although not easily explainable. Possibly, the high percentage of oleic acid in F cells could balance the low proportion of arachidonic acid, so establishing a percentage of unsaturated fatty acids of about 53% in F cells as well as in M cells. A definite steady state between saturated and unsaturated molecules could be a requisite for membrane fluidity and function [19].

In oxygen deprived conditions, glucose functions as the major energy-yielding substrate, by synthesis of ATP through the glycolytic pathway. In the present study, oxygen and glucose deprivation together are responsible for the modifications observed in the fatty acid composition of the phospholipids of H, M and F cell cultures. They induce different responses according to the types of cell which is submitted to them. F cultures, which were the least sensitive, maintained normal amounts and proportions of fatty acids in phospholipids. Inversely, M cells were utterly affected by the "ischemic" condition. They undergo a progressive loss of phospholipids, similar to the one observed by Chien *et al.* [20] in ischemic hearts. This loss may be considered as a manifestation of membrane injury, probably due to phospholipase activation [20, 21] and to impaired resistance of sarcolemma to enzymatic attack [22]. The reason why F cells were not susceptible to lytic phenomena would call for additional studies. Nevertheless, a difference in phospholipase activity seems to be present notwithstanding the difference found by Chajek *et al.* [23] between M and F cells as regards lipoproteinlipase.

In M cells, the most important loss of phospholipid fatty acids was observed in the polyenoic acids, thereby modifying fatty acid proportions. Arachidonic and docosahexaenoic acid proportions are reduced to levels which approximate the specific values of F cells. Otherwise as M cells stop beating under oxygen and glucose deprivation, they tend to reach the polyenoic profile of non-beating F cells. Myristic, palmitic and oleic acids, although reduced absolutely, were increased in percentages. This could be a consequence of the leakage of arachidonic acid, which could promote the reinsertion of saturated and monoenoic fatty acids [24].

The response of H cells to "ischemic" condition resemble those of F cells more closely than those of M cells: the fatty acid proportions shift to a disproportion between the percentages of monoenoic acids (which are reduced) and saturated acids (which are increased); the normal proportion of polyenoic fatty acids is maintained, although beatings stop. H cells do not undergo any phospholipid loss; on the contrary, they exhibit a 24% increase in total amount of fatty acids. These facts could suggest that, in mixed cell cultures, F cells tend to stabilize M cells.

In summary, heart muscle and non muscle cells in culture exhibited different proportions of phospholipid fatty acids: in M cells, polyenoic fatty acid proportions were higher, and mono- and dienoic fatty acid proportions were lower than in F cells. Moreover, M cells showed responses to simulated ischemia more sensitiviely. The model studied here appears a useful tool to investigate the effects of drugs on membrane composition in two different types of heart cells.

Acknowledgements—We thank Muriel Meier and Ghislaine Simonin for skilful technical assistance.

Institut de Pharmacologie
Laboratoire Associé du C.N.R.S.
n°206, 15, rue de l'Ecole de Médecine
75006 Paris, France

