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METABOLISM OF AFLATOXIN AND OTHER MYCOTOXINS IN RELATION TO THEIR TOXICITY AND THE ACCUMULATION OF RESIDUES IN ANIMAL TISSUES

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Abstract —— Mycotoxin metabolism is discussed in relation to (1) detoxification, (2) metabolic activation and mode of action, and (3) the public health problem arising from the possible accumulation of toxic residues in the tissues of farm animals consuming toxin-contaminated feeds. The aflatoxins, sterigmatocystin, ochratoxins A and B, the trichothecenes, and zearelenone are considered as far as our available knowledge permits. Mycotoxin metabolism may not only take place in the liver; the skin and gastro-intestinal tract may be alternative sites.

INTRODUCTION

In the paper for one of the poster sessions at this Symposium I have already referred to our limited knowledge of mycotoxin metabolism (Ref. 1). Even in the case of aflatoxin the relationship between metabolism and toxicity is still being explored and, by comparison, very little is known of this aspect of the metabolism of the growing number of mycotoxins that have now been implicated in clinical or experimental animal disease.

Despite the somewhat limited information available in some cases, I shall briefly consider the metabolism of the aflatoxins, sterigmatocystin, ochratoxins A and B, and the *Fusarium* toxins with regard to their mode of action and their potential for accumulating as toxic residues in animal tissues. Before doing this however, it may be useful to define a few terms that I shall be using.

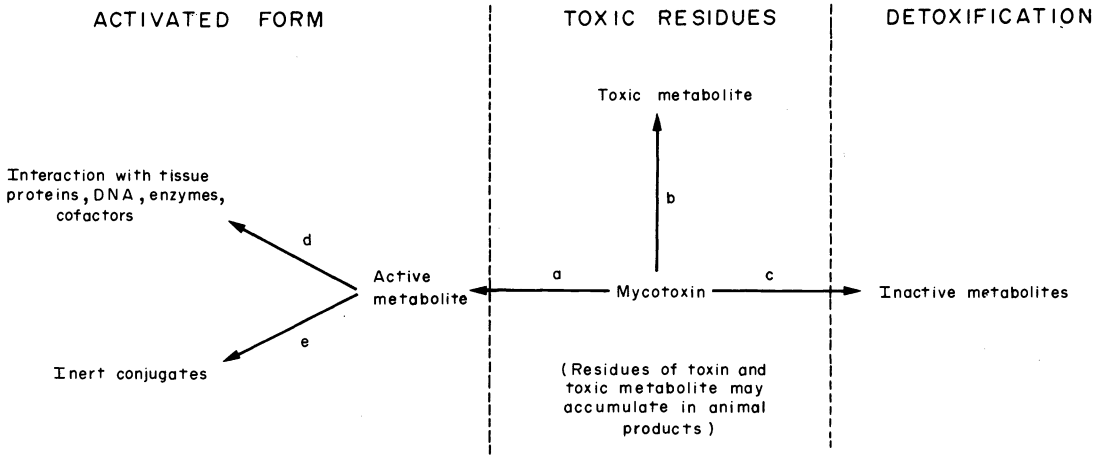
"Detoxification" will be taken to mean the biotransformation of a toxin, usually by the microsomal mixed function oxidases of the liver, to a metabolite that is either non-toxic when ingested by a second animal of the same or different species, or a metabolite that results from the conjugation of the toxin with cysteine, glutathione, or glucuronic, taurocholic or sulphuric acids in a further microsomal reaction to form more polar compounds that are rapidly eliminated from the body by excretion into the urine or bile for example.

A mycotoxin will be said to have the potential for accumulating "toxic residues" in body tissues when its metabolism *in vitro* or better still *in vivo* is known to be slow or, alternatively, when a significant pathway of metabolism results in the formation of one or more metabolites with no appreciable loss in toxicity and when such a product is slowly eliminated from the body. This potential problem is greatest when the animal in question is destined for use as human food.

"Metabolic Activation" differs from the above in that it involves biotransformation of the mycotoxin in question to at least one metabolite which, unlike the toxic metabolites just mentioned, is more toxic than the parent toxin and also plays a key role in the mechanism of toxic action in the appropriate target organ.

Clearly the precise outcome of these possible metabolic processes or biotransformations will depend upon their relative importance in a given animal species but, by definition, none of the toxins in question are detoxified so well that animals are entirely unaffected by toxin ingestion. In certain animal species however these "detoxifying" pathways appear to be better developed than in others. Metabolic activation, on the other hand, is a property not common to all mycotoxins and appears to be reserved for the most potent ones. Perhaps we can regard this as similar to the type of reaction which, in an entirely different context, Sir Rudolph Peters referred to as "lethal synthesis".

These generalised aspects of mycotoxin metabolism are summarised in Fig. 1 and the metabolism of some individual mycotoxins will now be considered in this general format but not in great detail so as to provide an overview of as many toxins as possible.



a = Activation usually occurs in the liver

b, c = Conversion to metabolites that are toxic or inert in a second animal species

d = Interaction with the target organ: liver in the case of 'activated forms' but other organs when the toxin does not undergo metabolic activation

e = Some activated toxin molecules may be detoxified (liver)

Fig. 1. A generalised scheme showing typical pathways of mycotoxin metabolism in the liver resulting in detoxification, the accumulation of toxic residues in tissues, and metabolic activation.

