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ACKNOWLEDGMENTS

The authors thank P. Egli for the radioactive compounds used in the binding studies, T. McCormick for the MS data, and H. Roberts for stability data.

Pharmacokinetics of Pentobarbital, Quinidine, Lidocaine, and Theophylline in the Thermally Injured Rat

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Received February 2, 1983, from the *Department of Pharmacology, Hahnemann Medical College and Hospital, Philadelphia, PA 19102.* Accepted for publication August 4, 1983.

Abstract □ Previous studies have shown that rats with 15% third-degree burns show a severe depression in various *in vitro* hepatic drug-metabolizing enzymes. This effect was assessed *in vivo* by measuring the disposition of four liver-metabolized drugs in 16% third-degree burned rats at 7 d postburn. Compared with pair-fed control rats, pentobarbital demonstrated a significantly prolonged clearance and elimination half-life without a change in volume of distribution. Quinidine demonstrated a significantly increased volume of distribution and a significantly decreased clearance without a change in elimination half-life. Lidocaine showed a significantly increased volume of distribution. Theophylline, which is only 50% metabolized in the rat, showed no changes in any pharmacokinetic parameters. The free drug fractions of quinidine and lidocaine were found to be significantly decreased at 1 d postburn and normal at 7 d postburn. These results warrant pharmacokinetic studies in human burn patients.

Keyphrases □ Pentobarbital—pharmacokinetics in thermally injured rats □ Quinidine—pharmacokinetics in thermally injured rats □ Lidocaine—pharmacokinetics in thermally injured rats □ Theophylline—pharmacokinetics in thermally injured rats □ Thermal injury—phramacokinetics of pentobarbital, quindine, lidocaine, and theophylline, rats

Thermal injury can produce numerous pathophysiological alterations in an organism which may modify the absorption, distribution, metabolism, and renal excretion of therapeutic agents (1). Such changes would be directly reflected in the derived pharmacokinetic parameters for a given drug (2). Recently, our laboratory has presented evidence of a generalized depression in various *in vitro* liver drug-metabolizing enzymes in rats subjected to 15–16% third-degree burns (3–5). Also, this phenomenon may occur in human thermal injury, as demonstrated by significantly decreased levels of D-glucaric acid in human burn victims (6). However, such data on drug metabolism in which *in vitro* liver homogenates and D-glucaric acid are used may not reflect the pharmacokinetic characteristics of a drug in an intact organism. Besides the activity of hepatic microsomal drug-metabolizing enzymes, other factors such as alterations in hepatic blood flow and changes in drug binding by plasma proteins may significantly contribute to the overall process of hepatic drug clearance (7). Thus, the present study was undertaken in a rodent model with a 16% third-degree burn to assess the effects of thermal injury on the *in vivo* disposition of four liver-metabolized drugs commonly used for burn patients.

EXPERIMENTAL SECTION

Animals—Adult male Sprague-Dawley rats[1] (weight, 220–250 g) received 16% full-thickness burns as described below. They were housed in plastic cages in groups of one or two over corncob bedding in a room maintained at 24 ± 0.5°C with a constant 12-h light and dark cycle. Pharmacokinetic experiments were done 7 d after the burning. Control rats housed alone were carefully pair-fed[2] daily with the burned rats to adjust for the slight anorexic effect of the burn wound.

Burn Procedure—A full-thickness burn was produced on the rats by the method of Arturson (8). This type of burn completely destroys all dermal appendages and epithelial elements, including sensory nerve endings, resulting in a virtually pain-free preparation (9). The animals were anesthetized with sodium pentobarbital (50 mg/kg) and shaved with electric clippers. Each rat was placed in a plastic mold with a hole cut out that corresponded to ~16% of the total body surface area. This value was arrived at by skinning burned rats and measuring the area of the burn and the total body area by tracing these areas onto graph paper and counting the squares. It was found that this particular mold consistently delivered an approximate 16% burn for animals in the weight range of 220–250 g. The burns were inflicted by placing the mold (containing the bare rat skin protruding through the hole) in 90 ± 1°C water for 20 s. This procedure produced a burn with sharp edges that did not impair the mobility of the animals. The animals awoke from the procedure in no apparent distress and did not require analgesics. At no time during the study did any burned animal exhibit gross signs of infection. Control animals were shaved and anesthetized but not immersed in water.

