[Jpn. J. Parasitol., Vol. 36, No. 1, 1–8, February, 1987]

Effects of Experimental Infection of Rats with *Giardia lamblia* on the Activities of Pancreatic and Brush Border Enzymes and on *in vitro* Absorption from the Intestines

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(Received for publication; September 6, 1984)

Abstract

Thirty albino (Wistar) rats were infected by oral administration of *Giardia lamblia* cysts (3×10^8 ml^{-1} day^{-1}), concentrated from the stools of human patients with giardiasis. Ten of these rats were administered Furazolidone (50 mg day^{-1} rat^{-1}) orally for 5 consecutive days. Twenty more rats were kept as uninfected and untreated controls. *In vitro* absorption studies, using loops of distal jejunum, revealed that the absorption of U-^{14}C-glucose was not affected, whereas that of U-^{14}C-fructose and α-D-mannose was increased, during infection. The absorption of ^{14}C-labelled individual amino acids and natural mixtures of amino acids was decreased. The estimation of activities of secreted pancreatic and intestinal brush border enzymes revealed that *Giardia* infection decreased the activities of amylase, phospholipase, trypsin, chymotrypsin, caboxypeptidase, sucrase, maltase, alkaline phosphatase and Na^+, K^+-ATPase. Furazolidone treatment reversed these effects on amylase, sucrase, maltase and alkaline phosphatase. The activities of other enzymes were not determined after treatment, except for carboxypeptidase and Na^+, K^+-ATPase, which were unaffected and decreased further, respectively. The histopathological examination of various parts of the small intestines, spleen, kidneys, lungs, liver and heart did not show adverse changes but the possibility of ultrastructural changes is not ruled out.

Key words: Experimental giardiasis, absorption of nutrients, brush border enzymes

Asymptomatic giardiasis occurs more frequently than the symptomatic one (Brodsky *et al.*, 1974), particularly in malinourished and immune suppressed children. Although symptoms related to dysfunction of intestine (Saha and Ghosh, 1977) and pancreas (Chawla *et al.*, 1975) have been reported, yet little is known about the biochemical, histopathological and functional alterations in the intestines after giardiasis. Therefore, in the present investigation, the effects of experimental infection of rats with *Giardia lamblia* (pre- and post-chemotherapy) on the activities of pancreatic and brush border enzymes and on *in vitro* absorption from the intestinal loops have been carried out only with single amino acids, whereas, in the present study, the absorption of balanced mixture of amino acids has also been studied.

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This work forms a part of the Ph. D. (Biochemistry) thesis of Miss R. Sood, submitted to the Punjab Agricultural University, Ludhiana in 1983.

Materials and Methods

Infection and treatment of rats: The rats used during this study were procured from the Small Animal Colony of Christian Medical College, Ludhiana, and kept in individual cages. The commercial diet (Hindustan Lever, Bombay) and water were offered *ad lib.* The rats were screened for a week, during the pre-experimental period, by examination of stools (Faust *et al.*, 1938) and few of them by post-mortem. The rats observed to be free from parasitic infestation were then used

for the experimentation.

The cysts of *G. lamblia* were concentrated from the stools of human patients by the method of Sehgal *et al.* (1976) using 33 per cent $ZnSO_4$ and suspended in normal saline to get 3×10^8 cysts ml^{-1} saline. Thirty albino (Wistar) weanling rats were each given 1.0–1.5 ml of the suspension of cysts described above, orally for 6 consecutive days. From three days after discontinuation of the administration of the inoculum, the faeces from each rat were screened by the method of Faust *et al.* (1938) for the presence of *G. lamblia* cysts. By this method, the infection was established in all rats within one week of discontinuation of administration of *G. lamblia* cysts. However, the pattern of excretion of cysts was not recorded as our main aim was to establish the infection. Immediately after this, ten of the infected rats were given each 50 mg Furazolidone (3-(5-nitrofurfurylideneamino) oxazolidin-2-one) day^{-1} orally for 5 consecutive days. The faeces were free from cysts of *G. lamblia* 15 days after discontinuation of the medication and it was at this stage, i.e., 33 days after the first inoculum for inducing infection, that the rats were sacrificed and the activities of digestive enzymes as well as histopathological studies were carreid out. Twenty more noninfected rats served as controls. The infected rats were sacrificed 15–20 days after the first inoculation.

