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SERUM AND TISSUE ENZYMES AND TRACE ELEMENTS IN HAMSTERS WITH SCHISTOSOMIASIS MANSONI AND/OR PROTEIN ENERGY MALNUTRITION*

By

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خمائر المصل والأنسجة والعناصر النادرة في الهامسترات المصابة بالبلهارسيا المعوية مع/أو سوء التغذية البروتيني

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و نادية مرقص

ينتشر مرض البلهارسيا في مجتمعات بشرية يعاني بعضها من مرض سوء التغذية البروتيني ، ومن هنا يتبّين أهمية تطوير نموذج حيواني تجريبي يمكن من خلاله دراسة الأثر المنفرد لكل من المرضين فضلا عن الأثر المشترك لكليهما معاً .

وتقدم الدراسة الحالية نموذجاً تجريبياً أمكن من خلاله دراسة التأثيرات المرضية للبلهارسيا المعوية التي تسببها الطفيليات من نوع **شستوسوما مانسوناي** ومرض سوء التغذية البروتيني من خلال تقدير التغيرات التي حدثت في عدد من الأنزيمات والعناصر المعدنية النادرة الموجودة في المصل وأنسجة الكبد والقلب والطحال والعضلات الهيكلية للقوارض التجريبية المعروفة باسم الهامسترات ، وقد أشارت النتائج التي تم التوصل إليها إلى أن التلف الناتج في الكبد عن سوء التغذية البروتيني يتزايد مع الاصابة بالبلهارسيا المعوية ، كما تمت مناقشة بعض الآليات المسئولة عن علاقة مرض البلهارسيا بالأنيميا وكذلك بعض العلاقات المتبادلة بين العناصر المعدنية النادرة في حالتي سوء التغذية البروتيني ومرض البلهارسيا المعوية في حالة وجود كل منهما بشكل منفرد أو وجودهما معاً .

Key Words: Hamsters, Serum and Tissue Enzymes and Trace Elements, Protein Energy Malnutrition and/or Schistosomiasis mansoni.

ABSTRACT

Schistosomiasis is mostly widespread in communities suffering from protein energy malnutrition. The development of an animal model for each condition is required for better understanding of changes in certain biochemical indices in pure schistosomiasis or pure energy malnutrition or both. Measurements of some enzymes as well as trace elements have been found to be valuable as diagnostic and prognostic indicators for tissue destruction. Accordingly, the present investigation reports measurements of some enzymes and trace elements in the sera and tissues of hamsters suffering from protein energy malnutrition and/or schistosomiasis. These biochemical parameters are considered indices for the involvement of the liver and other organs in protein energy malnutrition and/or schistosomiasis. The results obtained are discussed in relation to those of the other investigators. The most important finding is the severity of the damage produced by protein energy malnutrition on the liver compared with that of schistosomiasis mansoni. Moreover it appears that in golden hamsters, *Schistosoma mansoni* synergistically affects the liver damage produced by protein energy malnutrition.

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INTRODUCTION

Schistosomiasis is one of the most serious endemic diseases in several tropical and subtropical countries in Africa, South America and Asia, with about 200 million people suffering from the disease[1].

Many patients with severe schistosomiasis had since their birth diets deficient in proteins[2]. Also, infections with helminths may precipitate Kwashiorkor[3]. Accordingly, the development of an animal model for each condition would be a valuable prerequisite for the investigations needed to study the effects of either pure schistosomiasis or protein energy malnutrition.

Of the common laboratory rodents, mice and hamsters were found to be suitable experimental hosts for *Schistosoma mansoni,* one of the most important aetiological agents of schistosomiasis in Africa, South America and Western Asia[4,5]. Dewitt and Warren[6] reported that the lesions in the liver of mice infected with *Schistosoma mansoni* might not completely resemble those seen in man since mice were usually exposed to such large numbers of cercariae that they developed overwhelming infections that might be fatal before large amounts of fibrous tissues were formed. Later, it was demonstrated that mice, infected with as few as one pair of worms might develop significant hepatosplenic disease and could live almost indefinitely[7].

