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BIOCHEMISTRY OF SPORIDESMIN

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SUMMARY

The biosynthesis of sporidesmin labelled with carbon-14 from radioactive tryptophan, alanine, serine, glycine or methionine is reported. Sulphur-35 from labelled sulphate, methionine or cysteine is also incorporated into the toxin. A possible pathway for its biosynthesis is proposed.

The level of dietary protein influences the susceptibility of small animals to sporidesmin, the animals being more easily poisoned when fed a low-protein diet. The addition of casein, phosphopeptones prepared from casein, or synthetic DL-phosphoserine had a protective effect. Regression of the thymus gland in poisoned rats was observed, the extent of the regression being a measure of the severity of the illness.

TEN YEARS AGO in a symposium organized by the N.Z. Society of Animal Production, Percival (1959) concluded "many scientists have been attracted to what has become a narrow field of infinite length". While knowledge of *Pithomyces chartarum* has increased, and the chemistry of sporidesmin and the pathology of the diseased liver been well documented, there are few signs that the road leading to a complete biochemical explanation for sporidesmin toxicity is nearing the end.

This paper discusses some recent research into sporidesmin biosynthesis and poisoning carried out in the last two years at Ruakura by E. L. Hove, N. Towers and the writer.

BIOSYNTHESIS OF SPORIDESMIN

Using radioactive precursors added to growth medium, the biosynthesis of sporidesmin has been studied with the dual aims of understanding how the poison is synthesized and to develop methods for preparing labelled sporidesmin for studies on its absorption, excretion and metabolism in animals.

Sporidesmin labelled with carbon-14 was isolated from cultures grown on medium containing DL-tryptophan-^{14}C (methylene), L-alanine-U-^{14}C, L-serine-U-^{14}C, DL-serine-1-^{14}C, glycine-U-^{14}C, glycine-1-^{14}C, and L-methionine-^{14}C (methyl). Tryptophan and serine were the amino acids labelling

sporidesmin to the greatest extent. Sulphur-35 from sulphate-^{35}S, L-methionine-^{35}S or L-cysteine-^{35}S, and chloride-^{36} from Na^{36}Cl were also readily incorporated into the molecule.

This pattern of isotope incorporation suggests that the basic ring structure of sporidesmin is derived by tryptophan condensing with alanine through a peptide bond followed by ring closure. The radioactivity in the toxin from serine and glycine presumably results from the *de novo* synthesis of tryptophan from indole and serine, the latter being formed from glycine (White *et al.*, 1964). Supporting evidence is the observation that the addition of tryptophan to the culture medium reduces the incorporation of carbon-14 from glycine and serine into the toxin. Evidence for alanine being incorporated intact into sporidesmin is largely circumstantial, being based on the structure of the sulphur-containing ring in the poison.

The incorporation of carbon-14 from the methyl of methionine is probably due to the presence of methyl and methoxyl groups in sporidesmin. The biosynthesis of sporidesmolides by *P. chartarum* has been shown to involve the metabolism of methionine to methyl groups (Butler *et al.*, 1962) and the methyl of methionine has been shown to be the precursor of the N-methyl in another mycotoxin, gliotoxin (Winstead and Suhadolnik, 1960).

The experiments using sulphur-35 show that sporidesmin with high specific activity can be prepared from labelled sulphate, methionine or cysteine. Since radioactive sulphate is relatively cheap, this precursor is the most suitable isotope for preparing labelled sporidesmin (Brook and Matthews, 1960). So far there is no information on how the sulphur is incorporated nor whether the sulphur in the immediate precursor is organically bound.

The suggested route for biosynthesis of sporidesmin from these amino acids is, of course, largely speculative and confirmation of the synthesis must await the stepwise degradation of the toxin to measure the distribution of the radioactivity in the molecule.

The preparation of sulphur-35 sporidesmin has been valuable in determining how quickly the poison is absorbed from the digestive tract in rats and guinea-pigs, and in sheep, and has enabled measurements to be made of its rate and route of excretion. Preliminary studies have shown that unchanged sporidesmin-^{35}S can be isolated from faeces, bile and urine confirming earlier work (Mortimer and Stanbridge, 1968). The presence of sulphur-35 which could not be extracted from tissues, faeces and

urine with organic solvents, indicates the presence of sulphur-containing metabolites of the poison or bound forms of the toxin. This is being further investigated.

EFFECT OF DIET ON SPORIDESMIN TOXICITY

The level of protein in the diets of small animals has been found to influence the susceptibility of these animals to sporidesmin (Hove and Wright, unpubl.).

When rats fed on a diet containing 22% protein were dosed 20, 40, 60 or 80 μg of sporidesmin daily for three weeks, the death rates were 7, 45, 71 and 88%, respectively. Rats fed on a 9% protein diet were more easily poisoned, daily doses of 20 or 40 μg of poison killing 86 and 84%, respectively. Similarly, guinea-pigs dosed orally for 11 days at the daily rate of 0.1 mg/kg bodyweight were more easily poisoned on a 9% protein diet, 5/6 being dead within 23 days of the commencement of dosing, whereas 0/6 died in the group fed a 22% protein diet.

