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The use of protein hydrolysate improves the protein intestinal absorption in undernourished mice infected with *Schistosoma mansoni*

O uso de hidrolisado protéico aumenta a absorção intestinal de proteínas em camundongos desnutridos e infectados com *Schistosoma mansoni*

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Abstract *Patients residing in endemic areas for schistosomiasis in Brazil are usually undernourished and when they develop the hepatosplenic clinical form of the disease should usually receive hospital care, many of them being in need of nutritional rehabilitation before specific treatment can be undertaken. In the mouse model, investigations carried out in our laboratory detected a reduced aminoacid uptake in undernourished animals which is aggravated by a superimposed infection with* Schistosoma mansoni. *However, in well-nourished infected mice no dysfunction occurs. In this study, we tried to improve the absorptive intestinal performance of undernourished mice infected with S.* mansoni *by feeding them with hydrolysed casein instead of whole casein. The values obtained for the coefficient of protein intestinal absorption (cpia) among well-nourished mice were above 90% (either hydrolysed or whole protein). In undernourished infected mice, however, the cpia improved significantly after feeding them with hydrolysed casein, animals reaching values close to those obtained in well-nourished infected mice.*

Key-words*: Undernutrition. Schistosomiasis mansoni. Casein hydrolysate. Intestinal protein absorption.*

Resumo *Portadores de esquistossomose mansônica no Brasil são quase sempre desnutridos e, ao desenvolverem a forma hepatoesplênica da doença, geralmente necessitam de atenção hospitalar. Sob tais circunstâncias seria interessante uma reabilitação nutricional prévia ou paralela ao tratamento específico. Investigações conduzidas em nossos Laboratórios, utilizando camundongos, detectaram redução da absorção protéica entre animais desnutridos, a qual foi agravada por uma infecção superposta com* Schistosoma mansoni*. Contudo, em camundongos infectados, porém bem nutridos, tal disfunção não foi observada. Objetivou-se avaliar a absorção intestinal de camundongos desnutridos e infectados pelo* S. mansoni*, alimentados com caseína hidrolisada em comparação com caseína integral. Os resultados obtidos para o Coeficiente de Absorção Intestinal de Proteínas entre os camundongos bem nutridos foram superiores a 90% (tanto HID como INT). Entre os animais desnutridos, contudo, o aumentou significativamente quando se utilizou o hidrolisado protéico, atingindo valores muito próximos daqueles obtidos pelos camundongos infectados e alimentados com a dieta controle.*

Palavras-chaves: *Desnutrição. Esquistossomose mansônica. Hidrolisado protéico. Absorção intestinal de proteína.*

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Patients residing in endemic areas for schistosomiasis are usually undernourished[23]. Such patients when they develop severe clinical forms of the disease should receive hospital care and many of them are given special diets to improve their nutritional status before specific treatment can be established. Usually some dietetic formulae are given to the patients including those utilized in liver cirrhosis, but none take into consideration the singular liver and intestinal lesions related to schistosomiasis mansoni, in which the anatomical and pathophysiological pictures differ from those seen in other diseases. Previous investigations have detected both protein and lipid malabsorption in undernutrition[12 14 15 22 25] and schistosomiasis[4 7 8 9 10 11 18 19 20].

According to investigations by this laboratory[4 9], protein malabsorption occurs in undernourished mice and is aggravated when there is a superimposed infection with *S. mansoni*. However, well nourished infected mice fed control diets have no dysfunction. Of the factors involved in the pathogenesis of schistosomiasis and its course to the hepatosplenic clinical form, the parasite burden is considered to be the most important[2]. For this reason, the effects of two different cercarial burdens were examined in the present study.

Although the occurrence of protein malabsorption was demonstrated in undernourished infected mice[4 9], the cause of this dysfunction could not be determined. Enzyme deficiency was probably the main factor responsible.

This investigation was designed: 1) to evaluate whether a protein hydrolysate based diet (through its aminoacid component) would be better absorbed than a whole protein diet; and 2) to examine the effects of the intensity of infection upon the ability of malnourished infected mice to digest and absorb either dietetic whole protein or hydrolysed protein.