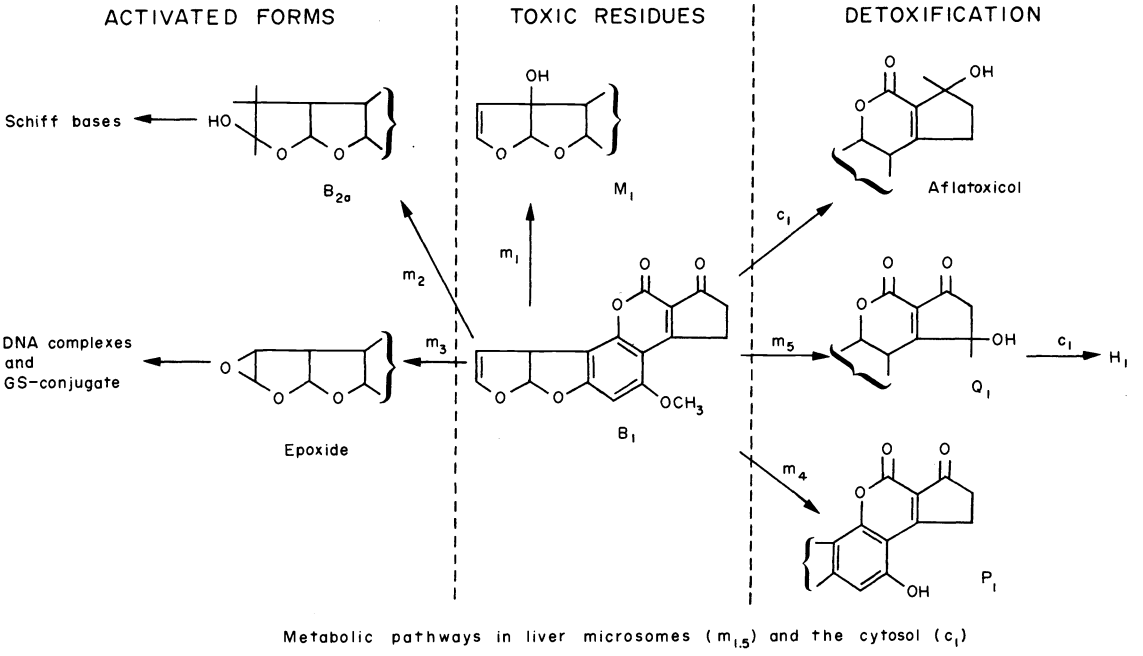


Fig. 2. Pathways for the metabolism of aflatoxin B_1 in the liver. The 2,3 dihydro analogue (aflatoxin B_2) is metabolised by reactions m_1, m_4, m_5 and c_1 and apart from c_1 and m_5 the G series of aflatoxins may be metabolised through the same pathways. GS denotes glutathione.

AFLATOXIN

As summarized in Fig. 2 aflatoxin B_1 metabolism may be considered under all three of the above headings.

Detoxification. Aflatoxin B_1 may be transformed in the liver into three hydroxylated metabolites (aflatoxicol, aflatoxin Q_1 and aflatoxin P_1) that are at least theoretically capable of conjugation to polar compounds suitable for rapid elimination from the body. To this extent these reactions involve detoxification and when tested in various *in vivo* systems for acute toxicity they are all appreciably less toxic than the parent toxin (see review, Ref. 2).

Aflatoxicol occupies a special position in that it is not formed in a microsomal mixed function oxidase reaction but by an NADP-linked dehydrogenase of the cytosol which also has 17-ketosteroid dehydrogenase activity. Further more this reaction is reversible to a greater or lesser extent and it has been proposed (Ref. 3 and 4) that this reversible reaction constitutes a "metabolic reservoir" of aflatoxin. Recently this suggestion has been developed further by Hsieh (Ref. 5) to provide a good correlation between this type of metabolism and an animal's susceptibility to aflatoxin poisoning.

It will also be noted from Fig. 2 that the same enzymic reduction of the cyclopentenone carbonyl group occurs in the case of aflatoxin Q_1 and probably this reaction is reversible.

Toxic residues. Aflatoxin is metabolised more rapidly by some animal species than others (Ref. 4) and consequently in "slow metabolising" animals (see classification in Ref. 4 and 6) aflatoxin B_1 can itself constitute a residue problem particularly in tissues of farm animals. Biotransformation to aflatoxin M_1 by microsomal enzymes (Fig. 2) is to some extent a detoxification since conjugates of this metabolite have been identified in bile (Ref. 7) and urine (Ref. 8) but because unconjugated M_1 is almost as toxic as B_1 it must still be regarded as a potential toxic residue. As is well known, a considerable public health problem exists in the excretion of aflatoxin M_1 in the milk of dairy cows consuming rations contaminated with aflatoxin.

