SPECIFICITY OF AMINO ACID TRANSPORT IN THE TAPEWORM *HYMENOLEPIS DIMINUTA* AND ITS RAT HOST

by A. J. MacInnis, D. J. Graff, A. Kilejian, and C. P. Read

ABSTRACT

The specificity of amino acid transport loci in *Hymenolepis diminuta* was determined by assaying reciprocal inhibitions of uptake in pairs of all possible combinations of fifteen amino acids with the exception of glutamic acid and tyrosine, where metabolism and solubility, respectively, prevented acquisition of data. From these results we propose that *H. diminuta* possesses at least six distinct loci, but with overlapping affinities. These loci were designated as follows: a) dicarboxylic amino acid preferring; b) serine preferring (*A* site in other organisms); c) leucine preferring (*L* site in other organisms); d) aromatic amino acid preferring; e) dibasic amino acid preferring; f) glycine preferring. Methionine shows high affinity for all loci except the dibasic amino acid preferring locus. Histidine uses both the aromatic and dibasic loci. When the various amino acids were used in the external medium to stimulate efflux, the results corroborated the loci proposed from the inhibitor studies. Fifteen amino acids were each studied as inhibitors of the uptake of each of ten amino acids by segments of the rat's intestine. These results indicated the presence of a locus with high affinity for the dibasic amino acids in the rat's intestinal mucosa, but lysine clearly has overlapping affinity for the systems transporting the neutral amino acids. The major difference observed between the tapeworm and its host was the strong inhibition of alanine uptake by leucine in the rat gut, whereas leucine had less effect on alanine uptake by the worm.

Austin MacInnis is Professor of Biology at the University of California, Los Angeles. D. J. Graff is Associate Professor of Zoology at Weber State College, Ogden, Utah. A. Kilejian is Associate Professor of Parasitology at The Rockefeller University. C. P. Read is deceased.

INTRODUCTION

Over the last ten years, a considerable body of data has accumulated which indicates that the mediated transport of amino acids into and out of cells involves several systems, some of which show overlapping affinities for individual amino acids. These affinities seem to differ from one cell type to another and from one organism to another, and may differ in various organs. Read et al. (1963) concluded that in the tapeworm *Hymenolepis diminuta* there are at least four membrane mechanisms for amino acid transport. These show respective preferences for diamino, dicarboxylic, and two groups of monoamino-monocarboxylic acids; the systems do not show complete specificity for the indicated classes of amino acids. The same authors also presented evidence suggesting that the two mechanisms (loci) for the transport of "neutral" amino acids seemed to resemble the *A* (alanine-preferring) and *L* (leucine-preferring) loci reported for Ehrlich ascites cells (Oxender and Christensen, 1963); these loci were later found in the human erythrocyte (Winters and Christensen, 1964).

Read et al. (1963) postulated that the absorption of a single amino acid from an amino acid mixture would depend on the relative concentrations of other amino acids and the effectiveness of individual amino acids as competitive inhibitors of adsorption of the single amino acid concerned. Experimental tests of this hypothesis have supported it in the cases of two tapeworm species (Read et al., 1963; Senturia, 1964) and two acanthocephalan species (Rothman and Fisher, 1964).

Transport loci's differing but overlapping specificities for amino acids may be presumed to involve (a) regulation of the intracellular pools of free amino acids in the intestinal parasite and in the host intestinal mucosa; (b) regulation of the composition of the extracellular pool of free amino acids in the gut lumen; and (c) the level of competition between host and parasite for available amino acids.

Since we still lack comprehensive data on the specificity of amino acid transport loci in an animal parasite, we have made a systematic examination of the transport interactions of a large number of amino acid pairs in *Hymenolepis diminuta* and in the intestinal mucosa of its rat host. Such data may furnish a more complete understanding of the number of qualitatively distinct amino acid transport loci in the parasite and allow an evaluation of differences between host and parasite.

MATERIALS AND METHODS

Organisms

Rat tapeworms, *Hymenolepis diminuta*, 10 days old (± 4 hours) were used as standardized for transport studies by Read et al. (1963). Rat gut tissues were obtained from male albino Holtzman rats. The animals were maintained

in the laboratory for about two weeks before use and weighed 160-190 g at the time of killing.

Methods with tapeworms

The inhibition of influx of each of 15 L-amino acids by each of the other 14 amino acids was determined using the methodology of Read et al. (1963). All incubations were of two-minute duration except for glutamic acid, with which one-minute incubations minimized metabolic efforts. Uninhibited influx was determined from incubations in 1 mM ^{14}C-amino acid in KRT (Krebs-Ringer tris-maleate buffered saline, Read et al., 1963); inhibited influx was determined from the uptake of 1 mM labeled substrate in the presence of 5 mM inhibitor in KRT. Influx in μmoles/gm ethanol-extracted dry weight/hr was determined by appropriate standards and calculations, from the radioactivity determined in the ethanol extract.

Percent efflux was determined by incubating the worms for one minute in 0.1 mM ^{14}C-labeled amino acid (in KRT), rinsing thrice in KRT, then placing the worms for two minutes in either KRT or 5 mM unlabeled amino acid in KRT, or directly into 70% ethanol. The amount of amino acid found in the sample placed directly into the ethanol was called the *initial influx*. The amount remaining in the samples after the subsequent two minutes in KRT was used to determine the passive efflux in KRT. The percentage of stimulated efflux was calculated by first subtracting the passive efflux of labeled amino acid leaked into KRT from the amount effluxed in the presence of 5 mM unlabeled amino acid in KRT, then dividing by the initial influx and multiplying by 100.

Other experiments with minor modifications in techniques are described in context.

Methods with Gut Tissues

The tissue accumulation method of Agar et al. (1954) was generally followed. Rats were killed by cervical dislocation, and a two-inch segment of the small intestine, the third to the fifth inch from the pylorus, was removed and immediately placed in cold Krebs-Ringer solution. The section was everted, rinsed, and cut into ring segments approximately $^1/_8$" wide. The segments were rinsed twice in cold buffer, then placed in Krebs-Ringer's-bicarbonate buffer (pH 7.4) containing 5 mM glucose (called KRGB hereafter) for at least one minute at 37° C before incubation in test solutions.