MATERIAL AND METHODS

Animals. Male Swiss weaning (21 to 25 days old) albino mice, weighing 10 to 15g were kept in individual cages under standardized conditions of light and temperature.

Infection. Infection was made by the percutaneous route with cercariae of the SL Brazilian strain of *Schistosoma mansoni* recently shed from *Biomphalaria glabrata* raised in our laboratory.

Diets. A control diet (CONT) was prepared to supply all the nutritional needs of mice[21], containing 22.6% protein (Table 1). The experimental diet (EXP) had a low protein content (5.2%) and all the components of the control diet, starch replacing the removed protein. For the absorption tests, diets Test 1 and Test 2 were prepared, the composition of which was the same as diets CONT and EXP, respectively, except for the substitution of whole casein by hydrolysed casein (N-Z-Case Plus, Sigma N4642). Diets were prepared in pellet form.

Experimental groups. A batch of 221 mice was initially formed. Only 152 animals survived until the end of the investigation due to spontaneous mortality induced by schistosomiasis infection and/or undernutrition. They were distributed into six groups, according to the type of the ingested diet (CONT or EXP) and to the cercarial burden (mice free of infection, infected with 40 cercariae, infected with 80 cercariae) as shown in Table 2.

Table 1 - Composition of the control diet (proteins, carboidrates, fat and vitamins water solubre and fat solubre).

		Percentage composition						
Ingredients	g%	prot	carb	fat	min	water soluble vits	fat soluble vits	fiber
Casein	27.1	22.0	-	-	-	-	-	-
Corn starch	57.4	0.6	50.4	0.1	0.1	-	-	-
Soybean oil	7.5	-	-	7.5	-	-	-	-
Mineral mixture[1]	4.0	-	-	-	4.0	-	-	-
Water soluble vits[2]	1.0	-	-	-	-	1,0	-	-
Fat soluble vits[3]	1.0	-	-	1.0	-	-	1.0	-
Cellulose	2.0	-	-	-	-	-	-	2.0
Total	100.0	22.6	50.4	8.6	4.1	1.0	1.0	2.0

1. Mineral mixture. $CaCO_3$: 600g; K_2HPO_4: 645g; $CaHPO_4.2\ H_20$: 150g; NaCl: 335g; $MgSO_4.\ 7H_20$: 204g; citrate Fe: 20g; $MnSO_4$: 10g; $ZnCl_2$: 1.5g; $CuSO_4$: 1.0g; KI: 0.2g; $CoC1_2$: 0.05g; $KAI\ (SO_4)_2$:0.1g; Na_2SeO_3 : 0.01g; NaF: 0.2g. 2. Water soluble vitamins: choline HCI: 10g; para-aminobenzoic acid: 5g; inositol: 1g; nicotinic acid: 0.5g; calcium pantothenate: 0.25g; riboflavine: 0.25g; thiamine HCI: 0.20g; pyridoxine HCI: 0.05g; folic acid: 0.05g; biotin: 0.01g, vitamin B12: 0.0005g; corn starch: q.s.p. 300g. 3. Fat soluble vitamins: retinol: 45,000mg; vitamin D2: 750mg; dl-alpha-tocopherol: 5,000mg; vitamin K: 5 mg; corn starch: q.s.p 100g.

Soon after weaning, three groups were fed with CONT diet and three others were offered EXP diet. Diets CONT and EXP were used throughout the investigation except for the last five days of the trial, when the CONT diet was replaced by Test 1 diet. Test 2 diet followed the use of the EXP diet for the respective group of mice.

Food and water were given ad libitum**:** after adaptation for a period of three days, subgroups of at least 10 mice on each diet were: a) infected with 40 or b) 80 cercariae of *S. mansoni,* and a third subgroup was kept free of infection. All the animals were weighed on a weekly basis.

