Biochemical Pharmacology, Vol. 37, No. 4, pp. 743–748, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

DRUG–PROTEIN CONJUGATES—XVI*

STUDIES OF SORBINIL METABOLISM: FORMATION OF 2-HYDROXYSORBINIL AND UNSTABLE PROTEIN CONJUGATES

J. L. MAGGS and B. K. PARK†

Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX, U.K.

(*Received* 4 *June* 1987; *accepted* 27 *August* 1987)

Abstract—The metabolism of sorbinil ((+)6-fluoro-spiro (chroman-4, 4′-imidazolidine)-2′,5′ dione), an aldose reductase inhibitor associated with immunological adverse reactions, was studied *in vivo* and *in vitro* with particular reference to the formation of protein conjugates of 2-hydroxysorbinil and their further metabolism. [8-^{3}H]Sorbinil was rapidly and extensively metabolized in the rat. 2-Hydroxysorbinil (2HSB) and a phenolic primary alcohol (2,4-imidazolidinedione 5-(2-hydroxyethyl)-5-(5-fluoro-2-hydroxyphenyl); IHFH) were its principal urinary metabolites; over 0–24 hr, they represented 17.0 ± 0.7% (mean ± SD, N = 4) and 7.1 ± 0.7% of the dose, respectively. [^{3}H]2HSB isolated from urine and re-administered was converted to IHFH. Chronic dosing with sorbinil (150 mg/kg × 5) induced 2-hydroxylation of the drug, the 0–24 hr urinary excretion of 2HSB increasing from 17.0 ± 0.7% to 24.7 ± 3.4% of the dose ($P < 0.05$ by Students' paired *t*-test). The biotransformation of 2HSB to IHFH was rationalized in terms of an open-chain aldehyde intermediate. Since aldehydes form both stable and unstable protein adducts, 2HSB was potentially a pro-reactive metabolite and initiator of the hypersensitivity reaction associated with sorbinil. However, [^{3}H]2HSB was neither metabolized by human liver microsomes nor underwent irreversible binding to the microsomal protein. Nevertheless, the mild reductant sodium cyanoborohydride, although without effect on microsomal binding of [^{3}H]2HSB, enhanced binding to human serum albumin. Formation of unstable Schiff base adducts was indicated.

The formation of protein conjugates by chemically reactive compounds [1, 2] and the reactive metabolites of stable compounds [3, 4] is an established fate of many xenobiotics *in vivo* and *in vitro* [5, 6]. Although generally a minor process, the covalent modification of critical cellular proteins is thought to initiate the tissue damage associated with several drugs and other chemicals [7–9]. In other instances, when the proteins that undergo modification are presumably not essential for the maintenance of cell viability, covalent binding has no apparent toxicological consequences [10, 11]. Certain adverse drug effects, involving hypersensitivity reactions, have been attributed to the production of immunogenic drug–protein conjugates [12]. If the drug moiety is antigenic, the conjugate may combine with the anti-drug antibody to form tissue-damaging immune complexes. The conjugates are usually regarded as chemically stable entities, and the clearance of covalently bound moieties such as 2,4-dinitrophenyl [13, 14] and 2-propenalyl [15] results from degradation of the protein. However, recent studies have demonstrated that acetaldehyde [16] and 16α-hydroxysterone [17] form unstable as well as stable protein adducts *in vitro*. Reversible conjugation occurs via Schiff base intermediates formed by reaction between the carbonyl compound and the C-6 amino groups of lysine residues [16–18]. Schiff bases readily dissociate but are stabilized by reduction [16–19]. Hence, in the absence of a reductant, the production of such adducts *in vitro* is likely to be at least underestimated if not undetectable; *in vivo* the intermediates may be stabilized by an endogenous reductant such as ascorbic acid [19]. Consequently a metabolic process which has toxicological implications might, in the case of compounds yielding carbonyl metabolites, escape detection in a standard extraction assay of irreversible protein binding *in vitro*.

The present studies of the metabolism of sorbinil ((+)6 - fluorospiro(chroman - 4,4′ - imidazolidine) - 2′,5′-dione), an aldose reductase inhibitor [20], were designed to investigate potential metabolic and chemical bases of the dominant adverse reaction associated with the drug, which has been tentatively ascribed to the formation of immune complexes [21]. This reaction takes the form of an erythematous rash with or without an accompanying fever. More serious toxic epidermal necrolysis and Stevens–Johnson Syndrome have occurred in isolated cases. 2-Hydroxysorbinil (2HSB) and a phenolic primary alcohol (IHFH) were found to be the principal urinary metabolites of sorbinil. It was suggested (R. Ronfeld, personal communication) that the latter was derived from 2HSB via an aldehyde intermediate (Fig. 3). Since aldehydes readily combine with proteins [15, 16], 2HSB was identified as a potential pro-reactive metabolite. In this paper the formation, further metabolism and protein conjugation of 2HSB

* For Part XV in this series, see Coleman *et al.*, *Biochem. Pharmac.* **37**, 737 (1988).

