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INHIBITION OF LIVER GLUTATHIONE *S*-TRANSFERASE ACTIVITY IN RATS BY HYPOLIPIDEMIC DRUGS RELATED OR UNRELATED TO CLOFIBRATE

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Abstract—The effects of *in vivo* administration of six hypolipidemic drugs on rat liver glutathione *S*-transferase activity were compared. This activity was measured with sulfobromophthalein (BSP), 1,2-dichloro-4-nitrobenzene (DCNB) or 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Except for the nicotinic acid derivative ethanolamine oxiniacate, all the compounds tested significantly reduced it, whether or not they were related to clofibrate. The hepatic glutathione concentration either remained unchanged or only increased slightly after treatment with the various drugs. When measured, the maximal excretion rate of bile BSP dropped significantly, but not that of phenol-3,6-dibromophthalein (DBSP). Hepatic dye uptake and storage were not impaired. These results show that hypolipidemic drugs of the peroxisome proliferator type inhibit rat liver glutathione *S*-transferase activity and may reduce transport of anions conjugated with glutathione before excretion.

The therapeutic class of hypolipidemic agents comprises compounds with various chemical structures, some of which are related to the structure of the reference clofibrate. Their mechanism of action has not yet been fully established even though several of their effects on lipid metabolism have been characterized. In addition to their hypolipidemic properties, these agents have been shown to produce, in rodents, an increase in liver weight and in the rough endoplasmic reticulum [1, 2], as well as alterations in mitochondria and microbodies [3–6]. According to certain reports [7, 8], the activities of several enzymes not involved in lipid metabolism are modified by clofibrate. For instance, bilirubin metabolism is favourably influenced by this drug, since induction of hepatic glucuronidation enhances the rate of plasma bilirubin disappearance in rats [8] and in man [9, 10]. In contrast to these effects, clofibrate reduces the biliary excretion of sulfobromophthalein (BSP) both in rats [11] and in patients with Gilbert's syndrome [9]. We recently showed in rats that the maximal excretion rate of bile phenol-3,6-dibromophthalein (DBSP) which is not biotransformed before excretion did not change after clofibrate treatment. The hepatic conjugation of BSP with glutathione (GSH) was reduced both *in vivo* and *in vitro*. These results suggested that clofibrate might reduce the hepatobiliary transport of BSP by lowering glutathione *S*-transferase activity in the liver [12].

Since hypolipidemic agents are widely used, we wondered whether inhibition of glutathione *S*-transferase activity was a property common to all these agents, and therefore tested a series of hypolipidemic drugs for their effects on BSP conjugation and transport by the liver.

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MATERIALS AND METHODS

Chemicals. BSP, 5-5'-dithiobis-(2-nitrobenzoic acid), 1-chloro-2,4-dinitrobenzene (CDNB) and GSH were purchased from Sigma Chemical Co (St Louis, MO). 1,2-Dichloro-4-nitrobenzene (DCNB) was obtained from Aldrich Chemical Co. (Belgium); DBSP was from SERB (France). Clofibride, fenofibrate, nafenopin, tibric acid, tiadenol and ethanolamine oxiniacate were respectively gifts from Fournier Frères (Gennevilliers, France), Fournier (Dijon, France), Ciba-Geigy (Basel, Switzerland), Pfizer (Orsay, France), Lafon (Maisons-Alfort, France) and Astra France (Nanterre, France).

Animals. Male Sprague–Dawley rats (220–250 g) (Charles River, France), fed with UAR standard chow, were given a daily i.p. injection for 10 days of one of the following: clofibride (20 mg.100 g body wt^{-1}), fenofibrate (5 mg.100 g body wt^{-1}), nafenopin (10 mg.100 g body wt^{-1}), tibric acid (20 mg.100 g body wt^{-1}), tiadenol (20 mg.100 g body wt^{-1}) or ethanolamine oxiniacate (50 mg.100 g body wt^{-1}). Compounds were injected in ethanol-propylenglycol (1:9). Paired control rats received the vehicle for 10 days. All experiments were performed 24 hr after the last drug dose.