Data were obtained in groups of sixteen samples from each rat, each $^1/_8$" gut segment constituting a sample. From each rat four samples were used to determine uninhibited influx of labeled amino acid; the remaining twelve samples were divided into three groups of four to determine the inhibited influx in the presence of each of three different unlabeled amino acids. This procedure provided an internal control so that variation among rats did not

significantly affect the data. The incubations were conducted in 50 ml beakers in a constant-temperature shaker bath at 37° C; 5% CO_2-95% air was gently bubbled through the solutions before and during incubation of the tissue. Uninhibited influx of labeled amino acid was determined from a one-minute incubation in 25 ml of 1 mM amino acid in KRGB. Inhibited influx was determined similarly, except that 5 mM inhibitor amino acid was present. After incubation the segment was rinsed thrice in buffered saline (room temperature), then placed in 70% ethanol to extract overnight. The amount of uptake was determined by removing a 0.5 ml aliquot of the ethanol extract and counting in a gas-flow Geiger counter. Conversion of radioactivity to μmoles was made with appropriate standards and calculations.

The uptake data for rat gut segments were based on total protein (serum-albumin equivalents remaining after ethanol extraction). Each gut segment was removed from the ethanol and digested in 5 ml of 1 N NaOH for one hour at 37° C. Total protein was determined by the method of Lowry et al. (1951).

To establish the validity of the experimental procedures with the rat gut tissues we made the following preliminary observations. We found that the uptake of alanine by each $^1/_8$" segment cut from a two-inch piece of gut was the same. We assumed that this was true for the other amino acids studied. A comparison by light and electron microscopy was made between gut tissue removed from the rat and immediately fixed in glutaraldehyde, and tissue which had undergone the experimental incubation before fixation. Tissue sections were prepared by the usual procedures for light and electron microscopy. Subsequent examination of the tissue revealed negligible effects attributable to the experimental procedures.

Statistical analyses

Correlation coefficients were calculated with the aid of an IBM 1620 computer, using a linear correlation coefficient program (IBM 6.0.038), as modified by John E. Simmons, Jr.

RESULTS AND CONCLUSIONS

Inhibition of Amino Acid Influx in *Hymenolepis*

We have determined the reciprocal inhibitory activity in the influx of amino acid pairs in all combinations of 15 amino acids into *Hymenolepis diminuta*. These data are summarized in figure 1. The vertical position of an amino acid in each column represents its activity as an inhibitor of the influx of the amino acid indicated at the base of the column. The columns in figure 1 were arranged to show relative increasing or decreasing inhibitory trends in the action of certain amino acids. Inspection of these data allows certain conclusions:

1. There is considerable overlapping in the affinity (inhibition) for various transport loci.

2. The most specific locus is the arginine-lysine system; histidine is the only other amino acid, of those tested, yielding a significant inhibition at this locus.

3. Methionine is a strong inhibitor at all transport loci other than the arginine-lysine locus.

4. There are obvious changes in the relative inhibiting activity of specific amino acids, looking from left to right in figure 1. These data show a progression from high inhibition by alanine, serine, threonine, and valine in the columns on the left to high inhibition by isoleucine, leucine, phenylalanine, and tyrosine on the right. The "crossing-over" trend of the inhibitions suggests the presence of more than one locus for the transport of monoamino-monocarboxylic acids, perhaps corresponding to the *A* and *L* systems described by Oxender and Christensen (1963) in Ehrlich ascites cells and by Read et al. (1963) in *H. diminuta*. Phenylalanine and histidine generally follow the trend of the *L* locus (leucine-preferring), except that phenylalanine is a better inhibitor of phenylalanine, tyrosine, and histidine influx than of leucine influx. From these observations, we concluded that there are at least two loci involved in the transport of neutral amino acids and a high probability of a third locus having a strong affinity for the aromatic amino acids.

A more precise way of examining the relative inhibitory activities of the amino acids studied involves the calculation of correlation coefficients. The coefficients for interactions of all pairs of 15 amino acids are shown in table 1. The inhibitory activities of phenylalanine and tyrosine on influx of other amino acids are more highly correlated ($r = 0.94 \pm 0.03$) than the inhibitions produced by leucine and phenylalanine ($r = 0.59 \pm 0.17$) or by leucine and tyrosine influx ($r = 0.54 \pm 0.18$). Leucine also shows a stronger association with alanine than does phenylalanine or tyrosine. Inhibitory effects of isoleucine are more highly correlated ($r > 0.90$) with effects of alanine, serine, threonine, methionine, valine, and leucine than with effects of phenylalanine or tyrosine ($r = 0.40$ and 0.35, respectively).

Also seen in table 1 is a high correlation ($r > 0.90$) between inhibitions effected by alanine and those by serine, threonine, methionine, isoleucine and, to a slightly lesser extent, valine ($r = 0.86$). These amino acids seem to react preferentially with a single locus. Isoleucine also seems to interact significantly with the locus involved in leucine influx.

In *H. diminuta*, proline has its highest correlation with valine. Inhibitions by glycine do not show high correlation with those of other amino acids tested, suggesting that there is a glycine-preferring locus; serine, threonine, and methionine clearly interact strongly with this locus (figure 1). Present and earlier data (Read et al., 1963) support the view that there is a monoamino-dicarboxylic acid-preferring locus.

