AMINO ACID ABSORPTION BY THE GUT OF THE BLACK BASS, *Huro salmoides*

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

---

by
Raymond Ray Crawford, Jr.
May, 1970

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ABSTRACT

The active transport of glycine, L-alanine, L-lysine, L-methionine, and L-proline were shown to occur in the gut of the black bass, Huro salmoides, using the tissue incubation technique of Agar (1954 and 1956). In defining active transport the following criteria were met: (1) the test amino acids were transported against their concentration gradients; (2) the transport mechanism proved to be dependent upon both metabolic energy and temperature; (3) the transport mechanism showed a molecular specificity because only the L-stereoisomer of alanine was transported against its concentration gradient; (4) chromatographic analysis indicated that the transported amino acids were structurally unchanged; and (5) saturation of the carrier mechanism was approached.

Certain generalizations can be made in comparing the carnivorous freshwater teleost (e.g., bass) and the omnivorous freshwater teleost (e.g., catfish) because there seems to be a difference in the ability of the gut of these two teleosts to absorb amino acids. The gut of the omnivorous teleost seems to have a greater ability to transport amino acids at lower concentrations. There also appears to be a regional difference in the amino acid absorption ability of the guts of the two types of fish. The maximum rate of absorption of amino acids occurred in medial region of the carnivorous bass, while the anterior intestine was reported to be the

optimal region of absorption in the omnivorous freshwater teleost.

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I.

INTRODUCTION

INTRODUCTION

Active transport is an experimentally well-established phenomenon found in the gut of all vertebrates studied to date. It is an energy-dependent process by which certain nutrients are absorbed against their concentration gradient. Additional characteristics of this process include: (1) a saturation phenomena; (2) some degree of molecular specificity; (3) the transported compound must remain structurally unchanged; and, (4) inhibition at both low and high temperatures (Wilson, 1962).

Gibson and Wiseman (1951) obtained the first evidence of active transport of amino acids in the rat small intestine by observing that there was a preferential absorption of the L-forms of amino acids from a racemic mixture. Since then the active transport of amino acids by the mammalian gut has been well characterized and several excellent reviews may be found: Edelman (1961), Wilson (1962), Wilbrandt (1963), Wiseman (1964), Csaky (1965), Benson and Rampone (1966), Heinz (1967), Rothstein (1968), and Wiseman (1968).

The presence of an active transport mechanism for amino acids in fish was first reported by Wilson (1957) who observed an accumulation of L-proline and glycine against a concentration gradient in the gut of a marine puffer, Sphaeroides maculatus. In other studies on marine teleosts Rout et al.

(1965) found that the gut of the winter flounder, Pseudopluronectes americanus, actively transported tyrosine and tryptophan against a concentration gradient. This aromatic amino acid transport mechanism did not show a stereospecificity in the flounder as had been found previously in mammals because active transport of both the D- and L-forms of tyrosine was observed.

L-tryptophan and L- and D-tyrosine were also reported to be transported by the gut of the marine killifish, Fundulus heteroclitus (Huang et al. 1967), which suggests that the gut of this fish is functionally similar to the winter flounder in its transport characteristics of aromatic amino acids. However, in studies on two freshwater lungfish, Cyclopterus lumpus and Propterus aethiopicus, a transport system for aromatic amino acids was not found (Huang et al. 1965).

Other marine fish in which intestinal transport of L-tryptophan has been found include: Myoxocephalus octodecimspinosus, Squalus acanthias, Lepidosirn paradoa, Hemitripterus americanus, Pollachius virens, and Macrozoares americans (Huang et al. 1965).

The goldfish, Carassius auratus, was shown to be similar to the hamster in the ability of its gut to actively transport L-threonine, L-alanine, L-serine, L-histidine, L-valine, L-methionine, L-phenylalanine and L-leucine (Mepham, 1966).

In addition, the anterior intestine of the goldfish, which would be analogous to the duodenum and jejunum of the mammalian intestine, was found to have the greatest capacity for transporting amino acids. The main difference between the hamster gut and the goldfish gut was the ability of the goldfish to produce concentration gradients 1.5 to 2 times as great as those produced in the hamster.

The upper intestinal regions of the freshwater catfish, Ictalurus nebulosus, and the marine scup, Stenotomus versicolor, showed the greatest amount of transport of L-leucine as evidenced by the largest serosal/mucosal ratios (Musacchia et at. 1961). Further studies on the catfish (Neff and Musacchia 1967), showed that under anaerobic conditions (gassing with 100% N_2) the undirectional flux of L-leucine was inhibited.

