THE INFLUENCE OF DIETARY FACTORS AND DRUG-PROCESSING ENZYMES ON SPORIDESMIN POISONING IN SHEEP — A PRELIMINARY REPORT

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SUMMARY

Studies on the excretion of sporidesmin by sheep showed that concentrations of unchanged sporidesmin excreted in the bile and urine accounted for the severity and pattern of lesions found in facial eczema. In the light of these findings, the pathogenesis of facial eczema is briefly reviewed.

In view of recent work on the effects of administered drugs and protein content of diet in altering the metabolism and excretion of foreign substances by the liver, it seemed pertinent to attempt to influence the metabolism and excretion of sporidesmin by this means. A preliminary report is given on two experiments which indicate that dietary factors can influence the effects of sporidesmin in a way deleterious to sheep. The experiments also suggest that some protective effect may be produced by prestimulation of the drug-processing enzymes in the liver.

THE experimental pathology of poisoning with sporidesmin, the major facial eczema toxin, has been studied both in animals and in *in vitro* systems (Mortimer, 1963; Mortimer and Collins, 1968; Mortimer and Stanbridge, 1968). While the major symptoms of the disease are due to loss of excretory functions of the liver, these studies have clearly shown that sporidesmin is not specifically a liver toxin. From these studies can be drawn the following conclusions which are pertinent to an understanding of the pathogenesis of facial eczema.

BIOLOGICAL PROPERTIES OF SPORIDESMIN

(1) Many types of cells, irrespective of embryonic origin, are susceptible to sporidesmin both *in vivo* and *in vitro*.

(2) Sporidesmin is cytotoxic *per se* and does not require activation by specialized metabolic processes in the intact animal.

(3) All evidence indicates that even small alterations to the molecular structure of sporidesmin lessen or destroy its toxicity (Mortimer and Collins, 1968).

(4) Irrespective of liver conjugatory mechanisms, sporidesmin is sufficiently water-soluble to attain within the animal aqueous concentrations which are cytotoxic.

(5) At low concentrations, it is a highly potent inflammatory substance, in certain circumstances its activity in initiating inflammation being comparable with histamine (Mortimer and Stanbridge, unpubl.).

(6) At higher concentrations, it causes irreversible changes in cells which result in their destruction.

As with practically all toxic compounds, the specific mechanisms of sporidesmin at the cellular level remain unknown.

Excretion of Sporidesmin in Relation to the Pathogenesis of Facial Eczema

The cytotoxic effect of sporidesmin on cell monolayer cultures *in vitro* is characteristic. This factor, together with its high cytotoxic activity (detectable to concentrations as low as 0.4 mμg per ml of media) has facilitated its detection and assay in biological fluids, such as serum, urine and bile, taken from sheep given realistic doses (1.0 mg per kg bodyweight) of sporidesmin by mouth (Mortimer and Stanbridge, 1968).

The major technical difficulty inherent in these studies was in obtaining repeated bile samples from conscious sheep without disturbing their normal bile homeostasis. A successful method was devised to catheterize permanently the total bile flow from the liver to the exterior and then return it to the common duct (Stanbridge and Mortimer, 1968). Using these model sheep, bile was obtained whenever necessary after dosing sporidesmin. Up to 20% of the sporidesmin given by mouth has been recovered unchanged from the bile in the first 24 hours after dosing. The concentrations of sporidesmin assayed in bile, urine and serum following oral dosage are given in Table 1. There was a hundredfold increase in the concentration of sporidesmin in its transference from serum to bile through the liver, while there was only a tenfold increase during its transference from serum to urine through the kidney. The relative concentrations in these three biological fluids would account for the severe inflammatory lesions found in the bile excretory system, the mild inflammatory lesions in the urine excretory tract, and the

TABLE 1: SPORIDESMIN ACTIVITY DETECTED IN BIOLOGICAL FLUIDS OF SHEEP GIVEN A SINGLE ORAL DOSE (1 MG/KG BODYWEIGHT)

Body Fluid	*Range of Time Detected*	*Time of Maximum Concentration (hr)*	*Maximum Concentration (μg/ml)*
Serum	15 min to 48 hr	0.25 to 12	0.2
Urine	4 hr to 48 hr	8 to 12	2
Bile	10 min to 72 hr	2 to 8	20

general lack of inflammatory lesions elsewhere in the animal where, in the absence of concentrating mechanisms, levels of sporidesmin similar to those found in serum would apply.

