reactions was found to be decreased in tumor-bearing animals when compared to non-tumor-bearing or control animals. Therefore, in addition to the impairment in the oxidative process of drug metabolism, decreases in the activities of these enzymes and the content of glutathione could impair the overall biotransformation and elimination of any drug being metabolized through the MFO enzyme system. This is further supported by the observation that the biological half-life of antipyrine was increased significantly in tumor-bearing animals. This condition can well result in prolonged duration of drugs in body circulation.

The findings reported here have definite implications in experimental chemotherapy. Several anti-cancer drugs including cyclophosphamide, procarbazine, methotrexate and mitomycin C are metabolized by liver microsomal enzymes [22–24]. Therefore, the pharmacokinetics and potential toxicity of these agents may be modified by the observed alterations in hepatic drug metabolism in tumor-bearing animals. The ability of tumor to interfere with hepatic drug-metabolizing capacity may be of clinical importance and should be given due consideration in the management of cancer treatment.

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The metabolism of aflatoxin B_1 by human liver

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The rates of NADPH-dependent metabolism by microsomes isolated from two male liver samples ranged between 1.0 and 2.7 nmoles AFB_1/min/mg microsomal protein and microsomes from a female liver gave rates of metabolism of 0.63 and 0.73 nmole/min/mg microsomal protein. The major aflatoxin metabolite detected by HPLC was aflatoxin Q_1 (approximately 70–90% of the soluble metabolites). Aflatoxin-8,9-dihydrodiol (10–30%) and aflatoxin M_1 were also detected as soluble products of microsomal metabolism.

The ability of human post microsomal supernatant to convert the aflatoxin B_1-8,9-epoxide to a glutathione conjugate was investigated. There was little evidence for the production of the glutathione conjugate in a system using human cytosol with quail microsomes or in human S9, or reconstituted S9 incubations.

Animal species differ markedly in their susceptibilities to both the acute and chronic toxicity of mycotoxin aflatoxin B_1 [1, 2], and there is evidence supporting a metabolic basis for the species differences to aflatoxin B_1 induced hepatotoxicity [3–5]. It is possible to estimate the extent of the activation to aflatoxin B_1-8,9-epoxide by microsomal metabolism by assaying AFB_1 dihydrodiol as its Tris complex on reverse phase HPLC [6]. A major detoxification product has been recently identified as a glutathione conjugate of the epoxide AFB_1–GSH [7].* Both activation and deactivation pathways have been investigated [4, 5]. These

* Abbreviations used: AFB_1, aflatoxin B_1; Tris, Tris-(hydroxymethyl)methylamine; AFQ_1, aflatoxin Q_1; AFM_1, aflatoxin M_1; AFP_1, aflatoxin P_1; AFB–GSH, 8,9-dihydro-8-(*S*-glutathionyl)-9-hydroxy-aflatoxin B_1; HPLC, high performance liquid chromatography; GSH, reduced glutathione.

studies have suggested that metabolic differences both in activation and deactivation pathways between species account for the relative susceptibilities to AFB_1 toxicity. Glutathione conjugation has also been associated with reduced macromolecular binding *in vivo* and *in vitro* [8, 9]. The major pathways of AFB_1 metabolism by hepatic microsomal and cytosolic fractions are given in Fig. 1. The formation of AFM_1, AFP_1 and AFQ_1 by microsomal fractions, since this is followed by their conjugation and excretion, can be regarded as detoxifying metabolism, as can the conversion of AFB_1 epoxide produced by microsomal activation to the AFB–GSH conjugate via cytosolic GSH-*S*-transferase enzymes [4, 5]. The macromolecular binding of the AFB_1 epoxide and AFB_1 dihydrodiol are considered to be responsible for the toxicity of the AFB_1 [3].

There is epidemiological evidence for the involvement of aflatoxin B_1 in human liver disease [10, 11]. Several studies have investigated human metabolism [12, 13] often concentrating on microsomal activation of AFB_1. This paper describes a study into activation and deactivation pathways using samples of human liver.

Materials and methods

Materials. Aflatoxin B_1 was obtained from Makor Chemical Company (Jerusalem, Israel). Adult male mice (C57/BL10) were supplied by Olac 1976 Ltd. (Shaws Farm, Blackthorn, Bicester, Oxon). Adult male Japanese quails were obtained from Lincolnshire Pheasantries (Tunby, Boston, Lincolnshire).

