A Long-term Intake of a Protein Hydrolysate Seems to Increase the Risk of Encephalopathy in Mice Infected with *Schistosoma mansoni*

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Previous investigations showed that Schistosoma mansoni *infection aggravates protein malabsorption in undernourished mice and this can be reverted by administration of casein hydrolysate. The present study was undertaken to evaluate the effects of ingestion of casein hydrolysate for long periods. Albino Swiss mice were divided into eight groups. Diets contained 5% (undernourished) or 20% (controls) casein levels. For each group there were sub-groups ingesting whole or hydrolysed casein for 12 weeks. Infection with* S. mansoni *developed in half of the animals under each diet. All undernourished mice developed malabsorption. Low albuminemia was detected in infected animals independently of the protein level in the diet. However, albuminemia was lower in infected controls than in undernourished non-infected mice, suggesting a deficient liver protein synthesis. Infected mice fed on a 20% protein hydrolysed diet exhibited low weight gain and high mortality rates. On the other hand, non-infected mice ingesting the same diet had the highest body weights. We are investigating the hypothesis that infected mice, even when fed normal diets, are unable to metabolise large amounts of amino acids due to the liver lesions related to schistosomiasis and as a result die of hepatic coma. In some of them, the excessive accumulation of ammonia in the blood enhances the outcome of an encephalopathy.*

Key words: schistosomiasis - undernutrition - aminoacids absorption - hydrolysed casein

Patients with severe Manson's schistosomiasis are usually undernourished (Coutinho 1976, Tavares Neto et al. 1988). The use of a specific diet in such situations could be recommended as an auxiliary therapeutic measure, but in order to be effective an appropriate knowledge about nutrient absorption and the metabolism of these patients would be required.

Previous investigations have detected malabsorption in cases of schistosomiasis mansoni in humans (El-Rooby et al. 1963, Fikry 1963, Fikry et al. 1966, Mott et al. 1971, Pucci et al. 1978, Nigro et al. 1984) and mice (Ferreira 1991, Coutinho et al. 1992a, Ferreira et al. 1993).

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Weanling mice fed a food blend with a low-protein content, which is commonly ingested by human populations in endemic areas in northeast Brazil, developed a type of malnutrition with similarities to the marasmatic form of calorie-protein malnutrition seen in humans (Coutinho et al. 1992b). In this experimental model, it was observed that dietary protein was not adequately absorbed mainly in infected undernourished animals and that *Schistosoma mansoni* infection had apparently no effect on well-nourished infected mice (Ferreira et al. 1993).

Assuming that some enzymatic digestive deficiencies were likely to exist in undernourished mice, leading to an imperfect hydrolysis, the effects of both whole casein and hydrolysed casein were investigated in a further experiment (Ferreira et al. 1994). That study demonstrated that undernourished infected animals fed hydrolysed casein had intestinal absorption rates equivalent to the controls.

The present investigation was designed to evaluate the effects of hydrolysed diets ingested for longer periods of time on *S. mansoni* infected undernourished and control animals.

Although the experiment is still under development, high mortality rates have been observed

in infected well-nourished mice fed hydrolysed casein. On the other hand their very low levels of serum albumin as compared to non-infected undernourished animals, suggest that mice became unable to metabolize the surplus intake of aminoacids during 12 weeks of infection, due to liver lesions related to schistosomiasis with the result that they progress to hepatic coma. In some animals, the excessive accumulation of ammonia in the serum enhances the outcome of a further encephalopathy.

MATERIALS AND METHODS

Animals - Twenty-one day old male weanling mice, weighing 9 to 12 g.

Diets - (a) 20% whole casein diet (purified diet of the American Institute of Nutrition - Reeves et al. 1993). This control diet had the following composition (g/100g): commercial casein 20; vegetable oil 7; corn starch 39,75; dextrin 13.2; sucrose 10; cellulose 5; Mineral Mix AIN-93G 3.5; Vitamin Mix AIN-93VX 1; L-methionine 0.15; L-cystine 0.15; Choline 0.25 and hydroquinone 0.0014. (b) 5% whole casein low-protein diet: identical to the control diet, except for a reduction in the casein level from 20 to 5%. Corn starch replaced the portion of removed casein. (c) 20% hydrolysed casein diet: similar to the control diet, except for the utilization of hydrolysed casein (N-Z-Case Plus, Sigma N4642) in place of whole casein. (d) 5% hydrolysed casein low-protein diet: identical to the 5% whole casein diet, except for the use of hydrolysed casein (N-Z-Case Plus, Sigma N4642).

Infection - A Brazilian strain of *S. mansoni* isolated from São Lourenço da Mata (State of Pernambuco, Brazil), maintained regularly in our laboratory was used. Each animal was infected percutaneously with 80 cercariae recently shed from *Biomphalaria glabrata* reared and infected in the laboratory. A total of 101 mice were experimentally infected with an additional 55 animals kept free of infection

Experimental groups - Mice were distributed into eight experimental groups, according to Table I.

