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EFFECT OF THIOACETAMIDE-INDUCED HEPATIC NECROSIS ON THE REGIOSELECTIVE METABOLISM OF *S*-WARFARIN BY RAT LIVER MIXED-FUNCTION OXIDASE ENZYMES

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Abstract—The biotransformation of *S*-warfarin was examined using liver microsomes prepared from rats 6–96 hr after treatment with a necrotizing dose (5.6 mmoles/kg) of thioacetamide. Four catalytically distinct classes of enzyme activity were observed which declined in activity with different half-lives after thioacetamide intoxication. *S*-Warfarin 7-hydroxylase activity was destroyed with a half-life of 16.6 ± 3.1 hr. 6-Hydroxylase activity was destroyed with a half-life of 25.3 ± 3.0 hr. 4′-Hydroxylase activity was destroyed with a half-life of 34.6 ± 4.8 hr, which paralleled the loss of total hepatic cytochrome P-450 with a half-life of 33.4 ± 3.6 hr. Production of an unidentified metabolite was not affected by thioacetamide intoxication during the first 48 hr. The ratio of rates of product formation were used as an alternative method to test the homogeneity of distinct enzyme catalytic activities. The ratio of measured responses (e.g. chromatographic peak heights) was used directly to determine the product ratios, provided that the rate of formation of each product was directly proportional to the experimentally measured response for each product. The use of product response ratios to discriminate between catalytic activities was inherently more precise because calibration errors were eliminated. Differences in the rates of destruction of warfarin hydroxylases provided further evidence of the multiplicity of hepatic mixed-function oxidases and suggested topographical differences in their location within the liver lobule.

Warfarin (**1**) is metabolized by liver microsomal mixed-function oxidases, which contain cytochrome P-450. The principal oxidative metabolites (Scheme 1) are 4′-hydroxywarfarin (**2**), 6-hydroxywarfarin (**3**), 7-hydroxywarfarin (**4**), 8-hydroxywarfarin (**5**), benzylic hydroxywarfarin (**6**), and dehydrowarfarin (**7**) [1, 2]. The *R*-isomer is metabolized to a greater extent than the *S*-isomer [3]. Effects of different chemical inducers on the metabolism of warfarin are regio- and stereoselective [3]. It has been suggested that this phenomenon may be useful as a probe of cytochrome P-450 isoenzymes [3–6]. In these previous studies the enzyme system was perturbed by inducers and inhibitors. We have employed a different perturbation method, namely selective destruction of the liver cells by a chemical agent, thioacetamide. This compound is known to cause centrilobular necrosis [7, 8]. Liver regeneration has been reported to begin 36 hr after treating rats with 50 mg thioacetamide/kg body weight [9]. Thus, both liver cell death and regeneration phases can be studied.

It has been suggested [10–12] that within the hepatic lobule the distributions of individual cytological substructures and enzymes vary with distance from the central vein. Some chemical agents destroy cells selectively [13] within the liver lobule. Thioacetamide [7, 8], carbon tetrachloride [7] and thiobenzamide [14] are known to cause centrilobular necrosis. On the other hand, allyl alcohol [13, 15] or its esters [16] cause periportal necrosis. Furosemide and its analogues, 2-hydroxymethylfuran and 2-acetylfuran [17, 18], normally cause centrilobular necrosis, but pretreatment with phenobarbital shifts the zone of necrosis from the centrilobular to midzonal region.

Warfarin, a single substrate, is metabolized to multiple products by cytochrome P-450 isoenzymes. The relative proportions of these isoenzymes are dependent on sex, age, nutrition, and induction condition [19–21]. Catalytically distinct isoenzymes can be characterized by measuring kinetic parameters (K_M, V_{max}) for selected substrates using isolated enzyme preparations. However, when isoenzymes are present in a mixture, such as cytochrome P-450 isoenzymes in crude microsomal preparations, conventional kinetic characterization is hampered if a substrate is acted upon by more than one isoenzyme. Substrates which yield multiple products are especially useful in this case, since kinetic differences in binding to isoenzymes are reflected in changes in the ratios of products formed [22]. This is true whether a single isoenzyme is responsible for forming only one product or many products [23]. Thus, if two products are formed by a single isoenzyme, the ratio of the rates of formation of the products will be a constant even though the concentration of either the substrate or the isoenzyme is varied. Inversely, if the ratio of the rates of formation of two products obtained from a single substrate is not constant when either the substrate or enzyme concentration is varied, then it is not possible for the two products to