In sporidesmin poisoning, the essential lesion is that of inflammation and necrosis of bile ducts and the lesion is attributed to the excretion and concentration of unchanged sporidesmin by the liver. This lesion appears within two days of poisoning. Injury to liver cells (hepatocytes) produced directly by sporidesmin at this time is slight and is quickly reversed. The severe injury to bile ducts is slowly organized by granulative repair tissue but, in the process, the bile ducts become obliterated, so initiating biliary obstruction which takes at least ten days to develop from the time of poisoning. Bile flow then ceases and excretory function is largely lost. Contemporaneously, jaundice and photosensitization develop, these symptoms being due, respectively, to retention in the circulation of bile pigments and phylloerythrin normally excreted in the bile. Retention of metabolic products as, for example, bile acids in the liver, then causes secondary injury to hepatocytes. However, even after prolonged biliary obstruction, many of the liver's metabolic activities still function in some degree so that, with most animals which succumb, death results from the severe stress of photosensitization and the resultant inability to graze, rather than from metabolic dysfunctions of the liver.

DRUG-METABOLIZING ENZYMES AND DIETARY FACTORS IN TOXIC DISEASES

It has been known for several years that laboratory and larger mammals rapidly develop a tolerance to barbiturate hypnotics and many other foreign substances. There is strong evidence that this is due to stimulation of liver microsomal enzymes which are concerned with meta-

bolizing or processing foreign substances (see Conney and Burns, 1962). Recently, McLean and McLean (1965, 1966) demonstrated that feeding a protein-free diet for several days to rats reduced their ability to metabolize exogenous foreign compounds to 10% normal. The metabolizing capacity of these protein-depleted rats could be increased to levels up to and in excess of normal by repeated injections of sodium phenobarbitone (Conney and Burns, 1962) or single injections of DDT (Fouts, 1963). They also showed that protein-depleted rats were more resistant to carbon tetrachloride (CTC) poisoning than were identically fed rats prestimulated with either phenobarbitone or DDT. This they explained by the suggestion that drug-processing enzymes in the liver metabolized CTC to a highly toxic product.

Seawright and McLean (1967) have since found that a group of sheep fed a low-protein (5.5%) diet was much more resistant to doses of CTC than was an identically-fed group given five daily injections of phenobarbitone before dosage with CTC. Considering dietary factors alone, a study of liver pathology and changes in liver enzymes by histochemical methods on sheep dosed with CTC has shown that there is considerably less liver injury and less disturbance of liver enzyme patterns in sheep pre-fed a low-protein (6%) diet than in sheep pre-fed a high protein (26%) diet (Mortimer and Manns, unpubl.).

In contrast to CTC poisoning, the evidence presented earlier indicates that (a) sporidesmin does not require processing by liver microsomal enzymes to acquire toxicity, (b) processing of sporidesmin by the liver might well reduce or destroy its toxicity, (c) the presence of non-metabolized sporidesmin in the bile is largely responsible for the bile-duct injury and the subsequent pathogenesis of facial eczema. Therefore, if the amount of unmetabolized sporidesmin excreted in bile could be altered either by (1) *stimulation* of processing enzymes by pre-feeding high-protein diet and/or by drug stimulation, or by (2) *depression* of processing enzymes in the liver by pre-feeding low-protein diet, then the induced changes in levels of processing enzymes would be reflected in severity of the ensuing disease process initiated by sporidesmin.

EXPERIMENTAL

Two experiments designed to produce wide differences in the amount of microsomal enzymes present in the livers of sheep before dosing them with sporidesmin have been

conducted. To avoid possible effects of nutrition on the course of the disease process once initiated, all groups after dosing with sporidesmin were placed in the same paddock which provided their sole diet.

The first experiment involved two groups of 12 sheep maintained indoors and a group of 10 sheep maintained on pasture. During the induction period of 14 days, one indoor group was fed *ad libitum* on silage made from maize, forage harvested at the green stage. The total crude protein content of the silage was 7.5%. The other indoor group was fed good quality hay *ad libitum,* plus a daily supplement of 170 g of proprietary sheep nuts (min. protein 20%) per head. This latter group also received five daily injections of phenobarbitone (40 g/kg bodyweight) in the second week of the feeding period, as a result of which two sheep died. At the end of the induction period, all remaining sheep in the three groups were dosed with 0.7 mg sporidesmin per kg bodyweight and placed together outdoors. It soon became apparent that the low-protein group were sick, inappetance became pronounced, and four deaths occurred before the 7th day. The first death in the phenobarbitone stimulated group was on the 32nd day and at this time there had been nine deaths in the maize-silage fed group and two deaths in the non-stimulated group fed throughout at pasture. On the 46th day after dosing, all remaining animals were recovering and the experiment was terminated. The results of the experiment are summarized in Table 2.

