MEMBRANE TRANSPORT 806

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INTRODUCTION

The presence of a large variety of more or less specific transport systems in the cytoplasmic membrane of living cells has long been established. It is generally believed that these transport systems are responsible for the relative constancy of the internal environment of the cell.

The transport systems for mammalian tissues appear to be constitutive and firmly bound to the membrane. In general the transport systems for most of the amino acids in microorganisms are constitutive, while for sugars only glucose transport activity appears to be constitutive (Kepes 1). The rest of the sugar transport systems are inducible. There seems to be a correlation between the ability of an amino acid to serve as a carbon or nitrogen source and the presence of additional transport systems that are inducible. A complete understanding of the molecular basis and the biological function of membrane transport will have to include the isolation and description of each component of the transport system. In recent years there has been an increased interest in the isolation and characterization of components of the transport systems.

Earlier kinetic studies using animal cells provided evidence for membrane mediation of solute transport (Christensen 2). The nature of the membrane components, which have been termed "carriers," is not well defined. These kinetic studies have, however, provided us with a description of the number and kinds of transport systems present in the membrane as well as a basis for recognition of the receptor sites.

In addition to the studies with animal cells the existence of transport mutants particularly in microorganisms, provides evidence that one or more components of the transport system is presumably protein in nature.

Combining the various approaches used in the past, several investigators are now isolating membrane proteins which retain receptor sites for the transported solutes.

The reader is referred to the following review articles written in the last few years on various aspects of membrane permeability; bacterial cells—Cohen & Monod (3), Kepes (1), Kepes & Cohen (4), Pardee (5), Kennedy (6), Kaback (7, 8), Lin (9, 10), Oxender (11, 12), Heppel (13, 14), Roseman (15, 16), Simoni (17); transport in mammalian cells—Heinz (18), Schultz & Curran (19); special kinetic problems—Christensen (20); broad coverage or application—Christensen (2), Hoffman (21), Stein (22), Tosteson (23), Kotyk & Janácek (24), and Hokin (25).

An extensive review of the transport mutations in man has been prepared by Hechtman & Scriver (26). Table 1 of that review lists the bacterial transport systems similar to those found in humans and Table 2 lists the transport mutations that have been identified for kidney and intestinal tissues. Peptide transport in microorganisms has been reviewed by Payne & Gilvarg (27) and Sussman & Gilvarg (28). It is impossible to review adequately the entire area of membrane transport in the space available; therefore, this review will be restricted to certain areas of research where the more indirect kinetic examination of transport is now

being combined with genetic and biochemical approaches. This combined approach has in the last few years greatly improved our understanding of the molecular basis of membrane transport.

General Considerations

Terminology.—In this review the term transport system will be used to refer to the total process of solute accumulation. Operationally an active transport system carries out a three-step process: binding of a solute to a receptor site, translocation of the complex across the membrane, and the coupling of the process to metabolic energy. The term *facilitated diffusion* will refer to the process of solute uptake where energy coupling (the third step in the transport process) either does not occur under physiological conditions or has been eliminated as a result of experimental manipulation.

The controversial term permease is in widespread usage with a variety of meanings. It was originally proposed to refer to the entire transport system (Cohen & Monod 3). More recently it was suggested that the stereospecific protein component be called a permease and the entire process be referred to as a permease system (Kepes & Cohen 4). One difficulty with the use of this term is the tendency to assume that a single protein is responsible for transport. The transport of sulfate into *Salmonella typhimurium* appears to involve four different gene products, one of which is the sulfate-binding protein (Dreyfuss & Pardee 29, Ohta, Galsworthy & Pardee 30). Since most transport systems appear to consist of a number of undefined protein components it would seem desirable to use more descriptive terms for the individual components as their functions become known and reserve the term permease to refer to the entire transport system. A more extensive discussion of the concept of the permease appears in a review by Kennedy (6).

Strain differences.—In comparing results obtained from various laboratories, differences in strains and growth conditions must be taken into account. This is particularly true for transport systems that are inducible, repressible, or otherwise subject to regulation. The study of galactose transport in *Escherichia coli* is complicated by the existence of three or four transport systems, at least three of which are inducible, each having different substrate and inducer specificities (Rotman, Ganesan & Guzman 31). The level of galactose transport in this organism appears to be influenced by endogenous induction in strains that are missing galactokinase (*galK*) or in regulatory mutants (*galR*) for the galactose operon. In addition, one or more of the four transport systems may be either constitutive or only partially inducible in a given strain (Lin 9, Lengeler et al 32).

In our study of the leucine transport system in *E. coli* K12 ATCC14948 which is repressed by certain amino acids, we found that the capacity to transport L-leucine was three to four times greater in glucose-grown cells than in glycerol-grown cells (84). Furthermore, with glucose-grown cells, glucose had to be added to the uptake medium to achieve the maximum rate of uptake of leucine.

The level of the constitutive and specific transport system for L-tryptophan in

E. coli which has been reported by Brown (33) appears to be subject to strain difference. Yanofsky (personal communication) has found that the level of the tryptophan-specific system in *E. coli* K12 (W3110) is four to five times that found in *E. coli* K12 (W1485). The increased uptake activity renders the W3110 strain more sensitive to the tryptophan analogue 4-methyltryptophan and provides a convenient method for selecting transport mutants for tryptophan.

The above illustrations serve to emphasize the importance of identifying the history of the bacterial strains being used and reporting the precise experimental conditions surrounding their use. Comparisons of the levels of a transport system or the influence of mutations on transport are therefore most meaningful for isogenic strains.

Inorganic Ion Transport

Sodium and potassium.—The important relationships between the alkali metal ions and the physiology of animal cells has long been recognized. The active transport of sugars and amino acids into many animal tissues has been shown to require the proper distribution of sodium and potassium ions. This subject will be discussed later in the section on energy coupling to achieve active transport.

Most animal cells have an active Na^+-K^+ transport system which allows them to maintain high levels of intracellular potassium ions and low levels of the sodium ion. Experimentally, the inward flux of K^+ is linked to the outward flux of Na^+, and both are inhibited by cardiac glycosides such as ouabain. Cell membrane preparations contain an adenosine triphosphatase activity which requires both Na^+ and K^+ and is inhibited by ouabain and other cardiac glycosides. Secretory tissues whose function calls particularly for alkali metal transport are rich in this ATPase. Attempts to solubilize the (Na^+-K^+) ATPase have met with extreme difficulty. Recently a lubrol-solubilized (Na^+-K^+) ATPase of 94,000 mol wt has been isolated and partially purified from bovine brain by Hokin & coworkers (Vesugi et al 34). The protein, phospholipid, and carbohydrate content of the solubilized preparation are 50%, 25%, and 2 to 3%, respectively. The activity is inhibited by ouabain. Kyte (34a) has obtained highly purified ATPase fractions which show the presence of equal amounts of only two polypeptide chains, one 84,000 and the other 57,000 daltons.

A phosphorylated intermediate of alkali metal ion-dependent ATPases has been isolated from 14 different sources. This intermediate appears to be a γ-glutamyl derivative (Post 37, Hokin 25).

A very extensive coverage of the subject of the (Na^+-K^+) ATPase and alkali metal ion transport has been made by Bonting (35). Additional reviews covering this area are the following: Heinz (18), Albers (36), Post (37), Hokin (25), and Whittam & Wheeler (37a).

The demonstration of an (Na^+-K^+) ATPase in *E. coli* cells that is sensitive to ouabain has been reported (Bonting 35). This activity apparently is only of minor significance to the cation transport process.

As is true for most organisms, bacterial cells accumulate and maintain high internal levels of potassium ions. The importance of potassium in the regulation

of protein synthesis has been reviewed by Lubin (38). Lubin & Kessel (39) isolated a mutant of *E. coli* B that grows slowly in 10^{-4} *M* K^+, but normally in the presence of 0.1 *M* K^+. The internal level of K^+ was one twenty-fifth the normal level at the lower K^+ concentration. This mutant is presumably an energy-uncoupled mutant (Lubochinsky, Meury & Stolkowski 40).

An extensive genetic analysis of potassium transport in *E. coli* K12 has been carried out by Epstein and co-workers (Epstein & Davies 41, Epstein & Kim 42). There are at least three saturable potassium transport systems in *E. coli*, and these seem to act independently of each other. The major transport system in terms of V_{max} is designated the TrkA system. It has a K_m around 1.5 m*M*. Mutants (*trkA*) have been isolated which lack this transport system.

A second transport system, designated TrkD, can nearly compensate for a loss of the TrkA system. The K_m value of potassium for the TrkD system is 0.5 m*M* and its V_{max} is around one-fifth that of the TrkA system. Mutants of the *trkD* gene lack this transport system.

A third transport system for potassium, designated Kdp, has a relatively low K_m value of less than 10^{-6} *M*. This system which is abolished in *kdp* mutants also has a V_{max} value about one-fifth that of the primary transport system (TrkA). The unusual feature of the Kdp system is that it is only operative when *E. coli* are grown in low K^+ media. This repressibility accounts for the failure to routinely observe this high-affinity system when cells are grown in K^+-containing medium. The high affinity of this third system plus a V_{max} adequate to maintain normal growth rates accounts for the observations that all *trkA* and *trkD* mutants that are *kdp*$^+$ grow normally in low potassium media. The TrkA and TrkD systems can maintain adequate cell K^+ levels when the external level is above 0.1 m*M*, but below this level the Kdp system becomes derepressed. Maximal derepression is only observed in K^+-starved cells. Early observations that K^+ starvation causes an increase in the K^+ exchange rates was presumably related to the induction of the Kdp system. Potassium uptake in a triple mutant, *trkA*, *trkD*, and *kdp*, is low and is linearly proportional to K^+ concentrations up to 105 m*M*. Additional mutations (*trkB*, *trkC*, and *trkE*) which alter the K^+ transport activity in *E. coli* have been analyzed genetically by Epstein & Kim (42). The relationship of these mutations to K^+ transport is being studied by Epstein and co-workers.

