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IMPAIRMENT OF HEPATIC GLUTATHIONE *S*-TRANSFERASE ACTIVITY AS A CAUSE OF REDUCED BILIARY SULFOBROMOPHTHALEIN EXCRETION IN CLOFIBRATE-TREATED RATS

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Abstract—Administration of clofibrate reduced the maximal excretion rate of bile sulfobromophthalein (BSP) in rats but left that of phenol-3,6-dibromophthalein (DBSP) unchanged. This decrease in liver transport of BSP was due to reduced bile excretion of conjugated BSP. Hepatic uptake and storage of this dye were not impaired. Liver glutathione *S*-transferase activity *in vitro*, measured with BSP, 1,2-dichloro-4-nitrobenzene (DCNB) or 1-chloro-2,4-dinitrobenzene (CDNB) was significantly reduced. This alteration in liver conjugating activity was probably not related to a modification of the hepatic GSH pool, since the GSH level was unchanged or only increased slightly after clofibrate treatment. Detection of this inhibition required at least two daily doses of clofibrate. Inhibition was dose-related and lasted for several days after cessation of the drug. In clofibrate-treated rats, Lineweaver–Burk plots showed a reduced V_{max} for both the BSP and GSH substrates. These results suggest that clofibrate decreases hepatobiliary transport of BSP by lowering glutathione *S*-transferase activity in the liver.

Elimination of sulfobromophthalein (BSP) from the plasma depends on several hepatic processes: they include the transfer of BSP from the plasma to the liver, its storage in the hepatocytes, its conjugation with reduced glutathione (GSH), the transport of the conjugated BSP from the liver cells into the bile, and the bile flow [1]. The alteration of one or several of these processes may change the rate of biliary BSP excretion. In previous studies, we observed that treatment of rats with clofibrate, a widely used hypolipidemic drug, enhanced both bilirubin conjugation [2] and its hepatic transport [3] but reduced biliary excretion of BSP. Conjugation of BSP with GSH is catalyzed by a cytosolic enzyme, GSH *S*-transferase B [4], which has been shown to be very similar if not identical with the anion-binding protein, ligandin [5]. After administration of clofibrate to rats, Fleischner *et al.* [6] noticed a fall in the hepatic concentration of this protein. Therefore, the reduction of the GSH-conjugating capacity of the liver might be the mechanism by which clofibrate reduces hepatobiliary BSP transport.

To determine whether or not BSP conjugation was impaired in rats after clofibrate treatment, we investigated the hepatic transport of BSP and of its analog, phenol-3,6 dibromophthalein (DBSP), which is not biotransformed before its excretion [7], as well as the activity of GSH *S*-transferases *in vitro*.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (220–250 g) (Charles River, France), fed with UAR standard chow, received 20 mg.100 g body wt^{-1} of clofibrate in propylenglycol for ten days, either *per os* or i.p., except in the dose–response study, when daily doses ranged from 10 to 40 mg.100 g body wt^{-1}. In the time-course study of the effects of clofibrate on liver GSH content and on BSP-GSH-conjugating activity, rats were treated with 20 mg.100 g body wt^{-1} i.p. for 1, 2, 3 or 4 days as indicated in the results. In each experiment, control rats received 0.1 ml.100 g body wt^{-1} of propylenglycol by the same route for the same period. All experiments were performed 24 hr after the last dose, unless otherwise specified.

Plasma dye disappearance. The initial disappearance rate from plasma of a single intravenous dose of BSP (5 mg.100 g body wt^{-1}), expressed as the first order rate constant, K_1, was determined from four carefully timed plasma samples obtained 2–8 min after dye administration.

Transport maximum and hepatic storage of dye. 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 [3]. Blood and bile samples were collected at 10-min intervals during the infusion. T_m was calculated from the concentration of dye measured in the bile samples between the 30th and 60th min of infusion. Hepatic storage of dye was directly estimated by determining the dye content of the liver which was removed at the end of the experiments.