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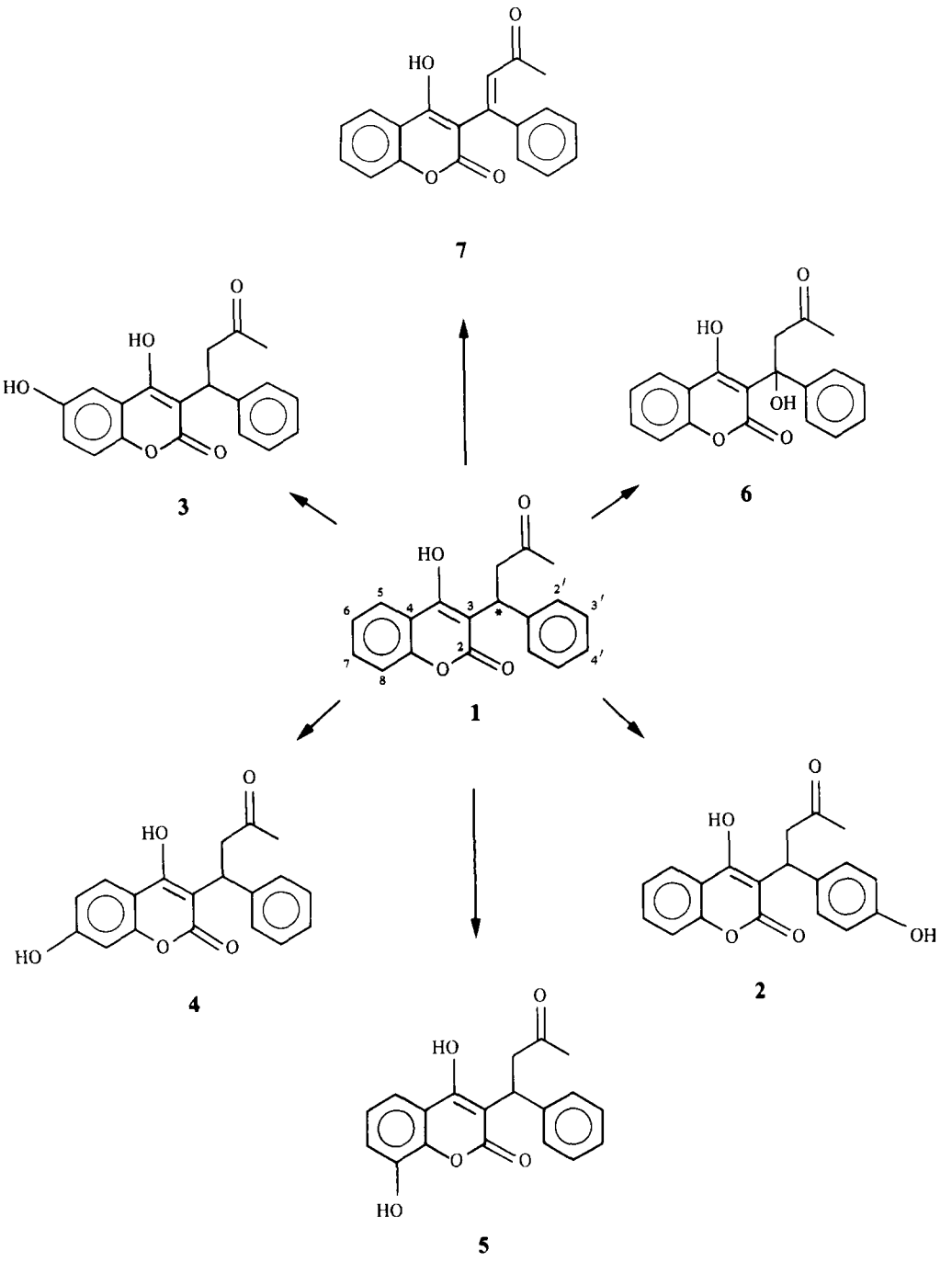

Scheme 1.

have been formed by a single enzyme. In this case either one product is formed by one enzyme and the second product is formed by another enzyme, or each product is formed to a different extent by two or more enzymes, or some other complex relation involving at least two enzymes exists. Thus, measurement of the ratios of the rates of formation of pairs of products from a single substrate can yield useful information about the multiplicity of an enzyme system if either the substrate concentration is varied or the concentration of enzyme active sites is perturbed.

