Formation of a complex between valine and intestinal mucosal lipid; its possible role in valine absorption

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ABSTRACT During intestinal absorption amino acids must traverse the lipid-rich epithelial cell membrane, possibly in a lipid-soluble form. In a search for such a form, we have determined the ability of lipid extracted from intestinal mucosa to bind valine. After incubation in a valine-containing medium this lipid (defined as the heptane-soluble fraction) contained, on the average, 3.63 μmoles of valine per 100 mg of lipid. Cyanide (0.002 M), 2,4-dinitrophenol (0.0002 M), and anaerobic conditions had little effect on this process. Valine uptake into the lipid fraction of mucosa was complete after 2.5 min.

Of a number of sugars and amino acids tested, isoleucine, methionine, and leucine were the most potent inhibitors of valine uptake into lipid. The inhibition by leucine appeared to be competitive. A similar uptake of glucose into the mucosal lipid was not inhibited by leucine, methionine, or isoleucine but was inhibited by galactose. Various phosphoglycerides (but not sphingomyelin) from other sources, used in place of mucosal lipid, were able to carry 20–150 times as much valine into heptane-soluble fraction as were other lipid classes. Some characteristics of the complex are similar to those of the valine transport system.

KEY WORDS rat · intestinal mucosa · ethanol–ether extraction · heptane-soluble lipid · valine–lipid complex · glucose–lipid complex · valine transport · metabolic inhibitors · competitive inhibition · phosphoglycerides

When an amino acid is absorbed from the intestine, the molecule must traverse the lipid-rich epithelial cell membrane. It seems likely that an amino acid must be in a lipid-soluble form at this point in the transport process. An amino acid may become soluble in lipid by combining with a lipid carrier molecule, such as a phospholipid, to form a complex capable of traversing the lipid membrane (1). However, the failure to identify such carriers for amino acid transport (2, 3) or to detect any permanent trace on the amino acid transported of the biochemical events that would have occurred during transport (4–6) argues against the existence of such small, mobile carriers, and it has been argued that the carrier-like behavior in membrane transport may be mediated by reactive sites embedded in the macromolecular constituents of the membrane (7, 8). We have extracted lipid from the intestinal mucosa and determined its ability to bind valine. The general properties of this association were investigated and compared to those of the intact, in vitro transport system for valine.

MATERIALS AND METHODS

Chemicals

The sources of the compounds used were as follows: L-valine, L-methionine, L-leucine, L-isoleucine, L-glutamic acid, glycine, and α-methyl-D-glucose (Calbiochem, Los Angeles, Calif.); D-glucose (Baker Chemical Company, Phillipsburg, N.J.); D-galactose, L-lysine monohydrochloride, 2-deoxy-D-glucose, and phosphatidyl inositide (Sigma Chemical Co., St. Louis, Mo.); xylose (Pfanstiehl Labs., Inc., Waukegan, Ill.); D-mannitol (Fisher Scientific Company, Chicago, Ill.); animal lecithin, vegetable lecithin, animal cephalin, phosphatidyl ethanolamine, α-tocopherol, and Vitamin A (Nutritional Biochemicals Corporation, Cleveland, Ohio); phosphatidyl serine, sphingomyelin, linoleic acid, linolenic acid, and arachidonic acid (Mann Research Labs., Inc., New York); oleic acid (Mallinckrodt Chemical Works, St. Louis, Mo.); tristearin and cholesterol

(Eastman Organic Chemicals, Rochester, N.Y.); palmitic acid and squalene (Matheson, Coleman, and Bell, Chicago, Ill.); peanut oil (Humco Lab., Inc., Texarkana, Tex.); and lard (Wilson & Co., Inc., Chicago, Illinois). Uniformly labeled L-valine-^{14}C was obtained from Nuclear-Chicago Corporation, Des Plaines, Ill. and was more than 99% pure when tested by dilution analysis, paper chromatography (*n*-butanol–water–acetic acid), and paper electrophoresis. Uniformly labeled L-lysine-^{14}C and glycine-^{14}C were also obtained from Nuclear-Chicago; they were reported to be at least 98% pure by dilution analysis, paper chromatography, and paper electrophoresis.

