Peroxidase and gamma-glutamyl transpeptidase activities during *Eimeria nieschulzi* (Apicomplexa) and/or *Nippostrongylus brasiliensis* (Nematoda) infections in the rat[1]

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Summary. Results suggest that malabsorption of amino acids which occurs during *Eimeria nieschulzi* and *Nippostrongylus brasiliensis* infections in rats is not due to impairment by intestinal inflammation of gamma-glutamyl transpeptidase activity.
Key words. Eimeria nieschulzi; Nippostrongylus brasiliensis; gamma-glutamyl transpeptidase; peroxidase.

It has been demonstrated that malabsorption of amino acids occurs in rats infected with the obligate intracellular protozoan, *Eimeria nieschulzi*[2], and in rats infected with the intestinal nematode, *Nippostrongylus brasiliensis*[3]. Malabsorption syndromes are usually associated with intestinal inflammation and Castro[4] has suggested that the immunological reactions in several helminth infections, including *N. brasiliensis*, affects epithelial cell differentiation and development as well as secretory, absorptive and digestive activities.

In 1978, Duszynski et al.[5], using a peroxidase assay as an indicator of inflammation due to myeloid derived leukocytes, demonstrated that at 2 and 16 days postinoculation (PI) with *E. nieschulzi*, the host intestinal mucosa was inflamed while at day 8 PI, inflammation was absent. They suggested that *E. nieschulzi* has the ability to suppress the inflammatory and/or immune response in the intestine of the rat host at day 8 PI. Bristol et al.[6], in 1983, demonstrated that when *E. nieschulzi* was administered to rats infected with *N. brasiliensis*, the nematode's patent period was lengthened significantly. This further supported the suggestion that *E. nieschulzi* suppresses the immune and/or inflammatory response since *N. brasiliensis* is normally rejected from the rat host about 14 days PI as a result of a combined inflammatory/immune response[6].

We were interested in determining if there is a decrease in the activity of gamma-glutamyl transpeptidase (GGT) activity during a *N. brasiliensis* infection that might correlate with intestinal inflammation and amino acid malabsorption. GGT is involved in the gamma glutamyl cycle, a postulated group translocation mechanism for the transport of amino acids[7]; experimental results obtained from rats strongly support this postulation[8]. Depressed activity of this enzyme due to the presence of inflammatory cells might explain why, in part, malabsorption of amino acids occurs during nippostrongylosis, We were also interested in knowing whether the presence of *E. nieschulzi*, which seems to depress the host's inflammatory response at 8 days PI, might alter the effect of the nematode's presence on GGT activities during concurrent infections.

Materials and methods. Specific pathogen-free outbred male Sprague-Dawley rats (Timco Breeding Laboratories, Houston, TX) weighing 100–150 g were inoculated per os with $10^5 \pm 6 \times 10^3$ sporulated oocysts of *E. nieschulzi* Dieben and/or s.c. with $4 \pm 0.24 \times 10^3$ L_3 larvae of *N. brasiliensis* Travassos. At the time of inoculation, oocysts were 2–4 months old while larvae were 14 days of age. The *N. brasiliensis* inoculum variability was 6% which is within the 95% confidence limit (6–9%) established by Keymer et al.[9] irrespective of inoculum size. Five groups of 10 rats each were inoculated according to the infection schedule in the table. All rats were housed in separate cages, maintained on a 12-h photoperiod and given food and water ad libitum.

On the day PI of sacrifice for each group, 2 infected and 2 uninfected rats were killed by cervical dislocation on each of 5 consecutive days. The abdomen was opened and the intestine flushed and the weight and length recorded according to the method of Duszynski et al.[5]. The intestines were divided into thirds and the middle 11-cm removed from each third. After each 11-cm segment was weighed, the mucosa was scraped with a glass microscope slide. The scrapings were homogenized in a pre-chilled VirTis tissue homogenizer in 4 ml cold 0.85% NaCl. For the peroxidase assay, 2 ml of the homogenate were further homogenized with 50 strokes in a pre-chilled Potter-Elvehjem homogenizer.

