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DIFFERENTIAL TOXICITY OF AFLATOXIN B_1 IN MALE AND FEMALE RATS: RELATIONSHIP WITH HEPATIC DRUG-METABOLIZING ENZYMES

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Abstract—It is well known that distinct differences in the metabolism of xenobiotics exist between males and females of the same species. Male and female rats were treated with a single intraperitoneal dose of aflatoxin B_1 (AFB_1): 1 or 3 mg/kg for males, 3 or 6 mg/kg for females. Comparative changes in hepatic drug metabolizing and plasma enzymes had been studied. The obtained results show that, at the common dose of 3 mg/kg, AFB_1 induced an 18% mortality in males and none in females. In the plasma, total bilirubin concentration as well as the activity of transaminases and alkaline phosphatase (ALP), utilized as indicators of liver damage, were highly increased in both males and females due to the treatment with 3 or 6 mg AFB_1/kg. In the female, the plasma features rapidly declined. In contrast, in the male, the effect of AFB_1 was prolonged. Hepatic determinations revealed a pattern difference of drug metabolizing enzymes and cytochrome P-450 between males and females. The results also show that in the male, most of the drug metabolizing enzyme activities were decreased until the ninth day with the 3 mg/kg treatment. So, we observed a decrease in the activities of UDP-glucuronosyltransferase (UDPGT) with *p*-nitrophenol as substrate (PNP) and GSH *S*-transferase, 40 and 53% respectively; while the activity of epoxide hydrolase was increased up to 170%. In the meantime, the concentration of cytochrome P-450 decreased by 69%. By contrast, in the case of the female, these decreases were only 14, 43 and 23% for the UDPGT, GSH *S*-transferase and cytochrome P-450, respectively. Moreover, these decreases occurred only during the first three days after treatment. Thereafter, these enzyme activities significantly increased above the control values. This study suggests that the induction of detoxicating enzymes, more important in the female (72% increase in the activity of UDPGT, 480% in that of epoxide hydrolase and 42% for GSH *S*-transferase), may have a protective role against AFB_1 metabolites and could explain, partly, the lower sensitivity of the female to the toxic effects of AFB_1.

AFB_1,† a mycotoxin produced by some strains of *Aspergillus flavus* and *Aspergillus parasiticus*, contaminates foodstuffs, induces hepatotoxic and hepatocarcinogenic effects in many animal species and probably also in humans [1, 2]. Males seem to be more sensitive than females of the same strain to the toxic action of the carcinogen [3, 4]. This sex difference is well known for a number of toxic substances such as warfarin, benzene and lead [5]. For the detection of toxic effects of xenobiotics, enzymes such as gamma-glutamyltransferase, transaminases and ALP are among the more sensitive and specific markers. They are generally measured in the plasma and they are used as indicators of liver injury. They reflect, for example, mechanisms of cellular release of proteins and their subsequent extracellular turnover or mechanisms of the neoplastic processes [6, 7]. Damaged cells lose their constituents including enzymes which are distributed all over the extracellular space. The rise of their activity in plasma has been shown to be in good correlation to the number of damaged cells. AFB_1 exerts toxic action after its metabolization to reactive derivative [8, 9]. To have a better understanding of the action of the mycotoxin and to explain the differential toxicity in male and female species, this study was carried out by comparing the effects of the carcinogenic compound on hepatic drug-metabolizing enzymes in male and female rats.

MATERIALS AND METHODS

Animals and treatment. Male and female Sprague–Dawley rats (Domaine des Oncins, St.-Germain-sur-l'Arbresle, France) weighing 160–180 g were kept on a 14 hr light and 10 hr dark cycle. The animals were allowed free access to food (UAR-Alimentation, Villemoisson, France) and tap water. Animals were given a single intraperitoneal dose of AFB_1 (Sigma, St. Louis, MO, dissolved in propylene glycol (Merck, Darmstadt, F.R.G.): 1 or 3 mg/kg for males; 3 or 6 mg/kg for females. Control rats received an equivalent volume of propylene glycol alone. Animals were sacrificed by decapitation at various intervals (12 hr to 20 days) after the single treatment. After exsanguination, the livers were quickly excised, washed with 0.25 M sucrose—1 mM Tris–HCl, pH 7.4, buffer and fractionated as described [11]. Each blood sample was collected using lithium heparin anticoagulant and centrifuged immediately; plasma was stored at 4° until assayed.