Sulfate.—Dreyfuss & Monty (43) showed that the transport of sulfate in the Gram-negative bacterium *S. typhimurium* was specified by more than one gene since they could isolate several different transport mutants. Further examination established the importance of three different cistrons to the sulfate transport system. A sulfate-binding protein has been isolated and purified from the shock fluid of *S. typhimurium* (Pardee et al 44). The protein has a mol wt of 32,000 and binds one mole of sulfate with a K_D value of 2×10^{-6} *M*. The binding is highly sensitive to the ionic strength. Initial studies of the structure of the sulfate-binding protein using X-ray diffraction have been reported by Langridge, Shinagawa & Pardee (45). These studies show that the protein has an axial ratio of 4:1. Cal-

culations based on the maximum yield of the binding protein suggest that there are about 10^4 molecules of the binding protein per cell. The binding protein has been shown to be internal to the cell wall since nonpenetrating inhibitors of sulfate binding and transport activities and antibodies to the binding protein are without effect on whole cells.

A genetic study of the sulfate transport system in *S. typhimurium* has been carried out by Ohta, Galsworthy & Pardee (30) using chromate resistance as a selection method for obtaining transport mutants. Many of the chromate-resistant mutations were found to be in the *cysA* region and to lack transport activity but have a variable amount of sulfate-binding protein. The sulfate-binding protein isolated from the various *cysA* mutants has wild-type properties, which suggests that although this gene is important for transport activity it is not the structural gene for the binding protein, but apparently codes for a second component of the transport system.

Other mutants with very low levels of both binding activity and transport were found to map as *cysB* mutants. The binding protein from *cysB* mutants also appears to be normal, indicating *cysB* to be a regulatory mutant. The structural gene for the sulfate-binding protein has not been identified, but the authors were able to show that the synthesis of the binding protein is closely connected to the *cysA* gene previously identified as a transport gene. The synthesis of the binding protein is regulated by the same mechanism that serves for sulfate transport and cysteine biosynthesis.

Phosphate.—Phosphate uptake into *E. coli* has been extensively studied by Medveczky & Rosenberg (46, 47). The uptake of phosphate can be partially abolished by osmotic shock treatment of *E. coli* which releases a phosphate-binding protein. This binding protein has been purified using an ion-exchange resin assay. The purified protein has a mol wt of 42,000 and binds one mole of phosphate with a K_D value of 8×10^{-7} *M*. The rapid uptake of phosphate is only observed in phosphate-starved cells although the level of the binding protein is not influenced by starvation. When cells were grown in the presence of 10 m*M* phosphate they showed no high-affinity transport activity for phosphate but still contained functional binding protein. These results suggest that a second required component of the phosphate transport system is regulated by the level of phosphate in the cell.

In a recent report, Medveczky & Rosenberg (47) described a milder shock treatment for *E. coli*. The transport activity could then be partially restored by incubating the cells with the phosphate-binding protein. The authors have isolated two different kinds of mutants with impaired phosphate uptake; one mutant lacks the binding protein and the other has normal amounts of binding protein. When these two mutants were subjected to the mild shock procedure the transport activity could only be restored in the mutant which originally lacked the binding protein. The other mutant presumably has a defect in a second component of the transport system. They also found that the purified binding pro-

tein stimulated the total phosphate uptake and incorporation into acid-insoluble material in spheroplast preparations. These experiments suggest that successful reconstitution of transport activity by restoring binding proteins may depend on the method used for shock treatment.

Bennett & Malamy (48) obtained evidence for two different transport systems for phosphate in *E. coli* K12. One of these systems also transports arsenate, and mutants of this system render the organism resistant to arsenate, but they still take up phosphate. A second class of mutants are arsenate resistant and no longer take up phosphate.

These results suggest that there are apparently several phosphate transport systems in *E. coli* and that the phosphate-binding protein isolated by Medveczky & Rosenberg may be associated with one of these, presumably a high-affinity transport system.

Glorieux & Scriver (49) have identified two transport systems in the human kidney that serve for phosphate transport, one of which is responsive to parathyroid hormone.

Calcium.—The mechanism of action of vitamin D on the intestinal uptake of calcium in the chick has been studied by Wasserman and co-workers (Wasserman, Corradino & Taylor 50, Wasserman 51). The first evidence that calcium-binding activity was induced by vitamin D came from studies on the distribution of labeled calcium in homogenates from rachitic and vitamin D-treated preparations. Vitamin D-treated preparations showed more calcium associated with the soluble phase. The binding material was a protein of mol wt 25,000 to 28,000 which binds one mole calcium, strontium, or barium per mole of protein with dissociation constants for the protein-cation complex of 2.6×10^{-5} *M*, 3×10^{-4} *M*, and 5.8×10^{-3} *M*, respectively. The calcium-binding protein has been found in the intestinal mucosa of the chick, rat, dog, cow, and monkey (Wasserman 51). The protein has been shown to be located in the region of the brush border and the goblet cells of the intestinal mucosa. The recent studies with the calcium-binding protein are summarized in a review by Wasserman (51).

Transport of calcium into vesicle preparations of sarcoplasmic reticulum has been extensively studied by Hasselbach & Makinose (52, 52a), Kanazawa et al (52b), and Martonosi et al (53–55). These vesicles contain a magnesium and ATP-dependent transport system for calcium which can produce and maintain large calcium gradients. The ATPase has been obtained from the microsomes by extraction with deoxycholate. Upon removal of the detergent from the microsomal preparation tiny vesicles are spontaneously re-formed which show energy-dependent calcium transport. Electrophoresis of the solubilized microsomes produces two inactive protein fractions which when recombined produce an active ATPase preparation.

Martonosi (55) has obtained evidence for a protein-bound phosphate intermediate formed during the hydrolysis of ^{32}P-labeled ATP by the microsomal preparation. Inhibition of the ATPase activity, calcium transport, and reduction

in the level of the intermediate all occur when hydroxylamine is added to the microsomes, which suggests an acyl phosphate as the intermediate, possibly one similar to that described for the Na^+-K^+ ATPase.

Rat liver mitochondria contain both high-affinity and low-affinity sites for calcium (Reynafarje & Lehninger 56). The high-affinity sites are believed to be part of the transport system sensitive to osmotic shock treatment. Soluble binding activity for calcium has been identified by Lehninger (57). The binding of calcium is inhibited by Sr^{2+}, Mn^{2+}, La^{3+}, but not by Mg^{2+}. The mol wt appears to be around 150,000. This binding activity for calcium has been implicated as a component of the high-affinity transport system located in the inner membrane of mitochondria.

Calcium transport appears to be similar to that found for the (Na^+-K^+) ATPase-driven alkali metal ion transport referred to earlier.

Amino Acid Transport

Glycine, alanine, and serine.—A large number of kinetic studies, both with animal cells and with microorganisms, have demonstrated the presence of inhibitory interactions between glycine, alanine, and serine, which suggests that they share a common transport system. More careful kinetic studies in both animal cells and microorganisms have revealed the presence of heterogeneity within this system. In 1963 we described a transport system (system A) in Ehrlich ascites cells that served especially for glycine, alanine, serine, and closely related amino acids, although also to some degree for almost all neutral amino acids (Oxender & Christensen 58). Later a careful kinetic study showed that the Na^+-dependent alanine uptake could not be completely inhibited by, for example, glycine, which led to the identification of a separate transport system that serves for alanine, serine, and cysteine (ASC system) and their 4- and 5-carbon homologs (Christensen, Liang & Archer 59). This latter system, while only of minor significance in the Ehrlich cell, is the major route of entry in certain other tissues, including nucleated and reticulated red blood cells (Eavensen & Christensen 60).

Davis & Maas (61) and Kessel & Lubin (62) used the strong inhibitory action of D-serine on the growth of *E. coli* W (ATCC9637) to select for D-serine-resistant mutants. These mutants showed an impaired ability to concentrate D-serine, glycine, and L-alanine but normal transport activity for other amino acids.

The transport systems for glycine, alanine, and serine in both Gram-positive and Gram-negative organisms are firmly attached to the cytoplasmic membrane and remain active after the cell wall has been removed. Kaback & Stadtman (63) studied the uptake of glycine into membrane preparations of the D-serine-resistant mutant of *E. coli* W. They reported that the residual uptake of glycine was not concentrative, but was subject to inhibition by DL-serine, DL-alanine, and DL-threonine.

Hechtman & Scriver (64) have isolated a mutant of *Pseudomonas fluorescens* deficient in the transport of L-alanine, L-proline, and β-alanine. Interestingly, although this mutant was resistant to 4-methyltryptophan, the transport activity for tryptophan and phenylalanine was not decreased. Tryptophan uptake was

measured at 10^{-3} *M* which may be too low to measure the specific transport system for tryptophan which has a K_m value of 10^{-6} *M*.

The antibiotic D-cycloserine (D-4-amino-3-isoazolidone) competitively inhibits the uptake of both D- and L-alanine in *Mycobacterium acapulcensis* (Mora & Bojalil 65) and in *Streptococci* sp (Reitz, Slade & Neuhaus 66). Certain D-cycloserine-resistant mutants are deficient in the transport of glycine, alanine, and serine (Wargel, Shadur & Neuhaus 67, 68), a transport defect apparently similar to that described for D-serine-resistant mutants.

Although many studies have suggested that these three amino acids are transported in bacteria by a common transport system, a careful examination of the kinetics of wild-type strains and the transport mutants of both *E. coli* K12 and W clearly show that more than one transport system serves for the transport of L-alanine (Piperno 69).

We have studied D-serine-resistant mutants of both *E. coli* K12[1] and W. In both strains 90 to 95% of the uptake of glycine, D-alanine, and D-serine is lost while only about 50% of the L-alanine transport is lost. These D-serine-resistant mutants are still capable of utilizing L-alanine as a carbon source. This allowed use of penicillin selection on L-alanine-containing media to isolate a double mutant that has now lost about 95% of the L-alanine transport capacity. The present results suggest that in *E. coli* one transport system serves for glycine, D-serine, D-alanine, and L-alanine. In addition, a second system serves for L-alanine and L-serine. The measurement of L-serine uptake presents a special problem since the small endogenous pool of L-serine is subject to rapid metabolic modification. At 37°C the initial rate of uptake is very rapid with steady-state levels being achieved in a few seconds.

In parallel studies Wargel, Shadur & Neuhaus (67, 68), using D-cycloserine-resistant mutants in both *E. coli* K12 and W, showed that D-cycloserine was transported by the system serving for glycine and D-alanine. The Lineweaver-Burk plots of glycine and D-alanine were found to be biphasic, which permitted discrimination of two types of transport mutants. The first-step mutant from *E. coli* K12 showed a loss of the high-affinity D-alanine-glycine transport. This mutation was found to be genetically linked to the *metB* locus. A second mutation resulted in the loss of the low-affinity line segment for glycine and D-alanine. The mutation was also near the *metB* locus, 0.5 min from the first mutation described. The results in our laboratory are essentially in agreement with those reported by Neuhaus and co-workers (67, 68).

