AMINO ACID POOLS OF *SCHISTOSOMA MANSONI* AND MOUSE HEPATIC PORTAL SERUM

by Harold L. Asch

ABSTRACT

Analyses were made of the free amino acid pools of adult male and female worms and worm pairs of *Schistosoma mansoni* and of the host (mouse) hepatic portal blood. Twenty-five identified and 10-13 unidentified ninhydrin-positive compounds were observed in the parasites. Most of the host blood amino acids were present at concentrations near the respective transport constants (K_t) for the parasite's uptake systems. Comparison of amino acid concentrations within worms to those present in hepatic portal blood suggests that several amino acids, especially glutamate, may be actively concentrated by the parasites. The results are discussed in terms of the physiology and biochemistry of the host-parasite relationship.

INTRODUCTION

Despite the rapidly growing body of information on the biochemistry and physiology of adult schistosomes, their nutritional requirements and mechanisms for obtaining nutrients are poorly understood. Therefore, a series of studies was undertaken to analyze the mechanisms by which these parasites absorb amino acids (Asch and Read, 1975a and b). In the course of these studies, the concentrations of free amino acids in worms and in the host mouse hepatic portal blood were determined.

MATERIALS AND METHODS

A Puerto Rican strain of *Schistosoma mansoni* was maintained in *Biomphalaria glabrata* (PR strain) as described previously (Asch and Read, 1975b). Mice were fed Purina chow *ad libitum*. At seven weeks post-exposure,

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mice were anesthetized with chlorobutanol (Hunter, 1960) and prepared for perfusion. The perfusion technique was that of Radke et al. (1962) as modified by Pappas and Asch (1972). To assay the amino acids following perfusion, 400-600 worms were rinsed thoroughly and allowed to sit overnight in 70% ethanol. The ethanol was removed, the worms were dried at 80° C for 15 minutes, and their protein content was determined by the method of Lowry et al. (1951). The ethanol was partitioned against three volumes of acidified chloroform and the water layer evaporated at 56° C to 0.3 ml under nitrogen. Amino acids in the sample were measured using a Technicon NC-1 Amino Acid Analyzer, with norleucine as internal standard. The techniques used did not account for potential non-physiological conversions such as glutamine to glutamate or cystine to cysteic acid. The concentrations of amino acids were expressed in terms of ml of tissue water and were calculated from the protein determinations. The ratio of wet weight:dry weight:alcohol extracted dry weight:total protein was 1.00:0.27:0.22:0.20 (P. Pappas, unpublished data; Asch, unpublished data).

For analysis of free amino acids in mouse hepatic portal blood, each mouse was anesthetized with chlorobutanol, the abdomen opened and the portal vein exposed. A small incision was made in the vein and the effusing blood was drawn into a heparinized syringe. Blood (0.4 ml) from one to three mice was centrifuged in a Microfuge (Beckman-Spinco, Palo Alto, California). The plasma was then deproteinized by adding 20 μliters of 50% trichloroacetic acid (TCA). The denatured protein was washed three times with 3% TCA and the washes combined with the supernatant. The preparation was partitioned against acidified chloroform; the water phase was evaporated under nitrogen at 56° C to 0.3 ml and then processed in the amino acid analyzer as described above. No difference was noted in plasma amino acid levels in the mouse when cervical fracture replaced anesthetization or when infected and uninfected mice were compared. Because anesthetization was more convenient and the distended portal veins of infected mice were more accessible, these techniques were employed routinely. Worms and mouse blood were collected between 9:00 A.M. and 11:00 A.M. from mice 7-8 weeks post-infection.

