Absorption of L-valine and D-glucose By the Midgut Gland of the Shrimp, *Penaeus aztecus*

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Barbara Ann Carr

December, 1972

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Abstract

An *in vitro* tissue accumulation technique was used to study the absorption of L-valine and D-glucose by the midgut gland of the shrimp, *Penaeus aztecus*. Evidence was found for the active transport of L-valine but not for D-glucose. However, both compounds were accumulated in significant amounts into the ethanol insoluble fraction of the tissue, indicating that they were anabolized.

The active transport of L-valine was indicated by the following: 1) significant differences between the accumulation rates into the ethanol soluble fraction of L-valine and D-mannitol at several initial ambient concentrations (D-mannitol is a sugar not known to be transported by the rat or hamster small intestine), 2) the inhibition of the transport mechanism by anaerobic conditions, 3) the almost total recovery of L-valine in a chemically unaltered form, 4) the competitive inhibition of the rate of accumulation of L-valine by another naturally occurring amino acid (L-leucine). and 5) the demonstration of first and zero order kinetics.

The movement of D-glucose into the midgut gland tissues was probably by diffusion as indicated by the fact that D-glucose was not accumulated against its concentration

gradient and the absence of significant differences between the rates of accumulation of D-glucose and D-mannitol into the ethanol soluble fraction.

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Introduction

Nutrition has been studied in many vertebrates but has been sadly neglected in most invertebrates. This is true of nutritional studies such as the study of absorption, and in particular, of the physiological mechanisms by which organic molecules are absorbed by various invertebrates.

The introduction shall consist of two parts. The first consists of a review of the structure and function of the crustacean digestive system. The second consists of a summary of current knowledge about amino acid and monosaccharide absorption in animals.

I

The phylum Arthropoda surpasses all others in ecological adaptation and sheer numbers of species and individuals. Among the major taxa which comprise the phylum Arthropoda is the class Crustacea, the only large class of Arthropoda which is primarily aquatic. The largest order of Crustacea is the Decapoda, which has been further subdivided into two suborders: the Natantia, comprising the shrimp (including the genus Penaeus) and the Reptantia, containing the lobsters, crabs and crayfish. Both groups are of commercial

importance to world fisheries.

The digestive system of the decapod (Natantia) consists of a fore-, mid- and hindgut and the midgut gland or hepatopancreas. The foregut is divided into an esophagus, cardiac stomach and pyloric stomach. Distal to the foregut is the midgut and its lateral diverticula, the midgut gland. The foregut is not presumed to have any absorptive function (Lockwood, 1967; van Weel, 1970) but is reported to be slightly permeable to glucose (Yonge, 1936). Dall concluded that the permeability to glucose in this region is insufficient to permit a significant amount of carbohydrate to be absorbed. The midgut or digestive gland is a mass of fine, blindly-ending tubules which unite into a common hepatopancreatic duct emptying into the anterior end of the midgut. Injection of olive oil and iron lactate into the cardiac stomach of Nephrops norvegicus (Norway lobster) and the subsequent appearance of these compounds in the midgut epithelium and the midgut gland led Yonge (1924) to propose an absorptive function for these areas, particularly the midgut gland. (It should be noted that these methods measure the absorption of macromolecules by either pinocytosis and/or phagocytosis and do not necessarily reflect active transport per se.) Dall (1967) found evidence of labelled material in the region of the midgut gland 0.5-1.0 hour

after feeding, but admitted that this was not conclusive proof of absorption in this area. The gland, however, does play an important role in the elaboration and secretion of digestive enzymes (Yonge, 1924; Lockwood, 1967; van Weel, 1970). Other investigators (Renaud, 1949; Skinner, 1965; Yamaoka & Scheer, 1970) have concluded that the gland may be an important storehouse of material, but this may vary according to species. Based upon experiments which demonstrated the absorption of macromolecules such as olive oil and iron lactate, van Weel (1970) and Lockwood (1967) concluded that the absorption of organic molecules was confined to the midgut and midgut gland. However, in those crustaceans with a short digestive tract, the midgut gland is probably the major absorptive organ (Yonge, 1924; Lockwood, 1967; van Weel, 1970). The hindgut is believed to have no absorptive function (Lockwood, 1967), although Croghan (1958) proposed the movement of NaCl across the epithelium of this region.

Surprisingly, experiments which would determine the absorption of organic molecules by active transport have never been performed.

II

The majority of absorption studies of both amino acids and monosaccharides have been performed utilizing mammalian

systems and, to a lesser extent, other vertebrates. Excellent reviews of this work have been compiled (Wilson, 1962; Wiseman, 1964; Benson & Rampone, 1966; Wiseman, 1968; Crane, 1968). The general consensus of these works is that, in most vertebrates, proteins and carbohydrate are digested to their constituent parts (amino acids and monosaccharides) and that the absorption of these small organic molecules is confined primarily to the small intestine (particularly the distal third). The absorption of amino acids and monosaccharides is believed to be due principally to active transport. There is an active transport system (carrier) specific for the naturally occurring sugars D-glucose and D-galactose. Recently, Gracey et al. (1972) suggested that D-fructose was actively transported by a carrier mediated mechanism present in the rat small intestine which is separate from that involved in the transfer of other actively transported sugars. The transport systems for amino acids have been tentatively divided into five separate systems (carriers): one each specific for 1) the basic amino acids, 2) the aliphatic (mono-amino-mono-carboxylic) amino acids, 3) the hetero-cyclic amino acids, and separate pathways for 4) proline and glycine, and 5) valine, leucine and isoleucine. There are no known transport systems for the absorption of acidic amino acids in animals.