Still unanswered is the question of whether there are specific transport systems for glycine, alanine, and serine. The effect of the carbon source on transport

[1] Wild-type *E. coli* K12 cells are not sensitive to D-serine since they contain inducible D-serine deaminase activity. Dr. Elizabeth McFall, New York University, has isolated mutants of *E. coli* that lack deaminase activity and are now sensitive to D-serine. A D-serine-resistant mutant isolated from the deaminase mutant was found to have a defect in the uptake of D-serine (Cosley, S. D., McFall, E. 1971. *Bacteriol. Proc.* p. 160, Abs P220). Dr. McFall kindly provided us with this D-serine-resistant mutant (*E. coli* K12 EM1302).

activity suggests that the transport of each amino acid can be altered or perhaps controlled independently (Wargel et al 68).

The model amino acid α-aminoisobutyric acid, a competitive inhibitor of glycine, alanine, and serine in mammalian tissues (Christensen, Parker & Riggs 70), is also actively transported by the Gram-positive organisms *Bacillus megaterium* and *Staphylococcus aureus* (Marquis & Gerhardt 71). α-Aminoisobutyric acid is actively transported by *E. coli* K12 with a relatively high K_m value of 4 m*M* (Piperno & Oxender 72). The mutual inhibitory action of α-aminoisobutyric acid, glycine, and alanine suggests that it is transported by the glycine-alanine-serine transport system described above.

Sodium ion is cotransported with α-aminoisobutyric acid in mammalian tissues (Inui & Christensen 73). Drapeau, Matula & MacLeod (74) and Wong, Thompson & McLeod (75) showed that the highly concentrative transport activity for α-aminoisobutyric acid into a marine pseudomonad sp. and into *Photobacterium fischeri* required sodium ion, although cotransport was not detected.

Proline.—In 1960 Lubin et al (76) isolated mutants from *E. coli* W which required from 250 to 500 mg of proline per liter to produce maximal rates of growth. A detailed study of this mutant showed that it lacked a specific proline transport system (Kessel & Lubin 77) although the transport of other amino acids was normal. Kaback & Stadtman (78) showed that the proline transport system is firmly attached to the membrane since cytoplasmic membrane fractions prepared from *E. coli* W6 accumulate proline in an energy-dependent process. The proline transport system in most microorganisms shows a high degree of specificity, with hydroxyproline the only other naturally occurring amino acid that competitively inhibits proline uptake. *P. fluorescens* apparently contains a transport system that serves for both proline and alanine (Hechtman & Scriver 64). The lack of success with attempts to solubilize the proline transport system from *E. coli* using disrupted membrane preparations (Kaback & Deuel 79) and osmotic shock techniques (Piperno & Oxender 80) indicates that it is firmly attached to the cell membrane.

Valine, leucine, and isoleucine.—A stereospecific transport system for leucine, isoleucine, and valine in *E. coli* K12 was described by Cohen & Rickenberg (81) and by Britten & McClure (82). In a kinetic analysis of amino acid uptake into *E. coli* K12 we found that the K_m values for entry of the three amino acids were around 10^{-6} *M* and that all three were taken up by a common transport system. In mammalian tissues the branched chain amino acids are usually transported by a general Na^+-independent transport system serving for a variety of neutral amino acids with large hydrocarbon side chains, such as phenylalanine and tryptophan. This transport system is referred to as the L system (Oxender & Christensen 58). In contrast to these results phenylalanine does not inhibit the uptake of leucine into *E. coli* even at 100 times the level of leucine.

When *E. coli* K12 is subjected to osmotic shock treatment in the cold (Neu & Heppel 83) the uptake of the branched chain amino acids is greatly reduced

(Piperno & Oxender 80). A binding protein (LIV-binding protein) has been isolated from the shock fluid which binds one mole of either leucine, isoleucine, or valine (Penrose et al 84). The dissociation constants for the binding proetin, determined by equilibrium dialysis, were indistinguishable from the K_m and K_i values for cellular uptake of leucine, isoleucine, and valine, which suggests that cellular uptake reflects the substrate specificities of the binding protein.

The presence of leucine in the growth medium represses the synthesis of the LIV-binding protein as well as the activity of the LIV-transport system. The synthetic model amino acid 2-aminocyclo-[2.2.1.]-heptane-2-carboxylic acid (BCH) which acts as a leucine analog in *E. coli*, provided the correct one of four isomers is used, is also bound to LIV-binding protein (Christensen et al 85). This same isomer serves as a specific model for the L transport system in a variety of animal cells (85).

The activity of the transport system and the synthesis of the LIV-binding protein are regulated by a system distinct from that serving for the biosynthetic pathway. The transport activity is not derepressed in mutants[2] that are derepressed for the biosynthetic enzymes for the branched-chain amino acids. We also examined a leucine auxotroph (*leu* 500)[3] of *S. typhimurium* which does not make any of the biosynthetic enzymes for leucine. This mutant shows normal levels of leucine transport and LIV-binding protein.

Recently we have isolated a regulatory mutant for leucine transport in *E. coli* K12 (Rahmanian & Oxender 86) which has derepressed levels of leucine transport and LIV-binding protein, but does not have derepressed leucine pathway enzymes. It makes two to three times as much binding protein and shows a corresponding increase in both the initial rate and the steady-state levels of leucine uptake when compared to the values observed for the parent K12 strain of *E. coli*. The binding protein has been purified from the mutant and shown to have kinetic and antigenic properties similar to those of the protein isolated from the parental strain. Only one binding protein for leucine could be detected in the shock fluid of both the parent and the mutant.

The LIV-binding protein has been purified and crystallized by using 2-methyl-2,4-pentanediol. The molecular weight appears to be 36,000 and it contains one cysteine residue. Heating the protein for 5 min at 100°C has little effect on the binding activity. It undergoes large and reversible conformational changes in the presence of 6 *M* urea (Penrose, Zand & Oxender 87). An extensive search for substrate-induced conformational changes using ORD and CD measurements and various fluorescent probes was not successful.

[2] Dr. E. Umbarger, Purdue University, has shown that mutants of *E. coli* K12 resistant to 4-azaleucine have derepressed levels of the biosynthetic enzymes for the branched-chain amino acids. This mutation maps near *gly*A on the *E. coli* chromosome. Dr. Umbarger has kindly provided us with *E. coli* mutant CU5002 which has derepressed levels of the biosynthetic enzymes for leucine.

[3] Dr. Paul Margolin, New York University, has provided us with a mutant *leu*500 of *Salmonella typhimurium* LT2 that does not contain detectable levels of the biosynthetic enzymes for leucine.

Anraku (88–90) carried out a separate and independent purification of the LIV-binding protein from *E. coli* strain 7 and studied its properties. His findings are in agreement with the results from our laboratory. Recently Furlong & Weiner (91) showed that a second transport system specific for leucine was present in *E. coli* strain 7 (derived from K10). A second binding protein (L-binding protein) specific for leucine and trifluoroleucine was purified and crystallized. The specific protein and the LIV-binding protein both have about the same molecular weight, dissociation constants, amino acid analyses, and are cross reactive antigenically. The synthesis of both proteins is repressed when leucine is added to the growth medium. The level of this leucine-specific binding protein apparently varies with the bacterial strain and growth conditions and has been difficult to isolate in the K12 strains presently being used in our laboratory.

Bussey & Umbarger (92, 93) have studied leucine uptake in a wild-type strain of yeast and in a trifluoroleucine-resistant mutant. Leucine-binding activity has been identified in extracts of the yeast but its purification has been hampered by activity losses.

Histidine.—Ames & co-workers (94–96) have carried out an extensive study of histidine transport in *S. typhimurium*. The kinetic plots suggest that at least two systems serve for histidine transport. One of these is described as a general aromatic transport system that his a low affiinity for histidine with a K_m value of 10^{-4} *M*. The other is a high-affinity specific transport system for histidine with a K_m of 3×10^{-8} *M*. The α-hydrazino analog of histidine was found to be an effective inhibitor of both growth and histidine uptake in *S. typhimurium* (Shifrin, Ames & Ames 97). A mutant *hisP* 1650, which is resistant to the inhibitory action of the analog, had a defective histidine-specific transport system. *HisP* mutants still retain the histidine-binding protein which can be isolated by osmotic shock treatment.

In an elegant study of the components of the histidine transport system reported by Ames & Lever (96), the high-affinity transport system has been resolved into three components: J, K, and P. The histidine-binding protein J is specified by the *hisJ* gene. Mutants in the *hisJ* locus lack the binding protein and are defective in histidine transport. Another class of mutants (*dhuA*) are histidine-requiring strains that have mutated so that D-histidine can be utilized as a source of L-histidine (Krajewska-Grynkiewicz et al 98). The D-histidine-utilizing mutant *dhuA* showed a fivefold increase in the level of the binding protein and the transport activity. The *hisP* locus codes for a protein P which is apparently necessary for the J protein to be operative in transport. A third component of histidine transport, K (not yet clearly identified), is proposed to work in parallel to the J protein and also requires the P protein to function in transport. The *hisJ*, *dhuA*, and *hisP* loci have been mapped and are in a cluster (near *purF*) on the *S. typhimurium* chromosome.

The increased J protein produced by the *dhuA* strain is indistinguishable from that produced by the wild type. The proteins have similar dissociation constants ($K_D\sim2\times10^{-7}$ *M*), also the same chromatographic behavior and isoelectric pH

(Ames, personal communication). The *hisJ* mutation has been shown to be the structural gene for the histidine-binding protein by the isolation of a binding protein that has altered temperature sensitivity. This mutant will be discussed later in a section on direct evidence for a role of the binding proteins.

Rosen & Vasington (99) have also isolated the histidine-binding protein from this same organism by osmotic shock treatment. The protein has a molecular weight of 25,000, a K_D value of 1.5×10^{-6} *M*, and a K_m value for histidine transport of 0.8×10^{-6} *M*. This histidine-binding protein would appear to be similar to the J protein under study by Ames & Lever (96) although the K_D reported by the latter authors is an order of magnitude lower (2×10^{-7} *M*) and the K_m of histidine transport two orders of magnitude lower ($\sim3\times10^{-8}$ *M*). Some of the discrepancies between the K_m and K_D values reported by the two laboratories may be a result of the methods used for uptake measurement. Ames and co-workers (94–96) have used a protein synthesis assay for histidine uptake in growing cells. The advantage of this assay procedure is that, at low levels of histidine where transport is rate limiting, protein synthesis acts as a "sink" for the entering histidine. This method does not allow the measurement of the maximum initial rate of uptake at higher levels of histidine where the uptake is faster than the incorporation into protein. A combination of methods may be necessary to establish more accurately the parameters of the histidine transport system.

