Specificities of Transport Systems and Enzymes

Selectivity of cells for exogenous compounds is enhanced by sequential action of membrane carriers and enzymes.

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The mammalian cell may be considered simply as a solution of enzymes surrounded by a membrane. An extracellular substance that is to be metabolized by intracellular enzymes must first penetrate the membrane. For most naturally occurring substances this penetration is achieved by so-called membrane carriers, proteins whose function is to facilitate the movement of small molecules across the membrane (*1*). It is generally recognized that without such membrane proteins many extracellular substances are not accessible to intracellular enzymes, and, if so, they remain functionally cryptic.

The high chemical specificity of enzymes and carriers is one of their most striking and important characteristics. It is reasonable on teleologic grounds to suppose that the specificities of enzymes and carriers are very nearly the same, in order to transfer suitable substrates to the enzymes. However, it will be shown that, in fact, the specificities of the carrier and the enzyme which act upon the same compound are distinctly different. One may thus inquire as to whether there is a biological explanation for this apparent mismatch in the requirements of the two proteins.

An analysis of the transport systems and enzymes of several chemical species has led to a rationale of the function of these differences. First, it will become clear that an extracellular molecule must satisfy two sets of structural requirements before it can be assimilated by the cell: one for the carrier, the second for the enzyme. Since these must be satisfied sequentially, as the substance moves first through the membrane, the molecule is screened in series for separate characteristics. In this way the two proteins, membrane carrier and enzyme, impose a combined specificity or selectivity of great exactitude. Second, it is postulated that, although multiple sites of binding of substrates to carriers usually exist, a principal requirement is for precisely that part of the substrate immediately adjacent to and including the functional group that will subsequently undergo enzymatic attack (this part of the substrate is referred to as its "reactive site"). The specificity for binding to the enzyme may or may not include the reactive site, but in general the structural requirements for this position are less restrictive than for other positions. From the nature of the chemical interaction between the carrier and the substrate it is further proposed that binding to the reactive site would probably block or prevent attack of the reactive site during transport. Thus, the carrier recognizes molecules susceptible to attack by enzymes at the reactive site and, at the same time, blocks reaction until it delivers the substrate to the enzyme. I designate the specificity of transport proteins (according to this hypothesis) as "reactive site–directed."

The characteristic differences between the specificities for the transport system and enzymes are illustrated by the examination of three widely variant groups of compounds. In each case, particular attention is paid to the role of the reactive substrate site. Two examples taken from studies on intestinal transport are the best reported with respect to transport specificities. The examination of the third system in polymorphonuclear leukocytes is a new study of the specificities of adenine transport and of adenine phosphoribosyltransferase, which catalyzes the conversion of adenine to adenosine monophosphate (AMP).

Neutral Amino Acid Transport by the Intestine

A widely variant, chemically, group of amino acids appears to be actively transported via a single membrane carrier (*2*). Such different amino acids as histidine, tyrosine, tryptophan, alanine, and methionine (as well as all other neutral amino acids) show mutual competition for transport. Moreover, in man, a single mutation brings about the loss of intestinal and renal transport systems for all these amino acids, as in Hartnup's disease (*3*). Thus, there is little specificity for the amino acid side chain, except that there is no tolerance for the introduction of charged groups (*4*).

In contrast to these rather loose requirements with respect to the side chain, the specificity of the transport system at the reactive alpha amino and carboxyl sites is quite exacting (Fig. 1). Thus, esterification of the carboxyl group (*4*) or decarboxylation (*5*) leads to a loss of affinity for transport. The alpha amino group is also essential, since the aminoacetyl and methyl derivatives and the deaminated compounds are not actively transported. Substitution of a methyl for an alpha hydrogen also leads to reduction in affinity for the carrier (*6*). Finally, only the L-stereoisomers are transported (*6*).

That the transport protein partially envelops the reactive site is suggested by its intolerance for bulk substitutions on the substrate (such as *N*-methyl and *N*-acetyl derivatives) and for replacement of the α-hydrogen. This envelopment would presumably prevent the approach or attack by a second molecule that occurs in the majority of enzyme reactions of amino acids. The α-carbon is not exchanged during transport (*4*), although this is but one of many conceivable forms of attack that could take place during transport.

The specificities of enzymes metabolizing amino acids are nearly mirror images of the requirements for transport. In general, the enzymes are stringent in their specificity for the side chain. One has only to mention the enzymes that activate amino acids for protein synthesis, which are essentially specific for individual amino acids (*7*).

On the other hand, there is considerably more tolerance for changes at the reactive site. As was mentioned, the the carbon atom is asymmetric at the

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reactive site, and the transport system is specific for the L stereoisomers. For enzymes in general, there is marked stereospecificity for reaction; moreover, the reaction frequently occurs at the optically active site. However, binding to the enzyme may be considered separately from the subsequent reaction as shown by the existence of compounds that cannot be attacked but that competitively inhibit normal conversions of substrate. Presumably such unattacked inhibitors are bound to the active enzyme site. If the magnitude of competitive inhibition is used as an index of binding affinity, it is found that, for many enzymes, the D stereoisomers of amino acids bind at least as well as the L forms. For example, analysis of the kinetics for hydrolysis by chymotrypsin indicates a Michaelis constant (K_m) of 5.3 millimolar for acetyl-L-tryptophanamide and an inhibition constant (K_i) of 2.7 millimolar for acetyl-D-tryptophanamide, although the latter compound is not attacked by the enzyme (*8*). The data for glutamine synthetase are also instructive: D- and L-glutamic acids are bound nearly equally, even though only the L form undergoes the complete reaction (*9*).