Biochemical Parameters—Estimations of the serum levels of proteins, enzymes, and other constituents were done on six 16% burned and six pair-fed control rats by sending 5 mL of serum from the pooled blood of two rats to a commercial laboratory[3]. The serum was analyzed with a standard serum multiple analyzer, with normal rat serum used as standards. Serum was ob-

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Rodent Chow; Ralston Purina Inc., St. Louis, Mo.
[3] Vet Path Veterinary Laboratories, Teterboro, N.J.

0022-3549/84/0800-1117$01.00/0

tained 7 d postburning or pair-feeding by needle puncture of the surgically exposed jugular veins in rats anesthetized with sodium pentobarbital (50 mg/kg ip).

Quinidine, Lidocaine, and Theophylline Pharmacokinetics—Both burned and pair-fed rats were anesthetized with 1.7 g/kg of urethane, an anesthetic that does not inhibit drug metabolism (10) and which has been reported to maintain hepatic blood flow at a level equal to that of an awake animal (11). The right and left jugular veins were surgically exposed by superficial skin incisions in the areas of both clavicles, and 25 mg/kg of quinidine (free base), 15 mg/kg of lidocaine hydrochloride, or 15 mg/kg of theophylline (18.75 mg/kg of aminophylline) was injected intravenously through the right jugular vein. Blood samples (0.4 mL) were taken at 0.5, 1, 2, 3, and 4 h postinjection for quinidine; at 10, 20, 30, 40, and 50 min postinjection for lidocaine; and at 1, 2, 3, 4, and 5 h postinjection for theophylline. Samples were obtained by venipuncture of the left jugular vein through the pectoris major muscle with a 1-mL heparinized tuberculin syringe.

Pentobarbital Pharmacokinetics—Both 16% burned and pair-fed rats were lightly anesthetized with ether, and their ventral tail veins were cannulated with a piece of heparinized polyethylene tubing[4]. After securing the tubing in the vein with silk ligatures, the animals were placed in restraint cages and allowed to become fully awake. The rats were kept in the restraint cages for at least 3 h before the pharmacokinetic experiment was run, since it has been shown that brief exposure to ether can inhibit drug metabolism in the immediate postexposure period (10). After this 3-h recovery period, 40 mg/kg of sodium pentobarbital was injected intravenously through the tail vein cannula. Once the rats were in deep anesthesia, they were removed from the restraint cages, and the right jugular vein was exposed as described above. Blood samples were taken at 0.5, 1.0, 1.5, 2.0, and 2.5 h postinjection by venipuncture of the jugular vein with a 1-mL heparinized tuberculin syringe. Samples ranged in volume from 0.6 to 1.0 mL and did not exceed 3.0 mL of blood before the withdrawal of the last sample. After the 2.0-h sample was taken, 1.0 mL of saline was injected into the rat through the tail vein cannula in an attempt to partially restore some of the blood volume.

Drug Analysis—Quinidine, lidocaine, and theophylline plasma concentrations were measured by commercially available enzyme immunoassay kits[5] as described previously (12). The plasma concentrations of pentobarbital were determined by a modification of the GC method of Watson and Kalman (13). A gas chromatograph[6] equipped with a hydrogen flame-ionization detector was used for this study. Glass columns (~183 cm × 4 mm i.d.), packed with 3% OV-17 Gas Chrom Q, were used under the following conditions: injection port temperature, 200°C; column temperature, 180°C; detector temperature, 215°C; carrier gas, nitrogen at a flow rate of 20 mL/min. The sensitivity settings were 2 × 1.

The extraction procedure was as follows. Into a 13-mL glass centrifuge tube with a ground glass stopper were placed 0.3–0.6 mL of unknown plasma and 100 μL of barbital standard (200 μg of barbital/mL of distilled water). Saturated potassium dihydrogen phosphate (0.6 mL) was added, and the tubes were shaken lightly. Dichloromethane (0.5 mL) was then added, the stopper was replaced, and the tube was inverted manually 40 times. The layers were separated by centrifuging at 4000 rpm for 5 min, and the protein-containing aqueous layer was removed by aspiration. The organic layer (3.0 mL) was then transferred to a second 13-mL glass centrifuge tube, and the solvent was evaporated under a stream of nitrogen in a water bath at 45–50°C. The sides of the tube were washed with 0.5 mL of dichloromethane, and the solvent was again evaporated off. Chloroform (50 μL) was added and swirled around the tube, and 2 μL of the reconstituted sample was injected into the gas chromatograph.