Collection and analysis of tissues: The heart, liver, lungs, kidneys, spleen, duodenum, proximal and distal jejunum were collected. These tissues were examined for histopathological changes (Luna, 1968). About 20 cm proximal jejunum was removed immediately after dissection of the sacrificed rats. The contents were gently pressed out into cold 0.1 M phosphate buffer (pH 7.5) for the assay of most of the enzymes and into 0.1 M tris-HCl buffer (pH 7.6) for the determination of trypsin and chymotrypsin. These intestinal contents were homogenized, centrifuged at 2000 rpm (0°C) for 10 min and the supernates were examined for pancreatic enzymes, viz., amylase (Dahlqvist, 1962), lipase (pH 8.0) and phospholipase (Mahadevan *et al.*, 1969, Lowry and Tinsley, 1976), trypsin (Walsh, 1970), chymotrypsin (Walsh and Wilcox, 1970) and carboxypeptidase (Appel, 1974). The empty intestines were then cut open, gently flushed with 0.1 M tris-HCl buffer (pH 7.6) and blotted. The mucosae were scrapped, with the help of a blunt knife, into the tris-HCl buffer containing 5 mM cysteine-HCl. These scrappings were homogenized, centrifuged at 2000 rpm (0°C) for 10 min and the supernates were used for the estimation of sucrase and maltase (Dahlqvist, 1968), alkaline phosphatase (King and Delory, 1939) and Na^+, K^+-ATPase (Kaplay, 1978). The protein, inorganic phosphates and mannose were estimated by the methods of Lowry *et al.* (1951), Ames (1966) and Dubois *et al.* (1956), respectively.

In vitro absorption of nutrients: 15 cm of distal jejunum was dissected, washed free of intestinal contents with ice cold Kreb's Ringer Phosphate buffer (pH 7.4). The method of Akedo *et al.* (1960) was used to determine the *in vitro* absorption of nutrients from these loops of distal jejunum collected from infected and control rats. To study absorption of hexoses, 40 μCi of U-^{14}C-fructose (Sp. Act. 107 mCi $mmole^{-1}$) or U-^{14}C-glucose (Sp. Act. 114 mCi $mmole^{-1}$) were diluted to 10 ml with Krebs Ringer Phosphate buffer containing 0.2 mg ml^{-1} fructose and/or 0.2% glucose, respectively. For absorption of mannose, 0.2 mg ml^{-1} cold mannose alone was used. To study the absorption of amino acids 70 μCi U-^{14}C-L-glutamic acid (Sp. Act. 150 mCi $mmole^{-1}$), U-^{14}C-L-leucine (Sp. Act. 240 mCi $mmole^{-1}$), U-^{14}C-lysine (Sp. Act. 126 mCi $mmole^{-1}$), U-^{14}C-proline (Sp. Act. 175 mCi $mmole^{-1}$) or 200 μCi U-^{14}C-L-glycine (Sp. Act. 80 mCi $mmole^{-1}$) were diluted to 5 ml with Kreb's Ringer Phosphate buffer, containing 2 mg ml^{-1} cold glucose and 0.2 mg ml^{-1} of respective cold amino acid. The absorption of natural mixture of ^{14}C-amino acids (100 μCi U-^{14}C-chlorella protein hydrolysate, Sp. Act. 40 mCi atom $carbon^{-1}$) was also studied similarly, but the concentration of mixture of cold amino acids added was 1 mg ml^{-1}. All the ^{14}C-com-