† Wellcome Senior Lecturer and author for correspondence.

are described. Some of the data have been presented in abstract form [22].

MATERIALS AND METHODS

Materials. [8-^{3}H]Sorbinil (10 Ci/mmol) was provided by Pfizer Central Research (Groton, CT). It was re-purified for *in vivo* studies by extraction into diethyl ether (Aristar™, peroxide-free; BDH, Poole, Dorset, U.K.) from 10 mM potassium phosphate buffer, pH 6.0. This material was radiochemically homogeneous (R_t 22 min) when analysed on a 5 μm C_8 column (25 cm × 0.46 cm i.d.; Spherisorb, HPLC Technology Ltd., Macclesfield, Cheshire, U.K.) using a non-linear gradient of acetonitrile (10% to 15% over 15 min, 15% to 55% over 10 min) in the above buffer; the flow rate was 1 ml/min. [8-^{3}H]Sorbinil was further purified for *in vitro* experiments by HPLC. Aliquots of an ethanol solution of the drug (45 μCi/mmol) were chromatographed on the C_8 column using a linear gradient of acetonitrile (10% to 50% over 20 min) in phosphate buffer. Eluate corresponding to sorbinil (R_t 14 min) was collected, combined, concentrated under a stream of N_2 and extracted three times with two volumes of ether. Combined extracts were evaporated to dryness and reconstituted in ethanol for storage. Unlabelled sorbinil, 2-hydroxysorbinil (2HSB) and 2,4-imidazolidinedione 5-(2-hydroxyethyl)-5-(5-fluoro-2-hydroxyphenyl) (IHFH) were also from Pfizer Central Research. Sodium cyanoborohydride was from Sigma Chemical Co. (Poole, Dorset, U.K.). NADPH (tetrasodium salt) was obtained from Boehringer Mannheim GmbH, Biochemica (Mannheim, F.R.G.). NCS solubilizer was a product of Amersham Corp. (Arlington Heights, IL). Scintillation fluid (Aqua Luma Plus™) was from Lumac/3M B.V. (Schaesberg, Netherlands). Human and bovine serum albumin (Fraction V) were from Sigma.

Animal studies. The metabolism of [8-^{3}H]sorbinil and its auto-induction were studied in male Wistar rats (200 g body wt.). [8-^{3}H]Sorbinil (150 mg/kg body wt; 12 μCi) in 0.5 ml polyethylene glycol 200 (PEG) was administered i.p. to two groups of four rats. The animals were housed in individual metabolism cages. Food and water were available *ad libitum*. Urine was collected every 24 hr for 72 hr. From 48 hr inclusive, one group received unlabelled sorbinil (150 mg/kg in PEG) i.p. every 24 hr for five days whilst the other received vehicle. A second dose of [8-^{3}H]sorbinil (150 mg/kg; 12 μCi; i.p.) was given seven days after the first. Urine was collected for 48 hr. The rats were killed by cervical dislocation, and their major organs removed, frozen in liquid N_2 and stored at −70° until analysed.

Duplicate aliquots of urine (50–100 μl) were assayed for radioactivity by liquid scintillation counting in 4 ml of scintillant. Total tissue radioactivity was determined according to Kitteringham *et al.* [13] following solubilization of portions of homogenized organs in NCS. Portions of liver (*ca.* 5 g) were used for preparation of microsomes as described previously [23]. Microsomes (8 mg protein) from each liver (yield, *ca.* 14 mg protein/g) were immediately solubilized in 3 ml 1 M NaOH and aliquots (700 μl) of the solutions assayed for radioactivity. Additional microsomes (8 mg protein) were precipitated with acetone, exhaustively extracted and solubilized for determination of radioactivity as detailed below.

Microsomal studies. Washed microsomes (105,000 *g* pellets) were prepared from the histologically normal livers of renal transplant donors [24]. Ethical approval for the study was granted by the Mersey Regional Ethical Committee and consent to removal of the livers obtained from the donors' relatives. Microsomal protein was assayed by the method of Lowry *et al.* [25] using bovine serum albumin as standard. Cytochrome P-450 content was determined according to Omura and Sato [26].