Traditional perturbers of enzyme activity can be classified into two types. Type-1 perturbers alter the concentration of enzyme active sites. These include agents which increase the rate of enzyme biosynthesis (inducers), agents which bind to the enzyme and render it catalytically inactive (competitive and non-competitive inhibitors), and agents which increase the rate of enzyme degradation or decrease the rate of enzyme biosynthesis (general metabolic poisons or inducers of catabolizing enzymes). Changes in the composition of isoenzyme systems caused by Type-

1 perturbers may be reflected in changes in the ratios of products formed from substrates which yield multiple products, although product ratios might coincidentally remain the same. However, if product ratios do change after subjecting the system to the effects of a Type-1 perturber, then this change is positive proof that more than one enzyme is involved in the synthesis of the product pair whose ratio was altered. Type-2 perturbers, on the other hand, temporarily or permanently alter the enzyme active site or change individual rate constants. These include agents which allosterically modify enzyme properties, changes in pH, changes in reaction temperature, changes in ionic strength, or changes in the rate-limiting step in the catalytic cycle due to depletion of essential cofactors. Because the relation of product ratios to Type-2 perturbers is completely unpredictable, these yield no useful information.

Selective intoxication of hepatic tissue is expected to alter the balance between cytochrome P-450 synthesis and catabolism in liver cells differently, depending on the severity of cellular intoxication. Selective hepatotoxicants such as thioacetamide [7, 8], therefore, may be classed at Type-1 perturbers of hepatic cytochrome P-450 mixed-function oxidases, if the whole liver is considered as the source of the enzymes being studied. Determination of the relative rates of formation of warfarin metabolites by microsomal enzymes prepared from partially intoxicated livers obtained after treatment *in vivo* with thioacetamide may be useful in studying the multiplicity of cytochrome P-450 in the liver considered as a whole. Additionally, comparison of the absolute rates of formation of warfarin metabolites with the average tissue concentration of cytochrome P-450 and the extent of hepatic damage may provide some insight into the distribution of warfarin-metabolizing enzymes within the liver lobule.

MATERIALS AND METHODS

Materials. All chemical reagents and solvents were of the highest commercial grade, obtained from various sources. NADP, succinic acid dehydrogenase and DL-isocitric acid trisodium salt type 1 were obtained from the Sigma Chemical Co. (St. Louis, MO). *S*-Warfarin, warfarin alcohols, and authentic 4′-, 6-, 7- and 8-hydroxywarfarin were obtained as previously described [1]. Dehydrowarfarin was the gift of Dr. L. S. Kaminsky. 3′-Hydroxywarfarin and 5-hydroxywarfarin were prepared in this laboratory [24].

Tissue preparation. Adult male Sprague–Dawley rats (270–310 g, Harlan Sprague Dawley, Madison, WI) were given food and water *ad lib*. and housed in stainless steel cages exposed to a 12-hr diurnal light cycle. The animals were treated with thioacetamide (5.6 mmoles/kg) and killed at 0, 6, 12, 24, 48 and 96 hr after treatment. The animals were fasted 18 hr prior to sacrifice. Treatments were timed so that all animals in an experimental replicate were killed within a 30-min interval on the same day. The experiment was replicated three times. Liver microsomes were isolated from each liver separately as previously described [25]. Microsomal pellets were resuspended in 80% 0.05 M Tris, 1 mM EDTA solution (pH 7.4): 20% glycerol (v/v) at a concentration of 0.4 ml resuspension fluid/g liver tissue and stored at −80° until required.

Cytochrome P-450 and protein determinations. The cytochrome P-450 concentration of microsomal suspensions was determined by the CO difference spectrum of dithionite-reduced microsomes as described by Omura and Sato [26]. A 0.1 ml aliquot of microsomal suspension was analyzed for protein by the method of Lowry *et al.* [27].