MARIANNE FREYSS-BEGUIN
ELISABETH MILLANVOYE-VAN BRUSSEL
GENEVIÈVE GRIFFATON
PAUL LECHAT

REFERENCES

1. T. J. C. Higgins, P. J. Bailey, D. Allsopp and D. A. Imhof, *J. Pharm. Pharmac.* **33**, 644 (1981).
2. E. Van Brussel and M. Freyss-Béguin, *Biol. Cell.* **35**, 153 (1979).
3. D. Acosta and C. P. Li, *In Vitro* **15**, 929 (1979).
4. D. Acosta and C. P. Li, *In Vitro* **16**, 93 (1980).
5. T. J. C. Higgins, D. Allsopp and P. J. Bailey, *J. molec. Cell. Cardiol.* **12**, 909 (1980).
6. A. Van Der Laarse, M. L. Graf-Minor and S. A. G. J. Witteveen, *Clinica. chim. Acta* **91**, 47 (1979).
7. D. G. Wenzel and J. W. Kleoppel, *Toxicology* **15**, 105 (1980).
8. M. Freyss-Béguin, E. Van Brussel and P. Lechat, *Biol. Cell.* **41**, 51 (1981).
9. E. Millanvoye-Van Brussel, M. Freyss-Béguin, G. Griffaton and P. Lechat, *Biochem. Pharmac.* **34**, 145 (1985).
10. G. Griffaton, M. Freyss-Béguin, E. Millanyove-Van Brussel, F. Dupuy, H. Millart, R. Lowy and P. Lechat, *C.R. Séances Soc. Biol.* **178**, 130 (1984).
11. I. Harary and B. Farley, *Exp. Cell Res.* **29**, 451 (1963).
12. M. Freyss-Béguin and E. Van Brussel, *Biol. Cell.* **37**, 111 (1980).
13. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
14. J. Folch, M. Lees and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1957).
15. J. Maclouf, B. Fruteau de Laclos and P. Borgeat, *Proc. natn. Acad. Sci. U.S.A.* **79**, 6042 (1982).
16. J. C. Dittmer and M. A. Wells, *Meth. Enzym.* **14**, 482 (1969).
17. G. W. Snedecor and W. G. Cochran, in *Statistical Methods*, 6th Edn. Iowa State University Press, Ames, Iowa (1967).
18. S. Gudbjarnason, B. Doell and G. Oskarsdottir, *Acta biol. med. germ.* **37**, 777 (1978).
19. P. Henkart, *Int. Rev. Cytol.* **60**, 121 (1979).
20. K. R. Chien, A. Han, A. Sen, L. M. Buja and J. T. Willerson, *Circulation Res.* **54**, 313 (1984).
21. W. B. Weglicki, K. Owens, C. W. Urschel, J. R. Serur and E. H. Sonnenblick, *Recent Adv. Stud. Cardiac Struct. Metab.* **3**, 781 (1973).
22. T. J. C. Higgins, P. J. Bailey and D. Allsopp, *J. molec. Cell. Cardiol.* **14**, 645 (1982).
23. T. Chajek, O. Stein and Y. Stein, *Biochim. biophys. Acta* **488**, 140 (1977).
24. W. Stern and M. E. Pullmann, *J. biol. Chem.* **258**, 11128 (1983).

Biochemical Pharmacology, Vol. 34, No. 14, pp. 2563–2566, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Inhibition of bovine brain monoamine oxidase by lead

(*Received* 10 *August* 1984; *accepted* 7 *December* 1984)

One of the clinical symptoms of lead poisoning is encephalopathy characterized by seizures and coma [1, 2]. Although the biochemical basis of this neurotoxic effect remains to be completely elucidated, it is known that the concentrations of several neurotransmitters are altered in the brains of animals exposed to lead [3, 4]. Of the catecholamines, norepinephrine is elevated after lead treatment [5, 6]. One possible explanation for this finding is that lead alters the activities of enzymes involved in norepinephrine metabolism. Recent studies in this laboratory have shown that lead inhibits phenylethanolamine-*N*-methyltransferase, the enzyme responsible for the conversion of

norepinephrine to epinephrine [7]. One major route of degradation of norepinephrine and other biogenic amines in brain is through the action of monoamine oxidase [8].

Monoamine oxidase (amine:oxygen oxidoreductase, deaminating, MAO*, EC 1.4.3.4) catalyzes the deamination of biogenic amines to the corresponding aldehyde. The enzyme exists in two forms which differ in their specificity for substrates and inhibitors. Type A MAO prefers amines, such as norepinephrine, which possess a hydrophilic aromatic ring, whereas type B MAO reacts with more hydrophobic amines such as benzylamine. Dopamine is a common substrate for both forms of the enzyme [9, 10]. Clorgyline and L-deprenyl are relatively specific inhibitors of the type A and type B enzyme respectively. Both forms of the enzyme are found in nearly equal proportions in the brain [11].

Both types of MAO are inhibited by *p*-chloromercuric benzoate, suggesting the presence of sulfhydryl groups which are necessary for enzyme activity [12]. Because heavy metals form mercaptides with sulfhydryls, it is reasonable to expect MAO to be inhibited by these metal ions. Indeed, Magour *et al.* [13] demonstrated that both forms of the enzyme are susceptible to micromolar concentrations of cadmium and copper. Gomes *et al.* [14] showed that MAO is inhibited by silver and that dialysis failed to restore enzyme activity. In the present study, we have investigated the effect of lead on both forms of bovine brain MAO.

