JOURNAL OF PHARMACEUTICAL SCIENCES

JANUARY 1979
VOLUME 68 NUMBER 1

RESEARCH ARTICLES

Effect of Salicylic Acid on Pharmacokinetics of Free and Plasma Protein-Bound Bilirubin in Experimental Unconjugated Hyperbilirubinemia

SVEIN ØIE and GERHARD LEVY ˣ

Received March 31, 1978, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication May 19, 1978.

Abstract □ The effect of salicylic acid injection and infusion on the concentrations of free and total (sum of free and bound) unconjugated bilirubin in plasma was studied in hyperbilirubinemic rats. *In vitro* addition of salicylic acid, 5–30 mg/100 ml, to rat plasma containing bilirubin, 12.7 mg/100 ml, caused a pronounced, salicylate concentration-dependent increase in the free bilirubin concentration. Normal rats were made hyperbilirubinemic by continuous intravenous infusion of bilirubin; the concentrations of free and total unconjugated bilirubin in plasma, and of total unconjugated bilirubin in whole blood, were determined as a function of time. Rapid intravenous injection of salicylate caused a rapid and pronounced decrease of total bilirubin concentrations in plasma and whole blood but had no apparent effect on the concentration of free bilirubin in plasma. Similar effects were obtained with slow infusions of salicylate, except that total bilirubin concentrations decreased gradually. These observations are consistent with the theory that the clearance rate of bilirubin by the body is proportional to the concentration of free bilirubin in plasma and that the steady-state concentration of free bilirubin depends only on the formation rate and intrinsic clearance of the pigment and is not affected by displacement of bilirubin from plasma protein binding sites. Occasional cases of acute apparent bilirubin intoxication were associated with unusually elevated concentrations of free bilirubin, reflecting a decreased intrinsic metabolic clearance of the pigment.

Keyphrases □ Salicylic acid—effect on pharmacokinetics of free and total bilirubin in hyperbilirubinemic rats □ Bilirubin, free and total—pharmacokinetics, effect of salicylic acid in hyperbilirubinemic rats □ Pharmacokinetics—free and total bilirubin, effect of salicylic acid in hyperbilirubinemic rats

The increased incidence of kernicterus and fatalities in premature infants treated with sulfisoxazole (1, 2) demonstrated the hazards of administering to hyperbilirubinemic neonates drugs that can displace the potentially neurotoxic heme pigment bilirubin from plasma protein binding sites. Studies in humans and animals (3–8) showed that drugs such as sulfisoxazole and salicylate, which displace bilirubin from plasma albumin, cause a redistribution of the pigment from plasma to extravascular tissues, including the brain. The concentration of free, diffusible (rather than total) bilirubin in plasma is, therefore, considered to be a key determinant of kernicterus in hyperbilirubinemic neonates (9). Many drugs displace bilirubin from binding sites on plasma albumin.

The displacement of bilirubin from albumin by a drug results in a permanent (disregarding stability considerations) increase in the concentration of free bilirubin in a closed *in vitro* system and may be thought to have a similar effect *in vivo*. However, according to recently developed pharmacokinetic theory (10, 11) and preliminary experimental data (12), such an increase should not occur. There is increasing evidence that the metabolic clearance rate of partly plasma protein-bound substances is proportional to their free (rather than total) concentration in plasma[1]. Under these conditions:

$$\text{total clearance} = \text{intrinsic clearance} \times \text{free fraction} \quad \text{(Eq. 1)}$$

where the intrinsic clearance is a measure of the activity of the enzyme system(s) responsible for the rate-determining step in the biotransformation process(es) and the free fraction is the ratio of free to total concentration of the substance in plasma.

According to Eq. 1, a plot of total clearance *versus* free fraction should be linear and go through the origin. This result has, in fact, been demonstrated in animals and humans for several extensively plasma protein-bound drugs (11, 14). This relationship also applies to bilirubin (12), indicating that the metabolic clearance rate of bilirubin is proportional to the concentration of free bilirubin in plasma.