Incubations. A solution containing 2.00 mg (6.49 μmoles) *S*-warfarin, 2.03 mg $MgCl_2 \cdot 6H_2O$, 3.83 mg NADP disodium salt, 4.56 mg isocitric acid, and 2 units of isocitrate dehydrogenase in 4.00 ml of 25 mM phosphate buffer (pH 7.4), containing 87.5 mM KCl, was incubated at 37° for 2 min in a 25-ml Erlenmeyer flask. The flask was shaken at 90–120 oscillations/min exposed to air. The microsomal suspension was diluted with 0.01 M phosphate buffered isotonic KCl solution (pH 7.4) to give a final protein concentration of 5.00 mg/ml. One ml was added to the mixed cofactors and substrate and incubated with continued shaking for 10 min. Acetone (3.0 ml) was added, and the incubation was continued for 2 min. The mixture was transferred to a 20 ml culture tube with a Teflon-lined screw cap. Then 0.5 M KH_2PO_4 (1.0 ml) was added, along with 0.4 ml of a solution containing 7.5 μg/ml 3′-hydroxywarfarin (internal standard) in 70% 44 mM potassium phosphate buffer (pH 7.4):30% acetonitrile (v/v). The mixture was vortexed and then centrifuged. The supernatant fraction was decanted and extracted with three 9.0-ml portions of cyclohexane to remove lipids and most of the excess warfarin. The aqueous phase was then extracted twice with 4.0 ml of 50% diethyl ether:50% ethyl acetate (v/v). The combined organic extracts were evaporated under N_2. The residue was redissolved in 0.4 ml of 70% 44 mM potassium phosphate buffer (pH 7.4):30% acetonitrile (v/v).

High pressure liquid chromatographic assay. The metabolite residue solutions (100 μl) were separated on a C-18 ion-pair column (Ultrasphere-IP, 4.6 × 250 mm, Beckman Instruments) using a mobile phase containing 70% 44 mM phosphate buffer (pH 7.4):30% acetonitrile to which was added 1.2 g/l cetyltrimethylammonium bromide. Warfarin and its metabolites were detected fluorometrically using a Kratos FS 950 detector equipped with a Hg lamp and 313 nm excitation filter used with a 340 nm cutoff emission filter. A linear calibration curve was obtained over the range 0–6 μg/ml for 4′- and 7-hydroxywarfarin and over the range 0–4 μg/ml for 6- and 8-hydroxywarfarin. Typical samples contained 0.1 to 3 μg/ml metabolites. Details of the assay procedure will be published elsewhere.

Statistical analysis. Peak height ratios were interpolated from calibration curves fitted by least-squares linear regression. Duplicate analyses for each animal tested were averaged before further analysis. Metabolite concentration/protein concentration ratios, metabolite peak height ratios and cytochrome P-450 concentration/protein concentration ratios for three separate experiments were compared by analysis of variance. It was necessary to use a logarithmic transformation of the data to

achieve homogeneity of variance. Treatment means were compared to control (time = zero) values by Tukey's test [28]. Half-lives were determined from least-squares linear regression analysis of the transformed data. All means are expressed ± standard errors.

RESULTS

Treatment with thioacetamide had no significant effect on liver weight as a fraction of body weight or on hepatic microsomal protein concentration.

Liver weights averaged 3.88 ± 0.09% (N = 15) of body weight. Microsomal protein concentrations averaged 3.8 ± 0.1 mg/g liver (N = 15).

Thioacetamide treatment caused a significant decrease in hepatic cytochrome P-450 content (Fig. 1). Loss of cytochrome P-450 began shortly after treatment and declined by an apparent first-order process to about 40% of its initial value with a half-life of 33.4 ± 3.6 hr. A minimum was reached 48–96 hr after treatment. In previous studies [29, 30], thioacetamide treatments were all begun simultaneously. The decline in cytochrome P-450 after thioacetamide (5 mmoles/kg) treatment previously observed [29] was partially masked by diurnal variation in hepatic cytochrome P-450 levels. It should be noted that in the present study all animals were killed within a 30-min interval, so that changes in cytochrome P-450 concentration are the result of thioacetamide intoxication and are not due to diurnal variations.