pounds were procured from BARC, Trombay (India). These preparations were filled into the uninverted loops of distal jejunum and incubated at 37°C in Kreb's Ringer Phosphate buffer (pH 7.4) and 1 ml samples from the serosal side were drawn at 30, 60, 90 and 120 min of incubation and put into the vial containing 10 ml scintillation fluid (Bray, 1960) consisting of Naphthalene, 60 g; PPO, 4 g; POPOP, 200 mg; CH_3OH, 100 ml; Ethylene glycol, 20 ml; and p-Dioxane to 1000 ml. The radioactivity was measured using Liquid Scintillation Spectrometer (Tricarb Model 3330; Packard Instruments Co. Inc., Downers Grove,

Table 1 Activities of pancreatic enzymes (Mean ± SD) in small intestinal contents of control, *Giardia* infected and Furazolidone treated rats

Enzyme activity	Control	Infected	Furazolidone treated
Amylase (μg maltose released min^{-1} mg^{-1} protein)	389.9±117.3 (209.8–525.5)	308.6±79.0 (148.8–380.0)	*437.7±116.1 (284.6–773.3)
Lipase (μ mole FFA released hr^{-1} mg^{-1} protein)	*4.93±2.3	5.13±1.1	ND
Phospholipase (μ mole FFA released 90 min^{-1} mg^{-1} protein)	*4.35±1.8	3.0±1.0	ND
Trypsin (Δ E min^{-1} mg^{-1} protein)	0.46±0.21 (0.20–0.78)	0.37±0.18 (0.13–0.62)	ND
Chymotrypsin (Δ E min^{-1} mg^{-1} protein)	0.47±0.39 (0.19–1.25)	0.38±0.18 (0.11–0.59)	ND
Carboxypeptidase (μg phenylalanine released 30 min^{-1} mg^{-1} protein)	1065.7±1017.8 (232.8–3310.8)	806.7±411.1 (233.3–1335.1)	785.8±489.2 (246.4–1575.0)

7–8 rats were used in each experiment; ND = not determined; Figures in parentheses represent the range.
*$P < 0.01$

Table 2 Activities (Mean ± SD) of enzymes of the small intestine brush border in control, *Giardia* infected and Furazolidone treated rats

Enzyme activity	Control	Infected	Furazolidone treated
Sucrase (μg glucose released hr^{-1} mg^{-1} protein)	17.9±2.4 (15.5–23.2)	15.9±2.7 (11.8–20.0)	†27.2±10.9 (15.2–51.1)
Maltase (μg glucose released hr^{-1} mg^{-1} protein)	59.4±15.6 (42.2–94.3)	51.9±8.1 (37.9–57.8)	57.0±14.0 (38.1–69.0)
Alkaline phosphatase (μg p-nitrophenol released min^{-1} mg^{-1} protein)	*25.2±9.2 (13.1–40.2)	17.4±2.7 (13.9–20.9)	*27.8±10.8 (15.2–46.9)
Na^+, K^+-ATPase (μg Pi released hr^{-1} mg^{-1} protein)	149.0±103.9 (52.7–338.8)	101.7±51.4 (41.4–209.2)	†37.5±19.0 (17.2–65.0)

Eight rats were used in each experiment. Figures in parentheses represent the range.
*$P < 0.01$
†$P < 0.05$

Illinois, USA). The mannose in the serosal side fluid was colorimetrically estimated by the method of Dubois *et al.* (1956). The rates of absorption of nutrients were calculated as follows:

$$\text{pmole } ^{14}\text{C nutrient absorbed min}^{-1} = \frac{\text{pmole nutrient ml}^{-1} \text{ serosal fluid}}{\text{Time of incubation (min)}}$$

The difference between pmole ^{14}C nutrient absorbed at a given time and that in the preceding 30 min was taken as the nutrient absorbed per 30 min. The values expressed in Tables 1, 2 and Figures 1, 2, 3 are mean of 2–5 experiments. The statistical significance of differences between the infected and control, and infected and Furazolidone treated rats were determined (Spiegel, 1961).