In summary, neutral amino acids are bound to the carrier principally at their reactive site, with rather nonspecific hydrophobic bonding to the variable side chain (*4*). Binding to the enzyme or enzymes is largely to complementary sites, being highly specific for the side chain and showing much greater tolerance within the reactive site. If there is extreme alteration at the reactive site, enzymatic attack cannot occur, of course, but partial reactions are not uncommon (*9*). A compound that has satisfied the requirements of the membrane carrier will have the appropriate configuration at the reactive site for enzymatic attack.

Sugar Transport by the Intestine

In this example a more precise comparison of transport and enzyme specificities can be made. Unlike the multiple metabolic fates of amino acids, the principal natural substrate of the sugar transport system is glucose, which has only one immediate fate, phosphorylation by the enzyme hexokinase. Although the available information is not so complete, some analysis can be made of the two other major naturally occurring monosaccharides, galactose and fructose.

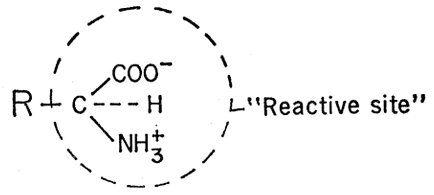


Fig. 1. For effective transport, R may vary from phenol to indole to imidazole to methyl, but must be uncharged. Structural requirements at the reactive site are quite specific, however. Enzymes, on the other hand, may show considerable tolerance within the reactive site (inversion of the optical configuration, for example) but are exacting in specifications for R.

Structural specificities for both the glucose transport system and hexokinase have been extensively studied (*10*). However, it is only recently that some of the detailed structural requirements have been clarified. On the basis of the capacity of isolated intestinal sacs to accumulate sugars against a concentration gradient, the principal structural requirements for transport were given to a first approximation, as depicted in Fig. 2a, namely, a pyranose ring of D configuration, and the hydroxyl group at position 2 (to be compared with the complete structure for glucose, Fig. 2b). Galactose, the epimer of glucose which has an inversion of the hydroxyl at carbon-4, is therefore a suitable substrate. The 2-hydroxyl in the position shown is considered absolutely critical since mannose, the 2-epimer of glucose, is not transported. However, there was also evidence that bulky groups at position 3 were not tolerated. Moreover, whereas inversions of hydroxyl groups at carbon-3 (D-allose) and carbon-4 (D-galactose) were tolerated, inversions at both carbon-3 and carbon-4 in the same molecule (D-gulose) were not (*11*).

Our special concern is with position 6. This is the reactive site; it is here that phosphorylation occurs as the first step in glucose metabolism. From Fig. 2a it would appear that substitutions at carbon-6 exert relatively unimportant effects on transport. However, some evidence has gradually developed which suggests the critical situation of position 6. First, although D-xylose, a five-carbon sugar homomorphic with glucose (hydrogen substituted for carbon-6 in glucose, Fig. 2b) was weakly transported, its affinity for the carrier was less than a hundredth of that of glucose or galactose (*12*). Moreover, whereas 6-deoxyglucose and 6-fluoro-6-deoxyglucose were well transported, 6-iodo-6-deoxy-D-galactose and 6-*O*-methylglucose were not (*11*). This immediately suggests that the carrier protein in fact closely envelops the 6-position, tolerating the smaller fluoro and hydrogen substitutions, but rejecting the more bulky iodo and methyl substitutions. More recently, Barnett, Jarvis, and Munday (*13*) have further clarified this interaction between the carrier protein and position 6. Their analysis was based on a test series of galactose analogs, the reasoning being that modifications in this compound (already inverted in the 4-hydroxyl compared to glucose) would be more likely to reveal the importance of a second alteration in the molecule. Transport was studied by means of a more sensitive method that depends on the accumulation of the compound in the intestinal tissue (*14*). Their results suggest that affinity decreases progressively, going from the small fluoro to the bulkier chloro, bromo, and iodo substitutions (Table 1). Most interesting is that 6-deoxygalactose is transported at approximately one-half the rate of the 6-deoxy-6-fluoro derivative. First, it may be emphasized that the van der Waals radii at the hydrogen and fluorine substitutions are nearly equal, so that the difference in affinity of these two derivatives is probably unrelated to their bulk. Alternatively it is suggested that adsorption of the substituent at position 6 to the carrier occurs via hydrogen bonding. Fluorine and oxygen could readily form hydrogen bonds, while this is clearly impossible for the deoxy derivative (although with respect to size, such formation falls within permissible limits) (Fig. 2c).

It would follow from the reactive site–directed hypothesis that the carrier protein has considerable specificity for position 6 as indicated above. First, the six-carbon sugar, glucose, has considerably higher affinity than the homomorphic five-carbon sugar, xylose; bulky substitutions are not tolerated (as in 6-*O*-methyl and the heavier 6-halogen in the sugars); and optimum binding depends on the formation of hydrogen bonding with a substituent at carbon-6 (6-fluoro-6-deoxy- versus 6-deoxygalactose). Since the carrier is intolerant of bulky groups at position 6, it appears that it is "fitted" tightly about this site and secured by hydrogen bonding (Fig. 2c). Such a conformation may shield the reactive site from approach and reaction with a second molecule.