[8-^{3}H]Sorbinil (10 μM or 100 μM, *ca.* 2 μCi) was incubated with microsomes (8 mg protein) and NADPH (1 mM, omitted from controls) in 4 ml 0.1 M sodium phosphate, pH 7.4. Incubations were performed in 25 ml flasks shaken in a water bath at 37° for 30 min. They were stopped by cooling on ice and extracted with ethyl acetate (5 ml × 3). Combined organic extracts were evaporated to dryness under N_2 at *ca.* 40° and reconstituted in methanol (400 μl) for HPLC analysis.

[^{3}H]2HSB and [^{3}H]IHFH (2.2 μM, 0.2 μCi), isolated from the urine of rats given [8-^{3}H]sorbinil and purified to radio-chromatographic homogeneity, were incubated with human liver microsomes (4 ml, 8 mg protein) and NADPH (1 mM) for 30 min. Radiolabelled material extracted into ethyl acetate was analysed by HPLC.

High-performance liquid chromatography. The urinary metabolites of [8-^{3}H]sorbinil and [^{3}H]2HSB, and material extracted from *in vitro* incubations, were chromatographed on the C_8 column (non-linear gradient) as described above. An LKB 2150 HPLC pump and 2152 HPLC controller (LKB, Bromma, Sweden) were used. Urine (50–70 μl; 10–90 × 10^3 dpm) was filtered (Minisart 0.2 μm, Sartorious GmbH, Göttingen, F.R.G.) before analysis. Eluate was monitored at 284 nm with an LC3 absorbance detector (Pye Unicam Ltd., Cambridge, U.K.), collected in 0.5 ml fractions and dissolved in scintillant (4 ml) for measurement of radioactivity. Radiolabelled components were chromatographically identified by co-elution with authentic unlabelled compounds. The urinary metabolites and unchanged sorbinil were assayed radiometrically. Recoveries of chromatographed radioactivity (0–24 hr collections) were 99 ± 4% (mean ± SD, N = 16). The assay precision (coefficient of variation for six analyses of one 0–24 hr collection) was 0.9% and 2.4% for [8-^{3}H]sorbinil and [^{3}H]2HSB, respectively.

Isolation, identification and re-administration of metabolites. Sorbinil, 2HSB and IHFH were isolated from 0–24 hr urine by HPLC. Urine (1 ml, containing *ca.* 7.5 μmol equivalents) from a rat given *ca.* 130 μmol [8-^{3}H]sorbinil was mixed with 0.1 M sodium phosphate buffer, pH 7.4, (1 ml) and extracted with ethyl acetate (5 ml × 3). Pooled extracts were evaporated to dryness under N_2 and reconstituted in 400 μl potassium phosphate (10 mM, pH 6.0)–methanol (9:1, v/v). Aliquots (40–80 μl) were chromatographed on the C_8 column. Eluate corresponding to the absorbance peaks (284 nm) of the three compounds was collected, concentrated *in*

vacuo to remove acetonitrile, adjusted to pH 7.4 with phosphate buffer and extracted with ethyl acetate (5 ml × 3). The unchanged sorbinil (450 μg), 2HSB (300 μg) and IHFH (100 μg) were purified to chromatographic (i.e. single component absorbing at 284 nm) and radio-chromatographic homogeneity by re-chromatographing them as before. Their identities were confirmed by direct-insertion electron impact mass spectrometry under standard conditions [27].

Larger amounts (*ca.* 2 mg) of [^{3}H]2HSB were isolated from the 0–24 hr urine of rats given [8-^{3}H]sorbinil (150 mg/kg). Urine (3 ml × 4) was mixed with 0.1 M sodium phosphate buffer (6:1, v/v) and each aliquot extracted with ethyl acetate (5 ml × 3). The metabolite was purified by the above procedure. [^{3}H]2HSB (1.6 mg, 0.6 μCi) dissolved in 0.5 ml PEG was administered i.p. to a male Wistar rat (200 g body wt). Urine was collected at 8 hr and 24 hr. Urinary metabolites were analysed, isolated and identified as before.

High specific activity [^{3}H]2HSB and [^{3}H]IHFH (25 μCi/μmol) for *in vitro* studies were isolated to radio-chromatographic homogeneity from 6 ml of 0–18 hr urine. [8-^{3}H]Sorbinil (0.96 mg, 100 μCi) in 0.54 ml PEG was given i.p. to a male rat. The above HPLC method yielded 35 μg [^{3}H]2HSB and 32 μg [^{3}H]IHFH (equivalent to *ca.* 70% recoveries from urine).