Table 2 - Mortality rates in undernourished and well-fed mice, regarding Schistosoma mansoni *infection and cercarial burden.*

	Mice (nº)			
Experimental groups	at the beginning of the experiment	spontaneous mortality	sacrificed at the end of the experiment	mortality rates (%)
Prot 22.6% (non-infected)	28	2	26	7.1[a]
Prot 22.6% (40 cercariae)	54	19	35	35.2[b]
Prot 22.6% (80 cercariae)	40	17	23	42.5[b]
Prot 5.2% (non-infected)	33	3	30	9.1[a]
Prot 5.2% (40 cercariae)	24	9	15	37.5[b]
Prot 5.2% (80 cercariae)	42	19	23	45.2[b]
Total	221	69	152	31.2

a; b indicate significant differences ($p<0.05$) regarding values in the last column (Chi-square test)

Whole protein intestinal absorption (Phase P1): this assay was performed by calculating the Protein Absorption Coefficient, according to the following formula:

$$\text{Protein absorption coefficient} = \frac{\text{nitrogen intake - fecal nitrogen}}{\text{nitrogen intake}} \chi 100$$

Mice were kept in individual stainless steel metabolic cages and the underlying trays were covered with a sheet of filter paper to absorb the urine and minimize faecal contamination with urinary nitrogen. For three consecutive days, the food intake was determined for each mouse in each group and the feces were collected for nitrogen determination. The assay started soon after the 20th week of infection. All the nitrogen determinations (diets and feces) were made through the Kjedahl's micromethod[13]. Faecal markers were considered to be unnecessary under the conditions of the present investigation.

Protein hydrolysate intestinal absorption (Phase P2): after the first assay (Phase P1) was completed, the same mice were submitted to phase P2, receiving Test 1 or Test 2 diets, both containing hydrolysed casein in the same levels as whole casein in diets CONT and EXP, respectively. After two days of adaptation, the same schedule was used as in the first assay, in order to evaluate the intestinal absorption of this protein formula by the mice.

Calculations regarding faecal metabolic nitrogen were not taken into account, since previous investigation using the same experimental model had shown a high equivalence between apparent and true intestinal protein absorption[9]. Thus, for the objectives of this study, its influence was assumed to be negligible on the outcome results.

Ethics: All the procedures followed were in accordance with the FIOCRUZ's (Oswaldo Cruz Foundation – Brazil) guidance for the care and use of laboratory animals.

Statistical analysis: Differences between the groups were determined through analysis of variance and Duncan's test[17]. Student's "t" test for paired samples was also used to compare P1 and P2 phases of the intestinal protein absorption test. Differences in mortality rates were determined by Chi-square test. The probability level considered significant was $p < 0.05$.

RESULTS

Weight curves. The weight curves of mice fed both CONT and EXP diets are shown in Figure 1. It can be easily noticed that the highest values are seen in the groups fed 22.6% protein. Infection with *S. mansoni* did not seem to have a notable influence on the weight of mice except from the 13th week of the infection period onward, when infected sub-groups ingesting the low-protein diet EXP (5.2% protein) had an apparent better performance as compared with the non-infected ones ingesting the same diet ($p<0.05$).

Protein intestinal absorption. The biological assays for determination of protein intestinal absorption showed that among the control groups (well nourished mice) the protein absorption coefficients were practically the same, independent of the protein source (either hydrolysed or whole protein). However, in the undernourished groups of animals, a difference could be detected between the two chemical formulae of dietetic protein, mice showing a better performance when fed with hydrolysed casein, reaching values very close to those displayed by mice fed the control (22.6% protein) diet (Table 3).

Pathology. As can be observed in Figure 1, undernourished infected mice showed a higher weight gain as compared to undernourished non-infected ones from the 13th until the 19th week of infection, with a trend to decline in the last week of the experiment. At the moment of necropsy, enlargement of the liver and spleen as well as presence of ascites were common gross findings.

Infected well-fed mice had similar findings regarding gross pathology, but in infected undernourished animals ascites seemed to be more severe.