For both assays, 0.1 ml aliquots were utilized. Protein determinations were made using the Bio-Rad assay. Peroxidase activity was measured by the method of Maehly and Chance[10] as modified by Duszynski et al.[5]. The increase in absorbance was measured at 470 mn at ambient temperature in a B & L Spectronic 70 spectrophotometer. One unit of peroxidase activity is defined as the quantity catalyzing the decomposition of 1.0 µmole of H_2O_2/min.

GGT activity was measured and international units calculated by the method of Rosalki and Tarlow[11]. The increase in absorbance was measured at 405 nm at ambient temperature in a B & L Spectronic 70. One unit of GGT activity is defined as the quantity liberating 1 µM of p-nitroaniline/min. Activity units for both enzymes were expressed per cm of intestinal length and per µg protein. Since statistical significance did not vary, the data are expressed per cm intestinal length as in the report by Duszynski et al.[5]. Data were statistically analyzed using the Mann-Whitney U-test and considered significant if $p \leqslant 0.05$.

Results and discussion. Results presented in the table show that GGT activity was decreased only at 8 days PI during an *E. nieschulzi* infection while peroxidase activities were elevated at all days examined indicating that infiltration by myeloid derived leukocytes occurred throughout both single and concurrent infections. The presence of elevated peroxidase levels at day 8 PI of the *E. nieschulzi* infection differs from that reported by Duszynski et al.[5]. The conflicting results can be explained, however, by the fact that the response is dose dependent[5] and may vary with rat and parasite strains. Further research in our laboratory demonstrated that with an inoculum of 1×10^6 oocysts the inflammatory response was depressed at day 8 PI. Lack of alter-

Gamma-glutamyl transpeptidase (units × 10^{-4}/cm small intestine) and peroxidase (units/cm small intestine) activities in rats infected with *Eimeria nieschulzi* and/or *Nippostrongylus brasiliensis* and uninfected control rats

Group (N = 10)	Infection schedule	Day PI sacrifice	Gamma glutamyl transpeptidase units × 10^{-4}/cm ($\bar{x} \pm$ SE)		Peroxidase units/cm ($\bar{x} \pm$ SE)	
			Parasitized	Control	Parasitized	Control
1	*E.n.*	2	4.46 ± 0.44	7.52 ± 0.48	0.74 ± 0.10*	0.36 ± 0.02
2	*E.n.*	8	7.28 ± 0.92*	12.68 ± 1.32	0.99 ± 0.15*	0.35 ± 0.06
3	*N.b.*	11	10.24 ± 1.00	7.80 ± 0.88	1.59 ± 0.24*	0.58 ± 0.10
4	*E.n.* on day 9 PI *N.b.*	11	8.56 ± 0.92	6.96 ± 1.12	1.17 ± 0.23*	0.64 ± 0.15
5	*E.n.* on day 3 PI *N.b.*	11	5.20 ± 0.68	5.84 ± 0.68	1.29 ± 0.17*	0.52 ± 0.08

* Differs significantly ($p \leqslant 0.05$) from corresponding control.

ation of GGT activities in all groups except 2 suggests that inflammation does not affect the activity of this enzyme. The decreased activity observed at day 8 PI, therefore, is probably a result of mechanical disruption of the intestinal epithelium due to *E. nieschulzi* oocysts being shed since peak oocyst production occurs at this time. Thus, although intestinal inflammation is known to affect absorptive and digestive activities[4], results presented here suggest that malabsorption of amino acids observed during *N. brasiliensis* and *E. nieschulzi* infections is not the result of impaired ability, due to inflammation, of GGT to catalyze reactions involved in amino acid transport and absorption.

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Suppression by the cyclohexanetrione Ro 31-0521 of retinoic acid-induced teratogenicity

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Summary. The cyclohexanetrione Ro 31-0521, which stimulates prostaglandin synthesis, inhibited retinoic acid-induced cartilage degradation in vitro and suppressed the congenital forelimb malformations in rats treated with retinoic acid on day 13 of gestation in a dose-dependent manner.
Key words. Retinoic acid; teratogenesis; cartilage; chondrogenesis; cyclohexanetrione.