As seen from the above, the amount of information on intestinal absorption of amino acids in fish is small. Furthermore, only the omnivorous freshwater teleosts have been studied. It was the purpose of this study, therefore, to extend the information on this large group of animals, the Pisces, and to compare the active absorption of amino acids in a carnivorous freshwater teleost, the black bass, with the omnivorous freshwater teleosts, the catfish and goldfish, which have been studied by other investigators.

II.

MATERIALS AND METHODS

METHODS AND MATERIALS

Large-mouth black bass, Huro salmoides, of both sexes ranging from 20 cm to 60 cm in length and weighing from 250 gm to 1500 gm each were obtained from a private lake in Harris County, Texas. These animals were maintained in aerated freshwater at a temperature of 24° until used. All animals were starved three to seven days before being sacrificed.

A. IN VITRO METHOD

1. Preparation of Tissue:

An in vitro method similar to the one by Agar et al. (1954, 1956) was used. The animals were sacrificed by a blow on the head and the viscera removed. The intestine was cut approximately 1 cm proximal to the anus and 1 cm distal to the pyloric sphincter and divided into three equal sections: proximal, medial, and distal. Each section was first cut lengthwise to expose the mucosal tissue and then crosswise to subdivide further each section into segments approximately 0.5 cm in length. A Ringer's solution at room temperature was used to keep the gut moist. The composition of the fish Ringer's solution is shown in Table I. (Forester and Taggert, 1950).

TABLE I

COMPOSITION OF FISH RINGER'S SOLUTION

Compound	Grams/Liter	mM/L
NaCl	5.8450	100.0
KCL	0.1865	2.5
$CaCl_2$	0.1665	1.5
$MgCl_2$	0.0952	1.0
NaH_2PO_4	0.0600	0.5
$NaHCO_3$	0.4201	5.0

2. Conditions of Incubation

The segments (@ ~ 400 mg wet weight) were placed in a 25 ml Erlenmeyer flask (two segments per flask) which contained 8.5 ml of fish Ringer's. This solution will hereafter be referred to as the "incubation media." These segments were allowed to preincubate for five minutes in a Dubnoff metabolic shaking incubator at 90 oscillations/minute and at a temperature of 30°C. The incubation solution was aerated continuously with O_2 - CO_2 (95%:5%).

An experimental period of 30 minutes were started by injecting 1 ml of fish Ringer's solution containing a test amino acid followed by 0.5 ml of fish Ringer's solution as a rinse through polyethylene tubing into the incubation medium. This brought the ambient solution up to a total volume of 10 ml and resulted in a test amino acid concentration of 0.1 mM/ml.

The amino acids used in this study were glycine, L-alanine, L-lysine, L-methionine, and L-proline. The movement of these amino acids into the tissue was followed by ^{14}C labeled amino acids at an activity of 0.13 microcuries/ml. All radioactive compounds were obtained from New England Nuclear Corporation. In addition, all incubation solutions contained 5.6 mM of D-glucose.

3. Tissue Extraction:

Incubation was terminated by removing the tissue from the incubation media, washing with 100 ml of fish Ringer's solution and placing it in 5 ml of 85% ethanol for 48 hours. Pilot studies indicated that 95% of the radioactivity extractable in 85% ethanol was removed from the tissues in the first extraction. The amino acids removed in this extraction represented the accumulation that occurred into the free amino acid pool.

4. Methods of Assay:

At the end of the extraction time, the gut segments were removed and dried in an oven at 100°C for 24 hours to obtain a constant weight. The amount of tissue water was determined by the difference in the tare weights of the tissue.

The amount of radioactivity in the incubation medium and in the extraction fluid was determined by placing 0.1 ml and 2.0 ml, respectively, of the solution in a ringed aluminum planchet and evaporating to dryness by an infrared light. The planchets were counted in a gas flow Geiger-Muller counter (Nuclear-Chicago C110B tube with a Nuclear-Chicago 186 scaler) using 97.3% helium - 1.7% butane as the ionizing gas.

5. Estimation of Insoluble Test Amino Acids:

After being dried and weighed, the gut segments

(@ ~150 mg dry weight) were placed in a Packard Soluene solution until all tissues were solubilized. The dissolved segments were counted in a Beckman scintillation counter (Model LS-150) using a cocktail mixture of six grams PPO in one liter toluene. This procedure would enable one to determine if any of the test amino acids were incorporated into a tissue fraction which was insoluble in 85% ethanol.