Tissue preparation. Human liver samples (5–10 g) were generously donated by the King's College Hospital Liver Unit (Sample 1) or from abroad (2 and 3). All were from accident victims whose organs may have been used for transplant. Sample 1 was removed to liquid nitrogen within hours after death and stored in liquid nitrogen for up to 4 months. Samples 2 and 3 were transported to the U.K. in an ice-cold vessel and they arrived in the laboratory partially frozen. They were stored at −70° for up to 4 months. Information supplied with the samples stated that sample 1 was from a young male (21 years), sample 2 was from a female (16 years) and sample 3 was from a male (17 years). Samples were removed from the deep frozen liver, thawed and used for the preparation of microsomes and post-microsomal supernatant. Aflatoxin assays and glutathione-*S*-transferase assays were carried out on the day of tissue preparation.

Microsomes, post-microsomal supernatant and 9000 *g* (S9) supernatant were prepared by the method described previously [3]. Quail microsomes and post-microsomal supernatant from quail and from mouse were prepared from fresh whole liver as described previously [4] and stored in aliquots at −70° until required.

Metabolic assays. Microsomal incubations using a microsomal suspension (400 μl) equivalent to 0.25 g wet weight of liver tissue, were carried out in duplicate or triplicate as previously described [3] in the presence or absence of NADPH. Microsomal plus supernatant incubations were essentially as for microsomal incubations alone except that the mixtures were supplemented with 5 mM reduced glutathione (GSH). Post-microsomal supernatant (400 μl) equivalent to 0.13 g wet weight of liver tissue was added prior to the addition of microsomes. 9000 *g*(S9) supernatant incubations involved supernatant (800 μl) equivalent to 0.26 g wet weight liver, and the mixtures were supplemented with 5 mM GSH.

HPLC analysis. Chromatographic analysis was achieved by the method described previously [6].

Results

A representative time course for the NADPH dependent metabolism of AFB_1 by human microsomes is shown in Fig. 2. The quantitative results of microsomal metabolism of AFB_1 by human liver samples are recorded in Table 1. Each individual experiment is recorded separately to illustrate the variation observed in the rate of metabolism, recovery of soluble aflatoxins (usually inversely proportional to the rate of metabolism), and the percentage of the metabolites recovered. In each experiment, quail microsomes prepared prior to this study, were used as a positive control for incubation conditions. The data from the quail incubations showed little experimental variation, suggesting that the variation in the human data was due to intra-liver variation in the mixed function oxidase activity.

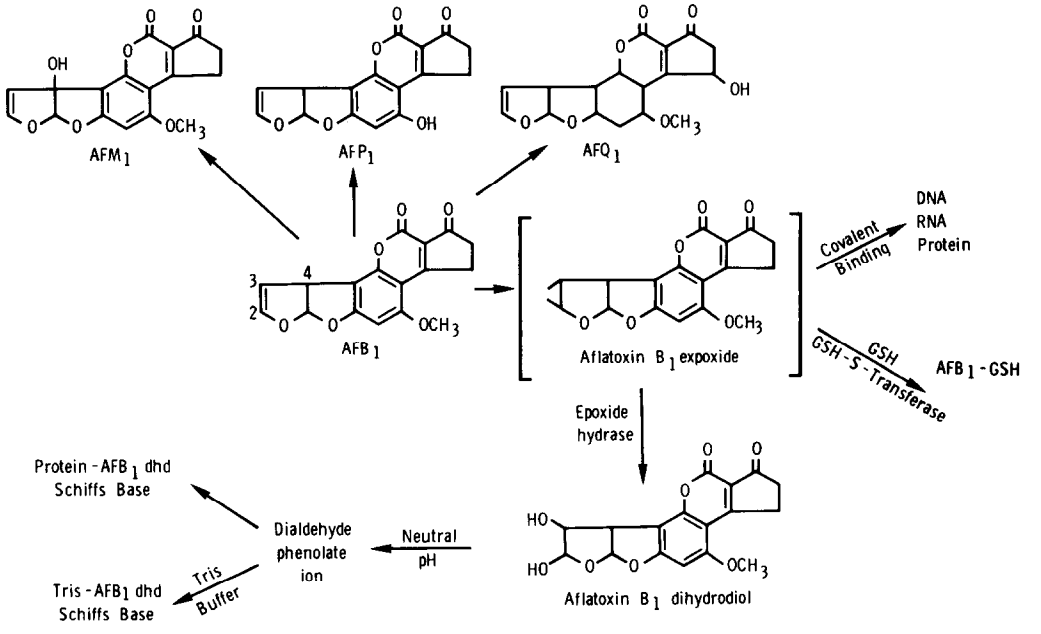

Fig. 1. Pathways of *in vitro* AFB_1 metabolism.