Intracellular Ca^{2+} mobilization and protein kinase C activation appear to be cellular mediators of various extracellular informational signals such as those of specific hormones and neurotransmitters[1-3]. In addition, Nishizuka[3] suggested that metabolites of the signal-induced turnover of inositol phospholipids, such as diacylglycerol, arachidonic acid and prostaglandins may be involved in the regulation of other homologous or heterologous cell types. We here report that the cyclohexanetrione Ro 31-0521 (fig. 1) which stimulates prostaglandin synthesis (N. A. Roberts, personal communication), inhibited retinoic acid-induced cartilage degradation in vitro and suppressed the congenital forelimb malformations in rats treated with retinoic acid on day 13 of gestation in a dose-dependent manner.

Materials and Methods. Female Fü-albino rats (outbred stock, Institute of Biological and Medical Research, Füllinsdorf, Switzerland) were mated overnight, and the females which had a vaginal plug the following morning were considered to be at day 1 of gestation.

Humeri from fetal rats at day 20 of gestation were prepared and incubated in Ham's F-10 nutrient mixture (supplemented with 10% fetal calf serum, 60 µg penicillin/ml, 100 µg streptomycin/ml and 10 mM HEPES, pH 7.4) in cell culture dishes in a humidified 5% CO_2 air atmosphere at 36 °C. Retinoic acid (dissolved in ethanol) and the cyclohexanetrione Ro 31-0521 (dissolved in dimethylsulfoxide) were added at the beginning of incubation. Equivalent amounts of the vehicles were added to the control cultures.

The amount of proteoglycans and/or glycosaminoglycans released from the bones into the medium was measured in aliquots of the medium by the alcian blue assay[4] with chondroitin sulphate as a standard. This amount is referred to here as proteoglycan release. The alcian blue-glycosaminoglycan complex was dissociated with 4% (w/v) sodium lauryl sulphate.

To estimate the change in length during the incubation period as the parameter for tissue breakdown[5], the length of the bones was measured under a reversed microscope, using a projecting prism, at a final magnification of 14.1 times, at the beginning and end of incubation.

For the studies in vivo retinoic acid and Ro 31-0521, both suspended in rape seed oil, were administered by oral intubation to pregnant rats using the application volume of 5 ml/kg. Controls received the vehicle only. Fetuses were obtained by laparotomy on day 21 of gestation. Fetuses were prepared for skeletal examination using a NaOH-alizarin red S staining procedure. Malformations of humeri and ulnae/radii were arbitrarily indexed 0–1 and 0–3, respectively, 0 representing controls (fig. 4). The nutrient mixture F-10 and fetal calf serum were obtained from GIBCO Europe, Glasgow, Scotland; all-trans-retinoic acid from F. Hoffmann-La Roche, Basle, Switzerland; the cyclohexanetrione Ro 31-0521 from F. Hoffmann-La Roche, Welwyn,

Suppression by Ro 31-0521 of forelimb malformations induced by retinoic acid on day 13 of gestation

Retinoic acid mg/kg	Compound	mg/kg	Number of dams	Number of fetuses	Malformation index Humerus	Ulna/radius
120	None		18	209	0.80 ± 0.07	1.24 ± 0.14
120	Ro 31-0521	20	9	108	0.42 ± 0.09*	0.38 ± 0.13*
120	Ro 31-0521	60	9	85	0.54 ± 0.13	0.24 ± 0.14*
120	Ro 31-0521	180	7	77	0.29 ± 0.14	0.02 ± 0.02*

Retinoic acid and Ro 31-0521, both suspended in rape seed oil, were administered orally to pregnant rats once on day 13 of gestation. Controls received rape seed oil alone. Fetuses were obtained by laparotomy on day 21 of gestation and processed for visualization of the skeleton with alizarin red S. Malformations of humeri and ulnae/radii were arbitrarily indexed from 0–1 and 0–3, respectively (fig. 4), and a mean malformation index was estimated per litter. No malformations of long bones were observed in controls and in the groups treated with Ro 31-0521 alone. Results are mean ± SE. For statistical analysis of the malformation indexes the U-test was used. * $p < 0.01$.