Metabolic activation is dependent upon the existence of a 2,3-vinyl ether double bond in the aflatoxin B_1 molecule (and possibly any aflatoxin derivative carrying this chemical function). The two proposals for metabolic activation are illustrated in Fig. 2 and more detail of the interactions by the activated forms of aflatoxin B_1 with cellular structures and constituents are given in Fig. 3.

The first proposal made in our laboratory (Ref. 9 and 10) was that, as the metabolic conversion of aflatoxin B_1 to its hemiacetal, B_{2a} appeared to be characteristic of livers of animal species susceptible to acute aflatoxin poisoning, it was in this form that it interacted with vital functions of the liver cell leading to hepatocellular necrosis. At physiological and alkaline pH values it was known that aflatoxin hemiacetal rearranges to dialdehydic phenolate resonance hybrid ions (Ref. 11) and in this form it reacted with amino-acids, peptides and proteins to form Schiff bases (see Fig. 3). An important distinction was made between metabolic formation and toxicity of this product within the hepatocyte and the known lack of oral toxicity of aflatoxin hemiacetal *in vivo*. The latter we felt, could be explained by its avid protein binding capacity and thus its sequestration by the deciduous cells of the gastrointestinal tract. In this way it would effectively escape significant absorption. Thus, intra-hepatocellular formation of the hemiacetal was the central feature of this hypothesis but equal emphasis has always been placed on the fact that this biotransformation is best developed in the normal livers of animal species with a high susceptibility to acute aflatoxin poisoning.

The second proposal originated by Garner and his colleagues (Ref. 12 and 13) was that livers of most, if not all, animal species are capable of converting aflatoxin B_1 to an unidentified toxic metabolite which could be assayed in a bacterial mutagenicity test. It was suggested that this metabolic product was the 2,3-oxide, that it had a transient existence and that it alkylated bacterial DNA. So far there is no direct evidence for the 2,3-oxide but it has been shown (Ref. 14) that when aflatoxin B_1 was incubated *in vitro* with liver microsomes and RNA or DNA, a nucleic acid adduct was formed which, on mild hydrolysis, yielded the 2,3-dihydrodiol of aflatoxin, indirectly suggesting prior formation of the 2,3-oxide. Further studies by Swenson and his colleagues (Ref. 15) have shown that synthetic aflatoxin 2,3-dichloride can mimic the biological effects of this presumed metabolite in all the appropriate bioassay systems.

Thus we have two distinct hypotheses. First, that aflatoxin B_1 may be activated by a metabolic conversion to hemiacetal which binds to various key enzymes and metabolically important liver cell structures. This we might call formation of the acute toxin. Second, that the toxin is activated to the hypothetical 2,3-oxide and because it is mutagenic it is also presumed to be the proximal carcinogen. Any 2,3-oxide unreacted with DNA might be hydrated enzymically in the liver to form the dihydrodiol of aflatoxin which would have

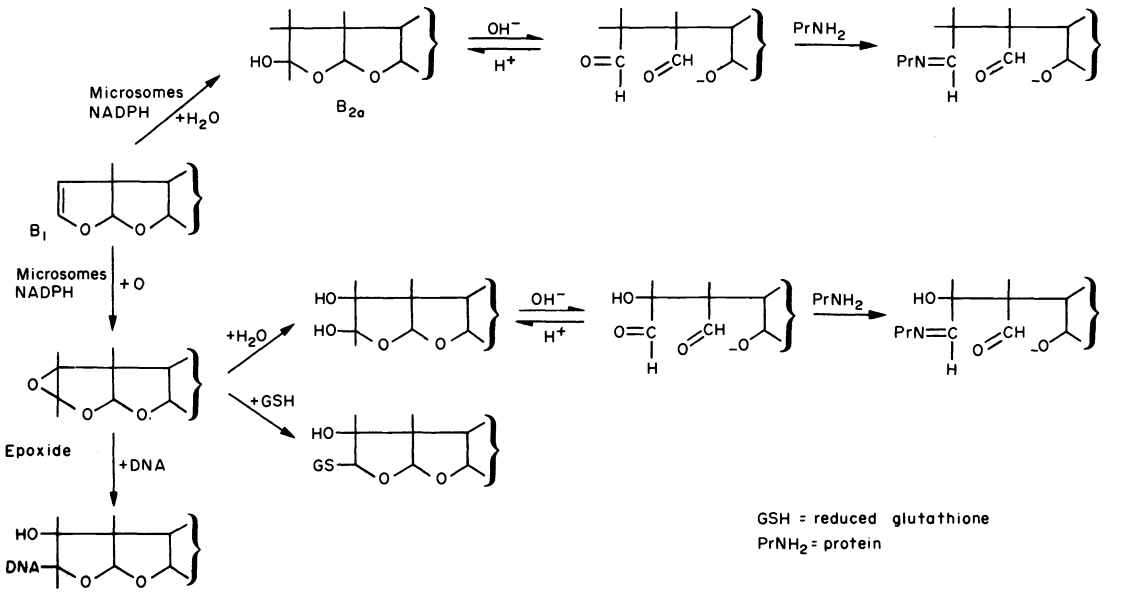


Fig. 3. Special aspects of aflatoxin B_1 metabolism (partial structures only) showing two routes for metabolic activation to the hemiacetal (B_{2a}) and the 2,3 oxide (epoxide). Interactions of these metabolites with protein and DNA and inactivation of the epoxide by enzymic reaction with glutathione are also indicated (see Ref. 4, 10 and 15).