Materials and methods

Materials. Serotonin HCl, benzylamine and DL-penicillamine were purchased from the Sigma Chemical Co., St. Louis, MO. Clorgyline was obtained from May & Baker Ltd., London. L-Deprenyl was obtained from Medimpex Northamerica, Inc., New York, NY. The Amersham Corp., Arlington Heights, IL, was the source of 5-hydroxy [side chain-2-^{14}C]tryptamine creatine sulfate (53 mCi/mmole).

Monoamine oxidase preparation and assay. A preparation of bovine brain mitochondria served as the source of MAO. Beef brain was obtained fresh, packed in ice, from the slaughterhouse. Mitochondria were prepared according to the method of Oguchi *et al.* [15]. The protein concentration was determined by solubilization of the membrane overnight in 1% sodium dodecyl sulfate followed by assay according to the method of Lowry *et al.* [16]. The final concentration of protein was 15 mg/ml.

Type A MAO was assayed according to the method of Honecker *et al.* [17] with certain modifications. Unless otherwise noted, the enzyme was exposed to 9 pmoles (0.3 nM, final concentration) L-deprenyl and $Pb(NO_3)_2$ or buffer for 30 min at 37° prior to addition of reaction mixture. This concentration of L-deprenyl inhibited 85% of type B MAO activity. This was the concentration recommended by Oguchi *et al.* [15] because higher amounts resulted in significant inhibition of type A MAO. In studies where chelators were used, these substances were added after the $Pb(NO_3)_2$ incubation and allowed to react for 30 min before the reaction mixture was added. The reaction mixture contained 0.157 μmole serotonin and 50 nCi [^{14}C]-serotonin in 0.01 M Tris–HCl (pH 8.0). The final volume was 300 μl. The reaction was allowed to proceed for 15 min at 37° and was stopped by addition of 200 μl of 2 N HCl. The product was extracted in 5 ml of toluene–ethylacetate (1:1 v/v) and 3 ml of this layer was taken for scintillation counting. The scintillation fluid consisted of 8 g PPO, 0.2 g POPOP, 1 liter toluene (scintanalyzed) and 1 liter Cellosolve (purified). Controls were treated with 200 μl of 2 N HCl prior to addition of reaction mixture.

Dilution experiments were performed by exposing 11.2 mg total protein to 4 μmoles $Pb(NO_3)_2$ for 30 min at 37°. The total volume was 3.47 ml. Two aliquots of 260 μl were removed for MAO assay. The remaining suspension was centrifuged at 8500 *g* for 20 min, the supernatant fraction was removed, and the pellet was resuspended in 2.6 ml Tris buffer. Two aliquots of 260 μl plus one of 300 μl (for protein determination) were removed, and the procedure was repeated. Assay controls were treated in similar fashion except that the $Pb(NO_3)_2$ was replaced by Tris buffer.

The assay for type B MAO was based on the method of McEwen and Cohen [18]. Incubation of the enzyme with $Pb(NO_3)_2$ and chelator studies were performed as described above except that L-deprenyl was omitted. The reaction mixture contained 1.5 μmoles benzylamine and 40 μmoles Tris–HCl (pH 7.2) in a final volume of 1 ml. Reaction proceeded for 45 min at 37° and was stopped by the addition of 100 μl of 60% perchloric acid. Cyclohexane (1 ml) was added, and the tubes were vortexed and allowed to stand for 15 min. A second mixing was performed, and the tubes were centrifuged for 15 min in an IEC clinical centrifuge. The absorbance of the cyclohexane layer was determined at 242 nm. Type B MAO activity was defined as the change in absorbance at 242 nm/min. An absorbance increase of 0.001/min corresponded to 73 pmoles of benzyladehyde formed/min [18]. Assay blank tubes were treated similarly but without benzylamine. The data represent the mean of duplicate samples that differ less than 12%.

Results and discussion

Both types of MAO are inhibited by $Pb(NO_3)_2$ (Fig. 1). Type A MAO was inhibited 50% by 1.0 mM (0.3 μmole) $Pb(NO_3)_2$, and inhibition was complete at 2.5 mM (0.75 μmole). The type B enzyme required 0.9 mM (0.9 μmole) and 1.2 mM (1.2 μmoles) $Pb(NO_3)_2$ to inhibit 50 and 100% of enzyme activity respectively. The reason for the sigmoidal shaped inhibition curve for type B MAO is unclear. It may reflect the binding of Pb^{2+} to sites other than those necessary for enzyme activity, but because a preparation of mitochondria was used as a source of enzyme, the nature of such sites cannot be ascertained. A purified enzyme preparation would be needed to study this effect.

