active transport has been found in vacuoles in vitro, this cannot be precluded in vivo. This would mean that transport phenomena measured "at the plasma membrane" may be a nett effect involving vacuoles and, this must always be taken into consideration when drawing conclusions.

Turning to other amino acids examined in the preparation of this thesis: lysine, asparagine and glutamine. Lysine has its own highly specific transport system (Grenson, 1966) with a $K_T$ value of $2.5 \times 10^{-5}$ M. It is also a substrate for the high-affinity arginine transport system as well as for the GAP (Table 1). In this respect much of the data described for arginine accumulation can be equally applied to lysine accumulation, although transport of lysine into sphaeroplasts and vacuoles has not been reported in so much detail.

As far as I know no specific transport systems for asparagine or glutamine have been described for Saccharomyces cerevisiae. However, when glutamine was demonstrated to be a substrate for the GAP (Grenson et al., 1970) the presence of a different transport system capable of glutamine transport could be inferred because glutamine uptake proceeded in a GAP-less mutant. The nature of this transport was not investigated further.
Peptides

Transport of peptides was first reported in *Saccharomyces cerevisiae* by Becker et al. in 1973, who demonstrated that methionine-containing peptides could be utilized by a methionine-requiring auxotroph. A similar study was performed by Marder et al. (1977). By using a double amino-acid auxotroph and mixtures of peptides, it was deduced that peptide uptake was distinct from amino-acid transport, that di- and tripeptides probably share a common system, and that peptides longer than \((\text{Leu})_3\) were not transported, indicating a size limit. However, owing to the methods and the limited range of peptides used, these must be only tentative generalizations.

Competitive inhibition of \((\text{Met})_3\) uptake was observed with Met-Ala-Met, Met-Met-Leu, \((\text{Ala})_3\), \((\text{Leu})_3\) and to a smaller extent by \((\text{Met})_2\), indicating, perhaps a shared transport system for these peptides (Becker and Naider, 1977). The value for \(K_p\) for \((\text{Met})_3\) uptake is \(8 \times 10^{-5}\) M and the transport is energy dependent. Within one minute of transport, \((\text{Met})_3\) is broken down to methionine residues, indicating absence of an intracellular pool of peptides in *Saccharomyces cerevisiae*.

The generalizations of Marder et al. (1977) are supported by the specific example of \((\text{Met})_3\) uptake, and by more recent work by Nisbet and Payne (1979). However, the possibility of further peptide-transporting systems has not been negated and some may yet be revealed.
Sugars

(i) Monosaccharides

In *Saccharomyces cerevisiae* transport of monosaccharides, by a process of facilitated diffusion, proceeds only up to a diffusion equilibrium (Cirillo, 1961; Fuhrmann et al., 1976). Evidence that phosphorylation of certain metabolizable sugars is necessary for transport, (Jaspers and Van Stevenink, 1975) is equivocal, and work by Kotyk and Michaljaničová in 1974 would indicate that phosphorylation is subsequent to transport.

It is interesting to note that in members of other yeast genera; namely *Torulopsis*, *Candida* and *Rhodotorula*, monosaccharide transport is an active process involving proton cycling (Kotyk and Höfer, 1965; Misra and Höfer, 1975). It is attractive to infer that this reflects an imposed evolutionary effect. Perhaps had *Saccharomyces* spp. not produced such a waste product as alcohol, it too may have had active monosaccharide transport.

According to Kotyk and Janáček (1975), the affinities of monosaccharides for transport processes are lower the more different the orientation of hydroxyl groups in the molecule is compared with glucose; the most significant position is C₃. For this system $K_T$ values vary from $5 \times 10^{-3}$ M for D-glucose, through $35 \times 10^{-3}$ M for D-galactose to $1000 \times 10^{-3}$ M for L-arabinose. These authors also state that there are two constitutive monosaccharide transport systems, one with low specificity which allows sugars to reach 40 to 50% of the cell water, and one of higher specificity which acts subsequently to the first and which permits penetration to the remainder of the cell water.
There is, in addition, an inducible monosaccharide carrier which shows preference for D-galactose and structurally related sugars (Kotyk, 1967). When this system is operative, the half-saturation constant for D-galactose transport is $4 \times 10^{-3}$ M. All three carriers exhibit counter transport.

(ii) Oligosaccharides

Some oligosaccharides (β-fructosides and α-galactosides) are split periplasmically by yeast and the resulting monosaccharides transported. But, where uptake of oligosaccharides does occur, it is an active process.

There are three inducible disaccharide transport systems in *Saccharomyces cerevisiae*. The first is induced by isomaltose, α-methylglucoside and probably other α-alkylglucosides. It can be readily separated into a constitutive facilitated diffusion and an imposed inducible 'active' part (Okada and Halvorsen, 1964). The second transport system has a wider inducer spectrum but it is primarily for maltose. Few of the other inducing substrates are transported (Okada and Halvorsen, 1963). This system is inactivated by D-glucose (Görts, 1969). The third system, reported to transport β-glucosides such as cellobiose (Kaplan, 1965), may involve periplasmic splitting and subsequently apparently active transport of D-glucose (Kaplan and Tercreiter, 1966).

Much evidence has been accumulated by Eddy and his co-workers
(Seaston et al., 1973; Brocklehurst et al., 1977; Eddy et al., 1977) to show that, with various strains of *Saccharomyces* spp., proton-coupled transport of α-thioethylglucoside, trehalose, maltose and sucrose does occur, and that there is a subsequent potassium ion efflux, probably due to a fall in the membrane potential when disaccharides accumulate on the inside of the membrane (Eddy et al., 1977).

Further work concerning energy requirements for maltose accumulation (Serrano, 1977) demonstrated that one proton is cotransported with maltose and that electroneutrality could be maintained by a concomitant efflux of potassium ions, or influx of a permeable ion such as thiocyanate.

### Anions

(i) **Phosphate**

Three transport systems have been reported for phosphate in *Saccharomyces cerevisiae*. According to Goodman and Rothstein (1957), the monovalent anion only is taken up. The reported $K_T$ values with respect to $H_2PO_4^-$ are: $5 \times 10^{-4}$ M (Borst-Pauwels and Jäger, 1969), $3 \times 10^{-5}$ M (Borst-Pauwels et al., 1975) and $6 \times 10^{-7}$ M, (Roomans et al., 1977).

Under almost all conditions, phosphate is accumulated irreversibly and a high intracellular level is maintained (25 mM) even under conditions of phosphate starvation. After a prolonged pre-incubation with glucose, however, a measurable efflux does
Phosphate transport is stimulated by K⁺ (Goodman and Rothstein, 1957). The authors suggested that, as phosphate was taken up, electroneutrality was maintained by efflux of hydroxyl ions. The resulting acidic internal environment could then be neutralized by a H⁺/K⁺ exchange.

Cockburn et al. (1975) observed a rapid absorption of protons with phosphate. The system involved was one with a $K_T$ value of $3 \times 10^{-5}$ M, and the yeast had been depleted of ATP. Depending on phosphate concentration and therefore rate of uptake, there were variously two or three protons absorbed for each $H_2PO_4^-$ ion. The authors inferred that phosphate was entering as a positively charged complex. Roomans and Borst-Pauwels (1979) report an influx of two protons per $H_2PO_4^-$ taken up, with a corresponding efflux of a single $K^+$.

A sodium-dependent cotransport of phosphate was revealed in the very high-affinity system ($K_T 6 \times 10^{-7}$ M; Roomans et al., 1977). There are two sites available to sodium ions and, apart from Li⁺ which has a lower affinity for these sites, no other cation will substitute. Estimations of intra- and extracellular sodium-ion concentrations would suggest that a gradient of sodium ions is not the driving force for phosphate transport but, by virtue of two Na⁺ combining with one $H_2PO_4^-$, the resulting positively charged complex would flow down the transmembrane potential.
electrical gradient.

An important contribution by the surface potential (biological membranes bear a net negative surface charge which attracts cations and repels anions) has been proposed by Roomans and Borst-Pauwels (1979) to explain the fact that, at pH 7.2, bivalent cations stimulated uptake of phosphate via the sodium-dependent system and, at pH 4.5, inhibited phosphate uptake via the proton-dependent system.

(ii) Sulphate.

From the very earliest work on sulphate transport into Saccharomyces cerevisiae, a link with subsequent metabolism was established. This has sometimes led to confusion in the interpretation of experimental evidence. In a report in 1959, Kleinzeiler et al. found that sulphate uptake was an active process. Working with nitrogen-deficient yeast, they showed that sulphate uptake was diminished but addition of ammonium salts could reproduce the original response. Later Kotyk (1959) discussed this in terms of a nitrogen-containing compound necessary for sequestering of sulphate once inside the cell, an opinion shared by Maw (1963).

A single transport system was found by Kotyk, (1959), with a $K_T$ value between $10^{-6}$ M and $10^{-5}$ M. In 1974, (McCready and Din, 1974), unaware of previous work, demonstrated a single transport system. In 1977 (Breton and Surdin-Kerjan) distinguished both kinetically and genetically two sulphate transport
systems, namely SPI (K_5 5 \times 10^{-5} M) and SPII (K_T, 3.5 \times 10^{-4} M). The authors proposed a co-operativity between the two systems as the sum of sulphate transporting activities in mutants deficient in one or other of the systems did not equal the activity of both in the parent.

Both sulphate transport systems are transinhibited by pre-accumulated sulphate and by early products of sulphate metabolism, such as adenosine 5'-phosphosulphate. Both systems are sensitive to selenate and chromate, which behave as competitive inhibitors of sulphate transport. The relative affinities of these ions are different in systems I and II.

(iii) Chloride.

Texts and reviews say that chloride ions if they are taken up at all are taken up passively and slowly, (Kotyk and Janáček, 1975; Rothstein, 1972; Rothstein, 1955; Conway, 1952). The only experimental evidence cited by these references is Fenn, W.O., 1942 unpublished data.

Cations

(i) Monovalent.

In yeast, certain cations such as H^+, K^+ and Na^+ are integrally involved in transport of other compounds both inwards and outwards. The mechanisms are often distinct from the mainstream monovalent-cation transport mechanisms, and therefore do
not complicate studies of the ion transports themselves.

There is one transport system to move all monovalent cations. Values for \( K_T \) for transport are: \( H^+ \), \( 1.8 \times 10^{-4} \text{M} \); \( K^+ \), \( 5 \times 10^{-4} \text{M} \); \( Rb^+ \), \( 1 \times 10^{-3} \text{M} \); \( Cs^+ \), \( 7 \times 10^{-3} \text{M} \); \( Na^+ \), \( 1.6 \times 10^{-2} \text{M} \); and \( Li^+ \), \( 2.7 \times 10^{-2} \text{M} \) (Armstrong and Rothstein, 1964). Each substrate is a competitive inhibitor of the others. Cation transport may be modified by binding of certain cations, mainly \( Ca^{2+} \), \( Cs^+ \) and \( H^+ \), to other sites located on the plasma membrane. The effect of this is a decrease in \( V_{\text{max}} \) value for uptake. Modification by cations affects transported substrates to different extents. For example, a low pH value inhibits Na transport up to two and a half times more than \( K^+ \) transport (Armstrong and Rothstein, 1967).

In addition to the system transporting monovalent cations inward, there is an outward system which has a different order of affinities. Both \( Na^+ \) and \( H^+ \) are extruded in preference to \( K^+ \) (Foulkes, 1956). The theory that there are two outward transporting systems, one each for \( Na^+ \) and \( H^+ \) (Conway et al., 1954), has been discredited, not only in \( Saccharomyces \) spp. but in bacteria too (Foulkes, 1956; Ennis and Lubin, 1961; Lubin and Ennis, 1964; Harold et al., 1967; Harold and Baarda, 1967). There is a certain amount of evidence that the inward and outward systems co-operate, and a \( K^+ - H^+ \) steady state may be achieved of the order of 100,000 (Rothstein, 1972: \( K^+_{\text{in}}/K^+_{\text{out}} \times H^+_{\text{out}}/H^+_{\text{in}} \)). Net movement of \( K^+ \) is normally balanced by an equal and opposite net movement of \( H^+ \) (Rothstein,
1955). The driving force for exchange of $H^+$ and $K^+$ is probably
electrically coupled to the electrogenic ejection of $H^+$, with
$K^+$ diffusing in, facilitated by a carrier (Riemersma and Alsbach,
1974; Peña, 1975).

(ii) **Bivalent.**

There is one system specifically for the transport of bi-
valent cations into yeast (Rothstein et al., 1958). Of the
cations investigated there is an affinity series: $Mg^{2+} > Co^{2+} >
Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+} > Sr^{2+}$ with $K_T$ values ranging from
$10^{-5}$ M to $10^{-3}$ M. An electrical balance is maintained by efflux
of two $K^+$ per bivalent cation absorbed.

In yeast extensively starved of carbon and nitrogen there is
little or no transport depending on the cation. However binding
to the cell surface, principally to carboxyl and phosphoryl
groups, does occur. Binding is readily distinguished from
transport as bound cations are exchangeable with others in
the medium, whereas transported cations are not (Rothstein and
Hayes, 1956).

Transport is stimulated by the presence of glucose and
phosphate (Jennings et al., 1958). The effect of phosphate depends
upon the transport of phosphate into the cell, but it is not due
to its presence in the medium, its presence in the cell or to
a simultaneous uptake. Uptake may occur before exposure to
bivalent cations. Somehow, perhaps by formation of an essential
phosphorylated intermediate, in the process of phosphate uptake
an increased capacity to transport bivalent cations is generated. If potassium ions are also added, a further stimulation occurs. This effect is indirect as potassium ions increase phosphate transport, which in turn increases the rate of bivalent cation transport.
THE EFFECT OF LIPIDS UPON MEMBRANE PROTEIN FUNCTION

Within the fluid mosaic model of Singer and Nicholson, (1972) it is the hydrophobic interactions between the lipids and the protein components which maintain the integrity of the membrane (Lenaz et al., 1975; Salton and Owen, 1976; Vanderkooi, 1974; Gitler, 1976). However, the amphipathic nature of individual lipid molecules and the diversity of lipids in biological membranes would suggest a more complex relationship.

There are some reports of the effects of lipids on transport of molecules across the bacterial plasma membrane. Using a variety of methods to bring about changes in the lipid composition of membranes, amino-acid uptake has been shown to be affected by phospholipid composition (Beepe, 1972; Ohta et al., 1977) while the effect of the degree of unsaturation in plasma-membrane phospholipids has been studied using unsaturated fatty acid-requiring auxotrophes of Escherichia coli (Cox et al., 1975).

The effect of plasma-membrane lipid composition on solute accumulation has been less extensively reported in eukaryotic organisms. A phosphatidylcholine-deficient mutant of Neurospora crassa has been shown to have a decreased capacity to accumulate L-lysine, thereby implicating a role for this phospholipid in L-lysine transport (Sherr, 1969). Also in Neurospora crassa, when grown under conditions of inositol deprivation, a decrease in activity of one of the glucose-transporting systems was observed. This would suggest a role for phosphatidylinositol in glucose transport (Scarborough, 1973).
Altering the ergosterol content of *Candida albicans* has been shown to affect the transport of some amino acids and not others (Singh et al., 1979), indicating, perhaps, an interaction between ergosterol and some amino-acid transport systems in this organism.

The physical state of lipids also affects the catalytic activity of membrane-bound enzymes, as demonstrated by thermally induced phase transitions (Fox, 1975; Melchoir and Stein, 1976). The enzyme reaction is more rapid within a liquid crystalline lipid environment than in one of gel-state lipids. This observation is the basis of the idea that the proximity and abundance of gel-state lipids may regulate protein function in biological membranes (Cronan and Gelmann, 1975; van Dijck et al., 1976; Cullis and De Kruijff, 1978; Cullis and De Kruijff, 1979). However, the unsaturated nature of most naturally occurring lipids results in transitions well below the normal physiological temperatures (van Dijck et al., 1976; Cullis and De Kruijff, 1978). This does not dismiss the possibility that individual gel-state lipid molecules could exert their influence within a liquid crystalline membrane. On the other hand, integral proteins have been shown to preferentially partition into fluid regions of the membrane (Grant and McConnal, 1974; Verkleij and Ververgaert, 1975; Kleeman and McConnal, 1976).

