in the current study the enzyme preparation was exposed to $Pb(NO_3)_2$ for a short time when compared to the chronic exposure that would be expected *in vivo*. Thus, it is possible that even lower amounts of Pb^{2+} could gradually bind to MAO and slowly decrease the number of active enzyme molecules. Animal studies would be needed to assess these possibilities.

The data obtained in the present study can be compared to that obtained with another isoenzyme, serum amine oxidase. The serum enzyme catalyzes the same reaction as MAO but is soluble and differs in its metal and cofactor requirements [26]. Lead reversibly inhibits serum amine oxidase, and the K_i is 46 μM [27].

In summary, type A and type B MAO were irreversibly inhibited by $Pb(NO_3)_2$. Type A and type B MAO were inhibited 50% by 1.0 mM $Pb(NO_3)_2$ (0.36 μmole Pb^{2+}/mg protein) and 0.9 mM $Pb(NO_3)_2$ (1.1 μmoles Pb^{2+}/mg protein) respectively.

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In vitro interconversion of aflatoxin B_1 and aflatoxicol by rat erythrocytes

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Aflatoxin B_1 (AFB_1), produced by some strains of *Aspergillus*, is a hepatotoxic and hepatocarcinogenic agent in a number of animal species [1, 2]. AFB_1 requires metabolic activation to exert its carcinogenic effect [3, 4]. Among the various metabolites of AFB_1, aflatoxicol (AFR_0) has been shown to possess the greatest mutagenic activity [5]. Thus far, most studies regarding the metabolism of AFB_1 to AFR_0 have focused on hepatic tissue; little is known about the metabolism of AFB_1 in the extrahepatic tissues. In this communication, the reversible interconversion of AFB_1 and AFR_0 catalyzed by rat erythrocytes is presented.

Materials and methods

Aflatoxin B_1 and AFR_0 were purchased from Makor Chemicals (Jerusalem, Israel). Enzymes and all chemicals were obtained from The Sigma Chemical Co. (St. Louis, MO, U.S.A.). Organic solvents were the products of Union Industrial Research Laboratories (Hsinchu, Taiwan, ROC).

Male Sprague–Dawley rats weighing about 150 g were used in the experiments. Venous blood was collected from the inferior vena cava of the rats in heparinized syringes. Erythrocytes were obtained by centrifuging at 600 *g* for

5 min. After washing three times with isotonic saline solution, the erythrocytes were resuspended in a volume of saline solution equal to that of the original volume of blood.

In the basic incubation system, AFB_1 or AFR_0 (5 μg) was dissolved in 20 μl of dimethyl sulfoxide and added to the tubes containing 0.5 ml of blood samples and 0.5 ml of buffer–saline solution (phosphate buffer, 50 mM, pH 7.4, containing 0.9% NaCl). The incubations were performed at 37° for 2 hr. At the end of incubation, the tubes were placed in an ice-bath to stop the reaction. Cold methanol (2 ml) was added with vigorous shaking to precipitate the proteins. The denatured proteins were removed by centrifuging at 8000 *g* for 30 min. The supernatant fluids were transferred to another tube and extracted three times with 1 ml of $CHCl_3$. The chloroform extractable fractions were dried under nitrogen gas.

The quantitation of AFB_1 and AFR_0 were carried out on a Micromeritics 7000B Liquid Chromatograph (Micromeritics Instrument Corp., Norcross, GA, U.S.A.). All analyses were performed on a μ-Porasil (Waters Associates Inc. Milford, MA, U.S.A.) column using chloroform–benzene–acetonitrile (75:7.5:1, by vol., containing 0.6% ethanol) as the mobile phase at a flow rate of 1 ml/min under 1,300 psi. The absorbance of the eluent at 325 nm was monitored to detect AFB_1 and AFR_0.

To study the factors which influence the interconversion of AFB_1 and AFR_0, variations were made in the basic incubation system. The metabolites formed in different incubations were determined by high performance liquid chromatography (HPLC) as described above. The factors being studied were plasma, glucose, incubation pH and incubation time (see Table 1 and figures for details).

The concentrations of NADP and NADPH in the erythrocytes and blood were determined by the method of Jorgensen and Rasmussen [6]. The extraction of NADP and NADPH was carried out by the method of Klingenberg [7].