The inhibition of type A MAO by Pb^{2+} is independent of the incubation time of enzyme with $Pb(NO_3)_2$ prior to enzyme assay. When 0.84 mg protein was exposed to 0.3 μmole $Pb(NO_3)_2$ for intervals between 0 and 60 min, the enzyme was inhibited 50% in all cases. In contrast, the

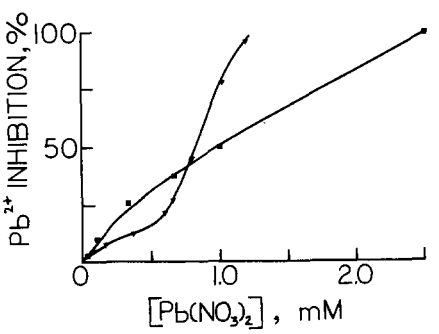

Fig. 1. Inhibition of MAO as a function of total $Pb(NO_3)_2$ concentration. The protein content per tube was 0.84 mg. Each point represents percent inhibition relative to control samples treated similarly but without $Pb(NO_3)_2$. Control samples for type A MAO produced 0.55 nmole product/min and type B MAO controls formed 0.56 nmole product/min. Key: (■–■) type A MAO, and (▼–▼) type B MAO.

* Abbreviations: MAO, monoamine oxidase; serotonin, 5-hydroxytryptamine; clorgyline, *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)propylamine HCl; L-deprenyl, selegilinium HCl or phenylisopropylmethylpropynylamine; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-[2-(5-phenyloxazolyl)]benzene; and Cellosolve, ethyleneglycol monoethylether.

Pb^{2+}-induced inhibition of type B MAO was dependent on the incubation time prior to enzyme assay. Exposure of 0.84 mg protein to 1 μmole $Pb(NO_3)_2$ for intervals between 0 and 30 min resulted in an increase in inhibition from 44 to 85%. Therefore, 30 min was chosen for the preincubation time in all cases. Controls, treated similarly but without $Pb(NO_3)_2$, showed no loss of enzyme activity during this period.

The effects of the MAO inhibitors clorgyline and L-deprenyl on the Pb^{2+}-induced inhibition of the enzymes were also studied. The absolute activity of type A MAO was decreased from 0.71 nmole product/min to 0.58 nmole product/min in the presence of 0.3 nM L-deprenyl. This reduction in activity (18%) was consistent with the findings of Oguchi *et al.* [15]. When 0.3 μmole $Pb(NO_3)_2$ was added, enzyme activity was inhibited 50% in both cases. The presence of 10 μM clorgyline reduced the total activity of type B MAO from 0.36 to 0.31 nmole product/min. However, the addition of 1 μmole $Pb(NO_3)_2$ inhibited the enzyme 84% regardless of the presence or absence of clorgyline.

We wished to determine if the inhibition of MAO was reversible or not. A plot of enzyme activity versus the total protein concentration in the presence of $Pb(NO_3)_2$ resulted in a line which intersected the x-axis to the right of the origin for both types of MAO (Fig. 2). This suggested that Pb^{2+} was acting as an irreversible inhibitor [19]. To confirm this we exposed the Pb^{2+}-treated enzyme to various compounds known to bind Pb^{2+} tightly (Table 1). DL-Penicillamine in equal mole ratio to $Pb(NO_3)_2$ was not able to reverse the Pb^{2+} inhibition of the type A enzyme. EDTA and phosphate, however, were able to partially restore activity. This finding is probably related to the association constants of the Pb^{2+}-complexes that are formed. Pb^{2+}-phosphate and Pb^{2+}-EDTA have K_a values of 10^{12} and 10^{18} [20, 21], respectively, whereas the value for DL-penicillamine is in the 10^8 range [22]. If the association constants of Pb^{2+} and enzyme functional groups are less than 10^{16}, then it is possible for phosphate and EDTA to partially restore enzyme activity. Studies using a purified enzyme preparation would be needed to determine the number of binding sites and the enzyme–Pb^{2+} association constants. For type B MAO none of the chelators was able to restore enzyme activity.