Preferential location of proteins within specific lipid environments as distinct from activation by lipids is more difficult to demonstrate. An approach has been to isolate lipid-protein complexes from membranes and then to compare the lipid
composition of the complex with the lipid composition of the membrane, revealing any preferential enrichments. Another approach is to compare membrane phase-transition temperatures of a series of integral proteins (Watson et al., 1973; ), and although this will not identify specific lipid environments, the different transition temperatures for the different proteins can be indicative of a heterogenous distribution of lipids throughout the membrane.

Where membrane-bound enzymes have shown a catalytic dependency upon specific lipids, it has usually been the phospholipid headgroup which has been the major determinant (Tanaka and Sakamoto, 1969; Lenaz, 1973; Fourcans and Jain, 1974). On the other hand preferential location of integral proteins is associated with fluidity, or rather the hydrophobic region of the membrane (Grant and McConnel, 1974; Verkleij and Ververgaert, 1975; Kleeman and McConnel, 1976). These two observations can be united. If phospholipid headgroups are regulatory effectors, then control of the lateral diffusion of proteins into regulatory regions is a function of the hydrophobic region. Cellular control of membrane lipid viscosity by adjusting the ratio of, for example, saturated to unsaturated fatty-acyl residues has been termed homeoviscous adaptation (Sinensky, 1974). Perhaps homeoviscous adaptation is the mechanism by which the detailed composition of lipids at the protein-lipid interface is determined.
MANIPULATION OF THE YEAST PLASMA-MEMBRANE COMPOSITION

In order to study the effect of composition upon the function of the plasma membrane, methods need to be devised which effect specific alterations to one class of molecule. Changes to plasma-membrane composition which have been reported mainly concern the lipid content, and so it is with this component of the plasma membrane that attention has been focused.

Changing cultural conditions, such as growth temperature and oxygen tension, has been shown to alter the lipid composition of Saccharomyces cerevisiae (Hunter and Rose, 1971; 1972; Jollow et al., 1968). Unfortunately the alterations are non-specific, affecting not only phospholipids and sterols, but also changing other aspects of metabolism such as energy generation. These methods are therefore of little direct use, but the data they provided are useful in determining more precise stratagems.

Four approaches can be considered for causing specific changes to plasma-membrane lipid composition in Saccharomyces cerevisiae. Foremost amongst these, and one which was used throughout the work reported in this thesis, is that which exploits the anaerobically induced requirement for a sterol and an unsaturated fatty acid (Andreason and Stier, 1953, 1954). Both of these requirements are fairly non-specific and so a wide variety of sterols (Proudlock et al., 1968) and fatty-acyl residues (Light et al., 1962) may be substituted into the plasma membrane. The effectiveness of this procedure can be seen by
the degree of enrichment obtained in the fatty-acyl residues within the membrane phospholipids. Both oleic and linoleic acid comprise 54% of these residues when supplemented into the growth medium (Thomas et al., 1978), whilst alterations to the other fatty-acyl residues are minimal. When media are supplemented with ergosterol, this sterol accounts for 75% of the free sterols in the membrane (Hossack et al., 1973). Supplementation with other sterols produces similar enrichments, for example cholesterol can account for 80% of free sterols within the plasma membrane.

A second approach involves aerobic growth in the presence of ethanolamine or choline (Ratcliffe et al., 1973) which leads to either a doubling of the phosphatidylethanolamine content or a trebling of the phosphatidylcholine content of the plasma membrane. The natural extension of both of these techniques, namely anaerobic growth in the presence of a sterol, an unsaturated fatty acid and either ethanolamine or choline has not been reported.

Thirdly, lipid-requiring mutants have been isolated, including unsaturated fatty acid-requiring auxotrophes (Resnick and Mortimer, 1966), saturated fatty acid-requiring auxotrophes (Schweitzer and Bolling, 1970; Schweitzer et al., 1971) and sterol requiring auxotrophes (Karst and Lacroute, 1974). Although this is potentially an excellent technique, the mutants tend to be leaky, and therefore unreliable.

Finally, lipid composition can be manipulated by the use of drugs which specifically prevent synthesis of membrane components
and therefore product a nutritional requirement for those components. For example SKF 4401-A will inhibit sterol synthesis. Growth in the presence of 3-chloropropane 1,2 diol, a glycerol analogue, has been shown to decrease the phosphatidylglycerol content of *Saccharomyces cerevisiae* (Bulman and Stretton, 1975). In 1972 Nomura and co-workers (Nomura et al., 1972a,b) demonstrated the inhibition of sterol and fatty acid synthesis by cerulenin, creating a nutritional requirement for these compounds. The chain length of exogenously provided fatty acid could still be increased (Omura, 1976). However these drugs have not been extensively exploited.

A criticism which can be levelled at all four techniques is that, although the plasma membrane can be enriched with a variety of specific lipids, it remains an enrichment and not an absolute replacement. Prudence must therefore be exercised in interpreting results obtained using such methods.
THE PLASMA MEMBRANE AND THE BREWING OF BEER

Many changes occur during the fermentation of wort. Central to these changes is the role played by the brewing yeast, typically either *Saccharomyces cerevisiae* or *Saccharomyces uvarum*. Upon pitching, there is an initial lag phase of about eight hours (Macleod, 1977), although this time can vary. This is followed by a rapid proliferation of the yeast for about four or five generations. During the lag phase of growth, many important physiological processes occur. All available oxygen disappears and the uptake of essential nutrients, for example amino acids, vitamins and various inorganic ions (Jones and Pierce, 1963, 1964; Cuts and Rainbow, 1950; Markham et al., 1966) begins. There is not, however, any appreciable utilization of carbohydrates. Throughout proliferation and up to the end of fermentation, wort sugars are taken up in an ordered sequence and converted to ethanol. At the same time a range of flavour compounds appears in the fermenting wort as a direct result of yeast metabolism.

Initial absorption of oxygen is probably associated with synthesis of sterols and unsaturated fatty acids (David and Kirsop, 1973), which are required in the form of, respectively, free sterol and phospholipid as membrane components, although sterol esters and triglycerides are also formed. Both sterols and unsaturated fatty acids have been shown to be obligate nutritional requirements for growth of *Saccharomyces cerevisiae* anaerobically (Andreasen and Stier, 1953, 1954). This requirement is fairly broad and so media supplemented with a wide variety of sterols and unsaturated fatty acids produce yeast cells with
plasma membranes enriched with the exogenously provided compounds. Wort itself has been shown to contain lipids, derived almost entirely from cereal grist (Forch and Runkel, 1974). Sterols, both free and esterified, account for 10% of the total lipid. In decreasing order of concentration the main unsaturated fatty acids present are linoleic, oleic and α or γ linolenic acids. Throughout the self imposed anaerobic growth of yeast during a fermentation, after appropriate metabolism, these exogenous lipids will appear as plasma-membrane components, producing a change in plasma membrane composition from the start to the end of fermentation.

Are these compositional changes throughout fermentation interfering with specific functions of the plasma membrane? In particular is the transport of materials into the yeast cell affected? Data obtained from a study of the effects on transport of specifically incorporated lipids into the plasma membrane of Saccharomyces cerevisiae will begin to illuminate the answer to this question.
MATERIALS AND METHODS
MATERIALS AND METHODS

Experimental Cultures

All experiments were conducted with *Saccharomyces cerevisiae* NCYC 366. The strain was maintained on slopes of MYGP medium (Wickerham, 1951) and was subcultured monthly. The medium contained per litre of water:

- Malt extract (lab m) 3 g
- Yeast extract (lab m) 3 g
- Glucose 10 g
- Mycological peptone (Oxoid) 5 g
- Agar 2 g

The medium was sterilized by autoclaving at 120°C for 15 minutes.

Growth of Cultures

Anaerobic cultures were grown by the method described by Alterthum and Rose (1973). The medium (pH 4.5) contained per litre of water:

- Glucose 100 g
- \((\text{NH}_4)_2\text{SO}_4\) 3 g
- \(\text{KH}_2\text{PO}_4\) 3 g
- Yeast extract (lab m) 1 g
- \(\text{MgSO}_4 \cdot 7\text{H}_2\text{O}\) 25 mg
- \(\text{CaCl}_2 \cdot 2\text{H}_2\text{O}\) 25 mg

and was supplemented with ergosterol (5 mg l\(^{-1}\)) and an unsaturated fatty acid (30 mg l\(^{-1}\)) as indicated in the text. The supplements were added as chloroform solutions prior to sterilizing and were stored under nitrogen gas at -20°C. One-litre portions of the media in two-litre round flat-bottomed flasks were sterilized by autoclaving.
at 115°C for one minute. Anaerobiosis was initiated and maintained by sparging with high-purity nitrogen, from which the last traces of oxygen had been removed by a Jencons Nilox oxygen scrubbing device through the flask whilst still hot, and throughout growth of the culture. The flasks were inoculated with 1 mg dry weight equivalent from a 24h liquid culture, and incubated with stirring at 30°C. Growth was followed by measuring extinction at 600 nm and measurements related to a dry weight curve. Organisms were harvested from late exponential-phase cultures (0.24 - 0.26 mg dry wt ml⁻¹) by filtration through a membrane filter (0.45 μm pure size; 50 mm diameter, Oxoid).

Control cultures lacking ergosterol were incubated with each batch of experimental cultures and, when growth in the control exceeded 0.10 mg dry wt ml⁻¹, experimental cultures were discarded. When proline-grown cells are referred to both the medium and conditions were identical to those already described except that ammonium sulphate was replaced by proline (3 g l⁻¹).

**Measurement of Rate of Solute Accumulation**

Organisms which were to be used to measure the rate of solute accumulation were washed with piperazine-N,N'-bis 2-ethanesulphonic acid (PIPES) (20 mM) buffer adjusted to pH 4.5 (pH 5.5 when uptake of Ca²⁺ was to be studied), suspended at 15 mg dry wt ml⁻¹ in the same buffer in a screw-capped centrifuge tube (250 ml), and the tube stored in an ice-water mixture after the headspace had been flushed with oxygen-free nitrogen gas. The suspension (15 ml)
used to measure the rate of solute accumulation consisted of PIPES buffer (pH 4.5 or 5.5) containing glucose (100 mM) except when the rate of uptake of D-glucose was to be measured, 0.5 mg dry wt organisms ml$^{-1}$ and solute in the range $10^{-6} - 10^{-1}$ M, including both radioactive and non-radioactive compounds. The concentration of radioactive compound ($\mu$Ci $\mu$mol$^{-1}$) in the solute solutions used were:

$L-[^{14}C]$ arginine monohydrochloride 0.05, $L-[^{14}C]$ asparagine 3.3, $^{45}$CaCl$_2$ 12.5, $D-[^{3}H]$ glucose 0.75 - 750, $L-[^{14}C]$ glutamine 3.3, $L-[^{14}C]$ lysine monohydrochloride 0.013 - 1.67, $K_2H_2^{32}PO_4$ 2.0, $Na_2^{35}$SO$_4$ 0.03 - 30 and $Na^{36}$Cl 0.001 - 100. Where ranges are indicated the tracer-carrier ratio was varied because of wide differences in the rate of accumulation at different solute concentrations.

The suspension was contained in a round bottomed Quickfit flask (100 ml capacity) (Fig. 1) fitted with a glass-stoppered sampling port. When preparing the suspension, the flask was maintained in a water bath at 30°C except when otherwise stated. Its contents were stirred magnetically, and the inside of the flask continuously flushed with oxygen-free nitrogen gas. The experiment was started by adding solute (tracer and carrier) to the flask, after which portions (1 ml) were removed at predetermined times, rapidly filtered through membrane filters (0.45 $\mu$m pore size; 25 mm diam; Millipore), and the filter and cells immediately washed with ice-cold PIPES buffer (pH 4.5 or 5.5) containing the experimental solute at the concentration included in the cell suspension. Filters were then transferred to scintillation vials containing Unisolve liquid scintillator No. 1 (Koch-Light, Colnbrook, Bucks, England; 7 ml). The radioactivity of the contents of vials was measured in a Packard Tricarb liquid scintillation spectrometer (model 3385), and the value for counts corrected for counting
Figure 1. Apparatus used to determine the rate of solute accumulation by *Saccharomyces cerevisiae* NCYC 366.
efficiency. Rates of accumulation were determined from plots of the time-course of accumulation for up to 3 min 15 s, although with some solutes, linearity was maintained with low concentrations for up to 30 min.

**Chromatography**

The fate of accumulated arginine was followed by monitoring the location of label in extracts of organisms which had accumulated L-[U-14C]arginine. Organisms were removed from suspensions as already described, and filters with organisms immersed in 10 ml water at 100°C for 10 min. The suspension was then filtered through a membrane filter (0.45 μm pore size; 25 mm diam; Millipore) and the radioactivity of the filtrate measured. The debris was then washed with arginine (200 μm), the filter with washed debris transferred to a scintillation vial and the radioactivity of the contents measured. Ninhydrin-positive compounds in the filtrate were separated by paper chromatography using the solvent systems: ethanol/water/urea (80:20:0.5, v/v/w), butanol/pyridine/water (315:175:240, by vol.) and phenol/water/25% (v/v) 25% aqueous ammonia (sp. gr. 0.88) (80:20:0.3, w/v/v). Chromatograms were dried, sprayed with ninhydrin (0.1%, w/v, in n-butanol saturated with water) and the location of spots marked. They were then exposed to photographic plates (Kodak Industrex C) for four weeks.

**Cold Osmotic Shock**

The cold osmotic-shock treatment to which *Saccharomyces cerevisiae*NCYC 366 was subjected was that of Patching and Rose...
(1971). Cells from a batch culture (240 – 260 mg dry wt l⁻¹) were harvested by centrifugation and suspended to a concentration of 1 mg ml⁻¹ in 10 mM KH₂PO₄, pH 4.5 at 30°C. Immediately after suspension the cells were pelleted by centrifugation and resuspended to 1 mg ml⁻¹ in 0.8 M mannitol with 10 mM EDTA, also at 30°C. As soon as the cells were suspended, they were pelleted by centrifuging and then resuspended to the same concentration in 0.5 mM MgCl₂ at 0°C, and again pelleted by centrifugation. The shocked cells were then examined as indicated in the Results section.