In the present study hamsters were used as experimental hosts for *Schistosoma mansoni.* They were mildy infected in order to produce an effect on the liver similar to that produced in human patients with hepatosplenic schistosomiasis[6,7,8].

Models of protein energy malnutrition have been also developed successfully in exprimental animals. In order to produce experimental Kwashiorkor where the caloric intake is much reduced, it was found that the animals have to be fed a diet in which the protein content is low enough to produce depletion without producing significant anorexia[9]. The morbid anatomy and histological changes in such animals were found to be similar to those occurring in Kwashiorkor in man [9,10]. Liver involvement has been reported in protein energy malnutrition[11,12]. Some other organs have been also reported to show different degrees of tissue destruction. These include gastrointestinal tract, some endocrine organs and kidneys, as well as the cardiovascular system[3, 11].

Schistosomiasis mansoni causes hepatic granulomatous inflammations in the liver and subsequently fibrosis[8, 13, 14,15,16,17]. Pulmonary granulomas[18], exocrine pancreatic function impairment [19]; skeletal muscle and tongue myodegradation and necrosis[20], kidney as well as spleen involvement[21] have been also reported. Measurement of some plasma enzyme has been found to be valuable diagnostic and prognostic indicators for tissue destruction[17, 22, 24, 25, 26, 27, 28, 29]. Aminotransferases, ceruloplasmin, cholinesterase and alkaline phosphatase are among the principal serum enzymes that proved to be useful in this respect[24, 25, 26, 27, 29].

Measurements of some trace elements have been also found to be important in this respect. This includes serum copper and iron levels that are found to be altered in some cases of liver involvement [26]. Cobalt absorption has been found to be also affected together with that of iron in patients with liver disease. Magnesium absorption has been also found to increase in cases of iron defeciency. Similalrly, copper can interfere with zinc absorption[30].

In the present investigation the levels of some enzymes and trace elements both in the serum and in the tissues of some organs have been taken as biochemical indices for liver as well as other organs involvement in schistosomiasis and/or protein energy malnutrition.

MATERIAL AND METHODS

Four groups, each of thirty, six weeks old golden hamsters (fifteen males and fifteen females in each group) were used. The hamsters in two of the four groups were each infected with 75 cercariae of an Egyptian strain of *Schistosoma mansoni* according to the methods described elsewhere [31]. One group of these infected hamsters was kept on a complete diet while the animals in the second group were maintained on a low protein, high carbohydrate diet. One group of the uninfected hamsters was given a complete diet while the other group was offered the low protein, high carbohydrate diet. The composition of the complete synthetic and that of the low protein high carbohydrate diets were similar to that used by Al-Nagdy *et al.* [32]. All the hamsters in each group were kept on the corresponding diet for ten weeks before they were sacrificed and investigated.

Schistosomes were collected from the mesenteric and the hepatic portal veins of hamsters infected with *S. mansoni* according to the method described by Saoud[31]. Schistosome counts did not vary significantly between the groups of infected hamsters investigated; these counts ranged 26 - 41 (mean: 29.7 ± 1.21).

Some enzymes and trace elements were estimated in the serum, liver, spleen, kidney, heart and muscular tissues. The enzymes included Aspartate amino transferase (AST)[33, 34] and Alanine aminotransferase (ALT) [33, 34], Cholinesterase (C.E)[35], Alkaline phosphatase (A.P)[36] and ceruloplasmin[37]. Copper, zinc, iron, magnesium and manganese were estimated using EEL atomic absorption spectroscopy[38]. The statistical analyses of the results were done using the Student's t-test[39].

RESULTS AND DISCUSSION

The results of the present investigation include two important findings. First, protein energy malnutrition (PEM) seems to be more destructive on the liver than schistosomiasis. Secondly, schistosomiasis increases the severity of liver damage caused by PEM. This is evident from the results obtained on growth, liver and tissues enzymes and/or trace element measurements in the sera or tissues of PEM and/or schistosomal hamsters.

