

## SHORT COMMUNICATIONS

### TISSUE $\gamma$ -GLUTAMYLTRANSPEPTIDASE ACTIVITY IN EXPERIMENTAL GIARDIASIS

R. K. SRIVASTAVA, S. R. DAS and B. RAY  
Microbiology Division, Central Drug Research Institute,  
Lucknow 226 001, India.

$\gamma$ -GLUTAMYLTRANSPEPTIDASE (E.C.2.3.2.2) activity has been detected in kidney and jejunum of normal, giardia infected and metronidazole treated giardia infected swiss mice. The enzyme activity was very much reduced in the tissues of infected mice in comparison with that of the normal. When the infection was cured by metronidazole treatment the enzyme activity returned to normal. This study shows that the *Giardia* infection impairs the " $\gamma$ -glutamyl cycle" for the absorption of amino acids, causing malabsorption.

Giardiasis is caused by *Giardia intestinalis*. The main pathological symptom includes malabsorption of the digested food materials<sup>1-4</sup>. Human giardiasis can be induced in experimental animals by inoculating the *Giardia* parasites<sup>3,5</sup>. ' $\gamma$ -glutamyl cycle' plays an important role in the transport of different biochemicals across the cellular membranes<sup>6,8,11</sup>. The major activity of the enzymes of the cycle is found in the kidney<sup>9,10</sup>. The enzymes of  $\gamma$ -glutamyl cycle catalyzes the synthesis and utilization of glutathione which involves in the uptake and release of amino acids from  $\gamma$ -glutamyl linkage<sup>12</sup>.  $\gamma$ -glutamyl cycle fulfills the requirements of amino acid transport system<sup>6-8</sup>.

$\gamma$ -glutamyltranspeptidase, a membrane bound enzyme of  $\gamma$ -glutamyl cycle, is predominantly found in the brush border of the proximal convoluted tubules of the kidney<sup>9,10,13</sup>. As the giardiasis is a disease attributed to malabsorption, it is worthwhile to study whether at the onset of the diseases, the enzyme  $\gamma$ -glutamyltranspeptidase has any alteration in its activity during the course of infection and after its cure with a known anti giardiasis drug treatment. The present work has, therefore, been undertaken to study  $\gamma$ -glutamyltranspeptidase activity in the kidney and jejunum of mouse during *Giardia intestinalis* infection and after its cure by metronidazole treatment.

Axenic *Giardia intestinalis* trophozoites were grown in TYI-S-33 medium of Diamond *et al*<sup>14</sup> for 72 hr at 37°C. The trophozoites were harvested and the infection was given to the *G. muris* free swiss mice (30 to

40 g) through intrajejunal inoculation of  $2 \times 10^5$  *G. intestinalis* trophozoites in 2 ml of TYI-S-33 medium. After 48 hr of postinfection the animals were divided into two groups. The drug 'Flagyl<sup>R</sup>' (metronidazole) (24 mg/kg/day/mouse) was fed to one group of 8 mice for 8 days. The other group did not receive any drug treatment. The uninfected and untreated mice were served as control. The faecal samples were examined in infected and in drug treated groups for the presences of *Giardia* trophozoites and/or cysts to monitor the course of infection and the cure of the disease. The kidney and jejunum were taken out after sacrificing the animals, weighed and homogenized in 0.1 M Tris-HCl buffer pH 8.0 containing 0.1% Triton X-100. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The residue was discarded and the supernatant was used as the enzyme source.

$\gamma$ -glutamyltranspeptidase activity was assayed according to the method of Naftalin *et al*<sup>15</sup>. The standard assay solution (1 ml) contained 0.05 M Tris-HCl buffer pH 8.0, 75 mM NaCl, 2.5 mM  $\gamma$ -glutamyl-*p*-nitroanilide, 20 mM glycylglycine (pH 8.0) and enzyme (0.5 to 1.0  $\mu$ g). After incubation of the mixture at 37°C for 20 min, 2.0 ml. of 10% acetic acid was added. The liberated *p*-nitroaniline was measured by diazocoupling method with N-(1-Naphthyl)-Ethylenediamine. The absorbance of the pink azo-dye was measured at 540  $\mu$ . The amount of liberated *p*-nitroaniline was measured with the help of standard curve. The protein content of the enzyme source was estimated according to Lowry *et al*<sup>16</sup>.

$\gamma$ -glutamyltranspeptidase activity was estimated in both the kidney and the jejunum of normal, giardia infected and with metronidazole treated mice which were pre-infected with *G. intestinalis*. It appears from table I that the enzyme activity was reduced significantly ( $p < 0.01$ ) in the kidney of *Giardia* infected mice but the activity of the same enzyme was recovered on the metronidazole treatment. The  $\gamma$ -glutamyltranspeptidase activity in jejunum of infected mice was also reduced, but less significantly than in kidney, as compared with healthy controls. On metronidazole treatment to the infected mice, the enzyme activity returned to normal in both the kidney and the jejunum.