Assessment of Changes in Cell Size

The volume distribution of populations of cells was determined using an electronic particle counter (Electrozone-Celloscope, model 111 LTS, Particle Data Inc., Elmhurst, Illinois, U.S.A). The counter was fitted with a 60 μm-diameter orifice. Signals from the counter were assessed in a multichannel pulse-height analyser (Nuclear Data Inc., Palatine, Illinois, U.S.A, series 1100) and the volume distribution in the suspension of cells plotted on an X-Y plotter (Hewlett-Packard Inc., Pasadena, California, U.S.A., model 7035B). The system was calibrated with standard latex spheres (2.03 and 9.79 μm diameter, Coulter Electronics, Luton, England). Suspensions of cells in 0.025 M KCl were diluted to about 10⁴ particles ml⁻¹ to avoid coincidence counting, and the suspension was drawn through the counter orifice until the analyser had assembled sufficient data to present a reasonable analysis (between 2.5 x 10⁴ and 5.5 x 10⁴ particles).
Retention of Solutes

Preloading of organisms with the experimental solute was achieved by following accumulation of radioactivity as already described. One difference was that the volume of the cell suspension was between 30 and 50 ml. At a specified time a known volume (10 - 15 ml) of cell suspension was removed and rapidly filtered (0.45μm pore size; 25 mm diameter; Oxoid) then immediately washed with PIPES buffer (1 x 10 ml; 30°C; pH 4.5 or 5.5) containing the experimental solute at the concentration included in the cell suspension. Membrane filters with cells were transferred to flasks (100 ml capacity) containing PIPES buffer (10 -15 ml; 30°C; pH 4.5 or 5.5) and, where specified, glucose (100 mM) and/or non-radioactive experimental solute. The contents of the flasks were stirred magnetically and were continuously flushed with oxygen-free nitrogen gas. Under these conditions cells became detached from the membrane filter within 15 to 20 seconds. The retention of radioactive solute by cells was then determined by removing samples (1 ml) at predetermined time intervals, which were filtered, washed and counted for radioactivity as already described.
**Proton Movements**

Changes in pH value of a suspension of washed cells during amino-acid absorption were detected with a combination glass electrode (type 401E7, Pye Unicam Ltd., Cambridge, England) containing 0.1M KCl solution. The electrode was coupled to a pH meter (PW 9409 digital pH meter, Philips, England), and recorder (Vitatron 2001, MSE Scientific Instruments, Crawley, West Sussex). Typically the cell suspension (30 ml) consisted of distilled water, yeast cells (between 5 and 10 mg ml⁻¹) and 2 deoxy-D-glucose (25 mM) with any alterations made as indicated in the Results section. It was held in a round-bottomed Quickfit flask (100 ml capacity) fitted with a glass-stoppered sampling port. When preparing the suspension the flask was maintained in a water bath at 30°C. The contents were stirred magnetically and the inside of the flask was continuously flushed with oxygen-free nitrogen gas. Changes in pH value were followed for about two minutes and the experiment started by adding radioactively labelled amino acid.

Amino acid uptake rates were determined as previously described. A full-scale deflexion on the recorder chart corresponded to 0.8 pH units. The system was calibrated by addition of exactly about 0.4 μ equiv. of HCl per ml of unbuffered cell suspension. Over a given range of 0.3 pH units the acid buffering capacity of the suspension remained constant between pH 4 and pH 5.

**Lipid Analysis**

Organisms to be used for lipid analysis were washed twice with water on the membrane filter, and lipid extracted by a
modification of the Folch et al. (1957) and Watson and Rose (1980) procedures. Freshly washed organisms (1 g dry wt) were mixed with methanol (20 ml) and the suspension was shaken in a Braun homogeniser (B. Braun, Melsungen, West Germany) for three period of 30 s at speed 2 (4000 rev. min⁻¹) after addition of 30 g glass beads (Glasperlen, B. Braun; 0.45 - 0.50 mm diam.). Chloroform was then added to the suspension to give the ratio 2:1 (v/v) chloroform/methanol, and the suspension was stirred on a flatbed stirrer for 2 h at room temperature (20 to 24°C). The suspension was then filtered through Whatman No. 44 filter paper, and the extraction procedure was repeated on the residue. The combined extracts were washed with 0.25 vol. 0.88% (w/v) KCl, and the mixture left to separate overnight at 4°C. The lower phase was removed, taken to dryness on a rotary evaporator, and the residue was immediately dissolved in chloroform/methanol (2:1, v/v). Samples were stored under nitrogen gas at -20°C.

Phospholipids were separated from other lipid classes in extracts by thin-layer chromatography on plates of silica gel H (0.5 mm thick) using a solvent system of hexane/diethyl ether/acetic acid (70:30:2, by vol.). Lipid bands were located by exposing plates to iodine vapour, and were marked with a pin. The phospholipid band was identified by reference to simultaneously run standards of phosphatidylcholine of phosphatidylethanolamine. Individual phospholipids were separated from extracts by thin-layer chromatography on plates of silica gel H (0.5 mm thick) using a solvent system of chloroform/methanol/acetic acid/water (25:15:4:2, by vol; Skipski et al., 1964). Using this procedure, phosphatidylinositol and phosphatidylserine were not completely separated, and fatty-acyl compositions were determined for the
combined fraction. *Saccharomyces cerevisiae*NCYC 366 resembles other strains of this species in containing appreciably more phosphatidylinositol than phosphatidylserine (Ratcliffe et al., 1973). Phospholipid spots were located by exposing plates to iodine vapour. The iodine was allowed to sublime at room temperature, and spots of lipid classes or individual phospholipids were scraped off plates, and fatty-acid methyl esters were prepared by refluxing the silica gel with BF₃ (14% w/v in methanol) for 10 min. After cooling, an equal volume of water was added, and the methyl esters were extracted into chloroform. The same procedure was used to prepare fatty-acid methyl esters from neutral lipid fractions containing sterol esters and di- and triacylglycerols. Fatty-acid methyl esters were analysed by gas-liquid chromatography on a column (2 m) of 15% EGSS-Y supported on 100 to 120 mesh Gas-Chrom P at 200°C. The injection port was at 210°C, and the carrier gas (N₂) flow rate was 60 ml min⁻¹. Percentage fatty-acid compositions were calculated by multiplying the retention time by the peak height on the trace.

**Chemicals**

All chemicals used were of Analar grade or of the highest grade available commercially. Ergosterol, myristic acid (cis-9-tetradecenoic acid), palmitoleic acid (cis-9-hexadecenoic acid), oleic acid (cis-9-octadecenoic acid), linoleic acid (cis-9-cis-12-octadecadienoic acid), cetoleic acid (cis-11-eicosaenoic acid), PIPES (piperazine-N,N'-bis 2-ethanesulphonic acid), CCCP (carbonyl cyanide m-chlorophenyl hydrozone), iodoacetamide, 2-deoxy-D-glucose, were purchased from Sigma Chemical Co., London,
England. DNP (2,4-dinitrophenol) was from B.D.H. Laboratory
Chemicals Division, Poole, England. Radioactively labelled compounds came from the Radiochemical Centre Ltd., Amersham, Bucks, England.
RESULTS
1. INITIAL STUDIES ON THE TRANSPORT OF L-ARGININE

1.1 Effect of Storage Time on Solute-Accumulating Ability of Organisms

Over a period of up to five hours, the viability of a cell suspension maintained in an ice-water mixture, as judged by plate counts using the medium of Wickerham (1952), did not change (Table 2). This was true for organisms enriched in either oleyl or linoleyl residues. However, the ability of organisms enriched in oleyl residues to accumulate L-arginine declined slowly over the five-hour period whereas, after about one hour, the arginine-accumulating ability of organisms enriched in linoleyl residues declined rapidly (Fig. 2). Comparable observations on the ability of organisms to accumulate other solutes were not made, although it was established that the ability of organisms enriched in either oleyl or linoleyl residues to accumulate any of the other solutes investigated hardly declined over the first hour of storage. All determinations were therefore made on organisms that had been stored in ice-water for not more than one hour, and usually considerably less.

1.2 Effect of Composition of Suspending Buffer on Arginine-Accumulating Ability of Organisms

For this investigation the pH value of all buffers was 4.5 and experiments were performed in the presence of 100 mM glucose. Of the buffers tested, namely 10 mM tris-(hydroxymethyl)-aminomethane/citrate, 10 mM tris-(hydroxymethyl)-aminomethane/HCl, 20 mM tris-(hydroxymethyl)-aminomethane/HCl, 20 mM morpholinopropane-sulphonic acid/HCl, 20 mM N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid/HCl, 20 mM piperezine—
Table 2. Viability, judged by plate counts of populations of *Saccharomyces cerevisiae* NCYC 366 stored at pH 4.5 in buffer and maintained under nitrogen within an ice-water mixture. Results are expressed as a percentage of the counts obtained at time 0 and are the mean values of three determinations.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Oleyl</th>
<th>Linoleyl</th>
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<td>0</td>
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<td>100</td>
</tr>
<tr>
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<tr>
<td>5</td>
<td>95</td>
<td>97</td>
</tr>
</tbody>
</table>
Figure 2. Change in the arginine-accumulating ability of populations of *Saccharomyces cerevisiae*NCYC 366 enriched in oleyl (●) or linoleyl (O) residues, when stored under nitrogen in an ice-water mixture. Values are presented as a percentage of the rate obtained at 0h.
N,N'-bis 2-ethansulphonic acid /HCl, 67 mM piperazine-N,N'-bis 2-ethansulphonic acid /HCl, 67 mM KH₂PO₄/K_HPO₄ only one had any effect upon the arginine-accumulating ability of organisms from a 100 µM solution of arginine and that was 67 mM KH₂PO₄/K_HPO₄. Further examination of this inhibition indicated that it may have been caused by potassium ions. These data are described in Section 5 of the Results section. Early experiments were carried out using 10 mM tris-(hydroxymethyl)-aminomethane/HCl buffer although subsequently the majority of experiments were done using 20 mM piperazine-N,N'-bis 2-ethansulphonic acid /HCl.

1.3 Effect of Temperature, Composition of Washing Solution and Number of Washings on the Arginine-Accumulating Ability of Organisms

Neither the number of washings (1 x 10 ml., 2 x 10 ml, 3 x 10 ml) nor the temperature of washing solution (4°C, 30°C) had any effect upon retention of radioactive solute by populations of *Saccharomyces cerevisiae* NCYC 366 accumulating L-arginine. This was found to be true whether cells were enriched with oleyl or linoleyl residues. However, washing solutions made up of buffer and arginine produced different results from washing solutions from which arginine was excluded (Fig. 3). The uptake rate when arginine was present in the washing solution was greater than when it was absent. There was a constant proportional difference between radioactivity retained by cells washed with arginine-containing solutions and cells washed with arginine-free solutions.
Figure 3. Effect of including L-arginine (0.2 mM) (●) or excluding arginine (○) from the washing solution (1 wash, 4°C) on Saccharomyces cerevisiae NCYC 366 accumulating L-arginine (0.2 mM).
1.4 Fate of L-Arginine Accumulated by Organisms

Hot-water extracts of organisms sampled from a buffered cell suspension accumulating L-arginine for up to 30 minutes revealed only one radioactive spot which had an identical RF value with that of L-arginine in all three solvent systems. Extracts from organisms incubated for 45 and 90 minutes revealed a second radioactive compound which was not identified (Fig. 4). However this compound did not correspond to ornithine, which is a catabolic product of arginine.

Radioactivity was detectable in the residue left after hot-water extraction from organisms incubated for 5 minutes although this represented less than 3% of the total accumulated. On further incubation the proportion of radioactivity in the residue increased until at 90 minutes this accounted for about 15% of the total accumulated (Fig. 5). Throughout the 90 minute incubation with glucose and arginine, there was no detectable increase in extinction at 600 nm, nor in total cell number as judged by haemocytometer slide counts.
Figure 4. Autoradiograph of hot-water extracts made from Saccharomyces cerevisiae NCYC 366 which had been accumulating L-[U-14C]-arginine for the following lengths of time: a, 10 minutes; b, 90 minutes; c, 30 minutes; e, 45 minutes; d, arginine alone as a reference standard.
Figure 5. Time course of appearance of radioactivity in hot-water extracts (▲) and the residue remaining after hot water extraction (■) of *Saccharomyces cerevisiae* NCYC 366 enriched with oleyl residues. (●) indicates the total radioactivity accumulated. Each point represents the average of two determinations.
2. PLASMA-MEMBRANE LIPID UNSATURATION AND TRANSPORT KINETICS IN SACCHAROMYCES CEREVISIAE

2.1 Effect of Lipid Unsaturation on the Kinetics of Amino-Acid Accumulation

2.1(i) Asparagine

L-Asparagine transport by systems other than the general amino-acid permease has not been previously reported for Saccharomyces cerevisiae. Within this study, asparagine transport was examined in populations of Saccharomyces cerevisiae NCYC 366 grown with ammonium ions as a nitrogen source. Enrichment of the plasma membranes of these organisms with either oleyl or linoleyl residues did not alter the kinetics of asparagine accumulation. Over the concentration range used, namely $10^{-6}$ M to $10^{-2}$ M, only one transport system was detected as determined by a direct linear plot, and by Woolf-Hofstee (Figs. 6 and 7) and Lineweaver-Burk plots. Woolf-Hofstee plots are used in preference to that of Lineweaver-Burk because an even distribution of points are obtained and weight is not given to accumulation velocities at low solute concentrations.

The $K_T$ and $V_{max}$ values for accumulation of asparagine were:

$K_T = 3.8 \times 10^{-4}$ M and $V_{max} = 14$ nmol (mg dry wt)$^{-1}$ min$^{-1}$.

2.1(ii) Glutamine

As described for asparagine, the literature contains no reports of L-glutamine transport by any system other than the general amino-acid permease into Saccharomyces cerevisiae. Between external glutamine concentrations of $10^{-5}$ M and $10^{-3}$ M the kinetics of glutamine accumulation by cells enriched with oleyl or with linoleyl residues were indistinguishable. One transport system was determined with a $K_T$ value of $3.3 \times 10^{-4}$ M and a
Figure 6. Woolf-Hofstee plot for asparagine accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Only one transport system can be distinguished. Points represent the mean value of at least three independent determinations.
Figure 7. Woolf-Hofsteet plot for asparagine accumulation by Saccharomyces cerevisiae NCYC 366 enriched in linoleyl residues. Only one transport system can be distinguished. Points represent the mean value of at least three independent determinations.
Figure 8. Woolf-Hofstee plot for glutamine accumulation by *Saccharomyces cerevisiae* WCYC 366 enriched in oleyl residues. Only one transport system can be distinguished, but there is considerable deviation from linearity at low glutamine concentrations. Points represent the average values of at least two independent determinations.
Figure 9. Woolf-Hofstee plot for glutamine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in linoleyl residues. Only one transport system can be distinguished, but there is considerable deviation from linearity at low glutamine concentrations. Points represent the average of at least two independent determinations.
value of 15 nmol (mg dry wt)$^{-1}$ min$^{-1}$. The deviation from linearity seen on Woolf-Hofstee plots at low glutamine concentrations (Figs. 8 and 9) has been observed for uptake of rubidium ions (Borst-Pauwels et al., 1971) and described as due to cooperative effects. This is considered in more detail within the Discussion section.

2.1(iii) Arginine

Woolf-Hofstee and Lineweaver-Burk plots for L-arginine accumulation in *Saccharomyces cerevisiae*NCYC 366 yielded lines of two linear segments irrespective of the fatty-acyl enrichment (Figs. 10 and 11). The $K_T$ and $V_{\text{max}}$ values calculated from these plots however, are very different for cells with plasma membranes enriched with oleyl residues than for cells with plasma membranes enriched with linoleyl residues.

The constants obtained by extrapolation of lines on Woolf-Hofstee and Lineweaver-Burk plots do not represent single uptake mechanisms, but necessarily represent some overlap. These values for oleyl enriched cells are $K_T$ $2 \times 10^{-5}$ M and $2 \times 10^{-2}$ M with $V_{\text{max}}$ values of 2 and 37 nmol (mg dry wt)$^{-1}$ min$^{-1}$ respectively. Linoleyl enriched cells have corresponding values of $K_T$ $1.3 \times 10^{-3}$ M and $9.5 \times 10^{-2}$ M with $V_{\text{max}}$ values of 5.5 and 220 nmol (mg dry wt)$^{-1}$ min$^{-1}$.