Studies on Intestinal Sacs

Male Wistar rats were fed Purina Labina pellets and water ad libitum. Animals weighing 200–300 g were selected and killed by decapitation; the small intestine was quickly removed, everted, and tied into sacs about 7 cm long according to the method of Wilson and Wiseman (9). Except as noted in individual experiments each sac was filled with 1.0 ml of a gassed medium (95% oxygen and 5% carbon dioxide) containing Krebs bicarbonate buffer, pH 7.4 (10), 3 mM L-valine, and 50,000–100,000 cpm/ml of radioactive L-valine. The sacs were placed in a 25 ml Erlenmeyer flask containing 5 ml of the same medium as inside the sac and incubated at 37°C for 60 min. The tissue uptake of valine is defined as the amount of valine (μmoles) present in 500 mg of intact intestinal tissue after the 1 hr incubation.

Isolation of Intestinal Lipid

The intestinal tissue used in the sac studies was washed free from surface radioactivity and the mucosa was scraped with a spatula. We homogenized the weighed scrapings with 25 volumes of hot ethanol–diethyl ether 3:1 in order to split the lipid from the lipoproteins and other complex forms (11). The homogenate was centrifuged, and the supernatant fraction was collected and dried under reduced pressure at temperatures no higher than 40°C. 12 ml of *n*-heptane was added to the residue with gentle heating (50°C) and the suspension was centrifuged. The supernatant fraction was collected and taken to dryness. The resultant residue was extracted with heptane, this time 6 ml, and the process repeated twice more, with 3 and 1.5 ml of heptane, respectively. The final heptane-soluble extract would be expected to contain any lipid-soluble valine present. As will be explained later, washing the heptane solution with water rapidly decomposed the valine–lipid complex.

Experimental Modifications

In many of the experiments described we did not use intestinal sacs as the source of the lipid and employed valine media of various concentrations added directly to tissue homogenates, heptane-soluble lipid, and known lipid compounds. These experimental modifications will be described in their proper context under Results.

Determination of Valine

The radioactivity of the various fractions was determined in a Tri-Carb liquid scintillation spectrometer, in a system containing xylene–dioxane–ethanol 5:5:3, naphthalene (40 g/liter), 2,5-diphenyloxazole (5 g/liter), and 1,4-bis[2-(5-phenyloxazolyl)] benzene (100 mg/liter) (Packard Instrument Co., Inc., Downers Grove, Ill.). Aliquots of the various fractions were taken for a determination of the dry weight. The amount of radioactivity per ml or mg of the various tissue fractions was used as a measure of the incorporation of valine into that fraction. The counting rates for each individual experiment were normalized so that 10,000 cpm/ml was equivalent to the initial concentration of nonisotopic valine. This procedure allowed for the conversion of radioactivity counts to amount of valine when desired, in order that data from identical experiments with different initial counting rates could be pooled.

Paper Chromatography

Ascending paper chromatography was conducted in butanol–acetic acid–water 80:10:10 and the chromatograms were sprayed with ninhydrin in ethanol (Sigma Chemical Co.). The radioactivity of areas of the chromatograms was determined by liquid scintillation counting.

RESULTS

It has been previously demonstrated in this laboratory that everted sacs from rat intestine can actively take up valine and transport it against a concentration gradient (12). In these experiments too, the valine was concentrated on the serosal side, although we do not report the observations. In order to determine whether valine is associated with lipid during its active transport, we determined radioactivity in extracts of the mucosa. Results such as those shown in Table 1 were consistently obtained. Approximately 2.4% of the total weight of the mucosa dissolved in the hot ethanol–ether; only 0.39 μmole of valine per 100 mg was associated with the insoluble residue. Of the dissolved material, 28% was not soluble in heptane; this portion contained the most valine on a weight-for-weight basis. The final heptane-soluble lipid contained 3.6 μmoles of valine per 100 mg; this suggests that a portion of the valine present in mucosal tissue during transport is in a lipid-soluble form. Attempts to backwash the heptane phase with water resulted in a rapid loss of radioactivity into the aqueous phase; this