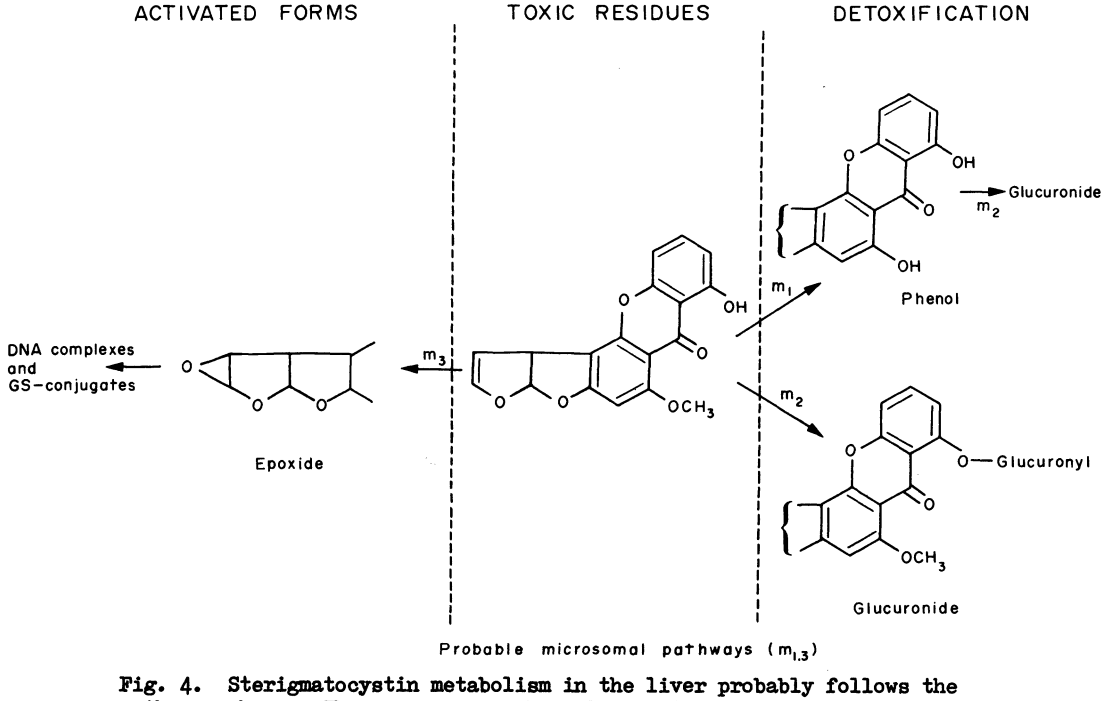


Fig. 4. Sterigmatocystin metabolism in the liver probably follows the pathways shown. There appears to be only one form of metabolic activation. GS denotes glutathione.

somewhat similar properties to the hemiacetal, binding with aminoacids etc. to form Schiff bases (Fig. 3). Conjugation with glutathione (GSH) might provide an alternative detoxification of the epoxide (Ref. 16).

STERIGMATOCYSTIN

Again this toxin can be considered under all three headings.

Detoxification. Relatively little is known of the metabolism of this toxin. At least two microsomal reactions are possible in the liver (demethylation and conjugation) and as glucuronides of sterigmatocystin have been identified in the urine of treated Vervet monkeys (Ref. 17) these two reactions are depicted in the Fig. 4. As in the case of aflatoxin, these reactions would be detoxifications only in the sense that more polar and more readily excreted forms of the toxin are produced.

Toxic residues. Little is known of the relative rates of sterigmatocystin metabolism by livers of different animal species, but clearly those metabolising it slowly are likely to accumulate this carcinogenic toxin in their tissues. By analogy with aflatoxin it is also possible that a 4-hydroxy compound is formed metabolically and if so this would presumably be toxic as the 2,3-vinyl ether grouping is generally assumed to be the active centre of the sterigmatocystin molecule.

Metabolic activation. Liver preparations which form the hemiacetal of the aflatoxin *in vitro* fail to carry out the equivalent transformation when sterigmatocystin is used as substrate (Ref. 9). Therefore this type of metabolic activation would seem to be unlikely. However, using the bacterial mutagenicity test, Garner and his colleagues (Ref. 12 and 13) have shown that the presumed 2,3-oxide is probably formed by liver microsomes (see Fig. 4).

OCHRATOXIN A

No active forms of this toxin are known so it can be discussed under two headings only.

Detoxification. The only metabolic reaction of ochratoxin A results in detoxification. Hydrolysis catalysed by carboxypeptidase A breaks the amide bond (Ref. 18) with the formation of the naturally occurring aminoacid phenylalanine plus ochratoxin α (Fig. 5). The latter isocoumarin carboxylic acid is very much less toxic than the parent toxin ochratoxin A in all acute *in vivo* and *in vitro* test systems (e.g. Ref. 19) and thus the complete toxin molecule appears to be required to achieve its toxic effect.

