However, 2 other fragments with 550,000 and 450,000 daltons mol.wt vary considerably in relative amount in strains and subspecies. The 550,000-daltons fragment accounts for 1% or less of the total satellite DNA in D. w. willistoni Tame and in D.w. quechua Belize, whereas it accounts for about 4% of the total satellite in D. w. willistoni Santa Marta and in D.w. quechua Lima. The 450,000daltons fragment accounts for a relative amount of 7-8% in D. w. willistoni Santa Marta and in the 2 strains of D. w. quechua, while it accounts for only 1-2% in D. w. willistoni Tame.

The 11 fragments observed in D. willistoni are present with the same mol.wts in the D. paulistorum strains, with the exception of the largest fragment which has a mol.wt of 1,200,000 daltons in D. paulistorum, instead of 760,000 daltons in D. willistoni. Besides this difference, the D. paulistorum Amazonian from Tame has a fragment with a mol.wt of 900,000 daltons that is absent in the other D. paulistorum strains.

The 550,000 daltons fragment is present in D. paulistorum satellite DNA in a relative amount of about 10% in the Amazonian semispecies from Caicara and Andean-Brazilian semispecies from Mirassol, but of 15% in the other 2 strains; these amounts are all consistently higher than those observed in D. willistoni. Furthermore an additional fragment of 510,000 daltons is present in D. paulistorum Amazonian Caicara and Andean-Brazilian Mirassol in a relative amount of 5%, and in a relative amount of 1% in the other 2 strains of D. paulistorum. The relative amount of the 450,000-daltons fragment, which is variable in D. willistoni, varies in D. paulistorum as well; it accounts in Andean-Brazilian Caicara for 13%, and for about 1% of the total satellite in the other 3 strains. Moreover, the 410,000daltons fragment is present in slightly different amounts in the D. paulistorum strains (see table for a summary).

In conclusion, even though the similarity in satellite-DNA digestion patterns in the strains studied reflects their close phylogenetic relationship, substantial differences exist between the 2 species, D. willistoni and D. paulistorum. Moreover, clear differences also exist between the strains of D. paulistorum and lesser differences between the strains of D. willistoni. However, strains of different subspecies or semispecies, which produce sterile hybrid males, are not consistently more different than strains of the same subspecies or semispecies, which produce fertile males and females. Hence, the sterility of the hybrid males cannot simply be explained in terms of the observed satellite DNA differences.

- This work was supported by grants 78.01852.65 from the CNR (Rome) and GM 22221 from the PHS (USA).
- M. Yamamoto and G.L.G. Miklos, Chromosoma 66, 71 (1978).
- P. M. B. Walker, Nature 229, 306 (1971). G. Corneo, Experientia 34, 1141 (1978).
- G. Corneo, Evol. Theory 1, 261 (1976).
- F.J. Ayala, M.L. Tracey, D. Hedgecock and R.C. Richmond, Evolution 28, 576 (1975).
- M. Cordeiro-Stone and S. C. Lee, J. mol. Biol. 104, 1 (1976).
- F. W. Studier, J. mol. Biol. 11, 373 (1965).
- E. Ginelli and G. Corneo, Chromosoma 56, 55 (1976).
- E. Ginelli, R. Di Lernia and G. Corneo, Chromosoma 61, 215 (1977)
- E. M. Southern, J. mol. Biol. 94, 51 (1975).
- P. Lebowitz, W. Siegel and J. Sklar, J. molec. Biol. 88, 105

Absorption of glycine and proline from the small intestine of rats infected with Eimeria nieschulzi

S.J. Ball, Christine E. Heading and B. Tranter

Department of Biology and Department of Paramedical Sciences, North East London Polytechnic, Romford Road, London E15 4LZ (England), 22 October 1979

Summary. The absorption of glycine and proline through the jejunum and ileum of rats with an Eimeria nieschulzi infection was impaired when the amino acids were presented to the mucosal surface as either a mixture or the dipeptide, glycyl-proline.

Coccidial infections in chickens affect the absorption of proteins, amino acids, lipids, carbohydrates and certain minerals (see reviews by Turk1 and Ruff2). Amino acid studies in infected chickens have been restricted to presenting the compounds in a free form. However, it is now generally accepted that a substantial proportion of dietary amino acid is absorbed from the gut in dipeptide form and hydrolysed to amino acid within the mucosal cells of the small intestines³. Therefore, we have studied the absorption from both free amino acid and dipeptide through the small intestines of rats infected with Eimeria nieschulzi, hitherto not previously examined.

Materials and methods. The amino acids used were glycine and proline, and the peptide was glycyl-proline. These substances were chosen because both glycine and proline are well absorbed when presented to the rat small intestine either as free amino acid⁴ or as glycyl-proline^{5,6}. In addition, proline and glycine can be quantitatively distinguished in experimental samples due to their different spectra after reaction with ninhydrin reagent, and although

some glycyl-proline can escape hydrolysis in the gut wall, the proportion in rats is small⁶.