Results and discussion

Our previous work* showed that rat blood is capable of catalyzing the interconversion of AFB_1 and AFR_0. In the present study, the same phenomenon was also observed. The washed erythrocytes did not transform AFB_1 into AFR_0; however, the transformation of AFR_0 to AFB_1 catalyzed by washed erythrocytes was much higher than that catalyzed by whole blood (Table 1). When rat plasma was added to the erythrocyte suspension, the transformation of AFB_1 to AFR_0 was observed. Conversely, the transformation of AFR_0 to AFB_1 was lowered upon addition of plasma to erythrocyte suspension. For studying the nature of the factor in the plasma that was required for the transformation of AFB_1, the plasma was boiled or dialyzed and then added to the erythrocyte suspension. The results show that boiled plasma also exhibited a stimulating effect on the transformation of AFB_1 to AFR_0; however, the stimulating effect of plasma was completely abolished upon dialysis (Table 1). Therefore, it may be speculated that the substance in the plasma which influenced the transformation of AFB_1 is a heat-stable small molecule.

To search for the small molecule required for the transformation of AFB_1, various compounds were added to the erythrocyte suspension and the transformation of AFB_1 was examined. It was shown that some sugars such as glucose, mannose and fructose were effective in stimulating the transformation of AFB_1 to AFR_0 catalyzed by rat erythrocytes (data not shown). As glucose is one of the most abundant sugars in animals, the effect of glucose on the transformation of AFB_1 to AFR_0 was examined further. As can be seen in Fig. 1, the transformation of AFB_1 to

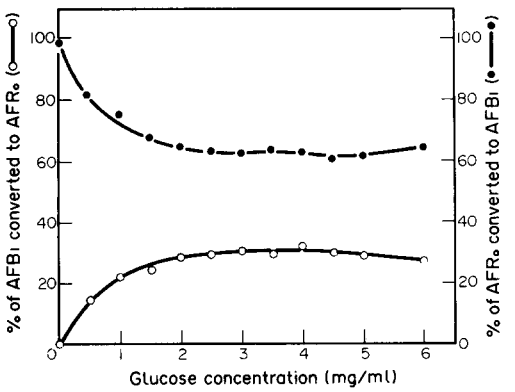

Fig. 1. Effect of glucose on the interconversion of AFB_1 and AFR_0 in rat erythrocytes. The incubations were performed at 37° for 2 hr in buffer–saline solution (pH 7.4).

* W. M. Chang, J. K. Lin, K. C. Wu and K. P. Hsiung, Fiftieth Annual Meeting Report, Chinese Chemical Society, Abstr. 50, p. 213 (1982).

Table 1. Interconversion of AFB_1 and AFR_0 by whole blood and erythrocytes

Blood sample	Substrate	Metabolite	%(Metabolite/Substrate)*
Whole blood	AFB_1	AFR_0	13.7 ± 3.4
	AFR_0	AFB_1	84.3 ± 2.2
RBC	AFB_1	AFR_0	ND†
	AFR_0	AFB_1	99.4 ± 0.5
RBC + plasma‡	AFB_1	AFR_0	15.1 ± 2.4
	AFR_0	AFB_1	86.5 ± 4.1
RBC + boiled plasma	AFB_1	AFR_0	14.0 ± 1.9
	AFR_0	AFB_1	87.3 ± 3.5
RBC + dialyzed plasma‖	AFB_1	AFR_0	ND
	AFR_0	AFB_1	99.2 ± 2.4
RBC + glucose¶	AFB_1	AFR_0	31.5 ± 1.9
	AFR_0	AFB_1	63.1 ± 4.6

* Values represent mean ± S.E. obtained from three determinations.
† Non-detectable.
‡ Buffer (0.3 ml) was replaced by plasma.
§ Plasma was boiled at 100° for 1 min.
‖ Plasma was dialyzed against distilled water for 3 hr.
¶ Final concentration: 3 mg/ml.

AFR_0 was proportional to the concentration of glucose in the incubation mixture. On the other hand, the transformation of AFR_0 to AFB_1 was found to decline upon addition of glucose.