GSH-conjugating activity in vitro. Livers were removed and perfused with ice-cold 0.9% saline and cytosols were obtained by two successive centrifugations (10,000 *g* for 10 min and 100,000 *g* for 60 min) from homogenates (20%, w/v) prepared in buffer containing 0.001 M EDTA, 0.03 M sodium phosphate and 0.25 M sucrose (pH 7.4). Specimens were stored at −18° when not used immediately. The protein concentration in the cytosol was determined by the method of Lowry *et al.* [13].

GSH *S*-transferase activity was determined using BSP [14], 1,2-dichloro-4-nitrobenzene (DCNB) or

1-chloro-2,4-dinitrobenzene (CDNB) [15] as substrate. All these enzyme assays were run under conditions of maximal initial velocity and were linear with time and the protein concentration.

Liver GSH was estimated according to Ellman [16].

Biliary transport capacity and hepatic dye storage. Rats were infused intravenously for 60 min with 215 nmol.min^{-1}.100 g body wt^{-1} of either BSP or DBSP after a priming dose of 2.15 μmol.100 g body wt^{-1}, as previously described [11]. Blood and bile samples were collected at 10-min intervals during the infusion. T_m (transport maximum) was calculated from the concentration of dye measured in the bile samples between the 30th and 60th min of infusion. Hepatic dye storage was directly estimated by determining the dye content of the liver, which was removed at the end of the experiments. Bile volume, the bile and plasma concentrations of BSP and DBSP, and liver dye content were determined as previously reported [12].

Statistical analysis. The data were compared by analysis of variance. When this analysis indicated a significant difference, the means were compared by Student's *t*-test. Multiple comparisons between several experimental groups and one control group were made using Scheffe's test.

RESULTS

Except for ethanolamine oxiniacate, *in vivo* administration to rats of all the hypolipidemic drugs tested significantly reduced the activity of liver glutathione *S*-transferase (Table 1). The structures of these drugs are shown in Fig. 1. Clofibric acid is included because it is the active metabolite of clofibrate and clofibride. As this figure shows, fenofibrate and nafenopin are structurally related to clofibrate, whilst tibric acid, tiadenol and ethanolamine oxiniacate are not.

With each of three substrates used, specific liver glutathione *S*-transferase activity dropped by 17–44%, depending on the compound tested. The concentration of glutathione, the co-substrate of the reaction, either remained unchanged or rose slightly. When results were expressed on a whole liver basis, they were different, since, as expected, some of the drugs produced significant liver enlargement. Thus, only one of the six drugs—clofibride—reduced total glutathione *S*-transferase activity, and ethanolamine oxiniacate slightly raised it. On the other hand, total GSH content was greatly enhanced by fenofibrate, tibric acid, tiadenol and nafenopin, the latter even doubled this content. Similar observations were made for cytosolic proteins (Fig. 2). Despite the above results, maximal biliary BSP excretion (T_m) diminished by about 20% in rats treated with clofibride, fenofibrate or tiadenol (Table 2). Basal bile flow was raised by clofibride and fenofibrate, but at T_m there was no difference in this respect between control and treated rats. Analysis of the bile obtained between the 30th and 60th minutes of BSP perfusion (i.e. at T_m) indicated that in the treated animals, the concentrations of total and conjugated BSP diminished. Therefore the contribution to T_m of con-

Table 1. Effects of six hypolipidemic drugs on hepatic glutathione and glutathione *S*-transferase activity

Treatment	Dose (mg.100 g body wt^{-1})	Liver wt/body wt (%)	Cytosolic protein (mg.g liver^{-1})	Reduced glutathione (μmol.g liver^{-1})	*Glutathione S-transferase* (nmol.min^{-1}.mg protein^{-1}) DCNB*	CDNB†	BSP‡
Control	none	4.47 ± 0.12	65.1 ± 1.1	6.6 ± 0.1	77.2 ± 2.2	1910 ± 90	50.8 ± 1.5
Clofibride	20	4.97 ± 0.17§	62.6 ± 1.0	6.2 ± 0.1	50.0 ± 1.6§	1395 ± 30§	35.3 ± 1.5§
Fenofibrate	5	6.42 ± 0.16§	68.4 ± 0.8§	7.6 ± 0.2§	49.2 ± 1.7§	1215 ± 60§	32.6 ± 1.2§
Nafenopin	10	8.11 ± 0.20§	67.3 ± 0.9	7.8 ± 0.3§	43.4 ± 1.8§	1135 ± 40§	29.8 ± 1.4§
Tibric acid	20	6.71 ± 0.14§	63.1 ± 0.9	6.9 ± 0.3	52.2 ± 2.8§	1421 ± 75§	34.5 ± 1.9§
Tiadenol	20	6.06 ± 0.14§	64.6 ± 0.5	7.5 ± 0.2§	63.9 ± 1.8§	1557 ± 35§	41.0 ± 1.5§
Ethanolamine oxiniacate	50	4.60 ± 0.08	66.3 ± 0.8	6.5 ± 0.1	81.3 ± 1.9	1912 ± 35	54.2 ± 1.6