Reductive stabilization of [^{3}H]*2HSB-protein adducts.* Stabilization of [^{3}H]2HSB-protein linkages by reduction was attempted with sodium cyanoborohydride, a mild reductant specific for Schiff bases [18, 28]. [^{3}H]2HSB (4.5 μM, 0.4 μCi) and the borohydride (500 μM) were incubated with either human liver microsomes or HSA (2 mg protein) in 2 ml 0.1 M sodium phosphate, pH 7.4. Reactions were conducted in capped test tubes at 37° for 18 hr and then extracted with ethyl acetate (5 ml × 3). The radiolabelled material in the extracts was analysed by HPLC.

Measurement of irreversibly bound radiolabelled material. Radiolabelled material irreversibly bound to microsomal protein and HSA was determined by exhaustive extraction. After extraction with ethyl acetate, incubations were mixed with acetone (0.9:1.0, v/v) and left at 5° for *ca.* 15 hr. Microsomal pellets were extracted by vigorous vortex mixing with acetone (5 ml × 2), methanol (5 ml × 2), methanol–water (7:3, v/v; 5 ml), cold 0.1 M sodium phosphate buffer (pH 7.4; 5 ml) and acetone–water (7:3, v/v, 5 ml × 2). HSA precipitates were extracted with acetone (5 ml × 3) and methanol (5 ml × 3). Supernatants were assayed for radioactivity to ensure complete removal of the reversibly bound radiolabelled material. The microsomal and HSA pellets were dissolved in 2 ml 1 M NaOH at 60° over 1–2 hr. The solutions were sampled for determination of radioactivity (500 μl aliquots) and protein (25–100 μl aliquots) [25].

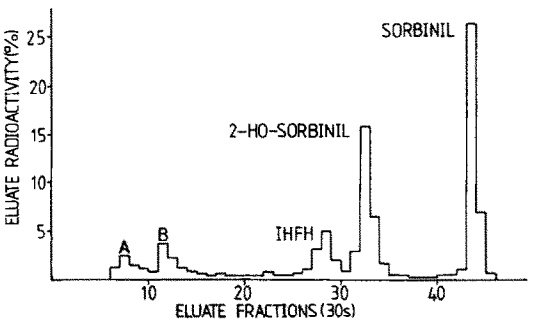

Fig. 1. High-performance liquid chromatogram of the urinary metabolites of [8-^{3}H]sorbinil in the rat. [8-^{3}H]sorbinil (150 mg/kg) was administered i.p. and urine collected for 24 hr. Urine was analysed on a 5-μm C_8 column eluted with a gradient of acetonitrile in phosphate buffer.

RESULTS

Metabolism of [8-^{3}H]*sorbinil and* [^{3}H]*2HSB* in vivo

Following the i.p. administration of 150 mg/kg [8-^{3}H]sorbinil to rats, 63.9 ± 1.2% (mean ± SD, N = 4), 7.0 ± 0.6% and 2.4 ± 0.4% of the radioactive dose was excreted in urine during the subsequent three days (total, 73.3 ± 0.8%). Unchanged drug, 2HSB and IHFH, identified preliminarily by co-chromatography with standards, were the principal radiolabelled urinary components (Fig. 1). These identifications were confirmed by mass spectrometry: molecular ions were present at *m/z* 236 (relative intensity (RI) 39%), *m/z* 252 (RI 30%) and *m/z* 254 (RI 14%), respectively. The fragment ions were identical to those obtained with the standards and were mostly ascribable to fragmentation of the hydantoin ring [29, 30]. The rats eliminated 20.8–23.3%, 16.4–17.8% and 6.3–7.9% of the dose as sorbinil, 2HSB and IHFH, respectively, in 0–24 hr urine (Table 1).

Table 1. Auto-induction of sorbinil 2-hydroxylation in the rat

Rats	Total radioactivity	Urinary excretion (0–24)		
		Sorbinil	2HSB	IHFH
Before sorbinil	63.9 ± 1.2	22.1 ± 1.1	17.0 ± 0.7	7.1 ± 0.7
After sorbinil	69.8 ± 7.1	18.2 ± 2.2**	24.7 ± 3.4*	7.3 ± 0.9
Before PEG	67.2 ± 3.7	24.5 ± 3.8	15.0 ± 1.0	12.0 ± 1.5
After PEG	69.9 ± 3.2	23.1 ± 3.5	16.3 ± 2.0	12.5 ± 0.8

Data are mean % ± SD of dose (N = 4). *P < 0.05, **P < 0.01, by Student's paired *t*-test. PEG, polyethylene glycol 200. [8-^{3}H]Sorbinil and unlabelled drug were administered i.p. to male Wistar rats in 150 mg/kg doses: 5 daily doses for induction. Sorbinil and metabolites were assayed by radiometric HPLC.