In contrast, the study of absorption of monosaccharides and amino acids in the invertebrate phyla have been confined to a few areas. The intestinal active transport of monosaccharides has been demonstrated in the chiton (Lawrence & Lawrence, 1967), the sea cucumber (D'Agostino & Farmanfarmaian 1960; Farmanfarmaian, 1969; Krishnan & Krishnaswamy, 1970; Krishnan, 1971), various echinoids (Bamford & James, 1972; Bamford et al., 1972) and the locust (Treherne, 1959). The few invertebrates in which intestinal active amino acid absorption has been demonstrated consist of a chiton (Greer & Lawrence, 1967), a sea cucumber (Lawrence et al., 1966), starfishes (Ferguson, 1964, 1968, 1971), an echinoid (Bamford & James, 1972) and a snail (Gilbertson & Jones, 1972).

Active transport is believed to be the primary mechanism for the absorption of small organic molecules in the majority of the vertebrates. In invertebrate phyla, however, the picture is not so clear. Some invertebrates seem able to survive using diffusion (sea urchin, Strongylocentrotus purpuratus) (Farmanfarmaian & Phillips, 1962; Boolootian & Lasker, 1964) or facilitative transport (sea cucumber, Thyone briareus) (Rundles & Farmanfarmaian, 1964) for the uptake of organic molecules. Others (the chiton) have developed extensive active transport systems to meet their needs (Lawrence & Lawrence, 1967; Greer & Lawrence, 1967;

Hanisch & Lawrence, 1972).

It has been stated previously that the midgut gland is probably the major organ of absorption in Crustacea, even though the experiments which have been performed to date have demonstrated the absorption of macromolecules by phagocytosis and/or pinocytosis. However the major mechanism of absorption of small organic molecules has been shown to be active transport in the majority of the vertebrates.

Statement of Problem

The present studies were undertaken to determine whether or not small organic molecules are actively transported by the midgut gland of a penaeidean shrimp (Penaeus aztecus) and thus determine the relative importance of this organ in the nutrition of this group of crustaceans.

Methods and Materials

Adult shrimp (penaeus aztecus) were obtained from the National Marine Fisheries Service, Galveston, Texas, Dow Chemical Company, Freeport, Texas, and the Ralston Purina Company, Crystal River, Florida. The specimens were kept in aerated artificial sea water and were not fed for at least 24 hours prior to their use in these experiments.

I. In Vitro Method

A. Preparations for and Conditions of Incubation

In preparation for these experiments, the animals were covered with ice and when sufficiently immobilized, the carapace superior to the midgut gland was cut away and the gland removed. The gland was then placed in ice cold (0-5 °C) shrimp Ringer's solution (see Table 1 for composition) and cut into sections approximately 10-20 mg in weight. The sections were then placed (2-6 sections per flask) into a 25 ml Erlenmeyer flask containing 2.5 ml of shrimp Ringer's solution. The flask was then placed in a Dubnoff metabolic shaking incubator to preincubate for 5 minutes at 28 °C and

TABLE 1
COMPOSITION OF SHRIMP RINGER'S SOLUTION

Compound	g/Liter	mM/Liter
NaCl	21.63	370
$MgSO_4-7H_2O$	4.93	20
KCl	1.12	15
$CaCl_2$	0.443	4
HCl*	0.0036	0.1

A constant pH of 7.21 was obtained after allowing the Ringer's to equilibrate to atmospheric air overnight. *One tenth ml of 1 N HCl was added per liter of shrimp Ringer's.

TABLE 2
COMPOSITION OF SCINTILLATION COCKTAIL I

Compound	Amount
Liquifluor (New England Nuclear)*	21 ml
BBS-3 (Beckman)**	42 ml
Toluene (Reagent grade)	500 ml

* Contains 100 g PPO (2,5-diphenyloxazole) and 1.25 g POPOP (p-Bis 2-(5-phenyloxazolyl)-benzene in a liter of toluene

** Contains a strong base

at a shaking rate of 100 oscillations per minute.

The test amino acid and monosaccharide used in this study were L-valine and D-glucose, respectively. Their movement into the tissues was followed by the use of tritiated (generally labelled) L-valine or D-glucose at an activity of 0.2 μCi per ml final volume. The movement of substances by diffusion alone was followed by the use of ^{14}C-universally labelled D-mannitol at an activity of 0.2 μCi per ml final volume. Radioactive compounds were obtained from New England Nuclear Corporation and ICN Corporation. All incubation solutions utilizing L-valine as the test compound contained D-glucose in an amount one tenth the concentration of L-valine.

Various initial concentrations of L-valine and D-glucose were used. The initial concentration of D-mannitol was always equal to the initial concentration of L-valine or D-glucose.

The experiment was initiated by the addition of 0.5 ml of shrimp Ringer's solution containing the desired amount of the test amino acid or monosaccharide and D-mannitol, bringing the total volume of the incubation medium to 3.0 ml. The sections were incubated for 15 minutes and the experiment was terminated by the removal of the tissue sections from the flask and subsequently rinsed in 10.0 ml of shrimp Ringer's solution.