TABLE 1 DISTRIBUTION OF VALINE IN MUCOSAL FRACTIONS DURING ITS TRANSPORT

Intestinal Fraction	Final Weight	Valine Uptake
	mg	*μmoles/100 mg*
Original mucosa	289.4 ± 16.76 (12)	3.29 ± 0.42 (12)
Tissue insoluble in ethanol–ether	42.5 ± 2.6 (12)	0.39 ± 0.02 (12)
Portion soluble in ethanol–ether, insoluble in *n*-heptane	7.0 ± 0.53 (12)	21.62 ± 3.83 (11)
Portion soluble in *n*-heptane	5.0 ± 0.46 (12)	3.63 ± 0.69 (11)

Everted sacs were incubated in a Krebs bicarbonate medium containing 3 mM valine-^{14}C. After 1 hr of incubation, solvent fractionation yielded fractions with dry weight and radioactivity as indicated. Means ± SEM (number of determinations in parentheses).

suggests that the association of valine with lipid is readily reversible.

The rapid rate of formation of the valine–lipid complex is shown in Fig. 1. Valine uptake reached a maximum level after 2.5 min.

Table 2 shows a comparison of the effects of metabolic inhibitors. Cyanide (0.002 M), 2,4-dinitrophenol (0.0002 M), and anaerobic conditions (gassing the medium with 95% N_2, 5% CO_2) reduced valine uptake into the lipid by 22, 19, and 28%, respectively. However, these same inhibitors reduced valine uptake by intact intestinal tissue 56, 54, and 69%, respectively, so that valine incorporation into lipid may not be dependent on enzymatic activity. To investigate this possibility we scraped approximately 1 g of mucosa from fresh rat intestine and inactivated its enzymes by homogenization in 25 volumes of hot ethanol–ether 3:1. 1 ml of the valine medium was then added directly to the homogenate. Table 3 shows that the uptake of valine into the lipid fraction under these conditions was about three times as great as

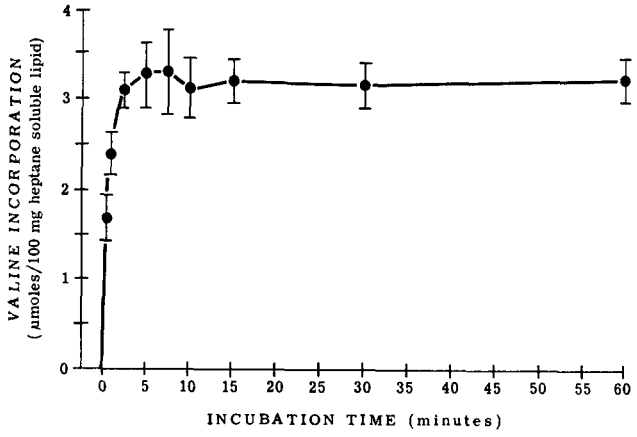

FIG. 1. Effect of time of incubation on the uptake of valine into the lipid. The sacs were incubated in a buffered 3 mM valine solution (Krebs bicarbonate, pH 7.4) for the indicated time periods. The sacs were removed, opened, and washed in ethanol–ether 3:1. The mucosa was scraped off, weighed, and homogenized in 25 volumes of fresh hot ethanol–ether 3:1. Of the material extracted, that soluble in heptane is represented here. Means ± SEM; n was at least 8.

TABLE 2 COMPARISON OF THE DECREASE IN VALINE UPTAKE BY EVERTED SACS AND IN VALINE UPTAKE INTO LIPID CAUSED BY METABOLIC INHIBITORS

Inhibitor	Tissue Uptake	Lipid Uptake
	% decrease	
Anaerobiosis	69.3 ± 2.9 (6)	27.8 ± 4.6 (3)
Dinitrophenol 2×10^{-4} M	53.9 ± 3.4 (4)	18.5 ± 4.3 (3)
Cyanide 2×10^{-3} M	55.9 ± 2.0 (4)	22.1 ± 3.9 (3)

Conditions are as indicated for Table 1 except that the sacs were incubated in the presence of the inhibitors or under anaerobic conditions. Means ± SEM (n in parentheses).

that obtained when intact tissue was used (Table 1). The addition of 1 ml of valine medium to 25 ml of the ethanol–ether solvent alone (control) did not result in the appearance of valine in the final heptane fraction.