The addition of rennet casein to the normal diet (22% protein) provided further protection to rats (Hove and Wright, unpubl.). In these experiments, casein was added to give a diet containing 37% protein. Rats dosed daily with 20, 40, 60 or 80 μg of toxin as above and fed the 37% protein diet had mortality rates of 0, 0, 13 and 38%, respectively, these figures being much lower than those reported above for the normal diet. Rabbits dosed with 20 μg of sporidesmin daily for ten days reacted similarly, 4/4 fed 22% protein dying within four weeks after dosing ceased, whereas 4/4 of the rabbits on the 37% protein diet were healthy and had gained weight.

The protection afforded by casein is not due to its role as a protein in supplying essential amino acids, since the addition of casein did not stimulate the growth of the animals, nor did the addition of other proteins in the form of gelatin or soya bean meal have any protective action.

The addition of casein ash, cystine, methionine, tryptophan, selenocystine or diphenyl paraphenylenediamine had no effect.

The role of casein in protecting small animals against sporidesmin appears to be a function of its phosphoprotein structure. The unique chemistry of the phosphoproteins depends on the bonding of phosphorus to certain amino acids, principally serine and threonine. The hydrolysis of casein with trypsin, followed by precipitation with lead acetate, yields a phosphorus-rich phosphopeptone fraction. The addition of this crude material to control

diets at a concentration of 1.3% (w/w) provides protection against sporidesmin equivalent to that of casein.

Since this phosphopeptone was active, DL-phosphoserine was tested at a concentration in the diet of 0.6% (w/w) and the results show that this substance, while not as effective as casein or the phosphopeptone, gave at least partial protection with 1/5 rats dying compared with 4/5 on the control diet. This protection was also reflected in the mean weights of these groups of animals, which were 181 g and 112 g, respectively.

An interesting observation from these experiments is the regression of the thymus gland during sporidesmin poisoning. This regression began when the animals started to lose weight and, in moribund or dead rats, only a few mg of gland remained compared with control animals in which the thymus gland weighed 500 to 600 mg. The relative size of the thymus has been found to be a good criterion of the severity of the poisoning and, in poisoned animals on the high casein diet, or on diets containing phosphopeptone or DL-phosphoserine, the glands were much greater than those on the control diet.

These results are the first to suggest that phosphoproteins and phospho-amino acids may have a nutritional function, but how these substances work is not known. Indeed, knowledge on the metabolic role of phosphoproteins such as casein, phosvitin from hen's egg yolk, or those found in tissues such as liver or brain, is very limited.

Other workers have reported that the severity of damage caused by some liver-damaging chemicals can be influenced by the protein content of the diet. Protein-depleted rats have increased resistance to carbon tetrachloride (McLean and McLean, 1966) whereas rats on low-protein diets are more sensitive to aflatoxin (McLean and McLean, 1967; Madhavan and Gopalan, 1965). The level of dietary protein is thought to influence the activity of microsomal detoxifying enzymes. A low-protein diet will reduce the level of these enzymes and with carbon tetrachloride this limits the production of toxic metabolites, whereas with aflatoxin a high level of microsomal activity will convert the mycotoxin to less toxic products. With sporidesmin, similar effects caused by protein may be occurring but, since the evidence for sporidesmin detoxification is inconclusive, further work is needed.

This protective effect by a phosphoprotein opens up a fascinating field for biochemical research. With regard to sporidesmin poisoning, it remains to be determined how the protection is effected and whether it can be applied

to ruminant animals in which the microbial activity in the rumen degrades much of the food protein. From a broader aspect, the metabolic role of phosphoproteins in nutrition and metabolism deserves further study.

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REFERENCES

Brook, P. J.; Matthews, R. E. F., 1950: *N.Z. Jl Sci., 3*: 591.
Butler, G. W.; Russell, D. W.; Clarke, R. T. J., 1962: *Biochim. biophys. Acta., 58*: 507.
Madhaven, T. V.; Gopalan, C., 1965: *Arch. Pathol., 80*: 123.
McLean, A. E. M.; McLean, E. K., 1966: *Biochem. J., 100*: 564.
————; ————, 1967: *Proc. Nutr. Soc., 26*: xiii.
Mortimer, P. H.; Stanbridge, T. A., 1968: *J. comp. Path., 78*: 505.
Percival, J. C., 1959: *Proc. N.Z. Soc. Anim. Prod., 19*: 77.
White, A.; Handler, P.; Smith, E. L., 1964: *Principles of Biochemistry*. McGraw-Hill Co., London. p. 541.
Winstead, J. A.; Suhadolnik, R. J., 1960: *J. Am. chem. Soc., 82*: 1644.