B. Tissue Extraction

Each section of tissue was removed and rinsed and immediately placed into 5.0 ml of 75% ethanol for 24 hours. Pilot studies indicated that at least 95% of the radioactivity extractable in 75% ethanol was removed on this first extraction. The D-glucose and L-valine thus removed was assumed to represent that portion accumulated into the free tissue monosaccharide or amino acid pool.

C. Methods of Assay

At the end of the extraction period, the gland sections were weighed and then dried in an oven at 100 C to obtain a constant dry weight. The difference in the weights of the wet and dry tissues represented tissue water.

Samples were taken of the incubation medium and the extraction fluid (0.2 ml and 1.0 ml, respectively) and placed into a scintillation vial containing 15.0 ml of scintillation cocktail I (see Table 2 for composition) to determine the amount of radioactivity present. The vials were then counted in a Beckman LS-150 liquid scintillation counter.

D. Estimation of Insoluble Test Compounds

To obtain an estimation of the amount of test compound incorporated into the ethanol insoluble fraction, a

representative group of tissue sections were solubilized with Soluene (Packard). Each tissue section (approximately 10-20 mg in weight) was placed into a scintillation vial with 1.0 ml of Soluene, and after 24 hours, 15.0 ml of scintillation cocktail II (6.0 gm of PPO in 1000 ml of toluene) was added. The vials were then counted in a Beckman LS-150 liquid scintillation counter to determine the amount of radioactivity present.

II Chromatography

The ethanol extraction fluid was analyzed by one dimensional descending paper chromatography to ascertain whether the test amino acid had undergone chemical alteration during the test period. Two hundred lambda of the ethanol extraction fluid was spotted on Whatman No. 1 filter paper and developed using a solvent of butanol: acetic acid: water (v/v 4:1:1). The dried chromatogram was sprayed with ninhydrin and redried. To determine the amount of radioactivity present in the chromatographic spots, a second chromatogram was spotted (also with 200 lambda) and sprayed with ninhyfrin. The areas which reacted with ninhydrin on both chromatograms were cut out and placed in scintillation vials containing 15.0 ml of scintillation cocktail I and counted in a Beckman LS-150 liquid scintillation counter.

The areas which reacted with ninhyfrin on the first chromatogram contained the activity which was present in the form of L-valine, while the second chromatogram contained all the activity present in the ethanol extraction fluid. A ratio of the counts from each chromatogram would therefore give a means of estimating the per cent recovery of the radioactivity from the ethanol extraction fluid as the original test amino acid.

III Calculation of Data

The accumulation of test compound into the midgut gland tissue represents that amount actively absorbed by the tissue plus the amount which enters by diffusion, The amount which enters by diffusion can be estimated by using D-mannitol in the incubation medium at the same initial concentration as the test compound. This value, subtracted from the total accumulation, gives an estimated amount of test compound which entered by an active mechanism (assuming that D-mannitol and the test compounds exhibit the same rate of diffusion).

IV Experimental Studies

The previously described in vitro technique was followed in all the experimental studies except when

modified as indicated.

A Concentration Studies

To study the effect of concentration upon the absorption of amino acids, L-valine was used in the incubation media at initial concentrations of 10^{-3}, 10^{-1}, 1.0, 2.5, 5.0, 10.0 30.0 and 100.0 μMoles/ml. Similarly, D-glucose was used at initial concentrations of 10^{-3}, 10^{-1}, 10.0, 15.0, 22.5, 30.0, 33.5, 50.0, 75.0, 100.0 and 112.5 μMoles/ml.

B Competitive Inhibition Studies

To determine the susceptibility of the system to competitive inhibition, a test solution of L-valine was used at a concentration of 1.0 μMoles/ml in the presence of L-leucine at a concentration 30 times that of L-valine (30.0 μMoles/ml).

C Energy Dependence Studies

To determine the dependence of the system upon metabolic energy, L-valine was used as the test compound at an initial concentration of 1.0 μMoles/ml. Anaerobic conditions were used to inhibit conditions dependent on aerobic respiration. Anaerobic conditions were maintained by bubbling the ambient solution with N_2 gas for 20 minutes prior to initiating the experiment and stoppering the

flask until the termination of the experiment.

Results

Chromatographic analysis was performed in those experiments where L-valine was used as the test compound, since there was an indication that it might be actively transported. There was no evidence for the active transport of D-glucose and thus the extractions from those experiments were not analyzed. Chromatographic analysis of the ethanol extraction fluid indicated that 90±5 % of the radioactivity present in the sample could be recovered as valine. This would indicate that the majority of L-valine absorbed into the free amino acid tissue pool was in its original chemical form and not significantly metabolized.