Regarding the growth of hamsters, it was found that the retardation of growth was much more pronounced in the PEM hamsters than in the schistosomal animals; the two conditions together were found to be even more effective on the retardation of growth (Table 1). Raiczyk and Hall[25] reported similar findings in mice infected with *Schistosomatium douthitti.* Kirsch *et al.*[9] noted that in experimental models of protein energy malnutrition, stunted growth was always found to be among the most important characteristics of the condition. Raiczyk and Hall[25] studied the pathophysiologic changes that occurred in mice experimentally infected with *Schistosomatium douthitti.* They observed that body weights were lower and liver weights were higher, resulting in significantly increased liver weight/body weight ratios.

Table 1

Weight gain or loss of hamsters in the different groups

Experimental Groups	Mean weight gained or loss in hamsters (Gm+)
Control Hamsters	+40.45
Protein Energy Malnourished Hamsters	+4.95
Schistosomal Hamsters	+19.25
Schistosomal Protein Energy Malnourished Hamsters	-16.85

Increases in serum levels of both AST and ALT are commonly observed in liver disease. Elevated serum AST and ALT levels indicate liver cell damage[26]. Raiczyk and Hall[25] reported that such an effect resulted from schistosomiasis in mice. The presently studied protein energy malnourished and the schistosomal hamsters exihibited significantly higher AST and ALT in their sera (Table 2), indicating liver cell damage.

In the present study it was found that PEM alone did not cause significant changes in the level of AST and ALT in the liver tissues of hamsters. However, when PEM was associated with *Schistosoma mansoni* infection, AST and ALT activities decreased significantly in the liver tissues (Table 3), indicating impairment in the ability of the liver to synthesize enough enzymes[40]. This result may indicate that schistosomiasis increases the severity of liver damage due to PEM.

Davidson *et al.*[3] stated that it was probable that an infection like schistosomiasis was more likely to lead to progressive liver disease if it acted in association with a damaged liver by chronic malnutrition. Coutinho *et al.* [41] even thought that in mice the effects of malnutrition, *per se,* were sometimes more detrimental to the host than those due to schistosomiasis.

Raiczyk and Hall[25] reported decreased ALT activities in the liver tissues of schistosomal mice associated with the increased serum activities. In the present investigation, PEM and/or schistosomiasis affect the levels of AST and/or ALT activities in organs other than those of the liver, indicating that the origin of the increased serum enzyme activities is from hepatic as well as extrahepatic origin (Tables 4,5,6,7). Most tissues including heart, skeletal muscles, kidney, lung, spleen and pancreas are rich in either one or both of these two enzymes[41].

PEM lowered significantly the serum levels of cholinesterase. Varley *et al.*[43] noted low serum cholinesterase values in malnutrition. They also noted that the serum activity of cholinesterase could be used, though to a rather limited extent as an indicator of liver disease, since the enzyme would be formed by the liver and thus would be reduced in liver cell damage. Presently, hepatic C.E. levels as well as those of heart, kidney and spleen tissues are found to be significantly lowered due to PEM in hamsters (Tables 2-7). Schistosomiasis decreased serum C.E. activity. This decreased serum activity was associated with decreased levels in hepatic and kidney tissues but not in the tissues of muscles, spleen and heart (Tables 2-7). This means that PEM causes depletion of the level of the enzyme in almost all organs investigated whereas schistosomiasis causes depression of the enzyme level in some organs while in others it is not affected. This indicates that the effect of PEM on C.E. activity is more pronounced than in schistosomiasis alone. It was found that in schistosomiasis, C.E. was the enzyme which was affected earlier than any other enzyme, where it was found to be reduced in mild, moderate and severe schistosomiasis [44, 45, 46, 47].