Equilibrium Dialysis Experiments—A modification of the method of Woo and Greenblatt (14) was employed. Sixteen percent burns were inflicted as described above, and serum from 1- and 7-d postburn rats was compared with pair-fed controls. Whole blood was obtained by allowing decapitated rats to bleed into a large beaker without anticoagulant. The serum obtained after centrifugation was stored at −20°C and thawed just before dialysis.

Quinidine and lidocaine were dialyzed against pH 7.4 potassium phosphate buffer with added sodium chloride, prepared by dissolving 14.11 g of K_2HPO_4, 2.59 g of KH_2PO_4, and 1.99 g of NaCl in distilled water to make 1 L of solution. Lengths (19 cm) of cellulose dialysis tubing[7], with a molecular weight cut off of 10,000–14,000, were boiled in distilled water for 4 h and then rinsed with cold distilled water. Serum (1 mL) was added to the tubing, and both ends were sealed with double knots. The resultant dialysis bags were then placed in culture tubes holding 10 mL of buffer containing either 1.6 μg/mL of quinidine (free base) or 1.6 μg/mL of lidocaine (free base). The culture tubes were placed in a shaking water bath at 37°C for 20 h to reach equilibrium. Serum and buffer quinidine and lidocaine concentrations were determined by the enzyme immunoassay method[5], and the free drug percentage was calculated by:

$$\text{percent free drug} = \frac{\text{drug concentration in buffer} \times 100}{\text{drug concentration in serum}}$$

Mathematical Calculations—Pharmacokinetic parameters were calculated by utilizing the one-compartment open model which assumes very rapid initial distribution of the drug (15). The plasma disappearance of all four drugs was considered to follow the exponential function $C_p(t) = Ae^{-\lambda t}$, where $C_p(t)$ is the plasma concentration at time t, A is the zero-time intercept, and λ is the elimination rate constant. The elimination half-life ($t_{1/2}$) was calculated by $t_{1/2} = 0.693/\lambda$, the volume of distribution (Vd) was calculated by Vd = dose/A, and the clearance (CL) was calculated by $CL = Vd \cdot \lambda$. The parameters were calculated for each animal by linear regression analysis of a semilogarthmic plot of drug concentration *versus* time. All data were compared by the unpaired Student's t test, with $p > 0.05$ being statistically insignificant.

Table I—Biochemical Parameters in 16% Burned *versus* Pair-Fed Control Rats[a]

Parameters	Control	Burn
Serum glutamic oxaloacetic transaminase, IU/L	175 ± 30	200 ± 18
Serum glutamic pyruvic transaminase, IU/L	57 ± 8	59 ± 11
γ-Glutamyltransferase, IU/L	3.5 ± 2.1	6 ± 3
Total Bilirubin, mg/dL	0.59 ± .25	0.52 ± .25
Albumin, g/dL	2.80 ± .10	1.96 ± .06[b]
Total Protein, g/dL	6.0 ± 0.3	4.8 ± 0.2[b]
Blood urea nitrogen, mg/dL	13.7 ± 0.6	14.6 ± 1.1
Creatinine, mg/dL	1.5 ± 0.3	1.3 ± 0.1
Blood urea nitrogen–creatinine	9.1 ± 0.4	11.2 ± 1.2

[a] Values are means ± *SD* of six burned and six control animals compared at 7 d postburn. [b] $p < 0.0005$.

RESULTS

Biochemical laboratory parameters for pair-fed control rats and 16% burned rats 7 d postburn are listed in Table I. The serum albumin and total protein concentrations were significantly lower in the burned rats as compared with