A Concentration Studies

T/A (terminal tissue concentration/ terminal ambient concentration) ratios are not reported since none were significant. There was a significant ($p < 0.01$) difference between the accumulations into the ethanol soluble fraction of L-valine and D-mannitol when both were present initially at ambient concentrations of 10^{-3}, 10^{-1}, and 1.0 μMoles/ml (Table 3). No significant difference was demonstrated when

TABLE 3: ACCUMULATION OF L-VALINE AND D-MANNITOL INTO THE ETHANOL SOLUBLE FRACTION OF THE MIDGUT GLAND OF P. AZTECUS

Initial Ambient Concentration (μMoles/ml)	Test Compound	No. of Obs.	Accumulation (μMole/g tissue wet weight)
10^{-3}	L-Valine	8	0.95×10^{-3}* ±0.14
10^{-3}	D-Mannitol	8	0.49×10^{-3} ±0.036
10^{-1}	L-Valine	6	0.0839* ±0.0089
10^{-1}	D-Mannitol	6	0.0418 ±0.0032
1.0	L-Valine	8	0.852* ±0.10
1.0	D-Mannitol	8	0.543 ±0.056
2.5	L-Valine	8	1.58 ±0.36
2.5	D-Mannitol	8	1.13 ±0.26
5.0	L-Valine	8	3.68 ±0.55
5.0	D-Mannitol	8	2.95 ±0.46
10.0	L-Valine	8	5.99 ±0.70
10.0	D-Mannitol	8	4.79 ±0.59
30.0	L-Valine	19	21.89 ±2.10
30.0	D-Mannitol	19	17.10 ±1.60
100.0	L-Valine	7	62.30 ±9.20
100.0	D-Mannitol	7	57.70 ±8.11

Temperature of incubation was 28°C. All values represent mean ± S.E.M. *Indicates L-Valine value is significantly ($p < 0.01$ as determined by comparison of paired observations by the two-tailed *t*-test) greater than the respective value for D-mannitol.

L-valine and D-mannitol were present together at ambient concentrations of 2.5, 5.0, 10.0, 30.0 and 100.0 μMoles/ml (Table 3). This indicated that the increase in the rate of accumulation of L-valine at these concentrations was due to diffusion and that L-valine and that L-valine and D-mannitol have very similar diffusion coefficients.

Net accumulation by active transport may be estimated by subtracting the D-mannitol values from the respective L-valine values and the lines thus obtained from the plot of net accumulation of L-valine vs. its initial ambient concentration have two distinct slopes (Figure 1). It may be inferred that the two lines thus obtained represent first and zero order kinetics, additional evidence for the presence of a carrier mediated system. All values for the accumulation of L-valine into the ethanol insoluble fraction were significantly greater than the respective values for D-mannitol, as indicated in Table 4. No value for the accumulation into the ethanol soluble fraction of D-glucose was significantly greater than the respective value for D-mannitol (Table 5). There was a significant difference between the accumulations into the ethanol insoluble fraction of D-glucose and D-mannitol at all initial ambient concentrations (Table 6). This data is also represented graphically in Figure 2. It should be noted, however, that the total accumulation of

Figure 1

Figure 1. A plot of net accumulation of L-valine (total L-valine accumulation minus total D-mannitol accumulation) into the midgut gland of Penaeus aztecus vs. initial ambient concentration of L-valine.