Toxic residues. Its precise mode of action and the reasons for its peculiar affinity for the proximal tubules of the kidney remain unknown but ochratoxin A binds strongly to protein and the affinity constant is higher for this toxin than the dechloro-derivative ochratoxin B (Ref. 20). As a protein binding agent ochratoxin A clearly presents a toxic residue problem. Pigs exposed to this toxin in their diet accumulate ochratoxin A in kidney, liver and muscle (in decreasing order) and the experimental regression equations of Krogh and his colleagues (Ref. 21) have been found by other experimenters (Ref. 22) to provide a fairly good means of predicting levels of these toxic residues.

TRICHOTHECENES

This group comprises over 30 toxins elaborated by Fusarium, Trichothecium, and Stachybotrys spp. for example and none appears to be metabolically activated. Their necrogenic action on the skin demonstrates that at least in this particular mode of action this is the case. Therefore these mycotoxins will also be considered under two headings only.

Detoxification. Assuming that the epoxide group is the "active centre" of the typical trichothecene molecule it is obvious that modifications of acyl or ester groups R_1, R_2, R_3, and R_4 (see Fig. 6) will only have modifying effects on toxicity. Thus there is evidence (Ref. 23) to show that T-2 toxin (R_1 = β-acetoxy; R_2 = acetoxy; R_3 = α-hydroxy; R_4 = α-isovaleroxy) is hydrolysed by liver tissues *in vitro* to the HT-2 toxin (R_1 = β-hydroxy) and like T-2 toxin itself it is still reactive in the dermal toxicity test. Only chemical modification of the epoxide will result in detoxification (see Fig. 6) although hydroxylated trichothecene mycotoxins are probably excreted as glucuronides (Ref. 24).

Chemical reduction with lithium aluminium hydride has been shown to destroy the toxic action of trichothecene toxins (Ref. 25) and so reduction in the liver or other tissues would be a detoxifying reaction. Enzymic modification with microsomal epoxide hydrase or the soluble glutathione S-epoxide transferase are obvious alternatives and it has been shown that crude liver homogenates containing both these enzymes do indeed destroy the toxic properties of T-2 toxin (Ref. 24), scirpene triacetate and diacetoxyscirpenol (Ref. 9 and 26).

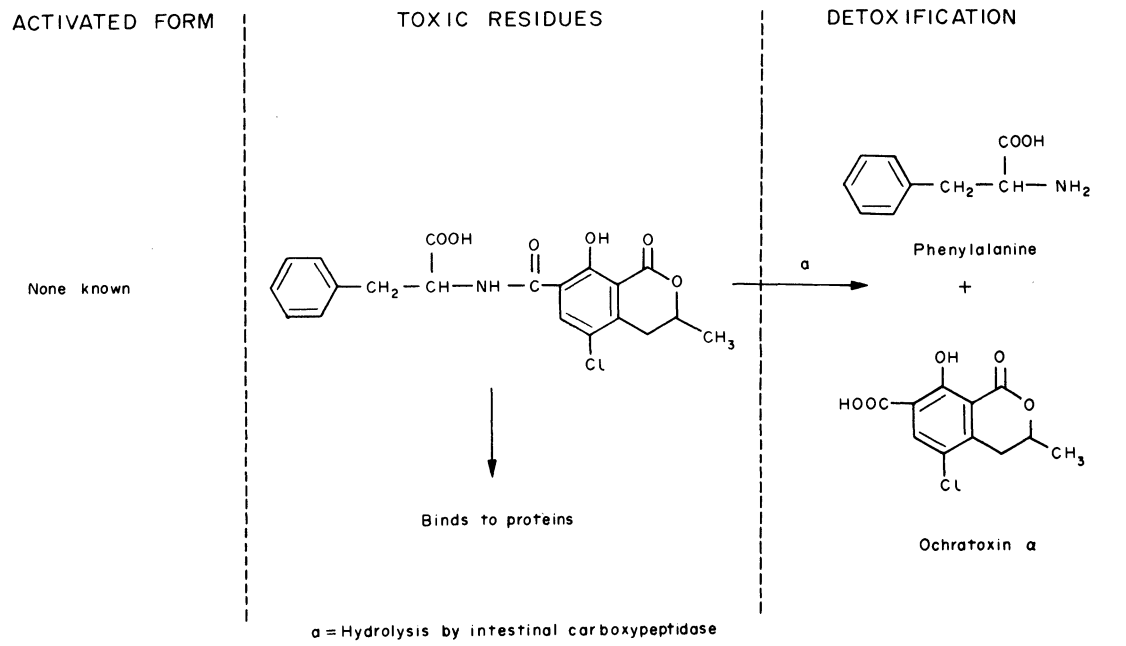


Fig. 5. Ochratoxin A is enzymically hydrolysed to phenylalanine and biologically inactive ochratoxin α mainly in the intestine but also in the liver.