TABLE 2: SUMMARY OF EXPERIMENT 1
SHEEP DOSED 0.7 MG SPORIDESMIN PER KG BODYWEIGHT

Group and Diet	*Induction Phase*		*Progressive Number of Deaths*		
	Phenobarbitone Stimulation	*Av. Weight Deviation (kg)*	*At 12 days*	*At 26 days*	*At 46 days*
A. Maize silage	No	— 0.2	4/12	8/12	11/12
B. Hay plus pellets	Yes	+ 1.5	0/10	0/10	2/10
C. Grazing pasture	No	—	1/10	2/10	4/10

Significance of differences in survivals:
A *v.* B, $P < 0.01$
A *v.* B + C, $P < 0.05$
Other comparisons not statistically significant.

The second experiment utilized better-defined high and low protein diets and also separated the effects of high protein diet from the effects of drug stimulation. For this purpose, three groups of 11 sheep were used; two of the groups received a low-protein pelleted diet and the third group received a high-protein pelleted diet for 14 days. The composition of the diets is given in Table 3. During the second week of the diet-induction period, one of the two low-protein fed groups was dosed with phenobarbitone (30 mg/kg) daily for five days. All three groups were then dosed with sporidesmin as in the first experiment and were then maintained on a diet of grass in the same paddock. This experiment was also terminated on the 46th day after dosing when remaining animals were recovering. The summarized results of the second experiment are given in Table 4.

TABLE 3: COMPOSITION AND CALCULATED CONTENT OF DIETS FED IN EXPERIMENT 2

Low Protein Diet (%)		*High Protein Diet (%)*	
Ground maize	45	Linseed cake meal	55
Ground barley straw	45	Grass meal	10
Molasses plus mineral premix	10	Barley meal	15
		Molasses plus mineral premix	5
% DOM = 62		= 64	
% D.C.P. = 4.5		= 22	
% C.F. = 17		= 7.5	

TABLE 4: SUMMARY OF EXPERIMENT 2
SHEEP DOSED 0.7 MG SPORIDESMIN PER KG BODYWEIGHT

	Induction Phase		*Progressive Number of Deaths*		
Group and Pelleted Diet	*Phenobarbitone Stimulation*	*Av. Weight Deviation (kg)*	*At 12 days*	*At 26 days*	*At 46 days*
A. Low protein	Yes	— 0.1	1/11	1/11	2/11
B. Low protein	No	— 0.6	1/11	1/11	4/11
C. High protein	No	+ 4.5	0/11	1/11	4/11

Differences between group treatments, in terms of survival, not significant.

DISCUSSION

The results presented are from preliminary experiments and a rigid interpretation is not possible at this stage. However, several points emerge. The first experiment showed that diet can significantly influence the susceptibility of sheep to sporidesmin, but in a manner which is not yet clear. Considering protein in its broadest undefined sense, low-protein diet, while protecting sheep from CTC poisoning, had no influence on sporidesmin-dosed sheep. While the first experiment may have indicated increased susceptibility owing to the low-protein content of the maize-silage diet, this interpretation was invalidated by the second experiment in which the extremely diverse levels of protein in the diets made no difference to the susceptibility of the groups. However, proteins differ greatly in quality and content of amino acids and in these experiments they were not defined.

With regard to the effects of prestimulation of the drug-processing enzymes in the liver, with the number of animals used the results were not statistically significant. Yet the results may indicate some protective effect, for deaths in the prestimulated groups in both experiments were 50% lower than in the respective non-stimulated groups.

The results are consistent with the hypothesis that sporidesmin, in contrast to CTC, requires no metabolism by the liver to exert its toxic effects, for the factors which are known to stimulate microsomal enzyme activity produced no increase in the toxic effects produced by sporidesmin.

Experiments are being conducted to clarify the factors involved in diet-induced changes in susceptibility, for they could have a bearing on the severity of outbreaks of facial eczema.

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