MANIPULATION OF LIVER METABOLISM IN RELATION TO RUMINANT TOXICOLOGY

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SUMMARY

In an experimental model the hepatotoxic effects of carbon tetrachloride to sheep were dramatically modified by pretreatments with hexachlorobenzene and carbon disulphide which are known, respectively, to increase and decrease the activity of liver drug-metabolizing enzymes. Using similar hexachlorobenzene pretreatments, significant protection was afforded to sheep experimentally poisoned with mycotoxic tremorgens in *Penicillium cyclopium* cultures and with sporidesmin, the mycotoxin responsible for facial eczema. Subsequently, in field trials, significant seasonal protection was afforded to sheep against outbreaks of perennial ryegrass staggers and facial eczema by prior treatment with hexachlorobenzene.

INTRODUCTION

Disruption of management systems, loss of production, overt disease and deaths in farm animals result from the ingestion of toxins of plant and fungal origin. Two important examples which plague graziers, particularly in New Zealand, are facial eczema and ryegrass staggers.

Mammals in general possess inbuilt mechanisms for the metabolism, detoxification and excretion of foreign compounds (xenobiotics). The major site of detoxification is the liver, where drug metabolizing enzyme (DME) systems, associated with hepatocyte microsomes, metabolize toxins to produce generally less harmful substances. The dynamic nature of enzyme systems permits manipulation of DMEs to either increase or decrease their capacity to detoxify xenobiotic compounds.

A potential and novel means of protection from toxic xenobiotics present in the grazing animal environment could be the stimulation of natural detoxifying mechanisms to a degree which would afford worthwhile protection throughout seasonal toxic hazards.

The following trials illustrate modification of susceptibility of sheep to certain toxic substances by means of pretreatments which either induce or suppress DME activity both in experimental models and in field exposure to facial eczema and ryegrass staggers over the season of danger.

EXPERIMENTAL AND RESULTS

Hexachlorobenzene (HCB) an inducer of DME activity (Turner and Green, 1974) was given in peanut oil by six intraperitoneal injections on alternate days to a total dose of 15 mg HCB per kg liveweight. For suppression of DME activity carbon disulphide (CS_2) diluted in peanut oil was given by stomach tube as a single dose of 0.05 ml per kg liveweight immediately prior to toxic challenge.

In the following experiments subjective assessments and scores relating to degree of liver injury or severity of neurological symptoms were made without prior knowledge of group identity. Levels of serum gamma glutamyl transpeptidase (GGT) activity were made to assess levels of liver injury in experiments where hepatotoxins were involved.

Manipulation of Carbon Tetrachloride Hepatotoxicity

While carbon tetrachloride (CCl_4) *per se* has low toxic activity to sheep it is, however, metabolized within the liver to form products of much greater hepatotoxic activity.

Two groups of six sheep were pretreated with either HCB or with CS_2 while a third (control) group received no pretreatment. Sheep of all three groups were then simultaneously given an equal dose of CCl_4. The toxic effects produced were assessed on change in serum enzyme levels and on mortality.

The protective effect of CS_2 (mediated by inhibiting the metabolism of CCl_4 to a more toxic metabolite) is seen in Fig. 1 where enzyme activities in that group of sheep deviated little from normal values. In the control (no pretreatment) group mean serum GGT values showed marked increases indicating liver injury over the first 48 hours. Thereafter values decreased and all sheep recovered. The HCB pretreated sheep showed more rapid increases in serum GGT levels and the toxic effect of CCl_4 was greatly augmented. In contrast to the control group, two sheep died within 12 hours and all were dead before 72 hours.

Protection of Sheep Dosed with Mycotoxic Agents

Sporidesmin-induced (Facial Eczema) Liver Injury

Two groups of ten sheep, a control group, and a group pretreated with HCB as previously described, were identically dosed with 0.4 mg sporidesmin per kg liveweight, spread over five days.

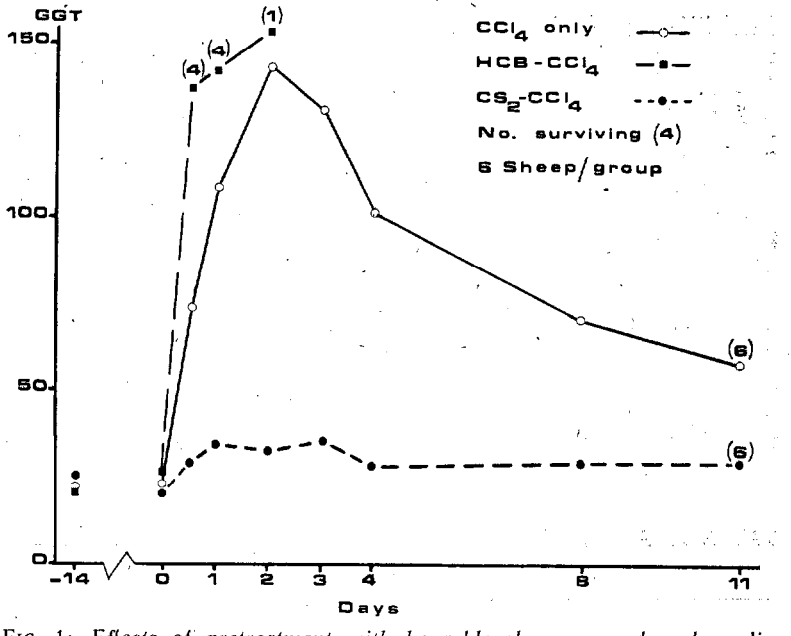

FIG. 1: *Effects of pretreatment with hexachlorobenzene and carbon disulphide in sheep poisoned with carbon tetrachloride.*

Severity of liver injury was evaluated by serum GGT levels and by visual assessment of degree of liver injury found in sheep at slaughter. Two sheep, both in the control group, became photosensitized and jaundiced. Mean serum GGT levels and mean scores of liver injury for the two groups are presented in Table 1.