The steady-state (plateau) concentration of total bilirubin in plasma, C_∞, is determined by the formation rate, R, and the total clearance of the pigment:

$$C_\infty = R/\text{total clearance} \quad \text{(Eq. 2)}$$

[1] Exceptions are those few (but clinically important) drugs and endogenous compounds whose elimination rate is limited by blood flow rate (13) or metabolic capacity.

0022-3549/79/0100-0001$01.00/0

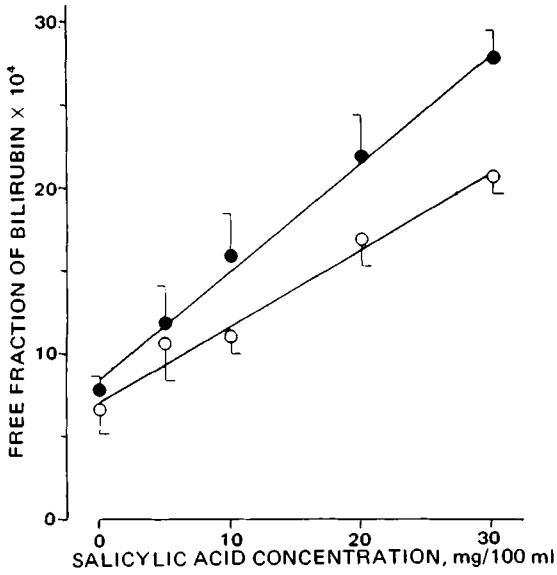

Figure 1—*Effect of various concentrations of salicylic acid on the protein binding of bilirubin at 37° in pooled plasma from four rats. Key: ●, 12.7 mg of total bilirubin/100 ml; and ○, 6.4 mg of total bilirubin/100 ml. Data are averages of five determinations with 1 SD shown in one direction only for clarity of presentation.*

The steady-state concentration of *free* bilirubin in plasma, $C_{\infty f}$, is determined by the formation rate and the *intrinsic* clearance of the pigment:

$$C_{\infty f} = R/\text{intrinsic clearance} \qquad \text{(Eq. 3)}$$

Equation 3 is obtained by substituting Eq. 1 into Eq. 2. Since total clearance is affected by changes in protein binding while intrinsic clearance is not so affected, a decrease in the plasma protein binding of bilirubin should decrease the steady-state concentration of total bilirubin but should have no effect on the steady-state concentration of free bilirubin in plasma. Administration of a drug that displaces bilirubin from plasma albumin should result in only a temporary perturbation of the steady-state situation until the "excess" bilirubin has been cleared by the body, unless that drug also modifies the intrinsic clearance (for example, by enzyme induction or inhibition) or the formation rate of bilirubin.

In the present study, this hypothesis was tested by determining the effects of a rapid injection and a slow infusion of salicylate on the concentrations of free and total bilirubin in the plasma and total bilirubin in whole blood of rats made hyperbilirubinemic by a continuous intravenous infusion of bilirubin. Contrary to common practice, the plasma concentrations of drug and bilirubin were maintained in a clinically realistic range (drug to albumin and bilirubin to albumin molar concentration ratios less than unity), and the concentration of free bilirubin was determined in undiluted plasma.

EXPERIMENTAL

Male Sprague–Dawley rats[2], 310–450 g, were maintained on a standard diet[3]. Two days before an experiment, a silicone rubber–polyethylene cannula was placed permanently in the right jugular vein to facilitate intravenous infusion, injection, and frequent withdrawal of blood samples (15, 16).

On the day of an experiment, a solution of bilirubin and sodium taurocholate (1:1.1 weight ratio in 0.7% sodium chloride adjusted to pH 7.4) was infused at a rate equivalent to 0.8 mg of bilirubin/kg/min (3.2 ml/hr/rat) for 15 min and then at 0.32 mg of bilirubin/kg/min for 105 min. Three rats then received an intravenous injection of salicylic acid, 67 mg/kg, as sodium salicylate in 0.5 ml of water, and the infusion was continued for 120 min with bilirubin, 0.32 mg/kg/min, and salicylic acid, 0.148 mg/kg/min. Three other rats received the same bilirubin infusion regimen for the first 2 hr, followed by an infusion of salicylic acid, 0.6 mg/kg/min, and bilirubin, 0.32 mg/kg/min, for the next 2 hr. Another four rats received both injection and infusion of salicylate in separate crossover experiments, about 2 weeks apart.