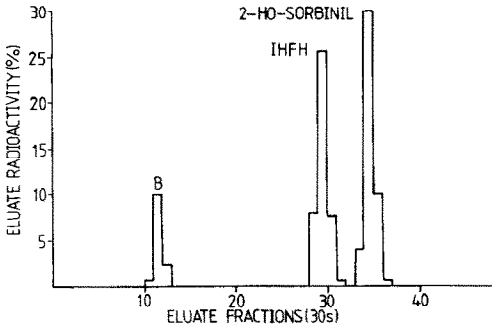

Fig. 2. High-performance liquid chromatogram of the urinary metabolites of [^{3}H]2HSB in the rat. [^{3}H]2HSB (8.0 mg/kg) was administered i.p. and urine collected for 8 hr.

Chronic dosing with unlabelled drug (150 mg per day × 5) significantly increased ($P < 0.05$) the 0–24 hr urinary excretion of 2HSB and decreased that of sorbinil without affecting the total excretion of radiolabelled material (Table 1). Vehicle alone was without effect.

[^{3}H]2HSB administered to a rat was metabolized to IHFH. During 0–8 hr and 8–24 hr after dosing, 61.0% and 11.7% of the administered radioactivity, respectively, was eliminated in urine. When the 0–8 hr urine was analysed by HPLC, unchanged 2HSB (R_t 17.5 min, 45% eluate radioactivity) and IHFH (R_t 15.0 min, 41% eluate radioactivity) were identified by co-chromatography with standards (Fig. 2). The identifications were substantiated by mass spectrometric analysis of the metabolites following isolation by HPLC: 2HSB yielded a spectrum with ions at m/z 252 ($M^{+\cdot}$, RI 27%), 234 ($[M\text{-}H_2O]^{+\cdot}$), 208, 192, 180, 164, 153 and 137 (base peak); IHFH yielded ions at m/z 254 ($M^{+\cdot}$, RI 24%), 236 ($[M\text{-}H_2O]^{+\cdot}$), 209, 193, 180, 164, 149 and 138 (base peak). An additional metabolite of 2HSB (R_t 6 min, 14% eluate radioactivity) was not identified but co-chromatographed with one (B) of two unidentified polar metabolites of sorbinil (A and B; Fig. 1).

Forty-eight hours after administration of [8-^{3}H]sorbinil, the lungs, heart, kidneys, spleen and brain each contained less than 0.1% of the dose whilst the liver contained 0.19–0.37% (mean 0.26%, N = 8). None of the hepatic radiolabelled material was irreversibly bound to the microsomal fraction: only very low levels of radioactivity (*ca.* 40–100 dpm/2 mg) were detected before and after exhaustive extraction of the protein.

In vitro *studies*

Neither [8-^{3}H]sorbinil nor its two metabolites ([^{3}H]2HSB and [^{3}H]IHFH) underwent detectable metabolism by human liver microsomes; the cytochrome P-450 contents of which (0.24–0.82 nmol/mg protein, N = 4) were within or, in the case of the highest content, above reported ranges [31, 32]. Unchanged radiolabelled compounds, identified by co-chromatography, were alone recovered from incubations containing NADPH. In addition, only background levels of radioactivity were associated with extracted microsomes incubated with the compounds.

Table 2. Formation of stable protein adducts of [^{3}H]2HSB by reduction *in vitro*

Incubation	Irreversibly bound [^{3}H]2HSB DMP/mg protein	% Incubated radioactivity
Control	240 ± 22	0.11 ± 0.01
Cyanoborohydride	978 ± 45	0.44 ± 0.02

Data are means ± SD (N = 3). [^{3}H]2HSB was incubated with HSA in the presence of sodium cyanoborohydride at 37° for 18 hr; reducing agent was omitted from control incubations.