On the other hand, if an assumption is made that the bilinear graphs represent the sum of two Michaelis-Menten uptakes, then the method of Neal (Neal, 1972) can be applied to derive $K_T$ and $V_{\text{max}}$ values for each individual mechanism. Values obtained in this manner are as follows. Oleyl enriched cells $K_T$ $1 \times 10^{-6}$ M and
Figure 10. Woolf-Hofstee plot for arginine accumulation by Saccharomyces cerevisiae NCYC 366 enriched in oleyl residues. Inset: Lineweaver-Burk plot for the same information. Two transport systems can be distinguished. Points represent the mean values of at least three independent determinations.
Figure 11. Woolf-Hofstee plot for arginine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in linoleyl residues. Inset: Lineweaver-Burk plot for the same information. Two transport systems can be distinguished. Points represent the mean values of at least three independent determinations.
2.1(iv) Lysine

Graphic analysis of L-lysine accumulation by Saccharomyces cerevisiae NCYC 366 (Figs. 12 and 13) yielded two transport systems, irrespective of the fatty-acyl enrichment. As seen with arginine however, kinetic constants obtained with oleyl enriched cells from these plots were different to those obtained with linoleyl enriched cells. For oleyl enriched cells linear extrapolation produced $K_T$ values of $1.10^{-5}$ M and $1.10^{-4}$ M with $V_{max}$ values of 4.2 and 6.1 nmol (mg dry wt)$^{-1}$ min$^{-1}$. Linoleyl enriched cells have $K_T$ values of $2.10^{-6}$ M and $1.10^{-4}$ M with corresponding $V_{max}$ values of 0.5 and 3.5 nmol (mg dry wt)$^{-1}$ min$^{-1}$. Utilising the method of Neal these values for single uptake mechanisms become as follows. $K_T$ values for oleyl enriched cells are $6.5.10^{-6}$ M and $2.8.10^{-4}$ M with $V_{max}$ values of 4.2 and 1.9 nmol (mg dry wt)$^{-1}$ min$^{-1}$ respectively and $K_T$ values for linoleyl enriched cells are $2.5.10^{-7}$ M and $1.1.10^{-4}$ M with corresponding $V_{max}$ values of 0.5 and 3.1 nmol (mg dry wt)$^{-1}$ min$^{-1}$.

2.2 Effect of Lipid Unsaturation on the Kinetics of Glucose Accumulation.

One D-glucose-transporting system was found for organisms with plasma membranes enriched with oleyl or linoleyl residues (Figs 14 and 15). However, the $K_T$ and $V_{max}$ values for these two cell types differed slightly. Oleyl enriched cells had a $K_T$ value of $5.0.10^{-3}$ M and $V_{max}$ value of 222 nmol (mg dry wt)$^{-1}$ min$^{-1}$.  

2.10$^{-2}$ M with $V_{max}$ values of 2 and 35 nmol (mg dry wt)$^{-1}$ min$^{-1}$, and linoleyl enriched cells with $K_T$ values of $1.5.10^{-5}$ M and $9.6.10^{-2}$ M, with $V_{max}$ values of 4.5 and 218 nmol (mg dry wt)$^{-1}$ min$^{-1}$.
Figure 12. Woolf-Hofstee plot for lysine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in oleyl residues. Inset: Lineweaver-Burk plot for the same information. Two transport systems can be distinguished. Points represent the mean values of at least three independent determinations.
Figure 13. Woolf-Hofstee plot for lysine accumulation by
Saccharomyces cerevisiae NCYC 366 enriched in linoleyl residues. Inset: Lineweaver-Burk plot for the same information. Two transport systems can be distinguished. Points represent the mean values of at least three independent determinations.
Figure 14. Woolf-Hofstee plot for glucose accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Only one transport system can be distinguished. Points represent the mean values of at least two independent determinations.
Figure 15. Woolf-Hofstee plot for glucose accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in linoleyl residues. Only one transport system can be distinguished. Points represent the mean values of at least two independent determinations.
Linoleyl enriched cells had a $K_T$ value of $5.5 \times 10^{-3} \text{M}$ and $V_{\text{max}}$ value of $268 \text{ nmol (mg dry wt)}^{-1} \text{ min}^{-1}$.

2.3 Effect of Lipid Unsaturation on the Kinetics of Inorganic Ion Accumulation

2.3(i) Phosphate

Calculations of $K_T$ and $V_{\text{max}}$ values for phosphate uptake by populations of *Saccharomyces cerevisiae* both by the direct linear plot and by the method of Lineweaver-Burk produced identical results whether plasma membranes were enriched with oleyl or linoleyl residues. The $K_T$ value was $3.0 \times 10^{-4} \text{M}$ and the $V_{\text{max}}$ value was $1.65 \text{ nmol (mg dry wt)}^{-1} \text{ min}^{-1}$. The method of Woolf-Hofstee however, (Figs. 16 and 17) showed disimilarities, namely although $K_T$ and $V_{\text{max}}$ values for cells enriched with oleyl residues were identical to those obtained by the other two methods, those values for linoleyl enriched cells were; $K_T 4.3 \times 10^{-4} \text{M}$ and $V_{\text{max}} 1.8 \text{ nmol (mg dry wt)}^{-1} \text{ min}^{-1}$.

2.3(ii) Sulphate

For this comparative study six sulphate concentrations were used, namely $2.9 \times 10^{-6} \text{M}$, $2.9 \times 10^{-5} \text{M}$, $2.9 \times 10^{-4} \text{M}$, $1.0 \times 10^{-3} \text{M}$, $1.0 \times 10^{-2} \text{M}$, $1.0 \times 10^{-1} \text{M}$. At concentrations of $2.9 \times 10^{-4} \text{M}$ and below, sulphate accumulation by populations of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues could not be distinguished from that of sulphate accumulation by cells with plasma membranes enriched in linoleyl residues. At concentrations greater than $2.9 \times 10^{-4} \text{M}$ retention of labelled sulphate by cells enriched with either residue was variable and was not useful for determining rates of accumulation. Both single (McCready
Figure 16. Woolf-Hofstee plot for dihydrogen phosphate accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Only one transport system can be distinguished. Points represent the mean values of at least two independent determinations.
Figure 17. Woolf-Hofstee plot for dihydrogen phosphate accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in linoleyl residues. Only one transport system can be distinguished. Points represent the mean values of at least two independent determinations.
Figure 18. Woolf-Hofstee plot for sulphate accumulation by *Saccharomyces cerevisiae* NCYC 366. Data obtained for both oleyl and linoleyl enriched cells are pooled owing to the wide scatter of points which did not enable any distinction to be made between them. Points represent the mean values of at least six independent determinations.
and Din, 1973) and dual (Breton and Surdin-Kerjan, 1977) sulphate transport systems have been reported in Saccharomyces cerevisiae. A graphical analysis of the present results is inconclusive in this respect (Fig. 18). Calculation of $K_T$ and $V_{\text{max}}$ values by the direct linear plot gives a single value for $K_T$ of $6.2 \times 10^{-5}$ M and $V_{\text{max}}$ 63 pmol (mg dry wt)$^{-1}$ min$^{-1}$.

2.3(iii) Calcium

No differences were found between cells of Saccharomyces cerevisiaeNCYC 366 enriched with either oleyl or linoleyl residues for calcium accumulation. One transport system was determined by the direct linear plot with a $K_T$ value of $1.4 \times 10^{-4}$ M and $V_{\text{max}}$ value of 13.7 pmol (mg dry wt)$^{-1}$ min$^{-1}$. However, these calculations were based upon observed initial rates of calcium retention which were composite functions of transport and binding. Evidence for this is presented within section 6 of the Results section. Results were not expressed on Woolf-Hofstee or Lineweaver-Burk plots because the variations of the results was large. Kinetic constants are therefore of only limited value.
<table>
<thead>
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<tr>
<td>Asparagine</td>
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</tr>
<tr>
<td>Glutamine</td>
<td>Arginine (low affinity)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Lysine (high affinity)</td>
</tr>
<tr>
<td>Sulphate</td>
<td>Lysine (low affinity)\textsuperscript{b}</td>
</tr>
<tr>
<td>Calcium</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

Table 3. Response of transport systems to a replacement of oleyl residues in the plasma membrane by linoleyl residues. \textsuperscript{a} and \textsuperscript{b}. These two systems are synonymous in \textit{Saccharomyces cerevisiae} (Grenson, 1966).
3. LIPID UNSATURATION AND THE EFFECT OF TEMPERATURE ON SOLUTE TRANSPORT.

The effect of fatty-acyl unsaturation on amino-acid accumulation was investigated by examining the effect of temperature on lysine accumulation by the high affinity system, and on asparagine accumulation. Arrhenius plots of these data are shown in Figs. 19, 20, 21, 22 and values derived from these plots are tabulated in Table 4. On Arrhenius plots, organisms with plasma membranes enriched in oleyl residues clearly show lines of two linear segments (Figs. 19 and 21), whilst organisms with plasma membranes enriched in linoleyl residues have a more poorly defined profile. Even so, it can be seen (Table 4), first that within identical plasma membranes, the transition temperatures for both the asparagine and lysine transport systems are different and that for the asparagine transport system at least, within compositionally distinct plasma membranes, the transition temperatures are again different.
Figure 19. Arrhenius plot for lysine (0.01 mM) accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Points represent the average of two determinations.
Figure 20. Arrhenius plot for lysine (0.01 mM) accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in linoleyl residues. Points represent the average of two determinations.
Figure 21. Arrhenius plot for asparagine (0.01 mM) accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in oleyl residues. Points represent the average of two determinations.
Figure 22. Arrhenius plot for asparagine (0.01 mM) accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in palmitoleyl-residues. Values plotted represent the average of two determinations.
<table>
<thead>
<tr>
<th>Amino-acid transport system</th>
<th>Fatty-acyl supplement</th>
<th>Transition temperature °C</th>
<th>Arrhenius activation energy kJ mol⁻¹</th>
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<tr>
<td></td>
<td></td>
<td>Above transition temperature</td>
<td>Below transition temperature</td>
</tr>
<tr>
<td>Lysine</td>
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</tr>
<tr>
<td>Lysine</td>
<td>Linoleyl</td>
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<td>-66</td>
</tr>
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<td>23.4</td>
<td>-30</td>
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</table>

Table 4. Values derived from Arrhenius plots (Figs 19, 20, 21, 22) of lysine and asparagine accumulation (0.01 mM) by *Saccharomyces cerevisiae* with plasma membranes enriched in oleyl or linoleyl residues.
4. PLASMA-MEMBRANE FATTY-ACYL CHAIN LENGTH AND TRANSPORT KINETICS IN SACCHAROMYCES CEREVISIAE

4.1 Effect of Palmitoleyl Residues on Accumulation of L-Asparagine and L-Lysine.

Four amino acid concentrations were used to determine $K_T$ and $V_{\text{max}}$ values for L-asparagine and L-lysine accumulation by *Saccharomyces cerevisiae*NCYC 366 with plasma membranes enriched in palmitoleyl residues. They were; 5, 10, 50 and 100 $\mu$M for asparagine and; 1, 5, 10 and 50 $\mu$M for lysine. Graphic analysis of accumulation rates at these concentrations are shown in Figures 23 and 24. For asparagine accumulation the $K_T$ and $V_{\text{max}}$ values were $6.2 \times 10^{-5}$ M and 3.3 nmol (mg dry wt)$^{-1}$ min$^{-1}$ respectively and for lysine accumulation they were $1.8 \times 10^{-5}$ M and 3.0 nmol (mg dry wt)$^{-1}$ min$^{-1}$.

4.2 Effect of Cetoleyl Residues on Accumulation of L-Asparagine and L-Lysine.

Accumulation velocities obtained for both asparagine and lysine accumulation were extremely variable. Consequently, Woolf-Hofstee plots of the information could not be interpreted. However, use of the Direct Linear Plot (Eisenthal and Cornish-Bowden, 1974; Cornish-Bowden and Eisenthal, 1974) enabled $K_T$ and $V_{\text{max}}$ values to be calculated. Figures 25 and 26 show the scatter of intersections obtained by use of the Direct Linear plot. For asparagine accumulation the $K_T$ and $V_{\text{max}}$ values were $3.5 \times 10^{-4}$ M and 2 nmol (mg dry wt)$^{-1}$ min$^{-1}$ respectively, and for lysine accumulation they were $8.1 \times 10^{-6}$ M and 2.7 nmol (mg dry wt)$^{-1}$ min$^{-1}$.
Figure 23. Woolf-Hofstee plot for asparagine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in palmitoleyl residues. Values plotted represent the average of two determinations.
Figure 24. Woolf-Hofstee plot for lysine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in palmitoleyl residues. Values plotted represent the average of two determinations.
Figure 25. Direct-linear plot for asparagine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in cetoleyl residues. Values plotted represent individual intersections of lines obtained from accumulation rates which were the average of two determinations.
Figure 26. Direct-Linear plot for lysine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in cetoleyl residues. Values plotted represent individual intersections of lines obtained from accumulation rates which were the average of two determinations.
5. PROPERTIES OF L-ARGININE ACCUMULATION WHICH ARE NOT DEPENDENT UPON PLASMA-MEMBRANE FATTY-ACYL UNSATURATION IN SACCHAROMYCES CEREVISIAENCYC 366

5.1 Effect of Cold Osmotic Shock on Arginine-Accumulating Ability of Organisms

The pattern of arginine uptake by populations of *Saccharomyces cerevisiae*NCYC 366 enriched in oleyl residues was altered when cells were subjected to cold osmotic shock (Fig. 27). Although arginine was retained by shocked cells, the amount retained did not increase after one minute. The amount of arginine retained by populations of shocked cells was proportional to the external arginine concentration between 0.3 and 3.3 mM arginine. Results identical to these were obtained with linoleyl-enriched cells.

5.2 Effect of Ammonium Ions and Proline on the Arginine-Accumulating Ability of Organisms

Growth of *Saccharomyces cerevisiae*NCYC 366 in a medium in which ammonium ions had been replaced by proline resulted in an increase in the initial rate of L-arginine accumulation at all concentrations of arginine examined (0.03 - 0.8 mM) (Fig. 28). Accumulation of arginine (0.2 mM) by proline-grown cells was more sensitive to the presence of ammonium ions in the uptake suspension than was accumulation by cells which had been grown with ammonium ions as their nitrogen source (Fig. 29). These observations were true whether the fatty-acyl enrichment of the plasma membrane was with oleyl or with linoleyl residues.
Figure 27. Effect of cold osmotic shock on accumulation of arginine (1.3 mM) by populations of *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues (•). Accumulation by populations which were not subjected to shock treatment is also shown (O). Values plotted are the average of at least two determinations.
Figure 28. Effect of growth with proline as nitrogen source (●) and ammonium ions as nitrogen source (O) on the subsequent initial rate of arginine accumulation (0.03-0.8 mM) by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues.
Figure 29. Effect of ammonium ions (2 mM) on rate of accumulation of arginine (0.2 mM) by *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues and which had been grown with proline (●) or ammonium ions as a nitrogen source (O). Ammonium ions (2 mM) were added to the suspension at 3 minutes.
5.3 Effect of Glucose and Potassium Ions on the Arginine-Accumulating Ability of Organisms

Inclusion of potassium ions (67 mM) had no effect on the rate of L-arginine (40 - 400 μM) accumulation by suspensions of Saccharomyces cerevisiae NCYC 366 when glucose (100 mM) was present. Omission of glucose and potassium ions lowered the rate of arginine accumulation at all arginine concentrations tested, although the proportional decrease was not the same at all arginine concentrations (Fig. 30). Omission of glucose when potassium ions (67 mM) were present resulted in almost total cessation of arginine transport (Fig. 31). The effect of potassium-ion concentration on uptake of arginine from a 100 μM solution by organisms with plasma membranes enriched in oleyl residues is shown in Fig. 32. Comparable responses were obtained whether plasma membranes were enriched with oleyl or linoleyl residues.