A brief examination of the specificity of hexokinase indicates that in several

respects it is the inverse of the sugar transport system (*10*). Most striking and in complete contrast to the sugar carrier is the tolerance for inversion of the hydroxyl at carbon-2 [mannose (the 2-epimer of glucose), which is inert in the transport system, has even higher affinity for hexokinase than glucose itself]. Substituted derivatives at carbon-2 (2-glucosamine and 2-deoxyglucose) also show high affinity for the enzyme. Fructose, the 2-keto glucose analog, is also phosphorylated by hexokinase, though it is not a substrate of the glucose carrier.

On the other hand, the hydroxyl at carbon-3 seems important in binding to hexokinase. Thus, 3-*O*-methylglucose, which is a satisfactory substrate for the transport system, is inert with respect to the enzyme, and allose, the 3-epimer of glucose, has very poor affinity for hexokinase but is also transported. Alterations at position 4 also show clear-cut differences. Thus, galactose (the 4-epimer of glucose), a good substrate for the transport system, is not phosphorylated by the enzyme.

Finally, at the reactive site, position 6, binding to hexokinase is affected by alterations at carbon-6, as is the transport system, although a detailed study of hexose derivatives is not available. The hydroxyl groups at carbons-1 and -3 are also considered critical for the attachment of the substrate to the enzyme (*10*), and are probably of greater quantitative importance. Thus, 6-deoxyglucose has a higher affinity than 1-deoxyglucose by an order of magnitude. The pyranose ring is less critical for binding to the enzyme than for transport as shown by 2-, 5-anhydro-D-mannitol which has a five-membered (furanose) ring and is a substrate for hexokinase, but is rejected by the carrier.

The complementarity of the specificities for sugar transport and phosophorylation may be summarized as follows.

Table 1. Effect of substitutions at position 6 on the initial rate of tissue accumulation of galactose derivatives (*13*).

Compound	Initial rate *
L-Galactose	11 †
D-Galactose	100
6-Deoxy-D-galactose	26
6-Deoxy-6-fluoro-D-galactose ‡	48
6-Deoxy-6-chloro-	26
6-Deoxy-6-bromo-	12
6-Deoxy-6-iodo-	11

* In arbitrary units normalized to 100 for D-galactose. The relative rates are approximations to the relative affinities for the carrier. † Lowest rate observed; this may reflect only passive diffusion or contamination of tissue and not activity of the carrier-mediated process. ‡ The van der Waals radii (*30*) are H, 1.2A; F, 1.35; Cl, 1.80; Br, 1.95; and I, 2.15A.

The most critical points for binding to the carrier are at carbons-1 (*15*), -2, -5 (D stereoisomer), and -6. A pyranose ring structure (six-membered ring including oxygen) is also essential. For the enzyme, the most significant binding sites are at carbons-1, -3, -4, -5 (D stereoisomer), and -6 to a lesser degree. The pyranose ring is not essential. In short, the carrier and enzyme have both unique and overlapping requirements. Acting sequentially, their combined specificity imposes stringent requirements at every carbon.

Adenine Transport by the Polymorphonuclear Leukocyte

In the two examples already discussed, the specificities of the transport systems are fairly broad. In the case of the neutral amino acids it is not clear which enzyme or enzymes should be considered for comparison with the membrane carrier even though the argument developed would seem to apply generally. Moreover, the experimental results for transport systems and the relevant enzymes are rarely available in equal detail for the same cell. The results reported here for both the adenine transport system and enzyme were obtained from the rabbit polymorphonuclear leukocyte. Adenine has only one immediate fate—phosphorylation by the enzyme adenine phosphoribosyltransferase (E.C. 2.4.2.7), which catalyzes the reaction, adenine + PRPP (*16*) ⇌ adenosine monophosphate + pyrophosphate.

Structural alterations in the sugars and amino acids were largely local in effect. Substitutions or alterations in the conjugated heterocyclic purine ring are considerably more complex. Physical effects may be exerted at some distance from the actual structural change. This fact forces us to consider in more detail the mechanism by which the enzyme brings about fusion between adenine and PRPP in order to understand what constitutes specificity for the reactive site.

Mechanism of transferase: The reaction would appear to involve the adsorption of both PRPP and adenine on the enzyme surface (*17*). It has been well-established that the natural substrate PRPP is in the α-D configuration (*18*), whereas AMP and other nucleotides are in the β configuration (*19*). Thus, the catalysis proceeds with inversion of configuration at carbon-1 of ribose. Consequently, and in analogy with bimolecular nucleophilic organic substitutions (S_N2), the reaction is envisioned as a nucleophilic attack by adenine on carbon-1 of PRPP (*20*) (Fig. 3).