RESULTS

The free amino acid content of mouse hepatic portal plasma (four assays), male worms (two assays) and worm pairs (one assay) are shown in table 1. Cysteic acid, taurine, and urea varied a great deal in their concentration. The variation was due probably to the coincidental elution of these compounds with unidentified ninhydrin-positive compounds and, in the case of cysteic acid, possible contributions from oxidation of cystine and cysteic acid. Although there was some variation in the absolute amounts of any one amino acid from one assay of portal plasma to another, the ratio of a given

TABLE 1

AMINO ACID POOLS OF *S.MANSONI* MALES AND WORM PAIRS AND OF DEPROTEINIZED MOUSE HEPATIC PORTAL PLASMA

COMPOUND	HUMAN PLASMA[1] (Peripheral)	MOUSE PLASMA[2] (Portal)	WORMS[3] Males	Males	Pairs
Aspartate	0.004	0.035 ± .016	ND	0.634	1.108
Threonine	0.127	0.367 ± .126	0.995	0.893	1.400
Serine	0.113	0.198 ± .060	0.748	0.754	1.167
Glutamate	0.051	0.097 ± .013	4.973	3.820	4.192
Proline	0.201	0.178 ± .031	1.283	1.090	1.175
Citrulline	0.021	0.097 ± .020	TRACE	0.126	0.067[r]
Glycine	0.294	0.361 ± .047	0.950	1.060	1.350
Alanine	0.263	0.629 ± .058	3.779	3.990	3.983
Valine	0.244	0.184 ± .073	0.236	0.285	0.380
Cystine	—	0.056 ± .026	TRACE	TRACE	TRACE
Methionine	0.023	0.026 ± .003	0.100[r]	0.100[r]	0.119[r]
Isoleucine	0.069	0.081 ± .015	0.121	0.140	0.207
Leucine	0.128	0.165 ± .040	0.255	0.330	0.454
Tyrosine	0.055	0.063 ± .016	0.140	0.155	0.143
Phenylalanine	0.054	0.069 ± .019	0.141	0.155	0.213
Ornithine	0.051	0.106 ± .035	0.099	0.100	0.228
Lysine	0.171	0.336 ± .082	0.615	0.397	0.706
Tryptophan	—	TRACE	0.020[r]	TRACE	0.038[r]
Histidine	0.060	0.141 ± .052	0.215	0.206	0.366
Arginine	0.089	0.048 ± .009	ND	0.080	0.021[r]
Ammonia	—	0.208 ± .029	0.307	0.080[r]	0.247
γ-aminobutyrate	—	0.015 ± .003	0.059	0.030[r]	0.046[r]
Cysteic acid	—	0.050 ± .026	0.310	0.130	4.460
Taurine	0.065	0.622 ± .197	3.914	0.260	1.867
Urea	—	5.004 ± 1.856	7.261	ND	ND

1. Obtained by averaging data from tables compiled by Diem and Lentner (1970), p. 574.
2. Mean of four determinations ± S.E. Values given as mM.
3. Single determinations. Values given as mM, based on calculation of worm water content from protein assay.

r. Rough estimate (due to small peaks).

ND. Not discernible (due to unresolvable peaks).

amino acid concentration to that of any other remained fairly constant. In addition to the 25 compounds listed, five to seven unidentified ninhydrin-positive compounds were detected in portal plasma. For the parasite, the same 25 compounds were identified in all three assays. Additionally, the numbers of unidentified ninhydrin-positive compounds found in males and worm pairs were ten and thirteen, respectively. Most of the parasite amino acids were present at concentrations similar to those in the host serum.

DISCUSSION

Robinson (1961) found thirteen free amino acids in *S. mansoni* adults but did not report any unidentified ninhydrin-positive compounds. The present report (table 1) indicates a larger number of amino acids plus a substantial number of unidentified ninhydrin-positive materials. Senft (1966) analyzed acid-hydrolyzed, lyophilized worms (containing both free and incorporated amino acids) and reported more than twenty ninhydrin-positive compounds (certain amino acids were omitted because of uncertainty of measurements). If one converts the free amino acid concentrations in *S. mansoni* reported by Chappell (1974) from μmoles/gm dry wt to μmoles/ml worm water, they are quite close to those shown in table 1.