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† Abbreviations: AFB_1, aflatoxin B_1; UDPGT, uridine diphosphate glucuronosyltransferase; PNP, *p*-nitrophenol; SO, sytrene oxide; BP-diol, benzo[*a*]pyrene diol; GSH, glutathione; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Microsomes and cytosol samples were kept at −20° for biochemical determinations.

Assays Protein was assayed by the method of Lowry *et al.* [12] using bovine serum albumin as the reference protein. The cytochrome P-450 content of the microsomes was determined [13] using an Aminco DW2 spectrophotometer. Microsomal epoxide hydrolase was measured by a fluorimetric method [14] with benzo[*a*]pyrene-4,5-oxide (IIT Research Institute, Chicago, IL) as substrate. UDPGT activity for both groups of substrates postulated by Wishart [15] was estimated by the method of Mulder and Van Doorn [16] adapted to the centrifugal fast analyser COBAS (Roche Bioelectronique) using PNP (Merck) and L-borneol (Aldrich, Beerse, Belgium) as aglycones. The GSH *S*-epoxide transferase activity was measured in the cytosol [17] with SO (Fluka AG, Basel, Switzerland) as substrate. Total bilirubin concentration was determined for plasma [18]. The activity of AST and ALT was measured at 30° by an optimized method [19, 20] and that of ALP by the method of Mathieu *et al.* [21] at the same temperature.

Data were analysed using the Student's *t*-test. That the significance of the results calculated on a g liver basis was the same as those expressed on a mg protein basis was checked.

RESULTS

Liver weight

Figure 1 shows the change in the liver weight of male and female rats treated with AFB_1. In the male, neither dose (1 or 3 mg/kg) induced a change for the first 3 days following AFB_1 administration. By days 3 to 9, especially with the 3 mg/kg treatment, AFB_1 decreases the liver weight. Maximum decreases were 18 and 40% at 1 and 3 mg/kg, respectively, and occurred by day 4 following treatment. In the female, the pattern of liver weight was similar in treated and control animals for the first 4 days after treatment. Thereafter, the liver weight was significantly enhanced by AFB_1 and was maximal (36% at 3 mg/kg; and 57% at 6 mg/kg) at the end of the experiment.

Plasma determinations in male and female rats

Figure 2 displays comparative changes in plasma features between male and female rats treated with the same dose of AFB_1 (3 mg/kg). The obtained results showed that generally, in the two sexes, the plasma levels of the studied constituents were maximal within the three days following the administration of the carcinogen. However, in the male, the total bilirubin concentration and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and ALP reached mean values 45, 60, 100 and 3 times that of controls, respectively, while in the female, these values were 17, 46, 189 and 4 times the controls.

Neither the bilirubin level nor the ALP activity in the male were affected by the 1 mg AFB_1/kg. On the contrary, the same dose slightly, but significantly, increased the activities of transaminases (65–290%)

In the female which was treated with 6 mg AFB_1/kg, the changes in plasma measurements were similar to that induced with the 3 mg/kg treatment. The bilirubin concentration, the AST, ALP and ALP activities were respectively 20, 77, 254 and 6 times the control mean values.

Drug-metabolizing enzymes in female rats

Cytochrome P-450. AFB_1 (3 mg/kg) was found to decrease (23% by day 2) the cytochrome P-450 content for the first 72 hr following treatment (Fig. 3). Thereafter, the variations induced by AFB_1 were within the limits of controls, although significant changes could be observed from the fourth day onwards.