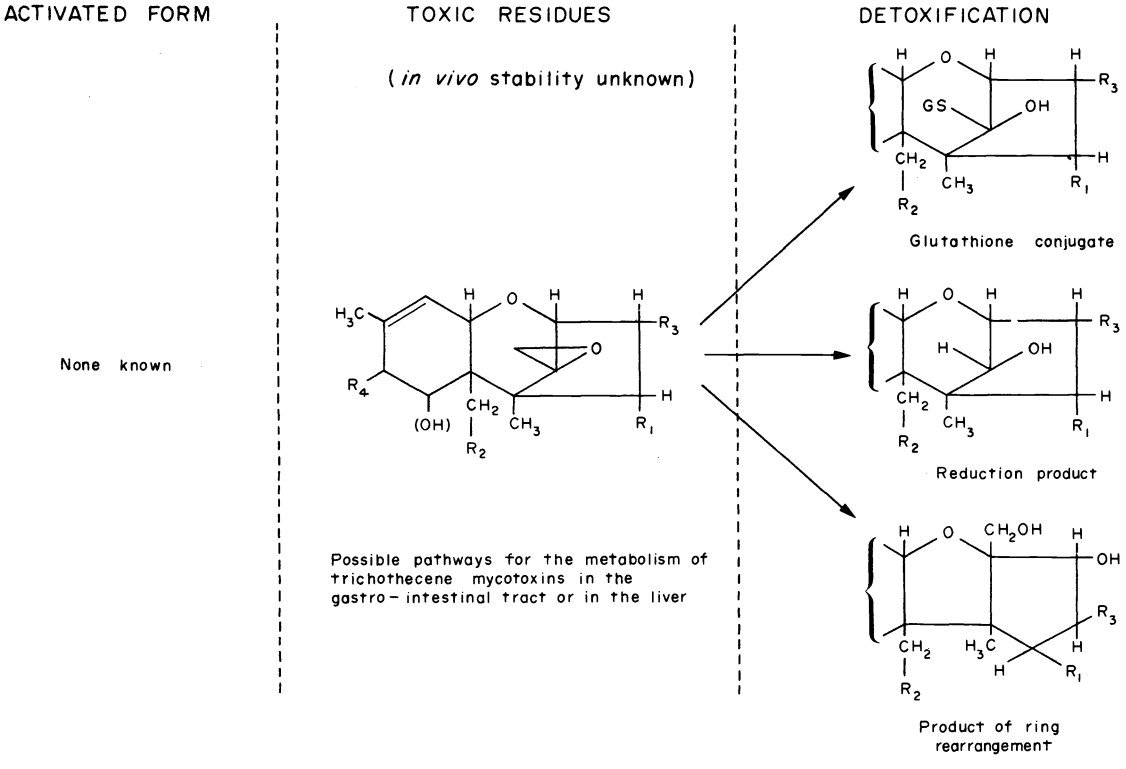


Fig. 6. Three possible pathways for the metabolism of 12,13-epoxy-Δ^9-trichothecene mycotoxins. In the generalised structure of these toxins given above R_1, R_2, R_3 and R_4 may be hydrogen, hydroxyl, esterified hydroxyl, keto (R_4 only), epoxide (replaces R_4 and adjacent OH group), or a macrocyclic bridge may link R_1 and the adjacent CH_3 group. The OH group adjacent to R_4 is replaced by hydrogen in some toxin molecules.

Ring rearrangement (Fig. 6) occurs under strongly acid conditions but our own unpublished experiments have shown that in the milder acidic conditions of the stomach this rearrangement is extremely unlikely.

Toxic residues. Nothing is known of in vitro stability or rates of metabolism of this group of toxins in different animal species but it has been shown that binding to sulphydryl groups of cysteine, glutathione and protein molecules occurs (Ref. 27). This may prove to be an essential part of the mode of action of these toxins but equally it may denote a potential residue problem when domestic animals have been exposed to trichothecene contaminated feeds.

ZEARALENONE

Another Fusarium toxin, the toxicology of zearalenone has received much attention but rather little is known of its metabolism and mode of action.

Detoxification. No pathways have yet been defined but at least two of the possible metabolites of zearalenone contain hydroxyl groups and presumably conjugates such as glucuronides could be formed by microsomal glucuronyl transferases (see Fig. 7).

Toxic residues. Again no information is available to identify animal species that metabolise zearalenone slowly but such a farm animal could possibly store toxic residues of the unchanged toxin with attendant health risks to the human consumer. Several metabolites of zearalenone are known to be formed by NAD/NADP-linked dehydrogenases present in the liver cell sap (Ref. 28) and these soluble enzymes probably convert the parent toxin into three oestrogenic compounds. If, as seems likely, reduction of the 8'-carbonyl group is essential for oestrogenic activity, perhaps this occurs at the site of action.

Zearalenol (or zeranol, Ralgro$^{(R)}$) is a widely approved anabolic preparation used for fattening cattle from which it is obvious that residues of this compound present very much less of a public health problem than diethylstilboestrol for example. Therefore it is probable that metabolites of zearalenone present only theoretical hazards as toxic residues, possibly in adipose tissues of animals that metabolise this toxin slowly.

Activated form. This has been discussed above in relation to general metabolism of this toxin.