Additional rats served as controls and received bilirubin infusion but no salicylic acid. Some of the control rats received an intravenous injection of 0.5 ml of 1.8% sodium chloride (approximately the same tonicity as the sodium salicylate injection solution) at 2 hr. All infusion equipment, including syringes and cannulas, was covered with aluminum foil to protect bilirubin from light.

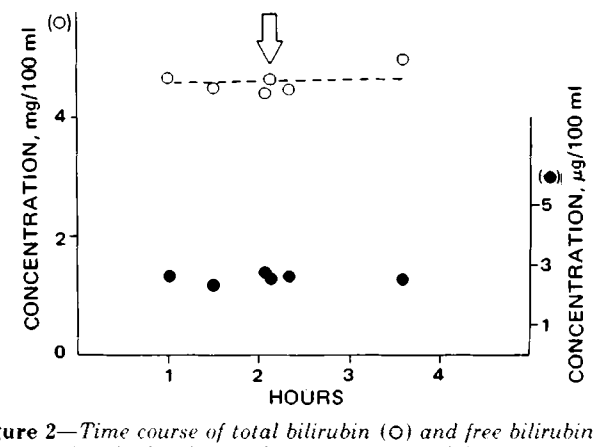

Figure 2—*Time course of total bilirubin (○) and free bilirubin (●) concentrations in the plasma of a rat infused with bilirubin at a rate of 0.80 mg/kg/min for 15 min and then at a rate of 0.32 mg/kg/min. The arrow indicates the time of injection of 0.5 ml of 1.8% NaCl. (Note the use of more than one scale in this and some of the following figures.)*

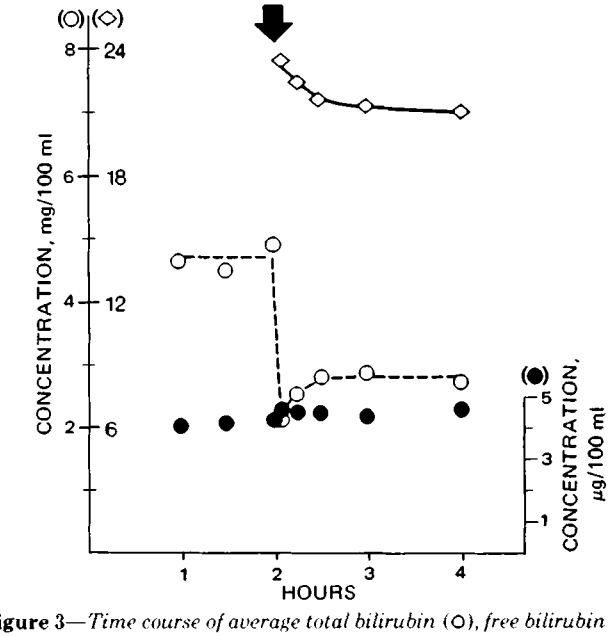

Figure 3—*Time course of average total bilirubin (○), free bilirubin (●), and salicylic acid (◇) concentrations in plasma of four rats infused with bilirubin, 0.8 mg/kg/min, for 15 min and then at a rate of 0.32 mg/kg/min until the end of the experiment. The arrow indicates the time of injection of salicylic acid, 67 mg/kg, which was followed by an infusion of 0.148 mg/kg/min.*

[2] Blue Spruce Farms, Altamount, N.Y.
[3] Charles River Formula 4RF.

Table I—Effect of Salicylic Acid Injection or Infusion on Total and Free Unconjugated Bilirubin and Salicylic Acid Concentrations in Plasma of Rats with Experimental Unconjugated Hyperbilirubinemia