Drugs were administered i.p. daily, for 10 days and rats were killed 24 hr after receiving the last dose. Results are means ± S.E.M. for eight rats. * 1,2-dichloro-4-nitrobenzene; † 1-chloro-2,4-dinitrobenzene; ‡ sulfobromophthalein. § Significantly different from control rats. $P < 0.05$.

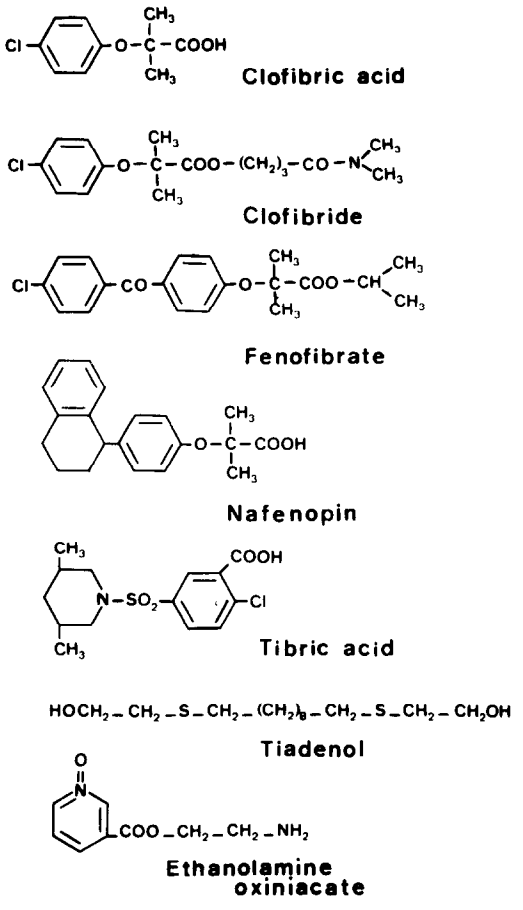

Fig. 1. Structure of the six hypolipidemic compounds studied, compared to that of clofibric acid.

jugated BSP decreased, a change accompanied by a concomitant rise in hepatic BSP (Table 3).

In line with these overall results, the maximal biliary excretion of DBSP, which is not conjugated prior to its excretion in rats, was not reduced by treatment with clofibride, fenofibrate or tiadenol (data not shown).

DISCUSSION

In the present work we investigated the inhibitory effect of a series of hypolipidemic drugs on liver glutathione *S*-transferase activity. Except for ethanolamine oxiniacate, all the drugs tested greatly inhibited this activity, whether or not they were chemically related to clofibrate. The mechanism of action of hypolipidemic drugs is still uncertain. However, in the rat, several of them have been shown to induce hepatomegaly associated with peroxisome proliferation and a rise in the activity of certain lipid metabolizing enzymes [4, 6, 17, 18]. Nicotinic acid belongs to a different class of hypolipidemic drugs and is thought to act mainly by inhibiting peripheral lipolysis; it does not produce hepatomegaly or peroxisome proliferation [19]. We therefore chose to study five hypolipidemic agents of the peroxisome proliferator type and one derivative of nicotinic acid.