6. Calculation of Data:

The total accumulation of the test amino acid into the free amino acid pool of the gut tissue represented that amount of amino acid which entered by both active transport and diffusion. The amount of amino acid entering by diffusion was estimated using D-mannitol and D-alanine in the incubation media at the same initial concentration as the test amino acids. This value was subtracted from the total accumulation, leaving only that amount of amino acid which entered the gut tissue by active transport. The diffusion values of D-mannitol and D-alanine using an initial ambient concentration of 0.1 mM/ml were 0.068 $\pm$ .015 mM/ml tissue water and 0.081 $\pm$ .003 mM/ml tissue water respectfully.

T/M ratios were determined for an indication of movement of the test amino acid against a concentration gradient. That is, the corrected tissue

accumulation values in mM/ml tissue water were divided by the concentration of the ambient solution in mM/ml. Ratios greater than one represented movement against a concentration gradient.

7. Chromatography:

The extracted test amino acids were checked for molecular structural rearrangement using paper chromatography. A specific "unlabeled" amino acid was added to its corresponding ethanol extraction and then the mixture spotted on a Whatman No. 1 filter paper. A developing solution composed of butanol, acetic acid, and water (4:1:1) was used. After the chromatographs had been developed and dried, they were sprayed with a ninhydrin solution to localize the cold amino acids. To test for radioactivity, sections of the chromatograph were cut out, placed in planchets, and counted in a gas flow Geiger-Muller counter.

B. EXPERIMENTAL STUDIES

The previously described in vitro technique was used in the following experimental studies except for the changes as indicated:

1. Concentration Studies:

To study the effect of concentration upon uptake of amino acids, L-alanine was used as the test compound at an initial concentration in the

incubation media of 10 mM/ml, 1 mM/ml, 0.1 mM/ml, or 0.01 mM/ml.

2. Time Studies:

In order to determine the length of time the gut would linearly absorb L-alanine, experimental period incubation times of 15 minutes, 30 minutes, 45 minutes, and 60 minutes were used.

3. Temperature Studies:

The influences of temperature on L-alanine absorption was determined by using ambient (bath) temperatures of 20°C, 30°C, and 40°C.

4. Mapping Studies:

There were no changes from the previously described <u>in vitro</u> technique (page 4).

5. Metabolic Inhibition Studies:

Two types of metabolic inhibitors were used to determine the dependence of the absorption of the test amino acids on metabolic energy: (1) the effect of anaerobia was determined by aerating the incubation media in each flask with 100% nitrogen instead of the O_2:CO_2 (95%:5%) mixture; and, (2) the effect of 2,4-dinitrophenol was determined by placing a concentration of 2×10^{-4}M of this substance in the incubation media.

III.

RESULTS

RESULTS

Analysis indicated that there was no significant incorporation of the test amino acids into a portion of the gut tissue which was insoluble in 85% ethanol. This does not definitely preclude that the test amino acids were not incorporated into tissue protein or polysaccharide. The percent recovery of the radioactivity indicated there was no significant catabolism of the test amino acids to CO_2 and water. Chromatographic analysis indicated that there was no conversion of the test amino acids to another form.

In the determination of a diffusion value, D-alanine gave essentially the same results as D-mannitol (page which suggested that the D-stereoisomer was not transported against a concentration gradient and that it moved across the tissue only by a diffusion process.

CONCENTRATION STUDIES

As can be seen in Table II, saturation of the carrier mechanism began to occur with a concentration of L-alanine greater than 0.1 mM/ml. There was approximately a ten-fold increase in tissue accumulation in all the regions when the ambient concentration was increased from 0.01 mM/ml to 0.1 mM/ml, but there was only a five-fold increase as the ambient concentration was increased to 1.0 mM/ml and 10.0 mM/ml.

TABLE II CONCENTRATION STUDIES: UPTAKE OF L-ALANINE BY THE GUT OF Huro salmoides

Segment	Ambient Conc. mM/ml	No. of Obs.	T/M	Tissue Accumulation mM/ml Tissue Water
Proximal	0.01	16	2.25 ± .20[+]	0.023 ± .002
	0.10	11	2.37 ± .29	0.227 ± .030
	1.0	14	1.62 ± .14	1.58 ± .15
	10.0	8	1.03 ± .07	10.2 ± .7
Medial	0.01	24	3.21 ± .20	0.032 ± .002
	0.10	18	4.76 ± .40	0.462 ± .041
	1.0	14	2.78 ± .13	2.81 ± .14
	10.0	8	1.41 ± .07	14.0 ± .7
Distal	0.01	19	2.62 ± .30	0.026 ± .003
	0.10	16	3.56 ± .33	0.340 ± .04
	1.0	4	1.96 ± .44	1.86 ± .46
	10.0	8	1.44 ± .08	14.1 ± .8

Incubation time of 30 minutes at a temperature of 30°C. [+] Values represent mean ± S.E.M.