Results

The rats used during this study did not show diarrhoea but all the infected ones excreted *G. lamblia* cysts in their stools. Random examination of the homogenates of the jejunum of infected rats, also showed the presence of *G. lamblia* trophozoites. These results demonstrated the establishment of human *G. lamblia* in the rats.

Enzymatic activities: The activities of pancreatic amylase, phospholipase, trypsin, chymotrypsin, and carboxypeptidase in the contents of the small intestine were decreased and that

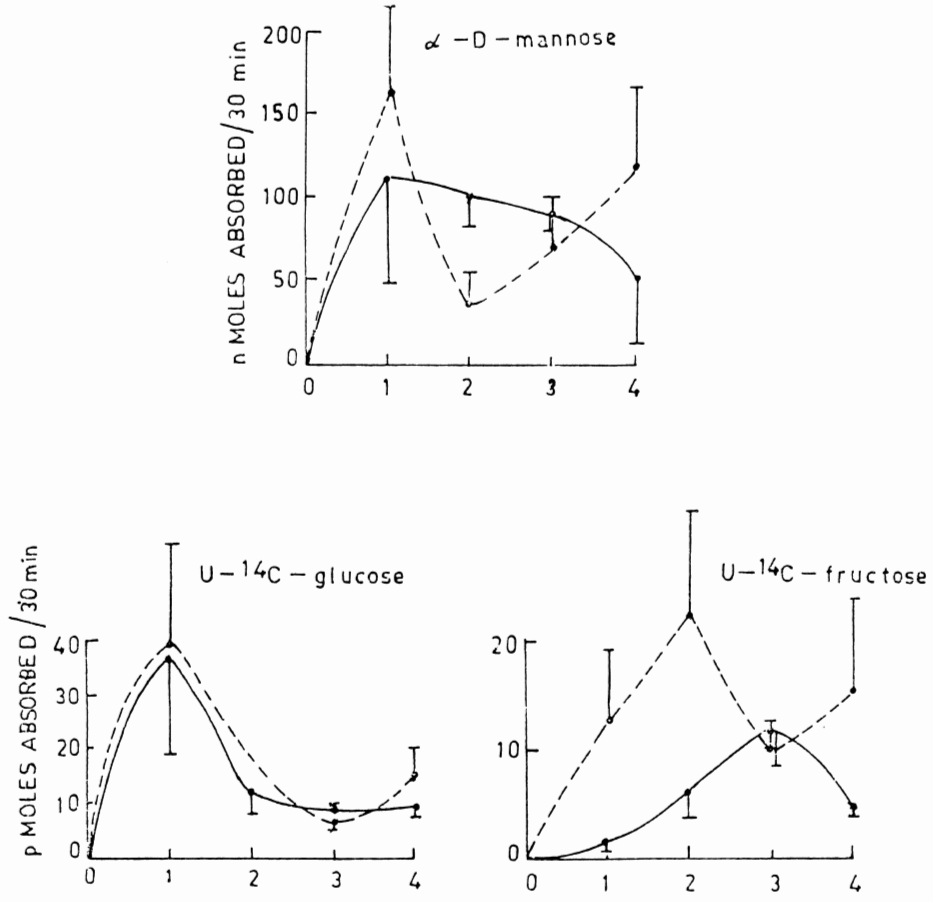

Fig. 1 Effect of *G. lamblia* infecton in rats, on the rate of *in vitro* intestinal absorption (pmole/30 min) of hexoses between 0–30 min (1), 30–60 min (2), 60–90 min (3) and 90–120 min (4). Control ——., infected . . . . . , SD is represented by vertical bar.

of lipase was slightly increased during infection period of 15–20 days after the first administration of inoculum for inducing infection (Table 1). The decrease was significant ($P < 0.10$) only in the case of phospholipase. Furazolidone treatment of infected rats for 5 consecutive days reversed the effects of infection on pancreatic amylase but not that on the carboxypeptidase (Table 1). Effects of the treatment on other enzymes was not studied. The activities of brush border enzymes, viz., sucrase, maltase, alkaline phosphatase, and Na^+, K^+-ATPase were decreased during infection (Table 2). Furazolidone treatment reversed all these effects except that the activity of Na^+, K^+-ATPase which was further decreased significantly (Table 2).