In previous studies [20] we demonstrated that the peroxisome proliferators tested here, like clofibrate and nafenopin [21, 22], raised the hepatic concentration of Z protein, also known as fatty acid binding protein (FABP), whereas the nicotinic acid derivative did not. Fleischner *et al.* [22] have shown that induction of Z protein by the hypolipidemic agents clofibrate and nafenopin was accompanied by a decrease in ligandin. Ligandin, first identified as a bilirubin and sulfobromophthalein-binding protein

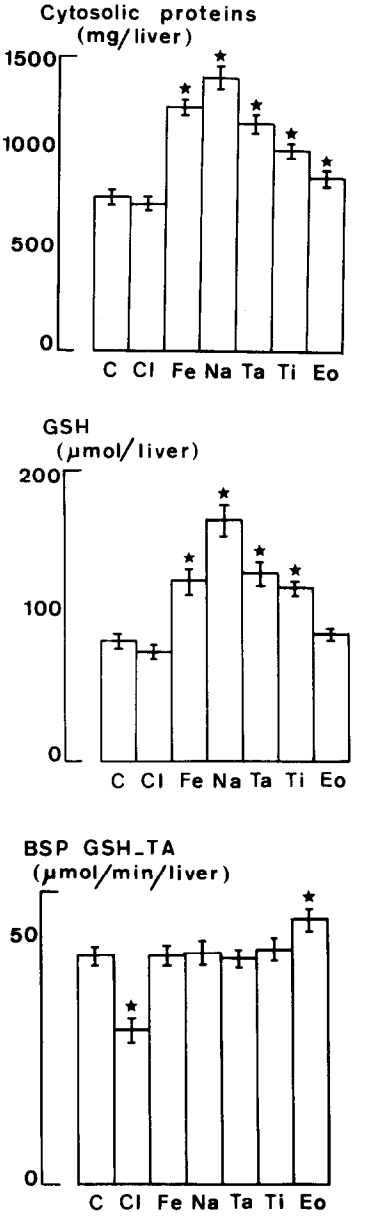

Fig. 2. Total cytosolic proteins, glutathione and BSP glutathione *S*-transferase activity in rat livers after i.p. administration for 10 days of various hypolipidemic drugs (C: Control, CL: Clofibride, Fe: Fenofibrate, Na: Nafenopin, Ta: Tibric acid, Ti: Tiadenol, Eo: Ethanolamine oxiniacate). Drug doses are identical with those given in Table 1. Values are means ± S.E.M. for eight rats. * Significantly different from control rats, $P < 0.05$.

Table 2. Effect of pretreatment of rats with clofibrate, fenofibrate or tiadenol on liver weight, bile flow and maximal bile secretion of BSP (T_m)

		Liver wt/ body wt (%)	Bile flow		Bile BSP concentration	
			before T_m (μl.min^{-1}.100 g body wt^{-1})	at T_m	at T_m (g.l^{-1})	T_m (μg BSP.min^{-1}.100 g body wt^{-1})
Control	(7)	3.46 ± 0.14	6.3 ± 0.3	8.9 ± 0.3	15.1 ± 0.3	133 ± 5
Clofibride	(6)	4.60 ± 0.17*	8.4 ± 0.5†	8.2 ± 0.4	13.5 ± 0.7‡	110 ± 5†
Control	(8)	3.25 ± 0.08	6.1 ± 0.2	7.1 ± 0.2	13.8 ± 0.3	98 ± 3
Fenofibrate	(9)	5.68 ± 0.07*	7.6 ± 0.4†	6.8 ± 0.5	11.4 ± 0.5†	76 ± 6†
Control	(7)	3.43 ± 0.13	6.1 ± 0.3	8.0 ± 0.4	14.9 ± 0.5	118 ± 5
Tiadenol	(7)	5.25 ± 0.13*	6.7 ± 0.3	7.8 ± 0.4	12.5 ± 0.5†	95 ± 4†

After a priming dose of 2.15 μmol.100 g body wt^{-1}, BSP was perfused for 60 min at a rate of 215 nmol.min^{-1}.100 g body wt^{-1} in rats previously treated i.p. for 10 days with a daily dose of 20 mg.100 g body wt^{-1} clofibride or tiadenol, or of 5 mg.100 g body wt^{-1} fenofibrate, and in control rats. Number of animals is indicated in parentheses. Results are means ± S.E.M. *$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$.