AP increased in the sera and hepatic tissues of PEM hamsters but decreased in muscular tissues (Tables 2-4). In schistosomiasis, AP increased in the serum together with that of liver tissues. Increased serum A.P. could be explained as being due to impairment of the liver as it increased in diseases of the liver and biliary tract[43]. Raiczyk and Hall[25] and Aboul Atta *et al.*[48] reported increased hepatic AP in schistosomal mice while Chernin *et al.*[24] reported increased serum AP levels in schistosomal mice. In the present study, schistosomiasis either alone or associated with PEM did not affect AP activity in the kidney, heart and spleen tissues (Tables 5-7). Regarding the muscular tissues, schistosomiasis alone did not affect AP but together with PEM it lowered its level as in PEM with no infection. This indicates that this effect is due to the failure of the enzyme protein synthesis due to protein deficiency rather than being due to the infection. The present results of hepatic ceruloplasmin activities also supports this assumption; schistosomiasis does not significantly affect hepatic ceruloplasmin whereas PEM together with schistosomiasis lower it significantly (Table 3). Failure of hepatic synthesis of ceruloplasmin has been reported in some cases of cirrhosis of the liver[43].

The association between schistosomiasis and anaemia has long been emphasized by various investigators[49]. Rodrigues and Galle [50] related the inflammatory processes that developed during the advanced stages of hepatic schistosomiasis to accumulation of siderosomes, capacity of the ferrous/ferric ions to unleash the formation of free radicals, peroxidation of membrane lipids and reduction of stability of the membranes of several components of the hepatolysosomal compartment. In the present work, serum iron content has been found to be significantly low in schistosomal as well as PEM hamsters. This effect is found, however, to be less pronounced in PEM than in schistosomiasis. The decrease in serum iron is even found to be

Table 2

Serum AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg and Ceruloplasmin Levels of Hamsters in the Different Groups
(mean ± S.E.)

GROUPS OF HAMSTERS	AST (R&F units/ml)	ALT (R&F units/ml)	C.E. I.U/ml	A.P. K&A units/ 100 ml	Cu ug%	Zn mg%	Fe ug%	Mg mg%	Ceruloplasmin mg/ml
Controls	35.54±2.91	27.40±7.99	1.09±0.03	3.22±0.48	96.6±0.3	2.82±0.06	104.4±1.93	2.8±0.04	16.37±1.29
PEM v.s. Controls, P. value	64.68±6.14 <0.001	106.7±1.75 <0.001	0.68±0.1 <0.01	5.118±0.25 <0.01	93.9±1.67 N.S.	3.07±0.13 N.S.	63.0±20.3 <0.05	2.4±0.11 <0.01	14.96±0.93 N.S.
Schistosomiasis v.s. Controls, P. value	104.8±12.17 <0.001	81.11±7.58 <0.001	0.81±0.06 <0.05	4.73±0.18 <0.01	42.0±3.74 <0.001	3.82±0.07 <0.001	47.68±3.79 <0.001	3.7±0.04 <0.001	15.18±2.66 N.S.
PEM ± Schistosomiasis v.s. Controls, P. value	64.69±6.14 <0.001	64.19±5.59 <0.01	0.68±0.1 <0.02	5.178±0.23 <0.002	80.0±2.74 <0.001	1.08±0.07 <0.001	100.0±2.74 N.S.	2.81±0.05 N.S.	16.70±0.97 N.S.
PEM v.s. Schistosomiasis, P. value	<0.02	<0.01	N.S.	N.S.	<0.001	<0.001	N.S.	<0.001	N.S.
PEM v.s. PEM + Schistoso-miasis, P. value	N.S.	<0.001	N.S.	N.S.	<0.002	<0.001	N.S.	<0.01	N.S.
Schistosomiasis v.s. PEM + Schistosomiasis, P. value	<0.001	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	<0.001	N.S.

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

Table 3

AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg, Mn and Ceruloplasmin Levels in the Liver Tissues of the Hamsters in the Different Groups (mean ± S.E.)