On the contrary, at the dose of 6 mg/kg, the cytochrome P-450 concentration decreased significantly from the 2nd to the 7th day after AFB_1 administration, reaching a maximum decrease of 34% by day 2. At the end of the experiment, the cytochrome P-450 levels in 6 mg/kg treated rats rose significantly above the controls.

Epoxide hydrolase. Figure 4 illustrates the effects of AFB_1 on the activity of epoxide hydrolase. Only the higher dose (6 mg/mg) caused a significant increase (60%) of the enzyme activity by the 12th hr after treatment. Thereafter, and whatever the dose administered, the carcinogen increased significantly the hydrolase activity from the first to the 20th day after injection. The maximum increases of the enzyme activity in both groups of 3 and 6 mg/kg treated animals were, respectively, 5 and 8 times higher than in controls.

UDP-glucuronosyltransferase. The activity of

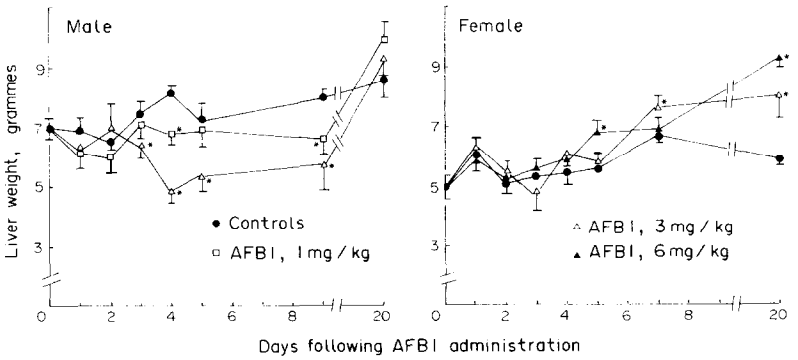

Fig. 1. Effect of AFB_1 treatment on liver weight of male and female rats. Values represent the mean ± S.D. for 3–6 animals. *Significantly different from controls ($P < 0.05$).

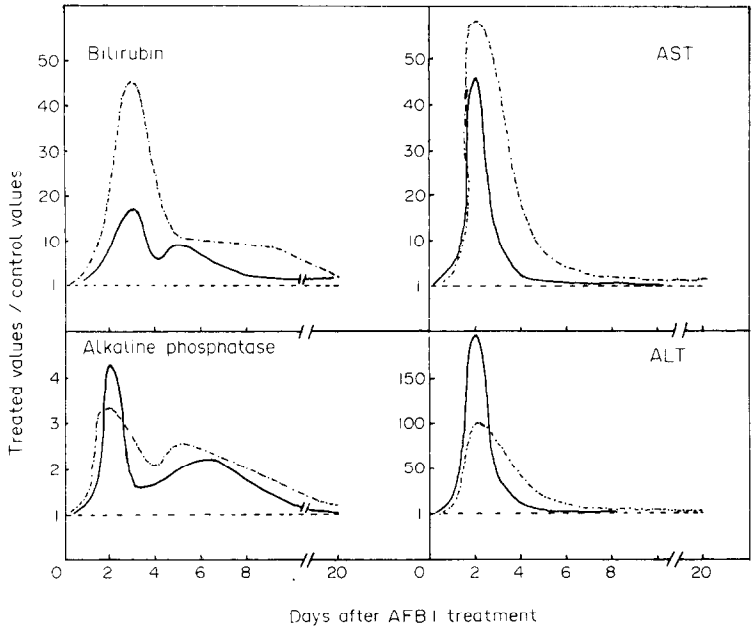

Fig. 2. Comparative changes in plasma parameters of male (. . . . .) and female (———) rats treated with the same dose (3 mg/kg) of AFB_1. Values were obtained from 3 to 6 animals.