Absorption of hexoses and amino acids: The *in vitro* absorption of U-^{14}C-fructose and α-D-mannose was increased during *Giardia* infection, whereas that of U-^{14}C-glucose was not affected much (Fig. 1). The time dependent rate of *in vitro* absorption of U-^{14}C-glucose (Fig. 1) decreased with increasing time of incubation after 30 min, both in the control and infected rat intestines. The rate of absorption of U-^{14}C-fructose (Fig. 1) initially increased upto 90 min in the control and upto 60 min in the infected rat intestines and thereafter it declined. The rate of absorption of unlabelled α-D-mannose decreased after 60 min and 30 min in control and infected rat intestines, respectively. However, the rates of absorption of hexoses increased slightly again in the infected rat intestines at the later stages of incubation.

The absorption of U-^{14}C-proline and U-^{14}C-amino acid mixture from U-^{14}C-chlorella protein hydrolysate was decreased significantly ($P < 0.01$) during infection (Fig. 2). The effect being much more ($P < 0.01$) pronounced in the latter. The rates of *in vitro* absorption of U-^{14}C-chlorella protein hydrolysate and of U-^{14}C-proline in the control rats, respectively, increased (Fig. 2) upto 60 min and 30 min of incubation, and declined thereafter. In case of infected rats the rate of absorption of U-^{14}C-chlorella protein hydrolysate increased upto 30 min and then remained low and constant, whereas that of U-^{14}C-proline declined. As compared to the control, the absorption of U-^{14}C-glycine, U-^{14}C-lysine and U-^{14}C-leucine was increased after infection (Fig. 3) whereas those of U-^{14}C-glutamic acid was slightly lowered after infection. The *in vitro* rates of absorption of U-^{14}C-glycine and U-^{14}C-lysine increased upto 30 min of incubation both in the control and infected rat intestines and declined thereafter (Fig. 3). The rates of absorption of U-^{14}C-leucine and U-^{14}C-glutamic acid in the control rats increased upto 60 min of incubation and then declined, whereas the

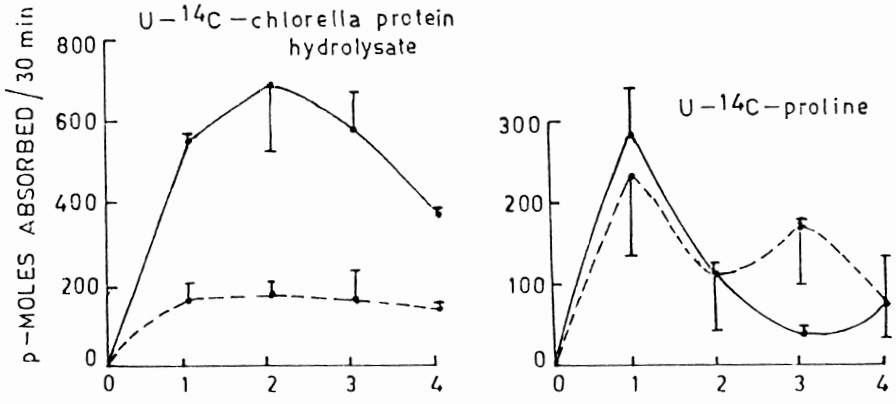

Fig. 2 Effect of *G. lamblia* infection in rats, on the rate of *in vitro* intestinal absorption (pmole/30 min) of U-^{14}C-proline and amino acid mixture of U-^{14}C-chlorella protein hydrolysate. Control ——., infected . . . . . , absorption between 0–30 min (1), 30–60 min (2), 60–90 min (3) and 90–120 min (4). SD is represented by vertical bar.