Stabilization of [^{3}H]*2HSB-protein adducts by chemical reduction*

Small amounts of [^{3}H]2HSB were irreversibly bound to HSA after an 18-hr co-incubation with sodium cyanoborohydride (Table 2). Enhancement of binding was not observed when the reductant (final concentration 40 μM) was added following an 18-hr incubation of [^{3}H]2HSB (4.5 μM) with HSA (1 mg/ml) (unpublished data). The cyanoborohydride did not produce any measurable irreversible binding of [^{3}H]2HSB to human liver microsomes. Small amounts of [^{3}H]IHFH (equivalent to 4% of incubated radioactivity), identified by co-chromatography, were recovered from incubations containing [^{3}H]2HSB, HSA and cyanoborohydride. Only [^{3}H]2HSB was extracted from control incubations.

DISCUSSION

2HSB, here shown to be the major urinary metabolite of sorbinil in rats, was further metabolized to IHFH, the major metabolite in man (R. Ronfeld, personal communication). The auto-induction of sorbinil 2-hydroxylation conforms with the observation that the drug (150 mg/kg) is a weak inducer of *p*-chloroanisole demethylation in the rat (R. Ronfeld, personal communication). 2HSB also yielded small amounts of IHFH *in vitro* when incubated with a mild reducing agent. These reactions can be rationalized in terms of 2HSB existing in an open-chain aldehyde form as well as the benzopyran form (Fig. 3). The aldehyde would be expected to undergo enzymic reduction to IHFH *in vivo*: the aldehyde and carbonyl reductases of human liver, of which alcohol dehydrogenase appears to be the major enzyme, reduce a broad range of xenobiotic carbonyl compounds [33]. Similar enzymes have been found in animals [34]. The formation of considerable amounts of IHFH is notable in view of the finding that sorbinil inhibits NADPH-dependent aldehyde reductase uncompetitively at low concentrations and non-competitively at high concentrations [35]. Generation of even small amounts of IHFH by sodium cyanoborohydride *in vitro* was unexpected because this reagent is reported to reduce Schiff bases, but not aldehydes, at neutral pH [18, 28]. However, the