A comparison of the free amino acid levels in worms to those in portal blood suggests that the parasites may concentrate several amino acids. Most notable of these is glutamate, which is approximately forty times higher in worms than in the surrounding blood. A potentially important source of error is the fact that some of the glutamate may have been derived by conversion from glutamine during preparation of the samples for analysis. Other than glutamate, the majority of amino acids appear to be maintained by the worms at concentrations only slightly greater than those found free in the blood. In a single experiment (Asch, unpublished data) no significant change was noted in glutamate concentration within male worms when they were incubated for two minutes in the presence of 0.05 mM glutamate. Thorough accumulation studies will be required to elucidate the extent, if any, of active transport involved in absorption of these amino acids.

The relatively high concentration of glutamate may not be due to specific accumulative transport mechanisms, but instead may result from accumulation of this compound as an end product of metabolism. Schistosomes take up as much as five times their weight in glucose per day (Bueding, 1950) and the high levels of glutamate, alanine, and aspartate may result from transamination of glucose metabolic end products. This hypothesis is supported by the report (Senft, 1963) that 6% of absorbed glucose is converted to alanine. These three amino acids may represent detoxification sinks for amino groups derived from amino acid metabolism, or they may be used as amino group donors for amino acid synthesis. The transaminases of schistosomes have

been examined (Garson and Williams, 1957; Huang et al., 1962; Conde del Pino et al., 1968) but their significance in the overall nutrition of these organisms is not known.

The concentrations of free portal plasma amino acids found here (table 1) are similar to those from mouse plasma (unspecified anatomical source, Senft, 1966), and slightly lower than those reported by Page et al. (1972) for cardiac plasma from mice that were hyperinfected, morbid, and starved. They are considerably greater than those reported for human peripheral blood (see Senft, 1966; Diem and Lentner, 1970). The importance of using amino acid concentrations of portal blood when discussing the nutrition of schistosomes must be emphasized. The increase in concentration of free amino acids in the blood following feeding (Van Slyke and Meyer, 1912) is first noticeable in the portal vein, where the highest levels in the blood are attained (Dent and Schilling, 1949; Denton and Elvehjem, 1954). Thus, the schistosomes may be exposed to varying concentrations of amino acids during the course of a day. This is not a source of error in the present studies because food was available *ad libitum* and mice were observed feeding to some degree at all hours, and because both blood and parasites were collected at the same time of day. In man, the natural host of *S. mansoni*, such fluctuations are a probable occurrence, especially in endemically infected populations whose individuals consume poor and restricted diets.

The hemodynamics of the portal blood may also affect the nutritional significance of the concentrations of amino acids available to the schistosomes (see Dent and Schilling, 1949). The total amount of amino acids actually presented to the parasites per unit time may be two or three orders of magnitude greater than the quantity in a few milliliters of stagnant blood. This may affect the rates of transport and metabolism as well as motility of the parasite.

Although the absolute concentrations of amino acids appear to rise following a meal, it is known that the ratio of one amino acid to another is well regulated (Dent and Schilling, 1949; Denton and Elvehjem, 1954). This appears to be consistent with the present findings that, although the absolute portal blood concentrations of a given amino acid varied from one determination to another, its ratio to any other given amino acid remained fairly constant. Most of the blood amino acids are present at concentrations near the respective transport constants (K_t) for the parasite's uptake systems (Asch and Read, 1975b). These findings signal an additional significance of the schistosome amino acid transport systems. The rate of entry of a given amino acid will be controlled by inhibitory interactions among the amino acids that compete with its transport. Since the ratios present in the parasite's milieu are constant, then, although an increase in absolute concentrations may cause higher uptake rates, the ratios of these rates among the amino acids whose uptake is mediated may remain fairly constant. This may be a controlling factor in the worm's biosynthetic capacities. Accordingly, these

transport systems may be viewed as parasitic adaptations to homeostatic mechanisms of the host (Read et al., 1963).

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