UDPGT with PNP as substrate (Fig. 5a) was significantly reduced (14 and 25%) for the first 48 hr following treatment with both doses of 3 and 6 mg AFB_1, respectively. The enzyme activity rose significantly above controls by day 4, to reach a maximum 72% increase at the dose of 3 mg AFB_1/kg, and 125% increase with the 6 mg/kg treatment.

When using 1-borneol as substrate (Fig. 5b), the enzyme activity increased by 25% 12 hr after administration of the lower dose of AFB_1 (3 mg/kg) and significantly decreased (15%) by day 2. At the dose of 6 mg/kg, AFB_1 decreased the activity of UDPGT for the first 72 hr after treatment, with a maximum decrease of 46%. From day 4, the activity of UDPGT (borneol) in treated rats (both groups) returned to control values.

GSH S-transferase. AFB_1 decreased (Fig. 6) significantly the activity of GSH S-transferase (43 and

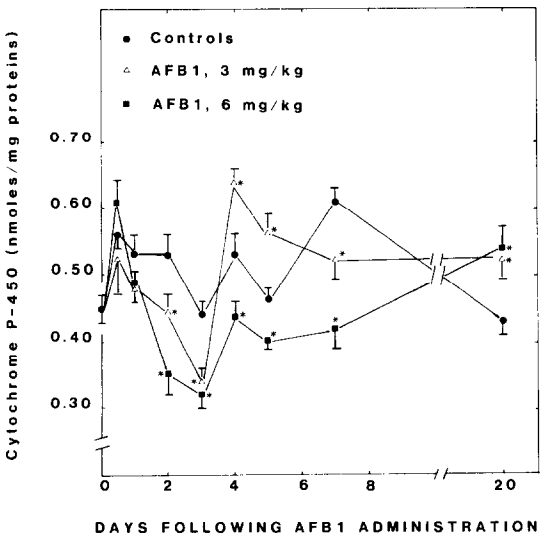

Fig. 3. Cytochrome P-450 content profiles in AFB_1-treated female rats. Values are the mean ± S.D. for 4–6 rats. * Significantly different from controls ($P < 0.05$).

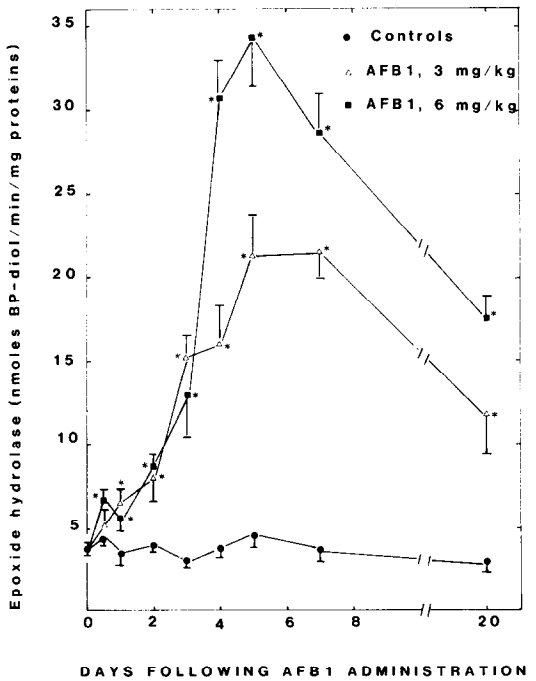

Fig. 4. Changes in the activity of epoxide hydrolase following AFB_1 administration to female rats. Points are the mean ± S.D. for 4–6 rats. *Significantly different from controls ($P < 0.05$).