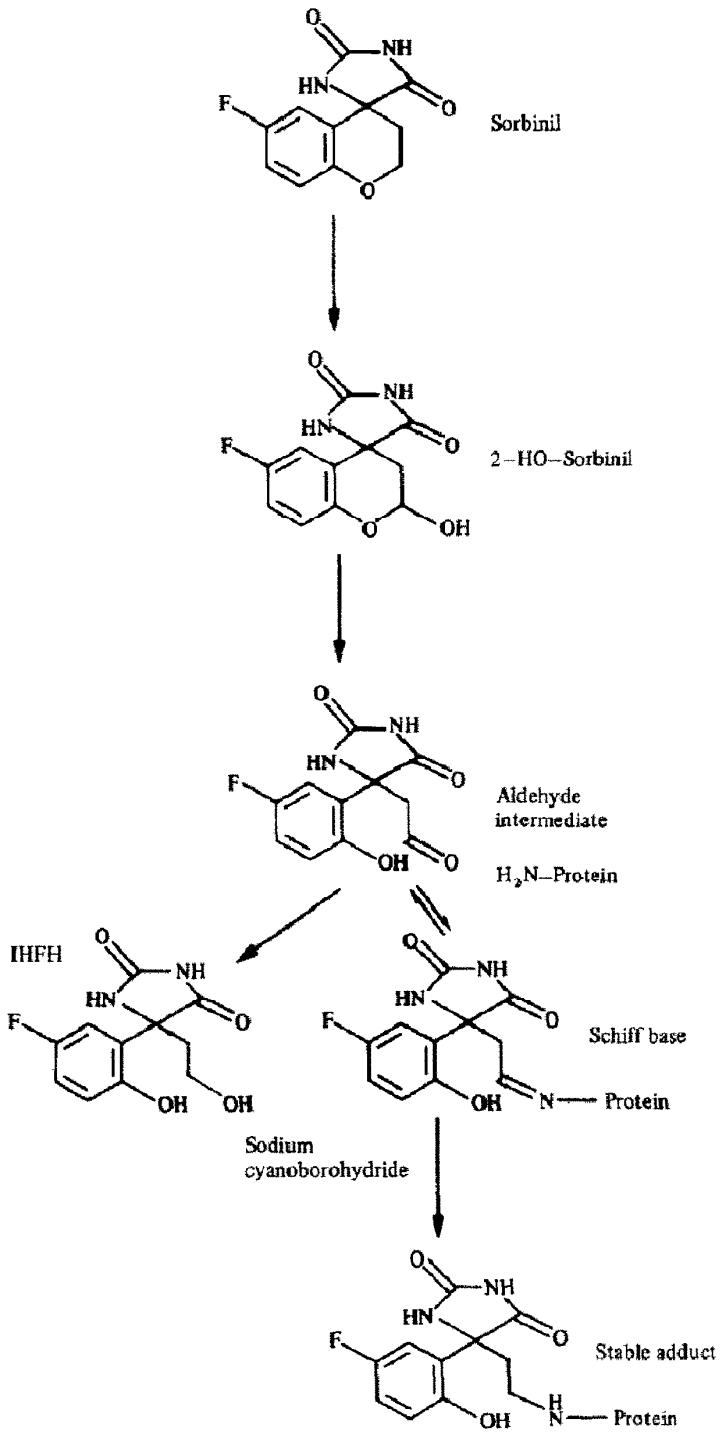

Fig. 3. Proposed metabolic and chemical scheme for the formation and stabilization of 2HSB-protein conjugates.

reaction may be attributable to the protracted incubation time.

The enhancement of irreversible binding of [^{3}H]2HSB to HSA by sodium cyanoborohydride indicated that the linkage was a Schiff base stabilized by reduction. A study of reductive methylation of proteins using this reagent [18] showed it to be highly specific: only the C-6 amino groups of lysyl residues and the C-1 amino terminus were modified by formaldehyde. Reductive alkylation of lysyl residues by aromatic aldehydes has also been demonstrated [36]. The lack of binding of 2HSB to microsomes might have been due to an absence of sufficiently reactive residues; in contrast, albumins have several reactive lysine moieties [19]. The reversible protein adducts formed by acetaldehyde [16, 19] and 16α-hydroxy-

estrone [17, 37] *in vitro* are conjugated by Schiff bases. In the case of acetaldehyde the stable adducts remain to be identified but are reported to involve lysyl residues [16]. Glucocorticoids such as cortisol and prednisolone also form stable adducts but their incorporation is not enhanced by cyanoborohydride [17] because their Schiff bases are not reduced [37]. Carbonyl compounds containing additional keto or hydroxyl groups, e.g. 16-hydroxyestrone and cortisol, may yield stable protein conjugates via rearrangements of Schiff bases [37].

Low levels of covalent modification of proteins, such as those seen with 2HSB under reducing conditions, may still have functional and immunological consequences. Thus the extent of enzyme inhibition by acetaldehyde depends upon the catalytic role of the modified lysyl residues rather than the degree of modification [16]. Chronic alcohol abuse in man is associated with increased titres of antibodies against acetaldehyde-protein adducts thought to arise from microsomal oxidation of ethanol [38]. The mild reducing action of sodium cyanoborohydride resembles that of the endogenous reductant ascorbic acid, which also reduces protein-linked Schiff bases *in vitro* but non-selectively [19].

In summary, we have shown that the major route of sorbinil's metabolism *in vivo* yields a metabolite, 2HSB, which forms reductively stabilized protein adducts *in vitro*. Whilst endogenous reductants might stabilize 2HSB-protein adducts, and hence generate potentially immunogenic conjugates, the absence of irreversibly bound hepatic residues 48 hr after administration of [8-^{3}H]sorbinil indicates that protein conjugation is normally a negligible route of sorbinil's metabolism *in vivo*. Consequently, a role for protein conjugation in the pathogenesis of the immunological adverse reactions of sorbinil remains to be established.

Acknowledgements—Financial support was provided by Pfizer Central Research, and we thank Dr J. Collier and Dr R. Ronfeld for their assistance. We are indebted to Professor M. L'E. Orme, Dr J. Colbert and the Royal Liverpool Hospital's renal transport team for providing human liver. Mass spectra were obtained by Dr R. J. Evershed and Mr M. C. Prescott, Department of Biochemistry.

