THE ROLE OF THE RYEGRASS ENDOPHYTE (ACREMONIUM LOLIAE) IN RUMINANT NEUROTOXICOSIS

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The neurotoxicosis ryegrass staggers causes difficulties in ruminant management particularly in parts of New Zealand but also occasionally in other parts of the world where ryegrass is the dominant pasture grass. In severe outbreaks mortality may occur but the syndrome is usually reversible. The principal neurotoxin is lolitrem B, a substituted indole-diterpenoid, detected only in perennial ryegrass containing the endophytic fungus Acremonium loliae (ref. 1,2). Lolitrem biosynthesis is predictably by the fungus since all other natural products containing the characteristic indole-diterpenoid moiety are fungal metabolites. Although lolitrem biosynthesis has not been demonstrated by A. loliae in axenic culture the indole-diterpenoid paxilline has been shown to be a metabolite of the endophyte both in submerged fermentation and occurring as a co-metabolite with lolitrem B in ryegrass seed (ref. 3). Assuming therefore that A. loliae is responsible for biosynthesis of at least the indole-diterpenoid part of the lolitrem molecule it is evident that the fungus has a direct role in making ryegrass potentially neurotoxic. Expression of neurotoxicosis will be conditional partly on the concentration of neurotoxins in grass and partly on the efficiency of elimination of ingested toxin. It is not possible to obtain lolitrem B biosynthetically-radiolabelled with $^{14}$C in order to follow its fate in animals. Thus analogous model systems provide the only way of predicting the route of elimination, and any molecular transformation in the fate of this xenobiotic. $^{14}$C-penitrem mycotoxins are eliminated almost exclusively in sheep bile without hepatic transformation but are transformed to at least two more-polar metabolites (ref. 4). However, penitrems are not necessarily ideal experimental models for lolitrems since the penitrem aromatic substituents are linked to the diterpenoid moiety giving additional structural rigidity. Thus, paxilline, being a putative biosynthetic precursor of lolitrem B and a simpler and chemically more stable molecule
than penitrem A, has been studied. $^{14}$C-Paxilline, prepared from Penicillium paxilli given 2-$^{14}$C-mevalonate, was incubated in sheep bile at 37°C overnight. Autoradiography of a thin-layer chromatogram of a chloroform extract of the incubate showed that paxilline is transformed to one more-polar compound, the structure of which has recently been elucidated from spectroscopic (mass and $^1$H NMR) evidence. The transformation involves addition of two atoms of oxygen across the 2,3 double bond of the indole moiety to open an eight-membered ring between the indole aromatic ring and the terpenoid moiety.

Since there are no obvious structural constraints precluding a similar transformation in lolitrem neurotoxins, this method of increasing polarity to reduce the incidence of entero-hepatic recycling, together with the probability of diminished biological activity, effectively enhances toxin elimination. Thus efficient hepatic function with respect to bile secretion will be a factor in protecting ruminants from otherwise toxic intake of tremorgenic neurotoxins.

REFERENCES