Analytical procedures. Bile volume was determined gravimetrically assuming that its density was 1.0 g.ml^{-1}. The concentration of BSP and DBSP in plasma and bile was estimated by absorbance at 580 nm after appropriate dilution of samples with 0.05 N NaOH. Free and conjugated BSP were separated on cellulose thin-layer chromatography plates as described by Whelan and Plaa [8]. The bands were

scraped off the plates and the two fractions were eluted from the cellulose with methanol. In both fractions, dye content was estimated by absorbance at 580 nm, as above.

To determine dye content in the liver, a 10% homogenate was prepared in distilled water, and 1 ml of saturated toluene sulfonate was mixed with each ml of homogenate. One millilitre of 50% trichloroacetic acid was added to precipitate the proteins, from which BSP had been displaced. After 20 min centrifugation at 2500 *g*, the supernatant was filtered through Whatmann No. 2 filter paper and its dye content estimated as specified above.

Liver, kidney and intestine cytosols were obtained by two successive centrifugations (10,000 *g* for 10 min and 100,000 *g* for 60 min) from homogenates (20%, v/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.* [9].

GSH *S*-transferase activity was determined using BSP [10], 1,2-dichloro-4-nitrobenzene (DCNB) or 1-chloro-2,4-dinitrobenzene (CDNB) [11]. In all cases, enzyme assays were conducted under the conditions required for zero-order kinetics as regards the cofactor and substrate concentrations; activities were linear in relation to the enzyme concentration and time. Liver GSH was estimated as described by Ellman [12].

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

Chemicals. BSP, 5–5′-dithiobis-(2-nitrobenzoic acid), 1-chloro-2,4-dinitrobenzene (CDNB) and GSH were purchased from Sigma Chemicals Co, St Louis, U.S.A. 1,2-Dichloro-4-nitrobenzene (DCNB) was obtained from Aldrich Chemical Co, Belgium and DBSP from SERB, France.

RESULTS

In vivo *studies.* Table 1 shows that in rats pretreated with clofibrate, maximal biliary BSP excretion (T_m) diminished significantly by about 23.5% ($P < 0.001$) compared to the controls. Although the rates of bile flow in the control and treated animals were slightly different before BSP infusion, they were similar at T_m. The choleretic effect of BSP was observed in both groups. Analysis of the bile obtained between 30 and 60 min (i.e. at T_m) indicated that the concentration of conjugated BSP, and therefore the contribution to T_m of this component, diminished in the treated animals. Despite a small but significant increase in the concentration of free BSP, its contribution to T_m was similar in both groups; the ratio of conjugated to total biliary BSP dropped significantly. These changes were accompanied by a concomitant rise in hepatic BSP (+54.7%).

The higher plasma levels of BSP observed in clofibrate-treated animals (data not shown) during dye perfusion could be due either to a depressed primary uptake process or to the diminished BSP transport from liver to bile, or both. To investigate these possibilities, we studied BSP elimination from plasma in one group of rats and perfused another group with DBSP. There was no significant difference between control and treated animals as regards the plasma elimination curves for BSP (K_1 was 0.187 ± 0.011 in controls versus 0.205 ± 0.013 in clofibrate-treated rats). This indicated that the initial clearance of dye was not affected by clofibrate.

Table 2 shows that clofibrate did not alter the biliary concentration, transport maximum (T_m) or the amount of DBSP in the liver.

In vitro *studies.* In clofibrate-treated rats, GSH *S*-transferase activity dropped significantly with the three substrates studied (Table 3). However, this decrease was greater with BSP as substrate (−32.3%) than with DCNB (−28%) or CDNB (−21%). The levels of liver GSH and cytosol proteins rose slightly. The decrease observed in the

Table 1. Effect of clofibrate treatment on the metabolism, transport maximum and bile and liver concentrations of BSP