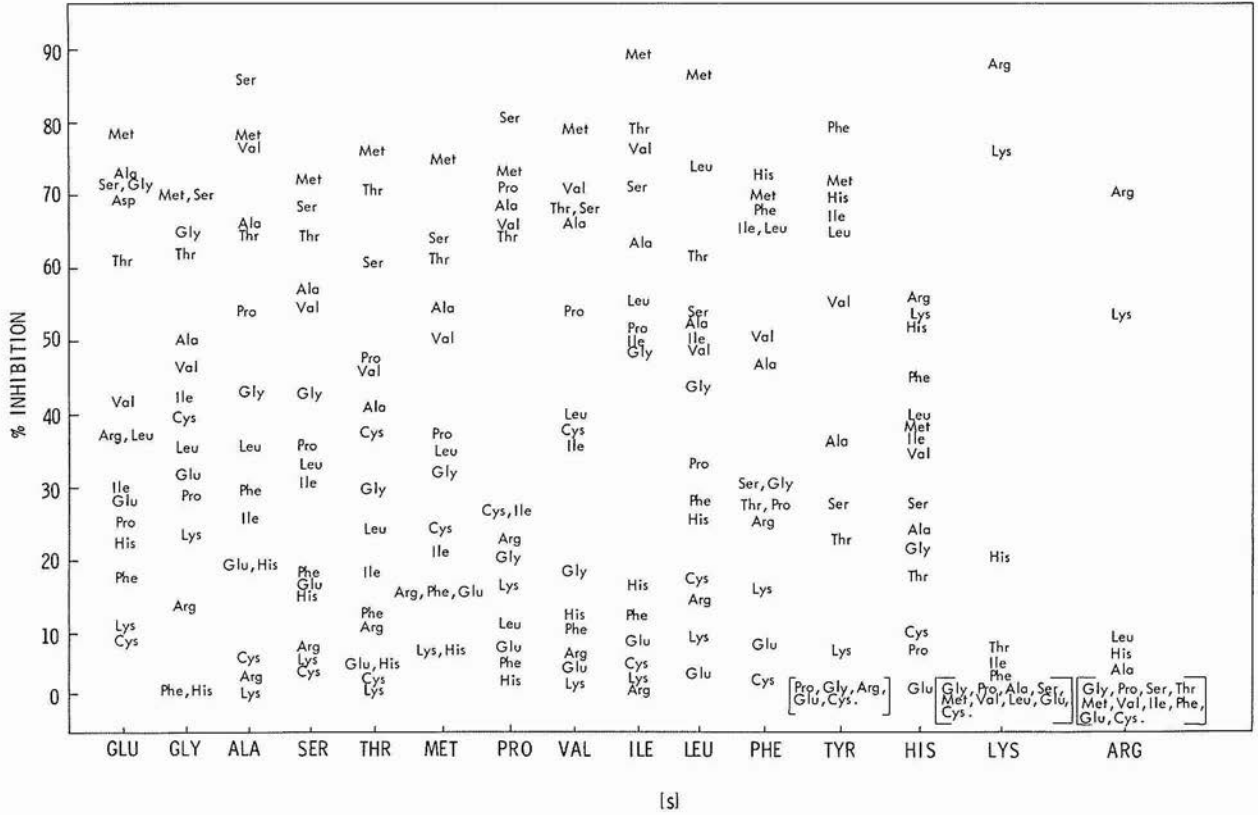

FIG. 1. COMPARISON OF INHIBITIONS by each of 15 amino acids on the uptake of amino acids by *H. diminuta*, substrate at 1 mM, inhibitor at 5 mM. The position of each inhibitor in the graph corresponds to the percentage of inhibition. Each inhibition was calculated from the mean of at least five samples (5 worms per sample). Uninhibited transport rates are listed in table 5.

TABLE 1

CORRELATION COEFFICIENTS OF THE PERCENTAGE OF INHIBITION BY 15 AMINO ACIDS ON VARIOUS AMINO ACIDS IN *H. DIMINUTA*

	Ala	Leu	Phe	Gly	Pro	Ile	Met	Ser	Thr	Val	Glu	Tyr	His	Arg	Lys
Glu	0.78	0.72	0.19	0.79	0.64	0.78	0.83	0.87	0.79	0.64	—	0.09	−0.11	−0.25	−0.30
Gly	0.68	0.65	−0.13	—	0.69	0.76	0.82	0.80	0.74	0.71	0.79	−0.12	−0.41	−0.38	−0.43
Ala	—	0.74	0.30	0.68	0.68	0.93	0.91	0.96	0.92	0.86	0.78	0.27	−0.21	−0.53	−0.56
Ser	0.96	0.83	0.30	0.80	0.83	0.97	0.95	—	0.95	0.88	0.87	0.27	−0.18	−0.48	−0.51
Thr	0.92	0.78	0.21	0.74	0.88	0.92	0.96	0.95	—	0.90	0.79	0.16	−0.20	−0.39	−0.42
Met	0.91	0.80	0.16	0.82	0.88	0.91	—	0.95	0.96	0.94	0.83	0.17	−0.26	−0.43	−0.49
Pro	0.68	0.55	−0.27	0.69	—	0.78	0.88	0.83	0.88	0.90	0.64	−0.55	−0.27	−0.27	−0.30
Val	0.86	0.78	0.23	0.71	0.90	0.90	0.94	0.88	0.90	—	0.64	0.25	−0.24	−0.49	−0.53
Ile	0.93	0.90	0.40	0.76	0.78	—	0.91	0.97	0.92	0.90	0.78	0.35	−0.16	−0.53	−0.57
Leu	0.74	—	0.59	0.65	0.55	0.90	0.80	0.83	0.78	0.78	0.72	0.54	0.61	−0.42	−0.48
Phe	0.30	0.59	—	−0.13	−0.27	0.40	0.16	0.30	0.21	0.23	0.19	0.94	0.54	−0.28	−0.26
Tyr	0.27	0.54	0.94	−0.12	−0.55	0.35	0.17	0.27	0.16	0.25	0.09	—	0.50	−0.34	−0.32
His	−0.21	0.61	0.54	−0.41	−0.27	−0.16	−0.26	−0.18	−0.20	−0.24	−0.11	0.50	—	0.59	0.62
Lys	−0.56	−0.48	−0.26	−0.43	−0.30	−0.57	−0.49	−0.51	−0.42	−0.53	−0.30	−0.32	0.62	0.98	—
Arg	−0.53	−0.42	−0.28	−0.38	−0.27	−0.53	−0.43	−0.48	−0.39	−0.49	−0.25	−0.34	0.59	—	0.98

There is evidence for a locus showing a strong preference for aromatic amino acids, which also exhibits a great affinity for leucine. Plotting the correlation coefficients for phenylalanine shows this clearly (figure 2). If the interactions of histidine with arginine and lysine are removed from consideration, the correlation of histidine with phenylalanine and tyrosine is much higher. Previous studies with a large series of aromatic amino acids furnish independent evidence for an aromatic amino acid-preferring locus (Read et al., 1963).

As an inhibitor, histidine (figure 1) is most effective against phenylalanine and tyrosine influx, but, unlike other neutral amino acids, histidine is also an effective inhibitor of lysine and arginine influx. The activity of other amino acids as inhibitors of histidine influx supports the conclusion of Woodward and Read (1969) that histidine is absorbed mainly through the aromatic and dibasic amino acid loci. In a separate experiment, we examined the interactions of phenylalanine, leucine, lysine, and arginine as inhibitors of histidine influx. The results (figure 3) show that when both a dibasic and a neutral amino acid are present as inhibitors, there is an additive inhibition of histidine influx, whereas the presence of two dibasic amino acids does not produce additional inhibition. This is further evidence that histidine has overlapping affinity for the otherwise highly specific aromatic and dibasic amino acid transport systems.