	Salicylic Acid Injection			Salicylic Acid Infusion		
Hours	Total Bilirubin Concentration, mg/100 ml	Free Bilirubin Concentration, μg/100 ml	Salicylic Acid Concentration, mg/100 ml	Total Bilirubin Concentration, mg/100 ml	Free Bilirubin Concentration, μg/100 ml	Salicylic Acid Concentration, mg/100 ml
1.0	4.88 ± 0.48[a]	3.96 ± 0.99	0	5.07 ± 0.90	3.78 ± 0.96	0
1.5	4.93 ± 0.77	4.12 ± 1.33	0	5.22 ± 0.94	3.70 ± 1.16	0
2.0[b]	5.30 ± 0.66	4.13 ± 1.27	0	5.19 ± 0.90	3.82 ± 0.95	0
2.08	1.87 ± 0.69	4.44 ± 1.05	24.8 ± 2.2	—[c]	—[c]	—[c]
2.25	2.37 ± 0.70	4.21 ± 1.38	22.2 ± 1.8	4.41 ± 0.89	4.17 ± 1.28	6.46 ± 1.51
2.5	2.86 ± 0.77	4.45 ± 1.64	21.1 ± 1.55	3.65 ± 0.83	3.91 ± 1.20	10.1 ■ 1.1
3.0	2.89 ± 0.39	4.26 ± 1.52	21.0 ± 1.2	3.15 ± 0.71	4.31 ± 1.37	14.3 ± 1.0
4.0	2.87 ± 0.57	4.24 ± 1.33	20.8 ± 0.8	2.47 ± 0.78	4.08 ± 1.63	20.6 ± 1.5

[a] All data are means ± *SD*, $n = 7$. Four of the seven rats in each group were used in crossover experiments. [b] Time of salicylate injection. [c] Blood sample was not obtained at this time.

Heparinized blood samples (0.6 ml) were collected at 1, 1.5, and 2 hr and then again 15, 30, 60, and 120 min after the salicylate injection or the start of the salicylate infusion. An additional blood sample was obtained 5 min after the salicylate injection. Plasma was separated by centrifugation and analyzed for free (17) and total unconjugated bilirubin (18) and for salicylic acid (19). In some cases, the erythrocytes were lysed by freezing, thawing, and addition of 1 ml of 6% ascorbic acid in 0.2 *M* phosphate buffer, pH 8.2, to 0.1 ml of erythrocytes. This solution was analyzed for total unconjugated bilirubin (18).

Considerable care was taken to obtain "mainstream" blood samples uncontaminated by the bilirubin infusion solution. The infusion pump was disconnected from the indwelling intravenous cannula, and the cannula was flushed with 0.1–0.2 ml of normal saline solution containing heparin, 20 units/ml (volume sufficient to fill the cannula without entering the bloodstream), using a syringe attached to the cannula with a three-way stopcock. The saline solution was then drawn back into the syringe, thereby filling the catheter with blood. About 0.5 ml of blood was then drawn into a second syringe, the blood sample of 0.6 ml was drawn into a third syringe, and the 0.5 ml of blood from the second syringe was reinjected and followed by about 0.1–0.2 ml of heparinized normal saline solution to empty the cannula of blood. The bilirubin infusion was then resumed. The entire procedure required about 1 min.

The concentration of free bilirubin was determined in undiluted plasma by measuring the rate of the peroxidase-catalyzed degradation of bilirubin by peroxide. A 0.1-ml aliquot of 0.1 *M* phosphate buffer, pH 7.4, containing 0.01 *M* edetate disodium was evaporated to dryness in a 10-ml centrifuge tube. Plasma, 0.1 ml, was added, and the dried buffer was dissolved in it by vortexing. Then 10 μl of peroxidase solution (8 purpurogallin units of horseradish peroxidase[4]/ml of 0.1 *M* phosphate buffer, pH 7.4) was added. The reaction was started by adding 5 μl of peroxide solution (0.2% ethyl hydrogen peroxide in 0.1 *M* phosphate buffer, pH 7.4) and stopped after 1–2 min by addition of 1 ml of 6% ascorbic acid in 0.2 *M* phosphate buffer, pH 8.2. The entire reaction was carried out in a water bath at 37°.

The concentration of total unconjugated bilirubin before and after the reaction was determined spectrophotometrically after chloroform extraction (18), using a bilirubin control[5] as the standard. *In vitro* studies of bilirubin binding in plasma were carried out in the same manner, except that about 25 μl of bilirubin solution (crystalline bilirubin[6] dissolved in water with the aid of a few drops of 2 *M* NaOH) was added to 1 ml of pooled plasma from several rats to obtain the desired bilirubin concentration, and salicylate was added as 5 μl of an aqueous solution/100 μl of plasma.