$\gamma$ -glutamyltranspeptidase, a member enzyme of ' $\gamma$ -glutamyl cycle', is a membrane bound enzyme and is abundant in the brush border of the proximal con-

**Table 1**  $\gamma$ -glutamyltranspeptidase activity in kidney and jejunum of normal, *Giardia* infected and metronidazole treated *Giardia* infected swiss mice.

	$\gamma$ -glutamyltranspeptidase (Specific activity) <sup>++</sup>	
	Kidney	Jejunum
Normal (10)†	1169 ± 88	22 ± 3.6
Experimental giardiasis(6)	737 ± 152 ( <i>p</i> < 0.01)*	14 ± 3.06 ( <i>p</i> < 0.1)**
Experimental giardiasis + Metronidazole treatment (24 mg/kg body wt/day for 8 days (8).	1108 ± 82 ( <i>p</i> < 0.7)*	19 ± 2.1 ( <i>p</i> < 0.7)**

\* when compared with kidney of normal mice.

\*\* when compared with jejunum of normal mice.

++ Specific activity of  $\gamma$ -glutamyltranspeptidase is defined as the nanomole of *p*-nitroamiline liberated/min./mg of protein.

† Number in parenthesis means the animals used in each system.

volute tubules of kidney<sup>6-8</sup>. The enzyme is also found in epithelia of the jejunum villi, salivary glands, bile ducts, seminal vesicles, liver etc<sup>9,10,17</sup>. It is well established that the translocation of the amino acids through  $\gamma$ -glutamyl carrier is one of the transport mechanism of the amino acids across the cell membrane<sup>6,12,18</sup>. Giardiasis is the disease causing malabsorption and the impairment of the transportation of the digested nutrient materials was found under both clinical and experimental conditions<sup>3,5</sup>. In the present study a considerable fall of  $\gamma$ -glutamyltranspeptidase activity in kidney and jejunum is found in experimental giardiasis and the enzyme activity is recovered after treatment with metronidazole. This result confirms that ' $\gamma$ -glutamyl cycle' is affected with giardiasis resulting to the malfunction of the transportation and absorption of the amino acids across the cell membrane. Metronidazole treatment led to the apparent cure of the disease with the concomitant restoration of the enzyme of ' $\gamma$ -glutamyl cycle' in the system, thereby the absorption of the amino acids become normal. The study suggests that the presence of the parasite is important and during the active infection the parasite produces certain factor(s) which disturb the normal functioning of the  $\gamma$ -glutamyl cycle activity in the host, thereby, creating malabsorption.

We wish to thank Dr Nitya Nand, Director, for his keen interest in the study, and also ICMR, New Delhi, for award of fellowship to RKS, one of authors.

3 April 1984

1. Anand, B. S., Mahmood, A., Ganguly, N. K., Rehani, M. M., Dilwari, J. B. and Mahazan, R. C., *Trans. R. Soc. Trop. Med. Hyg.*, 1982, 76, 616.
2. Anand, B. S., Kumar, M., Chakravarti, R. N., Sehgal, A. K. and Chuttani, P. N., *Trans. R. Soc. Trop. Med. Hyg.*, 1980, 74, 565.
3. Hoskin, L. C., Winawer, S. J., Broitman, S. A., Gottlieb, L. S. and Zamcheck, N., *Gastroenterology*, 1967, 33, 265.
4. Hartong, W. A., Gourley, W. K. and Arvantakis, C., *Gastroenterology*, 1979, 77, 61.
5. Stevens, D. P. and Roberts-Thomson, Ann. C., *Am. J. Pathol.*, 1970, 90, 529.
6. Orłowski, M. and Meister, A., *Proc. Natl. Acad. Sci. (USA)*, 1970, 67, 1248.
7. Tate, S. S., Ross, L. L. and Meister, A., *Proc. Natl. Acad. Sci. (USA)*, 1973, 70, 1447.
8. Tate, S. S. and Meister, A., *J. Biol. Chem.*, 1974, 249, 7593.
9. Albert, Z., Orłowski, M. and Szewczuk, A., *Nature (London)*, 1961, 191, 767.
10. George, S. G. and Kenny, A. J., *Biochem. J.*, 1973, 134, 43.
11. Van Der Werf, P., Orłowski, M. and Meister, A., *Proc. Natl. Acad. Sci. (USA)*, 1971, 68, 2982.
12. Meister, A., *Life Sci.*, 1974, 15, 177.
13. Glenner, G. G. and Folk, J. E., *Nature (London)*, 1961, 192, 338.
14. Diamond, L. S., Harlow, D. R. and Cannik, C. C., *Trans. R. Soc. Trop. Med. Hyg.*, 1978, 72, 431.
15. Naftalin, L., Sexton, M., Whilaker, J. E. and

- Tracey, D., *Clin. Chem. Acta.*, 1969, 26, 293.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 265.
17. Orłowski, M. and Szezeklik, A., *Clin. Chem. Acta.*, 1967, 15, 387.
18. Meister, A., *Science*, 1973, 180, 33.