In an examination of whether a lipid–amino acid complex existed for other actively transported amino acids, 1 mM lysine or 5 mM glycine was used in place of the 3 mM valine in the sac system. At the end of the 1 hr incubation both of these amino acids exhibited active transport. The lipid isolated from the mucosal homogenates contained 1.51 ± 0.23 μmoles of lysine and 8.45 ± 0.82 μmoles of glycine per 100 mg (mean ± SEM) on the basis of eight determinations each.

If the lipid of the intestinal mucosa is actually functional in the transport process, one might expect that the lipid present in the brush border of the epithelial cell would have a greater capacity to form a complex with valine than the lipid from the whole mucosal homogenate. Brush borders were isolated from rat intestine by the method of Harrison and Webster (13); their identity and purity were confirmed microscopically. Lipid was isolated from the brush border; its ability to complex valine was compared to that of an equal weight of unfractionated lipid isolated from mucosal homogenates. There was no statistically significant difference between the two preparations.

A characteristic property of the neutral amino acid transport system is the competition between its members

TABLE 3 UPTAKE OF VALINE BY ETHANOLIC MUCOSAL HOMOGENATE

	Fraction		
	Insoluble in Ethanol–Ether	Soluble in Ethanol–Ether, Insoluble in *n*-Heptane	Soluble in *n*-Heptane
	μmoles/100 mg		
1 ml of valine medium added to homogenate containing 1 g of mucosa and 25 ml of ethanol–ether 3:1	0.48 ± 0.11 (4)	8.60 ± 1.71 (5)	11.6 ± 1.22 (5)
1 ml of valine medium added to 25 ml of ethanol–ether 3:1	1.75 ± 0.36 (4)	11.0 ± 3.5 (4)	0 (4)

Initial valine concentration, 3 mM. Values ± SEM (n in parentheses).

TABLE 4 EFFECT OF VARIOUS POTENTIAL INHIBITORS ON THE UPTAKE OF VALINE INTO THE LIPID FRACTION

Potential Inhibitor	% of Control Uptake
L-Leucine	45.8 ± 5.8*
L-Isoleucine	32.8 ± 4.2*
L-Methionine	39.3 ± 7.8*
Glycine	94.1 ± 10.6
L-Glutamic acid	93.9 ± 2.7
L-Lysine	104.7 ± 9.4
D-Glucose	98.2 ± 6.7
D-Galactose	81.7 ± 4.0*
2-Deoxy-D-glucose	85.2 ± 2.5*
α-Methyl-D-glucose	86.3 ± 2.9*
Xylose	99.5 ± 5.0
Mannitol	92.3 ± 6.9

1.5 mg of heptane-soluble lipid, isolated from fresh mucosa and dissolved in 1 ml of ethanol–ether–heptane 12:4:1 was mixed with 0.1 ml of a Krebs medium containing 1 mM valine-^{14}C and 10 mM concentration of the compounds listed. The final concentration of valine in the heptane fraction was determined and expressed as a percentage of the uptake when valine alone (1 mM) was present. Values ± SEM (n = 6).

* Significantly different from controls ($P < 0.05$).

for a common tissue uptake site. It was therefore important, in assessing the possible physiological significance of the valine–lipid complex, to determine whether structurally similar amino acids would competitively inhibit complex formation. Table 4 shows the results of a study in which 1.52 mg of heptane-soluble lipid isolated from fresh mucosa and dissolved in 1 ml of ethanol–ether–heptane 12:4:1 was added to 0.1 ml of a Krebs medium (pH 7.4) containing radioactive valine at a concentration of 1 mM, and the indicated, potential competitors at 10 mM. The mixture was warmed, then evaporated to dryness, and the residue was extracted with heptane as described under Methods. Of the compounds tested, by far the most potent inhibitors of the uptake of valine were L-isoleucine, L-methionine, and L-leucine. The amino acids glycine, L-glutamic acid, and L-lysine did not decrease valine uptake.