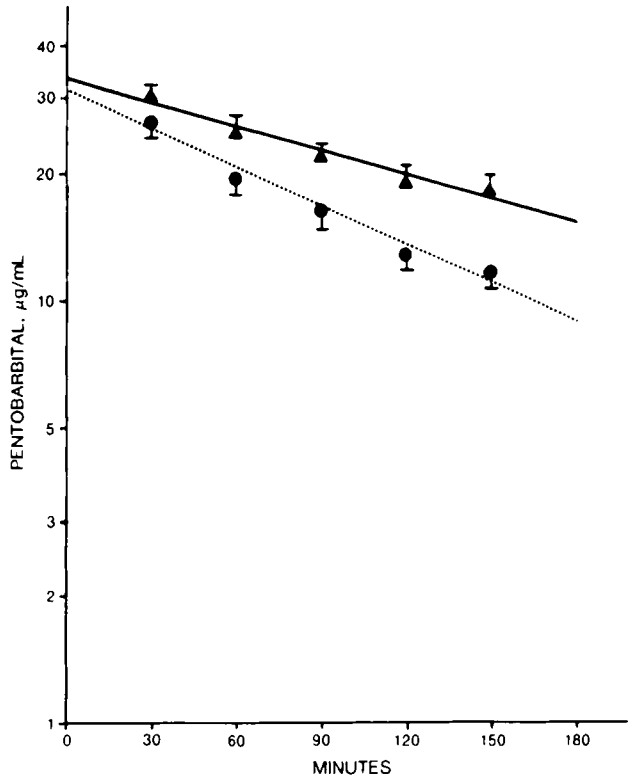

Figure 1—*Plasma disappearance curves of pentobarbital after a 40-mg/kg iv administration in 16% burned male rats at 7 d postburn (▲) and pair-fed control rats (●). Values are mean ± SE, with n = 5 or 6 at each point.*

[4] PE-50 tubing; Becton, Dickinson and Co., Parsippany, N.J.
[5] EMIT system; Syva Co., Palo Alto, Calif.
[6] Model 3920; Perkin-Elmer Corp., Norwalk, Conn.
[7] Fisher Scientific Co., King of Prussia, Pa.

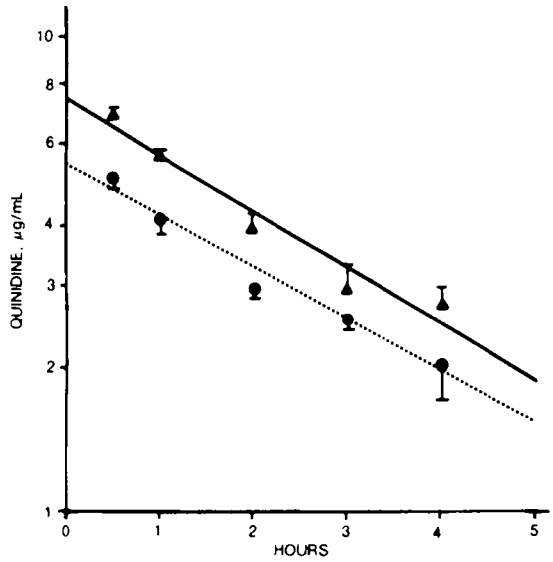

Figure 2—*Plasma disappearance curves of quinidine after 25 mg/kg (free base) iv administration in 16% burned male rats at 7 d postburn (▲) and pair-fed control rats (●). Values are mean ± SE, with n = 4 at each point.*

the control groups. There was no significant difference in the concentrations of the various liver enzymes measured between the two groups or in the concentrations of total bilirubin, creatinine, or blood urea nitrogen.

Figures 1–4 illustrate the respective average plasma disappearance curves for pentobarbital, quinidine, lidocaine, and theophylline for pair-fed controls and 16% burned rats 7 d postburn, and Table II shows the derived pharmacokinetic parameters and weight changes for these same animals. For each drug group tested, the burned rats gained a significantly lower amount of weight over the 7-d period than their corresponding controls, even though each control animal received the same amount of food daily as its paired burned animal. Thermal injury produced a significant 69% prolongation in pentobarbital elimination half-life and a correspondingly significant 43% reduction in total pentobarbital clearance. There was no significant difference in the volume of distribution observed for pentobarbital. For quinidine, the burned animals exhibited a significant reduction (24%) in total clearance and a significant reduction (25%) in volume of distribution, with an unchanged elimination half-life. The burned rats receiving lidocaine demonstrated a significant decrease (27%) in volume of distribution without significant changes in either total clearance or elimination half-life. For theophylline, thermal injury did not significantly alter total clearance, volume of distribution, or elimination half-life.