5.4 Effect of Metabolic Inhibitors on the Arginine-Accumulating Ability of Organisms

(i) 2,4-dinitrophenol and Carbonyl cyanide m-chlorophenyl hydrazone

Inhibition of L-arginine accumulation by 2,4-dinitrophenol and carbonyl cyanide m-chlorophenyl hydrazone was concentration dependent (Fig. 33). At 2,4-dinitrophenol concentrations greater than 0.5 mM, arginine accumulation by Saccharomyces cerevisiae NCYC 366 enriched with oleyl or with linoleyl residues was completely stopped. The same effect was obtained with carbonyl cyanide m-chlorophenyl hydrozone concentrations in excess of 0.2 mM.
Figure 30. Effect of the presence (○) and absence (●) of glucose (100 mM) on the initial arginine-accumulation rates over a range of arginine concentrations (0.04 - 0.4 mM) by Saccharomyces cerevisiaeNCYC 366 enriched in oleyl residues. Values plotted are the average of at least two determinations.
Figure 31. Effect of potassium ions (67 mM) on arginine (initial concentration 0.1 mM) accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched in oleyl residues, in the presence of 100 mM glucose (■) and in the absence of glucose (□). Values plotted are the average of three determinations.
Figure 32. Effect of potassium-ion concentration, in the absence of glucose, on the initial rate of arginine (0.1 mM) accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Points represent the average value of two determinations.
Figure 33. Effect of 2,4-dinitrophenol (●) and carbonyl cyanide m-chlorophenyl hydrazone (▲) concentration on the rate of arginine (initial concentration 0.2 mM) accumulation by *Saccharomyces cerevisiae* NCYC 366 enriched in oleyl residues. Results are expressed as a percentage of the rate obtained when the ionophore was absent and are the average values of at least three determinations.
(ii) Sodium Azide

At concentrations below 0.2 mM the effect of sodium azide on arginine accumulation was concentration dependent. However, at azide concentrations greater than 0.2 mM and less than 2 mM there was no increased inhibition (Fig. 34) of arginine accumulation by *Saccharomyces cerevisiae*NCYC 366 enriched with oleyl or linoleyl residues.

(iii) Sodium Fluoride

Inhibition of arginine accumulation by sodium fluoride did not exceed 40% within the concentration range examined (0.05 - 2 mM) (Fig. 35). The effect on cells which had been enriched with either of the fatty-acyl residues was very similar.

(iv) Iodoacetamide and 2-deoxy-D-glucose

Addition of iodoacetamide (10 mM) or 2-deoxy-D-glucose (50 mM) to suspensions of *Saccharomyces cerevisiae*NCYC 366 accumulating arginine in the presence of glucose did not affect the rate of accumulation within the time of the experiment (6 min). Pre-incubation of cells in buffered suspensions lacking arginine (10 min) with either of these compounds resulted in a decreased initial uptake rate at all arginine concentrations examined (0.04 - 0.4 mM) (Fig. 36). Cells enriched with oleyl or linoleyl residues behaved similarly in response to treatment with iodoacetamide or 2-deoxy-D-glucose.
Figure 34. Effect of sodium azide concentration on the initial rate of arginine (initial concentration 0.2 mM) accumulation by Saccharomyces cerevisiae NCYC 366 enriched in oleyl (●) or linoleyl residues (○). Results are expressed as a percentage of the rate obtained when sodium azide was absent and are the average values of at least three determinations. The curve describes the relationship between all shown values.
Figure 35. Effect of sodium fluoride concentration on the rate of arginine (initial concentration 0.2 mM) accumulation by *Saccharomyces cerevisiae*NCYC 366 with plasma membranes enriched in oleyl (■) or linoleyl (□) residues. Values plotted represent the average of two determinations.
Figure 36. The effect of 10 minute preincubation with 10 mM iodoacetamide (■) or 50 mM 2-deoxy-D-glucose (□) on the initial rate of L-arginine accumulation (between 0.04 and 0.4 mM) by Saccharomyces cerevisiae NCYC 366 with plasma membranes enriched in oleyl residues. The effect of preincubation with glucose (O) is also shown. Values plotted represent the average of two determinations.
5.5 Effect of Nitrogen Starvation and Arginine Accumulation on the Size of Organisms

Nitrogen starvation did not increase the initial rate of L-arginine accumulation, neither did it influence the total arginine accumulated by populations of Saccharomyces cerevisiae NCYC 366 with plasma membranes enriched in oleyl residues (Fig. 37). Comparable results were obtained with linoleyl enriched organisms. The virtual cessation of uptake after about 5 minutes indicated that there was no uncontrollable uptake. Throughout incubation with arginine (15 mM) there was no growth as measured by extinction at 600 nm. The size distribution of organisms which had been starved of nitrogen did not change (Fig. 37) although organisms which had been incubated with ammonium ions prior to incubation with arginine produced a diameter distribution which was centred around two sizes, namely 6.7 μm and 3.9 μm (Fig. 38). The average number of particles counted for each determination was about 35000.
Figure 37. Accumulation of arginine (initial concentration 15 mM) by *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues in the presence of glucose (initial concentration 100 mM) and KCl (initial concentration 25 mM). Arginine accumulated by cells which had been starved of nitrogen for 80 minutes are represented by (•) and arginine accumulated by cells which were incubated with ammonium ions for 80 minutes are represented by (O).
Figure 38. Effect of nitrogen starvation and arginine accumulation on the size of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues. In column (i) cells were incubated with ammonium ions (initial concentration 25 mM) for 80 minutes before addition of arginine (initial concentration 15 mM). In column (ii) cells were preincubated without a nitrogen source.
6. SOME ASPECTS OF ACCUMULATION AND RETENTION OF SOLUTES BY
SACCHAROMYCES CEREVISIAE

Within this section, with one exception, populations of Saccharomyces cerevisiae NCYC 366 whose plasma membranes were enriched with oleyl residues behaved in an identical manner to those whose plasma membranes were enriched with linoleyl residues. The exception is that where differences in transport kinetics have been shown (Section 2, p. 62) the rate of accumulation at a given concentration of solute is different for oleyl- and linoleyl-enriched cells. For this reason, data presented are for oleyl-enriched cells only unless otherwise indicated.

6.1 Amino Acids

(i) Arginine and Lysine

Linear uptake of L-arginine has already been described in Section 1 of the Results. This is a feature shared by L-lysine accumulation in Saccharomyces cerevisiae NCYC 366. During either arginine or lysine accumulation, replacement of radioactively labelled amino acid by unlabelled amino acid, or total removal of extracellular amino acid results in no loss of amino acid already accumulated (Fig. 39). Addition of 2,4-dinitrophenol does not change this (Fig. 39).

Effect of Growth Phase on Lysine-Accumulating Ability of Organisms

The initial rate of lysine accumulation from an 0.01 mM solution changed throughout batch growth (Fig. 40). During the exponential phase the initial rate of uptake increased to a maximum, and then declined. The decline continued into the onset of stationary phase.
Figure 39. Accumulation (▲) and retention of radioactively labelled ω-lysine by Saccharomyces cerevisiaeNCYC 366 when extracellular $^{14}$C-lysine (■) or removed altogether (□) at 8 and 9 minutes respectively. At 17 and 16 minutes 2,4-dinitrophenol (0.5 mM) was added.
Figure 40. Change in initial rate of lysine accumulation from an 0.01 mM solution (▲) during batch growth (▲) of *Saccharomyces cerevisiae*NCYC 366 with plasma membranes enriched in oleyl residues. Values plotted represent the mean of at least two independent determinations.
(ii) Asparagine and Glutamine

During the first three minutes, uptake of L-asparagine and L-glutamine at concentrations between 0.001 and 10 mM, was linear. Beyond this time at concentrations greater than 0.1 mM, the rate of accumulation decreases. An example is shown in Fig. 41. The final amount of amino acid accumulated is shown in Table 5.

At 0.01 mM amino acid, accumulation was linear throughout a thirty minute experiment.

(iii) Proline

The kinetics of L-proline accumulation were not examined in this study. However, preliminary experiments were performed, the results of which have a bearing on transport of other amino acids which were studied in more detail. Anaerobically, in the presence of glucose (100 mM), proline (0.04 mM) accumulation by *Saccharomyces cerevisiae* NCYC 366 proceeds in the manner described by Fig. 42. Addition of ammonium ions (4 mM) decreases the rate of proline accumulation (Fig. 43).
Figure 41. Accumulation of L-glutamine (initial concentration 0.1 mM) by Saccharomyces cerevisiae NCYC 366 with plasma membranes enriched in oleyl residues.
<table>
<thead>
<tr>
<th>Initial extracellular asparagine or glutamine concentration (mM)</th>
<th>Asparagine or glutamine accumulated (nmol (mg dry wt)$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>35 (±7)</td>
</tr>
<tr>
<td>1.0</td>
<td>70 (±12)</td>
</tr>
<tr>
<td>10.0</td>
<td>120 (±11)</td>
</tr>
</tbody>
</table>

Table 5. The amount of asparagine or glutamine accumulated by populations of Saccharomyces cerevisiae NCYC 366 in the presence of glucose at 30°C. Values in parentheses represent the standard deviation.
Figure 42. Accumulation of proline (0.04 mM initial concentration) by *Saccharomyces cerevisiae*NCYC 366 with plasma membranes enriched in oleyl residues and the effect of adding ammonium ions (4 mM) at 40 minutes.
6.2 Inorganic Ions

(i) Calcium

Following the retention of $^{45}$Ca$^{2+}$ by populations of *Saccharomyces cerevisiae*NCYC 366 with time revealed two distinct phases (Fig. 43). There was an initial, rapid, linear accumulation which lasted for about three minutes (0.01 mM Ca$^{2+}$ concentration); this was followed by a slower, linear accumulation which continued for up to forty minutes more. The process was not followed for longer periods of time. If cells which were accumulating $^{45}$Ca$^{2+}$ were removed from suspension and then resuspended in buffer, either in the presence or absence of glucose or $^{42}$Ca$^{2+}$, at the same concentration as in the original reaction mixture, then radioactivity was lost from the cells. The loss was identical for all of these conditions (Fig. 44). For a given preparation, the sum of $^{45}$Ca$^{2+}$ lost from cells plus the amount of $^{45}$Ca$^{2+}$ accumulated during three minutes of the slower phase of uptake, equalled that amount of $^{45}$Ca$^{2+}$ which was accumulated during the first three minutes.

(ii) Sulphate

Uptake of sulphate by *Saccharomyces cerevisiae*NCYC 366 does not proceed in a linear fashion. For example, removal of sulphate from an 0.029 mM solution was slower during the first one or two minutes than during the following ten minutes. From this time uptake continued at a slower rate for at least thirty minutes (Fig. 45). Resuspension of cells which had been accumulating $^{35}$SO$_4^{2-}$ (1.0 mM) resulted in loss of radioactivity from the cells (Fig. 46).
Figure 43. Accumulation of calcium ions (initial concentration 0.01 mM) by populations of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues.
Figure 44. Accumulation and retention of calcium ions (initial concentration 0.01 mM) by \textit{Saccharomyces cerevisiae} NCYC 366 with plasma membranes enriched in oleyl residues. At 21 minutes $^{45}\text{Ca}^{2+}$ were replaced with $^{42}\text{Ca}^{2+}$.
Figure 45. Sulphate accumulation (initial concentration 0.029 mM) by populations of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues.
Figure 46. Accumulation and retention of sulphate ions (initial concentration 0.029 mM) by *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues. At 10 minutes $^{35}$SO$_4^{2-}$ were replaced with $^{32}$SO$_4^{2-}$.
(iii) Dihydrogen Phosphate

Throughout a twenty minute period accumulation of dihydrogen phosphate (0.1 mM) by *Saccharomyces cerevisiae*NCYC 366 was linear (Fig. 47). Replacement of radioactively labelled dihydrogen phosphate with non-radioactive dihydrogen phosphate resulted in no loss of that dihydrogen phosphate which had already been accumulated. (Fig. 47).

(iv) Chloride

When either oleyl- or linoleyl-enriched populations of *Saccharomyces cerevisiae*NCYC 366 were incubated with Na$^{36}$Cl (0.001, 1 or 100 mM), anaerobically in the presence of glucose (100 mM) at pH 4.5 no increased retention of label occurred over a period of 60 minutes (Fig. 48). Neither removal of extracellular chloride ions, replacement of radioactive chloride by non-radioactive chloride ions nor the presence of 2,4-dinitrophenol (0.5 mM) had any effect upon retention of label. There was a correlation between the amount of label included in each experiment and the amount of radioactivity retained by cells. There was no correlation between the amount of chloride included in each experiment and the amount of radioactivity retained by cells.
Figure 47. Accumulation (■) and retention (□) of dihydrogen phosphate by *Saccharomyces cerevisiae*NCYC 366 with plasma membranes enriched in oleyl residues. Radioactively labelled dihydrogen phosphate was replaced by non-radioactive dihydrogen phosphate at 10 minutes.
Figure 48. Retention of radioactivity by populations of *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl residues when incubated at 30°C in the presence of glucose (initial concentration 100 mM) and Na\(^{36}\)Cl (initial concentration 1 mM).
7. PLASMA-MEMBRANE LIPID UNSATURATION AND PROTON MOVEMENTS IN

SACCHAROMYCES CEREVISIAE

7.1 Initial Observations on Proton Movements

When freshly harvested exponential-phase cells of Saccharomyces cerevisiae NCYC 366 with plasma membranes enriched in either oleyl or linoleyl residues were suspended in distilled water, under nitrogen gas, at a cell density between 5 and 10 mg dry wt. ml$^{-1}$, a pH value of between 5.5 and 5.9 was achieved rapidly. This value remained constant for at least 10 minutes. Addition of 2-deoxy-D-glucose (25 mM) to the cell suspension had no immediate effect. However about one minute after the addition, the pH value increased and continued to do so at a decreasing rate for about 30 minutes, until a steady value of between 6.4 and 6.6 was attained. Inclusion of glucose (200 mM) in the original cell suspension caused a decrease in pH which continued until a pH value of between 3.7 and 4.0 was achieved. Addition of more glucose at this time did not further lower the pH value. Addition of 2-deoxy-D-glucose (25 mM) at this point, or during the pH decrease caused by glucose, resulted, after about one minute delay, in an increase in pH value. The final pH value was between 6.1 and 6.7.

7.2 Stoichiometric Proton and L-Lysine Movements

An unbuffered suspension of Saccharomyces cerevisiae NCYC 366 with plasma membranes enriched in oleyl residues, adjusted to pH 4.4 and containing 2-deoxy-D-glucose (25 mM) had an acid influx rate of 2.54 nmol (mg dry wt$^{-1}$ min$^{-1}$) (average of four experiments). Addition of L-lysine (40 μM) decreased the acid influx rate to 2.25 nmol (mg dry wt$^{-1}$ min$^{-1}$) and there was a
simultaneous accumulation of lysine at a rate of 0.262 nmol (mg dry wt)$^{-1}$ min$^{-1}$ which continued for four minutes at which point titrating acid was added to the suspension (Table 6). There was no increased acid influx rate accompanying lysine accumulation but rather it seemed that some of the protons flowing into the cells were replaced by lysine molecules. There was an analogous situation when cells with plasma membranes enriched in linoleyl residues were used (Table 6).
<table>
<thead>
<tr>
<th></th>
<th>Acid influx rate before addition of lysine</th>
<th>Acid influx rate after addition of lysine</th>
<th>Decrease in acid influx rate upon addition of lysine</th>
<th>Lysine accumulation</th>
<th>Lysine/Proton ratio</th>
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<tr>
<td></td>
<td>nmol (mg dry wt)^{-1} min^{-1}</td>
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<td>1.58</td>
<td>0.15</td>
<td>0.132</td>
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Table 6. Proton movements associated with the anaerobic absorption of L-lysine by *Saccharomyces cerevisiae* NCYC 366 with plasma membranes enriched in oleyl or linoleyl residues.
8. ANAEROBIC GROWTH AND PHOSPHOLIPID COMPOSITION OF SACCHAROMYCES CEREVISIAE NCYC 366 SUPPLEMENTED WITH ERGOSTEROL AND ONE OF A VARIETY OF UNSATURATED FATTY ACIDS

8.1 Growth

The ability of Saccharomyces cerevisiae to grow anaerobically when supplemented with ergosterol (5 mg l⁻¹) and one of several unsaturated fatty acids (30 mg l⁻¹), namely myristoleic acid (14:1), palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2) and cetoleic acid (20:1) was determined (Figs. 49, 50, 51, 52 and 53). Myristoleic acid did not promote growth. Indeed the growth yield of a culture supplemented with myristoleic acid and ergosterol was lower than when supplemented with ergosterol alone (Fig. 50). Palmitoleic, oleic, linoleic and cetoleic acid increased growth yields relative to supplementation with just ergosterol. In all four cases the mean generation time (2.1 h) was identical, however the lag period with each fatty acid was different. For monounsaturated fatty acids, the longer the carbon chain was, the shorter the lag time. The lag period for linoleyl enriched cells was very similar to that obtained for palmitoleyl cells.