Clearly, it is position 9 of adenine which is the reactive site of attack by the enzyme. The further point to be made, based on the postulated mechanism of action, is that a particular electronic configuration at position 9 is required in order for nucleophilic attack to occur. Presumably adsorption of adenine to the enzyme promotes the nucleophilicity (electron density) of that

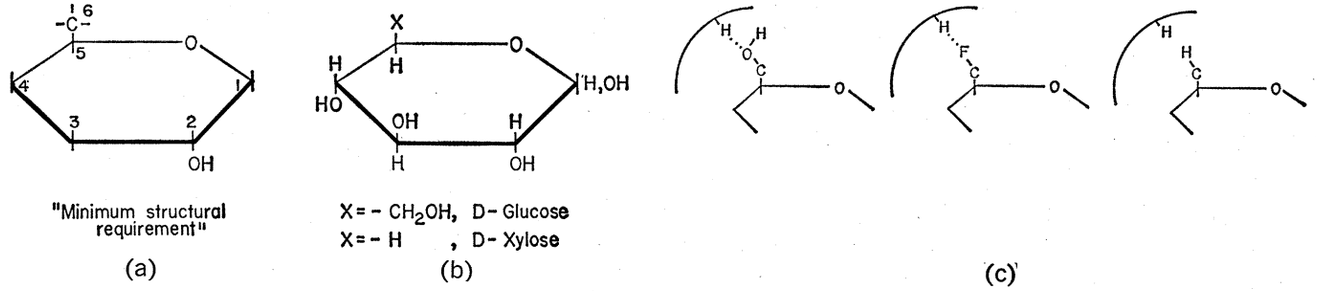


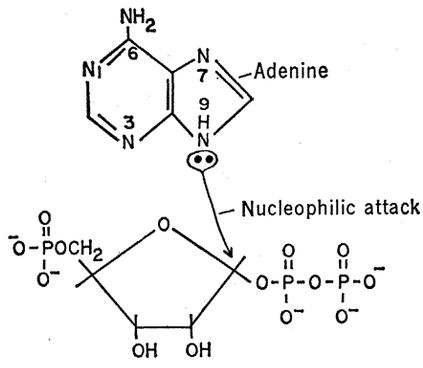
Fig. 2. (a) Principal structural features required for transport via the intestinal carrier. Carbon positions are numbered. (b) Ring (pyranose) structural formation for D-glucose and the five-carbon homomorphic sugar, D-xylose. (c) Closeness-of-fit of the carrier to position 6. Hydrogen bonding is possible for the glucose and the 6-fluoro derivative but not for the 6-deoxy sugars.

site (at least during the transition state), while at the same time facilitating its approach to carbon-1 of ribose. Considerable data indicate that the nitrogen in position 9 is protonated, as indicated in Fig. 3 (*21*). Calculations of the distribution of electrons in adenine suggest that they are present in high density at position 9 (*22*). However, in nonenzymatic reactions position 3 is most reactive, with the nitrogens at positions 1 and 9 showing lesser activity (*23*). The conformation of the enzyme active site therefore presumably shields position 3 while promoting activity at position 9.

With respect to specificity of the adenine carrier, adsorption to the transport protein, according to the reactive site–directed hypothesis, would be aimed at position 9, binding it in such a way as to block its reaction or to stabilize its configuration. There are, of course, other aspects of the carrier specificity which serve important functions, such as the selection of adenine as opposed to the other major naturally occurring purines.

The affinity or binding of the test compounds for the transport system was determined (*24*) by their capacity to inhibit the initial rate of adenine uptake by leukocyte monolayers. The affinity of a compound for the enzyme was tested by measuring its ability to inhibit the initial rate of adenine conversion to AMP (measured at limiting concentrations of adenine and saturating concentrations of PRPP). The inhibitors studied showed competitive kinetics with respect to adenine when either transport system or enzyme was used (*25*).

For most test compounds the results are reported as simply the percentage of inhibition at the indicated concentration. A good inhibitor is one that binds well. Where the values are of more critical importance in developing the argument, the K_i's were determined from reciprocal plots. Assuming that K_m and K_i are equilibrium constants, then K_i is the reciprocal of the association constant of the inhibitor with enzyme or carrier. (A high K_i corresponds to a poor affinity.) In order to compare the affinities of a given compound for the two proteins quantitatively, we must take account of the fact that the affinity of adenine itself is four times greater for the enzyme than for the carrier, K_m (enzyme) = 0.002 millimolar; and K_m (transport) = 0.008 millimolar. Since the relative affinity of a compound as compared with adenine is given by the ratio K_m/K_i (the K's are reciprocals of the affinities), the carrier and enzyme can be compared for the effects of a given compound by an evaluation of a second ratio, (K_m/K_i) for transport to (K_m/K_i) for enzyme. A value of less than 1 for this second ratio indicates a compound with relatively greater affinity for the enzyme than for the transport system; ratios greater than 1 indicate higher affinity for the transport system.



5—phospho—α—D—ribosyl pyrophosphate

Fig. 3. Nucleophilic attack by adenine on carbon-1 or ribose. The lone pair of electrons approach carbon-1 from the back side with respect to the α-pyrophosphate of the ribose ring.

The test compounds used in the analysis of specificity are grouped into three categories in which the adenine structure is modified: (i) substitutions at position 6—the amino group, (ii) additions at other positions, and (iii) modifications of the ring structure.