Four metabolites of *S*-warfarin (**1**) were identified by liquid chromatography. These included 4'-hydroxywarfarin (**2**), 6-hydroxywarfarin (**3**), 7-hydroxywarfarin (**4**) and small but unquantifiable amounts of 8-hydroxywarfarin (**5**). In addition, an unidentified metabolite was observed. This substance was intermediate in polarity between warfarin and the aromatic monohydroxylated metabolites. It did not comigrate with 3'-hydroxywarfarin, 5-hydroxywarfarin, either of the diastereomeric warfarin alcohols (formed by reduction of the side chain ketone group) [1], or with dehydrowarfarin (**7**) [2]. It may be benzylic hydroxywarfarin (**6**). Because no authentic standard for this metabolite was available, it was impossible to quantify exactly. Because its concentration could not be measured directly, it is reported as 4'-hydroxywarfarin equivalents, where one equivalent of unidentified metabolite has the same fluorescence intensity as a mole of 4'-hydroxywarfarin.

All of the warfarin-metabolizing activities of rat liver microsomes were affected by thioacetamide intoxication. Although comparisons between the activities remaining at individual times after treatment with thioacetamide did not demonstrate significant decreases from control (time = zero) values until 48–96 hr after treatment, nevertheless the data considered as a whole for each identified metabolite were negatively linearly correlated during the period 0–48 or 0–96 hr when the logarithm of the metabolic rate was plotted versus time after treatment. Consequently, the data were fitted to first-order pharmacokinetic models. The rate of 7-hydroxylation declined rapidly, with a half-life of 16.6 ± 3.1 hr ($r = -0.864$), to about 15% of its initial level during the 96-hr period studied (Fig. 2). The rate of 6-hydroxylation declined less rapidly, with a half-life of 25.3 ± 3.0 hr ($r = -0.919$). After 96 hr less than 10% of the initial 6-hydroxylase activity remained. The rate of 4'-hydroxylation declined at the same rate as the overall rate of loss of cytochrome P-450, with a half-life of 34.6 ± 4.8 hr ($r = -0.898$). At 96 hr after thioacetamide treatment, only 15% of the initial 4'-hydroxylase activity remained. The metabolic production of the unidentified metabolite was unaffected by thioacetamide intoxication for the first 48 hr after treatment; it then declined to about 30% of its initial level of activity by 96 hr.

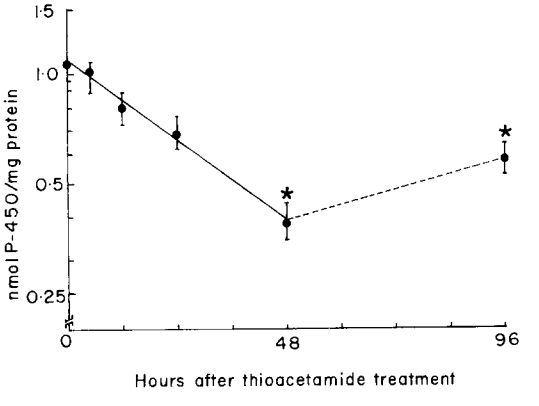

Fig. 1. Cytochrome P-450 concentration (nmoles/mg liver microsomal protein) after intraperitoneal administration of 5.6 mmoles/kg thioacetamide to Sprague–Dawley male rats. Each point represents the mean for three rat livers ± S.E. The fitted first-order kinetic model is indicated by a solid line (——); the broken line (---) indicates non-modeled behavior. An asterisk (*) indicates a significant difference between the average response at the time indicated and the untreated control group (time = zero) when each treatment time is considered individually.