OESTROGENIC TOXINS AND PSEUDO-STEROIDS

Zearalenone and its reduction products are uterotropic like oestradiol 17 β and, although these toxin molecules are not steroids, their oestrogenic activity must depend upon steroid-like interaction with appropriate receptor sites. This may involve pairs of hydroxyl or potential hydroxyl groups. Thus, as shown in Fig. 8, the macrocyclic zearalenone molecule could easily fold so that spacing of reactive groups a is equal to b. Aflatoxin also behaves as a pseudo-steroid in that it can occupy oestradiol-binding sites of the liver endoplasmic reticulum (Ref. 29) as well as active sites of liver 17-ketosteroid dehydrogenase (Ref. 30). These interactions may be explained in similar terms because measurements from molecular models show that the spacing of the potential phenolic hydroxyl and the enzymically reducible carbonyl group of aflatoxin is approximately equal to that of the two hydroxyls in oestradiol (b and c in Fig. 8).

EXTRA-HEPATIC METABOLIC ACTIVATION

Finally, on both sides of the Atlantic we have, for the past few years, devoted much time and effort to examining the metabolism of mycotoxins in the liver. However this should not be regarded as the sole site of in vivo biotransformation. For example, Newberne and his research group (Ref. 31) have shown that aflatoxin may be activated in the skin and our own preliminary observations (Ref. 32) suggest that, possibly, the hemiacetal is formed in a free radical type reaction under the influence of ultra violet radiation. The former group (Ref. 33) have now shown that irradiated rats are less likely than unirradiated aflatoxin-dosed rats to develop hepatomas. Could this possibly be because an upset in the balance of aflatoxin metabolism - more hemiacetal and less epoxide formed?

And then, what of the stomach and gastrointestinal tract? The acid conditions themselves, the action of digestive enzymes, and the gut flora are factors that have simply not been given sufficient attention. In the case of ruminants and other herbivores, microbial biotransformations surely cannot be without importance.

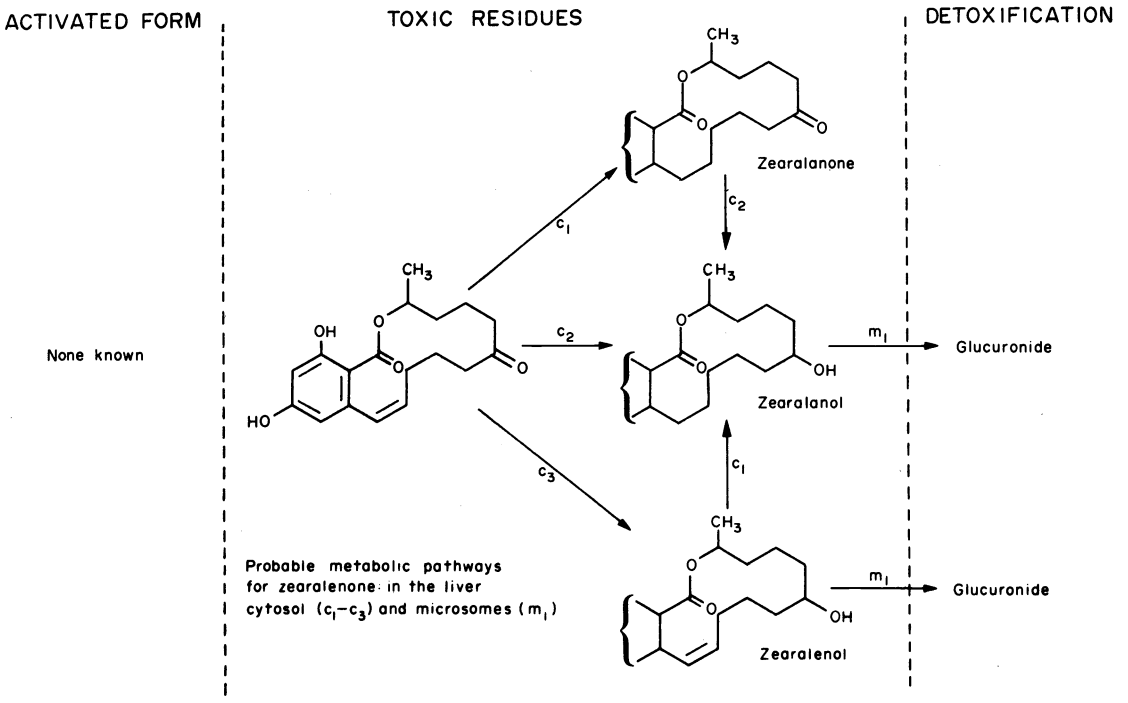


Fig. 7. Zearalenone (F_2) metabolism in the liver may follow the pathways shown.