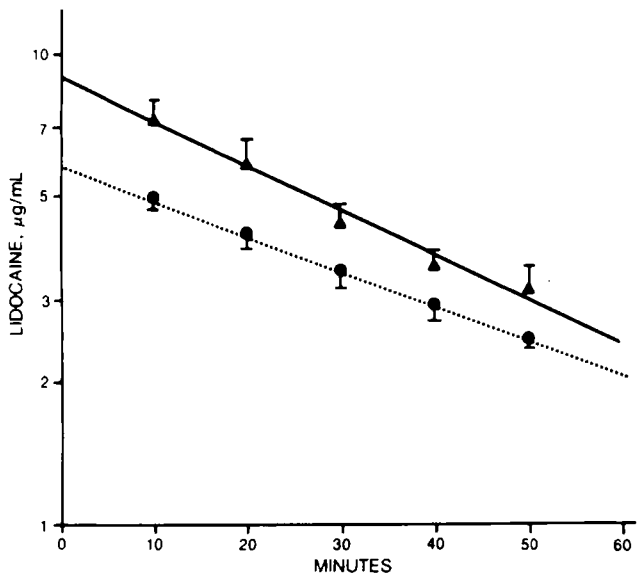

Figure 3—*Plasma disappearance curves of lidocaine after a 15-mg/kg (free base) iv administration in 16% burned male rats at 7 d postburn (▲) and pair-fed control rats (●). Values are mean ±SE, with n = 5 at each point.*

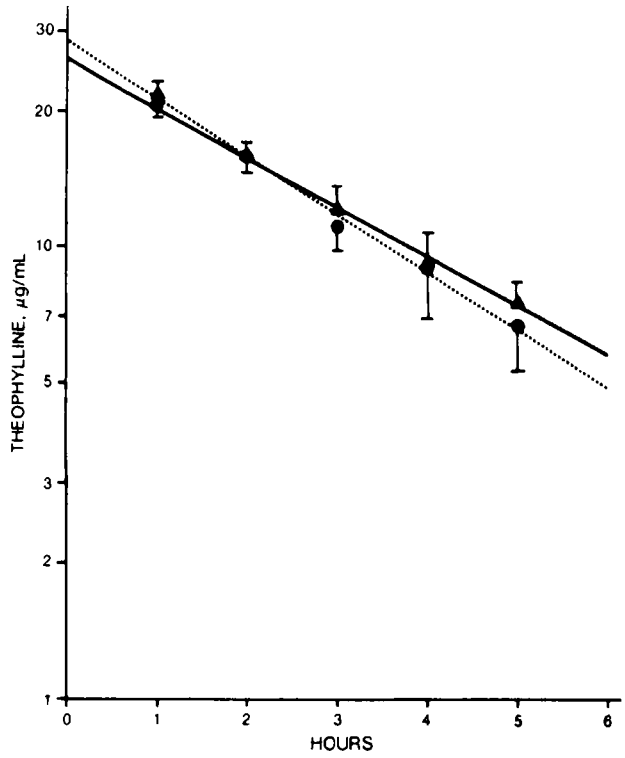

Figure 4—*Plasma disappearance curves of theophylline after a 15-mg/kg iv administration in 16% burned male rats at 7 d postburn (▲) and pair-fed control rats (●). Values are mean ± SE, with n = 4 at each point.*

To determine whether changes in drug protein binding contributed to the observed alterations in the volumes of distribution of quinidine and lidocaine at 7 d postburn, equilibrium dialysis studies were performed with quinidine and lidocaine in 16% burned and pair-fed control rats at 7 d postburn and also at 1 d postburn to serve as a comparison. At 7 d postburn, there was no significant difference in free drug percentages or total drug concentrations of either quinidine or lidocaine compared with controls (Table III). However, at 1 d postburn, there was a significant decrease (18%) in the free drug percentage of quinidine and a significant decrease (27%) in the free drug percentage of lidocaine, which resulted in a significant increase (20%) in total quinidine concentration and a significant increase (37%) in total lidocaine concentration.