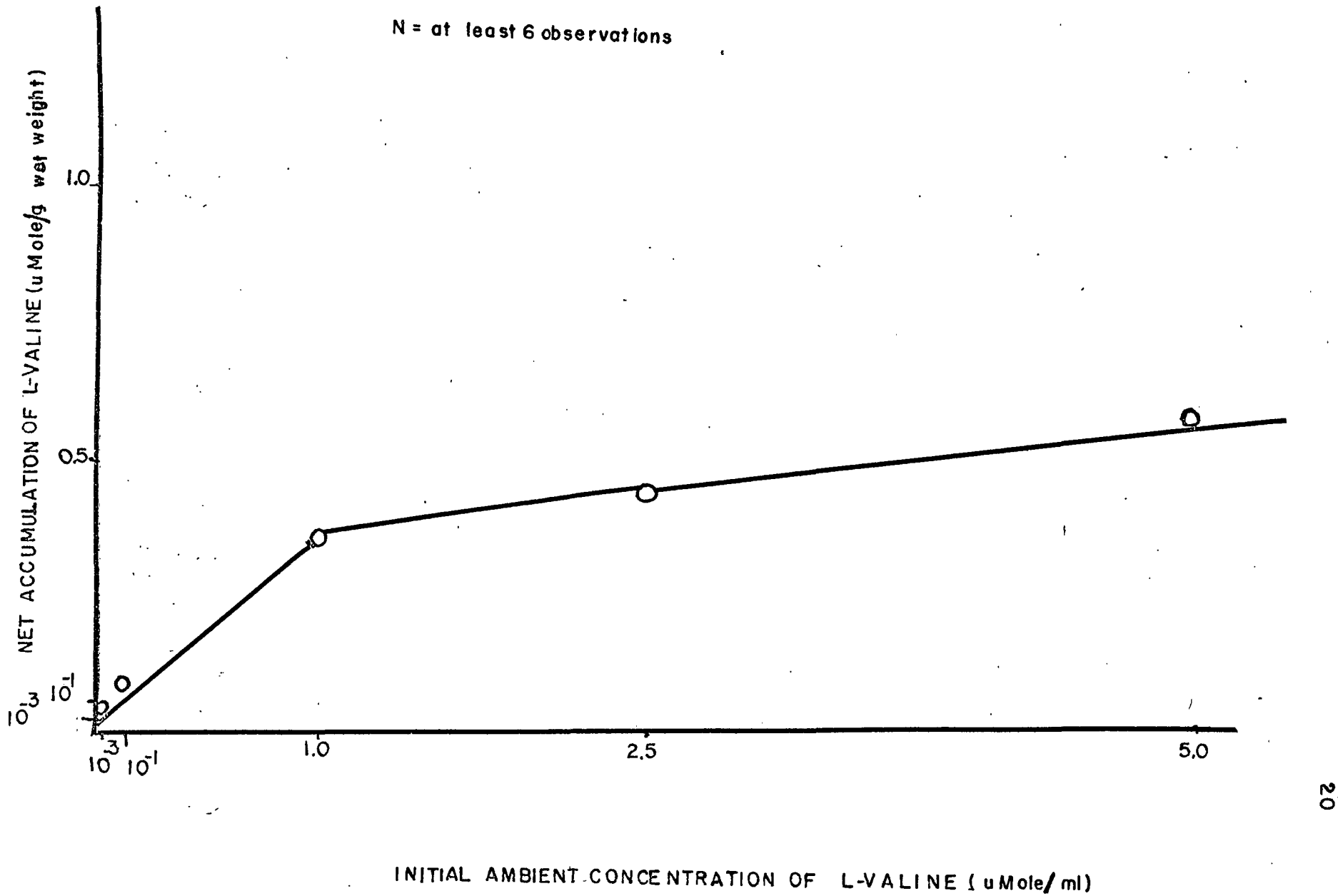

N = at least 6 observations
NET ACCUMULATION OF L-VALINE (u Mole/g wet weight)
1.0
0.5
10^{-3}
10^{-1}
10^{-3}
10^{-1}
1.0
2.5
5.0
INITIAL AMBIENT CONCENTRATION OF L-VALINE (u Mole/ ml)

TABLE 4: ACCUMULATION OF L-VALINE AND D-MANNITOL INTO THE ETHANOL INSOLUBLE FRACTION OF THE MIDGUT GLAND OF PENAEUS AZTECUS

Initial Ambient Concentration (µMoles/ml)	Test Compound	Accumulation (µMoles/g wet weight)
1.0	L-Valine	0.016 ±0.002
1.0	D-Mannitol	<0.001
2.5	L-Valine	0.044 ±0.006
2.5	D-Mannitol	<0.001
5.0	L-Valine	0.098 ±0.007
5.0	D-Mannitol	<0.001
10.0	L-Valine	0.28 ±0.12
10.0	D-Mannitol	<0.01

Temperature at incubation was 28°C. All values represent mean ± S.E.M. of 8 observations. All L-Valine values were significantly greater than respective values for D-Mannitol.

TABLE 5: ACCUMULATION OF D-GLUCOSE AND D-MANNITOL INTO THE ETHANOL SOLUBLE FRACTION OF THE MIDGUT GLAND OF PENAEUS AZTECUS

Initial Ambient Concentration (µMoles/ml)	Test Compound	No. of Obs.	Accumulation (µMole/g wet weight)
10^{-3}	D-Glucose	7	0.472×10^{-3} ±0.056
10^{-3}	D-Mannitol	7	0.456×10^{-3} ±0.049
10^{-1}	D-Glucose	8	0.053 ±0.0042
10^{-1}	D-Mannitol	8	0.050 ±0.0034
10.0	D-Glucose	5	6.22 ±0.17
10.0	D-Mannitol	5	6.93 ±0.38
15.0	D-Glucose	5	8.72 ±1.02
15.0	D-Mannitol	5	9.28 ±0.84
22.5	D-Glucose	5	10.0 ±1.7
22.5	D-Mannitol	5	10.7 ±1.9
30.0	D-Glucose	5	15.1 ±4.0
30.0	D-Mannitol	5	12.7 ±2.1
33.5	D-Glucose	5	14.6 ±2.4
33.5	D-Mannitol	5	15.7 ±2.5

(next page)

TABLE 5 (Continued)

50.0	D-Glucose	5	25.2 ±3.7
50.0	D-Mannitol	5	27.2 ±3.1
75.0	D-Glucose	5	34.2 ±8.2
75.0	D-Mannitol	5	32.3 ±7.8
100.0	D-Glucose	8	47.8 ±10.4
100.0	D-Mannitol	8	42.9 ±8.9
112.5	D-Glucose	5	55.7 ±3.8
112.5	D-Mannitol	5	56.1 ±4.1

Temperature of incubation was 28°C. All values represent mean ± S.E.M. No D-Glucose value was significantly different from the respective value for D-Mannitol.