Both the enzyme and carrier are quite stringent in their requirements for an amino group at position 6 (Table 2). However, there is a quantitative difference: a rather wide variety of analogs such as the 6-mercaptopurine **(5)** has appreciable affinity for the carrier. A detailed examination of the methyl and dimethyl substituted adenines, **6** and **7** shows that their relative affinity for the carrier is 20- to 40-fold that for the enzyme. Interestingly, both the phenyl- and benzylaminopurines **8** and **9** bind strongly to the enzyme and to the carrier, suggesting a region of favorable hydrophobic interaction of the substrate with both proteins (*26*).

The effect of substitutions in adenine (that is, purines in which the 6-amino group is left intact) is shown in Table 3. [The naturally occurring guanine and xanthine (**18** and **19**) are also included.] Except for substitutions at position 8, which will be discussed below, these derivatives in general show a reduced affinity for the proteins.

It is well to recall, however, that the loss of a single hydrogen bond of say 2.4 kilocalories is sufficient on purely thermodynamic grounds to reduce affinity a hundredfold. With this in mind, it is seen that substitutions at position 2 are fairly well tolerated by the transport system. Thus, the relatively bulky 2-methyl- (**11**) and 2-methylamino- (**15**) adenines are roughly fourfold better inhibitors than analogs methylated at other positions in the ring. However, 2-aminoadenine (**16**) is a poor inhibitor. These findings could be rationalized by supposing the existence of a hydrophobic carrier group that reacts favorably with analogs containing hydrophobic methyl groups and unfavorably with analogs substituted with the more hydrophilic amino groups. However, guanine (**18**) which also has a 2-amino group (and a poorly binding 6-hydroxyl group) has a higher affinity for the transport system than the diamino compound does, although this higher affinity is not so great as that of isoguanine (**17**). This slightly anomalous affinity of guanine may be related to a compensatory strengthening of binding at a conjugated, distant site.

The bicyclic structure is required for affinity to both the enzyme and the transport system (Table 4). The one exception is 4-amino-5-imidazole carboxamide **24**, which is a known substrate of the enzyme. Figure 4 emphasizes its structural similarity to adenine. By contrast, compound **24** has no detectable affinity for the carrier whatsoever. Thus, the enzyme tolerates the absence of carbon at position 2, whereas substitution at the same site leads to virtually inert analogs. In a general way then these specificities of enzyme and transport system are reciprocal: expansion at position 2 is tolerated by the carrier, but not the enzyme, whereas deletion of carbon-2 is tolerated by the enzyme but not the carrier. This latter requirement is perhaps best expressed as the necessity for a bicyclic structure for binding to the carrier.

Finally, what is the influence of position 9 on binding to the proteins? It would follow from the reactive site–directed hypothesis that the character of this site exerts an important influence on carrier binding. The fact that the nitrogen at position 9 is a member of a conjugated five-membered system for which alterations at any one site may

produce significant effects at another, greatly complicates the analysis.

In fact, one interpretation is that enzyme adsorption to the five-membered moiety is by way of π-bonding (that is, to the electron system as a whole without particular localization to a group or site). Thus, alterations of the ring to produce pyrazolo (**28**), triazolo (**27**), and then pyrrolo (**29**) derivatives, which progressively decrease the basicity (π-electron cloud) of the ring, are associated with parallel losses of affinity for the enzyme. The magnitude of these changes in density are damped by attraction of the π-electron–deficient pyrimidine ring, however. Substitutions which withdraw electrons from the imidazole ring as in the 8-bromo (**20**) and 8-mercapto (**19**) derivatives also induce appreciable losses in affinity. On the other hand, the 7- and 9-methyladenines (**13** and **14**) which donate electrons, are only very weak inhibitors. The interpretation of these substitutions by bromo and methyl groups is complicated by possible steric effects, however.

The affinity of the carrier for the same analogs follows a different pattern, which is not consistent with the π-bonding interpretation. Thus, whereas the pyrazolopyrimidine (**28**) suffers an appreciable loss in affinity for the carrier, the relative effect is a fifth of that incurred by the enzyme. 8-Bromo adenine (**21**) (a base-weakened derivative) which had poor affinity for the enzyme, was a good inhibitor of the transport system. Moreover, the pyrrolopyrimidine (**29**), a still weaker base, has a greater affinity for the carrier than the pyrazole derivative (**28**).

The relative affinity of the carrier for 7-deazaadenine is in fact 34 times that of the enzyme. It is suggested therefore that the nitrogen at position 7 is not so critical for binding to the carrier. Similarly, its relatively high affinity for 8-bromoadenine and the small decrease in binding of the pyrazolo (**28**) (where N is substituted for C at position 8) as compared with the pyrrolopyrimidine (**29**) suggests a minor role for carbon-8 as a binding site. By elimination therefore, and by virtue of the high affinity of 7-deazaadenine for the carrier, it would appear that the reactive site, position 9, forms complexes with the carrier.