The effect of sugars and mannitol on the uptake of valine into the lipid was determined as a test of the specificity of the process and in reference to the interaction between amino acid and sugar transport. D-galactose, 2-deoxy-D-glucose, and α-methyl-D-glucose appeared to be inhibitory while xylose and D-glucose were without effect. Mannitol, which has been shown to inhibit valine transport when present during preincubation (unpublished data), did not significantly reduce the uptake of valine. Results similar to those shown were obtained when the potential competitors were present at 5 mM. One explanation for the decrease in uptake of valine could be a decreased solubility of the valine in ethanol–ether–heptane in the presence of leucine, isoleucine, and methionine. To assess this possibility we added 0.1 ml of each mixture indicated in Table 4 to 1.0 ml of ethanol–ether–heptane, warmed the mixture, and allowed it to stand 20 min before centrifugation. Valine concentrations in the supernatant solutions from all the mixtures were the same.

Table 5 gives the results of a study in which valine uptake into the lipid was studied as a function of the relative proportions of valine and leucine. As the valine concentration increased in the presence of 0.91 mM leucine, the percentage of valine incorporation into the lipid increased. These results are consistent with a competitive inhibition between valine and leucine for the lipid. The percentage incorporation of valine into lipid from media containing 0.091 mM and 0.55 mM valine in the absence of leucine indicates that the efficiency of incorporation decreases with increasing valine concentration. A decrease in the efficiency of valine transport with increasing valine concentration has also been observed in whole tissue studies (12).

The apparent competitive inhibition of the uptake of valine into the lipid in the presence of various neutral amino acids raised the possibility that sugar uptake by the lipid may be inhibited by the presence of other sugars. The uptake of 1 mM D-glucose in the presence of various potential competitors was determined in the same way as for valine, and the results are given in Table 6. Although the results are not as clear-cut as with valine (Table 4), glucose uptake did decrease 20–30% in the presence of galactose, α-methyl-D-glucose, 2-deoxy-D-glucose, and fructose, but not xylose. In addition, glycine and glutamic acid lowered glucose uptake, but the neutral amino

TABLE 5 EFFECT OF VARYING THE VALINE CONCENTRATION ON THE UPTAKE OF VALINE BY LIPID IN THE PRESENCE OF LEUCINE

Amino Acid Concentration	Valine Uptake into Lipid	
μmole/ml	*μmole/ml heptane*	*% of optimum*
0.091 Valine	0.0240	26.4
0.091 Valine 0.91 Leucine	0.0064	7.0
0.18 Valine 0.91 Leucine	0.0146	8.1
0.36 Valine 0.91 Leucine	0.0432	12.0
0.55 Valine	0.125	22.7
0.91 Valine 0.91 Leucine	0.0936	10.3

0.1 ml of a Krebs medium containing valine or valine and leucine was added to 1 ml of heptane-soluble mucosal lipid (1.5 mg/ml) dissolved in alcohol–ether–heptane 12:4:1 to give the final amino acid concentrations listed in column 1. The solution was warmed and evaporated to dryness and the residue was extracted with heptane as described under Methods (volume of final heptane solution, 1.5 ml). Each value is the average of two determinations.

TABLE 6 EFFECT OF POTENTIAL INHIBITORS ON THE UPTAKE OF GLUCOSE INTO THE LIPID FRACTION

Potential Inhibitor	% of Control Uptake
D-Galactose (9)	67.3 ± 2.1*
α-Methyl-D-glucose (9)	82.0 ± 4.2*
2-Deoxy-D-glucose (7)	76.5 ± 3.0*
D-Xylose (7)	93.7 ± 4.2
D-Fructose (4)	80.4 ± 2.7*
Glycine (9)	79.1 ± 5.0*
L-Glutamic acid (9)	72.1 ± 4.2*
L-Methionine (9)	103.3 ± 5.5
L-Leucine (8)	117.1 ± 4.8*
L-Isoleucine (7)	116.5 ± 7.6*
L-Valine (7)	89.5 ± 4.0*

Conditions were exactly the same as those described for Table 4 except that the Krebs medium contained 1 mM glucose in the presence of 10 mM of the listed compounds. (n in parentheses.)
* Significantly different from controls ($P < 0.05$).

acids leucine, isoleucine, and methionine, which markedly lowered valine uptake, did not decrease glucose uptake into the lipid fraction. In fact, leucine and isoleucine appeared to stimulate the process.