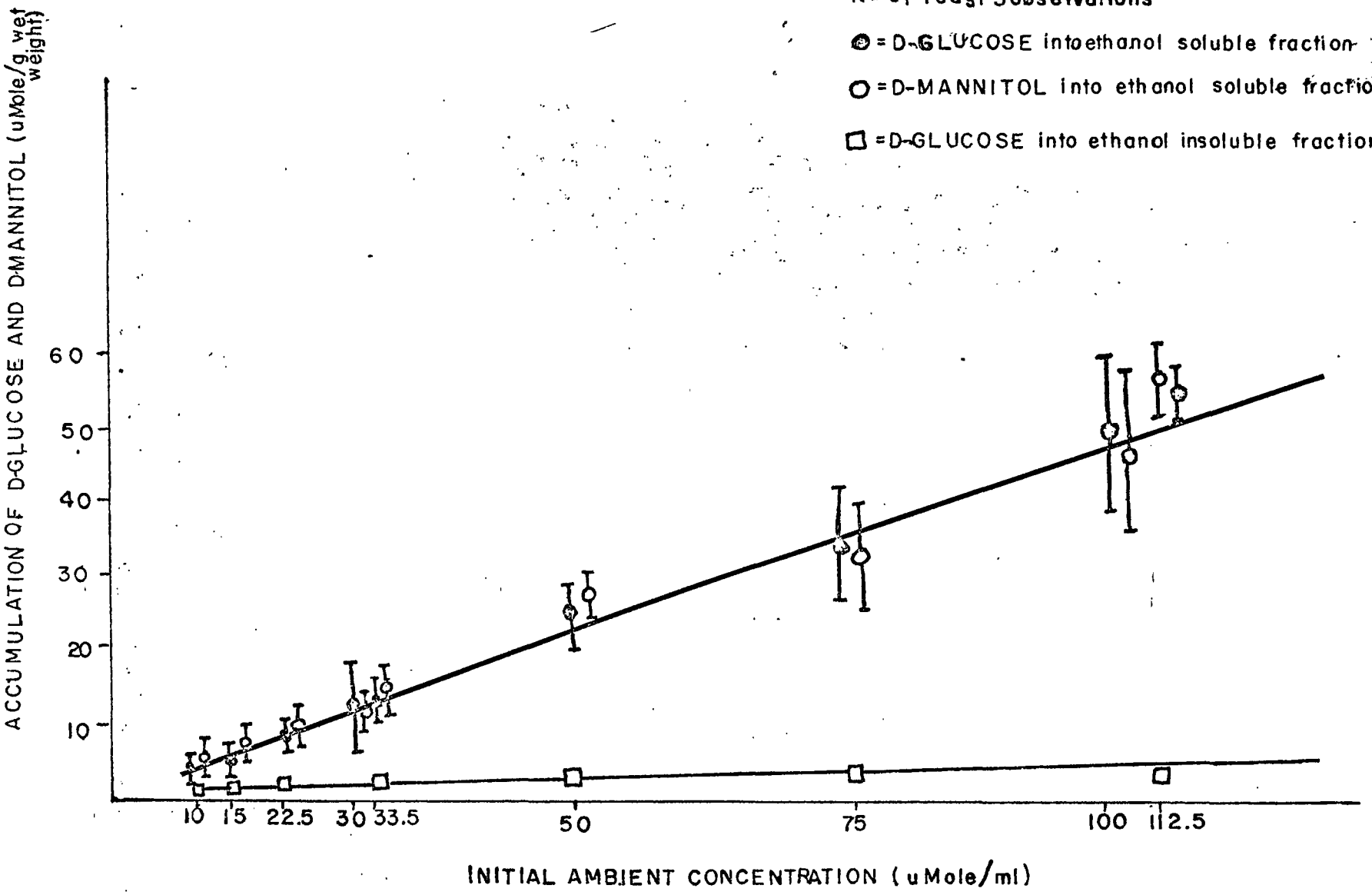
TABLE 6: ACCUMULATION OF D-GLUCOSE AND D-MANNITOL INTO THE ETHANOL INSOLUBLE FRACTION OF THE MIDGUT GLAND OF PENAEUS AZTECUS

Initial Ambient Concentration (µMoles/ml)	Test Compound	Accumulation (µMoles/g wet weight)
10.0	D-Glucose	0.40 ±0.02
10.0	D-Mannitol	<0.02
15.0	D-Glucose	0.56 ±0.02
15.0	D-Mannitol	<0.02
22.5	D-Glucose	0.85 ±0.07
22.5	D-Mannitol	<0.02
33.5	D-Glucose	0.94 ±0.02
33.5	D-Mannitol	<0.02
50.0	D-Glucose	0.94 ±0.05
50.0	D-Mannitol	<0.02
75.0	D-Glucose	2.59 ±0.21
75.0	D-Mannitol	<0.05
112.5	D-Glucose	3.09 ±0.01
112.5	D-Mannitol	<0.05

Temperature of incubation was 28°C. All values represent mean ± S.E.M. of 5 observations. All D-Glucose values were significantly greater than respective values for D-Mannitol.

Figure 2

Figure 2. A plot of accumulation of D-glucose and D-mannitol into the midgut gland of Penacus aztecus vs. initial ambient concenttation of D-glucose and D-mannitol. Open circles represent the accumulation of D-mannitol into the ethanol soluble fraction. Closed circles represent the accumulation of D-glucose into the ethanol soluble fraction. Open squares represent the accumulation of D-glucose into the ethanol insoluble fraction. The accumulation of D-mannitol into the ethanol insoluble fraction was insignificant and is not shown. Vertical lines indicate a range of two S.E.M's. The line was fitted by observation.


N = at least 5 observations
= D-GLUCOSE into ethanol soluble fraction
= D-MANNITOL into ethanol soluble fraction
= D-GLUCOSE into ethanol insoluble fraction
ACCUMULATION OF D-GLUCOSE AND D-MANNITOL (uMole/g wet weight)
60
50
40
30
20
10
10 15 22.5 30 33.5 50 75 100 112.5
INITIAL AMBIENT CONCENTRATION (uMole/ml)

D-glucose (ethanol soluble values plus ethanol insoluble values) was not significantly different from that of D-mannitol, indicating that the sugar was not actively absorbed.