Since phenylalanine does not interact with the dibasic locus and arginine does not interact with the aromatic locus, we reasoned that it might be possible to demonstrate linear changes in the relative numbers of these two transport systems along the tapeworm strobila. Accordingly, single 14-day-old worms were incubated for two minutes in the presence of 1.0 mM ^{14}C-arginine and 1.0 mM ^{3}H-phenylalanine. After rinsing, each worm was stretched on a thin glass plate sitting in a tray of crushed dry ice. The worms were completely frozen in about a second. Each frozen worm was then cut into pieces about 2 mm in length and placed in 70% ethanol. The following day, radiocarbon and tritium levels were determined in each ethanol extract. The ratio of radiocarbon to tritium was constant along the entire length of the strobila in each of five worms examined by this procedure and we concluded that the relative numbers of these two systems appeared constant.

An additional observation may be made concerning the data in figure 1. The horizontal sequence of columns selected to show the maximum number of different trends in competitions resulted in an arrangement of the transported amino acids in a structural series. The dicarboxylic amino acid, glutamate, is on the left edge and the dibasic amino acids, lysine and arginine, are on the right edge. Between the extremities is a series from left to right that shows, respectively, an increase in length of the carbon chain, addition of a hydroxyl group to the chain, branching of the chain, and finally the ring structures of phenylalanine, tyrosine, and histidine. Proline may appear to be

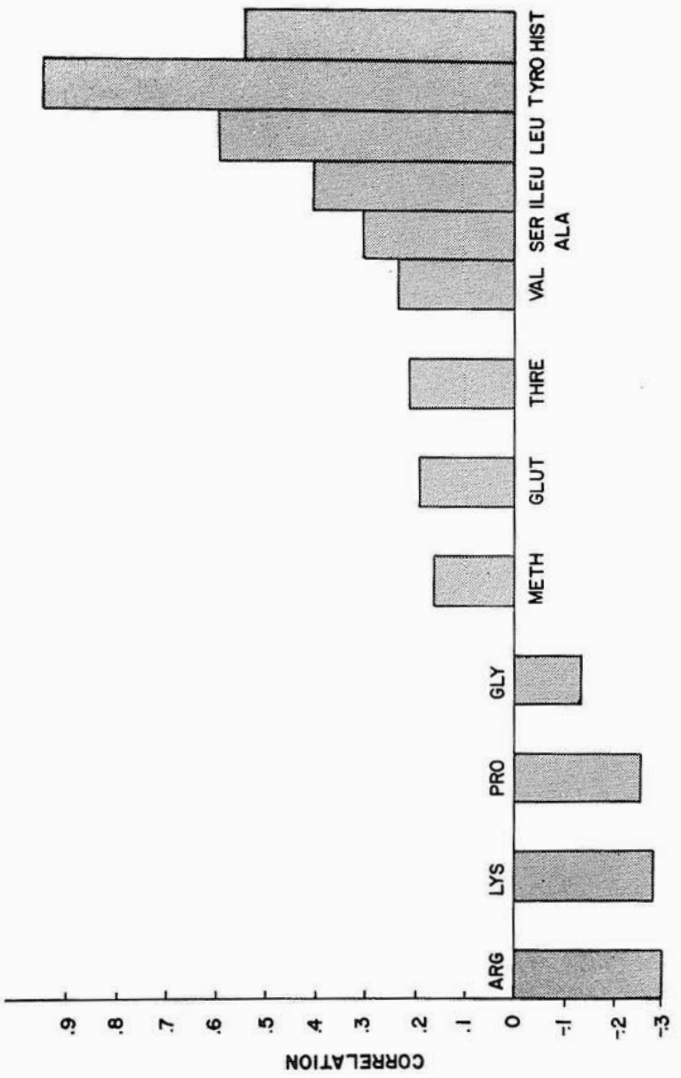

FIG. 2. PLOT OF CORRELATION COEFFICIENTS of inhibitions of each amino acid compared with inhibitions by phenylalanine in *H. diminuta*.

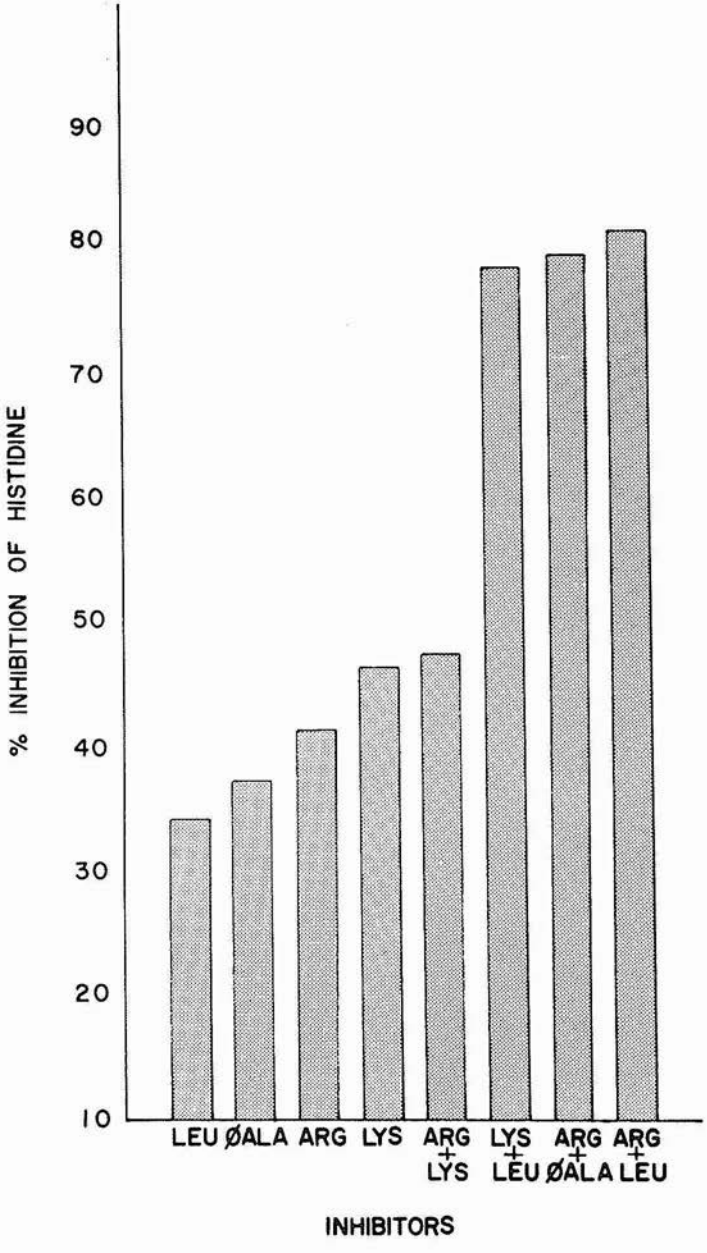

FIG. 3. COMPARISON OF THE EFFECTS of the presence of one (0.5 mM) or two (0.5 mM each) inhibitors on the uptake of histidine (0.1 mM) by *H. diminuta*.