	Control	Clofibrate-treated
Bile flow (μl.min^{-1}.100 g body wt^{-1})		
Before infusion	7.1 ± 0.3	8.8 ± 0.5*
At T_m	10.3 ± 0.5	9.7 ± 0.4
% conjugated BSP in bile at T_m	86.2 ± 0.6	78.8 ± 0.4‡
Bile BSP concentration at T_m (g/l)		
Total BSP	13.5 ± 0.4	10.9 ± 0.4‡
Free BSP	1.8 ± 0.1	2.3 ± 0.1†
Conjugated BSP	11.7 ± 0.4	8.6 ± 0.3‡
Transport maximum, T_m (μg BSP.min^{-1}.100 g body wt^{-1})	136 ± 5	104 ± 4‡
Contribution to T_m of free BSP	19 ± 1	22 ± 1
Contribution to T_m of conjugated BSP	117 ± 4	82 ± 4‡
Hepatic content (mg BSP. total liver^{-1})	6.4 ± 0.4	9.9 ± 0.4‡

After a priming dose of 2.15 μmole.100 g body wt^{-1} BSP, this agent was perfused for 60 min at a rate of 215 nmole.min^{-1}.100 g body wt^{-1} in seven rats previously treated *per os* with a daily dose of 20 mg. 100 g body wt^{-1} clofibrate for 10 days, and in six control rats. Results are means ± S.E.M.
* $P < 0.02$; † $P < 0.01$; ‡ $P < 0.001$.

Table 2. Effect of clofibrate treatment on the biliary concentration, transport maximum and hepatic content of DBSP

	Control	Clofibrate-treated
Bile flow (μl.min^{-1}.100 g body wt^{-1})		
Before infusion	6.1 ± 0.4	6.7 ± 0.3
At T_m	7.4 ± 0.4	7.6 ± 0.4
Bile DBSP concentration at T_m (g/l)	12.0 ± 0.3	12.4 ± 0.2
Transport maximum T_m (μg DBSP.min^{-1}.100 g body wt^{-1})	89 ± 4	94 ± 4
Hepatic content (mg DBSP.total liver^{-1})	10.7 ± 0.5	11.3 ± 0.6

After a priming dose of 2.15 μmole.100 g body wt^{-1} DBSP, this agent was perfused for 60 min at a rate of 215 nmole.min^{-1}.100 g body wt^{-1} in seven rats previously treated *per os* with a daily dose of 20 mg.100 g body wt^{-1} clofibrate for 10 days, and in seven control rats. Results are means ± S.E.M.

specific activity of GSH *S*-transferase was not compensated by the hepatomegaly produced by clofibrate (control rats: 31.6 ± 2.3 μmol BSP conjug./min/total liver versus clofibrate-treated rats: 24.0 ± 1.1 μmol BSP conjug./min/total liver; $P < 0.01$). This effect of clofibrate on hepatic BSP conjugating activity was shown to be dose-related (Fig. 1). A significant increase in the GSH concentration was noted for each dose studied. This effect was patent from the second day of treatment, maximal by day four and plateaued thereafter (Fig. 2).

We attempted to explain the above-mentioned decrease in BSP-GSH conjugating activity by determining the hepatic GSH content and BSP-GSH transferase activity at different times during the 96 hr following the last dose of the 4-day clofibrate treatment. As shown in Fig. 3, the GSH concentration was either unchanged or enhanced, whereas BSP-GSH transferase activity was significantly reduced until 96 hr.

To make sure that we noted all the early effects of clofibrate on GSH, the co-substrate of BSP conjugating activity, we examined the GSH level and the concomitant BSP-GSH-transferase activity in rat livers, 1, 2, 6 and 24 hr after injection of a single dose of clofibrate. As regards the levels of liver GSH and BSP-GSH-TA, there was no difference between control and clofibrate-treated rats. Finally, since fasting has been demonstrated to reduce hepatic GSH content, we determined BSP-GSH-conjugating activity in rats fasted for 42 hr prior to sacrifice. Table 4 shows that this enzyme's activity did not diminish in fasted control rats despite a significant