GROUPS OF HAMSTERS	AST R&F units/mg wet tissue	ALT R&F units/mg wet tissue	C.E. I.U/g wet tissue	A.P. K&A units/g wet tissue	ug/g tissue (wet weight)					mg/g wet tissue
					Cu	Zn	Fe	Mg	Mn	Ceruloplasmin
Controls	149.7±14.8	40.32±6.6	14.59±1.12	0.63±0.12	13.48±0.45	23.93±1.4	118.2±5.34	282.7±15.3	1.78±0.21	105.5±13.6
PEM v.s. Controls, P. value	188.6±22.3 N.S.	39.40±13.0 N.S.	5.8±1.05 <0.001	6.14±1.58 <0.01	5.79±0.44 <0.001	9.91±0.93 <0.001	54.58±2.06 <0.001	77.42±2.05 <0.001	1.30±0.02 N.S.	87.68±12.32 N.S.
Schistosomiasis v.s. Controls, P. value	147.8±6.7 N.S.	18.14±2.9 <0.01	4.5±0.28 <0.001	2.97±0.88 <0.02	5.84±0.43 <0.001	8.52±1.25 <0.001	36.9±1.92 <0.001	58.7±8.19 <0.001	1.67±0.06 N.S.	130.18±15.43 N.S.
PEM + Schistosomiasis, v.s. Controls, P. value	116.8±12.3 <0.05	24.50±3.5 <0.005	10.34±1.03 <0.001	3.63±1.13 <0.002	10.74±0.83 <0.02	9.04±0.29 <0.001	95.28±5.04 <0.02	181.06±41.4 <0.05	0.53±0.05 <0.001	60.95±7.47 <0.05
PEM v.s.Schistosomiasis, P. value	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.	<0.001	N.S.	N.S.	<0.05
PEM v.s. PEM + Shistosomiasis, P. value	<0.05	N.S.	<0.01	N.S.	<0.001	N.S.	<0.001	<0.001	N.S.	N.S.
Schistosomiasis v.s. PEM + Schistosomiasis, P. value	<0.05	N.S.	<0.001	N.S.	<0.001	N.S.	<0.001	<0.001	<0.001	<0.001

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

Table 4

AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg, Mn and Ceruloplasmin Levels in the Muscle Tissues of the Hamsters in the Different Groups (mean ± S.E.)

GROUPS OF HAMSTERS	AST R&F units/mg wet tissue	ALT R&F units/mg wet tissue	C.E. I.U/g wet tissue	A.P. K&A units/g wet tissue	ug/g tissue (wet weight)					mg/g wet tissue
					Cu	Zn	Fe	Mg	Mn	Ceruloplasmin
Controls	121.1±8.85	6.24±0.51	9.123±0.97	0.702±0.27	1.05±0.04	14.64±0.45	56.54±1.47	202.54±34.12	0.97±0.09	77.23±3.87
PEM v.s. Controls, P. value	39.95±0.09 N.S.	4.120±1.151 N.S.	6.960±1.21 <0.001	0.016±0.008 <0.01	1.78±0.086 <0.001	16.57±1.06 N.S.	57.54±3.59 N.S.	232.6±31.53 N.S.	0.56±0.12 <0.05	27.67±1.8 <0.001
Schistosomiasis v.s. Controls, P. value	86.89±1.01 <0.02	6.06±0.19 N.S.	6.656±1.095 N.S.	0.35±0.07 N.S.	1.01±0.02 N.S.	15.23±0.46 N.S.	56.32±1.44 N.S.	190.34±32.73 N.S.	0.97±0.11 N.S.	74.46±8.3 N.S.
PEM + Schistosomiasis v.s. Controls, P. value	46.43±1.72 <0.001	3.55±0.17 <0.001	7.480±0.779 N.S.	0.055±0.007 <0.05	1.84±0.24 <0.02	16.78±1.26 N.S.	68.46±12.04 N.S.	267.3±11.8 N.S.	0.88±0.03 N.S.	26.64±1.27 <0.001
PEM v.s. Schistosomiasis, P. value	<0.002	N.S.	N.S.	<0.001	<0.001	N.S.	N.S.	N.S.	<0.05	<0.001
PEM v.s. PEM + Schistosomiasis, P. value	N.S.	N.S.	N.S.	<0.002	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Schistosomiasis, v.s. PEM + Schistosomiasis, P. value	N.S.	<0.001	N.S.	<0.001	<0.01	N.S.	N.S.	N.S.	N.S.	<0.001

