tionally to the increase in distance and the running time similarly increased. Neither peak of JHE activity appears to be an artifact of electrofocusing media or conditions. It is possible that in the purification studies on *T. ni.* which obtained a single IEF peak[10,14] conditions used were not of sufficient resolution or one of the two JHE activities was lost during purification.

The results presented here indicate that previous IEF data used to construct and promote the popular *T. ni,* single protein model of JHE[7,8,15] should be reevaluated. Since the careful application of IEF methodology actually shows the presence of at least 2 discrete JHE activities, studies of JHE purification, structure, inhibition[16,17] and regulation should first carefully determine the nature of the JHE activity being studied.

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Glycine absorption from the small intestines of rats after secondary infections with *Eimeria nieschulzi*

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Summary. A second (challenge) infection of *Eimeria nieschulzi* in clinically immune rats did not produce weight gain depression but caused a decrease in the absorption of glycine from the ileum. The malabsorption due to challenge was equivalent to that caused by the primary infection which did cause weight loss.
Key words. Eimeria nieschulzi; coccidia; challenge infection; malabsorption; ileum; glycine.

As a result of epithelial damage, intestinal coccidiosis causes malabsorption of nutrients including proteins and amino acids in chickens[1,2] and in rats[3,4]. Absorption has usually been examined during the acute phase of the disease and to our knowledge, apart from a recent report[5] using *Eimeria acervulina* in chickens there has been no study of absorption resulting from challenge infections of immune animals. The present study compares the absorption of glycine from the ilea of rats during the acute and recovery phases of primary *E. nieschulzi* infections and secondary/challenge infections.

Male Wistar rats (120–150 g) were orally inoculated with sporulated oocysts of *E. nieschulzi* originally supplied by Dr Dawn Owen. In expt. 1 the primary and secondary infections were 10^5 oocysts and in expt. 2, the primary infection was 5000 oocysts and the second (challenge) infection was 5×10^6 oocysts. The oocyst dose in expt. 1 was chosen because it was known that the resulting infection caused malabsorption[3], and in expt. 2 doses of oocysts of the order of 5000 produce a good immune response[6]. Uninfected control groups provided the comparison for normal weight gains and the normal data on absorption, animals being sacrified at intervals throughout the experimental period. Control values for absorption remained constant throughout the experimental period and are represented in the figures under day 0. Glycine absorption through the ileum was measured in vitro by using a modified everted sac method[7] as described elsewhere[8] since it is believed to be a method particularly sensitive to damage of the epithelial layer. Pairs of rats were selected at random over the experimental period for absorption measurements conducted on 4–6 ileal sacs per animal. Pieces of ileum from each rat were processed for examination by light and scanning electron microscopy. The criterion of immunity to reinfection was taken to be the maintenance of normal weight gains linked to the absence of clinical signs.

In expt. 1, the amount of serosal glycine fell significantly during the primary infection (fig. 1) the lowest amount being recorded on day 7 post infection (p.i.) that is one day before the lowest weight gain depression. The weight gains shown are typical for this species of *Eimeria*. From day 8 p.i. the malabsorption decreased and although there was not a return to normal values during the recovery period there was no significant difference between the control value and those at day 21 p.i. when the second inoculation was administered. A similar pattern of depressed absorption followed the second infection (fig. 1) although there were no clinical signs of infection as shown by the maintenance of good growth. There was no obvious evidence by light microscopy of villous damage on day 21 p.i. or 5–8 days after the second infection but some flattening of villi was seen by scanning electron microscopy. It is of interest to note that 16 days after both the primary and secondary inoculations there was a further decrease in absorption. There was no evidence that this was caused by self-infection, and there is no satisfactory explanation at present. This double decrease in absorption did not occur in expt. 2 (fig. 2). In this experiment the weight gains were similar to those in expt. 1 and the absorption of glycine