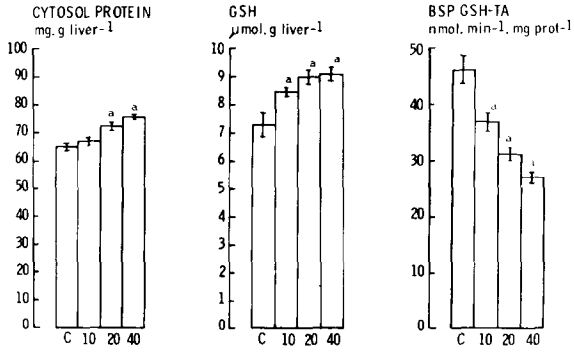

Fig. 1. Dose–responses to 4 days of i.p. clofibrate administration: effects on hepatic cytosol protein, reduced glutathione and BSP-GSH *S*-transferase. Each bar represents a different dose of 10–40 mg clofibrate.100 g body wt^{-1}; C: control rats. Values are means ± S.E.M. for six rats per dose. [a] Significantly different from controls ($P < 0.05$).

reduction in GSH content, but that it did diminish in rats pretreated with clofibrate, whether fasted or not. Nevertheless, on a whole liver basis, the BSP conjugating activity was depressed, since fasting reduces liver weight. In rats pretreated with clofibrate, the GSH concentration rose compared to that in control rats whether fed or fasted.

As GSH *S*-transferase activity is present in various organs involved in detoxication processes, we compared the effects of clofibrate on liver, kidney and small intestine. With CDNB as substrate, glutathione *S*-transferase activity remained unchanged in kidney (453 ± 14 nmol.min^{-1}.mg protein^{-1} in control rats

Table 3. Effect of clofibrate treatment on hepatic glutathione level and glutathione *S*-transferase activity

	Control	Clofibrate-treated
Cytosol protein (mg.g liver^{-1})	69.6 ± 2.5	76.7 ± 2.6*
Reduced glutathione (μmol.g liver^{-1})	5.5 ± 0.4	6.4 ± 0.2*
Glutathione *S*-transferase (nmole.min^{-1}.mg protein^{-1})		
1,2-Dichloro-4-nitrobenzene	53.3 ± 1.4	38.4 ± 1.6†
1-Chloro-2,4-dinitrobenzene	1190 ± 40	940 ± 50†
Sulfobromophthalein	38.7 ± 1.2	26.2 ± 0.8†

Rats were injected i.p. with a daily dose of 20 mg.100 g body wt^{-1} clofibrate for 10 days while control rats received propylenglycol. Each result is the mean ± S.E.M. of ten rats.
* $P < 0.05$; † $P < 0.001$.

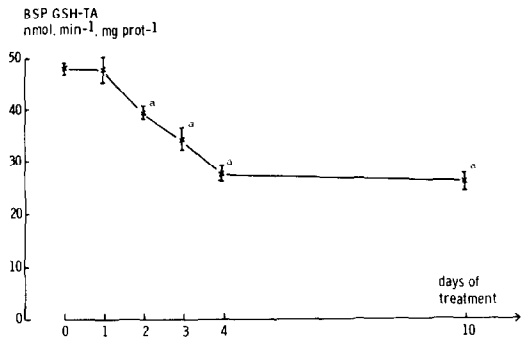

Fig. 2. Changes in hepatic BSP glutathione *S*-transferase activity after i.p. treatment of rats for 1, 2, 3, 4 or 10 days with 20 mg. 100 g body wt^{-1} clofibrate. Each point is the mean ± S.E.M. for six animals. [a] Significantly different from day 0, $P < 0.05$.

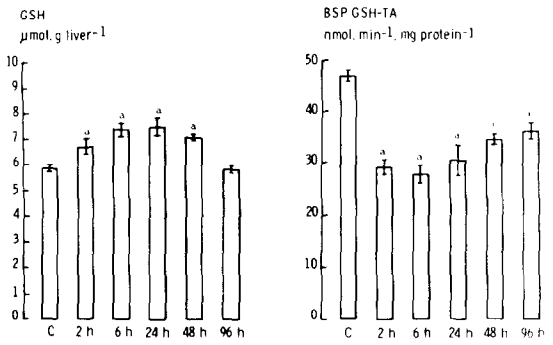

Fig. 3. Hepatic glutathione concentration and BSP glutathione *S*-transferase activity 2, 6, 24, 48 and 96 hr after i.p. administration to rats of 20 mg. 100 g body wt^{-1} clofibrate per day for 4 days. Values are means ± S.E.M. for five rats. [a] Significantly different from control rats (C). $P < 0.05$.