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

Table 5

AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg, Mn and Ceruloplasmin Levels in the Heart Tissues of the Hamsters in the Different Groups (mean ± S.E.)

GROUPS OF HAMSTERS	AST R&F units/mg wet tissue	ALT R&F units/mg wet tissue	C.E. I.U/g wet tissue	A.P. K&A units/g wet tissue	ug/g tissue (wet weight)					mg/g wet tissue
					Cu	Zn	Fe	Mg	Mn	Ceruloplasmin
Controls	177.19±21.6	12.33±1.36	18.93±1.57	0.012±0.004	1.16±0.35	7.93±0.12	51.0±0.71	176.12±2.33	1.07±0.03	92.01±11.05
PEM	28.3±3.7	6.65±0.48	12.49±1.17	0.015±0.004	0.77±0.032	2.52±0.43	47.56±13.16	187.8±2.57	0.62± 0.054	71.79±13.81
v.s. Controls, P. value	<0.001	<0.001	<0.002	N.S.	N.S.	<0.001	N.S.	<0.01	<0.001	N.S.
Schistosomiasis, v.s. Controls, P. value	154.7±16.2 N.S.	7.85±0.71 <0.01	18.98±1.62 N.S.	0.016±0.005 N.S.	1.38±0.086 N.S.	3.07±0.55 <0.001	65.15±2.02 N.S.	190.22±3.26 0.01	0.73±0.08 <0.01	118.39±18.89 N.S.
PEM + Schistosomiasis v.s. Controls, P. value	42.26±6.2 <0.001	4.32±0.99 <0.001	16.24±1.034 N.S.	0.019±0.006 N.S.	1.52±0.16 N.S.	5.56±0.4 <0.001	12.16±0.45 <0.001	191.67±4.54 <0.01	0.26±0.012 <0.001	91.66±18.73 N.S.
PEM v.s. Schistosomiasis, P. value	<0.001	N.S.	<0.01	N.S.	<0.001	N.S.	N.S.	N.S.	N.S.	N.S.
PEM v.s. PEM + Schistosomiasis, P. value	<0.01	<0.05	<0.05	N.S.	<0.002	<0.001	P<0.05	N.S.	<0.001	N.S.
Schistosomiasis v.s. PEM + Schistosomiasis, P. value	<0.001	<0.01	N.S.	N.S.	N.S.	<0.01	<0.001	N.S.	<0.001	N.S.

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

Table 6

AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg, Mn and Ceruloplasmin Levels in the Kidney Tissues of the Hamsters in the Different Groups (mean ± S.E.)