[23], is also recognized as a glutathione *S*-transferase [24, 25]. Although recent findings showed firstly, that ligandin (subunit YaYa) and glutathione *S*-transferase B (subunit YaYc) are two different proteins [26], and secondly, that unlike transferases A and C possessing Yb or Yb′ subunits they have negligible activity towards BSP [27], administration of clofibrate to rats reduced the capacity of the liver to conjugate BSP with glutathione [12]. The results for clofibrate and the other compounds tested in this study show that specific cytosolic enzyme activity was similarly inhibited with BSP, DCNB and CDNB, indicating that the activity of most of the transferases was reduced, since CDNB is a substrate for transferases A, AA, B, C and ligandin [27].

Expressed per total liver, glutathione conjugating activity was not different from control activity, except in the rats treated with clofibride. As Fig. 2 shows, total cytosolic proteins were greatly increased by some of the drugs, so that the inhibition of glutathione *S*-transferase activity by these drugs might have been masked by their general effect on the proteins, as glutathione *S*-transferases only account for 5% of cytosolic proteins [28]. This is why we studied the physiological effects of three hypolipidemic drugs on liver transport of BSP and its analogue, DBSP: clofibride, whose active metabolite is clofibric acid; fenofibrate, a compound related to clofibrate, and tiadenol which is not related to clofibrate; fenofibrate and tiadenol produced huge liver enlargement, so that, as already mentioned, BSP glutathione *S*-transferase activity, expressed per whole liver, did not change compared to control activity. Nevertheless, a reduction in maximal bile excretion of BSP was observed with all three drugs, concomitantly with a reduction in conjugated bile BSP and with an accumulation of liver BSP. In contrast, the rate of excretion of bile DBSP was identical in treated and control animals. Consequently, as previously shown for clofibrate [12], dye uptake and storage were not impaired by clofibride, fenofibrate or tiadenol. These findings do not exclude a reduction in the capacity of the glutathione *S*-conjugate transport system in the liver, either by some direct effect of hypolipidemic drugs [29] or by a competition between conjugated and unconjugated BSP, as demonstrated earlier [30, 31].

In line with our present observations, Awasthi *et al.* [32], who used ciprofibrate, another hypolipidemic agent which is a peroxisome proliferator and is related to clofibrate, showed that glutathione *S*-transferase activity in rat liver was inhibited *in vivo* and *in vitro* by this drug. Their studies with purified glutathione *S*-transferases indicated that the different forms and subunits of these enzymes were inhibited by ciprofibrate with CDNB as substrate. Although the inhibition was not competitive towards the first reaction substrate, it was found to be competitive with the second, i.e. glutathione. Awasthi *et al.* [32] concluded that ciprofibrate caused irreversible inhibition of glutathione *S*-transferases. This conclusion is not totally consistent with ours, since we previously demonstrated with clofibrate [12] that inhibition occurred after at least two daily doses, and that the effect of clofibrate lasted for four days after cessation of treatment. In addition, the reduced BSP-

Table 3. Effect of pretreatment of rats with clofibride, fenofibrate or tiadenol on biliary excretion of conjugated and unconjugated BSP and on liver dye storage

		Maximal biliary excretion (μg BSP.min^{-1}.100 g body wt^{-1}) Conjugated	Unconjugated	Liver content (mg BSP.total liver^{-1})
Control	(7)	119 ± 5	14 ± 1	11.2 ± 0.7
Clofibride	(6)	95 ± 4*	15 ± 1	15.8 ± 0.7†
Control	(8)	84 ± 3	14 ± 1	14.9 ± 0.8
Fenofibrate	(9)	62 ± 5*	12 ± 1	18.7 ± 0.5†
Control	(7)	105 ± 5	13 ± 1	14.1 ± 0.7
Tiadenol	(7)	83 ± 4*	12 ± 1	18.8 ± 0.3†

After a priming dose of 2.15 μmol.100 g body wt^{-1}, BSP was perfused for 60 min at a rate of 215 nmol.min^{-1}.100 g body wt^{-1} in rats previously treated i.p. for 10 days with a daily dose of 20 mg.100 g body wt^{-1} clofibride or tiadenol, or of 5 mg.100 g body wt^{-1} fenofibrate, and in control rats. Number of animals is indicated in parentheses. Results are means ± S.E.M. *$P < 0.01$; †$P < 0.001$.