Finally, because of the results of the chelator study with the type A enzyme, we wished to determine if enzyme activity could be restored by washing the Pb^{2+}-treated enzyme. When the enzyme was exposed to Pb^{2+} as described under Materials and Methods, the MAO activity was inhibited 40%. After the first wash the enzyme was inhibited 43% and after the second wash it was still inhibited 38%. Controls treated similarly but without $Pb(NO_3)_2$ showed no loss of enzyme activity throughout this experiment.

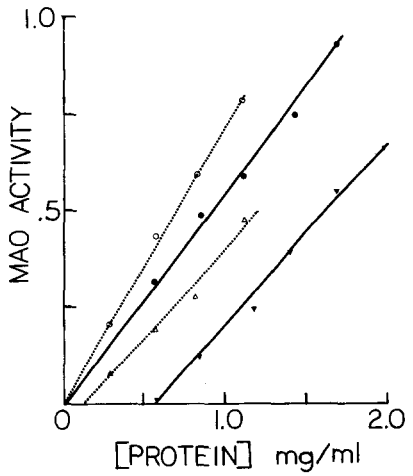

Fig. 2. MAO activity as a function of protein concentration. Enzyme activity is expressed as nmoles product formed/min. Type A MAO was assayed in the presence (△---△) or absence (○---○) of 0.3 μmole (1 mM) $Pb(NO_3)_2$. Type B MAO was assayed in the presence (▼–▼) or absence (●–●) of 1 μmole (1 mM) $Pb(NO_3)_2$.

Table 1. Effects of chelators on MAO activity

	$Pb(NO_3)_2$ (μmoles)	Chelator (μmoles)	% Inhibition*
Type A	0.3		57
		EDTA	
		0.3	0
	0.3	0.3	10
		DL-Penicillamine	
		0.3	3
	0.3	0.3	56
		Phosphate	
		0.3	3
	0.3	0.3	20
Type B	1.0		81
	1.2		85
		EDTA	
		1.0	30
	1.0	1.0	86
		DL-Penicillamine	
		1.0	17
	1.0	1.0	87
		Phosphate	
		1.0	0
	1.2	1.0	74

* Values are percent inhibition relative to control samples treated similarly but without $Pb(NO_3)_2$. When assayed for type A or type B MAO, these control samples producted 0.55 nmole product/min or 0.51 nmole product/min respectively. The protein content of all tubes was 0.84 mg.

It is not surprising that Pb^{2+} acts as an irreversible inhibitor of both types of MAO. The enzyme has sulfhydryl groups necessary for enzyme activity and Pb^{2+} binds to sulfhydryls very tightly [23]. Gomes *et al.* [14] found that, after exposure to silver nitrate or mercuric chloride, MAO activity could not be restored by dialysis, which suggests irreversible inhibition. Phenylethanolamine-*N*-methyltransferase, another enzyme with essential sulfhydryl groups, is irreversibly inhibited by Pb^{2+} [7]. However, the type A enzyme is more sensitive to Pb^{2+} than type B MAO when the Pb^{2+}:protein ratios are considered. A ratio of 0.36 μmole Pb^{2+}/mg total protein is required to inhibit 50% of type A MAO activity, while 1.1 μmoles Pb^{2+}/mg total protein is needed for 50% inhibition of the type B enzyme.

It would be of interest to know if Pb^{2+} could effectively inhibit MAO *in vivo*. It is difficult to draw conclusions based on the present *in vitro* experiments, but several points can be made. It took rather large amounts of $Pb(NO_3)_2$ (0.9 to 1.0 mM) to inhibit 50% of enzyme activity using a mitochondrial preparation as the source of enzyme. In rats exposed to 25 ppm Pb^{2+}, brain lead concentrations were approximately 2.5 μM [24]. Furthermore, in cases of fatal lead encephalopathy in humans, brain lead levels ranged from 5 to 85 μM [25]. This is considerably less lead than that needed to inhibit MAO in the present study. However, the values for rats and humans represent whole brain concentrations and do not take into account possible compartmentalization of lead into specific brain regions. If sufficient concentrations of unbound Pb^{2+} are present in the brain, then inhibition of MAO could occur. In addition,

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.

Acknowledgements—Acknowledgement is made to the donors of the Petroleum Research Fund, administered by The American Chemical Society, for support of this research. The authors are grateful to Ray K. Brown at Wayne State University, School of Medicine, Department of Biochemistry, for use of his facilities for several of the experiments.

