generation by sugar efflux and variable proton sugar stoichiometries.

**Energy-generating sugar efflux during the steady state**

The net uptake of a non-metabolized glucose analogue is linear for a few minutes and then slowly starts to level off to a plateau value because the influx of sugar is balanced by sugar efflux which is mainly catalysed by the sugar transport system. The rate of sugar exchange is very high and can be twice that of the net uptake rate. Despite this high sugar transport rate there is apparently a lowered energy demand for transport, since the respiratory increase per sugar transported is only half of the value during net influx conditions (Decker & Tanner, 1972). It was concluded that efflux of sugar partly generates 'energy', to 50% in aerobic conditions and up to 90% in anaerobic conditions (Komor et al., 1973). The mechanism, explained in present terms, is that a proton is transported out of the cells, at 50–90% probability, together with sugar during the steady-state situation.

The exit of a proton against the adversely oriented proton-motive potential difference at a high rate can be explained as a failure of the proton to dissociate fast enough on the internal side. As a result the exchange of sugar might occur and a back-shuttle of the proton–sugar complex over the small intra-membrane distance of the gate (Fig. 2) could occur without energetic problems. The slow association and dissociation of proton on the binding site or its access through the pore is a prerequisite for this interpretation.

**The proton/sugar stoichiometry**

For glucose and some glucose analogues such as 6-deoxyglucose the proton/sugar stoichiometry is 1. But there are significantly higher values for some analogues e.g. 1.5 for 3-O-methylglucose and 2 for 1-deoxyglucose, though all these sugars are transported by the same transport system. Since it seemed incomprehensible to assume a continuous variation in proton stoichiometry in the sense that the proton-binding site will accept different numbers of protons or even a fractional number, another explanation was sought. It was found that the stoichiometry of proton per sugar transport system was the same for all hexoses, but for 1-deoxyglucose there was an incomplete coupling of sugar to proton translocation, so that at a 50% probability the proton was translocated without sugar (Grüneberg & Komor, 1975). The appearance of unoccupied sugar-binding sites on the cytoplasmic side was measured as the rate of sugar efflux via the transport system. Thus the rate of 6-deoxyglucose efflux out of preloaded cells caused by 1-deoxyglucose addition was twice that of 1-deoxyglucose influx. This interpretation implies that the actual translocation step is fast compared with proton dissociation or another regulating step before translocation, which is easily explained if the translocation distance is short.

**Sugar transport by plants**

It is premature to generalize properties of the *Chlorella* system to other plants or even bacteria and animals, though some features seem to be of general importance.

The occurrence of proton as co-ion in sugar transport by plants reminds one of bacterial systems, whereas the generation of the ion-motive potential by a specially devised ATPase resembles animal systems.

There is an increasing body of evidence for the central role of proton–sugar symport in higher plants (*Ricinus*, maize, sugar cane) for cells and tissues which serve for long-distance translocation of sugar or storage (Komor et al., 1977; Heyser, 1980; E. Komor, M. Thom & A. Marettzi, unpublished work). A similar type of transport might also occur at the vacuolar membrane.


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**Active transport of peptides in bacteria**

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At first sight, peptides seem to be ideal substrates to use for investigating the fundamentals of active-transport processes in bacterial. Three useful features may be identified. First, it is relatively easy to produce peptides of different size, composition, and sequence for use in mapping the structural requirements of a membrane carrier. Second, peptides can both supply preformed amino acids for protein synthesis and act as sources of carbon and nitrogen, making it likely that their transport systems will be subject to a variety of regulatory influences. Third, at physiological pH values, different peptides may be neutral, cationic, or anionic, a feature that is of particular interest in studies of energy coupling to transport.