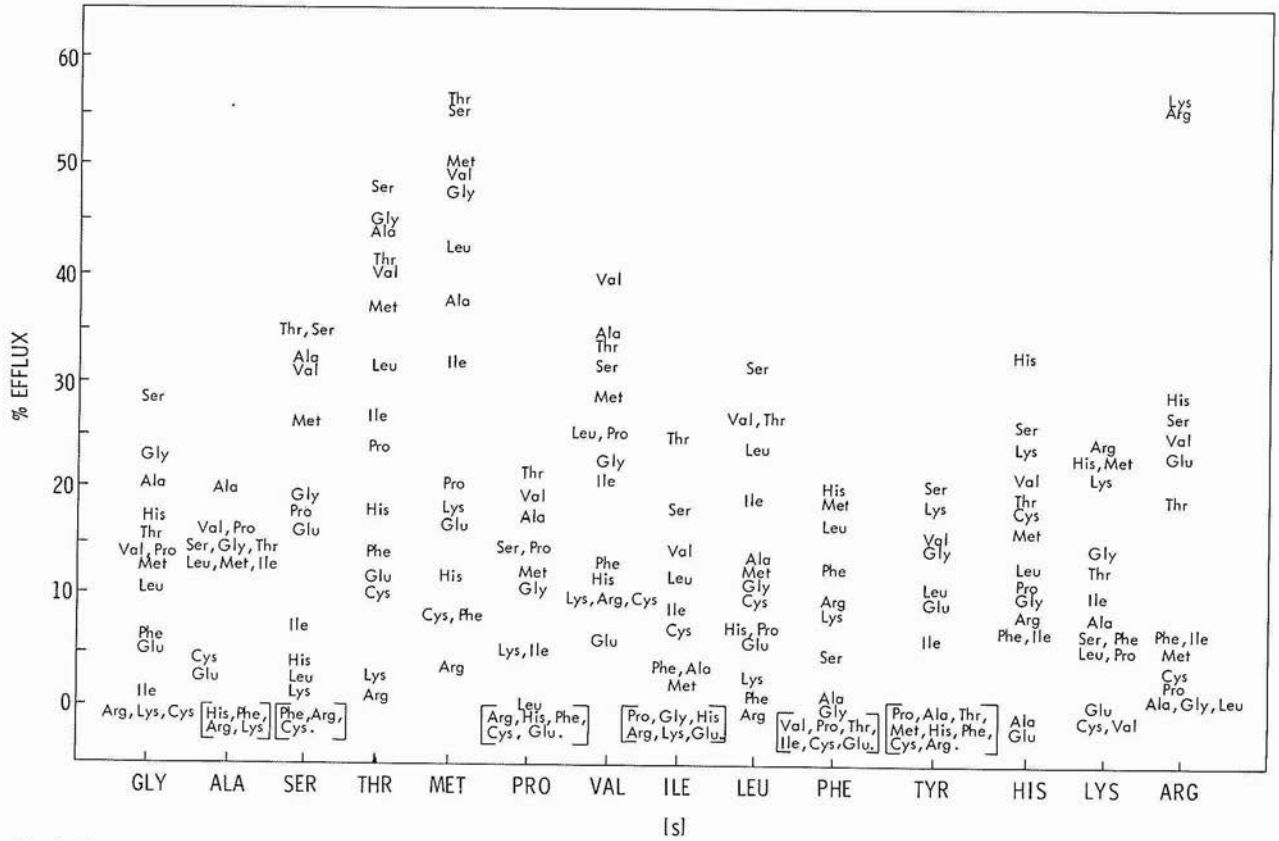

FIG. 4. COMPARISON OF EFFLUX of previously accumulated amino acid in the presence of single amino acids by *H. diminuta*. The position of each amino acid in the graph corresponds to the percentage of efflux (see methods for details). Each point was calculated from the mean of at least five samples.

an exception to this structural sequence. Its properties as an *imino* acid may account for its "fit" in the sequence, however, on the basis of the correlation coefficients of table 1, it seems logically placed between methionine and valine.

Efflux of Amino Acids from *Hymenolepis*

A number of investigations have shown that the presence of a solute in the surrounding medium may enhance the efflux of an analogous compound from the internal cellular pool. This has been shown to occur with amino acids in *H. diminuta* (Read et al., 1963; Hopkins and Callow, 1965; Kilejian, 1966; Woodward and Read, 1969; Arme and Read, 1969). We decided to examine systematically the relative activities of exogenous amino acids in enhancing outflow of previously absorbed amino acids. Such data might provide additional information on the specificity and overlapping affinities of transport loci and on the relative roles of loci in the efflux processes. The results of our experiments are shown in figure 4, and the correlation coefficients for the effects of individual external amino acids in enhancing efflux are given in table 2.

Generally, the same amino acid transport loci suggested by results from inhibition studies are supported by the data from efflux experiments. The interpretation of these data concerning the absolute value of efflux must be made with great caution. The absorbed amino acid was not, in any case, at a steady state level with regard to the internal pool. The relative activity of amino acids in promoting efflux should be valid for comparative purposes, however. Serine, threonine, alanine, and valine appear to be most effective in promoting efflux of other amino acids, suggesting that the locus shared by these amino acids is most active in efflux. Leucine and isoleucine are less effective than the above mentioned amino acids and phenylalanine is a weak effector of efflux. This suggests that the aromatic locus and the leucine-preferring locus are involved, to a relatively minor extent, in the efflux of neutral amino acids.

The efflux of glutamic acid was not examined because significant metabolism of this amino acid occurs in three minutes. The relative insolubility of tyrosine prevented examination of its activity as a stimulator of efflux at appropriate concentrations.