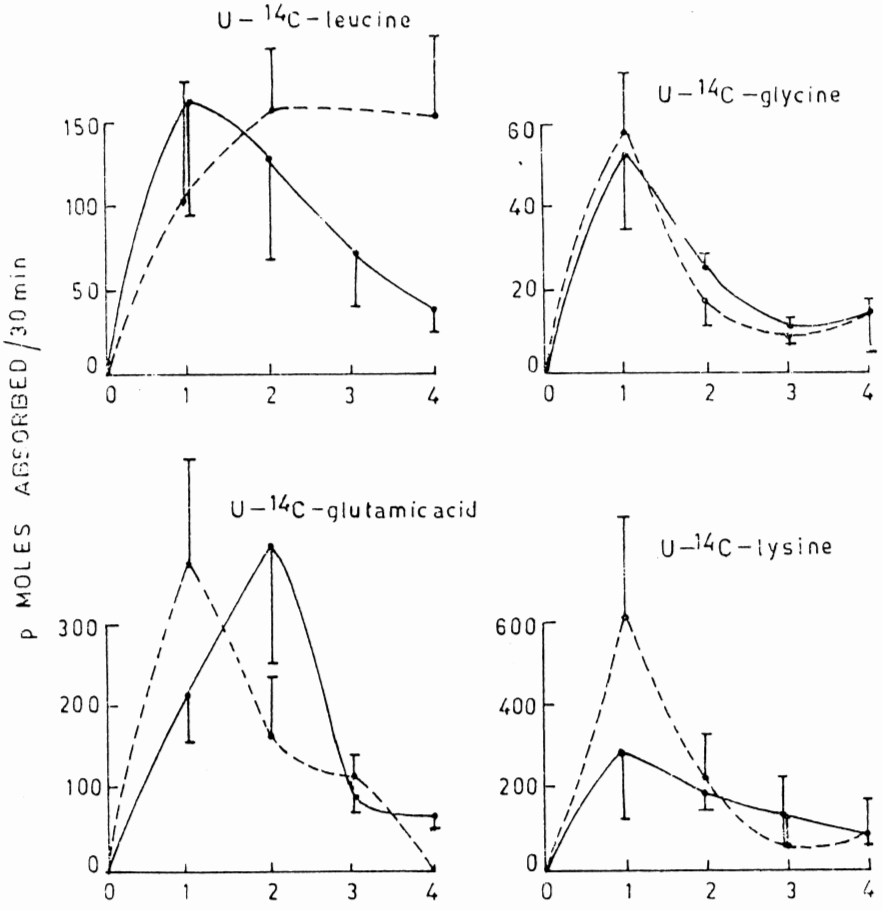

Fig. 3 Effect of *G. lamblia* infection in rats, on the rate of *in vitro* intestinal absorption (pmole/30 min) of individual amino acids between 0–30 min (1), 30–60 min (2), 60–90 min (3) and 90–120 min (4). Control ——., infected . . . . . , SD is represented by vertical bar.

rate of absorption of U-^{14}C-leucine in infected rat intestine did not decline after reaching a peak at 60 min of incubation and that of U-^{14}C-glutamic acid declined after 30 min of incubation.

Histopathological changes: No significant histopathological changes were observed (by light microscopy) in the duodenum, lungs, jejunum, liver, heart, spleen and kidneys.