Another indication that the carrier mechanism was beginning to become saturated was the increase in the T/M ratio as the ambient concentration was increased from 0.01 mM/ml to 0.1 mM/ml. A further increase in the ambient concentration resulted in a significant decrease ($p < 0.05$) in the T/M ratio.

The concentration of 0.1 mM/ml was used for all of the remaining experiments.

TIME STUDIES

There was a linear uptake of L-alanine from 15 minutes to 45 minutes (Figure 1). After 45 minutes the decrease in accumulation was presumably due to tissue damage because the incubation media became cloudy with cellular debris. Even though the greatest accumulation values were obtained at the 45 minute time period, the 30 minute period was chosen as the incubation time for all the subsequent experiments.

TEMPERATURE STUDIES

Figure 2 shows that the maximum accumulation of L-alanine occurred at an incubation temperature of 30°C which was considerably lower than the optimum temperature used in mammalian studies, and was indicative of the lower body temperature of these animals. Thirty degrees centigrade was used in the subsequent experiments.

FIGURE I. The effect of time on the uptake of L-alanine by the gut of Huro salmoides. Initial ambient concentration was 0.1 mM/ml. Incubation temperature was 30°C.

FIGURE 1

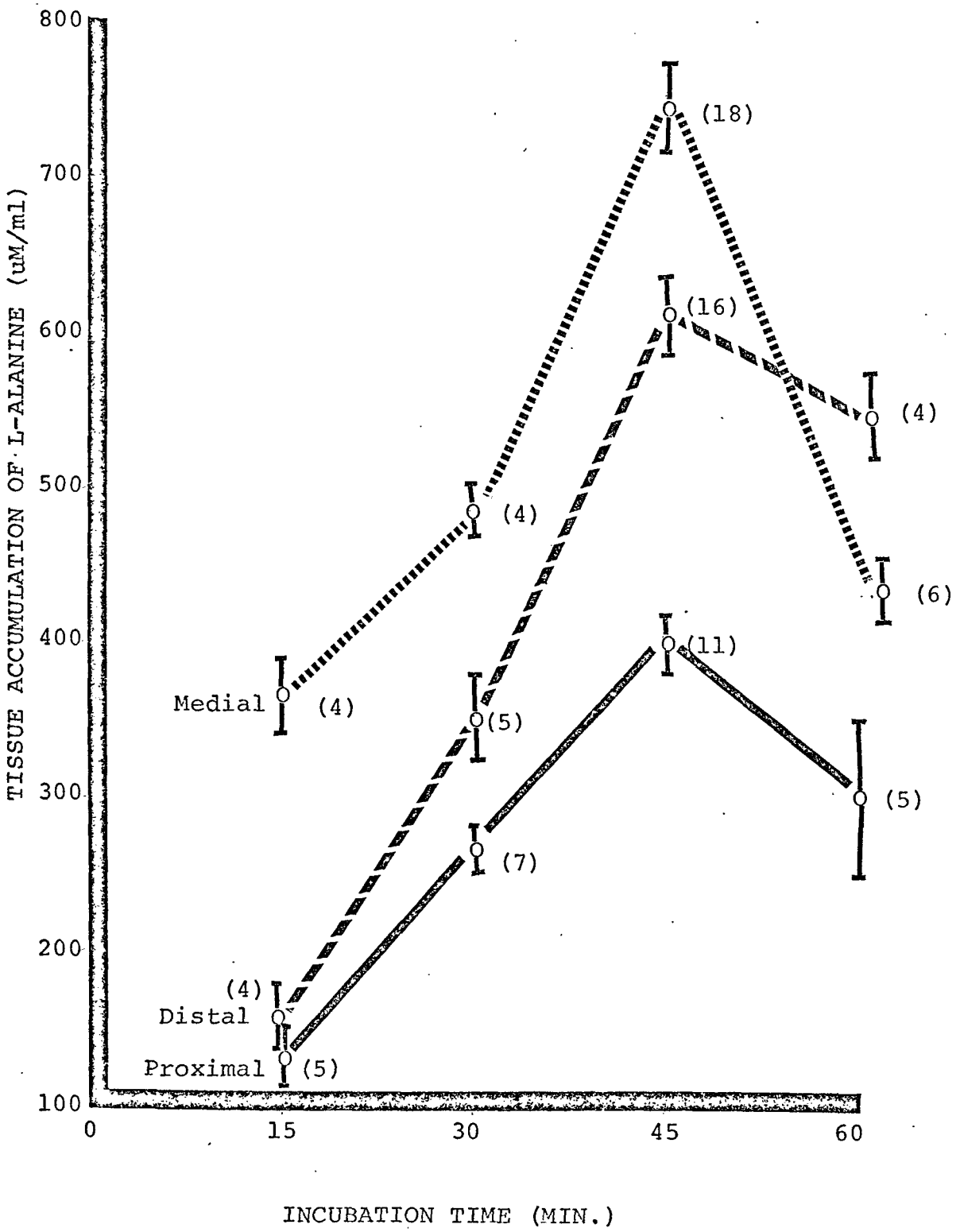
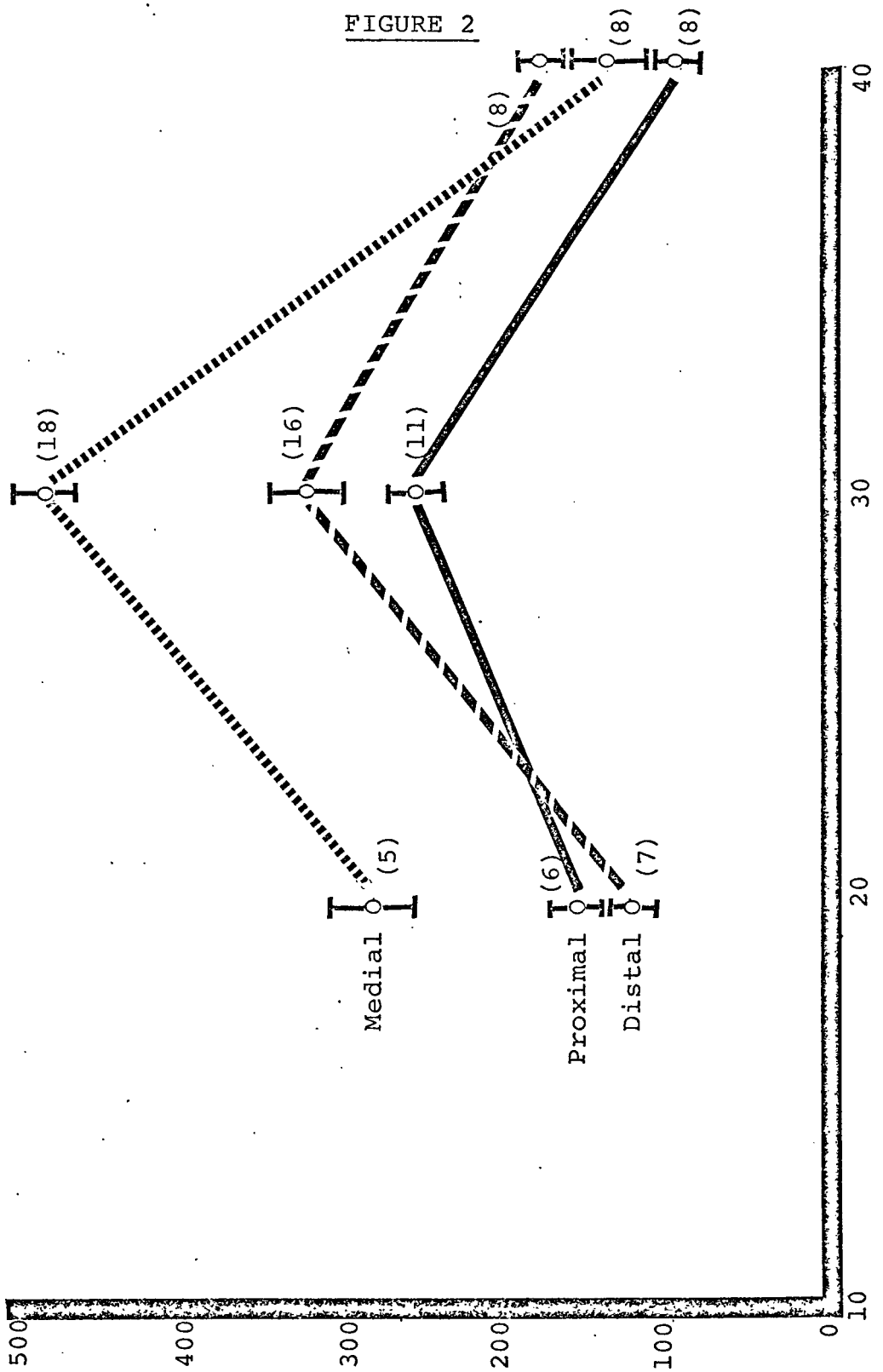