TABLE 1: ASSESSMENT OF SPORIDESMIN-INDUCED LIVER INJURY

	Mean Serum GGT[1]	*Liver Score*	
		No. Affected	*Mean Score*
Control	231	9	1.9
HCB	81	2	0.3
Significance	**	**	

10 Sheep per group: [1] results on day 26.

Tremorgen-induced Inco-ordination and Staggers

Tremoring, inco-ordination and collapse, symptoms typically seen in perennial ryegrass staggers, were produced in sheep and cattle orally dosed with cultures of the soil-inhabiting fungus

Penicillium cyclopium (di Menna *et al.*, 1976) which contained the neurotoxic tremorgens penitrems A and B (Wilson *et al.*, 1968).

A pilot experiment had indicated that prestimulation of DME in sheep afforded protection against the effect of tremorgenic cultures of *P. cyclopium* (P. H. Mortimer and P. G. Mantle, unpubl. findings). For confirmation, two groups of 20 sheep, one group of which had previously received HCB as described, were dosed over six days with tremorgenic *P. cyclopium* cultures (total individual dose 16 g air-dried weight) to produce severe tremors and inco-ordination. Severity of clinical symptoms of individuals was scored daily on a 0 to 5 scale similar to that described by Keogh (1973). Throughout the period of dosing the severity of symptoms increased but on cessation of dosing recovery was made in three days. The extent and severity of symptoms scored on day six are given in Table 2.

TABLE 2: ASSESSMENT OF EXTENT AND CLINICAL SEVERITY OF *P. CYCLOPIUM* INDUCED "STAGGERS"

	No. Affected	*Mean Score*
Control	18	2.35
HCB	5	0.30
Significance	***	

20 sheep per group: results on day 6.

Seasonal Protection of Sheep from Facial Eczema and Ryegrass Staggers

In the two previous experiments the effects of two widely different mycotoxic agents were lessened by prior augmentation of drug metabolizing enzymes by HCB. This provided further evidence that sporidesmin and penitrems are toxic *per se* and, in contrast to CCl_4, do not require metabolism in the liver to assume toxicity.

Therefore, trials were organized to determine if pretreatment of sheep with HCB could provide worthwhile protection throughout the common season during which facial eczema and ryegrass staggers occur. Groups of 50 sheep were equally divided and matched for weight, 25 were given HCB as previously described, and 25 served as controls. Matched groups were placed on trial sites which had been chosen for their known high probability for

the occurrence of one or both diseases. Results are presented from two of these sites where an outbreak of each disease occurred.

Seasonal Protection from Facial Eczema — Ohaupo Trial

The first clinical case of facial eczema was noted on 18-3-77, 28 days after this group was placed on the trial site. Subsequently, weekly serum samples were obtained from all sheep to determine GGT enzyme levels. In May, when all danger of toxic pasture had receded, the sheep were returned to Ruakura, slaughtered and degrees of liver injury contracted during the season were assessed. Mean values of serum GGT on day 53, and the mean liver injury scores found are presented in Table 3.

TABLE 3: ASSESSMENT OF LIVER INJURY IN NATURALLY OCCURRING FACIAL ECZEMA

	Mean Serum GGT[1]	*Liver Score*	
		No. Affected	*Mean Score*
Control	127	24	1.80
HCB	46	17	1.04
Significance	***	*	

25 Sheep per group: [1] results on day 53.

Seasonal Protection from Ryegrass Staggers — Te Akau Trial

Clinical cases were first apparent on 23-3-77, 19 days after this group was placed on trial. They rapidly assumed the proportions of a severe outbreak since, by day 32, 88% of the control group were clinically affected. In the HCB pretreated group recovery was rapid and virtually complete within 12 days from peak severity, whereas in the control group there was a protracted recovery over 32 days. Clinical assessments made at the height of the outbreak are presented in Table 4.

TABLE 4: ASSESSMENT OF EXTENT AND CLINICAL SEVERITY IN A FIELD OUTBREAK OF RYEGRASS STAGGERS

	No. Affected	*Mean Score*
Control	22	3.20
HCB	9	0.68
Significance	***	

25 Sheep per group: results on day 32.

DISCUSSION

The induction and repression of DME activity as means of modifying the action of toxins in experimental animals are well established (Allen and Seawright, 1973; Reynolds and Moslen, 1974; Gopinath and Ford, 1975). The experiments described here extend existing knowledge towards practical uses, for they provide convincing evidence that stimulation of DMEs in sheep can be sufficiently pronounced to give worthwhile protection against naturally-occurring toxic diseases over several months.

The toxic challenge provided in the field outbreaks of ryegrass staggers was of a very high level, yet HCB pretreatment reduced the outbreak to manageable levels and also curtailed its duration. With regard to facial eczema, the season did not produce such a high toxic challenge, for it induced mainly subclinical liver injury. In these circumstances HCB protection was significant, but protection from more severe challenges needs to be established.

In food animals carcass residues of HCB would present an unacceptable toxic hazard to man (Cam and Nigogosyan, 1963; de Matteis *et al.*, 1961; Cabral *et al.*, 1977). With the establishment of the protective principle in sheep using HCB we are now examining the practical use of a number of accepted food additives which are known to induce DME activity in laboratory animals.

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