B Competitive Inhibition Studies

One of the characteristics of an active transport system is its inhibition by compound(s) which structurally resemble the compound(s) being transported. In the presence of L-leucine, the accumulation of L-valine into the ethanol soluble fraction was significantly ($p < 0.01$) lower than the control L-valine value (Table 7). In addition the control value for the accumulation of L-valine into the ethanol soluble fraction was significantly ($p < .001$) greater than the respective value for D-mannitol, while in the presence of L-leucine, no significant differences between the two could be demonstrated. The values for the ethanol soluble accumulations of D-mannitol in the presence and absence of L-leucine were not significantly different from one another. The lack of effect on D-mannitol accumulation indicates that this effect was specific for the uptake of L-valine.

C Energy Dependence Studies

It has been previously shown that the rate of accumulation of L-valine into the ethanol soluble fraction was

TABLE 7: ACCUMULATION OF L-VALINE AND D-MANNITOL IN THE PRESENCE OF L-LEUCINE INTO THE ETHANOL SOLUBLE FRACTION OF THE MIDGUT GLAND OF P. AZTECUS

Inhibitor (Initial Concentration)	Test Compound	Accumulation (μMole/ g wet weight)
None	L-Valine	0.68 ±0.067 *
	D-Mannitol	0.49 ±0.046
L-Leucine (30.0 μMoles/ml)	L-Valine	0.49 ±0.049 †
	D-Mannitol	0.43 ±0.046

Temperature of incubation was 28°C. All values represent mean ± S.E.M. of 13 observations for the control value and 9 observations for the L-Leucine values. The initial ambient medium for each experiment contained 1.0 μMoles/ml of L-Leucine.
*Indicates that the value for L-Valine is significantly ($p<.001$ as determined by comparison of paired observations and the two-tailed t-test) greater than the respective value for D-Mannitol.
†Indicates that the value for L-Valine is significantly ($p<.01$ as determined by comparison of paired observations and the two-tailed t-test) less than the control L-Valine value.

significantly greater than the respective value for D-mannitol at an initial ambient concentration of 1.0 μMoles/ml (Tables 3, 8). However, with the imposition of anaerobic conditions, no significant difference between the rates of accumulation into the ethanol soluble fraction of L-valine and D-mannitol could be demonstrated (Table 8). Further, the rate of accumulation of L-valine under aerobic conditions was greater than under anaerobic conditions. These results indicate that the system was dependent upon aerobic metabolic energy. The accumulation of D-mannitol into the ethanol soluble fraction was not significantly altered by anaerobic conditions.

TABLE 8: EFFECT OF ANAEROBIC CONDITIONS ON THE ACCUMULATION OF L-VALINE AND D-MANNITOL INTO THE ETHANOL SOLUBLE FRACTION OF THE MIDGUT GLAND OF P. AZTECUS

Condition of Incubation	Test Compound	No. of Obs.	Accumulation (μMole/ g wet weight)
Aerobic	L-Valine	13	0.682 * ±0.067
	D-Mannitol	13	0.497 ±0.046
Anaerobic	L-Valine	7	0.54 ±0.13
	L-Mannitol	7	0.55 ±0.11

The initial ambient medium for each experiment contained 1.0 µMoles/ml each of L-Valine and D-Mannitol. Temperature of incubation was 28°C. All values represent mean ± S. E. M. *Indicates a significant difference ($p<0.05$) between the respective values of L-Valine and D-Mannitol as determined by comparison of paired observations and two-tailed t-test.

Discussion

The absorption of organic molecules by vertebrates is believed to be accomplished primarily by the physiological mechanism, active transport. Six of the major characteristics of active transport are:

1. The ability to move substances against their concentration gradients.

2. The dependence upon metabolic energy.

3. The compound transported is not chemically altered during transport.

4. The susceptibility to inhibition by compounds which structurally resemble the compound(s) being transported (competitive inhibition).

5. The specificity for compounds which are chemically similar.

6. The demonstration of first and zero order kinetics.

The movement of L-valine into the shrimp digestive gland tissues was probably by an active transport mechanism. This was suggested in this study by: 1) significant differences between the accumulations into the ethanol soluble fraction of L-valine and D-mannitol at several initial ambient concentrations, 2) sensitivity of the system to

inhibition by anaerobic conditions, indicating a dependence upon metabolic energy, 3) the recovery of over 90% of the L-valine in a chemically unaltered form from the ethanol extraction fluid, 4) the competitive inhibition of the system with the addition of a structurally similar compound and 5) the demonstration of first and zero order kinetics. D-mannitol is a sugar not known to be transported by either the rat or hamster small intestine (Crane & Mandelstam, 1960) and thus has been used in these experiments to determine what amount of a small organic compound is entering the tissue by passive diffusion. Also, since the rate of accumulation was not affected by anaerobic conditions and competitive inhibition, indications are that the significant reduction in the rates of accumulation of L-valine under these conditions was not due to the experimental technique. In addition, the significant accumulation of L-valine into the ethanol insoluble fraction of the midgut gland with no accumulation of D-mannitol indicates that L-valine was incorporated (anabolized) into the midgut gland tissues, while D-mannitol was not. This result was not surprising, since L-valine is usually included among the essential amino acids and thus can be expected to be incorporated, while D-mannitol is not known to be utilized by any animal.