Compared with adenine, 7-deazaadenine (**29**) while strongly bound to the carrier has a reduced electronic charge at position 9. If it were possible to synthesize a compound with a more comparable electronic charge at this position, an even higher affinity might be attainable. On the other hand, it is possible that the carrier actually favors the reduced charge and in this way would protect the adenine from attack. Specifically, it seems probable that nucleophilic attack requires that the enzyme intensify the electronic charge at the nitrogen in position 9, that is, the transition state would involve elevation of the charge density as compared to the substrate in free solution. The carrier, by favoring the pyrrole-like configuration, would tend to reduce or oppose such an increase in the nucleophilicity of position 9. If one were to generalize this argument, it would appear that,

Table 2. Inhibition of adenine analogs derived by substitution of the 6-amino group on the activities of adenine phosphoribosyl transferase and adenine transport. Substitutions for R refer to Fig. 4, top. For example, R = NH_2 in adenine.

Adenine analog		Enzyme			Transport			Relative affinity*
No.	R	Concn. (mM)	Inhibition (%)	K_i	Concn. (mM)	Inhibition (%)	K_i	
1	-H	1.0	39		1.0	30		
2	-OH	1.0	0		1.0	18		
3	$-OCH_3$	1.0	14		1.0	47		
4	-Cl	1.0	7		1.0	66		
5	-SH	1.0	12		0.1	22		
6	$-NHCH_3$	1.0	33	1.17	0.06	50	0.12	39
7	$-N(CH_3)_2$	1.0	5	9.5	0.8	50	1.6	24
8	$-C_6H_5$	0.3	52		0.25	67		
9	$-C_6H_5CH_2$	0.4	63		0.10	85		

* Relative affinity is measured by the ratio of (K_m/K_i) transport to (K_m/K_i) enzyme. A ratio greater than 1 indicates greater relative affinity for the carrier.

Table 3. Inhibition of adenine analogs derived by additions to the purine ring on the activities of adenine phosphoribosyl transferase and adenine transport.

Analog		Versus enzyme			Versus transport			Relative affinity*
No.	Name	Concn. (mM)	Inhibition (%)	K_i	Concn. (mM)	Inhibition (%)	K_i	
10	1-Methyladenine	2.0	0		1.0	30		
11	2-Methyladenine	1.0	−5		.25	35		
12	3-Methyladenine	2.0	0		1.0	32		
13	7-Methyladenine	1.0	48		1.0	12		
14	9-Methyladenine	1.0	0		1.0	41		
15	2-Methylaminoadenine	1.0	7		0.2	30		
16	2-Aminoadenine (2,6,-diaminopurine)	1.0	2		1.0	0		
17	2-Hydroxyadenine (isoguanine)	0.2	42		0.1	25		
18	2-Amino-6-hydroxy-purine (guanine)	0.72	32		0.1	25		
19	Xanthine	1.0	3		1.0	11		
20	8-Mercaptoadenine	1.3	35		1.0	71		
21	8-Bromoadenine			0.9			0.06	60

* Relative affinity is measured as in Table 2.

Table 4. Inhibition of adenine analogs derived by modification of the ring structure on the activities of adenine phosphoribosyl transferase and adenine transport.

Compounds		Versus enzyme			Versus transport			Relative affinity*
No.	Name	Concn. (mM)	Inhibition (%)	K_i	Concn. (mM)	Inhibition (%)	K_i	
			Pyrimidines					
22	4,5,6-Triaminopyrimidine	1.0	11		1.0	12		
23	2,5,6-Triaminopyrimidine	1.0	4		1.0	10		
			Imidazoles					
24	AICA†	1.0	56	0.4	1.0	5		$<<1$
25	Imidazole	20.0	8					
26	Histamine	2.0	8					
			Analogs					
27	8-Azaadenine	1.0	0		1.0	28		
28	4-Aminopyrazolo [3,4-d]pyrimidine			0.27			0.23	5
29	7-deazaadenine‡			1.21			0.14	34

* Relative affinity is measured as in Tables 2 and 3. † 5-Amino-4-imidazolecarboxamide. ‡ 4-Aminopyrrolo[2,3-*d*]pyrimidine.

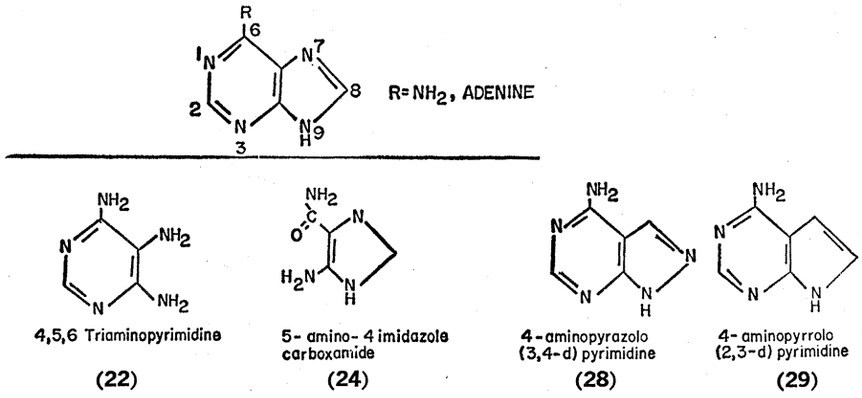


Fig. 4. Structural formulations of ring-modified analogs of adenine, which is at the top of the figure.

just as adsorption to enzymes may involve some strain which labilizes the bond to be attacked (*27*), adsorption to membrane carriers may, in effect, stabilize or prevent precisely such strain or distortion at the reactive site. For the sugar and amino acid transport proteins this took the form of close conformational envelopment or hydrogen bonding (or both) of the carrier to the reactive site.