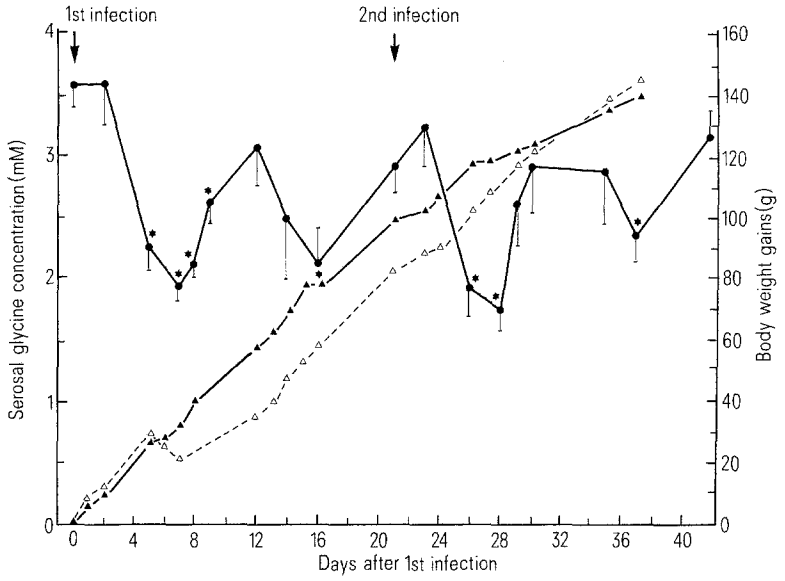

Figure 1 (expt. 1). Mean concentration of glycine (●) in serosal fluid of ileal sacs from rats at various times after a primary and secondary infection with *E. nieschulzi*. Vertical lines are ±SEM; for clarity single lines only have been drawn. *Values significantly different at $p < 0.01$ from that of the controls marked under day 0. ▲, Mean weight gains of uninfected control rats; △, mean weight gains of infected rats.

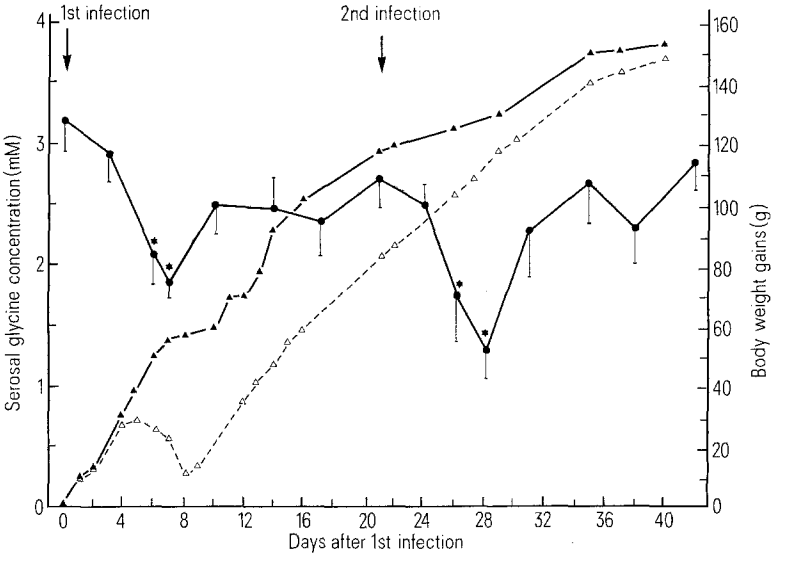

Figure 2 (expt. 2). As for figure 1 except the primary infection was 5000 sporulated oocysts of *E. nieschulzi* and the secondary (challenge) infection was 5×10^6 oocysts. Vertical lines are SEM and * = significant difference from controls (day 0) at $p < 0.01$.

showed the same trend although a lighter primary infection was used followed by a heavier second challenge.

The present work confirms previous findings[3,9] that a significant reduction in glycine absorption through the ilea of rats occurs as a result of *E. nieschulzi* infections. Also it demonstrates, that although rats show clinical immunity to second/challenge infections as judged by their good growth, the small intestine is not behaving normally since there are significant reductions in glycine absorption equivalent to that resulting from primary infections. The abnormality of intestinal functions in the presence of other signs of normality, although surprising, may be analogous to similar findings with amino acid absorption seen in rats adapted to removal of the proximal small intestine. Recent studies[10] have shown that in such rats gut morphology, biochemical activity and absorptive function do not all adapt at similar rates. Furthermore, the absorptive pattern for sugars appears to alter more quickly than that for amino acids.

Although the reason for the persistent abnormality in glycine absorption in the present study has not been investigated, one can conclude that the presence of clinical immunity in rats with secondary coccidial infections, does not imply normal physiological functions of the gastrointestinal tract.

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