Table 1. Primary metabolism of AFB_1 by human microsomes

Sample	Sex	Rate of AFB_1 metabolism (nmoles/mg protein /min)	Recovery of soluble aflatoxins after 30 min (% of initial AFB_1)	Production of metabolites (% of total metabolites at 30 min) AFB_1 diol	AFQ_1	AFM_1
1	M	2.7	35	27	73	trace
1	M	2.14	45	10	90	n.d.
1	M	1.5	50	18*	68*	14
2	F	0.63	59	20	77	n.d.
2	F	0.73	65	26*	74*	n.d.
3	M	1.027	46	26	65	trace
3	M	1.44	42	26*	72*	

n.d., not detectable.
* Only determined after 15 min incubation.
Quail microsomal metabolism of AFB_1 ranged from 1.22–1.55 nmole/mg protein/min.

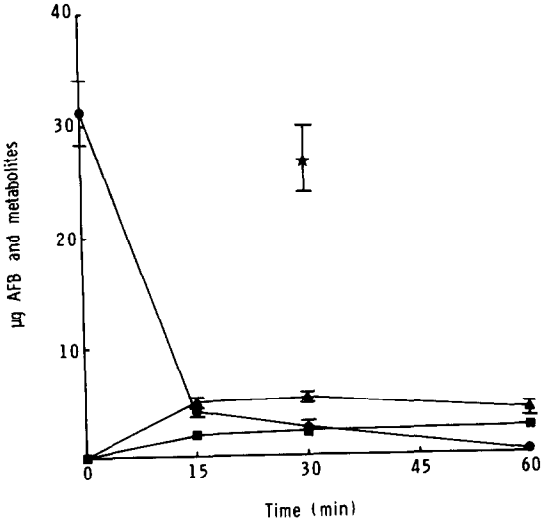

Fig. 2. Metabolism of AFB_1 by human microsomes in the presence (●) and absence (★) of NADP, and the production of AFQ_1 (▲) and AFB_1-dihydrodiol (■).

AFQ_1 was identified, by co-chromatography with authentic standard on reverse phase HPLC, as the major soluble aflatoxin metabolite produced by each of the three livers (Fig. 3a). This compound accounted for between 65 and 90% of the metabolites recovered, and after 15 min incubation approximately 15–20% of the added AFB_1 was detected as AFQ_1. AFB_1-8,9-dihydrodiol was detected as the Tris–diol complex previously described [6]. AFM_1 was also present in some of the incubations, accounting for up to 14% of the total metabolites recovered.

Secondary metabolism of AFB_1 by human liver was investigated using two procedures. The first involved a method described previously [4] whereby post-microsomal supernatant equivalent to 0.13 g wet weight of human liver was incubated with AFB_1 activated to AFB_1-8,9-epoxide, *in situ*, by quail microsomes. The efficiency of production of the epoxide by quail microsomes is illustrated in Fig. 3b in which the Tris–diol complex is detected as the major metabolite (Peak 2). The results of incubation of AFB_1 with quail microsomes and human supernatant are recorded in Table 2 and a representative HPLC trace is shown in Fig. 3d. In each of these experiments parallel incubations using quail microsomes and mouse supernatant were carried out as positive controls (Fig. 3g) and incubations, using quail microsomes and quail supernatant as a negative control (Fig. 3e). The metabolite profiles from the incubations of quail microsomes and human post-microsomal supernatant were found to resemble those resulting from incubations of quail microsomes and quail supernatant. The major metabolite in both cases was identified by co-chromatography, as AFB_1-8,9-dihydrodiol. Three human liver cytosols supplied frozen from an alternative source, also appeared to have little or no ability to form AFB_1–GSH in this system (results not given). In contrast, in incubations of AFB_1 with quail microsomes and mouse post-microsomal supernatant the only detectable soluble metabolite was AFB_1–GSH (Fig. 3g, Peak 5).