In view of the complexities of the histidine transport and the difficulty of showing complete loss of transport activity following osmotic shock treatment, there may be other transport systems for histidine, some of which are not sensitive to osmotic shock treatment.

Phenylalanine, tyrosine, and tryptophan.—A careful study of aromatic amino acid transport into *Salmonella typhimurium* by Ames (94) and Ames & Roth (95) showed that a common transport system served for all three of these amino acids. In addition, each of the aromatic amino acids was also transported by a specific system. The general aromatic transport system has a K_m value of 10^{-7} *M* for the amino acids while the K_m values for the specific systems are around 10^{-6} *M*. Interestingly, in this case the general transport system has a higher affinity than the specific system.

Mutants lacking the general aromatic transport system (*aroP*) in *S. typhimurium* have been isolated by selecting for resistance to azaserine or 5-methyltroptophan (Ames & Roth 95). The *aroP* is located near *proA* in *S. typhimurium*. We noted that *E. coli* K12 also contains a general aromatic transport system (Piperno & Oxender 72). Brown (33), in a thorough study of the transport of the aromatic amino acids in *E. coli* K12, showed that this organism has transport systems similar to those shown for *S. typhimurium* by Ames (94).

The general system, specified by the *aroP* gene which is located near the *leu* locus of the *E. coli* chromosome, transports all three aromatic amino acids with K_m values for each about 5×10^{-7} *M*. In addition, specific systems with K_m values about 2×10^{-6} *M* serve for the individual amino acids.

Brown (33) found that the aromatic amino acids were actively transported in

glucose-starved cells without externally added energy sources, although the addition of 2,4-dinitrophenol or sodium azide strongly inhibited the uptake and maintenance of the internal pool. Since the general transport system exhibits rapid exchange between internal and external amino acids (Brown 33, Piperno & Oxender 72) a large portion of the initial uptake may occur by an exchange reaction which is not energy dependent. When energy inhibitors are added, the internal pool is rapidly lost, thus decreasing the exchange component of uptake. During glucose starvation the internal pool of amino acids is usually maintained or may increase. The specific transport systems apparently do not show rapid exchange with external amino acids.

In a recent paper, Brown (100) has attempted to answer the difficult question of whether different internal pools are formed by the general and the specific transport systems. The author concluded that these systems contribute to a single internal pool which can supply amino acids for protein synthesis. The exodus of aromatic amino acids probably occurs largely through the general transport system. To do these experiments in wild-type cells one must block the general transport system by adding another of the aromatic amino acids. In view of the complications that could be produced by a rapid exchange process and the complexity of the experiment it is difficult to interpret these experiments clearly.

Guroff & Bromwell (101) have reported the isolation of a phenylalanine-binding protein from *Comamonas* sp. The relationship of this broad specificity binding activity to transport has not been well established. Kay & Gronlund (102) have studied aromatic amino acid transport systems in *Pseudomonas aeruginosa*. These authors have isolated aromatic amino acid transport mutants using a variety of analog inhibitors (Kay & Gronlund 103).

Tryptophan.—Tryptophan is transported by three different systems in *E. coli* K12. One of these systems is the general aromatic transport system described in the previous section. The K_m of tryptophan for this system is around 10^{-7} *M* (100). A second constitutive transport system is specific for tryptophan and has a K_m value of around 10^{-6} *M*. These systems have been described for *S. typhimurium* by Ames (94, 95) and for *E. coli* by Piperno & Oxender (72) and Brown (100).

The level of the specific tryptophan transport system varies in different strains of *E. coli*. In *E. coli* W1485 studied by Brown (100) the specific system is relatively low, and *aroP* mutants in this strain are slightly resistant to 4-methyltryptophan. This tryptophan analog enters the cell primarily by the specific tryptophan transport system. Yanofsky (private communication) has shown that the level of specific tryptophan transport in strain W3110 is around five times that found in W1485. Aromatic amino acid transport mutants of strain W3110 are still sensitive to certain tryptophan analogs which have been used to select double transport mutants for tryptophan (*E. coli* W3110, *aroP*, *trpP*). These mutants have extremely low tryptophan transport when grown on glucose which prevents the induction of the third transport system. Even when glycerol is used as a carbon source the double mutant requires levels of external tryptophan in excess of 200 mg/liter to produce maximum induction of tryptophan transport, presumably

because they do not have either of the high affinity transport systems that can maintain elevated internal levels of tryptophan (Oxender, unpublished results).

The third transport system for tryptophan in *E. coli* T_3A has been described by Boezi & DeMoss (104) and Burrous & DeMoss (105). Since it is inducible it is subject to catabolite repression and cannot be observed when glucose is used as a source of carbon. The K_m value for tryptophan is $\sim 10^{-5}$ *M*, and it is highly specific for tryptophan and certain of its methylated analogs. We find that when cells induced for tryptophan transport are subjected to osmotic shock treatment the transport activity is retained by the shocked cells. Binding activity for tryptophan can be identified in spheroplast and in membrane preparations of *E. coli* T_3A after induction by growth of the cells on tryptophan. Treatment of these membrane preparations with detergents partially solubilizes the binding activity (Oxender, unpublished results).

The transport of tryptophan by germinated conidia of *Neurospora crassa* is mediated by a neutral amino acid transport system studied by Wiley & Matchett (106, 107) and Matchett, Turner & Wiley (108). This neutral amino acid transport system has been designated system I by Pall (109, 110).

Wiley (111) has isolated and purified a tryptophan-binding protein obtained from *N. crassa* by osmotic shock treatment. The dissociation constant of this protein for tryptophan is 8×10^{-5} *M* which can be compared to the K_m value of 5×10^{-5} *M* for the uptake of tryptophan into whole cells. The binding activity of the protein for various amino acids decreased in the following order: phenylalanine > tryptophan > leucine. This order is consistent with the mutual competitive actions of these amino acids. This protein may well be a component of the neutral amino acid transport system (system I) of *N. crassa*. In the Ehrlich ascites tumor cell tryptophan is transported primarily by the L transport system (Oxender & Christensen 58).

Arginine and lysine.—Maas & co-workers (112, 113) showed the L-canavanine-resistant mutants of *E. coli* W have decreased transport activity for arginine, lysine, and ornithine, which indicates a common transport system for these three amino acids.

Wilson & Holden (114, 115) have obtained several specific arginine-binding proteins from *E. coli* W cells by osmotic shock treatment. The arginine transport activity for the shocked cells which was only reduced 25% could be partially restored by the addition of two of the purified proteins. They suggested that the arginine transport system is specific for arginine since no binding activity was seen for lysine.

In another laboratory three arginine-binding proteins have been isolated from *E. coli* by osmotic shock treatment (Rosen 116). One of these proteins binds lysine, arginine, and ornithine (LAO-binding protein), a result that may correspond to the original observations on the general basic amino acid transport system described by Maas (112). This general binding protein has a molecular weight of 30,000 and gives a K_D of 1.5×10^{-6} *M* for arginine, 3.0×10^{-6} *M* for lysine, and 5.0×10^{-6} *M* for ornithine.

The two additional binding proteins identified by Rosen (116) were specific for arginine and may correspond to the arginine-specific proteins reported by Wilson & Holden (114, 115). The general transport system serving for arginine, lysine, and ornithine is similar to that described in mammalian tissues by Christensen (117) and Christensen & Liang (118). They have referred to the corresponding system as the Ly^+ system in the Ehrlich cell.

Since lysine transport is partially inhibited by arginine in intact *E. coli* and not in shocked cells, it appears that lysine shares a transport system with arginine and ornithine for which there is a common binding protein. Apparently lysine is also taken up by a separate system unaffected by osmotic shock. The lysine-specific transport system has been identified in *E. coli* (Rosen 116). The specific system appears to have a lower affinity for lysine than the general or LOA transport system. Normally only the LOA transport system is lost by osmotic shock treatment of *E. coli* while the lysine-specific system is retained in shocked cells. Rosen has been able to alter growth conditions so that the general transport system was completely repressed. Under these conditions the lysine-specific system was reduced by osmotic shock and a binding protein specific for lysine was isolated. This binding protein is labile at 4°C and sensitive to ionic strengths above 0.02.

Glutamine.—A repressible transport system and a corresponding binding protein for glutamine have been described for *E. coli* by Weiner et al (119, 120). This protein (mol wt 29,000) is specific for glutamine and has a K_D of 3×10^{-7} *M*. The K_m value for glutamine uptake is 0.8×10^{-7} *M*. Furthermore, glutamine (1 μM) causes a shift in the fluorescence spectrum of the tryptophan residues of the binding protein. The dissociation constant as determined by change in fluorescence is also around 10^{-7} *M*. Using a stopped-flow apparatus, these authors found that the k for the forward or binding reaction is 9.8×10^{7} M^{-1} sec^{-1} and the k for the back or dissociation reaction is 16 sec^{-1}. A study of the latter properties of the glutamine-binding protein may provide insight into the mechanism of the translocation step in transport.

A mutant has been isolated which has a threefold higher initial rate of glutamine transport and three times as much binding protein. Other mutants, resistant to glutamylhydrazide, have been isolated which have only 10% of the transport activity and about 10% of the binding protein.

In mammalian systems glutamine appears to be transported by the neutral amino acid transport systems referred to as the A and ASC systems (Oxender & Christensen 58).

Glutamic acid.—Many of the early studies on glutamic acid uptake into microorganisms have been summarized in a 1962 review by Holden (121). Marcus & Halpern (122, 123) have identified several genetic loci that are responsible for glutamate uptake in *E. coli* K12. The glutamate transport system of *E. coli* K12 is specified by three genes: *gltC* and *gltS*, which are closely linked and are located between *pyrE* and *tna* loci on the chromosome; and *gltR*, which is responsible for

the formation of a repressor of the transport system is located near the *metA* gene. Wild-type *E. coli* K12 cannot grow on glutamate since the synthesis of the glutamate transport system is partially repressed. Glutamate-utilizing mutants (*gltC*) can be readily isolated and have been shown to have increased glutamate transport activity. Since the K_m of glutamate transport is 1×10^{-5} *M* for both the mutant and parent strains, the authors concluded that *gltC* was a regulatory gene for glutamate transport.