versus 430 ± 12 in treated rats) and decreased in small intestine to the same extent as in liver (172 ± 14 nmol. min^{-1}. mg $protein^{-1}$ in control rats versus 119 ± 3 in treated rats; $P < 0.01$), after a daily oral dose of 20 mg. 100 g body wt^{-1} clofibrate for 4 days.

Kinetic studies of liver BSP-GSH *S*-transferase activity showed that the decrease in this enzyme's conjugating activity was mainly due to a decrease in the apparent V_{max} for both BSP and GSH. No difference in K_m values was observed (Fig. 4). Similar results were obtained for DCNB-GSH conjugation (data not illustrated).

DISCUSSION

Administration of clofibrate to rats significantly reduced both maximal biliary excretion of BSP and the *in vitro* GSH conjugating activity in the liver. *Per os* administration of clofibrate also inhibited this activity in the intestine, but did not affect it in the kidney. These results agree with those of Clifton and Kaplowitz who showed that the response to phenobarbital or 20 MC was organ-specific [13]. The reduced biliary excretion of BSP observed here was concomitant with a decrease in the conjugated BSP in the bile and with an accumulation of BSP in the liver. The contribution of free BSP to the maximal rate of total BSP secretion in the bile was unchanged.

These effects of clofibrate were compared to its effects on the hepatic transport of another dye, DBSP; it is believed that this agent is not metabolized prior to its excretion in the bile, at least in the rat [7]. Here, the rate of DBSP excretion in the bile was identical in clofibrate-treated and control animals and no hepatic dye accumulation was noticed. As the initial rate of BSP elimination from the plasma remained unchanged, the main effect of clofibrate on transport of BSP in the liver was obviously related to the reduction of the dye metabolism. The results of Whelan *et al.* [14], Varga *et al.* [15] and Zigmond and Solymoss [16] indicate that BSP conjugation facilitates dye transport into the bile and is the rate-limiting step in the *in vivo* transfer of BSP from the blood to the bile. However, a different conclusion was reported by Klaassen and Plaa [17], who demonstrated that the increased BSP excretion in the bile of phenobarbital-treated rats was not due to enhanced BSP conjugation, but rather to an increase in bile flow. In another study, Siddik *et al.* [18] demonstrated, in vitamin A-deficient rats, that increased enzyme conjugation led to enhanced dye excretion. Conversely, acute inhibition of BSP-conjugating activity by benziodarone reduced both BSP-GSH excretion in bile and the T_m of BSP [19].

Interestingly, in our study, the 32% decrease in

Table 4. Effect of diets and clofibrate treatment on the hepatic glutathione level and on sulfobromophthalein glutathione *S*-transferase activity

	Liver wt/ body wt (%)	Cytosolic protein (mg. g $liver^{-1}$)	Reduced glutathione (μmol. g $liver^{-1}$)	BSP glutathione *S*-transferase (nmol. min^{-1}. mg $protein^{-1}$)
Fed control rats	4.1 ± 0.1	58.8 ± 1.0	6.3 ± 0.4	41.9 ± 1.2
Fasted control rats	2.9 ± 0.1*	61.8 ± 2.5	5.0 ± 0.2*	41.2 ± 1.5
Fed rats pretreated with clofibrate	5.5 ± 0.3†	65.0 ± 0.8†	7.2 ± 0.1†	31.5 ± 1.0†
Fasted rats pretreated with clofibrate	3.9 ± 0.1*†	62.5 ± 1.6	6.0 ± 0.3*†	34.0 ± 8.0†

Rats were either fed a normal standard diet or fasted for 42 hr before being killed. 20 mg. 100 g body wt^{-1} clofibrate was administered i.p. daily for 4 days. Results are means ± S.E.M. for six rats. * Different from value for fed rats, $P < 0.05$; † Different from value for control rats, $P < 0.05$.