Inhibition of Amino Acid Influx in Rat Gut

The relative activities of various individual unlabeled amino acids as inhibitors of the influx of each of ten labeled amino acids into rat gut tissue are shown in figure 5. The correlation coefficients for the inhibitions produced among pairs of amino acids are presented in table 3. Although these data are limited, they clearly indicate the presence of more than one amino acid transport system and also suggest that there are overlapping affinities for amino acids by these systems. For example, while alanine is more effective

TABLE 2

CORRELATION COEFFICIENTS OF THE EFFECTS AS EXCHANGERS OF 14 L-AMINO ACIDS ON VARIOUS AMINO ACIDS IN *H. DIMINUTA*

	Phe	Leu	Ala	Ser	Gly	Pro	Thr	Val	Met	Ile	Arg	Lys	Tyr	His
Gly	−0.04	0.54	0.64	0.75	—	0.62	0.84	0.65	0.66	0.27	−0.32	−0.07	0.25	0.20
Ala	−0.45	0.69	—	0.78	0.64	0.80	0.87	0.91	0.78	0.43	−0.57	−0.35	0.15	−0.18
Ser	−0.38	0.65	0.78	—	0.75	0.90	0.83	0.82	0.81	0.52	−0.21	−0.19	0.20	0.05
Thr	−0.20	0.79	0.87	0.83	0.84	0.77	—	0.90	0.91	0.53	−0.51	−0.19	0.25	0.07
Met	−0.17	0.86	0.78	0.81	0.67	0.75	0.91	0.86	—	0.65	−0.30	−0.01	0.41	0.16
Pro	−0.44	0.58	0.80	0.90	0.62	—	0.77	0.87	0.75	0.51	−0.17	−0.10	0.15	0.11
Val	−0.26	0.78	0.91	0.82	0.65	0.87	0.90	—	0.86	0.62	−0.40	−0.24	0.17	0.06
Ile	−0.28	0.84	0.43	0.52	0.27	0.51	0.53	0.62	0.64	—	−0.11	−0.35	0.21	0.31
Leu	−0.23	—	0.69	0.65	0.54	0.57	0.79	0.78	0.86	0.84	−0.28	−0.34	0.42	0.30
Phe	—	−0.23	−0.45	−0.38	−0.04	−0.45	−0.20	−0.26	−0.16	−0.28	0.18	0.58	−0.20	0.33
Tyr	−0.20	0.42	0.15	0.21	0.25	0.15	0.25	0.17	0.41	0.21	0.29	−0.17	—	0.30
His	0.33	0.31	−0.18	0.05	0.20	0.11	0.07	0.06	0.16	0.31	0.38	0.28	0.30	—
Lys	0.59	−0.23	−0.35	−0.19	−0.07	−0.10	−0.19	−0.24	−0.01	−0.35	0.49	—	−0.17	0.28
Arg	0.18	−0.28	−0.57	−0.21	−0.32	−0.17	−0.51	−0.40	−0.31	−0.11	—	0.49	0.29	0.38

TABLE 3

CORRELATION COEFFICIENTS OF THE INHIBITORY ACTION OF 10 L-AMINO ACIDS ON VARIOUS AMINO ACIDS IN RAT GUT TISSUE

	Glu	Gly	Ala	Ser	Met	Pro	Val	Leu	Phe	Lys
Glu	—	0.27	0.39	0.35	0.40	0.12	0.33	0.61	−0.24	0.38
Gly	0.27	—	0.11	−0.08	0.16	−0.25	0.13	0.11	−0.24	0.51
Ala	0.39	0.11	—	0.56	0.43	0.32	0.63	0.52	0.59	0.39
Ser	0.35	−0.08	0.56	—	0.70	0.30	0.67	0.36	0.62	0.08
Met	0.40	0.16	0.43	0.70	—	0.28	0.56	0.42	0.38	0.42
Pro	0.12	−0.25	0.32	0.30	0.28	—	0.58	0.39	0.07	0.09
Val	0.33	0.13	0.63	0.67	0.56	0.58	—	0.60	0.42	0.32
Leu	0.61	0.11	0.52	0.36	0.42	0.39	0.60	—	0.52	0.23
Phe	0.56	−0.24	0.59	0.62	0.38	0.07	0.42	0.52	—	0.29
Lys	0.38	0.51	0.39	0.08	0.42	0.09	0.32	0.23	0.29	—

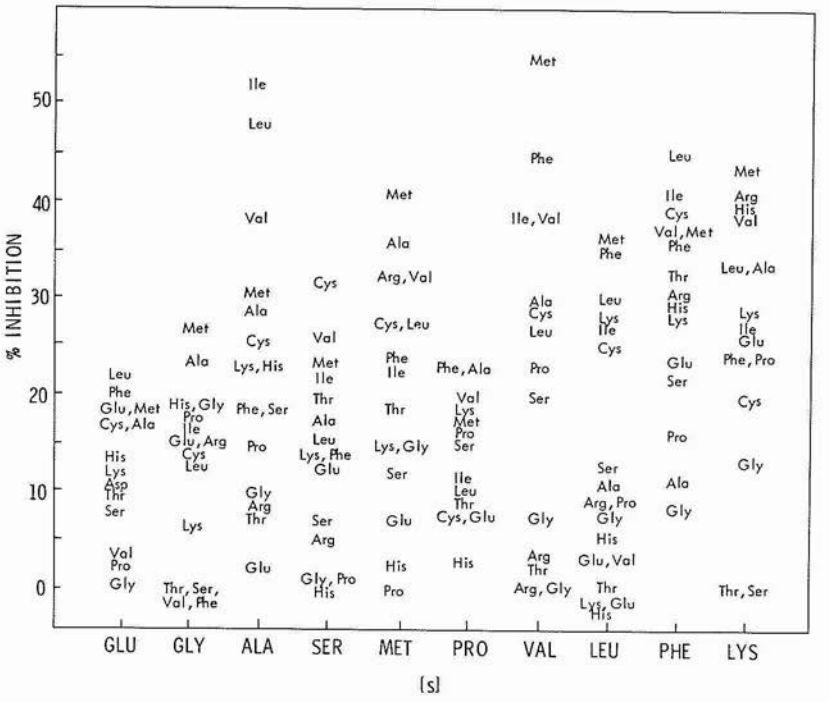

FIG. 5. COMPARISON OF INHIBITIONS by each of 15 amino acids on the uptake of various amino acids in segments of rat gut tissue. The position of each inhibitor in the graph corresponds to the percentage of inhibition. Substrate concentration at 1 mM, inhibitor at 5 mM. Each inhibition was calculated from the mean of at least four samples. Uninhibited transport rates are listed in table 5.

than leucine as an inhibitor of glycine, methionine, and proline influx, the converse is true in the relative effects of leucine and alanine on the influx of leucine, alanine, and phenylalanine. Likewise, valine and alanine have very similar effects on proline influx, but these two amino acids differ sharply in their effects on glycine influx.