Intestinal absorption of whole protein and/or protein hydrolysate - This function was studied by a balance technique and the coefficient of protein absorption (CPA) was determined according to the formula below:

$$CPA = \frac{\text{Nitrogen intake - Fecal nitrogen}}{\text{Nitrogen intake}} \times 100$$

After the 12th week of the experiment, animals were kept in individual metabolic cages during five consecutive days. Food intake was weighed along this period. Trays under the cages were covered with filter paper to absorb urine, in order to reduce contamination of the feces with urinary nitrogen. Feces collected during the assay (five days) were dried in a 105°C oven until a constant weight was obtained. Fecal nitrogen was determined by the Kjedahl micro-method. The difference between nitrogen intake and fecal nitrogen expresses the absorbed nitrogen. Multiplication of this value by factor 6.25 gives a value for the amount of absorbed protein.

TABLE I

Distribution of mice according to the type of diet and infection with *Schistosoma mansoni*

Diet	Infection	No. of mice
20% whole casein	No	13
20% hydrolysed casein	No	13
5% whole low-protein	No	14
5% hydrolysed low-protein	No	15
20% whole casein	Yes	17
20% hydrolysed casein	Yes	29
5% whole low-protein	Yes	27
5% hydrolysed low-protein	Yes	28
Total	-	156

Biochemical determinations - Soon after the absorption assays were completed, mice were sacrificed by exsanguination through the axillary vein under light anesthesia with ether. Blood was collected in test tubes and centrifuged at 3.500 rpm during 10 min to obtain serum for determination of albumin in an automatic analyzer (bromocresol ftaleína method). Ammonia was determined by the L-glutamate desidrogenase method - Sigma 170. All the biochemical measurements were performed at the Central Laboratory of State of Alagoas (State Secretary of Health).

Protocol - Twenty-four hours after weanling, mice were infected with *S. mansoni* and divided into eight experimental groups (Table II). Food and water were given *ad libitum*. The animals were weighed weekly. The biological intestinal absorption assays were performed 12 weeks after infection. Mice were then sacrificed by exsanguination through the axillary vein under light ether anesthesia. Collected blood was centrifuged and sera used for biochemical determinations of albumin and ammonia. Liver samples were collected for histological study and fixed in 10% neutral formalin.

RESULTS

The experiment started with 156 mice, however 56 animals died spontaneously during the experimental period (Table II).

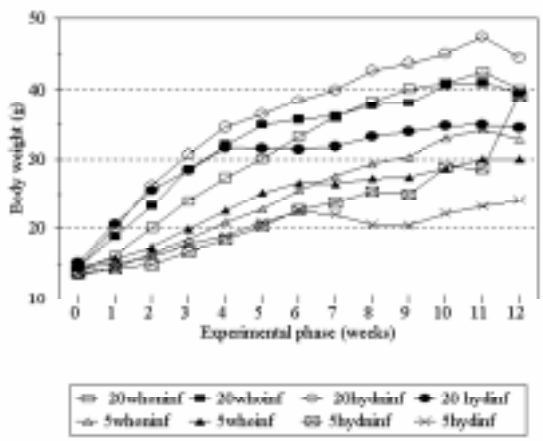
TABLE II

Mortality rates regarding type of diet and *Schistosoma mansoni* infection

Experimental groups (Diets)	Number of mice			
	At the beginning of the experiment	Spontaneous death	At the end of the experiment	Mortality rates (%)
20% whole casein (non-infected)	13	0	13	0.0
20% hydrolysed casein (non-infected)	13	0	13	0.0
5% whole casein (non-infected)	14	3	11	21.4
5% hydrolysed casein (non-infected)	15	1	14	6.7
20% whole casein (infected)	17	5	12	29.4
20% hydrolysed casein (infected)	29	15	14	51.7
5% whole casein (infected)	27	14	13	51.9
5% hydrolysed casein (infected)	28	18	10	64.3
Total	156	56	100	35.9

The weight curves of mice from the different experimental groups can be seen in the Figure.

It can be observed that the weight curves of mice ingesting 20% casein diets showed much higher values than the weight curves from mice ingesting 5% casein diets. An exception to this was the group of well nourished infected animals ingesting a 20% hydrolysed casein diet. From the 5th week of infection onward, their body weights were observed to drop progressively and at the end of the experimental period their weights were similar to those of non-infected undernourished mice. However, non-infected mice fed a hydrolysed 20% casein diet showed normal body weight gains. The same results were detected among undernourished animals fed a 5% hydrolysed casein diet. Superimposed infection was responsible for the lowest figures in these animals, as compared to the other groups.



Weight curves of mice regarding type of diet and infection with *Schistosoma mansoni*.
20whoninf: 20% whole casein (non-infected); 20whoinf: 20% whole casein (infected); 20hydninf: 20% hydrolysed casein (non-infected); 20 hydinf: 20% hydrolysed casein (infected); 5whoninf: 5% whole casein (non-infected); 5whoinf: 5% whole casein (infected); 5hydninf: 5% hydrolysed casein (non-infected); 5hydinf: 5% hydrolysed casein (infected).

Absorption rates and biochemical data can be seen in Table III.

