Colonization by *Salmonella typhimurium* and *Shigella flexneri* III of the gastrointestinal tract of mice treated with β-2-thienylalanine and streptomycin

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Mice fed β-2-thienylalanine (β-2-T) by oesophageal tube were no more susceptible to gastrointestinal tract colonization by *Salmonella typhimurium* or *Shigella flexneri* III than control mice fed water. In both β-2-T-fed and water-fed groups, the increasing dosage of *S. typhimurium*, in logarithmic increments to groups of mice, resulted in increasing numbers of these bacteria detectable on dilution plates from organ homogenates. Colonization by *S. flexneri* III only occurred at a dosage of 10⁸ bacteria for both groups. Pretreatment with 50 mg streptomycin allowed 10³ *Salmonella* or 10⁴ *Shigella* to colonize both β-2-T- and water-fed groups.

Coliforms, inhibited by β-2-T under certain conditions *in vitro*, were found in equal numbers in both groups. No obvious differences were noted in either types of other bacteria detected or numbers recovered from the two groups.

No gross behavioural changes were noted in mice fed β-2-T and not challenged with pathogenic bacteria, and no pathological changes were noted in hepatic or splenic tissues. With increasing *Salmonella* dosage, collections of polymorphonuclear leucocytes, which were almost focal, and increased numbers of giant cells were noted in splenic red pulp areas, in both groups.
INTRODUCTION

The phenylalanine (Phe) analogue, β-2-thienylalanine (β-2-T), increases urinary excretion of Phe in rats without being metabolized to CO₂ or being incorporated to a great extent into proteins (Godin and Dolan, 1967). There is increased renal excretion of Phe when β-2-T is infused in adult monkeys (Lines and Waisman, 1970a). β-2-T reduces intestinal absorption of Phe in monkeys with much less fall in serum tryptophan levels than that produced by cycloleucine or p-chlorophenylalanine (Lines and Waisman, 1970b). No long-term effect on growth or development of β-2-T-treated infant monkeys has been noted (Lines and Waisman, 1973), and β-2-T depresses the serum Phe level after an oral Phe dose in phenylketonuric (PKU) patients (Krips and Lines, 1972). It interacts with Phe at the intestinal mucosa in the rat by inhibiting the specific Phe transporter, while not inhibiting transport of tyrosine (Tyr) (Wapnir and Lifshitz, 1974). The interaction appears to be a cell surface phenomenon, rather than an intracellular competition (Munro and Clark, 1958). β-2-T is therefore being considered as a possible treatment agent for phenylketonuria.

As part of a study of possible analogue-induced side effects, β-2-T-inhibition of bacterial growth has been studied. An assay has been developed which differentiates gram-negative genera according to whether the organisms are inhibited by β-2-T or not. *Escherichia* and *Shigella* strains are inhibited by β-2-T, while *Salmonella*, *Enterobacter*, *Proteus*, *Citrobacter*, and *Pseudomonas* strains are not inhibited in the assay (Brown and Lines, 1976). By increasing the Mg⁺⁺ concentration in the assay medium, inhibition can be abolished for all gram-negative genera (Brown and Lines, 1978), and can be modified for *Bacillus subtilis* in the Guthrie and Susi (1963) PKU assay (Brown and Lines, 1978; Brown, Elliott and Lines, 1979). Phe also reverses bacterial inhibition by β-2-T *in vitro* (Beerstecher and Shive, 1946; Dittmer et al., 1946; Drea, 1948; Dunn and Dittmer, 1951). Only 7% of anaerobic isolates from faeces of PKU patients are inhibited by β-2-T *in vitro*, and the addition of Phe abolishes growth inhibition for these bacteria (Brown, Vesey, Tannock, Bell, Elliott and Lines, in preparation). Indications are then that β-2-T, while competing for intestinal absorption of Phe and reducing blood levels of Phe, may have little effect on the indigenous bacteria during *in vivo* treatment with β-2-T. However, infant monkeys treated long-term with this agent may be predisposed to *Shigella* gastroenteritis (Lines and Waisman, 1973). In order to assess such *in vivo* effects, mice have been treated with β-2-T. If β-2-T were to seriously affect growth of host indigenous bacteria, then pathogenic bacteria might be expected to colonize such experimentally treated animals more easily than water-fed control animals of the same litter. Since *Escherichia* are inhibited by β-2-T *in vitro* (Brown and Lines, 1976), there may be a reduction in recovery of coliforms from β-2-T-fed animals as compared to water-fed controls. Streptomycin is known to reduce gram-negative bacterial populations, allowing *Salmonella typhimurium* to colonize mice at much lower dosages than in un-