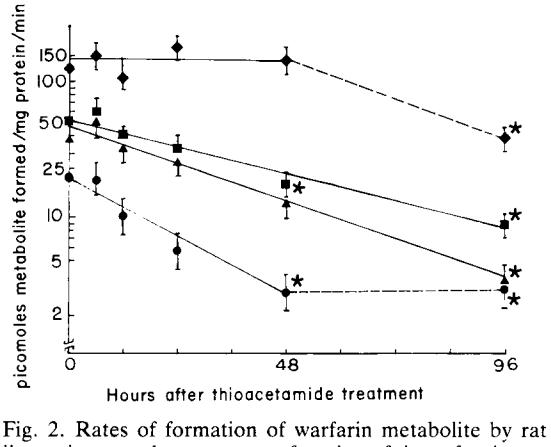

Fig. 2. Rates of formation of warfarin metabolite by rat liver microsomal enzymes as a function of time after intoxication by thioacetamide (5.6 mmoles/kg). Key: (●) 7-hydroxywarfarin; (▲) 6-hydroxywarfarin; (■) 4'-hydroxywarfarin; and (◆) the unidentified metabolite. Values plotted represent the mean (± S.E.) rate of formation for three rat livers at each time point in pmoles per mg of microsomal protein per min except for the unidentified metabolite, which is reported as picoequivalents of fluorescence intensity equal to one mole of 4'-hydroxywarfarin. Other plotting symbols are as defined in Fig. 1.

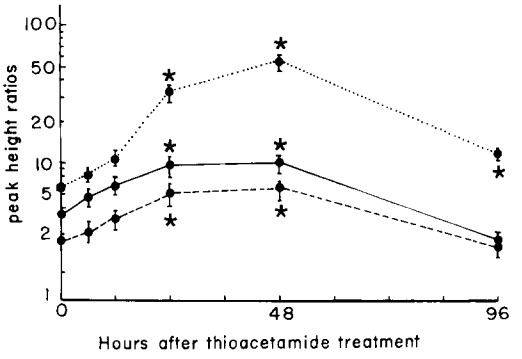

Fig. 3. Chromatographic peak height ratios obtained from extracts of incubations of *S*-warfarin with liver microsomal enzymes prepared from rats treated with thioacetamide (5.6 mmoles/kg). The solid line (——) represents the ratio of 6-hydroxywarfarin to 7-hydroxywarfarin, the dashed line (– – –) the ratio of 4′-hydroxywarfarin to 7-hydroxywarfarin, and the dotted line (· · · ·) the ratio of the unidentified metabolite to 7-hydroxywarfarin. Asterisks (*) indicate the times after treatment for which the average peak height ratio was significantly different from untreated controls (time = zero). Each point represents the mean ratio (± S.E.) for three rat livers.

Since chromatographic peak height ratios were directly proportional to the metabolite concentrations for 4′-, 6- and 7-hydroxywarfarin, and are assumed to be directly proportional for the unidentified metabolite, the peak height ratios for each pair of metabolites were compared (Figs. 3 and 4). In every case, statistical analysis confirmed that the ratios were different from the ratios obtained in untreated control preparations for at least one time point after thioacetamide intoxication. The rates of formation of 4′- and 6-hydroxywarfarin and the unidentified metabolite became proportionally higher than the rate of 7-hydroxylation as the intoxication progressed through the first 48 hr, confirming that the half-life for loss of 7-hydroxylase activity was significantly shorter than the half-lives for loss of the other catalytic activities (Fig. 3). Similarly, a slight but significant relative increase in 4′-hydroxylase activity compared to 6-hydroxylase activity was observed (Fig. 4), confirming that the half-life for loss of 6-hydroxylase activity was significantly shorter than the half-life for loss of 4′-hydroxylase activity. The large relative increase in the amounts of the unidentified metabolite reflects the fact that the enzyme system producing this compound remains essentially unaffected by thioacetamide intoxication during the first 48 hr.

DISCUSSION

We have shown previously [22, 23] that a single enzyme that metabolizes a substrate to multiple products will always yield the same ratio of products independent of both enzyme and substrate concentration; changes in product ratios after perturbing the system in ways which do not alter the active sites of enzymes are indicative that more than one enzyme is involved.

Enzyme concentrations can be affected by agents which either increase or decrease their rate of biosynthesis or by agents which either increase or decrease their rate of degradation. Previous studies [3, 31] have shown that catalytically different hepatic mixed-function oxidase enzymes can be selectively induced by a number of agents, as measured by changes in the stereoselectively and regioselectivity of warfarin metabolism. All of the warfarin hydroxylase activities measured required the participation of cytochrome P-450, as shown by studies using inhibitors [31, 32] and purified cytochromes [5, 6].