Concerning the aspect of the intestinal content, in a few animals, a slight degree of diarrhea was detected

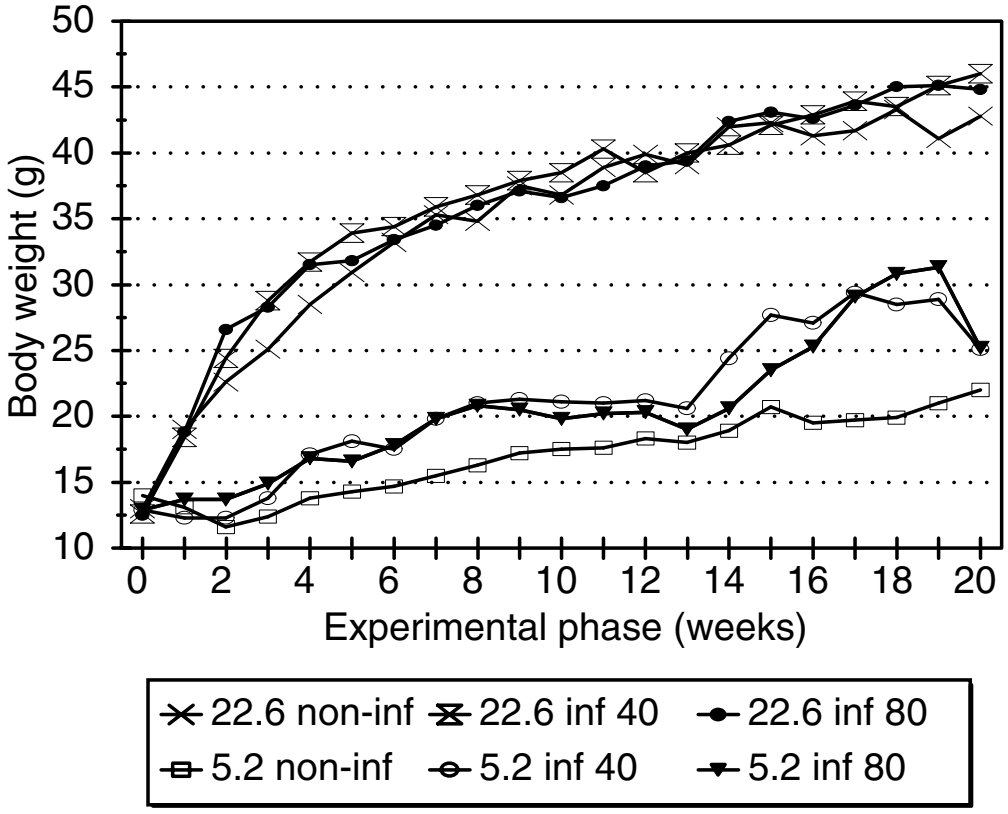

*Figure 1 - Weight curves of mice by type of diet and cercarial load (*S. mansoni*).*
Legend: 22.6 or 5.2: refers to control (22.6% protein) or experimental (5.2% protein) diets; non-inf = mice free of infection; 40 or 80 = experimental groups infected with 40 or 80 cercariae of S. mansoni, respectively.

Table 3 - Coefficient of protein intestinal absorption in mice fed either whole or hydrolysed casein diets, infected with different cercarial burdens.

		Whole protein				Hydrolysed protein				
Experimental groups	N	N ing	N exc	N abs	CPIA	N ing	N exc	N abs	CPIA	p-value
Prot 22.6% (non-infected)	26	0.7573	0.0674	0.6899	91.1 ± 3.56[(a)]	0.6750	0.0513	0.6237	92.4 ± 3.08[(a)]	NS
Prot 22.6% (40 cercariae)	35	0.9873	0.0701	0.9172	92.9 ± 4.96[(a)]	0.6506	0.0514	0.5992	92.1± 3.88[(a)]	NS
Prot 22.6% (80 cercariae)	23	0.6211	0.0590	0.5621	90.5 ± 2.84[(a)]	0.4354	0.0431	0.3923	90.1 ± 3.80[(a)]	NS
Prot 5.2% (non-infected)	30	0.0762	0.0163	0.0599	78.6 ± 8.54 [(b)]	0.1263	0.0120	0.1143	90.5 ± 11.13[(a)]	S
Prot 5.2% (40 cercariae)	15	0.1018	0.0172	0.0846	83.1 ± 6.09 [(b)]	0.1195	0.0153	0.1042	87.2 ± 3.94[(a)]	S
Prot 5.2% (80 cercariae)	23	0.0828	0.0188	0.0640	77.3 ± 10.6 [(b)]	0.0954	0.0166	0.0788	82.6 ± 5.03[(c)]	S

N = nitrogen; ing = ingested; exc = excreted; abs = absorbed.

a, b, c indicate significant differences ($p<0.05$) regarding values in each column for CPIA (Duncan's test).

p-value: (Student's "t" test for paired samples, regarding values for CPIA in the same line).