glutathione conjugating activity in clofibrate-treated rats was mainly due to a decline in the apparent V_{max} for both substrates. Consequently, although Awasthi *et al.* clearly showed the inhibitory effect of ciprofibrate *in vitro* in the millimolar range of concentrations, we believe that the reduced glutathione conjugating activity in the liver is more likely to be due to a reduction in the amount of enzyme, for three reasons: firstly, previous results showed that there was no accumulation of clofibrate *in vivo*, even after several days of treatment [33], so that the hepatic concentration of clofibrate was presumably too low to interfere with the activity of the glutathione S-transferases; secondly, no inhibition was detectable 1, 2, 6 or 24 hr after injection of a single dose of either 20 mg.100 g body wt^{-1} clofibrate [12] or 20 mg.100 g body wt^{-1} nafenopin (personal observations), and thirdly, treatment with the hypolipidemic drug Wy-14,643, which is a peroxisome proliferator, caused both increase and decrease in the hepatic concentrations of several mRNA species coding for peroxisomal and non-peroxisomal proteins [34]. Among the species whose concentrations fell significantly were those coding for two proteins with molecular weights of 24,000 and 25,000 respectively. Although they have not been identified, they might well be the Yb and Yc subunits of glutathione S-transferase (respective molecular weights: 23,500 and 25,000) [35, 36]. In that case, the increase in Z protein might be balanced by a decrease in glutathione S-transferases.

In vivo, inhibition of glutathione S-transferase activity has also been shown with phenoxyacid herbicides [37], which are also peroxisome proliferators [38]. This last observation raises the possibility that a relationship exists, on the one hand, between the inhibitory effect on glutathione S-transferase activity of the hypolipidemic drugs of the peroxisome proliferator type, and on the other, the peroxisome proliferation caused by these drugs. Other compounds such as acetylsalicylic acid [39] and phthalates [40] have also been shown to cause similar proliferation. There is probably a link between the peroxisome proliferating effect and the enhancement of hepatic Z protein in the case of several drugs, including tiadenol, clofibric acid and acetylsalicylic acid [41]. Lastly, it is difficult to define the relationship between the structure of hypolipidemic drugs and their inhibition of glutathione S-transferase activity, since both aryloxic acid derivatives and compounds not related to them, such as tiadenol, produce similar effects.

In conclusion, hypolipidemic drugs of the peroxisome proliferator type inhibit liver glutathione S-transferase activity in rats, and may reduce hepatic transport of anions conjugated with glutathione before excretion. These findings might also apply to other species, especially man, since some degree of peroxisome proliferation was recently shown to occur in the liver of patients taking clofibric acid [42].

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REFERENCES

1. R. Hess, W. Stäubli and W. Reiss, *Nature, Lond.* **208**, 856 (1965).
2. R. Beckett, R. Weiss, R. E. Stitzel and E. J. Cenedella, *Toxic. appl. Pharmac.* **23**, 42 (1972).
3. D. J. Svoboda and D. L. Azarnoff, *J. Cell Biol.* **30**, 442 (1966).
4. P. B. Lazarow, *Science* **197**, 580 (1977).
5. T. Osumi and T. Hashimoto, *J. Biochem.* **83**, 1361 (1978).
6. P. Van Veldhoven, P. E. Declercq, L. J. Debeer and G. P. Mannaerts, *Biochem. Pharmac.* **33**, 1153 (1984).
7. N. J. Lewis, D. T. Witiak and D. R. Feller, *Proc. Soc. exp. Biol. Med.* **145**, 281 (1974).
8. A. Foliot, J. L. Drocourt, J. P. Etienne, E. Housset, J. N. Fiessinger and B. Christoforov, *Biochem. Pharmac.* **26**, 547 (1977).
9. K. Kutz, H. Kandler, R. Cugler and J. Fevery, *Clin. Sci.* **66**, 389 (1984).
10. A. Lindenbaum, X. Hernandorena, M. Vial, C. Benattar, J. C. Janaud, M. Dehan, A. Foliot, R. Leluc and J. C. Gabilan, *Arch. Fr. Pediatr.* **38**, 867 (1981).
11. F. Jean, A. Foliot, C. Celier, E. Housset and J. P. Etienne, *Biochem. biophys. Res. Commun.* **86**, 1154 (1979).