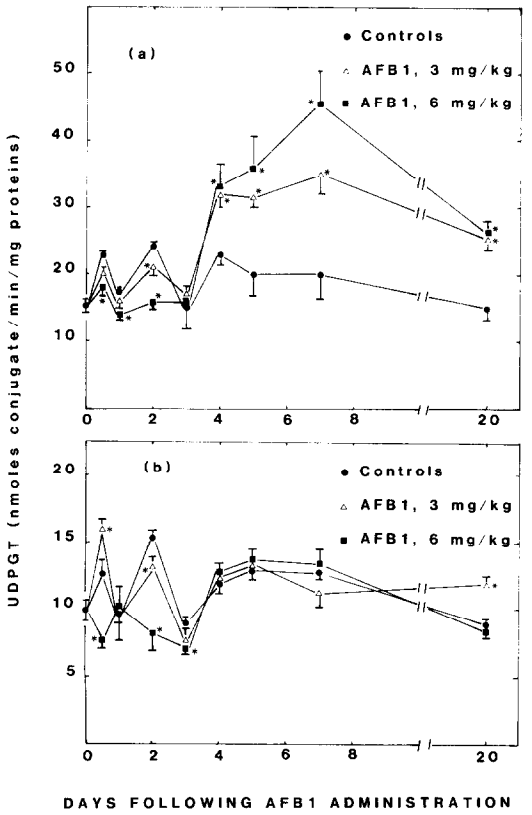

Fig. 5. Relationship between AFB_1 dose administered and the activity of UDP-glucuronosyltransferase: (a) with PNP as group I planar substrate; (b) with 1-borneol as group II bulky substrate. Results are the mean ± S.D. for 4–6 female rats. *Significantly different from controls ($P < 0.05$).

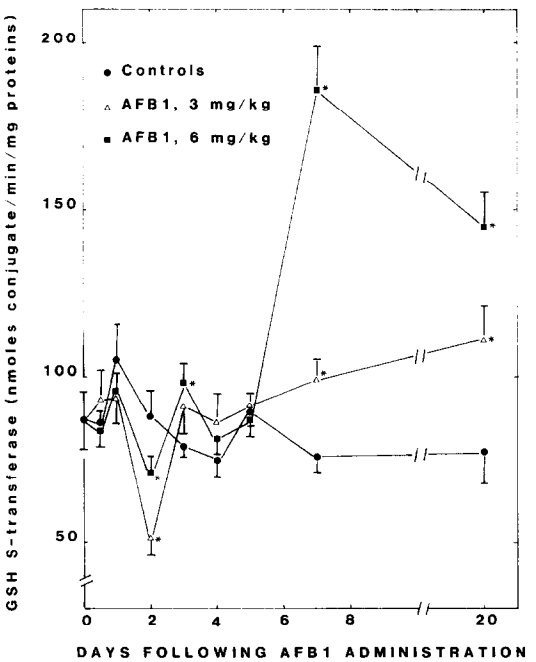

Fig. 6. Effect of AFB_1 upon GSH *S*-transferase activity in female rats. Values represent the mean ± S.D. for 4–6 animals. *Significantly different from controls ($P < 0.05$).