Which component of the lipid was responsible for valine uptake was determined next. 15 mg of each compound listed in Table 7 was dissolved in 15 ml of hot ethanol–ether and added to 1 ml of buffered 3 mM valine-^{14}C. After centrifugation, the concentration of valine in the heptane phase was determined. The phosphoglycerides were able to carry 100 times as much valine into the lipid fraction as most of the other lipids tested; oleic, linoleic, and arachidonic acids were moderately

TABLE 7 ABILITY OF DIFFERENT LIPID SPECIES TO CARRY VALINE INTO THE LIPID FRACTION

Compound	Uptake of Valine into Lipid
	μmoles/ml final heptane fraction
Animal lecithin (5)	0.976 ± 0.098
Vegetable lecithin (2)	0.933
Animal cephalin (3)	0.638 ± 0.043
Phosphatidyl ethanolamine (4)	0.846 ± 0.116
Phosphatidyl serine (4)	0.754 ± 0.204
Phosphatidyl inositide (4)	0.984 ± 0.185
Sphingomyelin (4)	0.0006 ± 0.0001
α-Tocopherol (4)	0.011 ± 0.002
Vitamin A (3)	0.006 ± 0.001
Cholesterol (6)	0.004 ± 0.001
Squalene (3)	0.0009 ± 0.0003
Lard (4)	0.003 ± 0.0002
Peanut oil (14)	0.007 ± 0.0002
Tristearin (4)	0.0006 ± 0.0003
Palmitic acid (10)	0.006 ± 0.002
Oleic acid (9)	0.043 ± 0.007
Linoleic acid (8)	0.034 ± 0.008
Linolenic acid (6)	0.009 ± 0.002
Arachidonic acid (4)	0.050 ± 0.010

15 mg of each of the lipids was dissolved or homogenized in 15 ml of hot ethanol–ether. 1 ml of the buffered valine (3 mM) solution was added to this homogenate and the final concentration of valine in the heptane fraction determined. Means ± SEM (n in parentheses).

effective. Little difference between the various phosphoglycerides could be seen. The lack of activity with tristearin suggests that the phosphoryl-base portion of the phospholipid functions as the valine carrier, but the lack of activity with sphingomyelin, which contains a phosphoryl choline moiety, indicates that a disubstituted glyceride structure may also be required. The highly unsaturated compound squalene had very low activity, so that unsaturation is not a prerequisite for valine complexing activity.

The valine–lipid complex was found to be very labile in an aqueous environment. When the heptane fraction containing the complexed lipid was taken to dryness and 0.3 ml of water was added with gentle warming (40°C), a portion of the residue was solubilized in the water. Paper chromatography of the water-soluble extract gave six ninhydrin-positive spots. The only radioactive spot had an R_f corresponding to that of free valine.

DISCUSSION

The results of this study show that lipid from intestinal mucosa can bind valine under a variety of experimental conditions. The importance of lipid complexes in the metabolism of amino acids has been suggested by a number of studies. Hendler (14) has proposed that an amino acid–lipid complex is an intermediate in the synthesis of protein in hen oviduct and may represent an important

stage during the metabolism of the amino acids. Similar complexes have been found in *Drosophila* (15), rat liver microsomes (16), *Bacillus megaterium* (17), broad-bean plants (18), and *Penicillium chrysogenum* (19).

A functional role of lipid, especially phospholipid, as an intermediate in transport has been suggested by LeFevre, Habich, Hess, and Hudson (11). These workers described the formation of phospholipid–sugar complexes from "ghosts" of human erythrocytes and concluded that parallels exist between the properties of mediated sugar transport in the red cell and those of the complex. The methods used in LeFevre's studies were similar to those we used.