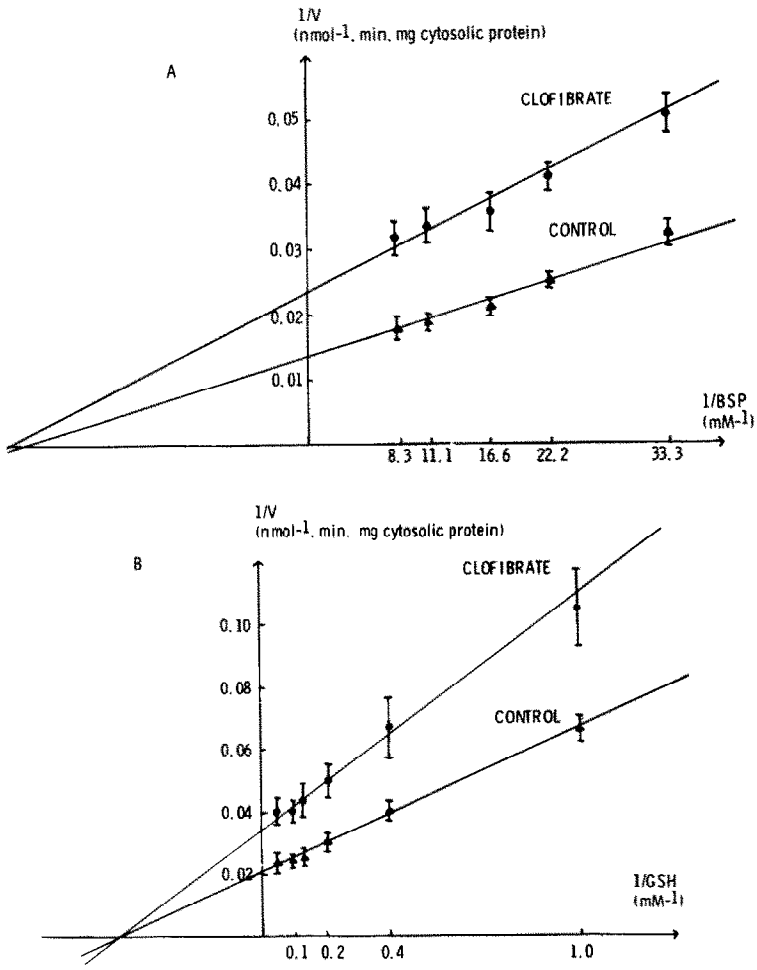

Fig. 4. Effects of clofibrate on the GSH conjugation of BSP. Cytosol was isolated from rats killed 24 hr after the last of 4 daily i.p. doses of 20 mg.100 g body wt^{-1} clofibrate. The figure shows a double reciprocal plot of the amount of BSP-GSH formed, A: in the presence of various concentrations of BSP. Control rats exhibited a V_{max} of 76.9 nmoles BSP-GSH formed.min^{-1}.mg protein^{-1} and a K_m of 39.5 μM versus a V_{max} of 42.6 and a K_m of 35.7 in treated rats; B: in the presence of various concentrations of GSH. Control rats exhibited a V_{max} of 47.6 nmoles BSP-GSH formed.min^{-1}.mg protein^{-1} and a K_m of 2.22 mM versus a V_{max} of 29.0 and a K_m of 2.20 in treated rats.