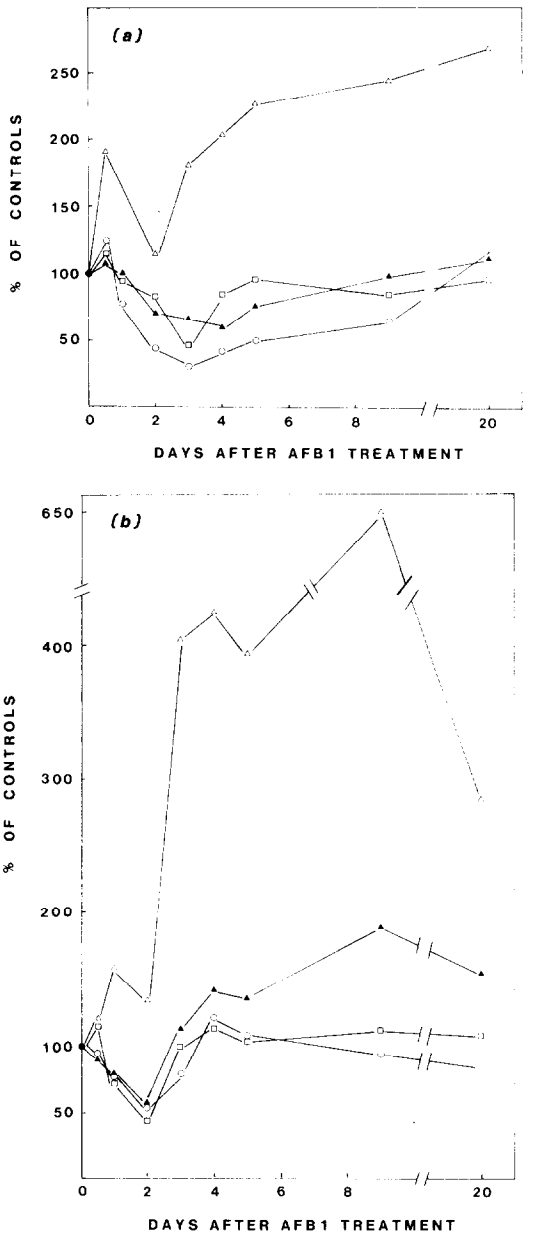

Fig. 7. Changes in the cytochrome P-450 content (○——○) and in the activities of epoxide hydrolase (△——△), UDPGT (PNP) (▲——▲), GSH *S*-transferase (□——□) in male (a) and female (b) rats treated with the same dose (3 mg/kg) of AFB_1. Results were obtained from 3 to 6 animals.

20% at 3 and 6 mg AFB_1/kg respectively) by the 2nd day after treatment. Thereafter, the enzyme activity rose above the control values reaching maximum 42 and 144% increases at the doses of 3 and 6 mg/kg, respectively.

Drug-metabolizing enzymes in male rats

At the dose of 3 mg/kg, most of the drug-metabolizing enzyme activities, in the male, except that of epoxide hydrolase, were lessened by AFB_1 for the first 9 days following treatment (Fig. 7a). The results

showed that there was a 69% decrease in the cytochrome P-450 content, 40 and 53% in the activities of UDPGT (PNP), GSH *S*-transferase, respectively.

DISCUSSION

In this study, at the dose of 1 mg/kg, AFB_1 had no lethal effect, while the 3 mg/kg treatment induced an 18% mortality in male rats but not in females. Only the dose of 6 mg/kg caused a 25% mortality in female rats. These results are in agreement with literature data [1, 3, 22, 23] and were characterized in males, by an important decrease (40%) of liver weight (Fig. 1). On the contrary, for females, AFB_1 led to an increase in the liver weight. The toxocity of the carcinogen was at its top level by days 2 or 3 as shown by the plasma levels of bilirubin and the activities of AST, ALT and ALP, which are considered to be good indicators of hepatotoxicity. According to the same criteria, the toxic effects were more prolonged in males. Interestingly, in females after the toxic period, which was characterized by the drop of drug-metabolizing enzymes, a fast recovery of hepatic function was found. This period took place as soon as three days after treatment and was accompanied with a decline of plasma toxicity markers, an increase of liver weight and the return of hepatic drug-metabolizing enzyme activities to levels similar to or higher than those of controls. In males, the restoration of hepatic function was slower, and occurred only between days 9 and 20 following AFB_1 administration.

In males, the 3 mg/kg treatment decreased most of the hepatic drug-metabolizing enzyme activities for the first 9 days following administration. This decrease was less important in the female and it occurred only for the first three days after treatment. Moreover, from the fourth day forwards, there was an induction of detoxicating enzyme activities in the female rat liver. Chronic administration of repeated small doses of AFB_1 has been found to increase the cytochrome P-450 content and the activities of hepatic drug-metabolizing enzymes. However, these small doses have no effect of the plasma enzyme activities [24]. This suggests that AFB_1 effect on drug-metabolizing enzymes is well related to the degree of AFB_1 toxicity. Gurtoo and Dahms [25] have reported the possible involvement of different cytochromes P-450 in the metabolism of AFB_1 via various pathways. The fact that the cytochrome P-450 content decreases regardless of sex and the dose of AFB_1 suggests that acute AFB_1 treatment had a direct destructive effect on the cytochrome P-450 system.