8.2 Fatty-Acyl Composition of Phospholipids from Saccharomyces cerevisiae

Phospholipids from organisms grown in the presence of palmitoleic acid were enriched with residues of this fatty acid to a slightly greater extent than phospholipids from organisms grown in the presence of oleic acid (Table 7), the value for which was slightly lower than that previously reported (about 54%) for anaerobically grown Saccharomyces cerevisiae NCYC 366 by Thomas
Figure 49. Time-course of growth of *Saccharomyces cerevisiae* NCYC 366 anaerobically in 1 l portions of medium supplemented with ergosterol at 5 mg l⁻¹ plus myristoleic acid at 30 mg l⁻¹ (▲) or with ergosterol at 5 mg l⁻¹ (▲). Values plotted are the average of three determinations.
Figure 50. Time-course of growth of *Saccharomyces cerevisiae* NCYC 366 anaerobically in 1 l portions of medium supplemented with ergosterol at 5 mg l\(^{-1}\) plus palmitoleic acid at 30 mg l\(^{-1}\) (▲) or with ergosterol at 5 mg l\(^{-1}\) (△). Values plotted are the average of three determinations.
Figure 51. Time-course of growth of *Saccharomyces cerevisiae* NCYC 366 anaerobically in 1 l portions of medium supplemented with ergosterol at 5 mg l$^{-1}$ plus oleic acid at 30 mg l$^{-1}$ (●) or with ergosterol at 5 mg l$^{-1}$ (▲). Values plotted are the average of three determinations.
Figure 52. Time-course of growth of *Saccharomyces cerevisiae* NCYC 366 anaerobically in 1 l portions of medium supplemented with ergosterol at 5 mg l⁻¹ plus linoleic acid at 30 mg l⁻¹ (▲) or with ergosterol at 5 mg l⁻¹ (△). Values plotted are the average of three determinations.
Figure 53. Time-course of growth of *Saccharomyces cerevisiae* NCYC 366 anaerobically in 1 l portions of medium supplemented with ergosterol at 5 mg l\(^{-1}\) plus cetoleic acid at 30 mg l\(^{-1}\) (▲) or with ergosterol at 5 mg l\(^{-1}\) (△). Values plotted are the average of three determinations.
et al. (1978). However, phospholipids from organisms grown in the presence of cetoleic acid were enriched to a much smaller extent (about 20%). Fatty-acyl composition of phospholipids grown in the presence of myristoleic acid and of linoleic acid were not determined.
<table>
<thead>
<tr>
<th>Fatty acid supplement</th>
<th>Palmitoleyl</th>
<th>Oleyl</th>
<th>Cetoleyl</th>
</tr>
</thead>
<tbody>
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<td>60.10</td>
<td>ND</td>
<td>4.80</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>8.25</td>
<td>11.10</td>
<td>16.15</td>
</tr>
<tr>
<td>C₁₈:₁</td>
<td>1.35</td>
<td>49.70</td>
<td>ND</td>
</tr>
<tr>
<td>C₂₀:₁</td>
<td>ND</td>
<td>ND</td>
<td>18.55</td>
</tr>
</tbody>
</table>

ND not detected

Table 7. Fatty-acyl composition of phospholipids from *Saccharomyces cerevisiae* NCYC 366 grown anaerobically in the presence of palmitoleic, oleic or cetoleic acid

The values quoted are the averages of two independent analyses
DISCUSSION
Plasma Membrane Lipid Composition and Transport Kinetics

in *Saccharomyces cerevisiae*NCYC 366

The first conclusion which can be drawn from the study is that the transport kinetics as described by $K_T$ and $V_{max}$ values, of two substrates, namely L-arginine and L-lysine were altered when oleyl residues were replaced by linoleyl residues in the plasma membrane whilst the transport kinetics of five other substrates, namely L-asparagine, L-glutamine, dihydrogen phosphate, sulphate and calcium were not altered. The kinetics of D-glucose accumulation were in neither category as $V_{max}$ values differed and $K_T$ values were almost identical. However, this is unimportant to the main argument. What do the two transport systems which were changed have in common? Both substrates are accumulated by two transport systems (Grenson et al., 1966; Grenson, 1966; Chan and Cossins, 1976) although this is open to an alternative interpretation. Professor Per Nisson (University of Bergen, Norway) has pointed out that the Woolf-Hofstee plots for arginine accumulation (Figs. 11 and 12) are too angular to account for the sum of two systems both of which adhere to Michaelis-Menten kinetics. He suggested that the lower affinity, high velocity, accumulation of arginine was the result of a conformational change induced in the arginine-transport system by a threshold concentration of arginine. This produces an entirely new transport system, the kinetics of which are described without any contribution from the transport system that worked at lower arginine concentrations. The abrupt nature of the switch produces the sharp angle obtained on Woolf-Hofstee plots. In the absence of genetic information, which was not easily obtainable with *Saccharomyces cerevisiae*NCYC 366 as it is a brewing strain of uncertain ploidy, the possibilities of one or two arginine-transporting systems could not be resolved. Whichever is the case, arginine and lysine have a common transport system, the high affinity arginine-transporting system,
and the low affinity lysine-transporting system are one and the same (Grenson et al., 1966; Grenson, 1966).

A relevant question is whether replacement of oleyl residues by linoleyl residues within the plasma membrane affects the shared transport system in a similar manner when it is transporting arginine to when it is transporting lysine? This question can only really be answered when the meaning of the kinetic constants is known. With solute transport, a process more complex than that for which the Michaelis-Menten principles were originally derived, the half saturation constant \( K_{m} \) need not represent the true dissociation constant of the carrier. Reasons for this have been considered (Kotyk and Kleinzeller, 1967; Kotyk, 1967b) based upon measurements of monosaccharide transport into and out of yeast cells. These authors concluded that if the mobility of the free carrier was different to the mobility of the loaded carrier, that difference would be reflected in the \( K_{m} \) value. Their argument assumed that transport is mediated by a mobile carrier, which is a model which can be used to explain monosaccharide transport, but is not necessarily applicable to the transport of amino acids by Saccharomyces cerevisiae. Later work concerning amino acid transport in Saccharomyces cerevisiae directly (Kotyk et al., 1971b) led to the suggestion that for uphill transport, the \( K_{m} \) value would include parameters of the metabolic coupling reaction. For the particular comparisons made throughout this thesis however, any contribution by a metabolic coupling reaction is likely to be applicable in all cases, and would therefore not interfere with conclusions which were drawn. Accepting these criticisms, it is not unreasonable to assume that the \( K_{m} \) value reflects the affinity that the transport system has for its substrate and that the \( V_{\text{max}} \) value represents the maximal rate of work that can be done by the
system as a whole. Therefore the influence plasma-membrane lipid unsaturation has upon these values, will be viewed in this context.

For dual transport systems, the $K_T$ and $V_{\text{max}}$ values calculated from kinetic plots may prove misleading as there must occur overlapping of concentrations at which each transport system works. Use of the equations derived by Neal (1972) therefore, could provide information more directly relevant to individual transport systems. Applying this method to distinguish the transport system responsible for both arginine and lysine accumulation one finds that, with both substrates, the $V_{\text{max}}$ value is increased when oleyl residues are replaced by linoleyl residues within the plasma membrane, and that the affinity the transport system has for lysine, as measured by $K_T$, is increased, whilst with respect to arginine, it is decreased. As increased plasma-membrane lipid unsaturation leads to increased fluidity, it could be argued that inclusion of linoleyl residues would enable the transport system to work faster. The fact that $K_T$ values with respect to arginine and lysine were moved in opposite directions is not necessarily surprising as arginine and lysine are structurally very different. It is possible that the specific change effected to plasma-membrane lipid unsaturation produces changes at only one part of the active site of the transport protein or proteins. This could be the region which recognises the $\delta$-guanidino group of arginine and the $\delta$-amino group of lysine, although this remains merely speculation. I now consider the arginine and lysine transport systems which are not shared. With linoleyl-enriched cells the low-affinity arginine-transport system showed an increased $V_{\text{max}}$ value relative to oleyl-enriched cells, whilst the high-affinity lysine-transport system had a decreased $V_{\text{max}}$ value.
which suggests that the relationship between plasma-membrane lipid unsaturation and the rate of solute transport is more subtle than the simple fluidity idea would predict, or at least it cannot be invoked for every case.

When membrane/protein interactions are considered it is important to distinguish between the proximity of lipid molecules to proteins and the interactions they may have if they are there. One possible approach is to exploit the physical properties of lipid molecules rather than the chemical properties, for example melting temperatures. Within any one membrane, for example, the yeast mitochondrial membrane, different enzymes can have different transition temperatures on Arrhenius plots (Watson et al., 1973). This can be interpreted in terms of a heterogeneous distribution of lipids within the membrane, such that the variety and type of lipid molecules in the vicinity of the enzymes decide the transition temperatures. Unfortunately the precise nature of the lipid annuli surrounding these enzymes cannot be determined in this manner. However, given the situation that an intrinsic protein is kinetically sensitive to a specific membrane compositional change, for example the high-affinity lysine-transport system, and another is kinetically insensitive to the same, specific compositional change, for example that responsible for asparagine accumulation, then I contend that data obtained from Arrhenius plots could be very useful in determining possible associations between these proteins and the lipid molecules which have been included within the plasma membrane.

Within the oleyl-enriched plasma-membrane, both lysine- and
asparagine-transport systems show well defined breaks on Arrhenius plots which give different transition temperatures for each system. Within the linoleyl-enriched membrane the breaks are not nearly so well defined, but following Arrhenius plots from a low to a higher temperature, the point at which deviation from linearity first occurs might be taken as the temperature at which lipid molecules begin to melt around the transport proteins. Once more these temperatures are different for the lysine- and asparagine-transport systems. So within two compositionally distinct plasma membranes, both transport systems have distinct transition temperatures.

Rather than comparing two transport systems in one membrane, one can compare the behaviour of any one transport system in two different membranes. The transition temperatures for lysine transport across the oleyl-enriched and the linoleyl-enriched plasma membrane were very similar. But, if a distinction is to be used, then the transition temperature for the oleyl-enriched plasma membrane is higher than that for the linoleyl-enriched plasma membrane. However, this is the result which was expected, as doubly-unsaturated linoleyl residues melt at a lower temperature than mono-unsaturated oleyl residues.

Transition temperatures for asparagine transport across oleyl- and linoleyl-enriched plasma membranes were quite distinct. Very surprisingly, the transition temperature obtained with oleyl-enriched cells was lower than that obtained with linoleyl-enriched cells. This may have dramatic implications regarding the distribution of lipid molecules throughout the plasma membrane of
How might these results be explained? The kinetic sensitivity of the lysine-transport system to the specific alteration effected to the plasma membrane of *Saccharomyces cerevisiae* NCYC 366 suggested that different lipid molecules may surround this transport system in the two membranes. As the transition temperature is, if anything, lowered when oleyl residues are replaced by linoleyl residues, I suggest that phospholipids with linoleyl residues form the annulus in linoleyl-enriched membranes and phospholipids with oleyl residues occur in the annulus in oleyl-enriched membranes.

On the other hand, the kinetic insensitivity of the asparagine-transport system to replacement of oleyl residues by linoleyl residues suggests that there is either no reaction with fatty-acyl residues on phospholipids in the surrounding annulus or that the composition of the annulus is specifically controlled. An elevated transition temperature for asparagine transport by linoleyl-enriched cells relative to oleyl-enriched cells suggests the latter possibility. The higher transition temperature is indicative of the appearance of higher melting-point components around the asparagine transport system in linoleyl-enriched plasma membranes as compared with the oleyl-enriched plasma membranes. Implicit in this conclusion is an exclusion of linoleyl residues from the lipid annulus. So which fatty-acyl residues might be included?

In aerobically-grown *Saccharomyces cerevisiae* NCYC 366, the major fatty-acyl residues present in the membrane phospholipids
are palmityl, palmitoleyl and oleyl residues (in anaerobically-grown cultures, palmitoleyl and oleyl residues are replaced by the unsaturated fatty-acyl residues provided, namely oleyl or linoleyl residues (Thomas et al., 1978). Ignoring other minor fatty-acyl residues and the contribution sterols may have, in either membrane there are two main fatty-acyl residues to locate. In the very simple model shown in Figure 54a, the asparagine transport system is surrounded by phospholipids containing palmityl and oleyl residues. When phospholipids with oleyl residues are replaced in the annulus by phospholipids with linoleyl residues (Figure 54b) the transition temperature would be expected to decrease, which was not the case. Figure 54 shows a situation which may prevail. Total exclusion of phospholipids containing linoleyl residues is not required to increase the transition temperature. It is only necessary to decrease the number of linoleyl residues relative to the number of oleyl residues which were present in the annulus of the asparagine-transport system, in oleyl-enriched cells.

So far no mention has been made of the functional requirements that transport systems may have for specific fatty-acyl residues on phospholipids. It was thought that light could be thrown on this by enriching plasma membranes with palmitoleyl or cetoleyl residues, and comparing kinetic data from these organisms with that already obtained for oleyl- and linoleyl-enriched organisms.

Enrichment of phospholipids by palmitoleyl residues was about 60% and therefore was comparable with enrichment by both oleyl and
Figure 54. Possible arrangements of phospholipids around the asparagine transport system of *Saccharomyces cerevisiae* NCYC 366.

P represents phospholipids containing palmityl residues.

O represents phospholipids containing oleyl residues.

L represents phospholipids containing linoleyl residues.
linoleyl residues. On the other hand, cetoleyl residues accounted for only about 20% of the total fatty-acyl residues in phospholipids extracted from organisms grown in the presence of cetoleic acid, which precluded these cells from being included in the comparison.

In this comparison, low concentrations of lysine and asparagine were used, such that for lysine accumulation only the high-affinity system was examined, with minimal interference from the low-affinity system. Values for $K_T$ and $V_{max}$ were therefore compared with those obtained for oleyl- and linoleyl-enriched organisms before the equations devised by Neal (1972) were used.

The affinity of the lysine-transport system within palmitoleyl-enriched populations of *Saccharomyces cerevisiae* was slightly decreased relative to oleyl-enriched populations, but considerably decreased relative to that obtained with linoleyl-enriched organisms. The $V_{max}$ values with membranes enriched in mono-unsaturated residues were higher than with linoleyl-enriched membranes. Oleyl and palmitoleyl residues are the major naturally occurring unsaturated fatty-acyl residues in the plasma membrane of aerobically-grown *Saccharomyces cerevisiae* and their presence or absence have both beneficial and detrimental effects on the operation of the lysine-transport system. Unfortunately it is not possible to distinguish between the possibilities that the presence of monounsaturated residues increased $V_{max}$ values and decreased affinity for lysine, or that doubly unsaturated residues decreased the $V_{max}$ value and increased the affinity. There is also a third possibility that the differences
seen when linoleyl residues replace either of the monounsaturated residues are the result of an absence of monounsaturated residues.

Owing to the limited cetoleyl enrichment of the plasma-membrane, kinetic data obtained for lysine and asparagine transport by cetoleyl enriched organisms have been regarded as not comparable with those data obtained from organisms with other fatty-acyl enrichments. However, cetoleyl-enriched plasma membranes do not contain Δ^9 unsaturated fatty-acyl residues and so one could ask whether the presence of Δ^9 unsaturated residues specifically influences asparagine and lysine transport. The $K_T$ and $V_{max}$ values for asparagine and lysine transport by *Saccharomyces cerevisiae*NCYC 366 enriched in palmitoleyl or oleyl residues are both increased, albeit only slightly, relative to values obtained with cetoleyl-enriched organisms. It is possible, therefore, that a double bond at position 9 in the fatty-acyl chain can be considered stimulatory in terms of $V_{max}$ whilst inhibitory in terms of $K_T$.