RESULTS

Bilirubin added *in vitro* to pooled rat plasma in concentrations of 6.4 and 12.7 mg/100 ml was 99.935 and 99.925% bound, respectively, to plasma proteins. Salicylic acid decreased the protein binding of bilirubin in a concentration-dependent manner (Fig. 1). At a concentration of 30 mg/100 ml, salicylic acid decreased bilirubin binding to 99.794 and 99.721%, respectively. These changes are equal to 3.2- and 3.7-fold increases in the free fraction of bilirubin in plasma containing 6.4 and 12.7 mg of bilirubin/100 ml, respectively.

The bilirubin infusion schedule resulted in an average plasma concentration of total bilirubin of about 5 mg/100 ml, which, with three exceptions that are not included in the tabulation for reasons explained subsequently, remained constant in any one animal for the duration of the experiment (Fig. 2) or until salicylate was administered (Table I). The total bilirubin concentration in plasma decreased rapidly and substantially with salicylate injection and then increased slightly to a new plateau (lower than preinjection) as salicylate concentrations declined to a steady state (Fig. 3 and Table I). Salicylate concentrations were at a maximum immediately after injection, with a subsequent distribution phase, and then approached a plateau due to continuing infusion of the drug. Despite the significant changes in the concentrations of salicylate and total bilirubin, the free bilirubin concentration remained essentially constant throughout the experiment.

Slow infusion of salicylate over 2 hr resulted in a gradual decrease in the total bilirubin concentration, but the concentration of free bilirubin again remained essentially constant at all times (Fig. 4 and Table I). Both methods of salicylate administration were designed to, and did, produce about the same drug concentration in plasma at 4 hr, but the injection lowered the total bilirubin concentration by a maximum of 65% while the infusion caused a maximum decrease of 52% on the average (Table I). This difference was due to the higher salicylate concentration immediately after drug injection (*i.e.*, the predistributive concentration maximum) since the relationship between salicylate and total bilirubin concentrations in plasma was the same with both modes of salicylate administration (Fig. 5).

The rapid decrease of total bilirubin concentrations in plasma following injection of salicylate was accompanied by a significant increase in the concentration of bilirubin in erythrocytes (Table II). However, the bilirubin concentration in whole blood decreased (Fig. 6), indicating that some bilirubin was redistributed from blood to extravascular tissues. The elevated bilirubin concentration in the erythrocytes persisted for the duration of the experiment, suggesting that diffusion of the pigment out of the erythrocytes is very slow, perhaps due to precipitation of bilirubin in these cells.

Three rats (one injection control, one receiving salicylate injection, and one receiving salicylate infusion) developed signs of bilirubin intoxication and were excluded from the results summarized in Table I. At about the 3rd hr of bilirubin infusion, the skin of these animals became noticeably yellow, their breathing slowed, and they became prostrate. The time course of bilirubin concentrations in the plasma of one of these animals is shown in Fig. 7. Total and free plasma bilirubin concentrations increased substantially and continuously after the sodium chloride (control)

Table II—Effect of Salicylic Acid Injection on Total Unconjugated Bilirubin Concentration in Erythrocytes of Rats with Experimental Unconjugated Hyperbilirubinemia

Hours	Bilirubin Concentration[a], mg/100 ml
1.0	0.57 ± 0.24
1.5	0.58 ± 0.21
2.0[b]	0.61 ± 0.18
2.08	1.55 ± 0.83[c]
2.25	1.84 ± 0.65[c]
2.5	1.90 ± 0.60[c]
3.0	2.08 ± 0.63[c]
4.0	1.87 ± 0.55[c]

[a] Mean ± *SD*. $n = 4$. [b] Time of salicylate injection. [c] Significantly different from control period ($p < 0.02$).

[4] Sigma type I.
[5] Dade Division, American Hospital Supply Corp., Miami, Fla.
[6] Sigma.