GROUPS OF HAMSTERS	AST R&F units/mg wet tissue	ALT R&F units/mg wet tissue	C.E. I.U/g wet tissue	A.P. K&A units/g wet tissue	ug/g tissue (wet weight)					mg/g wet tissue
					Cu	Zn	Fe	Mg	Mn	Ceruloplasmin
Controls	51.6±10.05	7.96±0.88	33.26±7.77	0.04±0.015	6.22±0.58	16.0±0.35	37.94±2.19	158.58±0.54	1.77±0.06	188.07±29.59
PEM v.s. Controls, P. value	37.9±4.8 N.S.	7.13±0.61 N.S.	7.08±0.60 <0.002	0.023±0.009 N.S.	15.65±0.83 <0.001	31.29±1.61 <0.001	42.3±10.23 N.S.	345.73±20.7 <0.001	2.13±0.72 N.S.	86.33±15.99 <0.01
Schistosomiasis v.s. Controls, P. value	16.75±0.5 <0.01	4.30±0.48 <0.01	16.11±2.5 <0.05	0.0076±0.001 N.S.	10.47±0.32 <0.001	15.73±2.69 N.S.	56.80±3.45 <0.002	323.5±23.5 <0.02	2.33±0.1 <0.001	109.48±7.99 <0.05
PEM + Schistosomiasis, v.s. Controls, P. value	8.0±0.08 <0.001	3.67±1.31 <0.02	9.27±0.49 <0.01	0.0122±0.004 N.S.	9.93±0.08 <0.001	23.25±4.13 N.S.	83.5±0.92 <0.001	618.54±8.37 <0.001	0.871±0.22 <0.01	60.96±13.02 <0.01
PEM v.s. Schistosomiasis, P. value	<0.05	<0.001	<0.01	N.S.	<0.001	<0.001	N.S.	<0.01	N.S.	N.S.
PEM v.s. PEM + Schistosomiasis, P. value	<0.001	<0.05	<0.02	N.S.	0.001	N.S.	<0.01	<0.001	N.S.	N.S.
Schistosomiasis v.s. PEM + Schistosomiasis, P. value	N.S.	N.S.	<0.02	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	<0.01

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

Table 7

AST, ALT, C.E., A.P., Cu, Zn, Fe, Mg, Mn and Ceruloplasmin Levels in the Spleen Tissues of the Hamsters in the Different Groups (mean ± S.E.)

GROUPS OF HAMSTERS	AST R&F units/mg wet tissue	ALT R&F units/mg	C.E. I.U/g wet tissue wet tissue	A.P. K&A units/g wet tissue	ug/g tissue (wet weight)					mg/g wet tissue
					Cu	Zn	Fe	Mg	Mn	Ceruloplasmin
Controls	10.25±0.14	1.05±0.56	16.18±0.059	0.114±0.059	2.82±0.54	11.08±1.94	209.57± 9.31	144.45±20.7	0.258± 0.02	104.53±17.03
PEM v.s. Controls, P. value	6.675±1.4 N.S.	1.305±0.23 N.S.	8.323±1.39 <0.05	0.0516±0.017 N.S.	2.84±0.56 N.S.	13.77±1.82 N.S.	497.35± <0.001	384.11± <0.001	0.024± <0.001	101.84±21.63 N.S.
Schistosomiasis v.s. Controls, P. value	4.42±0.9 <0.01	0.55±0.03 N.S.	16.66±4.39 N.S.	0.014±0.0007 N.S.	2.32±0.07 N.S.	14.13±2.3 N.S.	276.6±29.7 <0.05	192.87± <0.05	0.311± N.S.	111.4±17.22 N.S.
PEM + schistosomiasis v.s. Controls, P. value	5.5±0.7 <0.01	0.488±0.19 N.S.	11.44±2.94 N.S.	0.0068±0.003 N.S.	7.63±0.73 <0.001	5.89±0.67 <0.05	374.28± <0.02	391.9±53.2 <0.002	0.026± <0.001	76.26±21.3 N.S.
PEM v.s. Schistosomiasis, P. value	N.S.	<0.01	N.S.	<0.05	N.S.	N.S.	<0.001	<0.001	<0.02	N.S.
PEM v.s. PEM + Schistosomiasis, P. value	N.S.	<0.02	N.S.	<0.05	<0.001	<0.01	<0.05	N.S.	N.S.	N.S.
Schistosomiasis v.s. PEM + Schistosomiasis, P. value	N.S.	N.S.	N.S.	N.S.	<0.001	<0.01	N.S.	<0.01	<0.02	N.S.

R & F = Reitman and Frankel units, K & A = King and Armstrong units, N.S. = Not significant at the 5% level,
PEM = Protein energy malnutrition.

insignificant when schistosomiasis was associated with PEM (Table 2).