For lysine accumulation the effects of enrichment with Δ^9 mono-unsaturated fatty-acyl residues relative to cetoleyl enrichment, are reversed by linoleyl enrichment. Both $K_T$ and $V_{max}$ values are decreased for linoleyl enriched organisms. A case could now be made to dismiss the possibility that kinetic changes in lysine accumulation resulting from linoleyl enrichment are due to an absence of Δ^9 monounsaturated fatty-acyl residues as data suggest that the presence of both residues on plasma membrane phospholipids can affect kinetics of lysine accumulation, but do so in a manner opposed to each other.
As more information becomes available concerning the lipid requirements for transport systems, it becomes increasingly obvious that enrichment with specific fatty-acyl residues, whether natural plasma-membrane components or not, can have dual effects upon the operation of transport systems. Any one residue, or indeed absence of a specific residue, can both increase affinity and decrease rate of work or vice versa. Under different conditions, both effects could be beneficial. In a nutritionally rich environment a high $V_{max}$ value could have an advantage whilst during substrate limitation a low $K_m$ value is more desirable.

It is a reasonable assumption, that the phospholipid environment of transport systems contain many different fatty-acyl residues, competing in their effects at various positions around the transport systems to greater or lesser extents. The composition of a lipid annulus is likely to be determined by the affinity the protein has for those lipid molecules, despite the effects that those lipids may have on the function of the protein. Nevertheless, it is difficult to rationalize the large number of lipid molecules in the plasma membrane as a matrix in which proteins operate at the whim of the random lipid jostling. It is not impossible that the multiplicity of lipid molecules form the fine tuning of transport regulation, the control of which is by proximity and abundance of specific phospholipids, although it is perhaps just as difficult to imagine how such movements of phospholipids might be achieved.
Effect of Storage Time on Solute-Accumulating Ability of Organisms

The differences found between oleyl-enriched and linoleyl-enriched populations of *Saccharomyces cerevisiae* when stored in an ice-water mixture were an interesting if fortuitous discovery. The rationale behind the experiment had been to find out whether mid to late exponential-phase growth organisms could be stored in order that transport kinetics could be studied within only a few batches of cells.

The initial slow decline was common to both oleyl-enriched and linoleyl-enriched cells and was therefore probably independent of plasma-membrane composition. This could reflect the residence time of proteins involved with transport in the membrane and be part of membrane-component turnover. On the other hand it is reasonable to suppose that the subsequent rapid decline in arginine-transporting ability by linoleyl-enriched cells, which was absent from oleyl-enriched cells, was a direct result of the alteration in plasma-membrane composition. Other data which are available suggest that linoleyl-enriched plasma membranes are less stable than oleyl-enriched plasma membranes. For example α-mannosyltransferase activities of plasma-membrane preparations enriched with linoleyl residues were more variable than those activities of oleyl-enriched preparations, which might be explained by a greater loss of components (protein and/or phospholipid) from the more fluid membranes (L. Julia Douglas, unpublished observation). Furthermore sphaeroplasts prepared from cells enriched in linoleyl residues leaked protein faster than sphaeroplasts with membranes enriched in oleyl residues (Diana M. Belk and A.H. Rose, unpublished observations) although
whether the proteins were released from within the sphaeroplasts, or were plasma-membrane components is not known. Either way, one may conclude that a membrane which contains an increased proportion of unsaturated residues, that is one which is more fluid, has a greater inherent instability. However difficulties arise with the knowledge that, within several batches of cells, the timing of the sudden decline in arginine-transporting ability was remarkably constant. An explanation which invokes an instability in more fluid membranes is somewhat untenable. As far as is known enrichment of *Saccharomyces cerevisiae* with oleyl or linoleyl residues only affects membrane-related phenomena. One must conclude either that the decline in arginine-transporting ability is precipitated by a time-dependent aspect of metabolism which is common to cells enriched in either residue but is only manifest in linoleyl-enriched organisms, for example achievement of a critical pool size which may result in component protein loss from the less stable plasma membrane. Alternatively one may site the plasma membrane directly and suggest a more rapid leakage of molecules through the linoleyl-enriched plasma membrane which results again in a critical pool size or even an ionic imbalance across the plasma membrane. This could result in loss of activity of the transport system rather than loss of component protein. The more attractive suggestion is that which exploits the increased permeability of the linoleyl-enriched plasma membrane since it is known that an increased proportion of unsaturated residues in the yeast plasma-membrane results in a more rapid passive permeability rate of many molecules (Suomalainen and Nurminen, 1976). If an ionic imbalance was the cause of the loss of arginine-transporting activity upon cold storage, then both leakage of protons into the cells and
leakage of potassium ions out of the cells are likely candidates. At $30^\circ$C I found no differences in proton influx rates between oleyl- and linoleyl-enriched populations of *Saccharomyces cerevisiae* NCYC 366. In order to maintain electroneutrality one could infer that the potassium ion efflux rates were also identical in cells enriched with either residue. This does not exclude the possibility that proton- and potassium-ion permeability are different when cells are stored in an ice-water mixture. In the absence of further data however, any attempt to explain the sudden loss of arginine-transporting ability is purely conjecture.

**Effect of Cold-Osmotic Shock on Arginine-Accumulating Ability of Organisms**

Arginine accumulation by *Saccharomyces cerevisiae* probably involves several distinct reactions, namely binding to a periplasmically located protein (Opekarova et al., 1975), transfer to the transport system, transport, and then passage into vacuoles (Boller et al., 1975). The role that the plasma-membrane ATPase plays must also be considered. During cold osmotic shock treatment, the periplasmic arginine-binding protein is likely to be lost, but this should not account for total loss of arginine-accumulating ability which was observed. In sphaeroplasts (Boller et al., 1975) formation of which is also likely to result in loss of the arginine-binding protein, transport of arginine still proceeds. Therefore some additional component of the arginine-transport system must be affected by cold osmotic shock.

With a highly empirical procedure such as cold-osmotic shock,
the experimental procedure used must be carefully defined if comparisons are to be made with results of other workers. Rate of cooling final temperature achieved, the osmotic stress involved as well as recovery conditions are some of the parameters which probably affect the shock procedure. The procedure I used exactly reproduced that used by Patching (1971) and therefore comparisons which are to be drawn with his work are valid.

Patching (1971) found that cold-osmotic shock treatment of *Saccharomyces cerevisiae* NCYC 366 caused neither lysis nor complete disruption of the cell's permeability barrier. In the same study, cold-osmotic shock was shown to lower the ability of cells to accumulate both glucosamine and 2-aminoiso butyrate. Total loss of transporting activity, as was observed for arginine accumulation, was not reported. These three transport processes are not known to share any specific components and so, for example, the collapse of an electrochemical gradient would probably not account for the results. On the other hand, these three transport processes do have a common location, namely the plasma-membrane. Together these data suggest that cold-osmotic shock treatment of *Saccharomyces cerevisiae* affects many individual targets within the plasma membrane. The magnitude of these effects may depend on the firmness of the association of these targets in the plasma membrane. One could be forgiven for expecting linoleyl-enriched organisms to be more sensitive to cold osmotic shock treatment than oleyl-enriched organisms because, in the light of data obtained from cold storage of *Saccharomyces cerevisiae* NCYC 366, a shadow of uncertainty was thrown upon the firmness of association between the arginine-transport...
system and the linoleyl-enriched plasma membrane. Unfortunately no such differences were observed; therefore the fatty-acyl composition of the plasma-membrane would not appear to be a major determinant of cold-osmotic shock sensitivity of arginine transport. Perhaps administration of a more gentle shocking procedure would not result in total loss of arginine-transporting activity and hence provide differential effects.

Effect of Arginine Accumulation on the Size of Organisms

Explosive, vacuolar absorption of glycine by \textit{Saccharomyces uvarum} leading to lysis of the cell has been reported (Indge et al., 1977). Arginine also accumulates in vacuoles and so it was thought possible that explosive absorption might also occur with arginine. Indeed if explosive absorption was to occur, then the composition of the plasma membrane would play a central role in determining first an increase in cell size and secondly the tendency to lyse. However, neither explosive absorption of arginine nor an increase in the size of cells enriched with either oleyl or linoleyl residues was observed (Fig. 36, Fig. 37).

The first conclusion is that no size increase was observed because explosive absorption did not occur, therefore there was no osmotic pressure manifest in the vacuole. In retrospect there can be many reasons why explosive absorption did not occur. Lysine transport is controlled by feedback inhibition (Morrison and Lichstein, 1976). Often transport phenomena related to arginine are found to apply for lysine and \textit{vice versa}. Morrison and Lichstein (1976) have also shown that preloading \textit{Saccharomyces cerevisiae}
with arginine will prevent lysine accumulation. For these reasons it seems reasonable that the same feedback mechanism will operate for arginine accumulation, thereby preventing explosive absorption. But let us assume that the feedback mechanism did not operate.

In cell free preparations, Boller et al. (1975) showed that arginine only entered the vacuole in exchange for arginine that was already there, a process not compatible with concentration of arginine molecules. However the same workers also demonstrated a considerable concentration difference within intact organisms between arginine in vacuoles which was high and arginine in the cytoplasm which was low. These two observations are inconsistent and one must assume that in vivo either the exchange mechanism is not so rigid, or that a molecule other than arginine can substitute in this process. Dürr et al. (1979) showed that the amount of polyphosphate in yeast cell vacuoles could be equated with the amount of arginine also present in vacuoles, with the negative charges of the polyphosphate being cancelled out by the positive charge of arginine. In this form the arginine is osmotically inactive and so would not contribute to the swelling of vacuoles, and therefore it would seem unlikely that explosive absorption of arginine would occur. On the other hand increase in vacuole but not cell size has been reported (Indge et al., 1977) in the presence of arginine and lysine, but this was in preparations in which glycine was concentrated in the cytoplasm and not in the vacuoles. Also, no mention was made of vacuolar polyphosphate contents in these preparations.
Some Aspects of Accumulation and Retention of Solutes by *Saccharomyces cerevisiae*

Whether the kinetics of solute accumulation were affected by the switch from oleyl to linoleyl residues in the plasma membrane or not, it was thought that such a compositional change could play a role in retention of solutes which had been accumulated. No differences were found however between oleyl- or linoleyl-enriched organisms in this respect with any of the solutes examined including arginine and lysine. There were differences, however, in retention of different solutes, which in themselves posed some interesting questions.

Neither arginine nor lysine was lost from cells of *Saccharomyces cerevisiae* when presented with a variety of conditions (Fig. 38), namely presence and absence of the relevant amino acid, or energy source, or metabolic inhibitors. This is consistent with the generally held view that amino acids are accumulated irreversibly against a concentration gradient (Kotyk and Janácek, 1975). A notable exception to this is efflux of glycine by preparations of *Saccharomyces carlsbergensis* reported by Seaston *et al.* (1975). In this work the efflux rate was low and it was suggested that it would probably not be detectable by measurement of radioactivity retained by cells. Therefore, one experiment was performed which looked for increased radioactivity with time in filtrates of organisms which had previously accumulated lysine, but this proved negative. Following the same line, phosphate was also shown not to leak from *Saccharomyces cerevisiae*NCYC 366 under a similar variety of conditions (Fig. 47). Uptake and retention of sulphate and calcium ions were, however more interesting and gave results.
Anaerobic accumulation of inorganic sulphate by *Saccharomyces cerevisiae* appears to be in three distinct stages (Fig. 45). Stage one comprises a slow uptake for one or two minutes, stage two a rapid uptake for five to ten minutes, and stage three, a decrease in rate lasting for at least twenty minutes. The fate of inorganic sulphate was not followed and so the following explanation is only speculation. Observations similar to those obtained in stage one have already been reported (Kotyk, 1959) when it was observed in nitrogen-deficient and anaerobic yeast. It was interpreted as the time taken for formation of some carrier which was either exhausted or formed more slowly under these conditions. The time scale would suggest that the term 'carrier' does not refer to a transport protein, but rather to a compound involved in assimilation of inorganic sulphate once it has crossed the plasma membrane. The lag period would then be due to trans-inhibition of transport by immediately accumulated intracellular sulphate (Breton and Surdin-Kerjan, 1977). Provision of more 'carrier' would alleviate this inhibition, and this is conceivably what is happening in stage 2, limited perhaps by the maximum permissible rate of sulphate transport. Stage three is more difficult to explain. Under conditions of different carbon and nitrogen availability, Kotyk (1959) showed that within ten minutes of the onset of sulphate uptake, only about 3% of radioactively labelled sulphur atoms were found as inorganic sulphate, much of the rest appearing as free amino acids. The pathway incorporating sulphate-sulphur atoms into amino acids involves at least five enzymes (Schlossmann and Lynen, 1957; Wilson
et al., 1961; Dreyfuss and Monty, 1963; Roy and Trudinger, 1970). It is not unreasonable to postulate that the rate of reaction of one of these enzymes is slower than that of transport such that, after an early rapid uptake of sulphate, the concentration of the substrate for the slow enzyme increases. Feedback mechanisms would then operate, and the rate of sulphate uptake would be retarded.

An obvious explanation for loss of radioactivity from cells which had been accumulating labelled sulphate when challenged with non-radioactive sulphate (Fig. 46) would be either counter transport of labelled sulphate out of the cell or leakage from the cell. Neither of these possibilities is satisfactory, however as first, the same radioactivity loss was seen when cells are resuspended without sulphate so that it is not the result of counter transport. Second, one may assume that accumulation of unlabelled sulphate is proceeding as it would have continued to do had labelled sulphate not been replaced by unlabelled. If this is the case, then loss of radioactivity must represent loss of sulphate which normally occurs throughout sulphate accumulation. The initial rate of radioactive loss approximates to the rate of sulphate accumulation measured at that time. I contend that an accumulation process which is accompanied by a rapid efflux of substrate is unlikely. I know of no reports concerning leakage of large quantities of sulphur-containing compounds from Saccharomyces cerevisiae under these conditions. One possible candidate however, is hydrogen sulphide which has been shown to be produced by yeast under conditions of nitrogen limitation (M. Stratford, personal communication). However this is known to come from sulphite and not sulphate. Most probably the loss of
radioactivity accounts for that sulphate which has progressively become bound to the outside of the cell. One might disagree since dihydrogen phosphate did not behave in the same way (Fig. 47). However, sulphate carries two negative charges whilst dihydrogen phosphate is only singly charged.

Accumulation and retention of calcium ions are in some ways very similar to those for sulphate, but there was no lag period before rapid uptake occurred (Fig. 43). Loss of radioactive calcium ions from populations of Saccharomyces cerevisiae when challenged with non-radioactive calcium ions (Fig. 44), together with the amount of calcium ions accumulated within three minutes of the slow uptake period, almost exactly equalled the amount represented by three minutes of rapid accumulation. For this reason I propose that the initial rapid uptake of calcium ions is a net result of transport and binding to the cell surface.

The experiments performed with calcium ions formed part of a survey of a number of different solutes, to determine whether or not there were any differences between the kinetics of calcium-ion accumulation by oleyl-enriched or linoleyl-enriched cells. They were not an examination of calcium transport as such, although this would be worthy of pursuit. The binding-accumulation relationship was not studied further, or in enough detail to apply to the kinetic data. Kinetic parameters determined were based upon initial rates of calcium-ion retention, and are therefore composite functions. Therefore values quoted for $K_T$ and $V_{max}$ do not have their traditional meaning. Recently, a paper by Borbolla and Peña
(1980) has considered just such problems concerning calcium-ion transport by yeast. By consideration of a binding component a very high affinity system for calcium transport has been revealed.