NS = non-significant; S = significant.

during the first month of the experiment, faeces of normal aspect having been observed afterward.

Mortality rates. Mortality rates did not differ significantly between undernourished and well-fed mice, deaths being mainly due to *S. mansoni* infection. The apparent differences on account of cercarial burdens in the groups of undernourished and well-fed mice were not statistically significant (Table 2).

DISCUSSION

The influence of the diet was quite evident, low-protein fed mice showing the lowest values regarding body weight curves. However, schistosomiasis infection did not seem to influence the animals' growth under the conditions of this experiment, except from the 13th week of infection onward, when infected subgroups in spite of ingesting the low-protein diet EXP (5.2% protein) showed higher weight gains than non-infected ones ingesting the same diet ($p < 0.05$). This was likely due to the development of ascites, portal hypertension and hepatosplenomegaly observed by us and several authors in the chronic stage of murine schistosomiasis[1 6]. In addition, the detection of low levels of serum albumin has been reported by several investigators[3] not only as a result of protein malnutrition but also due to some degree of hepatic derangement occurring during schistosome infection.

The biological assay performed in this investigation aimed to measure the amount of absorbed nitrogen in the intestine of undernourished and well-fed mice after ingesting two different protein formulae.

The use of faecal markers was considered to be unnecessary, because the animals ingested the same diets (CONT or EXP) all over the study except for the last five days of the experiment, including a two-day period for adaptation to diets Test 1 and Test 2, before data collection was initiated. The methodology used in this work had been previously described by Waterlow & Wills[26], Holemans & Lambrechts[12], among other investigators.

The data obtained in this investigation concerning protein absorption appear to conflict in some ways with previous results from our laboratory[4 9]. However, in past experiments, only the acute phase of murine schistosomiasis (nine weeks of infection) was studied; while the present investigation had been extended to the chronic phase of the disease (20 weeks of infection). In addition, in past investigations undernutrition was induced in mice by offering a human diet from endemic areas of Northeast Brazil (Regional Basic Diet - RBD), which is multideficient as well as having a low protein content[24]. In this study, a casein based diet was selected at levels of 5.2% (low-protein) and 22.6% (normal-protein). The undernutrition in the experimental model using the RBD was more severe than in the pure casein induced model, because the casein diets used have a higher biological value and also were supplemented with vitamins and minerals not utilized in the RBD induced model of murine undernutrition[5].

In previous studies[4 9], infection did not affect protein intestinal absorption in well nourished mice, but it aggravated malabsorption detected among undernourished animals. In the present investigation, however, the reduced absorption was related only to undernutrition. Mice in the RBD model absorbed about 40% of the ingested protein and in the casein model they absorbed around 80%.

When instead of dietary whole protein the animals started being fed a protein hydrolysate, undernourished infected animals exhibited absorption rates close to the control mice, except for those with very heavy schistosomal infections (80 cercariae), although they presented a significant improvement in their absorptive performance.

These results suggest that the reduced protein intestinal absorption detected in malnourished animals is probably related to decreased protein hydrolysis[15 25], since administration of hydrolysed protein was able to improve significantly the absorption rates of undernourished mice. Information is lacking regarding the quantity and composition of the pancreatic juice in undernourished infected mice. It is known that in murine schistosomiasis pancreatic damage is frequently seen and may be particularly severe in some cases[16].

The infective cercarial load seemed to interfere in some way with the absorption of protein hydrolysate, except in well-nourished hosts. Undernourished mice infected with the heaviest cercarial load (80 cercariae) improved considerably absorption while on hydrolysed casein although they did not reach the same absorption rates detected for well-nourished animals. However, undernourished mice either non-infected or infected with 40 cercariae attained values close to those of well-nourished infected controls.

In spite of the favourable results, data reported here were only based on short-term experiments in laboratory animals, further studies being necessary before the use of protein hydrolysates can be recommended as an auxiliary dietetic formula in the treatment of patients with schistosomiasis.

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