### EFFECT OF EXTRACTS OF *ARTABOTRYS UNCINATUS* AND *ALLIUM SATIVUM* ON *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE*

MICHAEL GRAINGE, LESLIE BERGER\*  
and SALEEM AHMED†

*Department of Plant Pathology, University of Hawaii;*

\**Department of Microbiology, University of Hawaii;*

†*East-West Center, Honolulu, Hawaii, 96848, USA.*

AQUEOUS extracts of several plants were tested for activity against *Xanthomonas campestris* pv. *oryzae* the causative agent of bacterial leaf blight, a serious disease of rice in South and South-East Asia. Extracts from the cloves of *Allium sativum* (garlic) and from the leaves of *Artabotrys uncinatus* (ylang ylang) were obtained using a Carver press and subjecting the plant material to 10,000–15,000 lb/in<sup>2</sup> pressure. The liquid obtained was filtered through a Whatman #1 filter and then filter sterilized using 0.22 micrometer filters.

The pathogen was streaked onto tetrazolium chloride agar<sup>1</sup> plates using cotton swabs dipped into 24 hr cultures of luria broth. Sterile water at pH 5 and pH 7 was used in place of the plant extracts as controls. Eight replications were used.

After 2–3 days the inhibition zones were recorded. The two plant extracts that produced the largest inhibition zones were those of *A. sativum* (68 mm) and *A. uncinatus* (64 mm). The pH of the extract was 5.8 for *A. sativum* and 5.3 for *A. uncinatus*. No inhibition zones were produced on the control plates.

Garlic is a well known bactericide, but in a recent review of the literature on pest control materials from 1600 plants, *A. uncinatus* was not listed as possessing bactericidal properties<sup>2</sup>. However, this plant belongs to the family *Annonaceae* which has several *Annona* spp. known to possess pest control properties<sup>3</sup>. The leaves of *A. uncinatus* have also been found to possess antifungal activity against several plant pathogenic fungi<sup>4,5</sup>.

1. Fahy, P. C. and Persley, G. J. (Eds) *Plant Bacterial Diseases: A Diagnostic Guide*, Academic Press, New York, 1983, p. 346.
2. Grainge, M., Ahmed, S., Mitchell, W. C. and Hylin, J. W., *Plant Species Reportedly Possessing Pest-Control Properties—A Database*, Resource Systems Institute, East-West Center, Honolulu, Hawaii, USA, 1984.
3. Jacobson, M., *Insecticides from Plants—A Review of the Literature, 1954–1971*. U.S. Dept. of Agriculture, Agriculture Handbook No. 461, 1975, 6.
4. Misra, S. B. and Dixit, S. N., *Acta Botanica Indica*, 1979, 7, 147.
5. Misra, S. B. and Dixit, S. N., *Indian J. Mycol. Plant Pathol.*, 1979, 9, 250.

### SOME NEW PTERIDOPHYTIC REMAINS FROM THE LOWER GONDWANA ROCKS OF HINJRIDA GHATI, ORISSA

D. D. PANT, P. C. SRIVASTAVA and P. K. DAS

*Department of Botany, University of Allahabad, Allahabad 211 002, India.*

THE first report of pteridophytic plant fossils from Hinjrada Ghati section near Handappa made by Khan<sup>1</sup> included *Phyllothea indica*, *Schizoneura gondwanensis*, *Sphenophyllum speciosum*, *Sphenopteris polymorpha* and *S. hughesii* but lately Chandra and Rigby<sup>2,3</sup> have added a few lycopsid, sphenopsid forms besides some ferns including *Pantopteris gracilis* to the previously reported forms from this bed. However, during a recent visit to this locality the authors discovered the impressions of a pinna with fertile pinnules of a fern and a whorl of a new species of *Sphenophyllum* Koenig<sup>4</sup> which are described hereunder.

*Sphenophyllum utkalensis* sp. nov.

**Diagnosis:** Node showing an almost symmetrical whorl of six leaves; leaves sessile, triangular with pointed angular shoulders, distal margin of each leaf showing a deep apical notch, veins repeatedly dichotomised, median veins proceeding straight to distal margins but lateral ones arched sideways; internodes not seen.

Holotype No. 90066 of Divya Darshan Plant Collection at present located in Botany Department of Allahabad University.