Marcus & Halpern (123) have also isolated a temperature-sensitive regulatory mutant for glutamate transport. This mutant produces a thermolabile repressor which is inactivated at 42°C, thereby derepressing the transport system and permitting the mutant to grow on glutamate at 42°C but not at 30°C.

Cystine.—E. coli W contains two transport systems for L-cystine (Leive & Davis 124, Berger, Weiner & Heppel 125). A general transport system serves for cystine and α-ε-diaminopimelic acid and related amino acids. The K_m of this system for cystine is 3×10^{-7} *M* and it is lost upon osmotic shock treatment of the cells. A second specific system for cystine has a K_m value of 2×10^{-8} *M* and is present after shock treatment.

A cystine-binding protein corresponding to the general system has been isolated from *E. coli* W which binds cystine and diaminopimelic acid. The dissociation constant for the binding protein is 2×10^{-7} *M* for cystine and the molecular weight is 28,000. The binding activity is not inhibited by *p*-mercuribenzoate, N-ethyl maleimide, or glutathione, which suggests that disulfide interchange is not involved in cystine binding.

A mutant, D2, which transports both cystine and diaminopimelate five times more rapidly than its parent, has been isolated by Leive & Davis (124). Another mutant strain, D2W, examined by Berger, Weiner & Heppel (125), only had the general transport system. Osmotic shock treatment completely reduces the transport activity for cystine in this mutant, confirming the earlier interpretation in wild-type cells that the general transport system was sensitive to osmotic shock treatment while the specific cystine transport system was insensitive.

Regulation of amino acid transport activity.—As indicated earlier, most of the amino acid transport systems are constitutive. In *E. coli* the activity of most of these systems is relatively unaffected by variations in the internal level of the amino acids. In cases where the amino acid can be used as a carbon or nitrogen source transport activity is often found to be inducible. Glutamate (Halpern & Lupo 126) and tryptophan transport (Boezi & DeMoss 104) serve as examples of inducible amino acid transport systems in *E. coli*. Thorne & Corwin (127) reported that a regulatory gene for tryptophan transport in *E. coli* was located in or near the *trp* operon. Rose & Yanofsky (128) have shown that the mRNA does not contain major unidentified structural genes either before the operator-proximal E gene or after the operator-distal A gene. However, deletion mutants where the deletion extended into the *tonB* region of the chromosome show de-

creased transport activity for tryptophan and a variety of other amino acids, which implies that *tonB* mutants may have a general membrane defect (Yanofsky, personal communication).

The active transport systems of yeast and fungi are extremely sensitive to the growth conditions and composition of the suspending medium used for uptake studies. Ammonium ions seem to have a regulatory effect on the general amino acid transport systems in yeast (Gits & Grenson 129) and in fungi (Pall 109, Thwaites & Pendyala 130, Benko, Wood & Segel 131). When these organisms are using ammonium ions as a source of nitrogen the general transport systems are repressed and in the absence of ammonium ions, high-capacity general amino acid transport systems are induced.

The stimulation of the trans flux of a solute through the cell membrane that occurs when certain solutes are preloaded into animal cells has been well documented (Heinz & Walsh 132, Oxender & Christensen 58). This phenomenon, sometimes called accelerative exchange (Stein 22), is believed to reflect a faster translocation process for the carrier-solute complex than for the empty carrier. Conversely, Heinz & Durbin (133) pointed out on theoretical grounds that if the empty carrier translocation process were faster than that for the carrier-solute complex "transinhibition" would result.

Transinhibition of amino acid uptake has been observed in *Penicillium chrysogenum* by Benko, Wood & Segel (134), in yeast by Crabeel & Grenson (135), in *Streptomyces hydrogenans* by Ring, Gross & Heinz (136), in *N. crassa* by Wiley & Matchett (106) and Pall (137, 138), and in the Ehrlich cell by Oxender & Christensen (58). Transinhibition apparently serves to stop excessive accumulation of amino acids and to conserve cellular energy.

Sugar Transport

The transport of glucose into the red blood cell occurs by a process of facilitated diffusion (Stein 22) while in many other tissues the active transport of sugars requires the cotransport of sodium ions (Schultz & Curran 19, Stein 22). As pointed out by Kepes (1) most of the sugar transport systems in microorganisms except the one for glucose are inducible. *E. coli* and *S. typhimurium* apparently transport sugars by three different processes: facilitated diffusion, active transport and group translocation. *Staphylococcus aureus*, on the other hand, apparently transports all sugars by the group translocation process (Egan & Morse 139, Simoni 17).

Glucose.—The transport of glucose in most microorganisms is mediated by the PEP-sugar phosphotransferase system, which will be discussed later. Although glucose transport is constitutive, it is affected by the nutritional state of the organism (Hoffee & Englesberg 140, Hoffee, Englesberg & Lamy 141). α-Methylglucoside uptake into *E. coli* is apparently inhibited by ATP production (Kessler & Rickenberg 142).

Two transport systems serving for glucose, one of which is repressed by glucose, have been described in *N. crassa* (Scarborough 143, 144, Schneider &

Wiley 145, 146, Neville, Suskind & Roseman 147). Cell membranes are fairly impermeable to phosphate esters; however, in recent years transport systems for certain phosphate esters have been identified. L-α-Glycerophosphate can be taken up by *E. coli* (Hayashi, Koch & Lin 148). Mutants that grow with glucose 6-phosphate, but not glucose, as a carbon source have been isolated by Hagihira, Wilson & Lin (149) and Fraenkel, Falcoz-Kelly & Horecker (150). An inducible transport system for the hexose phosphates was identified in these mutants by Pogell et al (151) and Winkler (152). The properties of this transport system were investigated by Winkler (153) and Dietz & Heppel (154–156). External ester is required for induction of the transport system. Mutants which lack both glucose-6-P dehydrogenase and hexose phosphate isomerase accumulate up to 0.05 *M* glucose-6-P inside the cell when fed glucose, yet this high level of the ester does not induce the transport system. Induction of the transport system in these mutants allows glucose-6-P to exit from the cells, presumably by a reversal of the uptake process. Winkler (152, 153) showed that the esters of glucose, fructose, and mannose were substrates as well as inducers for the hexose phosphate transport system. Fructose-6-P must first be converted to Glucose-6-P and then exit from the cell to produce induction.

Galactose.—Galactose is reported to have at least four distinct transport systems with different K_m values in *E. coli.* These transport systems are characterized by different substrate and inducer specificities (Rotman & co-workers 31, 157).

Role of lactose transport system (TMG-I): Galactose induces the galactose and β-methylgalactoside transport system in *E. coli* provided galactokinase is missing so that endogenous galactose will accumulate (Wu 158).

The growth of K12 strains of *E. coli* that are unable to metabolize galactose is inhibited by galactose, and mutants selected for resistance to this galactose toxicity are always found to be lactose transport mutants. On the other hand, they still retain the β-methylgalactoside transport system. Strains of *E. coli* ML selected for galactose resistance have lost both transport systems. These results imply that the level of the β-methylgalactoside transport system is higher in ML strains than in K12 strains of *E. coli;* therefore, a double transport mutant is required to produce resistance to galactose in ML strains.

In 1960 Horecker, Thomas & Monod (159, 160) described what they took to be an inducible exit system for galactose in a galactokinase mutant of *E. coli.* Wu (161) has suggested that this phenomenon could be ascribed to the lactose transport system. The inducible β-methylgalactoside transport system appears to be constitutive in galactokinase mutants, presumably as a result of endogenous galactose formation. Since this latter high-affinity system can maintain a high internal level of galactose, the induction of the lower-affinity lactose transport system by addition of galactose to the medium serves to lower the internal level of galactose. Because the exit is stimulated in this way, it is probably not useful to use the terminology "inducible exit system."

Role of melibiose transport system (TMG-II): A second transport system in

E. coli K12 usually found only at lower temperatures has been called the TMG-II, but is now termed the melibiose transport system since it is induced by melibiose and galactinol. In galactokinase and lactose transport mutants of K12 this system can be induced at 25°C by 1 m*M* galactose (Leder & Perry 162).

β-Methylgalactoside transport system: This is a low-K_m transport system which acts on β-methylgalactoside, galactose, D-fucose, glucose, and β-glycerol-galactoside. This system is induced by galactose and D-fucose and appears to be the same as that studied by Buttin (163, 164) and Horecker & co-workers (159, 160, 165). A reexamination of *E. coli* strain B78A *galR* shows only the β-methyl-galactoside transport system (Lengeler, private communication).

A galactose-binding protein has been isolated from *E. coli* by osmotic shock treatment (Anraku 88, 89, 166). The purified protein shows a K_D of 10^{-6} *M* for galactose and glucose and has a mol wt of 35,000. Boos (167) and Boos & Sarvas (168) have studied the role of the binding protein in transport. Recently Boos & Gordon (169) and Boos et al (170) have shown that the galactose-binding protein can exist in two forms with different affinities for galactose. The presence of galactose causes the protein to undergo a conformational change which results in decreased binding activity for the substrate. The conformational change has been studied by gel electrophoresis and by fluorescence measurements. Binding protein from a transport-negative mutant does not undergo the substrate-induced conformational changes. These studies of substrate-induced conformational changes may provide insight into stages of membrane transport subsequent to the binding step.

Convincing evidence has been presented to show that the galactose-binding protein is genetically linked to the β-methylgalactoside transport system which is specified by the *mglP* gene located near the *his* locus on the chromosome of *E. coli* (Lengeler et al 32, Boos & Sarvas 168). The latter report describes a separate regulatory gene *mglR* for the *mglP* transport system which is distinct from the *galR* gene which regulates the gal operon.

Specific galactose transport system: Rotman & co-workers (31, 157) have described a transport system reported to be specific for galactose with a high K_m value of about 10^{-4} *M*. Galactose uptake by this system was inhibited by glucose, mannose, and arabinose. This transport system has only been clearly described in *E. coli* W4345 which lacks both galactokinase and the lactose transport system. *E. coli* W4345 can grow on melibiose at 37°C in the presence of 10^{-3} *M* galactose, which suggests that perhaps the melibiose transport system may be partially inducible at 37°C in this strain (Lengeler, personal communication).