Agents which cause destruction of enzymes should be equally useful in classifying related enzymes based on differences in their catalytic activity. However, partial destruction may accomplish allosteric modification of the enzyme, altering its enzyme activity without destroying it completely. Such alteration might occur as the result of chemical modification of the protein. Although thioacetamide metabolites covalently bind to liver proteins [33] with formation of acetimidolysine derivatives [34], the catalytic activity of microsomal cytochrome P-450 towards benzphetamine demethylation is unaffected *in vitro**. Furthermore, all of the covalent modification of liver proteins occurs within the first 6 hr following thioacetamide administration [30]. Allosteric modification of cytochrome P-450 enzymes does not seem to explain the observed differences in the rates of destruction of warfarin hydroxylase activities because allosteric modification, if it occurs, must be

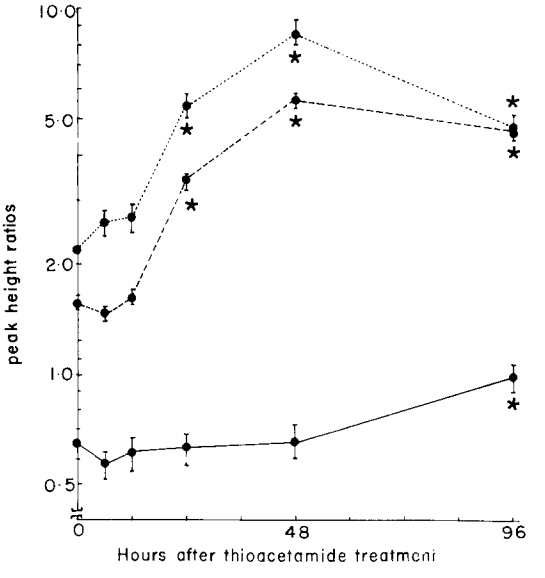

Fig. 4. Chromatographic peak height ratios obtained as described in Fig. 3. The solid line (——) represented the ratio of 4′-hydroxywarfarin to 6-hydroxywarfarin; the dashed line (– – –) and the dotted line (· · · ·) represent the ratio of the unidentified metabolite to 6-hydroxywarfarin and to 4′-hydroxywarfarin respectively. Each point represents the mean (± S.E.) for three rat livers. Asterisks (*) indicate the times after treatment for which the average peak height ratio was significantly different from untreated controls (time = zero).

* W. R. Porter and R. A. Neal, unpublished data.

complete within 6 hr. The observed changes in warfarin hydroxylase activity must therefore reflect progressive declines in total liver tissue concentrations of active enzymes which retain their respective catalytic activities unaltered by thioacetamide treatment. Thus, changes in warfarin metabolite ratios indicate that at least two different cytochrome P-450 species are involved in warfarin metabolism.

Kaminsky *et al.* [4] have shown that two cytochrome P-450 isoenzymes purified from uninduced rat liver microsomes are capable of forming substantial amounts of 7-, 6- and 4′-hydroxylated metabolites from *S*-warfarin (P-450 UT-A and P-450 UT-C). P-450 UT-C was found to be regioselective for 7-hydroxylation; both the 4′- and 6-hydroxylated products were formed at a rate equal to 40% of the rate of 7-hydroxylation, whereas other products, such as benzylic hydroxywarfarin, 8-hydroxywarfarin and dehydrowarfarin, were formed in much smaller amounts from *S*-warfarin by this partially purified isoenzyme. An isoenzyme with similar catalytic activity, P-450 PB-C, was isolated from phenobarbital-induced rat liver microsomes, except that its regioselectivity for 7-hydroxylation of *S*-warfarin was slightly more pronounced. However, since these particular preparations were not homogenous, it is possible that the major isoenzyme present in preparations UT-C and PB-C is predominantly an *S*-warfarin 7-hydroxylase, and that the other products detected result from contamination with other P-450 isoenzymes. Additionally, an impure isoenzyme preparation from phenobarbital-induced rat liver, P-450 PB-A, had a similar spectrum of catalytic activity as the impure P-450 UT-A preparation.