The metabolism of AFB_1 by human post-mitochondrial supernatant was investigated on at least one occasion for each liver sample, using either 9000 *g* supernatant or a reconstituted S9. Qualitatively the metabolic profile produced by these S9 incubations resembled that of human microsomal metabolism (Fig. 3c and 3h). AFQ_1, AFB_1-8,9-dihydrodiol and AFM_1 were identified by co-chromatography. A small shoulder on the HPLC peak of AFM_1 was observed in some experiments and had the correct retention time for AFB_1–GSH. The identity of the material was not confirmed, nor was it possible to quantify accurately, but the amount present was very low. If this HPLC component is AFB_1–GSH its production does not appear to compete with AFB_1-8,9-dihydrodiol production to any great extent. In incubations of AFB_1 with human microsomes and mouse supernatant, AFB_1–GSH was detected (Fig. 3f, Peak 5) and apparently the formation of this material competed with AFB_1-8,9-dihydrodiol production.

Discussion

Human microsomes show a rapid NADPH dependent metabolism of AFB_1. A comparison with quail microsomes included as a quality control, suggested that male human microsomes have the ability to metabolize AFB_1 at a high rate compared with some laboratory animals examined previously [4]. AFQ_1 appears to be the major detectable metabolite produced by human microsomal metabolism in contrast to mouse, male rat, guinea pig and quail in which AFB_1-8,9-dihydrodiol is the major soluble metabolite.

The formation of AFQ_1 has been observed by other workers [12, 13] and may be considered to be a detoxifying step, although the fate of the AFQ_1 produced is unclear at present. The amount of AFB-8,9-dihydrodiol in human microsomal incubations detected as the Tris–diol complex in the soluble metabolites is fairly low. Activation via the

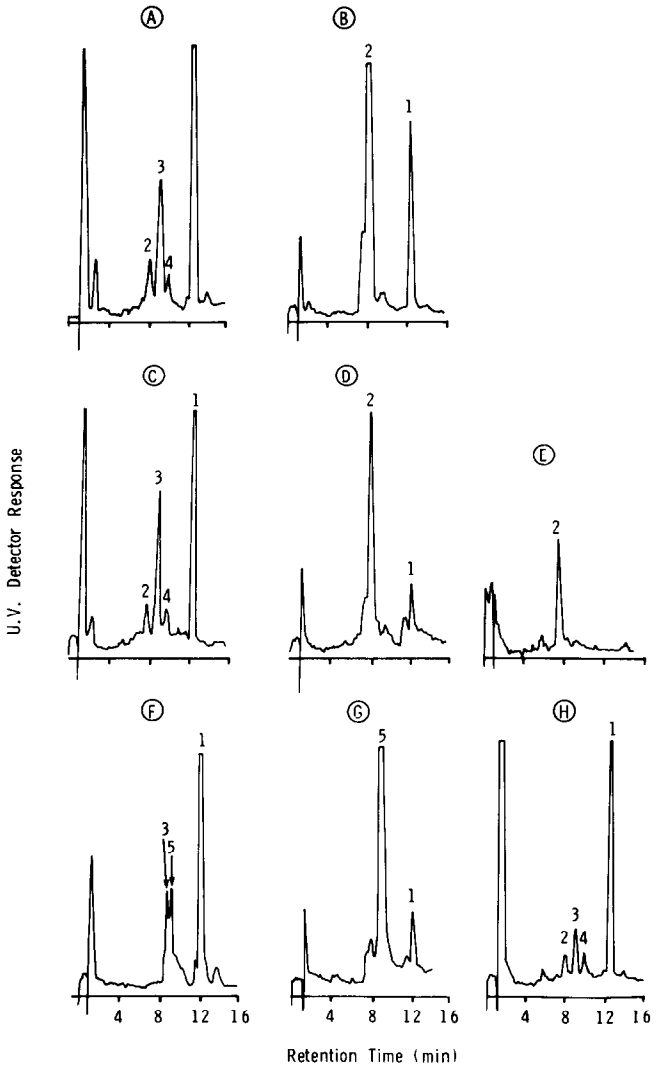

Fig. 3. Reverse phase HPLC chromatograms of AFB_1 and metabolite produced by incubations of (A) human microsomes, (B) quail microsomes, (C) human reconstituted S9, (D) quail microsomes + human post-microsomal supernatant, (E) quail microsomes + quail post-microsomal supernatant (0.25 × injection volume of other chromatograms shown), (F) human microsomes + mouse post-microsomal supernatant, (G) quail microsomes + mouse post-microsomal supernatant, (H) human post-mitochondrial supernatant (S9). Peak 1 is AFB_1, 2 is AFB_1-dihydrodiol, 3 is AFQ_1, 4 is AFM_1, 5 is AFB_1–GSH.

formation of AFB_1-8,9-epoxide therefore appears not to be a major pathway. However recovery of soluble aflatoxins after 30 min incubation with human microsomes was low, particularly in the rapid metabolizers, suggesting that there was a substantial metabolism-dependent binding to macromolecules, which may proceed by an alternative mechanism to epoxide interaction.