Treatment with AFB_1 increased by more than 5-fold the activity of epoxide hydrolase in female rats, against only 2-fold increase in males. Thus, the AFB_1-epoxide formed could be more rapidly metabolized in females than in males. Moreover, it is well known that AFB_1 is much less bound to female than male rat liver subcellular fractions [26]. Among the metabolites of AFB_1, are aflatoxin M_1, a hydroxylated derivative considered to be as toxic as AFB_1 [27, 28], and AFB_1-epoxide which forms covalent bonds with proteins and nucleic acids [29, 30]. Epoxides, highly reactive metabolites, are responsible for the toxic and carcinogenic effects of most of the chemical compounds [31, 32], and the role of epoxide hydrolase in the transformation of these epoxides into dihydro-diols, generally not toxic, has been demonstrated [33, 34]. Moreover, GSH *S*-epoxide transferase was less affected and even increased in females treated with AFB_1. Conjugation to GSH via the GSH *S*-transferases has been established as an important pathway of detoxification in the excretion of toxic metabolites of AFB_1 [35].

It must be underlined that the basal activities of hepatic drug-metabolizing enzymes were higher in the male than in the female. On the contrary, after AFB_1 treatment, the levels of enzyme activities were higher in females. For example, the maximal activity of epoxide hydrolase following administration of 3 mg AFB_1/kg dose was 21.5 ± 1.6 nmoles BP-diol/min/mg proteins in the female (basal activity: 3.7 ± 0.6), against 15.0 ± 1.0 nmoles BP-diol/min/mg proteins in the male (basal activity: 5.9 ± 1.0) treated with the same dose of AFB_1. These observations lend a strong support to the argument that the detoxification system is more efficient in females than in males.

Many xenobiotics or endogenous compounds are metabolized in the liver to glucuronides which are excreted in urine as generally inactive compounds. The increase of UDPGT activity by up to 72% in females, versus a 40% decrease in males, might contribute to a more important excretion of toxic AFB_1 derivatives as glucuronoconjugates. The importance of phase II enzymes thus becomes clear. Bock *et al.* [36] have recently reported the role of glucuronidation in the inactivation and elimination of polycyclic aromatic hydrocarbons. However, its role in the glucuronidation of AFB_1 has not yet been elucidated and needs further investigation.

The present study has underlined the sex-related difference in AFB_1 toxicity between male and female rats. It reveals also the pattern difference of drug-metabolizing enzymes in the two sexes. The induction of detoxicating enzyme activities in the female may have a protective role against AFB_1-metabolites and could explain, in part, the higher sensitivity of the male to the toxic effects of AFB_1 than the female. This result suggests also that the $t_{1/2}$ of AFB_1 may be much shorter in females.

Sex differences have also been described in humans exposed to aflatoxin consumption, and the incidence of hepatocarcinomas probably due to AFB_1 is 2–5 times higher in men than in women [37–39]. The underlying mechanisms of this difference are unknown but are likely to involve hormonal regulation of the rate of AFB_1 metabolism or of the detoxification of active derivatives. Androgens have been reported to increase drug metabolism whereas estrogens have either no effect or are inhibitory [40–42]. The liver is not only the main centre of estrogen catabolism but is also a target organ for these compounds. Hepatocytes have cytosolic and nuclear receptors which make them, at least partially, estrogen dependent. Righter *et al.* [43] have shown that testosterone treatment enhances AFB_1 toxicity in male rats. Moreover, AFB_1 metabolism in immature male and in immature female rats was also reported to increase after testosterone admin-

istration [44]. These results suggest that the gonads may have a determinant role in the toxicity of AFB_1. Rather than the hormones themselves, it seems more and more evident that the hypothalamo-pituitary axis is directly implicated in the regulation of drug metabolizing enzymes [45].

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