**Methods of measuring peptide transport**

In spite of these features, peptides have been used by few investigators of transport processes. The explanation for this seems clear: commercial radioactively labelled peptides are generally unavailable and it is not easy therefore to study transport in the conventional way by measuring radioactivity accumulated by bacteria. This difficulty has meant that, until recently, information on peptide uptake in micro-organisms has come mainly from experiments in which the utilization of peptides by amino acid auxotrophs was monitored. This indirect way of studying transport, which allows only limited characterization of the process, has recently been critically appraised (Payne, 1980). Results obtained with bacteria, yeast and other fungi, animals and higher plants are given in several recent reviews (Payne, 1976, 1980; Payne & Gilvarg, 1978; Becker & Naider, 1980; Matthews & Payne, 1980; Wolfinbarger, 1980). To try to overcome the particular methodological problems in studying peptide transport, we have developed two fluorescence techniques that allow the transport of any peptide to be measured directly. In both procedures, peptide uptake is assessed by measuring the rate at which substrates are removed from the medium. In the first (Payne & Bell, 1979), the incubation medium is sampled periodically,
filtered to remove the micro-organisms, and samples of the filtrate made to react with dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride. The dansyl derivatives are separated by two-dimensional t.l.c. and their amounts determined from the intensities of the fluorescent spots. Substrates that are accumulated intracellularly can be determined similarly by using cell extracts. In the second method (Nisbet & Payne, 1979), samples of the filtered incubation medium are made to react with fluorescamine to give fluorescent peptide derivatives that are quantified from the fluorescence of the solution. This latter method has been automated so that it is now possible to monitor transport continuously (J. W. Payne & T. M. Nisbet, unpublished work). Thus, by using a pumping system, the incubation mixture is continuously sampled and filtered to remove the micro-organisms, the filtrate is made to react with fluorescamine and assayed by using a fluorimeter equipped with a flow-cell and recorder.

**Amino acid and peptide exodus**

When whole cells are used to study transport, one is always aware of the potential influence of cellular metabolism on the transport of substrates, and this is well illustrated with some of the substrates. Thus, when the dansyl procedure is used to analyse the incubation medium during peptide uptake by bacteria (e.g. *Escherichia coli*, *Salmonella typhimurium*, *Streptococcus faecalis*), it is observed that the peptides are rapidly hydrolysed within the bacteria and, immediately, extensive exodus of the cleaved amino acid residues occurs (Payne & Bell, 1979). Peptide uptake proceeds to completion concomitantly with continuous amino acids exodus. However, this exodus is not uniform, being more extensive for some residues (e.g. glycine and alanine) than for others (e.g. tyrosine and tryptophan), whereas no exodus is seen for some residues (e.g. glutamic acid, glutamine, asparagine and serine); metabolically related molecules may also undergo exodus (Payne & Bell, 1977, 1979). This combination of selective exodus and continuous peptide transport appears to be nutritionally more efficient than regulating peptide uptake. In contrast with the observations in bacteria, no comparable exodus is seen with the yeast *Saccharomyces cerevisiae*, presumably because of its greater capacity to store free amino acids (Nisbet & Payne, 1979).

Recently, exodus of peptide residues has also been observed (J. W. Payne, unpublished work). It occurs when only partial peptide uptake is possible. Thus, in *E. coli*, the rates of exodus of L-Ala-L-Ala-D-Ala, L-Val-L-Val-D-Val, and Gly-Gly-D-Leu are about 40, 20 and 25% of the rates for the corresponding L-stereoisomers. The peptide bonds involving the C-terminal D-residues are resistant to hydrolysis and consequently intact L-Ala-D-Ala, L-Val-D-Val, and Gly-D-Leu accumulate within the cell (it should be noted that uptake of these free dipeptides is undetectable); as tripeptide uptake continues, these dipeptides and the respective N-terminal amino acids undergo selective exodus. In the second example, by using peptidase-deficient mutants of *S. typhimurium* (Miller & MacKinnon, 1974), tripeptides are readily taken up but some are only partly cleaved and the unhydrolysable dipeptide fragments undergo exodus. In strain TN 273 pepN pepD pepB, uptake of Met-Leu-Gly, Gly-Pro-Ala, and Gly-Leu-Gly leads to exodus of Leu-Gly, Pro-Ala, and Leu-Gly respectively, and, in strain TN 272 pepN pepD pepB, uptake of Leu-Gly-Gly, Gly-Pro-Ala, and Ala-Gly-Gly leads to exodus of Gly-Gly, Pro-Ala and Gly- Ala respectively. In several instances, the presence of a significant intracellular pool of intact dipeptide inhibits growth. These observations provide a useful tool with which to investigate differences between the mechanisms of uptake and exodus. Uptake of these tripeptides is by the oligopeptide transport system ('opp') and it seems likely that dipeptide exodus may be largely via the dipeptide permease ('dpp'); studies using opp+ dpp- stains should be particularly helpful in this connection.