The points of difference between the specificities of the adenine carrier and enzyme may be summarized as follows. Both carrier and enzyme are relatively exacting for substituents at position 6 (amino group) although there is greater spatial tolerance by the carrier; carbon-2, or perhaps a bicyclic structure, is essential for binding to the carrier but not to the enzyme. On the other hand, substitutions at carbon-2 are not tolerated by the enzyme, although such substitutions are permitted within certain limits by the carrier. Finally, the pyrrole-like position 9 is a significant point of binding to the carrier, rather than to the enzyme, in which π-bonding or interactions with positions 7 and 8 seem more critical.

Biological Significance

The effect of transport and enzyme specificities acting sequentially provides for more rigorous screening of extracellular substances. The reactive site–directed element of carrier specificity (which is frequently not possessed by enzymes) insures that only those substances which have the functional-group configuration necessary for enzymatic attack are admitted to the cell, excluding potentially dangerous unreactive competitive compounds. Once inside the cell, the compounds are sorted out by enzymes into pathways, the specificity for which is determined by nonreactive–site structural elements. Essentially, molecules coming from without the cell are screened successively for different information, the net providing presumably a highly selective advantage. For example, the toxic glucose analog 2-deoxyglucose is rejected for absorption by the intestinal epithelial cell, since it has extremely low affinity for the sugar transport system; however, if this system is bypassed, the analog is rapidly assimilated by hexokinase, for which it is a perfectly satisfactory substrate.

The above generalizations offered have been formulated on the basis of three systems of transport. A bacterial transport system has not been analyzed not only because comparable information is sparse, and a different biological principle may well be involved, but also because in several bacterial systems both transport and enzymatic events appear to be compressed into a single membrane phase (*28*), a circumstance which greatly complicates the analysis.

In such systems, the increased selectivity of transport carrier and enzyme acting sequentially would not be operative. Certainly, little is known of the chemical events involved in membrane transport in any cell. However, the indirect arguments advanced suggest that reactive site–directed specificity of membrane carriers may protect or prevent attack at the reactive site during transport. Such protection would in effect bring about the separation of transport and chemical change, and its gain would be in the fine control over the assimilation of exogenous substances. The difference in specificities between carrier and enzyme make it unlikely that the carrier catalyzes a reaction at the reactive site. The reactive site–directed specificity of membrane carriers would more likely protect or prevent attack at the reactive site during transport.

The possibility that covalent bond formation occurs at other substrate sites during transport certainly exists, but supporting evidence is lacking.

It should be added finally that a compound may be so modified structurally that it may enter the cell by way of alternative transport systems. For example, fructose, which is not a substrate for the intestinal glucose carrier, is transported by way of another system. Again, hypoxanthine, which is a poor substrate for the adenine carrier, enters by a distinctly separate carrier (pathway) (*29*). However, such alternative routes often do not exist, and, in any event, such pathways may provide for only very slow rates of entry.

The foregoing analysis certainly raises many questions on the evolution and integration of transport and enzyme systems, but, in addition, may provide some useful guidelines for the design of chemotherapeutic analogs which, like physiologic substrates, must first penetrate the cell membrane in order to exert their effects on intracellular enzymes.

Summary

The specificities of membrane carriers and enzymes which act on the same chemical group of compounds are both overlapping and complementary; they are distinctly different. Consequently an exogenous substance must satisfy two sets of requirements sequentially (first the carrier's as it penetrates the membrane, then the enzyme's) before it is assimilated into the cell or before it may inhibit the assimilation of other compounds. This double screening makes for an effective specificity of greater exactitude than either enzyme or carrier acting separately. One characteristic of the specificity for transport appears to be a requirement for that part of the substrate molecule which would undergo enzymatic attack once inside the cell. This element of specificity is designated reactive site–directed. The interaction of the carrier protein with the reactive site tends to limit transport to molecules capable of undergoing attack, while simultaneously preventing the attack during translocation through the membrane.