12. A. Foliot, D. Touchard and C. Celier, *Biochem. Pharmac.* **33**, 2829 (1984).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. J. Goldstein and B. Combes, *J. Lab. clin. Med.* **67**, 863 (1966).
15. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **149**, 7130 (1974).
16. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
17. C. R. Mackerer, *Biochem. Pharmac.* **26**, 2225 (1976).
18. H. P. Grauert, J. K. Reddy, W. S. Kennan, G. L. Sattler, V. S. Rao and H. K. Pitot, *Cancer Lett.* **24**, 147 (1984).
19. O. M. Bakke and R. K. Berge, *Biochem. Pharmac.* **31**, 3930 (1982).
20. A. Foliot, *Actual. Chim. Ther.* **6**, 123 (1979).
21. A. Foliot, B. Christoforov, E. Housset, J. P. Petite, J. P. Ploussard and J. P. Etienne, *Biol. Gastroenterol.* **7**, 332 (1974).
22. G. Fleischner, D. K. J. Meijer, W. G. Levine, Z. Gatmaitan, R. Gluck and I. M. Arias, *Biochem. biophys. Res. Commun.* **67**, 1401 (1975).
23. A. J. Levi, Z. Gatmaitan and I. M. Arias, *J. clin. Invest.* **48**, 2156 (1969).
24. N. Kaplowitz, I. W. Percy-Robb and N. B. Javitt, *J. exp. Med.* **138**, 483 (1973).
25. W. H. Habig, M. D. Pabst, G. Fleischner, Z. Gatmaitan, I. W. Arias and W. B. Jakoby, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3879 (1974).
26. J. D. Hayes, R. C. Strange and I. W. Percy-Robb, *Biochem. J.* **181**, 699 (1979).
27. B. Mannervik and H. Jensson, *J. biol. Chem.* **257**, 9909 (1982).
28. W. B. Jakoby, *Adv. Enzymol.* **46**, 383 (1978).
29. D. Sorrentino, V. Licko, N. M. Bass and R. A. Weisiger, *Gastroenterol.* **88**, 1696 (1985).
30. J. L. Barnhart and B. Combes, *Am. J. Physiol.* **231**, 399 (1976).
31. Z. Gregus, F. Fischer and F. Varga, *Biochem. Pharmac.* **26**, 1951 (1977).
32. Y. C. Awasthi, S. V. Singh, S. K. Goel and J. K. Reddy, *Biochem. Pharmac.* **123**, 1012 (1984).
33. J. R. Baldwin, D. T. Witiak and D. R. Feller, *Biochem. Pharmac.* **29**, 3143 (1980).
34. B. Chatterjee, W. F. Demyan, N. D. Lalwani, J. K. Reddy and A. K. Roy, *Biochem. J.* **214**, 879 (1983).
35. N. M. Bass, R. E. Kirsch, S. A. Tuff, I. Marks and S. J. Saunders, *Biochim. biophys. Acta* **492**, 163 (1977).
36. T. D. Boyer, W. C. Kenney and D. Zakim, *Biochem. Pharmac.* **32**, 1843 (1983).
37. E. Hietanen, K. Linnainmaa and H. Vainio, *Acta pharmac. toxicol.* **53**, 103 (1983).
38. H. Vainio, J. Nickels and K. Linnainmaa, *Scand. J. Work Environ. Health* **8**, 70 (1982).
39. Z. Hruban, M. Gotoh, A. Slesers and S. F. Chov, *Lab. Invest.* **30**, 64 (1974).
40. T. J. B. Gray, B. G. Lake, J. A. Beamand, J. R. Foster and S. D. Gangoli, *Toxicol.* **28**, 167 (1983).
41. Y. Kawashima, S. Nakagawa, Y. Tachibana and H. Kozuka, *Biochim. biophys. Acta* **754**, 21 (1983).
42. M. Hanefeld, C. Kemmer and E. Kadner, *Atheroscl.* **46**, 239 (1983).