The major phenobarbital-inducible form of cytochrome P-450, purified to apparent homogeneity, predominately catalyzes the 4′- and 6-hydroxylation of *S*-warfarin [4]. It is present in uninduced rat liver, as judged by immunohistochemical studies [35]. Furthermore, it is nonuniformly distributed in the hepatic lobule, with the highest concentration appearing in cells of the centrilobular region, with declining levels in cells further removed from the central vein. Similarly, the major 3-methylcholanthrene-inducible form of cytochrome P-450, purified to apparent homogeneity, exclusively catalyzes the formation of 6-, 8- and small amounts of 7-hydroxylated metabolites from *S*-warfarin [4]. It is also present in uninduced rat liver, as judged by immunohistochemical studies [35]. It is also nonuniformly distributed on the hepatic lobule, but periportal and midzonal cells contain essentially the same amount of this isoenzyme, although still slightly less than the amount found in centrilobular hepatocytes.

The observed metabolic fate of *S*-warfarin upon incubation with microsomes obtained from thioacetamide-intoxicated rat liver suggests that several of the various cytochrome P-450 isoenzymes previously purified from this rat strain are nonuniformly distributed in the hepatic lobule. The loss of 7-hydroxylase activity (mediated by P-450 UT-C primarily) exactly parallels the previously reported [29, 30] development of centrilobular necrosis, suggesting that this isoenzyme is primarily confined to the cells surrounding the central vein. The 4′- and 6-hydroxylase activity, mostly associated with the P-450 UT-A and PB-B isoenzymes, decayed more slowly following thioacetamide treatment, suggesting that a significant portion of this enzyme activity is located on hepatocytes farther from the central vein, in agreement with immunohistochemical data for P-450 PB-B [35]. The enzyme responsible for the unidentified metabolite has not been characterized previously, but is hardly affected by thioacetamide treatment until tissue regeneration begins (after 48 hr). This suggests that this enzymic activity is localized in the periportal region; the decline in activity noted during the regeneration phase may be due to commitment of tissue protein precursor reserves to new cell growth.

The direct comparison of product ratios, as measured by chromatographic peak height ratios, provides important diagnostic information. Provided that the measured experimental response (i.e. chromatographic peak height) is directly proportional to metabolite concentration (which must be established using calibration curves), the ratio of measured responses for two metabolites will equal the ratio of rates of product formation. The direct comparison of response ratios has the advantage that errors introduced by the use of calibration curves are eliminated.

Examination of Figs. 3 and 4 shows the magnitude of the change in product ratios. For example, 24–48 hr after thioacetamide treatment, the remaining hepatic mixed-function oxidase activity was five to ten times more regioselective for producing the unidentified metabolite than 7-hydroxywarfarin compared to untreated controls. During this same time period, the regioselectivity of the remaining 4′- and 6-hydroxylases compared to 7-hydroxylase activity was doubled compared to untreated controls. Thioacetamide intoxication had a less pronounced effect on 4′- and 6-hydroxylases compared to each other, with the maximum change in regioselectivity occurring at 96 hr, at which time both enzyme activities had been mostly destroyed. However, formation of the unidentified metabolite was enhanced over 4′- and 6-hydroxylase activity about 4-fold 48 hr after thioacetamide treatment when compared to untreated controls.

In conclusion, agents which promote enzyme catabolism can be just as useful as agents which selectively induce enzyme biosynthesis in studies of enzyme multiplicity. Mixed-function oxidase catalyzed metabolism of *S*-warfarin, as measured by the rates of 4′-, 6- and 7-hydroxylation, declined by first-order kinetic processes after poisoning the liver with thioacetamide. The regioselective activities declined at different rates. 7-Hydroxylase activity was lost most rapidly and was clearly separate from the other catalytic activities. Both 4′- and 6-hydroxylase activities were lost at about the same rate as the average rate of destruction of cytochrome P-450, although small differences were observed. Formation of the unidentified metabolite was unaffected by thioacetamide poisoning during the first 48 hr after treatment and declined only during the period in which tissue regeneration was beginning.

The direct examination of product ratios as a function of time after thioacetamide treatment confirms

the conclusions derived from a pharmacokinetic interpretation. More precise estimates of the changes in catalytic activity can be obtained since calibration errors are eliminated.

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