References and Notes

1. The terms membrane carrier, carrier, and transport system are used interchangeably in this article to signify a system obeying saturation kinetics, which facilitates the movement of specific compounds across the cytoplasmic membrane.
2. For other cell types two or more transport systems may be involved for this broad group, but even here several rather diverse amino acids share a common carrier [see H. N. Christensen, *Advan. Enzymol.* **32**, 1 (1969)].
3. C. R. Scriver, *N. Engl. J. Med.* **273**, 530 (1965).
4. E. C. C. Lin, H. Hagihara, T. H. Wilson, *Amer. J. Physiol.* **202**, 919 (1962). There is some variation in the affinity for individual amino acids which is positively correlated with the lipophilic nature of their side chains. This was interpreted [T. H. Wilson, *Intestinal Absorption* (Saunders, Philadelphia, 1962), p. 123] as being due to their increased solubility in the lipid membrane. It is equally well interpreted as being related to a favorable interaction with hydrophobic groups of the carrier protein.
5. D. Nathans, D. F. Tapley, J. E. Ross, *Biochim. Biophys. Acta* **41**, 271 (1960).
6. This specificity is not absolute. D-Methionine shows definite, though very low, affinity for the neutral amino acid carrier (*4*). Also, elimination of the α-hydrogen may yield compounds with detectable, albeit much reduced, affinity. In Ehrlich ascites tumor cells, *N*-methyl derivatives may be transported by a second carrier system that may be specialized for this cell type; compare (*2*).
7. See, for example, R. W. Holley, J. Apgar, B. P. Doctor, J. Farrow, M. A. Marini, S. H. Merrill, *J. Biol. Chem.* **236**, 200 (1961).
8. E. C. Webb, *Biochem. Soc. Symp.* **19** (1960).
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10. Some minor variations in organ specificities for hexokinase are known. Brain has been studied most completely [A. Sols and R. K. Crane, *J. Biol. Chem.* **210**, 581 (1954)]. The intestinal enzyme appears to be quite similar [A. Sols, *Biochim. Biophys. Acta* **19**, 144 (1956)]. There is also variation in the structural requirements for glucose transport in various tissues, the most important of which is the lack of requirement for the 2-hydroxyl in other tissues, such as erythrocytes [R. G. LeFevre and J. K. Marshall, *Amer. J. Physiol.* **194**, 333 (1958)]. The importance of the 3- and 4-positions in binding to the enzyme were emphasized by C. F. Lange and P. Kohn [*J. Biol. Chem.* **236**, 1 (1961)].
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15. Interactions with position 1 are also interesting with respect to the physiological substrate galactose, which is also transported via the "glucose" system. This substrate is phosphorylated by galactokinase (E.C. 2.7.1.6) at position 1, and this is its reactive site. The fact that several structural modifications are permissible for transport can lead to the erroneous conclusion that binding to this site is not critical. Thus, 1-deoxy-D-glucose and a score of α- and β- (1-substituted) alkyl glucosides are transported. However, some puzzling exceptions are aurothio-D-glucose and β-thiomethyl glucoside, and these are not transported [B. R. Landau, L. Bernstein, T. H. Wilson, *Amer. J. Physiol.* **203**, 237 (1962)]. One interpretation of these data is that the thio derivatives could not be hydrogen bonded to a carrier protein, and this is strengthened by an analysis of various galactose derivatives (*13*). 1-Deoxygalactose and methyl-β-D-thiogalactopyranoside are not transported, but α-D-galactopyranosyl fluoride is. Since the deoxy or thio derivatives cannot form hydrogen bonds, whereas the fluoro analog can, it is suggested that hydrogen bonding to the one position is essential for transport. Only limited information is available on the specificity of galactokinase [F. Alvarado, *Biochim. Biophys. Acta* **41**, 233 (1960)]. The data available do emphasize the complementary nature of galactokinase and sugar transport specificities. Thus, derivatives at carbon-2 such as 2-deoxygalactose and talose (the 2-epimer of galactose) which are rejected by the transport system are phosphorylated by galactokinase. On the other hand glucose, the 4-epimer of galactose, is rejected by galactokinase.
16. PRPP, 5-phospho-α-D-ribosyl pyrophosphate (Fig. 3).
17. The kinetics of product inhibition indicate that the catalysis proceeds as an ordered sequential reaction. PRPP adds first to the enzyme, then adenine; PP_i leaves, then AMP [A. Kornberg, I. Lieberman, E. S. Sims, *J. Biol. Chem.* **215**, 417 (1955); R. D. Berlin, *Arch. Biochem. Biophys.* **134**, 120 (1969)].
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25. The actual concentrations used were (mmole/liter): tris hydrochloride, 50, *p*H 8.29; magnesium chloride, 2.0; PRPP, 0.1; and adenine, at its apparent K_m, 0.002. The enzyme was prepared from rabbit polymorphonuclear leukocytes isolated from peritoneal exudates. The cells were subjected to sucrose lysis after the method of Cohn and Hirsch [*J. Exp. Med.* **112**, 983 (1960)], and the enzyme was assayed directly from the soluble 8000*g* supernatant fraction. The AMP product was stable in this extract, which also contained no adenase, guanase, or xanthine oxidase. The enzyme specific activity was 0.02 unit per milligram of protein (1 unit = 1 micromole converted per minute). The sensitive assay was performed by chromatographic separation of substrate and product [R. D. Berlin and E. R. Stadtman, *J. Biol. Chem.* **241**, 2679 (1966)]. Paper chromatography of the reaction mixtures in three solvent systems indicated that adenine and AMP were the only radioactive compounds present.
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Information Theory in Biology after 18 Years

Information theory must be modified for the description of living things.

Horton A. Johnson

Several years ago *Science* published an article by E. N. Gilbert entitled "Information theory after 18 years" (*1*). In reviewing the contribution of information theory to communications engineering, Gilbert, though still hopeful, found that up to that time it had flourished on promises alone.

In biology the role played by information theory has been even more disappointing. Its introduction into biological thought in the early 1950's promised a calculus uniquely suited to the mathematical description of living systems. Now, after 18 years of symposia and published articles on the subject, it is doubtful whether information theory has offered the experimental biologist anything more than vague insights and beguiling terminology.

After reviewing some of these hopes and disappointments, I shall point out some new directions in which information theory might evolve to become a useful general calculus for biology.

Early Expectations

During the past 100 years, in which physical sciences have enjoyed such a profitable interplay between experiment and mathematical theory, the life sci-

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