the *in vitro* conjugation rate of BSP after clofibrate treatment (Table 3) was similar to the 23.5% decrease in T_m (Table 1). Furthermore, this drop in T_m was entirely due to the decline in the proportion of conjugated BSP appearing in the bile. When the effect of clofibrate on BSP conjugation was characterized *in vitro*, the resulting reduction in GSH-conjugating activity (i.e. specific activity) was not compensated by either an increase in total cytosolic proteins or by liver enlargement. This inhibitory effect of clofibrate was shown to be dose-dependent, requiring two days to occur and lasting several days after drug administration had stopped. These data are consistent with the possibility that clofibrate has a direct effect on BSP conjugating enzyme. In addition, the reduced BSP-GSH conjugating activity in clofibrate-treated rats was mainly due to a decline in the apparent V_{max} for both substrates. This decline did not seem to be related to a decrease in the level of liver GSH, since it was either unchanged or increased after several days of treatment. The inhibitory effect of clofibrate on GSH *S*-transferase preceded the rise in liver GSH. Consequently, the mechanism of this rise could be a reduction in GSH consumption by the liver, since the GSH conjugating activity diminished with all the substrates studied. In animals fasted for 42 hr, hepatic GSH declined but GSH conjugating activity was not modified. Administration of clofibrate similarly reduced BSP conjugation, whether or not the animals were fasted. The reduction in the biliary transport of BSP observed in fasted rats is more likely to be due, as Combes believed [20], to a reduction in available GSH than to a decline in BSP conjugating activity.

Levi *et al.* in 1969 [21] described two cytosolic proteins, Y and Z, which bind bilirubin, dyes and numerous organic anions and are thought to facilitate their transfer from blood to liver. These proteins are modified by physiopathological states [22, 23] and also by drug administration. Y protein is induced by phenobarbital [24] and to a lesser degree by certain other chemicals, whilst Z protein is induced by

hypolipemic drugs [6, 25]. Both are reduced by estrogens [23]. Fleischner *et al.* demonstrated that when Z protein was induced by hypolipemic compounds there was a concomitant reduction in Y protein or ligandin [6]. Therefore, as ligandin is probably identical with the main BSP-conjugating enzyme, GSH *S*-transferase B [26], our results suggest that the effect of clofibrate on hepatic transport of BSP is consistent with a reduction of this enzyme. If one accepts this assumption, ligandin may participate in the hepatic transport process in two ways: as an intracellular binding protein in the hepatic uptake of organic anions, and as an enzyme that further metabolizes some of these compounds. In our BSP perfusion experiments, BSP storage in the liver does not seem to be altered, since the dye accumulates in the liver. Consequently, the capacity of the cytosolic proteins Y and Z to bind BSP should be equal in the controls and clofibrate-treated rats as confirmed by the fact that perfusion of unconjugated dye (DBSP) did not alter hepatic dye storage.

In this connection, the heterogeneity of ligandin has been shown by Bass *et al.* [27], since purified ligandin was found to consist of the two subunit species Ya and Yc, with molecular weights of 22,000 and 24,000. These authors demonstrated that phenobarbital treatment increased the concentration of Ya but had little effect on Yc. This raises the question of the respective roles of the ligandin subunits Ya and Yc in the binding of ligands and metabolizing of compounds. In the case of clofibrate, one may wonder whether it does not have an elective effect on the metabolizing function but not on the ligand binding. Previous Sephadex gel chromatography studies of BSP binding to liver cytosol from rats treated with clofibrate [6, 25] revealed normal binding to the Y protein peak containing ligandin and other glutathione-transferases, despite increased binding to the Z protein peak.

The present results for hepatic transport of BSP are in contrast with those of Meijer *et al.* [28] who reported that clofibrate treatment did not affect biliary excretion of anions. Nevertheless, in the same study another hypolipemic drug, nafenopin, which increased Z protein and reduced Y protein in rat liver, was demonstrated to affect the overall hepatobiliary transport of numerous organic anions like bilirubin, BSP and even DBSP. In this experiment, the levels of DBSP in the plasma were higher than in the controls, while those in the liver were lower. These findings differ from our present observations with clofibrate and DBSP. This difference might be attributable either to a more pronounced effect of nafenopin on ligandin, or to quite a different mechanism.

Our results suggest that clofibrate lowers the hepatic transport of BSP in rats by reducing GSH-conjugating activity; this diminished capacity of liver to transport BSP could result from a reduction in the quantity of conjugated BSP available for excretion, or from competition for biliary excretion between conjugated BSP and free BSP as shown by Barnhart *et al.* [29], or both. Comparison of the effects of clofibrate on the hepatic transport of bilirubin [3] and of BSP led us to conclude that the biliary secretory rate of compounds undergoing biotransformation before excretion would mainly depend on the metabolic rate of the liver.