The effect of glucose in stimulating the transformation of AFB_1 to AFR_0 catalyzed by erythrocytes may be related to the hexose monophosphate shunt (HMP) in the erythrocytes. After entering erythrocytes, glucose can be phosphorylated by hexokinase to form glucose-6-phosphate and then can pass into the HMP shunt with the regeneration of NADPH, which is required for the transformation of AFB_1. To verify this explanation, the concentrations of NADP and NADPH in the erythrocytes were measured. As shown in Table 2, the ratio of NADPH to NADP in whole blood was higher than in washed erythrocytes. When whole blood was incubated at 37° for 2 hr, the ratio of NADPH to NADP was approximately the same as that of fresh blood. However, when erythrocytes were incubated under the same conditions, the ratio of NADPH to NADP declined. When glocose was added to the erythrocyte suspension and incubation at 37° for 2 hr, the ratio was several-fold higher than in the absence of glucose. This result clearly demonstrates that glucose enhanced the regeneration of NADPH in the erythrocytes and, consequently, the transformation of AFB_1 was also enhanced.

When the toxins were incubated with erythrocyte suspension in buffer solutions of different pH, the maximum transformation of AFB_1 to AFR_0 was found at pH 6.5 to 7.5, and the maximum transformation of AFR_0 to AFB_1 was found at pH 7.0 to 8.0 (Fig. 2). It has been shown that the HMP shunt in erythrocytes is interrupted upon lysis of the cells [8]. When the incubation was performed at pH 4.0 or 9.0, hemolysis of the erythrocytes was observed. Thus, as can be seen in Fig. 2, the transformation of AFB_1 and AFR_0 declined rapidly at these pH levels.

When the incubations were carried out at physiological pH, the transformation of the toxins depended on the incubation time. Figure 3 shows that a 2-hr incubation was required for the maximum transformation of AFB_1 and AFR_0. When the concentration of substrate (AFB_1 or AFR_0) was below 500 ng/ml, the maximum transformation of the toxins was achieved within 30 min (data not shown). Owing to the difficulty encountered in measuring nanogram levels of aflatoxins by our analytical system, we used 5 μg of AFB_1 and AFR_0 in the incubation system. Thus, all incubations in the present experiment were performed for 2 hr to obtain a maximum toxin transformation.

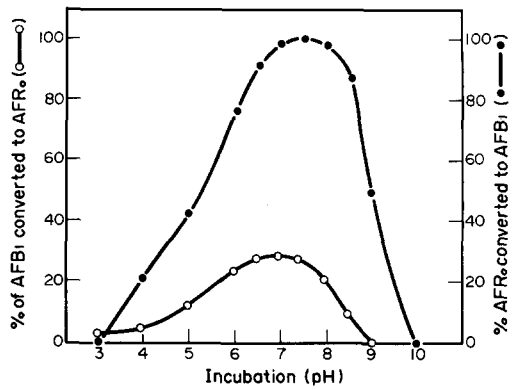

Fig. 2. Effect of pH on buffer–saline on the interconversion of AFB_1 and AFR_0 in rat erythrocytes. Aflatoxin B_1 or AFR_0 (5.0 μg) was incubated with 0.5 ml of red cells in buffer–saline at various pH levels. Glucose was added into the incubation mixture at the level of 3 mg/ml to study the conversion of AFB_1 to AFR_0. Buffers used: citric acid–Na_2HPO_4 buffer (pH 3.0 to 6.0), sodium phosphate buffer (pH 6.5 to 8.0) and diethanolamine–HCl buffer (pH 8.5 to 10.0).

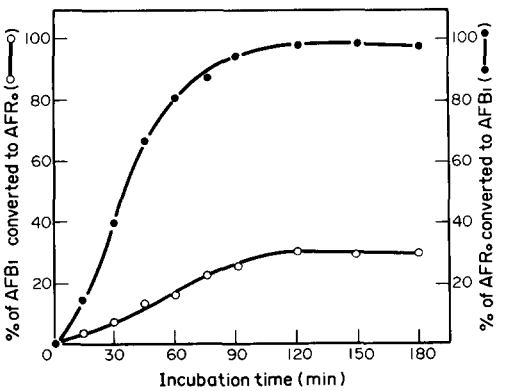

Fig. 3. Effect of incubation time on the interconversion of AFB_1 and AFR_0 in rat erythrocytes. The incubations were performed at 37° for various times. Glucose was added to the incubation mixture at the level of 3 mg/ml to study the conversion of AFB_1 to AFR_0.