Table II—Pharmacokinetic Parameters of Various Drugs in 16% Burned *versus* Pair-Fed Control Rats[a]

Parameter	Control	Burn
	Pentobarbital (40 mg/kg iv)[b]	
$t_{1/2}$, min	102.9 ± 27.8	173.9 ± 40.1[c]
Vd, L/kg	1.21 ± 0.16	1.20 ± 0.19
CL, mL/min/kg	8.59 ± 2.39	4.94 ± 0.90[c]
Percent weight change[d]	+17.5 ± 2.6	+4.1 ± 9.6[c]
	Quinidine (25 mg/kg iv)[e]	
$t_{1/2}$, h	2.65 ± 0.57	2.63 ± 0.48
Vd, L/kg	4.58 ± 0.47	3.45 ± 0.24[f]
CL, mL/min/kg	20.5 ± 3.3	15.5 ± 2.8[g]
Percent weight change[d]	+15.9 ± 3.5	+3.0 ± 4.4[f]
	Lidocaine (15 mg/kg iv)[h]	
$t_{1/2}$, min	38.6 ± 4.7	34.5 ± 6.5
Vd, L/kg	2.52 ± 0.27	1.84 ± 0.42[c]
CL, mL/min/kg	46.7 ± 9.1	37.5 ± 9.4
Percent weight change[d]	+10.4 ± 3.7	+1.8 ± 6.2[g]
	Theophylline (15 mg/kg iv)[e]	
$t_{1/2}$, h	2.19 ± 0.38	2.33 ± 0.36
Vd, L/kg	0.50 ± 0.05	0.57 ± 0.10
CL mL/min/kg	2.70 ± 0.66	2.83 ± 0.66
Percent weight change[d]	+14.0 ± 5.1	−1.2 ± 12.2[g]

[a] Mean ± *SD* measured 7 d postburn. [b] *n* = 5 for the controls, 6 for the burn group. [c] Significantly different from the controls at $p < 0.01$. [d] Calculated by [(wt. at day 7 − wt. at day 0)/wt. at day 0] × 100. [e] *n* = 4 for both groups. [f] Significantly different from the controls at $p < 0.005$. [g] Significantly different from the controls at $p < 0.05$. [h] *n* = 5 for both groups.

Table III—Equilibrium Dialysis Results in 16% Burned *versus* Pair-Fed Control Rats [a]

	Control	Day 1 Postburn	Day 7 Postburn
	Percent Free Drug		
Quinidine	29.9 ± 5.1	24.4 ± 2.3[b]	31.1 ± 2.4
Lidocaine	52.5 ± 6.0	38.1 ± 1.8[c]	52.5 ± 4.8
	Total Drug Concentration, μg/mL		
Quinidine	4.13 ± 0.71	4.96 ± 0.45[b]	3.98 ± 0.48
Lidocaine	2.46 ± 0.24	3.38 ± 0.23[c]	2.48 ± 0.18

[a] Mean ± *SD*; $n = 6$. [b] Significantly different from the controls at $p < 0.05$. [c] Significantly different from the controls at $p < 0.0005$.

DISCUSSION

In this study, it has been demonstrated that thermal injury can alter pharmacokinetic parameters for various liver-metabolized drugs in rats. Previous studies in this laboratory have shown that third-degree burns can depress various components of the hepatic mixed-function oxidase system in rats (3-5) and D-glucaric acid excretion in humans (6). A manifestation of this effect *in vivo* would be a decrease in the total clearance of a drug. However, as stated previously, in addition to levels of hepatic drug-metabolizing enzymes, total clearance can also be affected by changes in hepatic blood flow and free drug fraction (7).

The significant reduction (43%) in pentobarbital clearance can probably be attributed to a reduction in the level of liver enzymes that metabolize pentobarbital. Although a decrease in the free fraction of pentobarbital could also cause a decreased total clearance (7), this event was unlikely since albumin is the main serum protein that binds pentobarbital (16), and it has been shown that the serum albumin concentration is decreased in the burned rats (Table I). This decrease in serum albumin should cause, if anything, an increased and not a decreased free drug concentration (7). Also, the observed total plasma pentobarbital clearance of 8.59 mL/min/kg in control rats is considerably less than the estimated literature values for hepatic plasma flow of ~40 mL/min/kg in similar rats (17). Thus, it can be assumed that pentobarbital clearance is relatively independent of hepatic blood flow changes in the rat (7).

For quinidine, there was a significant decrease (24%) in total clearance ($p < 0.05$), and for lidocaine there was a 20% reduction in total clearance ($p < 0.1$). Although these two drugs have total clearances in humans which are dependent on liver blood flow and drug-metabolizing enzyme activity (7), the relationship of total clearance to renal clearance, liver blood flow, and enzyme activity is unknown in rats. Thus, it is not possible to attribute the observed differences in total clearance for these drugs in the burned rats to any one particular factor. However, changes in drug protein binding did not play a part, as both drugs demonstrated unchanged free drug fractions at 7 d postburn (Table III).