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REFERENCES

1. C. D. Klaassen and G. L. Plaa, *Am. J. Physiol.* **213**, 1322 (1967).
2. A. Foliot, J. L. Drocourt, J. P. Etienne, E. Housset, J. N. Fiessinger and B. Christoforov, *Biochem. Pharmac.* **26**, 547 (1977).
3. F. Jean, A. Foliot, C. Celier, E. Housset and J. P. Etienne, *Biochem. biophys. Res. Commun.* **86**, 1154 (1979).
4. B. Combes and G. S. Stakelum, *J. clin. Invest.* **40**, 981 (1961).
5. W. H. Habig, M. J. Pabst, G. Fleischner, Z. Gatmaitan, I. M. Arias and W. B. Jacoby, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3879 (1974).
6. G. Fleischner, D. K. F. Meijer, W. G. Levine, Z. Gatmaitan, R. Gluck and I. M. Arias, *Biochem. biophys. Res. Commun.* **67**, 1401 (1975).
7. N. B. Javitt, *Proc. Soc. exp. Biol. Med.* **117**, 254 (1964).
8. F. J. Whelan and G. L. Plaa, *Toxic. appl. Pharmac.* **5**, 457 (1963).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. J. Goldstein and B. Combes, *J. Lab. clin. Med.* **67**, 863 (1966).
11. W. H. Habig, M. J. Pabst and W. B. Jacoby, *J. biol. Chem.* **149**, 7130 (1974).
12. G. L. Ellman, *Archs Biochem. Biophys.* **82**, 70 (1959).
13. G. Clifton and N. Kaplowitz, *Biochem. Pharmac.* **27**, 1284 (1978).
14. G. Whelan, J. Hoch and B. Combes, *J. lab. clin. Med.* **75**, 542 (1970).
15. F. Varga, E. Fischer and T. S. Szily, *Biochem. Pharmac.* **23**, 2617 (1974).
16. G. Zsigmond and B. Solymoss, *J. Pharmac. exp. Ther.* **183**, 499 (1972).
17. C. D. Klaassen and G. L. Plaa, *J. Pharmac. exp. Ther.* **261**, 361 (1968).
18. Z. H. Siddik, R. Drew and T. E. Gram, *Biochem. Pharmac.* **29**, 2583 (1980).
19. B. G. Priestly and G. L. Plaa, *Proc. Soc. exp. Biol. Med.* **132**, 881 (1969).
20. B. Combes, *J. clin. Invest.* **14**, 1214 (1965).
21. A. J. Levi, Z. Gatmaitan and I. M. Arias, *J. clin. Invest.* **48**, 2156 (1969).
22. L. B. Stein, S. Mishkin, G. Fleischner, Z. Gatmaitan and I. M. Arias, *Am. J. Physiol.* **231**, 1371 (1976).
23. H. Reyes, A. J. Levi, Z. Gatmaitan and I. M. Arias, *J. clin. Invest.* **50**, 2242 (1971).
24. H. Reyes, A. J. Levi, Z. Gatmaitan and I. M. Arias, *Proc. natn. Acad. Sci. U.S.A.* **64**, 168 (1969).
25. A. Foliot, B. Christoforov, E. Housset, J. P. Petite, J. P. Ploussard and J. P. Etienne, *Biol. Gastroentérol.* **7**, 332 (1974).
26. J. N. Ketley, W. H. Habig and W. B. Jacoby, *J. biol. Chem.* **250**, 8670 (1975).
27. N. M. Bass, R. E. Kirsch, S. A. Tuff, I. Marks and S. J. Saunders, *Biochim. biophys. Acta* **492**, 163 (1974).
28. D. K. F. Meijer, J. Bognacki and W. G. Levine, *Naunyn-Schmiedeberg's Arch. Pharmac.* **290**, 235 (1975).
29. J. L. Barnhart and B. Combes, *Am. J. Physiol.* **231**, 399 (1976).