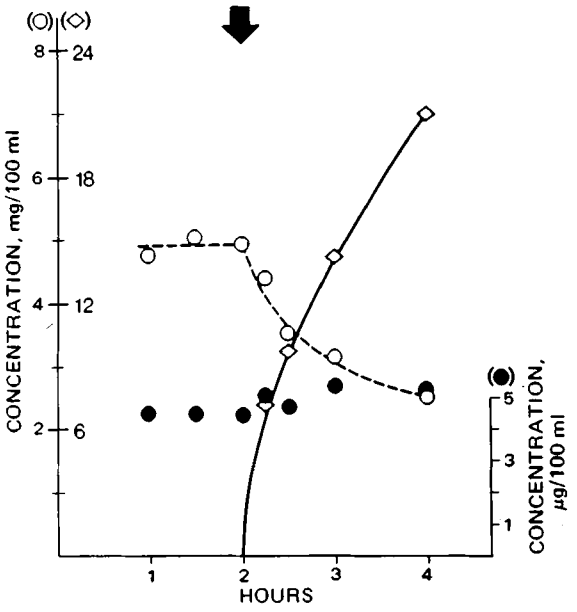

Figure 4—*Effect of salicylic acid infusion (0.6 mg/kg/min, starting at the time shown by the arrow) on average free and total bilirubin concentrations in plasma of four rats. Bilirubin infusion rate and symbols are as in Fig. 3. Note the similarity of presalicylic acid bilirubin concentrations in the salicylic acid infusion (this figure) and injection experiments (Fig. 3), which were carried out on the same animals in a crossover study.*

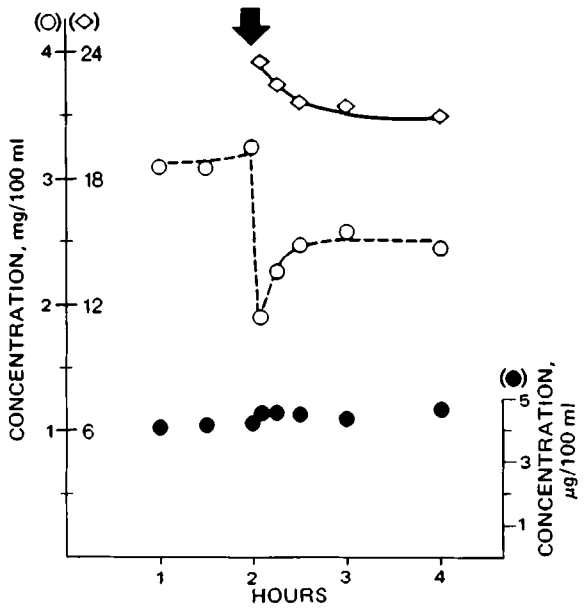

Figure 6—*The same experiment as in Fig. 3, except that open circles represent average total bilirubin concentrations in whole blood rather than in plasma. A similar but more gradual decrease of total bilirubin concentrations in whole blood was observed when salicylate was administered by slow infusion without initial rapid injection.*

injection. The concentration of free bilirubin increased relatively more than the concentration of total pigment, indicating a decrease in the protein binding of bilirubin.

This decrease is more readily apparent in Fig. 8, which shows the free fraction values as a function of total bilirubin concentration in pooled plasma from four normal rats and in plasma of the sick rat. The free fraction values of bilirubin in the intoxicated rat were normal for the first 150 min of the experiment (during which the animal appeared healthy) but then rose substantially above normal as the animal began to show signs of intoxication.

A different picture was apparent in another rat with signs of bilirubin intoxication. The free and total bilirubin concentrations in the plasma of that animal, which received a sodium salicylate injection at 2 hr, remained relatively constant over 3 hr while the skin of the animal became increasingly yellow. However, the concentration of free bilirubin and the free fraction value of bilirubin were about five times higher than in the normal animals. The albumin and total protein concentrations in the plasma of this intoxicated animal were only 2.24 and 6.75 g/100 ml, respectively, compared to an average of 3.75 and 7.96 g/100 ml, respectively, in 42 normal animals from the same source (20).

DISCUSSION

Salicylate, in therapeutically realistic concentrations, had a pronounced displacing effect on protein-bound bilirubin in undiluted rat plasma. The bilirubin concentrations were in the range encountered in jaundiced neonates and did not exceed the capacity of the strong binding sites of albumin in plasma.