The data from these experiments suggest that the optimum concentration for the absorption of L-valine is in the

vicinity of 1.0×10^{-3} M for the midgut gland of Penaeus aztecus. This level is similar to that reported for the hamster (i.e., 1.0×10^{-4} M) using similar tissue accumulation methods (Wiseman, 1968).

The data in this study suggest also that the movement of D-glucose into the gland tissues was probably by diffusion. This was indicated by 1) the fact that D-glucose was not moved against its concentration gradient and 2) the lack of significant differences between the rates of accumulation into the ethanol soluble fraction of D-glucose and D-mannitol at any initial ambient concentration. This information, coupled with the linear progression obtained with the plot of rate of accumulation vs initial ambient concentration of both compounds, would seem to indicate that D-glucose was not transported by the midgut gland tissues. D-glucose was anabolized to a significantly greater degree than D-mannitol, as indicated by the significant accumulation of D-glucose but not D-mannitol into the ethanol insoluble fraction. Again, this is not surprisong, as D-glucose plays an important role in the metabolism of the cell, while D-mannitol, as mentioned before, is not known to be utilized.

Much of the literature to date has emphasized the role of the midgut gland in the absorption of small organic molecules. The results from these experiments would indicate

that amino acids are actively absorbed by the tissues of the midgut gland while monosaccharides are absorbed primarily, if not entirely, by diffusion - a conclusion that stands at least partially in contradiction to the literature. Similarly, amino acids but not monosaccharides were reported to be transported in the holothurian, Stichopus parvimensis (Lawrence et al., 1966). Interestingly, a contemporary study (Morgan, 1971) showed that the midgut of Penaeus aztecus actively transported both L-valine and D-glucose but not D-mannitol. A comparison of the total absorptove ability of both organs indicates that the midgut and not the midgut gland has the greater absorptive capability for amino acids and monosaccharides at the same ambient concentration. This strongly suggests that the midgut is the more significant absorptive organ, and that if the midgut gland is important for the absorption of amino acids and monosaccharides, it is only secondarily.

Unfortunately, large S.E.M.'s were obtained in the course of these experiments. The large S.E.M.'s probably reflect the following variables. Animals were obtained at all seasons of the year, and thus seasonal variations in the animals may have contributed to fluctuations in the results. In addition, the animals were in different molting stages (i.e., the majority were in intermolt, with a few individuals in premolt). It should be noted that

physiological changes are taking place even within the intermolt stage and thus variations in the results may also reflect these changes. The best results would have been obtained if animals at one stage of molting were used and if the experiments had been performed during one season.

It must be noted also that the midgut gland in this species is small in relation to the rest of the animal, at least in the large (11-20 cm in length) adults used in these experiments. This is in contrast to the information available about other decapods, where the gland is described as being large and diffuse (Yonge, 1924; Dall, 1964; Dall, 1967). However Dall reports variation in the size of the digestive gland between two penaeidean species (1964) and reflects that this difference may be related to the manner in which the animal obtains food. The decapods are reported to be predators, scavengers and/or detritus feeders, and variations in size of the gland may be due to this. The digestive gland has been observed to undergo cyclic changes in response to the molt cycle (Skinner, 1965; Yamaoka & Scheer, 1970). Variations in size and perhaps physiology have also been observed in similar organs of other vertebrates: the digestive caeca of the starfish change in size and function (Allen & Giese, 1966) and the digestive gland of the chiton Katharina tunicata changes in size and perhaps physiology (Lawrence, Lawrence & Giese, 1965), both in response to the

seasons.

The literature to date on crustacean nutrition has emphasized the role ofthe midgut gland in the absorption of organic nutrients, but this study has shown that, while the midgut gland is capable of actively transporting amino acids, it lacks this capacity for D-glucose. Thus it must be concluded that the midgut gland is only secondarily of importance in the absorption of small organic molecules.

Considering that this is the second time that amino acids but not monosaccharides have been reported to be actively transported by the same tissue, it is interesting to speculate whether this is an indication that amino acid transport is the more primitive of the two processes or, alternatively, whether monosaccharide transport has become degenerate in these tissues.

Summary

An *in vitro* tissue accumulation technique was used to study the absorption of L-valine and D-glucose by the midgut gland of the shrimp, *Penaeus aztecus*. Evidence was found for the active transport of L-valine but not for D-glucose. However, both compounds were accumulated in significant amounts into the ethanol insoluble fraction of the tissue, indicating that they were anabolized.

The active transport of L-valine was indicated by the following: 1) significant differences between the accumulation rates into the ethanol soluble fraction of L-valine and D-mannitol at several initial ambient concentrations (D-mannitol is a sugar not known to be transported by the rat or hamster small intestine), 2) the inhibition of the transport mechanism by anaerobic conditions, 3) the almost total recovery of L-valine in a chemically unaltered form, 4) the competitive inhibition of the rate of accumulation of L-valine by another naturally occurring amino acid (L-leucine) and 5) the demonstration of first and zero order kinetics.

The movement of D-glucose into the midgut gland tissues was probably by diffusion as indicated by the fact

that D-glucose was not accumulated against its concentration gradient and the absence of significant differences between the rates of accumulation of D-glucose and D-mannitol into the ethanol soluble fraction.

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