The apparent lack of ability of human post-microsomal supernatant to convert AFB_1-8,9-epoxide produced *in situ* by quail microsomes to AFB_1–GSH can be interpreted in several ways:

In the case of each of the three samples of human liver and three post-microsomal supernatants that we have examined no glutathione *S*-transferase activity towards AFB_1-8,9-epoxide has been detected.

Several possibilities exist for this absence of activity: 1. An absence of the appropriate isoenzymic activity. In previous studies quail [4] and chicken [7] liver were found to have no detectable ability to catalyse the formation of AFB_1–GSH and the activities in cytosol fractions isolated from guinea pig and male rat were also very low [4]. Clearly therefore there is a variation in the presence of the appropriate conjugation activity between species. 2. Loss of enzymic activity during storage or sample preparation. Care was taken to maintain the liver samples at low temperatures, and the high microsomal cytochrome P-450 activity toward AFB_1 metabolism suggests that a loss of all drug metabolising activities had not occurred. However, a selective loss of AFB_1-glutathione-*S*-transferase activities can not be ruled out and further studies are needed on this aspect. 3. Incompatibility of quail microsomes with human post-microsomal supernatant. The quail microsomes are used as the source of AFB_1-8,9-epoxide *in situ*. This method has proved successful in producing AFB_1–GSH when com-

Table 2. Production of AFB–GSH and AFB_1-8,9-dihydrodiol by quail microsomes, post-microsomal supernatant and 5 mM GSH

Source of post-microsomal supernatant	AFB_1-8,9-dihydrodiol (nmoles)	AFB_1–GSH (nmoles)
Human liver (1) (male) N = 11	24.6 ± 3.6	0.9 ± 1.30
Human liver (2) (female N = 4	16.6 ± 1.1	0
Human liver (3) (male) N = 4	18.8 ± 3.4	0
Mouse liver N = 10	1.1 ± 0.9	85.6 ± 9.3

± S.D.
N = Number of incubations.
128 nmoles AFB_1 per incubation.

bined with post-microsomal supernatant from mouse, female rat, and to a limited extent from male rat and guinea pig, which suggests that an incompatibility of human cytosol with quail microsomes as a possible explanation for the absence of AFB_1–GSH formation is perhaps unlikely. Furthermore when incubations were performed using 9000 *g* supernatant (S9) from human liver, or a reconstituted system using human microsomal suspension and post-microsomal supernatant (reconstituted S9) there was also little evidence for extensive AFB_1–GSH formation, although a small shoulder on the AFM_1 peak in some incubations may be AFB_1–GSH. AFB_1-8,9-dihydrodiol production appeared to be fairly similar to that observed in human microsomal incubations suggesting that any glutathione conjugating activity present in human S9 incubations did not compete extensively with the AFB_1-8,9-dihydrodiol pathway. There is good experimental evidence to show that in the presence of high GSH-*S*-transferase activity, AFB_1–GSH formation competes with that of diol and with macromolecular binding as illustrated by the high recovery of soluble aflatoxins in the presence of mouse supernatant. Finally when human microsomes were incubated with AFB_1 and C57 mouse supernatant, AFB_1–GSH was detectable and AFB_1-8,9-dihydrodiol was not. Therefore, in the presence of high glutathione-*S*-transferase activity in the mouse [4], AFB_1-8,9-epoxide produced by human microsomes is accessible, and capable of being converted to AFB_1–GSH.

In conclusion human liver metabolizes AFB_1 rapidly to AFQ_1 and probably to a lesser extent to AFB_1-8,9-epoxide. If AFQ_1 production is a detoxification pathway, primary metabolism would suggest that humans may be less susceptible to AFB_1 hepatoxicity than other rapid metabolizers of AFB_1 such as quail, that produce almost exclusively AFB_1-8,9-epoxide. However the apparent lack of capacity for AFB_1–GSH production by human post-microsomal supernatant suggests that any AFB_1-8,9-epoxide produced is likely to undergo macromolecular binding rather than detoxification. There is a need to extend these studies to a wider range of samples to determine the variation in these functions in different human populations.

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