Discussion

Digestive enzymes: The decreased levels of the pancreatic enzymes (Table 1) amylase, phospholipase, trypsin, chymotrypsin and carboxypeptidase in the intestinal contents of rats infected with *Giardia,* indicated pancreatic malfunctioning either by inflammation of pancreas and/or pancreatic duct or by blockage of the latter by migration of the *G. lamblia.* Pancreatitis induced by this organism has been reported by Sheehy and Holley (1975). Other workers (Chawla *et al.,* 1975, Gupta and Mehta, 1973, Birg, 1963) have also reported decreased levels of trypsin, but there are no reports on the activities of other pancreatic enzymes. The increased excretion of fat during giardiasis, observed by Amini (1963) and Leonbrua and Cruz (1968) may be explained partially on the basis of decreased formation of lysophospholipids, which are important in solubilization and absorption of fats from the

small intestines (Palumbo *et al.*, 1962; Hoskins *et al.*, 1967). Imbalance in the levels of intestinal hormones for secretion of pancreatic enzymes during giardiasis has been suggested by Gupta and Mehta (1973) but has been refuted by Chawla *et al.* (1975).

Disaccharidases are bound to the brush border membranes, whereas Na^+, K^+-ATPase is situated laterally (Harper *et al.*, 1979). Considerable decrease (Table 2) in the activities of these enzymes indicates that the intestinal membrane architecture is modified during giardiasis and is partially reversed after treatment with Furazolidone for 5 days and the elimination of infection. The lack of the histopathological lesions observed by light microscopy may not necessarily be indicative of normal status, as Tandon *et al.* (1974) have demonstrated ultrastructural changes by electron microscopy (EM) in cases of giardiasis. In the present study the tissues were not examined by EM, so it is difficult to say anything about the ultrastructural changes.

Absorption of hexoses and amino acids: Giardiasis induced increase (Fig. 1) in the rates of absorption of U-^{14}C-fructose and α-D-mannose, which are not absorbed by active transport (Wiseman, 1964), during the early stages of absorption again indicated damage to the intestinal mucosa. It is well recognized that α-D-glucose and free L-amino acids are absorbed by Na^+-dependent active transport (Harper *et al.*, 1979). It is also established that L-amino acids in a mixture affect each others' absorption (Wiseman, 1964). However, in most of the investigations (Anand *et al.*, 1980; Ganguli *et al.*, 1982) absorption of only individual amino acids has been studied during giardiasis. In the present investigation the absorption of amino acids from a protein hydrolysate has also been studied (Fig. 2). The higher levels and rates of absorption of U-^{14}C-amino acids mixture as compared to individual amino acids and the giardiasis induced decrease in the absorption of natural mixture of amino acids also indicated damage to the intestinal mucosa, which affects the permeability of the intestinal cell membranes. This decrease in absorption of natural mixture of amino acids may also be due to the competition between the *G. lamblia* sticking to the intestinal surface and the mucosa itself.

The time dependent changes (Figs. 1, 2, 3) in the absorption of various nutrients from the intestines of control and infected rats indicated that the substrates which are absorbed faster in initial stages (fructose, mannose, glycine, lysine in infected rats and proline in control rats) have lower rates of absorption in later stages of incubation, whereas those with lower rates of absorption in initial stages show either prolonged low rates of absorption or the absorption is relatively higher in the later stages of incubation. These results indicate that the substrates may become limiting factor and limitation differs between control and infected rat intestines.

It is apparent that biochemical (may be ultrastructural as well) lesions were produced, but the period of infection was not sufficient to produce histopathological changes. From the foregoing, it can be concluded that in rat, the *G. lamblia* decreases secretion of pancreatic enzymes as well as absorption of actively transported nutrients, but does not produce diarrhoea or lesions observable with ordinary microscope.

Acknowledgements

The authors are thankful to Dr. Rattan Singh, Prof-cum-Head, Department of Biochemistry and Dr. Balwant Singh, Prof. and Head, Dept. of Vet. Pathology for providing necessary facilities. The authors are also thankful to Dr. B. D. Sabharwal, Prof. and Head, Dept. of Pathology, Dayanand Medical College for providing faecal samples of Giardiasis patients. R. S. thanks Council of Scientific and Industrial Research, New Delhi for award of a fellowship.

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