**Disadvantages of using radioactive peptides for transport studies**

The rapid intracellular metabolism and selective amino acid exodus that accompanies peptide uptake seems likely to invalidate many conclusions based solely on conventional assays that measure radioactivity accumulated by bacteria from radioactively labelled peptides [see Payne (1980) for discussion]. We have confirmed this expectation in several instances. For example, Fig. 1 shows an analysis of Ala-[14C] Ala uptake by *E. coli* based on conventional radiotracer techniques and the manual fluoroscience assay (Nisbet & Payne, 1979). The latter assay reveals the steady, continuous transport of peptide at a rate of about 52 nmol min⁻¹ mg of protein⁻¹. In contrast, monitoring radioactivity within the cells shows an initial rapid uptake that apparently slows within about 30 s and quickly reaches a plateau value. Various conclusions might normally be made from this type of kinetic curve. In fact, knowing that alanine exodus is occurring even within the first 30 s, it is clear that, after initial mixing with the intracellular pool of non-radioactive alanine, the rate of [14C]alanine exodus quickly approaches the rate of dipeptide uptake; when these rates become equal, the resultant apparent steady state is actually one of dynamic flux. Subsequently, the rate of exodus may exceed uptake and the radioactivity within the bacteria declines. We have found exactly similar results in *Strep. faecalis*. In a second example, uptake of [14C]Gly Phe and Gly-[14C]Phe by *E. coli* was studied under similar conditions to those of Fig. 1. Fluorescamine assay gave (not surprisingly) the same rate of uptake (30.5 nmol min⁻¹ mg of protein⁻¹) for the two labelled substrates. By using the radiotracer method, initial rates of 12 and 23 nmol min⁻¹ mg of protein⁻¹ were obtained for [14C]Gly-Phe and Gly-[14C]Phe respectively, and the kinetics of
uptake for these substrates during a 3-min incubation were apparently markedly different. The uptake rate is apparently lower when labelled glycine is used, partly because of greater loss of label to $^{14}$CO$_2$ and also because of the relatively more rapid exodus of glycine than phenylalanine. Perry & Abraham (1979) have reported analogous findings with Staphylococcus aureus. It appears to be a general finding, therefore, that the use of radioactively labelled peptide substrates can provide misleading results on the kinetics of transport in bacterial cells.

Stoichiometry of energy coupling for peptide transport

In Strep. faecalis, glycolysis is the sole source of metabolic energy, oxidative phosphorylation and respiration are absent, and a proton-translocating ATPase generates the proton motive force across the membrane (Harold, 1977). Furthermore, after incubation in buffer alone at 37°C for about 10min, its endogenous energy reserves are depleted. These features make this organism very suitable for studies of energy coupling to transport. We find that peptide transport in Strep. faecalis is dramatically inhibited by arsenate, dinitrophenol, and dicyclohexylcarbodi-imide. In cells starved for energy as described above, peptide transport can be made absolutely dependent on added glucose, and by studying how the amount of peptide transported varies with added glucose, the energy consumption for transport can be measured. By using a range of peptides, an apparent transport stoichiometry ($TS_p$) of 0.15–0.40 mol of peptide transported/mol of glucose is found. This apparent stoichiometry is a marked underestimate of the real efficiency, because there are other endogenous energy-utilizing processes occurring. The rate at which these processes consume the added glucose in the absence of peptide transport can be measured by adding the glucose first, and at various times thereafter adding peptide and measuring the amount accumulated. This experiment shows that the amount of peptide transported decreases linearly with the period of prior glucose incubation, meaning that glucose is consumed endogenously at a constant rate (about 450 nmol·min$^{-1}$·mg of protein$^{-1}$). These findings explain the observed linear relationship between $TS_p$ and rate of transport; the slower the uptake the smaller is $TS_p$. We also find that when the cells are transporting peptides, glucose is consumed more quickly, indicating that transport takes a significant proportion of the available energy. Therefore, on the assumption that the endogenous rate of energy consumption is maintained during peptide transport, a true transport stoichiometry ($TS_t$) could be obtained. This (probably oversimplified) assumption requires investigation before the true efficiency of the transport process can be calculated, and at the present time it seems as if the continuous fluoroscamine method is the only way to study this. Finally, although $TS_t$ has not yet been determined, it is clear that it is the same for di, tri, tetra and penta-peptides, an important conclusion that emphasizes the efficiency of peptide transport vis-à-vis amino acid transport.

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