Protein deficient diets lowered egg production of schistosomes[2, 51, 52] which were found to be a major factor in the production of anaemia[49]. Accordingly, protein deficiency would be thus expected to protect the host to some extent against the development of anaemia. In support of this assumption are the present findings regarding serum copper when PEM does not change it significantly whereas schistosomiasis with or without PEM significantly decrease it.

The decreased serum iron in the infected hamsters is found to be associated with significant reduction in hepatic iron stores (Table 3) with no effect on heart and muscle stores (Table 4,5). Again, the hepatic iron content in the infected PEM animals has been found to be higher than in case of schistosomiasis alone, thus providing further confirmation to the hypothesis that protein deficiency protects the animal against production of anaemia. Animals with schistosomiasis either alone or with PEM have increased spleen iron content (Table 7) which could be due to the increased destruction of the anaemic red blood cells in the spleen[53, 54]. Increased iron content of the kidney tissues in hamsters with schistosomiasis and schistosomiasis together with PEM is also observed (Table 6). This could be due to precipitation of the iron in the kidney tissues during its increased excretion following increased destruction of the anaemic red blood cells. PEM in hamsters has also lowered serum iron that is associated with depleted hepatic iron stores and increased levels in spleen; a picture similar to that observed in schistosomiasis (Tables 2,3,7).

The results of the serum zinc levels obtained presently support the fact that PEM aggrevates the effects of schistosomiasis since it decreases only in hamsters suffering from schistosomiasis and PEM together (Table 2). Varley *et al.*[43] indicated that serum zinc decreased in liver cirrhosis. However, hepatic and cardiac zinc stores decreased significantly in the hamsters suffering from PEM and schistosomiasis either occurring separately or together (Tables 3 and 5). Some of the effects observed presently regarding serum and tissue zinc contents might be due to the known association between copper and zinc absorption. Copper can interfere with zinc absorption by competing for binding sites of the albumin molecules in the intravascular space[30].

The increased serum magnesium levels in the presence of decreased hepatic values observed presently in hamsters infected with schistosomiasis could be probably due to renal disorders which mask the state of hypomagnesaemia resulting from schistosomal hepatic effect. The decreased renal clearance of magnesium seems to cause accumulation of the metal in body tissues. In the present study, infected hamsters together with PEM and PEM hamsters had significantly higher Mg levels than normal in the tissues of the heart, kidney and spleen. Increases in serum Mg can occur in both chronic and acute renal diseases especially if there is oliguria although it may be low in advanced renal disease[33]. In patients with renal failure, hypermagnesemia can be an important medical problem[30] and kidney lesions have been reported to occur in schistosomiasis[21,55] and in PEM[11].

In some types of liver disorders, the depressed hepatic zinc content was found to be associated with decreased hepatic manganese values. Butt *et al.*[56] reported decreased hepatic manganese content in patients with hepatic necrosis. Boxter and Smith[57] noted significant decreases in manganese uptake in the acutely damaged livers.

Barak *et al.*[58] found that a choline -deficient diet produced deficiency in liver manganese, an enzyme activator related mostly to hepatic function. Presently, schistosomiasis aggrevated by PEM in hamsters lowered significantly hepatic manganese content, a condition which did not occur in PEM or schistosomal hamsters (Table 4). Again this is another indicator that PEM aggrevates the effects on the liver tissues due to schistosomiasis.

One type of fatty liver that has been studied extensively in rats is due to a deficiency of choline which has been therefore called a lipotropic factor. A deficiency of Vitamin E enhances the hepatic necrosis of the choline deficiency type of fatty liver[42]. Yamini *et al.* [20] observed low hepatic Vitamin E values in a Brazilian tapir (*Tapirus terrestris*) in Michigan (USA) suffering from granulomatous hapatitis associated with eggs of schistosomes. Protein deficiency can also cause this type of fatty infiltration of the liver[26].

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