Department of Chemistry
The University of Detroit
Detroit, MI 48221, U.S.A.

LATHA K. UNNI*
MARY LOU CASPERS†

REFERENCES

1. O. David, J. Clark and K. Voeller, *Lancet* **2**, 900 (1972).
2. M. A. Wessel and A. Dominski, *Am. Scient.* **65**, 294 (1977).
3. A. T. Modak, S. T. Weintraub and W. B. Stavinoha, *Toxic. appl. Pharmac.* **34**, 340 (1975).
4. T. C. Dubas, A. Stevenson, R. L. Singhal and P. D. Hrdina, *Toxicology* **9**, 185 (1978).
5. M. Golter and I. A. Michaelson, *Science* **187**, 359 (1975).
6. N. K. Satija, T. D. Seth and D. S. Tandon, *Toxicology* **10**, 13 (1978).
7. M. L. Caspers, *Biochem. Pharmac.* **31**, 1985 (1982).
8. A. J. Rivett, B. J. Eddy and J. A. Roth, *J. Neurochem.* **39**, 1009 (1982).
9. J. P. Johnson, *Biochem. Pharmac.* **17**, 1285 (1968).
10. D. W. R. Hall, B. W. Logan and G. H. Parsons, *Biochem. Pharmac.* **18**, 1447 (1969).
11. R. F. Squires, in *Monoamine Oxidases-New Vistas* (Eds. E. Costa and M. Sandler) (*Adv. Biochem. Psychopharmac.* **5**), p. 355. Raven Press, New York (1972).
12. M. Harada, K. Mizutani and T. Nagatsu, *J. Neurochem.* **18**, 559 (1971).
13. S. Magour, O. Cumpelik and M. Paulus, *J. clin. Chem. clin. Biochem.* **17**, 777 (1979).
14. B. Gomes, G. Naguwa, H. G. Kloepfer and K. T. Yasunobu, *Archs Biochem. Biophys.* **132**, 28 (1969).
15. K. Oguchi, S. Kobayashi, T. Uesato and K. Kamijo, *Biochem. Pharmac.* **31**, 1515 (1982).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. H. Honecker, W. Christ, B. Muller-Oerlinghausen and H. Coper, *J. clin. Chem. clin. Biochem.* **14**, 453 (1976).
18. C. M. McEwen, Jr. and J. D. Cohen, *J. Lab. clin. Med.* **62**, 766 (1963).
19. I. M. Segel, *Enzyme Kinetics*, pp. 127–34. John Wiley, New York (1975).
20. L. G. Sillen and A. E. Martell, *Stability Constants of Metal–Ion Complexes*, p. 187. The Chemical Society, London (1964).
21. L. G. Sillen and A. E. Martell, *Stability Constants of Metal–Ion Complexes*, p. 273. The Chemical Society, London (1964).
22. L. G. Sillen and A. E. Martell, *Stability Constants of Metal–Ion Complexes*, p. 438. The Chemical Society, London (1964).
23. F. R. N. Gurd and P. E. Wilcox, in *Advances in Protein Chemistry* (Eds. M. L. Anson, K. Bailey and J. T. Edsall), Vol. 11, p. 351. Academic Press, New York (1956).
24. I. A. Michaelson and M. W. Sauerhoff, *Toxic. appl. Pharmac.* **28**, 88 (1974).
25. J. N. Cumings, *Heavy Metals and the Brain*, p. 118. Blackwell Scientific Publications, Oxford (1959).
26. K. T. Yasunobu, H. Ishizaki and N. Minamiura, *Molec. cell. Biochem.* **13**, 3 (1976).
27. L. K. Unni and M. L. Caspers, *Biochem. Pharmac.* **33**, 2149 (1984).

* Present address: Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield, IL 62708.

† Address all correspondence to: Dr. Mary Lou Caspers, Department of Chemistry, The University of Detroit, 4001 West McNichols Road, Detroit, MI 48221.

Biochemical Pharmacology, Vol. 34, No. 14, pp. 2566–2569, 1985.
Printed in Great Britain.

0006–2952/85 $3.00 + 0.00
Pergamon Press Ltd.

In vitro interconversion of aflatoxin B_1 and aflatoxicol by rat erythrocytes

(*Received* 11 *January* 1984; *accepted* 18 *December* 1984)

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