Male Wistar rats (200-250 g) were each orally inoculated with approximately 200,000 sporulated oocysts of a strain of E. nieschulzi supplied by Dr Dawn Owen, and 5 days later absorption from the small intestine was assessed in both infected and an equal number of uninfected controls, using the everted sac method⁷. Samples were analyzed by a ninhydrin-based method. Infection was confirmed by microscopical examination and pieces of intestine were taken from some rats for histological examination by lightand stereoscan electron microscopy. There observations showed that by the 5th day of infection many epithelial cells of the villi contained schizonts (figure 1, e) and that a change had occurred in villous architecture, from a spatulate shape (figure 1, a and b) to a more shortened and thickened appearance (figure 1, c and d) but with little or no cellular breakdown.

The effects of infection upon absorption can be seen in figure 2. In 6 of the 8 comparisons made, poorer absorption

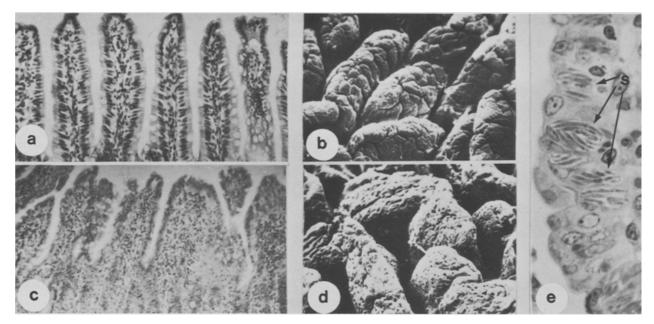


Fig. 1. a Section (\times 80) and b surface view (\times 200) of uninfected rat ileum. c Section (\times 80) and d surface view (\times 200) of rat ileum infected with E. nieschulzi. e Section of epithelial cells of villus of rat ileum showing schizonts (s) of E. nieschulzi with mature merozoites (\times 660).

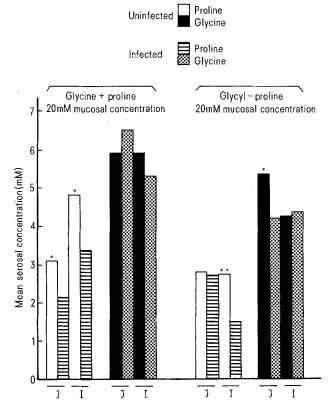


Fig. 2. Amounts of proline and glycine detected in serosal fluid after 30 min. Uninfected control values marked with asterisks are significantly higher than corresponding infected values. (*p < 0.05; **p < 0.01). J=jejunum; I=ileum. (10 infected and 10 uninfected rats were used; values are the mean of 6 readings).

was shown from the tissues of the infected rats and 4 of these differences were significant⁸. Infection appeared to have impaired absorption from both the jejunum and ileum. In no case was absorption significantly greater from the infected compared with the uninfected tissue. The absorption of glycine was always significantly greater than proline irrespective of the region of the intestines examined, the form of presentation or the presence or absence of infection. Regardless of infection, the absorption of both amino acids from the ileum was better when they were presented in the free rather than the peptide form (p < 0.05). From the jejunum there was no significant difference in this respect, except in the case of glycine across infected tissue (p < 0.05). Serosal samples examined by TLC did not contain detectable peptide, i.e. < 0.5 mM. The substances selected here and the methods adapted for their measurement have proved suitably sensitive in rats to enable them to be used as parameters for the study of the effects of coccidiosis upon amino acid absorption.

- D.E. Turk, in: Avian Coccidiosis, p.227. Ed. P.L. Long, K.N. Boorman and B.M. Freeman. British Poultry Science Ltd, Edinburgh 1978.
- 2 M.D. Ruff, in: Avian Coccidiosis, p.281. Ed. P.L. Long, K.N. Boorman and B.M. Freeman. British Poultry Science Ltd, Edinburgh 1978.
- 3 D.M. Matthews, Physiol. Rev. 55, 538 (1975).
- 4 B.G. Munck, Biochim. biophys. Acta 120, 97 (1966)
- 5 R.C. Heading, H.P. Schedl, L.D. Stegink and D.L. Miller, Clin. Sci. molec. Med. 50, 607 (1977).
- 6 D.J. Boullin, R.F. Crampton, C.É. Heading and D. Pelling, Clin. Sci. molec. Med. 45, 849 (1973).
- 7 T.H. Wilson and G. Wiseman, J. Physiol. 123, 116 (1954).
- 8 M.M. Quenouille, in: Rapid Statistical Calculations, p.14. Charles Griffin & Co. Ltd, London 1959.