Additional evidence for this fourth transport system has appeared from other laboratories. Boos (167) initially found two galactose-binding proteins in the shock fluid. They purified the protein that is associated with the β-methylgalactoside transport system. There may be a second galactose-binding protein that can be removed by shock treatment in certain bacterial strains. Kerwar, Gordon & Kaback (171) have described an inducible galactose transport system in isolated membrane vesicles from *E. coli* ML3 which can be stimulated by electron

donors (discussed later). This strain of *E. coli* is *lac-y*$^-$ and was grown at 37°C. Under these conditions only the β-methylgalactoside transport system should be operative; however, the conditions for preparing vesicles reduces the level of the galactose-binding protein to undectable levels as judged by Ouchterlony immunodiffusion plates. The transport activity shown in vesicles gave a K_m value of 10^{-5} *M* which is near the value reported for the galactose-specific transport system (Rotman, Ganesan & Guzman 31).

A comprehensive study of various galactose transport mutants in a given strain would be useful to help identify the number of distinct transport systems for galactose.

Galactose transport into yeast has been extensively studied by Cirillo & co-workers (Kuo, Christensen & Cirillo 172, Kuo & Cirillo 173). An inducible transport system permits the entry of galactose into the yeast cell by a process of facilitated diffusion. Once inside the sugar is phosphorylated by the enzyme galactokinase. Free galactose can be detected in galactokinase mutants.

Arabinose.—The active transport of L-arabinose into *E. coli* B/r has been described by Novotny & Englesberg (174). This system is induced by L-arabinose and also transports D-fucose and D-xylose. Use of arabinose depends on the product of the arabinose C gene which permits the expression of three arabinose-utilizing genes linked to it and a transport gene located elsewhere on the chromosome. The C gene product acts positively to permit expression of the arabinose operon. This gene product has recently been identified by Wilcox et al (175).

Osmotic shock treatment released an arabinose-binding protein from *E. coli* B/r (Hogg & Englesberg 176). A similar arabinose-binding protein has been isolated from extracts of *E. coli* K12 by Schleif (177). The K_D value for the binding proteins was found to be $\sim 10^{-6}$ *M* in both laboratories. The binding protein also binds D-fucose and D-xylose, in agreement with the results found for transport into whole cells. Mutants have been isolated that have decreased levels of binding protein and decreased transport activity for arabinose (Schleif 177). In addition, certain transport-negative mutants contain normal levels of binding protein, which suggests that additional components are required for transport activity.

Lactose.—β-galactosides are actively transported into *E. coli* when the *y* gene of the *lac* operon is functional (Kepes & Cohen 4, Kennedy 6). In the presence of inhibitors of the production of metabolic energy the β-galactoside transport system becomes a facilitated diffusion system and can exhibit exchange with substrates of the transport system (Winkler & Wilson 178, Koch 179). Recently Wong, Kashket & Wilson (180) have described a mutant that appears to have lost the ability to accumulate thiomethylgalactoside (TMG) but still gives rise to facilitated diffusion of TMG. Genetically this appears to be a defect in the *lacY* gene which results in an M protein that can not be coupled to the normal energy source.

The *lacY* gene specifies a membrane-bound protein (M protein) which is

essential for the uptake of β-galactosides (Fox & Kennedy 181). The M protein can be extracted from membrane preparations using detergents such as Triton X-100 or sodium dodecyl sulfate. The apparent molecular weight is 31,000 (Jones & Kennedy 182). Thiodigalactoside (TDG), an effective inhibitor of lactose transport, gives a K_i value of 5×10^{-5} M as determined in whole cells and 7×10^{-5} M when determined in cell-free extracts. Melibiose and TDG protect the M protein from attack by N-ethylmaleimide while lactose and thiomethylgalactoside do not. These observations led Kennedy to postulate that the M protein has two binding sites for sugars. One of these sites contains an active sulfhydryl group and the second, nearby site is also rendered inactive when sulfhydryl reagents inactivate the first site. The reader is referred to an article by Kennedy (6) for a more complete discussion of the lactose transport system. There is the suggestion from these studies that a single gene product (M protein) may possess both the substrate recognition site and the site for coupling to a cellular energy source.

Phosphotransferase system.—Kundig et al (183) and Kundig & Roseman (184) described a PEP-dependent phosphotransferase system in *E. coli.* The phosphoryl group of phosphoenolpyruvate is transferred to a nitrogen atom of the histidine residue of a low molecular weight protein (HPr) in the presence of a cytoplasmic enzyme (Enzyme I), yielding a phosphorylated derivative of HPr. The phosphoryl group is then transferred in a second enzymatic reaction to the sugar in the presence of a family of sugar-specific, membrane-bound enzyme complexes (Enzyme II). HPr (mol wt 9700) and Enzyme I are soluble proteins and the Enzyme II complex appears tightly bound to the membrane. The Enzyme II complex for the constitutive glucose system has been fractionated into three components, two proteins (IIA and IIB) and phosphatidylglycerol, all three of which are required for phosphotransferase activity in place of the particulate fraction (Roseman 15). Further fractionation of the soluble protein fraction by electrofocusing leads to the isolation in homogeneous form of three IIA proteins, each specific for one of the three sugars: glucose, mannose, and fructose. The protein designated IIB tends to aggregate in the absence of detergent and has not been extensively characterized. It gives a band corresponding to 36,000 mol wt when subjected to gel electrophoresis in the presence of sodium dodecyl sulfate and urea.

Reconstitution of the phosphotransferase system was dependent on the order of addition of the isolated components. Maximum activity was achieved when IIA was combined with IIB and then the lipid added.

In addition to the sugar-specific system (IIA, IIB) for glucose described above, a second constitutive glucose system has recently been demonstrated (Kundig & Roseman, personal communication). In this case one of the two required sugar-specific proteins is found in the supernantant fluid and has been designated Factor III. The membrane-bound IIB protein of this latter system is not the same as that found for the first glucose transport system described above.

Factor III has a mol wt of 20,000 and appears to dissociate into subunits

about the size of the HPr component. The transport system for glucose using Factor III has a K_m value tenfold less than the first glucose transport system described which uses the IIA component.

The pattern of the phosphotransferase system appears to be fairly similar in *E. coli* and *S. typhimurium* and somewhat different from that found in *S. aureus* (Morse & co-workers 139, Nakazawa et al 185). Almost all sugars, including lactose, appear to be transported by the phosphotransferase system in *S. aureus*. The lactose transport system in this organism requires a soluble Factor III^{lac} and a membrane-bound Enzyme II^{lac} which are sugar specific in addition to Enzyme I and HPr. Factor III has been shown to be a phosphorylated intermediate in the reaction. It has been highly purified and has a mol wt of 34,000 (Simoni 17).

A role of the phosphotransferase system in sugar transport was first reported by Kundig et al (186). Transport activities for α-methylglucoside (α-MG) and thiomethylgalactoside (TMG) were reduced in shocked cells and could be restored by adding back purified HPr. TMG-6-P measured in these experiments has been suggested to arise from transport as free TMG by *lac Y* system with subsequent phosphorylation (Kennedy 6). Genetic evidence has been presented to show that Enzyme I or HPr mutants exert a pleiotropic effect on the uptake of several sugars (Egan & Morse 139, Roseman 15, 16, Simoni 17).

The physiological behavior of HPr and Enzyme I mutants in *E. coli* has been presented by Saier et al (187), who suggest that genes that code for Enzyme I and HPr components are part of an operon concerned with sugar uptake and utilization. HPr mutants exert a polarity effect on Enzyme I levels. The authors' summary suggests that (*a*) none of the Enzyme I mutants of *E. coli* that have been isolated can use mannitol, mannose, or fructose, but their ability to use other sugars such as glycerol, lactose, maltose, and melibiose varies; (*b*) all HPr mutants are "leaky" and can grow slowly on several sugars not phosphorylated by the phosphotransferase system; (*c*) certain sugars that are not transported by the phosphotransferase are, nonetheless, not utilized by Enzyme I and HPr mutants. This latter property is believed to result from a hypersensitivity of these mutants to catabolite repression. *E. coli* mutant strain 1101, which lacks HPr, and strain 1103, lacking Enzyme I, can not grow on lactose unless a supplement of cyclic AMP is provided. Phosphorylation of glycerol, maltose, lactose, and melibiose by the phosphotransferase system has not been demonstrated in *E. coli*.

Although one cannot question the importance of these reactions to the metabolism of sugars, their precise role in transport has not been established.

Can Enzyme I or HPr mutants carry out facilitated diffusion? Can the Enzyme II complex produce mediated fluxes of the free sugars? The membrane-bound components, Enzyme II complexes, are sugar specific and could possibly be capable of carrying out facilitated diffusion (Roseman 16). It has not, however, been clearly demonstrated and certain evidence suggests that it does not occur with α-methylglucoside (Kaback 188). The latter report suggests that the phosphotransferase system of *E. coli* shows a preference for external glucose even though the cells were preloaded with glucose under conditions where it remained

as the free sugar. If facilitated diffusion did occur and the flux of free sugar was rapid, an internal phosphorylating system would appear to prefer external sugar since the relatively large external pool would rapidly reverse the initial starting gradients of the labeled sugars.

In another study, Winkler (152, 189) showed that labeled α-methylglucoside phosphate is easily lost from the cells and appears outside the cell as the free sugar, which suggests that facilitated diffusion or mediated exit can occur with the free sugar. The presence of unlabeled substrate in the medium appears to accelerate the exit of labeled sugar. The increased loss produced by external substrates is believed to result from an inhibition of the "recapture" of sugar in the periplasmic space by the transport system as opposed to an increased exodus (Robbie & Wilson 190). Winkler (189) has shown that α-methylglucoside is lost from the cells with a half-time for exit of 2 min. The limiting factor in the exit process is a phosphatase activity which can be inhibited by fluoride ions. A model for α-methylglucoside transport incorporating phosphorylation, dephosphorylation, and a "recapture process" has been used by Winkler to explain the steady-state levels of the sugar.

Preloading cells with unlabeled α-methylglucoside had no effect on the initial rate of uptake of labeled sugar, which indicates either that accelerative exchange does not occur for this system or that the cells already contain a saturating level of substrate for this transport system. The question of whether the Enzyme II complex produces mediated fluxes could be more definitively answered with studies on mutants lacking various components of this process.

