external methyl α -glucoside. Specificity studies, as measured in competition experiments, indicate that glucose, 2-deoxyglucose, 6-deoxyglucose and galactose are transported by the same system. Independent experiments, based on the fact that 2-deoxyglucose, 6-deoxyglucose and galactose are substrates for glucose dehydrogenase, confirm that these substrates are taken up by whole cells. These findings support the claim by Romano *et al.* (1970) that the phosphoenolpyruvate-dependent phosphotransferase system is not involved in glucose transport in this organism. Isotopic experiments with both 2-deoxyglucose and galactose have shown that these compounds are actively transported into glucose-grown cells. In both cases the major intracellular material was the unaltered substrate, although their hexonic acid derivatives could be detected extracellularly. Our results are contradictory to those of Eagon (1971), who has proposed that 2-deoxyglucose is not actively transported but enters by passive diffusion, the concentration gradient being maintained by the intracellular activity of glucose dehydrogenase. However, we have also shown that mutants defective in glucose dehydrogenase exhibit unimpaired 2-deoxyglucose transport. These discrepancies can, we believe, be explained on the basis of the cold-shock phenomena described by Farrell & Rose (1968), since previous investigations have not taken this into account.

Both the concentration and the activity of the methyl α -glucoside transport system have been shown to be under metabolic control. Succinate and gluconate inhibit the activity of the transport system, the nature of the inhibition suggesting that it is due to some metabolic product. Uptake is severely repressed in citrate- and succinate-grown cells.

These results will be further discussed.

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Transport of Non-Electrolytes in Yeast

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In spite of the fact that various yeast species have been exploited for thousands of years in generally beneficial fermentation processes and that some have recently proved their economic worth as producers of protein from waste water and even from petroleum, our exact knowledge of the ways they take up their carbon and nitrogen substrates is rather meagre. The greatest amount of information by far is available from baker's yeast, *Saccharomyces cerevisiae*, but some heuristically significant findings have been made in other species, like *Saccharomyces carlsbergensis* and *Rhodotorula glutinis*.

The uptake of materials by yeasts does not proceed to any significant extent by simple diffusion, there being in all the cases examined specific receptors residing in the outer membrane, the plasmalemma, which, either themselves or in concert with other membrane components, translocate the substrate into the cytoplasm. This is so for the uptake of both monosaccharides and amino acids, the two classes of non-electrolytes studied in some detail. However, other features of the transport process differ rather fundamentally, and they are dealt with separately below.

Monosaccharides are transported apparently by systems of rather broad specificity (Cirillo, 1961), although, even so, two or more may exist in a given species (Kotyk, 1967). The systems may be either constitutive or inducible by growth or incubation with a suitable inducer (Cirillo, 1968; Kotyk & Haškovec, 1968). Whereas in the genus *Saccharomyces* monosaccharides are taken up by a process of facilitated diffusion (although some phosphorylation during the membrane passage may take place in the case of D-glucose and other, closely related, sugars; van Steveninck, 1969), in other yeast species the process is a typical primary active transport, proceeding apparently in coupling with an exergonic metabolic reaction, but without interaction with an active transport, say, of ions (Kotyk & Höfer, 1965; Deák & Kotyk, 1968). All these uphill transports are distinguished from the 'permease' transport systems in bacteria by their extremely tight coupling with the energy-producing reaction. In fact, there is no reliable way of suppressing the 'uphill' part of the process without blocking transport altogether (Höfer & Kotyk, 1968). It may be envisaged that the movement itself of the carrier is metabolically linked. Another distinguishing feature, even with active transports, are the relatively high half-saturation constants (at least 10^{-4} M), implying relatively low affinities for substrate. This drawback is somewhat

compensated for by the rather high maximum rates of uptake.

The uptake of amino acids proceeds invariably uphill by processes governed by permeases with relatively narrow specificities, even if they overlap from case to case (see, e.g., Grenson, 1967). Thus any amino acid is transported by more than one membrane agency. The steady-state intracellular/extracellular concentration ratios may attain values of 1000:1, and here, too, the transport in most species studied appears to proceed without obligatory coupling with the transport of another molecule or ion (Kotyk *et al.*, 1971), although Eddy *et al.* (1970) find stoichiometric relationships between the transport of glycine and K^+ in *Saccharomyces carlsbergensis*. The most striking feature of amino acid transport (in all the species studied) is the complete absence of efflux from the major pool (cf. Eddy, 1963). This may obviate the use of metabolic energy for maintaining the intracellular amino acid pool, by simply making the carrier molecule (apparently through a change of conformation) incapable of carrying substrate outward. In addition to this lack of efflux, the cells possess a regulatory mechanism that diminishes the influx at high intracellular amino acid concentrations [cf. Ring *et al.* (1970) for *Streptomyces hydrogenans* and Pall (1971) for *Neurospora crassa*]. This regulatory 'trans' inhibition does not affect the same site as that involved in uptake, there being both qualitative and quantitative differences in the activity spectra of various amino acids (Kotyk & Říhová, 1972).

Hence it appears that the molecular mechanism of the uptake process is more complex than a mere sequence of binding-translocation-dissociation. This complexity is displayed both 'across' the membrane, as a result of the various regulatory influences (e.g. the negative effect of glucose 6-phosphate on sugar uptake in baker's yeast; Azam & Kotyk, 1969) and of possible participation of enzymes catalysing the binding and/or the dissociation of the substrate from the carrier proper, and 'along' the membrane, where interactions between the transport systems for different classes of non-electrolytes, e.g. amino acids and sugars (Poncová & Kotyk, 1967) or sugars and polyols (Haškovec, 1971), have been found.

This complexity, together with the relatively low affinities, hindered attempts to isolate the essential components of the transport systems from yeasts, in contrast with the work in bacteria (see Heppel, 1969). So far, a galactose-binding fraction has been isolated from yeast plasmalemma by Haškovec & Kotyk (1969) and a pure membrane component showing affinity for glucose-type sugars has since been obtained from solubilized yeast membranes in this laboratory. It is a lipoprotein with a molecular weight of 10^5 , the lipid part containing ergosterol, phosphatidylethanolamine, phosphatidylcholine and

a trace of lysophosphatidylcholine. It has a dissociation constant with glucose of more than $10^{-3}M$, and also binds D-fructose, L-sorbose, D-xylose and D-arabinose, but not L-arabinose and L-alanine (Horák & Kotyk, 1972). Its function in the transport process *in vivo* has yet to be investigated.

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Some Applications of the Ion-Gradient Hypothesis to the Problem of the Mechanism of Amino Acid Transport

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The possibility that mouse ascites cells may concentrate amino acids from a Ringer solution, not by utilizing ATP directly, but by making use of the energy inherent in the ionic gradients of Na^+ and K^+ that are normally present across the plasmalemma, was first suggested by Riggs *et al.* (1958). Similar models, in which Na^+ and perhaps K^+ are co-substrates with a specific amino acid or carbohydrate, which accumulates without being chemically transformed, have since proved applicable both to pigeon erythrocytes and to various other mammalian systems such as the intestine. Alternative explanations involving a direct link between the hydrolysis of ATP