800
700
600
500
400
300
200
100
0
15
30
45
60
TISSUE ACCUMULATION OF L-ALANINE (uM/ml)
INCUBATION TIME (MIN.)
(18)
(16)
(4)
(4)
(6)
Medial
(4)
(5)
(11)
(5)
(7)
(4)
Distal
Proximal
(5)

FIGURE II. The effect of temperature on the uptake of L-alanine by the gut of Huro salmoides. Initial ambient concentration was 0.1 mM/ml. Incubation time was 30 minutes.

FIGURE 2


(18)
(16)
(11)
(8)
(8)
(8)
(5)
(6)
(7)
Medial
Proximal
Distal
500
400
300
200
100
0
10
20
30
40
TISSUE ACCUMULATION OF L-ALANINE (uM/ml)
INCUBATION TEMPERATURE (°C)

MAPPING STUDIES

The greatest net accumulation occurred in the medial regions of the gut with all the test amino acids studied (Table III). Fractional T/M ratios were obtained in the proximal and distal regions for all of the test amino acids except glycine in the distal regions, and L-alanine in both the proximal and distal regions.

T/M ratios greater than one indicated movement against a concentration gradient and represented direct evidence for the presence of active transport. Ratios less than one do not indicate the absence of active transport since these values were obtained by subtracting a diffusion value from the total accumulation value which represented both diffusion and active transport.

METABOLIC INHIBITION STUDIES

As seen in Table IV, accumulation was significantly decreased ($p < .05$) when 100% N_2 was bubbled through the ambient solution. However, at the termination of the experiment, a pH change from 7.5 to 8.4 was found indicating that the decrease could be due to a pH effect since amino acids are charged molecules. This finding necessitated the use of a different type of metabolic inhibitor.

When the metabolic energy source was inhibited by DNP (Table III), accumulation was markedly and significantly

TABLE III MAPPING STUDIES AND INHIBITION STUDIES

(Effect of Dinitrophenol: 2×10^{-4}M)

Amino Acid	Segment	Accumulation µM/ml Tissue Water No Inhibitor	Accumulation µM/ml Tissue Water With Inhibitor	T/M No Inhibitor	T/M With Inhibitor
Proline	Proximal	26 ± 2 (4)+	4 ± 1 (5)	.26 ± .02 (4)	.041 ± .011 (5)
	Medial	115 ± 5 (5)	53 ± 3 (4)	1.12 ± .05 (5)	.53 ± .03 (4)
	Distal	64 ± 1 (4)	41 ± 1 (3)	.66 ± .02 (4)	.43 ± .02 (3)
Lysine	Proximal	82 ± 3 (4)	44 ± 2 (6)	.92 ± .03 (4)	.49 ± .02 (6)
	Medial	114 ± 6 (7)	80 ± 2 (4)	1.23 ± .04 (7)	.88 ± .02 (4)
	Distal	50 ± 2 (5)	30 ± 2 (5)	.56 ± .02 (5)	.32 ± .02 (5)
Glycine	Proximal	93 ± 7 (3)	26 ± 2 (3)	.93 ± .06 (3)	.23 ± .02 (3)
	Medial	327 ± 19 (7)	90 ± 5 (4)	3.19 ± .19 (7)	.93 ± .05 (4)
	Distal	255 ± 8 (4)	54 ± 2 (4)	2.63 ± .09 (4)	.57 ± .02 (4)
Methionine	Proximal	44 ± 2 (2)	28 ± 4 (3)	.45 ± .01 (2)	.30 ± .04 (3)
	Medial	162 ± 10 (3)	64 ± 9 (3)	1.50 ± .06 (3)	.68 ± .09 (3)
	Distal	83 ± 2 (3)	19 ± 5 (2)	.84 ± .02 (3)	.20 ± .06 (2)

Incubation time of 30 minutes at a temperature of 30°C. Initial ambient concentration of test amino acids was 0.1 µM/ml. + Values represent mean ± S.E.M. and number of observations in parenthesis ( ).