The nature of the type of bonding between the valine and the lipid is important in assessing a possible physiological role of the complex in transport. It has been shown that during the transport of amino acids the same apparently unchanged molecules that is taken up at the mucosal side appears in the serosal medium (20). This finding eliminates the possibility that a lipid–amino acid combination involving a primary covalent linkage such as that found in *O*-L-lysyl phosphatidyl glycerol (21) is functional in transport. The ease of release of the valine from the lipid on contact with an aqueous medium is not compatible with the properties of amino acid incorporation into phosphatidopeptides (22, 23). The choice of the present lipid extraction method, in which aqueous backwash steps could not be used successfully, was dictated by this extreme lability of the complex to aqueous conditions. If the amino acid–lipid is functional in transport, it would be necessary that this binding involve hydrogen bonding, electrostatic attractions, or interaction between lipophilic residues (24).

If the valine–lipid complex is functional in valine transport, certain predictions can be made about its characteristics. An inhibition of valine incorporation into the lipid would be expected in the presence of known competitive inhibitors of valine transport. Leucine, isoleucine, and methionine, which competitively inhibited valine transport in the sac (12), also inhibited the formation of the complex in an apparently competitive manner (Tables 4 and 5). Glutamic acid, which is transported by an acidic amino acid pathway (25), lysine, which is transported by a basic amino acid pathway (26), and glycine, which is transported by a glycine-preferring pathway (27) did not inhibit either valine incorporation into the lipid complex (Table 4) or valine transport in the sac (12).

The inhibition of amino acid transport in the intestine by various sugars has been reported (28–31). Alvarado (31) has presented evidence to support a common carrier mechanism for the intestinal transport of amino acids and sugars. The small inhibition of valine incorporation caused by galactose and α-methyl-D-glucose (Table 4) could be connected with this phenomenon. The decrease in the incorporation of glucose into the lipid in the presence of other actively transported and structurally similar sugars (Table 6) is similar to the characteristics of glucose transport in the intestine. However, the most important finding of the glucose experiments was that the uptake of glucose into the lipid fraction was not decreased in the presence of leucine, isoleucine, and methionine. This absence of inhibition suggests that the marked inhibition of valine uptake into the lipid caused by these amino acids is due not to a nonspecific alteration in the chemical or physical characteristics of the lipid, but to chemical competition for a specific site on the lipid.

If the valine–lipid complex is functional in active transport, an amino acid–lipid complex should exist for every actively transported amino acid. Both lysine and glycine were incorporated in the lipid fraction during their passage across the mucosa.

The extremely rapid incorporation of the valine into the lipid, which seems to be complete after 2.5 min (Fig. 1), is compatible with a role in active transport since 10 min is required for maximum saturation of the intestinal tissue during active valine transport (12). This kinetic pattern suggests that lipid plays a role in transport, but so far we have not confirmed this role by determining the rate of turnover of the valine–lipid complex.

On the basis of an involvement of the lipid in transport one would expect the lipid isolated from the brush border to be better able to form a complex with valine than the lipid isolated from whole mucosal homogenates. However, the results showed no difference in activity between these lipids, which suggests that the binding does not depend on one specific lipid present only (or to a greater extent) in brush border tissue.

In contrast to valine transport in the intestine the formation of the complex is not dependent on an energy-consuming metabolic process or physiological conditions. Although the everted sacs incubated with metabolic inhibitors contained 55–70% less valine than the controls, the uptake of valine into the lipid fraction derived from these sacs was decreased only about 20% by these inhibitors (Table 2). This comparatively slight inhibition of uptake is probably due to the decrease in the amount of valine in the tissue available for binding rather than an inhibition of an energy-requiring step.

Both the results with different lipid species (Table 7) and the presence of ninhydrin-positive spots on the paper chromatogram of the hydrolyzed complex favor the phosphoglycerides as being the binders of amino acids. These phosphoglycerides are concentrated in the membranes and have the solubility characteristics required of a hypothetical carrier.

These findings suggest a relationship between the valine–lipid complex and valine transport, but certainly

leave many questions unanswered. There is no direct evidence of the existence of the valine–lipid complex prior to the evaporation of the alcohol–ether extract. Some amino acid–lipid complexes have been reported to be an artifact of the extraction procedure (32), although it now appears that the extent of this artifact formation is slight (18). Despite the experimental limitations described and despite several inconsistencies between the properties of the complex and those expected of a transport carrier, the numerous similarities in these properties merit a more detailed characterization of the complex.

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