**Expression of the General Amino-Acid Permease**

Whenever amino-acid transport into *Saccharomyces cerevisiae* is studied, a contribution to that transport by the general amino-acid permease must be considered. *Saccharomyces cerevisiae*NCYC 366, a brewing strain, is not suitable for genetic manipulation. Therefore evidence concerning expression of the general amino-acid permease is indirect. Cells of *Saccharomyces cerevisiae*NCYC 366 which had been grown with L-proline as their nitrogen source accumulated L-arginine more rapidly than cells which had been grown with ammonium ions as their nitrogen source (Fig. 27), and the accumulation was more sensitive to the presence of ammonium ions in the proline-grown culture (Fig. 28). This suggests that the general amino-acid permease was not expressed by cultures of *Saccharomyces cerevisiae*NCYC 366 when grown with ammonium ions. It is not, however, conclusive proof. It is interesting to note that the decreased rate of arginine accumulation obtained when ammonium ions were added to suspensions of proline-grown cells was less than the rate of arginine accumulation by ammonia-grown cells. One must infer that expression of the arginine transport system, perhaps by variation of the peaking time (Section 6; Fig. 39) of the arginine-transport system, is controlled by the available nitrogen source.
As for transport of proline, only a very few experiments were done, and these employed cells which had been grown with ammonium ions as their nitrogen source. Throughout a 60 minute period, the rate of proline accumulation gradually increased (Fig. 41), probably reflecting the induction of a proline-transport system (Magaña-Schwencke and Schwencke, 1969) and not the general amino-acid permease as might have been thought. It should be noted too that the rate of lysine accumulation (Fig. 38) did not increase over a 25 minute period. An interesting consequence of this finding is that the proline-transport system not only has its synthesis repressed by ammonium ions (Schwencke and Magaña-Schwencke, 1969; Magaña-Schwencke and Schwencke, 1967) but has its activity inhibited by ammonium ions (Fig. 42), a fact not referred to by Schwenke and Magaña-Schwencke (1969).

Accumulation of L-Asparagine and L-Glutamine

The literature contains little information on the accumulation of L-asparagine or L-glutamine by *Saccharomyces cerevisiae*. The most interesting is that of Dunlop and Roon (1975) and Dunlop et al. (1976) who, whilst examining the properties of a cell wall-bound asparaginase (asparaginase II), isolated a mutant which was shown to be allelic with other mutants isolated by the methods of Rytka (1975) and therefore designated 'GAP minus'. Cells of the wild type would accumulate L-asparagine, while cells of the mutant would not. This work emphasises a point raised by Grenson et al. (1970) that L-asparagine is transported by the GAP, a point which up till then rested upon the competitive inhibition by L-asparagine of known GAP substrates. However, whilst I have never doubted that L-asparagine could be transported by the GAP, one could infer from
Dunlop and co-workers that L-asparagine is only transported by the GAP.

I examined the kinetics of L-asparagine and L-glutamine accumulation in *Saccharomyces cerevisiae* NCYC 366 under conditions which would not permit expression of the GAP. If one accepts this, then accumulation of L-asparagine must proceed by a transport system other than the GAP.

There are some important points which may clarify the apparent contradiction. Dunlop and Roon (1975) examined L-asparagine accumulation by nitrogen-starved stationary-phase cells, and I used mid to late exponential-phase cells. In *Saccharomyces cerevisiae* NCYC 366, accumulation of L-lysine has been shown to depend on the age of the culture, a fact agreed by John Woodward (personal communication), who has shown a similar culture-age dependency for proline transport and for the GAP. It is not unreasonable to suppose that the system responsible for asparagine transport could display a similar culture-age dependency, and would therefore not be detectable in stationary-phase cells. Indeed more recent work has shown that asparaginase II activity varies throughout batch growth (Pauling and Jones, 1980). Another point which must be borne in mind is that the mutant isolated by Dunlop and Roon (1975) which could not transport L-asparagine and was subsequently stated to be GAP negative, might have contained a second mutation for L-asparagine transport. This possibility is quite attractive because the lone selective pressure used to isolate the mutant was resistance to D-asparagine when grown with proline as the main nitrogen source. One might speculate further and
suggest that regulation of the putative L-asparagine transport system is such that it is only expressed in the presence of ammonium ions. Therefore, under conditions of nitrogen depletion as were used to induce asparaginase II activity, the transport system would not be detectable. A prediction of this model is that the system would not transport D-asparagine.

In *Saccharomyces cerevisiae*NCYC 366 there is a possibility that asparaginase II could interfere with L-asparagine transport in two ways. It could lower the concentration of L-asparagine available for the transport system which would mean that kinetic data were calculated at incorrect L-asparagine concentrations. Alternatively, its activity could give rise to sufficient L-aspartic acid such that accumulation by the dicarboxylic amino-acid transport system would become significant. The magnitude of both effects hinges upon the asparaginase II activity. The method used to measure free aspartic acid would have detected an asparaginase II activity of as little as 1 nmol (mg dry wt)$^{-1}$ min$^{-1}$. Even at a concentration of 1 mM L-asparagine no such rate was found and therefore the contribution asparaginase II may have made to L-asparagine transport was negligible.

**Plasma-Membrane Lipid Unsaturation and Proton Movements in *Saccharomyces cerevisiae***

It had been established that the kinetics of lysine and arginine accumulation by *Saccharomyces cerevisiae* were sensitive to changes in plasma-membrane lipid unsaturation. This raised the question of whether the energy coupling for these transport systems was altered in any way. Initial experiments were to find out whether changes in
composition of the plasma membrane altered its permeability to protons. The very similar acid influx and efflux rates obtained with oleyl- and linoleyl-enriched cells suggested that there were no differences between them in this respect (Section 7.1 of the Results). But it was also possible that the proton stoichiometrics had been altered for these uptake systems.

In the presence of 2-deoxyglucose, anaerobic preparations of *Saccharomyces cerevisiae* NCYC 366 showed a decrease in acid influx rate when lysine was accumulated, and this decrease was equivalent to the rate of lysine accumulation. This was contrary to expectation, as previous reports (Seaston et al., 1973; Cockburn et al., 1975) had shown that lysine was accumulated by *Saccharomyces carlsbergensis* NCYC 74 depleted of ATP in association with one or two proton equivalents, depending on the transport system involved. On the other hand, Eddy and Nowacki (1971) had shown that, in the absence of the metabolic inhibitors, antimycin A and 2-deoxyglucose, preparations of yeast cells showed a net acid excretion when lysine was accumulated. The mechanism of this excretion is not understood, but is thought to involve the cycling of protons. In energy-depleted yeast lysine proton absorption is balanced electrically by a simultaneous efflux of potassium ions (Eddy and Nowacki, 1971), a movement which is driven by absorption of protons. How might the known facts be used to account for the behaviour of *Saccharomyces cerevisiae* NCYC 366?

At pH 4.5, lysine carries one net positive charge; electically therefore it is equivalent to one proton. Throughout lysine accumulation, the rate of positive charge influx (lysine together with protons) was equivalent to that of the rate of positive charge influx
(just protons) immediately before lysine accumulation. I contend that the limiting factor could be the rate at which another positively charged species, potassium ions for example, could flow out of the yeast cell. Potassium-ion movements were not measured. However in Section 5.3 of the Results, accumulation of arginine, a molecule not unlike lysine, was inhibited in the absence of an energy source by extracellular potassium ions, indicating perhaps a regulatory role for this ion. On the other hand, proton ionophores (Section 5.4 of the Results) could completely prevent arginine accumulation, demonstrating a dependence on the proton gradient across the plasma membrane. With this in mind, one cannot reject the possibility that some proton cycling is still occurring in the presence of 2-deoxyglucose. If this was so then the results obtained would not represent the true quantitative movement of protons. However, *Saccharomyces cerevisiae* with plasma membranes enriched in oleyl or linoleyl residues responded in an identical manner with respect to proton movements. For this reason this line of approach was not continued.


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APPENDIX
PLASMA-MEMBRANE LIPID UNSATURATION CAN AFFECT THE KINETICS OF SOLUTE ACCUMULATION BY SACCHAROMYCES CEREVIAE

M.H.J. KEENAN and A.H. ROSE
Zymology Laboratory, School of Biological Sciences, Bath University, Claverton Down, Bath BA2 7AY, Avon, England

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1. Introduction

Active transport of solutes has been studied in a variety of eukaryotic micro-organisms, including several species of yeast [1]. These studies have concentrated largely on two aspects of the transport process, namely the multiplicity and specificity of the systems involved, and the manner in which the transport processes are energized. Active transport of sugars and amino acids [2,3] into yeasts has been shown to involve several different carrier systems, which exhibit a wide range of specificities and substrate affinities [2]. Accumulation of inorganic cations and anions by yeasts has been less extensively studied, although it appears that the transport systems involved may be fewer in number and more specific [4]. There is evidence that accumulation of sugars, amino acids [5-8] and other solutes, including phosphate [4], by yeasts is energized not directly by ATP but instead in response to an electrochemical gradient of protons formed across the plasma membrane [9,10]. Van Steveninck’s suggestion [11] that accumulation of certain sugars involves group-translocation, in which the sugars are phosphorylated, is inconsistent with the data reported by Eddy and his colleagues [3] and by Kotyk and Michaljaničová [12].

Nothing has so far been reported on the role of plasma-membrane lipids in transport of solutes into yeasts. The present paper describes the effect of plasma-membrane lipid unsaturation on accumulation of arginine by Saccharomyces cerevisiae. To effect specific alterations to the lipid composition of the yeast plasma membrane, the study exploited the anaerobically induced requirement of S. cerevisiae for an unsaturated fatty acid and a sterol [13,14] both of which requirements are fairly non-specific [15,16], as described by Hossack and Rose [17].

2. Materials and Methods

S. cerevisiae NCYC 366 was maintained on slopes of malt extract-yeast extract-glucose-mycological peptone-agar [18]. The yeast was grown anaerobically in medium (1 litre) supplemented with ergosterol (5 mg/l) and either oleic or linoleic acid (30 mg/l) as previously described [19]. Cells were harvested from mid exponential-phase cultures (0.24-0.26 mg dry wt/ml) by centrifugation, the centrifuge tubes and headspaces being flushed with high-purity nitrogen gas from which the last traces of oxygen had been removed by a Nilox scrubber [19]. After washing with water, cells were suspended in 10 mM Tris-citrate buffer (pH 4.5) at 15 mg dry wt/ml. During the course of an experiment, the suspension of washed cells was kept in an ice-water mixture. The headspace in the tube was flushed with oxygen-free nitrogen gas. Over a period of up to 5 h, the viability of the suspension, as judged by plate counts [18], did not change. The reaction mixture (usually 15 ml) used to measure the rate of arginine accumulation consisted of 10 mM Tris-citrate buffer (pH 4.5) containing 0.5 mg dry wt cells/ml, 100 mM glucose and the stated concentration of solute. The suspension was contained in a round-bottomed flask (100 ml; Quickfit) fitted with two glass-stoppered sampling ports. Initially, Tris-citrate buffer containing glucose
was added to the flask and the solution stirred magnetically at 30°C. The inside of the flask was continually flushed with oxygen-free nitrogen gas. A portion (0.5 ml for 15 ml reaction mixture) of cell suspension was added to the flask, the contents of which were allowed to equilibrate for a further 5 min. The appropriate volume of a solution of L-arginine, containing L-[U-\(^{14}\)C]arginine (0.05 \(\mu\)Ci/\(\mu\)mol arginine; chromatographically pure; Radiochemical Centre, Amersham, England), was then added to the flask. Samples (1 ml) were removed from the suspension at predetermined time intervals, rapidly filtered through membrane filters (0.45 \(\mu\)m pore size; 25 mm diam.; Millipore) and the filter and cells immediately washed with ice-cold Tris-citrate buffer (10 ml) containing L-arginine at the concentration used in the cell suspension. Filters were transferred to scintillation vials containing Unisolve liquid scintillator No. 1 (Koch-Light, Colnbrook, Bucks, England; 8 ml). The radioactivity of the contents of vials was measured in a Philips liquid scintillation analyser, and the value for counts corrected for counting efficiency.

3. Results

Cells grown anaerobically in the presence of oleic or linoleic acid have plasma membranes enriched to the extent of approx. 54% of the total with the exogenously provided fatty-acyl residue [20]. Plots of amount of arginine accumulated against time were linear over the first 20 min for cells grown in the presence of either fatty acid and suspended in buffer containing arginine up to 1.0 mM. However, plots obtained using suspensions containing 5–200 mM arginine showed that the rate declined with time, the more so the higher the concentration of arginine. Plots of the initial velocity of accumulation against arginine concentration were biphasic for cells enriched in either fatty-acyl residue (Fig. 1). However,
the curves differed in shape, indicating different kinetics for arginine accumulation in the two types of cell. Woolf-Hofstee plots [21] showed that cells enriched in oleyl residues had a high-affinity accumulation system (apparent $K_m$ $2 \times 10^{-5}$ M; apparent $V_{max}$ 2 nmol min$^{-1}$ mg dry wt.$^{-1}$) and a lower affinity system (apparent $K_m$ $2.1 \times 10^{-2}$ M; apparent $V_{max}$ 37 nmol min$^{-1}$ mg dry wt.$^{-1}$). Cells enriched in linoleyl residues had an accumulation system with an apparent $K_m$ of $1.3 \times 10^{-3}$ M (apparent $V_{max}$ 5.5 nmol min$^{-1}$ mg dry wt.$^{-1}$) and another with an apparent $K_m$ of $9.5 \times 10^{-5}$ M (apparent $V_{max}$ 220 nmol min$^{-1}$ mg dry wt.$^{-1}$).

Radioautography of paper chromatograms of boiling-water extracts of cells, enriched in either fatty-acyl residue and containing $[^1^4C]$arginine, revealed that the only radioactively labelled compound in the extracts cochromatographed with arginine.

The effect of the ionophores 2,4-dinitrophenol (DNP) and carbonylcyanide m-chlorophenylhydrazzone (CCCP) was studied by incorporating the reagent in cell suspensions at 1.0 mM for 10 min at 30°C before a portion was added to the reaction mixture, or by adding the compound to a reaction mixture (final concentration 1.0 mM) after 6 min incubation. Both compounds had identical inhibitory effects on the rate of arginine accumulation in mixtures containing either 200 $\mu$M or 50 mM arginine.

Preliminary experiments have shown that the kinetics of accumulation of L-lysine differ in cells enriched in oleyl and linoleyl residues. On the other hand, accumulation of phosphate ions shows similar kinetics in cells enriched in either residue.

4. Discussion

Chan and Cossins [22] have reported that a strain of S. cerevisiae, grown aerobically in medium containing ammonium ions, possessed two systems for accumulation of arginine. The apparent $K_m$ and $V_{max}$ values reported for these systems were similar, although not identical, with those obtained in the present study for cells enriched in oleyl residues. Chan and Cossins [22] did not report on the lipid composition of their strain of S. cerevisiae but, from data reported by other workers [23], it would probably have C$_{16:1}$ and C$_{18:1}$ as the major fatty-acyl residues. Our data show, however, that the kinetics for arginine accumulation are considerably altered following introduction of doubly unsaturated fatty-acyl residues into the yeast plasma membrane although both uptake systems responded identically to DNP and CCCP.

Although the possibility that synthesis of transport proteins is affected by the fatty-acyl composition of the plasma membrane cannot be eliminated a more likely explanation is that activity of membrane-bound transport proteins is influenced by the nature of the surrounding fatty-acyl residues. The effect could result from the greater fluidity in membranes containing the doubly unsaturated residue. Alternatively, the effect could be more specific, with activity of the high-affinity system requiring the presence of monounsaturated fatty-acyl residues around the proteins involved. There are numerous reports in the literature of a requirement for lipids by various enzymes [24]. Few of these reports have described the effect of lipids on the kinetics of the enzyme reaction concerned, but where it has been studied [24,25] the conclusion has been that lipids probably do not affect the conformation of the protein.

Finally, since intracellular vesicles have been shown to concentrate amino acids in S. cerevisiae, particularly basic amino acids [26], the effects of fatty-acyl unsaturation on apparent $K_m$ and $V_{max}$ values reported herein could well be the result in part of changed rates of accumulation of arginine in intracellular vesicles.

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