DISCUSSION

Role of Binding Proteins in Transport

As presented above, there are a large number of transport systems under study, and in many cases specific components are being isolated and identified through a combination of genetic and biochemical approaches. The binding proteins isolated mainly from Gram-negative bacteria have been proposed as components of the transport systems for a variety of amino acids, sugars, and ions.

Most evidence linking the binding proteins with solute transport is indirect. Summaries of the indirect lines of evidence along with some of the alternative interpretations that can be made to these arguments are listed below.

(*a*) Osmotic shock treatment causes a loss in transport activity and at the same time binding activity can be recovered in the shock fluid. The osmotic shock treatment, however, can cause a loss of a variety of essential small molecules, some of which may be necessary for energy production. Cells usually need to be incubated with an energy source to eliminate an effect of energy depletion on the transport measurements. Solutes known to be insensitive to osmotic shock can be used as controls (i.e. proline or alanine).

If the binding proteins are membrane components why are they so easily

released from the membrane? In our experience the lowest yields of the leucine-binding protein (LIV) are obtained from exponentially growing cells. The transport activity, on the other hand, is the highest in this growth phase. The binding protein is recovered in highest yields from stationary cultures where transport activity is lowest. This information, taken with the unusually large amounts of binding proteins found in bacterial cells, suggests the possibility that protein removed by shock treatment may represent a protein component of the transport system that had already come out of the cytoplasmic membrane into the periplasmic space and that some of the protein may remain firmly attached to the membrane where it can function in transport.

(*b*) Kinetic constants for cellular transport and binding activity are similar. Many kinetic measurements of transport do not yield linear plots either because of the difficulty of getting initial rates or because multiple transport systems are present. Furthermore, the K_m values for uptake are equivalent to dissociation constants for the receptor sites only if the initial binding step is not the rate-limiting step in uptake; i.e., the Michaelis-Menton equilibrium assumption must be shown to apply.

(*c*) A parallel regulation of transport activity and the ability to synthesize the binding protein has been observed. Coordinate regulation of several operons at different loci on the chromosome is a common feature of certain metabolic pathways.

(*d*) Binding proteins have been localized in the cell envelope. The cell envelope is a complex multilayered structure and with the current techniques available it is impossible to establish unequivocally the cellular location of the binding proteins.

The above summary shows that a large body of data implicates a role of the binding proteins in transport, although the evidence is indirect. Some reports of successful reconstitution of transport systems and the identification of the structural genes for the binding proteins represent more direct evidence for a role of specific proteins in transport systems. Some of these studies will be discussed below.

Restoration of transport activity.—Many laboratories report lack of success during attempts to add purified proteins back to shocked cells; however, some success has been reported. Anraku (90) has reported partial restoration of galactose and leucine transport by combining the purified binding protein and a second protein fraction that could be obtained from the shock fluid by ammonium sulfate treatment. Our laboratory has been unsuccessful, however, in attempts to restore leucine transport by adding the purified protein back.

Wilson & Holden (115) also showed some restoration of the lowered arginine transport in *E. coli* W by adding two binding fractions back to shocked cells; however, the shock treatment only caused a 20–25% reduction in the original transport activity.

Corradino & Wasserman (191) report that they are able to stimulate calcium

transport in embryonic chick intestine by adding calcium-binding protein to the medium.

As we have already noted, phosphate transport in *E. coli* is reported to be partially restored in shocked cells by adding back the purified phosphate-binding protein (Medveczky & Rosenberg 47). The transport activity could not be restored in mutants that contain normal binding protein but were defective in some other component of the transport system. The accumulated ^{32}P was shown to be inside the cells and not simply adsorbed to the surface by measuring the increased incorporation of phosphate into organic phosphate compounds.

These studies show only limited success obtained in restoration attempts. In view of the poor reproducibility of measures of uptake in shocked cells and the presence of multiple transport systems, more definitive evidence and extension of the techniques to other systems appear necessary to establish clearly the reconstitution of transport activity by purified protein components.

Evidence from genetic studies.—Genetic studies have implicated the binding proteins in transport but few laboratories have been able to identify the structural genes for the various binding proteins. Other factors than binding protein have been implicated by genetic studies. For example, three different cistrons are concerned with sulfate transport although none of these appears to code for the binding protein (Ohta et al 30).

Boos & Sarvas (168) and Lengeler et al (32) have carried out an extensive study of the relationship of the galactose-binding protein (GBP) to the β-methylgalactoside transport system in *E. coli*. These authors showed that the two features were genetically linked since they were equally co-transducible with the *his* marker. They examined 102 *his*$^+$ transductants and 75 showed transfer of both transport activity and binding protein synthesis for galactose. Interestingly, the 27 remaining cases showed no galactose transport and did not make the binding protein. In a recent study Lengeler et al (32) extended these studies and showed that the β-methylgalactoside transport system is regulated by a *mglR* gene separate from the *galR* gene which regulates the galactose operon. Synthesis of the binding protein and the transport activity were coordinately regulated. A more direct indication of a role of the GBP in transport was obtained in an examination of revertants for negative galactose chemotaxis by Hazelbauer and Adler (personal communication). They obtained a revertant that showed about a hundredfold less sensitivity to galactose chemotaxis than did the wild-type strain. This revertant was shown to have altered transport activity and an altered binding protein. The peptide map of the isolated binding protein was different from that of the wild-type galactose binding protein (W. Boos, personal communication). These experiments provide more direct support for a role of the binding protein in galactose transport.

Ames & Lever (96) have identified the *hisJ* gene of *S. typhimurium* which specifies a histidine-binding protein. This protein is a component of one of the transport systems for histidine. Revertants of *hisJ* mutants have been isolated,

and in all cases they are able to produce J protein and have recovered transport activity for histidine.

One of these revertants induced by the frameshift mutagen ICR 191 in a *hisJ* mutant which was itself induced by ICR 191 produces an altered binding protein for histidine. The altered protein is more temperature sensitive and has different mobilities on DEAE and hydroxylapatite columns when compared to *hisJ* protein from the parent strain. This revertant is also temperature sensitive to L-histidine transport and for growth on D-histidine (Ames, personal communication). These data provide a direct demonstration of a role of the histidine-binding protein in histidine transport in *S. typhimurium*.

What role do the binding proteins play in transport? Some of the following roles have been considered.

(*a*) Most logically, they have been proposed as bearing the receptor site detected for the corresponding transport system. Why are binding proteins detected for certain bacterial cells but not for many other organisms? Why are binding proteins found for certain amino acids and sugars and not others? Why do most of them come from Gram-negative bacteria?

(*b*) They may serve as an initial sequestering agency located in the periplasmic space which can then pass the solute to the membrane-bound transport system. The trouble with this hypothesis is that the binding protein will not increase the concentration of the free solute in the periplasmic space and will only compete with the receptor site of the membrane-bound transport system for solute.

(*c*) Binding proteins may serve to transfer solutes from the outer membrane to the inner membrane through the periplasmic space. This hypothesis supposes that protein-bound substrates will move across the periplasmic space faster than the free substrate. No hypothesis as to the character of this space supports such a supposition.

Translocation

The second step in transport is usually referred to as the translocation step. In this step the "solute-carrier complex" is believed to undergo some type of a conformational change which transfers the solute through the membrane. This may or may not be an energy-dependent process. In animal cells most amino acid and sugar-transport systems can carry out exchange diffusion in the presence of inhibitors of metabolic energy. The same is true for many amino acids and certain sugars in the *E. coli* cell.

Are the binding proteins potential candidates for undergoing conformational changes? Some support for this hypothesis has been obtained. First of all, most binding proteins are extremely stable and can be reversibly denatured. The thermodynamically stable conformation possesses the high binding activity (Penrose et al 87). Boos & Gordon (169, 170) showed that the galactose-binding protein can exist in two conformational states, one with higher affinity for galactose than the other. The substrate binding converts one form to the other. The

change in conformation can be followed by changes in the fluorescence of the protein. Weiner & Heppel (120) have shown that glutamine induces conformational changes in the glutamine-binding protein which can be monitored by fluorescence measurements.

These studies indicate that certain binding proteins possess some of the properties necessary for participation in the translocation step or second step in membrane transport.

Nature of Energy Delivery to Solute Transport

Active transport of solutes requires metabolic energy and therefore cells must possess processes for coupling energy production to the transport systems. When inhibitors of cell energy production are added to the uptake medium many active transport systems become facilitated diffusion systems.

Loss of energy coupling to certain transport systems appears to occur during the final stages in the maturation of the rabbit reticulocyte. During the maturation process all protein synthetic activity is lost, and the uptake of glycine and alanine by the isolated cells becomes nonconcentrative and is no longer sensitive to metabolic energy inhibitors (Winter & Christensen 192). Not all active transport systems are lost during the maturation process since the mature erythrocyte exhibits a very active (Na^+-K^+) ATPase which serves to maintain a high internal potassium ion level.

The active transport systems for sugars and amino acids by many animal cells show a requirement for sodium ions. The sodium ions have been shown to be cosubstrates with the sugars and amino acids (for reviews see Heinz 18, Schultz & Curran 19, Stein 22, Christensen 193).

Since sodium ion serves as a cosubstrate in the transport of certain amino acids and sugars and the (Na^+-K^+) ATPase maintains a gradient of the alkali metal ions in the cell, the question can be asked: Do the alkali metal ion gradients as suggested by Riggs, Walker & Christensen (194) serve to drive the active transport of sugars and amino acids in animal cells? Crane (195, 196), using intestinal tissue; Vidaver (197, 198), using pigeon erythrocytes; and Eddy (199, 200), using ascites cells, all showed that in the presence of metabolic energy inhibitors the direction of active transport of the cosubstrate could be reversed by reversing the sodium ion gradient.

Schafer & Jacquez (201, 202) and Schafer & Heinz (203), using Ehrlich, ascites cells showed, however, that if the sodium ion gradient is reversed and metabolic energy production is not inhibited, α-aminoisobutyric acid is still actively transported into the cell. Kimmich (203a) showed that intestinal cells can accumulate sugar for short periods against a Na^+ gradient. It appears that if the ATP level in the cell is maintained the active transport process for amino acids cannot be reversed by reversing the ion gradients. The active transport of amino acids and sugars in animal cells is coupled both to ion gradients and to metabolic energy production. It remains to be determined just what percentage of the energy for active transport is derived from the two sources under various physiological

conditions. Perhaps the fraction can vary, depending on particular circumstances.