TABLE III (con't.) MAPPING STUDIES AND INHIBITION STUDIES

(Effect of Dinitrophenol: 2×10^{-4}M)

Amino Acid	Segment	Accumulation uM/ml Tissue Water No Inhibitor	Accumulation uM/ml Tissue Water With Inhibitor	T/M No Inhibitor	T/M With Inhibitor
Alanine	Proximal	227 ± 29 (11)	62 ± 4 (4)	2.37 ± .29 (11)	.63 ± .04 (4)
	Medial	462 ± 41 (18)	154 ± 5 (6)	4.76 ± .40 (18)	1.56 ± .05 (6)
	Distal	340 ± 40 (16)	130 ± 5 (5)	3.56 ± .33 (16)	1.32 ± .05 (5)

Incubation time of 30 minutes at a temperature of 30°C. Initial ambient concentration of test amino acids was 0.1 uM/ml. + Values represent mean ± S.E.M. and number of observations in parenthesis ( ).

TABLE IV INHIBITION STUDIES: EFFECT ON N_2 ON L-ALANINE TRANSPORT BY THE GUT OF Huro salmoides

Segment	No. of Obs.	Tissue Accumulation Without N_2 $\mu M/ml^2$ Tissue Water*	Tissue Accumulation With N_2 $\mu M/ml$ Tissue Water	T/M Without N_2	T/M With N_2
Proximal	11	227 ± 29[+]	169 ± 17	2.37 ± .29	1.81 ± .18
Medial	18	462 ± 41	243 ± 26	4.76 ± .40	2.50 ± .29
Distal	16	340 ± 40	268 ± 35	3.56 ± .33	2.69 ± .35

* These data taken from Table III, Alanine.

Incubation time of 30 minutes at a temperature of 30°C. Initial ambient concentration of L-alanine was 0.1 $\mu M/ml$. [+] Values represent mean ± S.E.M. Starting pH 7.5, terminal pH of media 8.5.

decreased ($p < .05$) in most cases to less than half the control values. DNP seemed to affect all regions of the gut adversely, indicating that an active accumulation process was operating in all the regions of the gut.

IV.

DISCUSSION

DISCUSSION

Active transport of amino acids in the gut of the black bass (Huro salmoides) has been shown, the evidence being: (1) paper chromatographic analysis of the test amino acids indicated that no structural alterations occurred during transport, (2) inhibition of the metabolic energy by DNP significantly inhibited the accumulation of the test amino acids, (3) temperatures 10°C above and below 30°C inhibited the transport mechanism, (4) the test amino acids were moved against their concentration gradients, (5) the transport mechanism showed a specificity for the L-stereoisomers of alanine as compared to the D-steroisomer, and (6) saturation of the carrier sites was approached.

The use of D-alanine to find a diffusion value served two purposes, first it provided a measurement of the rate of diffusion for the test amino acids, and second it fulfilled the specificity criteria for active transport. Lower concentrations of D-alanine were not tested to see if this stereospecificity requirement is absolute as it has been reported to be in the mammalian gut. D-methionine is the only D-amino acid which has been reported to be transported in the mammalian gut (Jervis and Smith, 1960).

There are three well documented absorption pathways for amino acids in the rat and hamster gut: (1) a general

pathway for neutral amino acids, (2) a general pathway for basic amino acids, and (3) a second pathway for proline and aromatic neutral amino acids in addition to pathway 1 (Lin et al. 1962). Two other pathways have been suggested: (1) a second pathway for glycine (Akedo and Christensen, 1962; Christensen, 1962; Newey and Smith, 1963; and Newey and Smith, 1964), and (2) a pathway for leucine, isoleucine, and valine (Hagihira et al. 1960). Although competitive inhibition studies were not carried out on the bass gut, the transport data suggest that perhaps the first four of the above pathways were present. Valine and leucine were not tested so it is not known if a fifth pathway might exist.

This present study necessitated use of the tissue incubation technique instead of the everted sac method because the muscular characteristics of the bass gut made it impossible to evert without stripping off the mucosal layer. Since the everted sac method was used by other investigators, a quantitative comparison to omnivorous fish was difficult. The reason for this was that everted sac data include both an active accumulation into the tissue and a passive diffusion into the serosal compartment while the tissue incubation data include only active accumulation into the tissue. In addition, radioisotopes were used to follow the movement of the test amino acids in the bass gut while ninhydrin analysis methods were used by other investigators.