REFERENCES

1. J. L. Maggs, N. R. Kitteringham, P. S. Grabowski and B. K. Park, *Biochem. Pharmac.* **35**, 505 (1986).
2. G. N. Wogan and N. J. Gorelick, *Environ. Health Perspect.* **62**, 5 (1985).
3. J. L. Maggs, P. S. Grabowski and B. K. Park, *Biochem. Pharmac.* **32**, 2793 (1983).
4. S. D. Nelson, *J. Med. Chem.* **25**, 753 (1982).
5. M. A. Trush, E. G. Mimnaugh and T. E. Gram, *Biochem. Pharmac.* **31**, 3335 (1982).
6. N. R. Kitteringham, J. L. Maggs, C. Lambert, J. Colbert, M. L'E. Orme and B. K. Park, *Br. J. clin. Pharmac.* **24**, 274 (1987).
7. J. A. Hinson, in *Reviews in Biochemical Toxicology* (Eds. E. Hodgson, J. R. Bend and R. M. Philpot), Vol. 2, pp. 103–129. Elsevier, New York (1980).
8. M. R. Boyd, *CRC Crit. Rev. Toxicol.* **8**, 103 (1980).
9. M. B. Genter, G. Szakál-Quin, C. W. Anderson, D. C. Anthony and D. G. Graham, *Toxic. appl. Pharmac.* **87**, 351 (1987).
10. A. J. Streeter, S. M. Bjorge, D. B. Axworthy, S. D. Nelson and T. A. Baillie, *Drug. Metab. Dispos.* **12**, 565 (1984).
11. A. J. Streeter and T. A. Baillie, *Biochem. Pharmac.* **34**, 2871 (1985).
12. B. K. Park, J. W. Coleman and N. R. Kitteringham, *Biochem. Pharmac.* **36**, 581 (1987).
13. N. R. Kitteringham, J. L. Maggs, S. Newby and B. K. Park, *Biochem. Pharmac.* **34**, 1763 (1985).
14. J. L. Maggs, N. R. Kitteringham and B. K. Park, *Drug. Metab. Dispos.* **14**, 142 (1986).
15. L. J. McGirr, M. Hadley and H. H. Draper, *J. biol. Chem.* **260**, 15427 (1985).
16. T. J. Mauch, T. M. Donohue, R. K. Zetterman, M. F. Sorrell and D. J. Tuma, *Hepatology* **6**, 263 (1986).
17. R. Bucala, J. Fishman and A. Cerami, *Proc. natn. Acad. Sci. U.S.A.* **79**, 3320 (1982).
18. N. Jentoft and D. G. Dearborn, *J. biol. Chem.* **254**, 4359 (1979).
19. D. J. Tuma, T. H. Donohue, V. A. Medina and M. F. Sorrell, *Archs Biochem. Biophys.* **234**, 377 (1984).
20. R. Sarges and M. J. Peterson, *Metabolism* **35**, Suppl. 1, 101 (1986).
21. J. Jaspan, *Drugs* **32**, Suppl. 2, 23 (1986).
22. J. L. Maggs and B. K. Park, *Br. J. Pharmac.* **89**, 851P (1986).
23. J. L. Maggs, P. S. Grabowski and B. K. Park, *J. Steroid Biochem.* **19**, 1273 (1983).
24. H. S. Purba, J. L. Maggs, M. L'E. Orme, D. J. Back and B. K. Park, *Br. J. clin. Pharmac.* **23**, 447 (1987).
25. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
26. R. A. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
27. J. L. Maggs, P. S. Grabowski and B. K. Park, *Xenobiotica* **12**, 657 (1982).
28. R. F. Borch, M. D. Bernstein and H. D. Durst, *J. Am. chem. Soc.* **93**, 2897 (1971).
29. R. A. Corral, O. Orazi, A. M. Duffield and C. Djerassi, *Org. Mass. Spectrom.* **5**, 551 (1971).
30. K. K. Midha, K. W. Hindmarsh, I. J. McGilveray and J. K. Cooper, *J. Pharm. Sci.* **66**, 1596 (1977).
31. A. R. Boobis, C. E. Hampden, S. Murray, P. Beaune and D. S. Davies, *Br. J. clin. Pharmac.* **19**, 721 (1985).
32. P. H. Beaune, P. G. Kremers, L. S. Kaminsky, J. De Graeve, A. Albert and F. P. Guengerich, *Drug Metab. Dispos.* **14**, 437 (1986).
33. T. Nakayama, A. Hara, K. Yashiro and H. Sawada, *Biochem. Pharmac.* **34**, 107 (1985).
34. R. L. Felstad and N. R. Bachur, *Drug Metab. Rev.* **11**, 1 (1980).
35. K. S. De Jongh, P. J. Schofield and M. R. Edwards, *Biochem. J.* **242**, 143 (1987).
36. M. Friedman, L. D. Willams and M. S. Masri, *Int. J. Peptide Protein Res.* **6**, 183 (1974).
37. R. Bucala, P. C. Ulrich, B. T. Chait, F. A. Bencsath and A. Cerami, *J. Steroid Biochem.* **25**, 127 (1986).
38. M. Hoerner, U. J. Behrens, T. Worner and C. S. Lieber, *Res. Commun. Chem. Path. Pharmac.* **54**, 3 (1986).