Table 2. Concentrations of NADP and NADPH in rat erythrocytes

Blood samples	NADPH* (μM)	NADP* (μM)	NADPH/NADP
Whole blood, freshly drawn	23.9 ± 0.8	6.4 ± 1.2	3.73
RBC, washed	18.4 ± 2.7	11.5 ± 2.3	1.60
Whole blood, incub. at 37°, 2 hr	21.8 ± 3.1	5.6 ± 1.9	3.89
RBC, incub. at 37°, 2 hr	10.6 ± 1.4	16.0 ± 1.9	0.66
RBC + glucose† incub. at 37°, 2 hr	24.3 ± 1.9	2.1 ± 0.6	11.57

* Values represent mean ± S.E. obtained from three determinations.
† Glucose was added to the red cell suspension at the level of 3 mg/ml.

The work of Wong and Hseih [9] showed that AFR_0 is the major metabolite in rat plasma when AFB_1 is administered intravenously or orally, and that the concentration of AFR_0 in plasma is maximal at 5 min after dosing. Wong and coworkers postulated that the AFR_0 in rat plasma is produced by the action of liver cytoplasmic AFB_1-reductase. As a matter of fact, AFB_1-reductase activity in rat liver cytosol is relatively low compared with that of erythrocyte. Probably, the *in vivo* formation of AFR_0 in rat plasma is catalyzed by the action of erythrocyte AFB_1-reductase. Since AFB_1 is carried in the blood from the gastrointestinal mucosa to the target organ, the transformation of AFB_1 in hepatic tissues or in target organs may be modified by the action of enzyme(s) present in the erythrocytes. Recently, the AFB_1-reductase of chicken liver was partially purified by Chen *et al.* [10]. The molecular weight of the enzyme was estimated to be 46,500, and the activity of the enzyme was inhibited 50–70% by some steroid hormones. It seems that the AFB_1-reductase in the liver cytosol is attributable to a steroid-metabolizing enzyme. However, the intrinsic role of the AFB_1-reductase in erythrocytes *in vivo* warrants further investigation.

In summary, the rat erythrocyte was shown to be capable of catalyzing the interconversion of AFB_1 and AFR_0. The transformation of these toxins was modified by glucose, owing to the variation in the concentration of NADP and NADPH in the erythrocytes.

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Xenobiotic imprinting of hepatic metabolic enzyme systems: effect of neonatal 3-methylcholanthrene administration*

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The cytochrome P-450-dependent polysubstrate monooxygenase system (PSMOS) is the primary hepatic enzyme system responsible for the activation and/or detoxication of both endogenous and exogenous substrates. In many cases, the detoxication is due to the fact that increased polarity of the product facilitates excretion. In other cases, the product may be highly electrophilic, resulting in covalent binding to nucleophilic sites on tissue macromolecules and thus in increased toxicity. The activity of this system can be increased or induced by many different classes of compounds. These inducers include the barbiturates, pesticides, steroids and polycyclic hydrocarbons, among others.

Certain endogenous steroids have major influences on this enzyme system. An often studied example of this is the role of testosterone in regulating PSMOS activity in the rat. An adult male rat has higher PSMOS activities than does the female. These sex differences usually appear after 30 days of age. Depriving male rats of testosterone by castration soon after birth prevents these sex differences [1, 2]. This property of testosterone, whereby its presence during the neonatal period results in alterations later in the life of the animal, is known as neonatal imprinting [3].

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This work with testosterone led to studies to determine if imprinting could be evoked by the administration of a nonsteroidal xenobiotic that is known to induce the PSMOS. A logical choice for such an agent was phenobarbital (PB). This barbiturate is one of the most extensively studied inducers of PSMOS. Using PB in various dosing protocols and determining PSMOS activity by different methods, it has been shown by us and others that PB administration during the neonatal period imprints or programs rat hepatic metabolic enzyme activities [4–6].

We previously reported that PB administration during the early neonatal period resulted in elevated enzyme activities in both male and female rats at 140 days of age [4]. These changes include higher P-450 levels, increased P-450 reductase activity, increased metabolism of ethoxycoumarin, higher rates of glucuronyltransferase activity, and increased binding of carcinogens to DNA both *in vitro* and *in vivo*.

Since evidence now exists that compounds from two different inducer classes, i.e. steroids and barbiturates, can imprint the PSMOS, the possibility exists that neonatal administration of a potent inducer from a different inducer class could program PSMOS activity in adulthood. We chose 3-methylcholanthrene (3-MC) as representative of the polycyclic hydrocarbons which induce forms of P-450 distinct from those induced by PB.