We may recall that lysine clearly has overlapping affinity with systems involved in the transport of neutral amino acids, as reported previously by Arme and Read (1969). Also, glycine and lysine show a higher correlation coefficient with one another for the inhibition of the influx of other amino acids than either of them shows with any other amino acid tested. Hampton (1970) found lysine to interact strongly with the neutral amino acid transport systems in *Trypanosoma cruzi*, a protozoan parasite of mammals. These systems differ sharply from *Hymenolepis diminuta*'s. The marked inhibition of lysine influx produced by arginine and histidine suggests that there is a locus showing strong affinity for basic amino acids.

Proline shows a low correlation coefficient with most amino acids tested, with greatest relation to the branched chain amino acids, valine and leucine. Note in figure 5 that leucine, isoleucine, valine, and cysteine follow the same general pattern as inhibitors of the influx of other amino acids.

DISCUSSION

Examination of the relative effects of various individual amino acids on the influx and efflux of other amino acids indicates that *Hymenolepis diminuta* has several amino acid transport loci, differing qualitatively but with considerable overlapping affinity for various amino acids. From our data and previous studies, we can postulate six amino acid transport systems for *H. diminuta*, as summarized in table 4.

In rat gut, there appear to be an alanine-preferring system, a leucine-preferring system, a glycine-preferring system, and a basic amino acid-preferring system. More extensive study is required to identify other systems that may be present. As with *Hymenolepis*, there is considerable overlap of affinity for the amino acids examined. The proline-preferring system studied by Hagihira et al. (1961) is probably identical with what we have referred to as the leucine-preferring system. As shown by Hagihira et al., this system exhibits affinity for branched chain compounds that may be regarded, in the broad sense, as analogues of leucine, valine, or isoleucine.

It is also interesting to compare the amino acid transport systems of the symbiotic tapeworm *Hymenolepis diminuta* with those of the host's intestinal mucosa. First, we must point out that our studies of the rat intestinal mucosa were made with unparasitized host tissue. Although we recognize that the presence of the tapeworm might modify the characteristics of transport systems of the mucosa, it seems probable that they would only be changed in the quantitative sense. With that assumption, there are clearly very significant differences between the parasite and the intestinal mucosa.

The most striking differences between the inhibitions in the rat and worm are the effects of leucine and isoleucine on alanine uptake. It may be seen from the data obtained with the rat gut segments (figure 5) that leucine and isoleucine are effective inhibitors not only of leucine but also of alanine (cf. figure 1). In the rat gut, alanine is not as good an inhibitor of leucine or phenylalanine as it is in the worm. In the rat gut, valine is a relatively good inhibitor of alanine, but not of leucine, yet valine appears as a good inhibitor of phenylalanine. Valine's effect as an inhibitor of the various amino acids does not follow the same pattern in the worm. These results demonstrate an obvious difference in the nature of the *A* and *L* loci in host and parasite.

The screening of interactions in amino acid transport by examining single inhibitor-to-substrate ratios is useful, but this approach has limitations. For example, it furnishes no evaluation of whether there may be multiple binding

TABLE 4

SIX AMINO ACID TRANSPORT SYSTEMS FOR *H. DIMINUTA*

LOCI	DICARBOXYLIC	GLYCINE	SERINE	LEUCINE	PHENYLALANINE	DIBASIC
MAIN AMINO ACIDS USING THE LOCUS	aspartic glutamic methionine	glycine methionine	serine alanine threonine methionine valine proline	leucine isoleucine methionine	phenylalanine tyrosine histidine methionine	arginine lysine
OVERLAPPING AMINO ACIDS	serine alanine glycine	serine threonine alanine	glycine	glycine serine threonine alanine valine	leucine isoleucine	histidine

sites on one or more systems. Further, it does not detect other kinds of interaction, such as stimulation, which may occur at other inhibitor: substrate ratios. Such interactions are known to occur in *Hymenolepis* in the absorption of purines and pyrimidines (MacInnis et al., 1965; MacInnis and Ridley, 1969; Pappas et al., 1973) and of long chain fatty acids (Chappell et al., 1969). Ruff and Read (1974) have recently demonstrated such interactions in the absorption of amino acids by the hemoflagellate *Trypanosoma equiperdum.*

The screening method used in our study does not distinguish non-productive binding, i.e., binding without subsequent transport, which is known to occur with purines and pyrimidines in *H. diminuta* (Pappas et al., 1973), with amino sugars in *Trypanosoma gambiense* (Southworth and Read, 1972), and with sugars in *Schistosoma mansoni* (Uglem, personal communication). If a compound binds but is not transported through a particular locus, it may still exhibit competitive inhibition. This may account for the discrepancies between K_t (Michaelis constant) and K_i (inhibition constant) observed in amino acid transport in *H: diminuta* by Read et al. (1963). K_i values could be used to predict the rate of absorption of a single component from a mixture of amino acids, whereas K_t values did not yield satisfactory predictions. This is exactly what would be expected if some amino acids undergo non-productive binding on certain transport loci. Thus the free pools of amino acid in the gut may influence the nature of competitive inhibitions, non-productive binding yielding inhibition and other factors not yet delineated. Of the latter, we can predict that energy sources such as glucose will also be of importance. CO_2, pH, NH_4, Na, K, and Ca will also impinge on this competition. What effect do these items have in regulating protein synthesis? Are they coupled to the regulation of RNA synthesis? Questions such as these must be answered before a satisfactory model of host-parasite interaction can be proposed. A beginning on this complex problem may be attempted, however, by summarizing some of the data we have accumulated on amino acids. In table 5 we have listed, in order of rank, various properties and effects of amino acids in the tapeworm and in the rat's gut. Presently, they suggest at least one obvious conclusion: The tapeworm has evolved remarkably well-adapted systems that favor its growth in competition with the host. A look at table 5 reveals, for example, that the rank orders of the amino acids as inhibitors in the worm and rat are not correlated. One would expect that many of the parameters examined would be correlated with the size of the free pools. Yet no simple correlations of pool size with exchange rate, initial uptake, or protein synthesis are evident. These data should simplify future studies by delineating aspects most likely to provide decisive information on host-parasite competition. Perhaps they will also reveal patterns of value to future investigators. One of the most important, as yet unanswered, questions concerning amino acid flux is whether or not the tapeworm modifies its environment in ways that benefit its own welfare.