Mice fed 5% casein diets (low-protein diets) showed absorption rates significantly lower than animals fed 20% casein diets fed animals. Neither the protein chemical formula (whole, hydrolysed) nor infection with *S. mansoni* influenced the results with these animals.

Hypoalbuminemia was detected in all infected mice fed 20% and 5% whole and/or hydrolysed casein, this finding certainly related to infection with *S. mansoni*.

Regarding the serum levels of ammonia, significant differences were not detected among the experimental groups, with the exception of the infected mice fed with 20% hydrolysed casein. In this group, serum ammonia values were high when compared to controls (20% hydrolysed casein fed, non-infected mice). This difference was statistically significant ($p < 0.05$).

TABLE III

Coefficients of protein absorption (CPA) and biochemical data of mice fed whole casein/hydrolysed casein diets, as related to infection with *Schistosoma mansoni* (Mean ± SD)

Experimental groups	N	CPA (%)	Albumin (g/dl)	Ammonia (mg/dl)
20% whole casein (non-infected)	13	90,6 ±4,01[a]	5,63 ± 2,4[a]	1,54 ± 1,3[a]
20% hydrolysed casein (non-infected)	13	90,5 ± 3,48[a]	4,77 ± 2,5[a]	1,10 ± 0,3 [a,b]
5% whole casein (non-infected)	11	82,09 ± 6,66[b]	6,31 ± 5,5[a]	1,09 ± 0,5 [a]
5% hydrolysed casein (non-infected)	14	79,05 ± 9,90[b]	4,72 ± 2,6[a]	0,95 ± 1,0 [a]
20% whole casein (infected)	12	88,7 ± 3,11[a]	3,09 ± 0,4[b]	1,28 ± 0,2 [a]
20% hydrolysed casein (infected)	14	87,6 ± 3,62[a]	3,07 ± 0,7[b]	1,38 ± 0,3 [a,c]
5% whole casein (infected)	13	83,6 ± 8,45[b]	3,18 ± 1,2[b]	1,41± 0,8 [a]
5% hydrolysed casein (infected)	10	76,8 ± 7.49[b]	3,05 ± 1,5[b]	1,18 ± 0,4 [a]

a; b; c; d: different letters indicate significant differences regarding values in each column ($p < 0.05$).

DISCUSSION

The observation that none of the non-infected mice fed a 20% casein diet showed malabsorption, raises the possibility that the low performance detected in the 20% hydrolysed casein infected group was explained by their reduced capacity to adequately metabolize the surplus of absorbed aminoacids due to the hepatic lesions, since mice started loosing weight five weeks post infection, when oviposition of *S. mansoni* in murine infection is known to occur. In this same group, higher mortality rates were detected when compared with the other groups fed with 20% casein.

Among the undernourished animals (5% casein, low-protein diet), the hydrolysed casein fed infected group also showed high mortality rates.

The 20% hydrolysed diet given to non-infected mice allowed a higher weight gain when compared to non-infected animals fed 20% whole casein and no mortalities occurred. It seems likely that the liver of infected mice became unable to metabolize the excessive nitrogen derived from the absorbed aminoacids or, more specifically, they lost the ability to metabolize ammonia into urea. In effect, serum ammonia levels detected in the infected group fed 20% hydrolysed casein were significantly higher than those in the non-infected group fed 20% hydrolysed casein.

It is known that the accumulation of ammonia in the blood may precede the outcome of an encephalopathy (Pittella 1981, Campollo et al. 1992). The physiopathology of this metabolic disorder is still not very well understood, although it is believed that the reduction of alphaketoglutarate impairs the Krebs' cycle (Orten & Newhaus 1984). When there is an excess of ammonia in the brain, a considerable amount of it takes part in the formation of glutamine. This substance (unlike glutamate, which may partially cross through the cellular membrane) easily diffuses into the blood circulation, removing two molecules of ammonia, as follows:

alpha-ketoglutarate + NH_3 « glutamic acid
glutamic acid + NH_3 « glutamine

Glutamine may release ammonia to the liver, which is then used in the synthesis of nitrogen compounds or is excreted by the kidneys as a cation (NH_4^+). Any impairment in these physiological mechanisms may be responsible for the increase in the ammonia levels in the blood, which is injurious to the brain.

According to the majority of investigators, even in the chronic phase of human schistosomiasis mansoni, if there are no associated pathologies, hepatic insufficiency does not occur (Andrade 1968, Baptista & Geraldo 1985, Silva & Lima 1985, Borges & Manoukian 1987). However, Baptista and Geraldo (1985) reported an indirect involvement of the central nervous system in schistosomiasis as a consequence of hepatic insufficiency and recommended that this fact must be emphasized to allow a better comprehension about the repercussions of the liver injury on the brain.

We are currently investigating the effects of a long-term intake of hydrolised protein in mice infected with *S. mansoni*. Even when fed balanced diets, in this experimental model, the infected liver seems to be unable to metabolize the surplus of ingested aminoacids, with the result that the mice die from hepatic coma. In light of these findings, new studies are being undertaken before the use of such diets can be recommended as an auxiliary dietetic treatment for human patients.

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