For theophylline, the unchanged total clearance may not be due to a lack of effect of thermal injury on drug-metabolizing enzymes, since the disposition of theophylline differs markedly in rats as compared with humans, in that 46% of the drug is excreted unchanged by the kidneys in rats (18), as compared with less than 10% in humans (19). Thus, the renal route in rats may compensate for any reduction in hepatic clearance.

Since the concentrations of quinidine, lidocaine, and theophylline were measured by enzyme immunoassay, the possibility exists that certain metabolites in rat plasma may cross-react with the parent compounds in the assay and, thus, cause falsely higher values for intact quinidine, lidocaine, or theophylline to be reported. If such a situation were present, the values for drug concentrations and pharmacokinetic parameters in both burned and control rats would be altered slightly from their true values. However, any difference in these values would still be preserved. The only situation that could occur which would make the observed differences between burned and control rats artifactual would be that in which the burned rats metabolized the parent compound through a different metabolic pathway, producing different metabolites from the controls with a higher cross-reactivity for intact drug. Although such a situation cannot be completely ruled out, its occurrence seems unlikely.

For both the basic drugs, quinidine and lidocaine, there were significant decreases in volume of distribution in the burned animals at 7 d postburn (Table II). Increased plasma protein binding of these drugs was not the causative factor for these changes (Table III). A possible explanation is a displacement of drug from tissue-binding sites by substances released at the burn site. For these same drugs, there was a significant decrease in the free drug fraction and a significant increase in total drug concentration when serum taken from rats 1 d postburn was used for equilibrium dialysis studies. Quinidine and lidocaine are examples of drugs that bind more strongly to a specific plasma protein, α_1-acid glycoprotein, than to albumin (20). A number of inflammatory conditions, including burns, have been shown to elevate α_1-acid glycoprotein levels in humans (21) and to increase the plasma protein binding of various drugs (20). The results shown in Table III are consistent with the observation of a rapid rise in α_1-acid glycoprotein levels 24 h postburn in the male rat and a fall to normal levels by 7 d postburn.

In recent years, it has become increasingly apparent that various methodological artifacts could cause errors in the interpretation of data from protein binding experiments with equilibrium dialysis (22). Of particular relevance to this study is the possibility that the free drug fractions reported here after an 18-h dialysis may be larger than those after a shorter dialysis period due to possible deterioration of α_1-acid glycoprotein with time or dilution of this protein by an osmotic influx of water during the dialysis. Although no dialysis experiments were done at shorter time intervals, the control free drug fraction of quinidine reported here (30%) compares favorably with a literature value of 31% in the rat done over a shorter dialysis interval (23). However, the control free fraction of lidocaine (53%) is higher than an estimated literature value of 44% in the rat based on saliva-plasma lidocaine levels (24) and, thus, may have been influenced by the aforementioned factors.

Despite careful daily pair-feeding of burned animals with control animals, all of the burned rats in the four drug groups had a significantly slower rate of weight gain compared with the pair-fed controls (Table II). This finding is consistent with the hypermetabolic response and increased basal metabolic rate found previously both in burn patients and experimental animals (25).

The pathophysiological alterations caused by thermal injury can produce variable changes in the kinetic and dynamic behavior of drugs in humans. An increased elimination rate was found for the renally eliminated aminoglycosides gentamicin (26), tobramycin (27), and amikacin (28). This effect was probably due to a burn-induced increase in the glomerular filtration rate (27). For the nondepolarizing neuromuscular blocking agents tubocurarine (29) and metocurine (30), two- to fivefold increases in plasma drug concentrations were required to give normal muscle relaxation in burn patients. This effect could not be explained by increased drug protein binding and was probably related to a burn-related change in the neuromuscular junction receptors (31). The liver-metabolized drug diazepam has recently been shown to have a reduced free clearance in burn patients (32); however, all patients in this study received cimetidine, a drug known to inhibit diazepam clearance (33). Thermal injury in humans has also been shown to lower plasma protein concentrations (34) and to alter the protein binding of a number of drugs (35). A pharmacokinetic study in 15% burned rats has shown that thermal injury can increase the free phenytoin fraction with a corresponding increase in total phenytoin clearance (36). However, no calculation of free phenytoin clearance was done.