A recent report presents evidence that the active transport system for melibiose in *S. typhimurium* carries out cotransport with sodium ions (Stock & Roseman 204). It was necessary to use special precautions and plastic vessels to maintain sodium-free media for demonstrating the sodium dependence of the melibiose transport system. Furthermore, reextrusion of the Na^+ entering the cell with melibiose quickly obscured the cotransport, a factor that may have permitted it to be overlooked in other cases, as in the experiments of MacLeod (74, 75).

What is the nature of energy coupling in the active transport systems of bacteria?—Oxidative phosphorylation does not appear to be the energy source for active transport of amino acids into *E. coli* membrane vesicles since ATP and phosphoenolpyruvate do not stimulate nor does arsenate inhibit, proline transport (Kaback & Milner 205, Klein, Dahms & Boyer 206). These vesicle preparations cannot carry out oxidative phosphorylation (Klein, Dahms & Boyer 206).

Pavlasva & Harold (207) proposed that a proton gradient in the membrane provides the energy for transport. *E. coli* W1895 actively transports thiomethylgalactoside under *anaerobic conditions*, and this transport can be inhibited by a variety of uncouplers of oxidative phosphorylation. The authors concluded that the inhibitors acted by dissipating the proton gradients across the cell membrane. West (208) has provided support for this hypothesis by showing that protons migrate through the membrane during the active transport of lactose in *E. coli*. In related studies Eddy & Nowacki (209) have shown that active transport of amino acids in yeast is coupled to the migration of H^+ vs K^+.

Mitchell (210) has extensively reviewed possible coupling relationships between transport and chemical reactions. His review presents the arguments for the hypothesis that active solute transport may be chemiosmotically coupled to metabolism.

Recently, Kaback and associates (Kerwar, Gordon & Kaback 171, Barnes & Kaback 211, 212, Kaback & Barnes 213, Konings, Barnes & Kaback 214, Short, White & Kaback 215) have greatly extended their earlier studies on energy coupling to active transport in membrane vesicles. They first showed that the transport of a wide variety of amino acids and sugars by *E. coli* membrane vesicles is tightly coupled to D-lactic acid dehydrogenase (Kaback & Milner 205, Barnes Kaback 211). This membrane-bound, flavin-linked dehydrogenase is coupled to the reduction of oxygen via a cytochrome system also present in the membrane of the vesicles. In *S. aureus* the electron donor appears to be exclusively the α-glycerolphosphate dehydrogenase (Short, White & Kaback 215). α-Glycerolphosphate dehydrogenase induced by glycerol can act as an electron donor for active transport of lactose and of glucose-6-P in *E. coli* (Dietz 216).

Hirata, Asano & Brodie (217) and Konings & Freese (218) have shown that artificial electron donors such as ascorbate coupled to the redox dye such as phenazine methosulfate (PMS) are very effective in stimulating amino acid trans-

port in the membrane vesicles. Ascorbate plus PMS has proved twice as effective as D-lactate in supporting transport in *E. coli* vesicles (Konings, Barnes & Kaback 214).

Kaback has postulated for a working model that "transport carriers" are electron transfer intermediates between the dehydrogenases and cytochrome b_1. The carriers contain sulfhydryl groups, a circumstance believed to account for the inhibition of transport by sulfhydryl reagents. The reduced form of the carriers has low affinity but can oscillate within the membrane, a process which may give rise to facilitated diffusion. Oxidation of the carriers during the process of electrons passing through the chain provides the energy to convert the low-affinity form to the high-affinity form.

The importance of the energy source observed by Kaback for active transport of certain sugars and amino acids in vesicles has been clearly established; however, the mechanism by which this energy flow is coupled to transport has not been clearly identified. Additional studies are necessary to establish clearly that the dehydrogenase-stimulated transport activity is not a special feature of membrane vesicle preparations but serves as a physiologically important energy source in whole cells. The possibility that the electron transport system is more directly related to the "integrity of the membrane" should be more clearly eliminated. The isolation and study of mutants for the dehydrogenases or other components of the electron transport chain ought to prove useful for establishing the relationship of these energy sources to membrane transport. A more extensive review of energy coupling to active transport has been prepared by Klein & Boyer (218a).

Assembly of Transport Systems

A considerable body of genetic data suggests that many of the transport systems are multiple component complexes, and phospholipids appear to be essential components of certain transport systems.

Are the transport systems assembled in the cytoplasm and inserted in the membrane, or do the individual components enter the membrane? If the latter is true, do the components enter randomly or at fixed loci? The isolation of bacterial mutants with defective phospholipid synthesis has greatly aided the study of membrane assembly and function. Mutants of *E. coli* K12 that require unsaturated fatty acids for growth have been isolated by Silbert & Vagelos (219), Henning et al (220), Schairer & Overath (221), Esfahani, Barnes & Wakil (222), and Wilson, Rose & Fox (223). Glycerol-requiring mutants of *Bacillus subtilis* (Mindich 224, 225), *E. coli* (Hsu & Fox 226), and *S. aureus* (Mindich 227) have also been isolated. These mutants have been used independently to control the synthesis of phospholipid and protein components of the membrane, thus altering its chemical composition. Hsu & Fox (226) and Wilson & Fox (228) reported greatly lowered activity of the lactose transport system of *E. coli* when induction was attempted in the absence of phospholipid synthesis, although the M protein was synthesized in normal amounts and incorporated into the membrane. These studies, coupled with earlier studies by Fox (229), suggest that the assembly of

the functional lactose transport system requires the simultaneous synthesis of protein and phospholipid components. In another study, Mindich (227), found that the activity of the lactose transport system of *S. aureus* is only 30 to 50% of the normal level in the absence of phospholipid synthesis. More recently Willecke & Mindich (230) reported that the inducible-citrate transport system in *B. subtilis*, previously described by Willecke & Pardee (231), could be induced in the absence of phospholipid synthesis. These studies imply that for the lactose transport system the M protein and lipid components must at least be synthesized simultaneously. A lipoprotein complex may have to be formed prior to insertion into the membrane, or the individual molecules may simply need to be incorporated into the same area at the same time to prevent the formation of less favorable complexes with other membrane components.

Is the lateral migration of macromolecules within the membrane significant, and does it play a role in the assembly of transport systems? Frye & Edidin (232) showed that when mouse cells (c11D) were fused to human cells (VA-2) using Sendai virus the surface antigens were 90% intermixed within 40 min, which suggests that the mammalian cell surface is fluid enough to allow rapid lateral diffusion of macromolecules. Hubbell & McConnell (233) have used spin-labeled steroid phospholipids to show that there are fluid regions in a large number of biological membranes. In extensive studies on membrane vesicles prepared from egg phosphatidylcholine, McConnell and his colleagues have measured the lateral diffusion and the inside-outside transitions (flip-flop) of phospholipids. Kornberg & McConnell (234) found that the phospholipid molecules pass from one monolayer to another less frequently than 2×10^{-5}/sec at 30°C. These results exclude the possibility of inside-outside transitions of phospholipids being responsible for mediated carrier fluxes. The elementary step for lateral diffusion, however, is at least eight orders of magnitude more frequent (Kornberg & McConnell 235). More recently, DeVaux & McConnell (236) have measured the frequency for the elementary step and found it to be $\sim10^{7}$ sec^{-1}. If lateral diffusion of bacterial membrane lipids occurs at this same rate, a phospholipid molecule could diffuse from one end of a 1 μ-long bacterium to the other in a time of the order of 1 sec (236). They suggested that not all classes of lipids would be expected to show the same lateral diffusion, and perhaps unsaturated lipids may be concentrated near transport sites due to a special affinity for the proteins in this region. The possibility of rapid lateral diffusion of both proteins and phospholipids suggests the interesting hypothesis that the construction of macromolecular complexes or "mosaics" may result from a random insertion of components followed by lateral migration. The affinities of the various components for each other would result in the formation of thermodynamically stable complexes.

Possibly, in certain biological membranes, barriers to lateral diffusion of macromolecules exist. Restriction of the surface diffusion would permit identification of possible fixed sites of membrane synthesis. Studies on membrane synthesis in *Bacillus megaterium* have indicated that new membrane is primarily end-localized (Morrison & Morowitz 237), while membrane assembly in *E. coli* ap-

pears to be a random insertion of protein and lipid components (Tsukagoshi, Fielding & Fox 238, Wilson & Fox 239). The rapid lateral migration of membrane components would, however, preclude the possibility of identifying fixed sites for membrane synthesis.

SUMMARY

Current progress in our understanding of membrane transport is being achieved through a combination of genetic and biochemical studies. While these studies help to establish the diversity and complexity of various transport systems they also provide a description of some of the membrane components concerned with transport processes. Another approach not reviewed here considers the composition and structure of the membrane.

The active transport of variety of ions, including sodium, potassium, and calcium, is closely coupled to membrane ATPases, through which they derive their energy. In most cases the ion transport systems are firmly bound to the membrane, although calcium-binding proteins have been identified from intestinal tissue and mitochondria.

The amino acids and some of the sugars are actively transported in various animal tissues and bacteria without metabolic modification. In the animal cells these transport systems are Na^+-dependent. The melibiose transport system in *S. typhimurium* has also been shown to be Na^+ dependent. Since these transport systems also serve for sodium ions they may derive energy for active transport from the ion gradients, although evidence suggests they can also derive energy from other metabolic sources even when the ion gradients are reversed.

As shown with vesicle preparations, active transport of a variety of sugars and amino acids is markedly stimulated by certain electron donors, which suggests that the membrane-bound electron transport system serves as a source of energy for active transport in bacteria.

The identification of binding proteins for a variety of ions, sugars, and amino acids which appear to play a role in certain high-affinity transport systems permits a study of the receptor site in isolation. These systems appear to be lost during osmotic shock treatment or during membrane vesicle formation. Several binding proteins show substrate-induced conformational changes which may provide insight into the translocation step of the transport process.

The transport of certain sugars into *E. coli* and most, if not all, of the sugars into *S. aureus* appears to occur by a group translocation process accomplished by phosphoryl transfer through a phosphotransferase system. Phosphorylation of the sugar either accompanies the transport process or is tightly coupled to it since the phosphorylated sugars are accumulated prior to the appearance of the free sugars.

In light of the complexity and diversity of the transport systems, a careful integration of kinetic studies, biochemical isolation, and mutational analysis will be helpful for complete understanding of the molecular basis of membrane transport.

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