With an initial ambient concentration of L-alanine at 10 mM (0.01 mM/ml), the proximal segment of the bass gut had a tissue/medium (T/M) ratio of 2.37 which was comparatively close to the serosal/mucosal (S/M) value of 2.58 reported by Mepham and Smith (1966) using the same concentration in the anterior intestine of the goldfish. Larger T/M ratios were obtained in the bass gut with higher concentrations (0.1 mM/ml) of L-alanine, however concentration studies were not reported for the goldfish.

In the same study, S/M values were not reported on the goldfish rectum, although it was reported that the anterior intestine of the goldfish had a greater transfer of L-alanine from the mucosal to the serosal compartment than the rectum. However, in the bass the ability of the different regions of the gut to transport L-alanine was medial > distal > proximal.

It may be noted that due to the relative shortness of the goldfish gut and the technique used, Mepham and Smith (1966) were able to divide the gut only into two equal sections which they called the anterior intestine and the rectum. The anterior intestine of the goldfish would thus approximate the proximal plus one-half the medial section of the bass gut. If the mid-region of the goldfish gut did have the highest rate of transport, it is quite possible that it would overshadow any effects seen in either the anterior intestine or rectum.

Neff and Musacchia (1967) reported that the middle intestinal segment of the freshwater catfish had the highest levels of mucosal absorption and serosal increase of L-leucine at the three levels of concentrations studied. However, there was not a constant progression of the transport activity in the different regions of the catfish gut. At 2.5 mM L-leucine, the order of the intestinal segments with the highest levels of mucosal decrease and serosal increase was medial > proximal > distal while at both 5 and 10 mM the order of progression was medial > distal > proximal.

The reported S/M ratios for the catfish gut do not seem consistant with the ability of different gut regions to transport L-leucine (Neff and Musacchia, 1967). There was a consistant decreasing progression of transport activity of the intestinal regions at all three concentrations: proximal > medial > distal.

The optimal concentration for obtaining the highest T/M ratio in the bass was 40 times greater than that used in the catfish. In addition, the T/M ratios in the bass gut reflected the ability of the different regions to transport the test amino acids. At all concentrations of L-alanine studied in the bass there was a constant progression of the different regions: medial > distal > proximal. This progression was constant for both the T/M ratios and the ability of the different regions to accumulate L-alanine.

The data suggest that there is a difference between the carnivorous teleost and the omnivorous teleost in the ability of the different regions of their guts to transport amino acids. The order of transport activity of the intestinal regions in omnivorous fish has been reported to be proximal medial distal while in the carnivorous fish the order of the intestinal regions is medial distal proximal. In addition the omnivorous teleost has a greater capacity to transport amino acids at lower concentrations than the carnivorous teleost. This is in agreement with the type of diet of the two animals, for one would expect that the diet of the omnivorous teleost would routinely result in a lower concentration of protein and amino acids being consumed as compared to the carnivorous teleost. Furthermore, the ability of the bass gut to transport a wide range of concentrations of L-alanine could be advantageous if there was a fluctuation in the protein content of the diet of the animal.

V.

SUMMARY

SUMMARY

The active transport of glycine, L-alanine, L-lysine, L-methionine, and L-proline were shown to occur in the gut of the black bass, Huro salmoides, using the tissue incubation technique of Agar (1954 and 1956). In defining active transport the following criteria were met: (1) the test amino acids were transported against their concentration gradients; (2) the transport mechanism proved to be dependent upon both metabolic energy and temperature; (3) the transport mechanism showed a molecular specificity because only the L-stereoisomer of alanine was transported against its concentration gradient; (4) chromatographic analysis indicated that the transported amino acids were structurally unchanged; and (5) saturation of the carrier mechanism was approached.

Certain generalizations can be made in comparing the carnivorous freshwater teleost (e.g., bass) and the omnivorous freshwater teleost (e.g., catfish) because there seems to be a difference in the ability of the gut of these two teleosts to absorb amino acids. The gut of the omnivorous teleost seems to have a greater ability to transport amino acids at lower concentrations. There also appears to be a regional difference in the amino acid absorption ability of the guts of the two types of fish. The maximum rate of absorption of amino acids occurred in medial region of the carnivorous bass, while the anterior intestine was reported to be the

optimal region of absorption in the omnivorous freshwater teleost.

VI.

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