TABLE 5

COMPARISON OF RANK ORDER OF VARIOUS PROPERTIES AND EFFECTS OF AMINO ACIDS IN THE TAPEWORM AND RAT GUT TISSUE

Uptake rates by *H. diminuta* from 0.1 mM substrate are from one-minute incubations used to calculate efflux rates shown in table 4. Protein synthesis rates were determined from aliquots of TCA precipitates of homogenized worm tissue.

	H. diminuta Uptake Rate S = 0.1 mM μmoles / gm / hr		*H. diminuta* Uptake Rate S = 1.0 mM μmoles / gm / hr		Rat Gut Uptake Rate S = 1.0 mM μmoles / gm / hr		*H. diminuta** Free Pool	Rat Gut* Lumen	*H. diminuta* x̄ % Inhibition of Other Amino Acids		Rat Gut x̄ % Inhibition of Other Amino Acids		*H. diminuta*** % of Total Uptake From 2 hr Incubation Incorporated Into Protein	
1	met	57	thr	167	met	169	ala	glu	met	64	met	32	ile	74
2	thr	57	ala	164	val	147	glu	gly	thr	49	ile	28	phe	55
3	val	56	val	140	phe	146	gly	ala	val	48	leu	26	thr	55
4	ala	43	met	139	leu	144	pro	lys	ser	46	phe	23	val	52
5	ser	39	ile	130	ser	134	ser	asp	ala	41	cys	23	arg	50
6	leu	32	pro	127	pro	121	leu	ser	leu	36	val	23	lys	44
7	pro	31	ser	122	gly	120	lys	leu	ile	32	ala	23	leu	38
8	gly	27	gly	117	ala	92	val	arg	gly	32	lys	19	tyr	37
9	arg	23	leu	92	glu	65	asp	pro	pro	32	arg	15	ser	36
10	his	19	phe	86	lys	57	his	val	arg	25	his	14	his	32
11	ile	19	his	51			ile	thr	his	24	pro	12	asp	24
12	lys	18	thr	37			met	ile	lys	21	ser	11	gly	16
13	phe	18	lys	34			phe	phe	phe	21	glu	11	pro	15
14	tyr	14	arg	26			thr	his	glu	10	thr	9	ala	8
15	glu		glu	18			arg	met			gly	8	glu	6

* From J. E. Simmons

** MacInnis, Graff, Fisher and Read, unpublished

x̄% Average percentage

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (AI 13830, AI 01384, 5 T01 AI 00106 to Clark P. Read; AI 00070 and NSF 8753 to A. J. MacInnis).

We wish to express our deep gratitude to Mr. William Kitzman for his exceptional assistance with these experiments. His skills, comradeship, and good humor provided a unifying force over nearly two decades in Clark Read's laboratory. To Evelyn Hake we extend similar thanks for her selfless devotion to science, assistance with these experiments, and innumerable contributions to the parasitology group at Rice beginning in the Chandler era.

REFERENCES CITED

Agar, W. T., F. J. R. Hird, and G. S. Sidhu
1954 The uptake of amino acids by the intestine. Biochimica et Biophysica Acta **14**:80-84.

Arme, C. and C. P. Read
1969 Fluxes of amino acids between the rat and a cestode symbiote. Comparative Biochemistry and Physiology **29**:1135-1147.

Chappell, L. H., C. Arme, and C. P. Read
1969 Studies on membrane transport V. Transport of long chain fatty acids in *Hymenolepis diminuta* (cestoda). Biological Bulletin **136**:313-326.

Hagih a, H., E. C. C. Lin, A. H. Samiy, and T. H. Wilson
1 51 Active transport of lysine, ornithine, arginine and cystine by the intestine. Biochemical and Biophysical Research Communications **4**:478-481.

Hampton, J. R.
1970 Lysine transport in the culture form of *Trypanosoma cruzi*: Kinetics and inhibition of uptake by structural analogues. International Journal of Biochemistry **1**:706-714.

Hopkins, C. A. and L. L. Callow
1965 Methionine flux between a tapeworm (*Hymenolepis diminuta*) and its environment. Parasitology **55**:653-666.

Kilejia A.
19 Formation of the L-proline pool of the cestode, *Hymenolepis diminuta*. Experimental Parasitology **19**:358-365.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall
1951 Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry **193**:265-275.

MacInnis, A. J., F. M. Fisher, Jr., and C. P. Read
1965 Membrane transport of purines and pyrimidines in a cestode. Journal of Parasitology **51**:260-267.

MacInnis, A. J. and R. K. Ridley
1969 The molecular configuration of pyrimidines that causes allosteric activation of uracil transport in *Hymenolepis diminuta*. Journal of Parasitology **55**:1134-1140.

Oxender, D. L. and H. M. Christensen
1963 Evidence for two types of mediation of neutral amino acid transport in Ehrlich cells. Nature **197**:765-767.

Pappas, P. W., G. L. Uglem, and C. P. Read
1973 The influx of purines and pyrimidines across the brush border of *Hymenolepis diminuta*. Parasitology **66**:525-538.

Read, C. P., A. H. Rothman, and J. E. Simmons
1963 Studies on membrane transport with special reference to host-parasite integration. Annals of the New York Academy of Science **113**:154-205.

Rothman, A. H. and F. M. Fisher, Jr.
1964 Permeation of amino acids in *Moniliformis* and *Macracanthorhynchus* (Acanthocephala). Journal of Parasitology **50**:410-414.

Ruff, M. D. and C. P. Read
1974 Interactions of amino acids in membrane permeation in *Trypanosoma equiperdum*. In preparation.

Senturia, J. B.
1964 Studies on the absorption of methionine by the cestode, *Hymenolepis citelli*. Comparative Biochemistry and Physiology **12**:259-272.

Southworth, G. C. and C. P. Read
1972 Absorption of some amino acids by the haemoflagellate, *Trypanosoma gambiense*. Comparative Biochemistry and Physiology **41A**:905-911.

Winters, C. G. and H. N. Christensen
1964 Migration of amino acids across the membrane of the human erythrocyte. The Journal of Biological Chemistry **239**:872-878.

Woodward, C. K. and C. P. Read
1969 Studies on membrane transport VII. Transport of histidine through two distinct systems in the tapeworm *Hymenolepis diminuta*. Comparative Biochemistry and Physiology **30**:1161-1177.