In conclusion, the results of this study and previous investigations suggest that the complex dynamic state of thermal injury can affect numerous aspects of drug disposition and action. Further pharmacokinetic studies with liver-metabolized drugs in burn patients are needed to determine whether altered hepatic drug metabolism plays a part in the disposition of drugs in human thermal injury.

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ACKNOWLEDGMENTS

This study was supported by a grant from the Hahnemann Medical College and Hospital Biomedical Research Support Grant No. 5-S07-RR05413 and certified by the Hahnemann Medical College Animal Welfare Committee as meeting the guidelines of the National Institutes of Health for the care and use of laboratory animals.

The authors thank Mrs. Linda Bush and Ms. Joanne Addario for their help in the preparation of this manuscript.

Comparative *In Vivo* and *In Vitro* Studies of Phenytoin Protein Binding and *In Vitro* Lipolysis in Plasma of Pregnant and Nonpregnant Rats

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Received June 17, 1983, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication October 3, 1983.

Abstract □ This investigation was designed to determine the cause of the changes in drug protein binding that occur in rat plasma, particularly in plasma from pregnant animals, during *in vitro* drug-protein binding measurements. *In vivo* estimates of phenytoin binding in plasma were obtained from steady-state CSF-plasma concentration ratios in pregnant and nonpregnant rats. Immediate ultrafiltration of heparin- or EDTA-anticoagulated plasma yielded phenytoin free fraction values that were in good agreement with *in vivo* estimates for nonpregnant rats but that were about one-third higher than *in vivo* estimates for pregnant animals. *In vitro* free fraction values tended to increase during incubation of plasma and/or during equilibrium dialysis. The concentrations of the four major endogenous free fatty acids were similar in plasma of pregnant and nonpregnant rats if determined immediately after blood collection. Six hours of incubation at 37°C caused fatty acid concentrations to increase about fivefold and twofold in heparin-anticoagulated plasma from pregnant and nonpregnant animals, respectively. The corresponding increases in EDTA-anticoagulated plasma were only about twofold and 1.14-fold, respectively. These changes were associated with decreased plasma protein binding of phenytoin. The *in vivo* differences between pregnant and nonpregnant rats with respect to phenytoin binding in plasma are not due to differences in fatty acid concentrations, but the *in vitro* differences are due primarily to corresponding differences in free fatty acid concentrations if extensive *in vitro* lipolysis occurs.

Keyphrases □ Phenytoin—plasma protein binding, lipolysis, pregnant and nonpregnant rats □ Lipolysis—*in vitro* and *in vivo* comparisons, plasma protein binding, pregnant and nonpregnant rats □ Anticoagulants—heparin, EDTA, plasma protein binding, *in vitro* and *in vivo* comparisons, pregnant and nonpregnant rats

Pregnancy is known to be associated with quantitative changes of drug biotransformation (1) and drug-protein binding in plasma or serum (2). Properly designed studies of the effect of pregnancy on the pharmacokinetics of drugs, therefore, require measurement of unbound drug in plasma, to differentiate between pharmacokinetic changes due solely to drug binding alterations and those due to altered intrinsic clearance or to a combination of both types of effects. Unfortunately, the drug-protein binding characteristics of plasma from pregnant rats and, to a lesser extent, from pregnant women are quite unstable (3, 4). A lack of appreciation of this problem can lead to serious misinterpretations in pharmacokinetic studies, particularly in the assessment of intrinsic drug clearance and drug concentration–pharmacological activity relationships.

This investigation was designed to determine the causes of *in vitro* alterations in protein binding of drugs in plasma from pregnant and nonpregnant rats and to assess the effects of the *in vitro* anticoagulant and of the methodology used for protein binding determinations. The study protocol also permitted a comparative determination of the effect of pregnancy on the total clearance and *in vivo* plasma protein binding of phenytoin in rats.

EXPERIMENTAL SECTION

Pregnant (day 20 of gestation) and nonpregnant Lewis rats[1] (the latter ≃ 200 g) received a loading dose of phenytoin, 14.7 mg/kg, by rapid injection

[1] Charles River Breeding Laboratories, Wilmington, Mass.

0022-3549/84/0800-1121$01.00/0