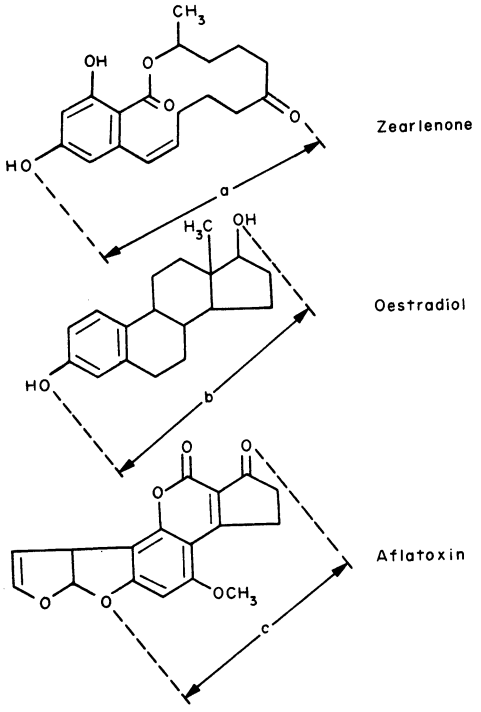


Fig. 8. A possible molecular basis for the pseudo-steroid activities of aflatoxin and zearalenone. Hydroxyl or potential hydroxyl groups of these toxin molecules and oestradiol 17 β may be identically spaced (a = b = c) when interacting with common receptor sites.

REFERENCES

1. D.S.P. Patterson, in IUPAC Symposium Mycotoxins in Foodstuffs, Paris in press (1976).
2. D.S.P. Patterson, in Mycotoxic fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook, T.D. Wyllie & L.G. Morehouse (eds), Marcel Dekker Inc., New York, in press. (1976).
3. D.S.P. Patterson and B.A. Roberts, Fd Cosmet. Toxicol. 9, 829-837 (1971).
4. D.S.P. Patterson, Fd Cosmet. Toxicol. 11, 287-294 (1973).
5. D.P.H. Hsieh, UJNR Symposium Mycotoxins in Human and Animal Health, Maryland, in press (1976).
6. R.I. Krieger, A.S. Salhab, J.I. Dalezios and D.P.H. Hsieh, Fd Cosmet. Toxicol. 13, 211-219 (1975).
7. O. Bassir and F. Osiyemi, Nature, Lond. 215, 882 (1967).
8. E.C. Harland and P.T. Cardeilhac, Am. J. vet. Res. 36, 909-912 (1975).
9. D.S.P. Patterson, Biochem. Soc. Trans. 1, 917-922 (1973).
10. D.S.P. Patterson, in Biochemical Mechanisms of liver injury, T.F. Slater (ed), Academic Press, London, in press (1976).
11. A.E. Pohland, M.E. Cushmac and P.J. Andrellos, J. Ass. off. analyt. Chem. 51, 907-910 (1968).
12. R.C. Garner, E.C. Miller, J.A. Miller, J.V. Garner and R.S. Hanson, Biochem. biophys. Res. Commun. 45, 774-780 (1971).
13. R.C. Garner, E.C. Miller and J.A. Miller, Cancer Res. 32, 2058-2066 (1972).
14. D.H. Swenson, J.A. Miller and E.C. Miller, Biochem. biophys. Res. Commun. 53, 1260-1267 (1973).
15. D.H. Swenson, J.A. Miller and E.C. Miller, Cancer Res. 35, 3811-3823 (1975).
16. H.G. Raj, K. Santhanam, R.P. Gupta and T.A. Venkitasubramanian, Chemico-Biol. Interactions 11, 301-305 (1975).
17. P.G. Thiel and M. Steyn, Biochem. Pharmac. 22, 3267-3273 (1973).
18. M.J. Pitout, Biochem. Pharmacol. 18, 485-491 (1969).
19. F.S. Chu, I. Noh and C.C. Chang, Life Sci. 11, 503-509 (1972).
20. F.S. Chu, CRC Crit. Rev. Toxicol. 2, 499-524 (1974).
21. P. Krogh, N.H. Axelsen, F. Elling et al, Acta. path. microbiol. Scand., Sect. A., Suppl. 246, 1-16 (1974).
22. D.S.P. Patterson, B.A. Roberts and B.J. Small, Fd Cosmet. Toxicol., in press (1976).
23. R.A. Ellison and F.N. Kotsonis, Appl. Microbiol. 27, 423-424 (1974).
24. N.R. Kosuri, cited by J.R. Bamburg and F.M. Strong, in Microbial Toxins Vol VII, S. Kadis, A. Ciegler and S.J. Ajl (eds) Academic Press, New York, pp. 207-292 (1971).
25. J.R. Bamburg, Mycotoxins of the trichothecane family produced by cereal molds, PhD Thesis, Univ. Wisconsin, University Microfilms Inc., Ann Arbor, Wisconsin (1969).
26. P.M.D. Foster, private communication (1976).
27. Y. Ueno and H. Matsumoto, Chem. Pharm. Bull. 23, 2439-2442 (1975).
28. Y. Ueno, S. Aykaki, N. Sato and T. Ito, IUPAC Symposium, Mycotoxins in Foodstuffs, Paris, in press (1976).
29. D.J. Williams and B.R. Rabin, Nature, Lond. 232, 102-105 (1971).
30. D.S.P. Patterson and B.A. Roberts, Experientia, Basel 28, 929-930 (1972).
31. P.M. Newberne, W-C.M. Chan and A.E. Rogers, Toxicol. appl. Pharmac. 28, 200-208 (1974).
32. D.S.P. Patterson, Symposium Mycotoxines et la Contamination des Chaines Biologiques, Toulouse, in press (1975).
33. P.I. Joseph-Bravo, M. Findley and P.M. Newberne, J. Toxicol. environ. Hlth. 1, 353-376 (1976).