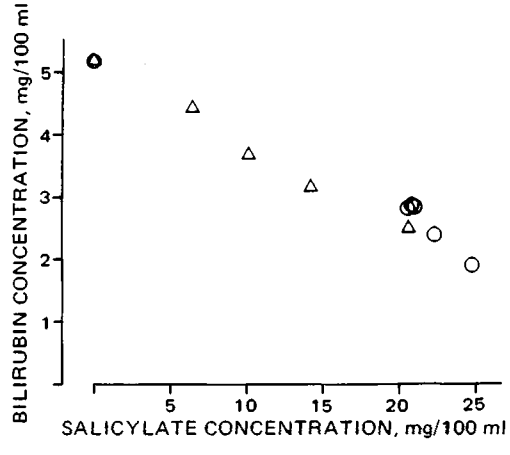

Figure 5—*Relationship between plasma concentrations of total bilirubin and salicylic acid in rats infused (△) or injected (○) with salicylic acid and infused with bilirubin. Each data point represents the mean of seven animals.*

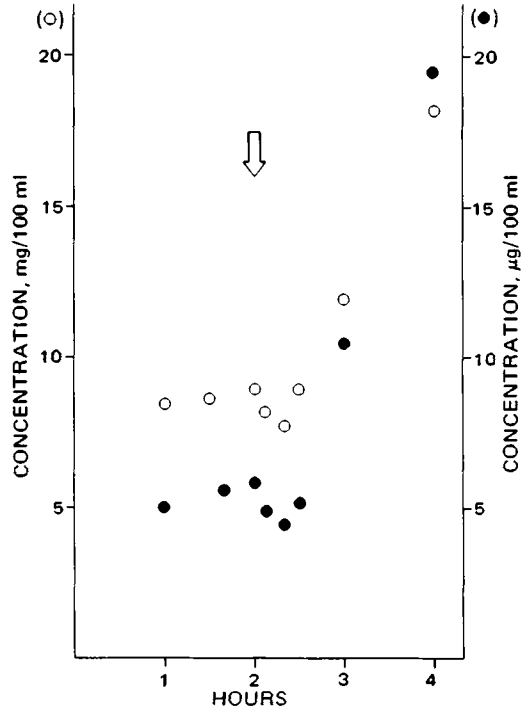

Figure 7—*Time course of total (○) and free (●) bilirubin concentrations in plasma of a rat infused with bilirubin and showing toxic symptoms. The arrow indicates the time of injection of 0.5 ml of 1.8% NaCl.*

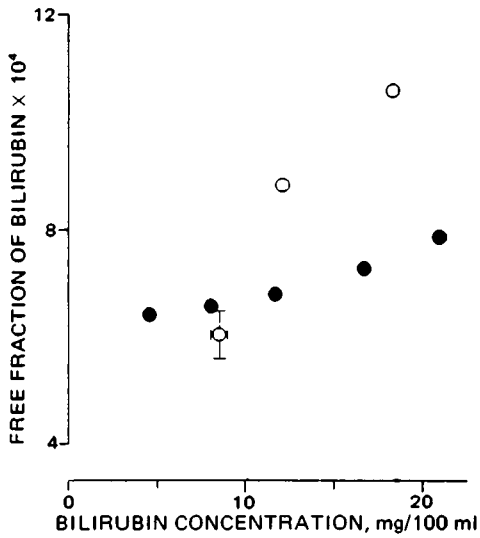

Figure 8—*Free fraction of bilirubin as a function of total bilirubin concentration in pooled plasma from four rats (●) and in plasma of the sick rat described in Fig. 7 (○). The open circle with bars represents the mean value ± 1 SD of the first six pairs of data points in Fig. 7.*

Rapid intravenous injection of salicylate to rats made hyperbilirubinemic by a constant infusion of bilirubin caused a rapid and pronounced decrease of plasma total bilirubin concentrations and a concomitant increase of bilirubin in the erythrocytes. This redistribution of bilirubin was not limited to the blood since the total bilirubin concentration in blood decreased. Thus, salicylate caused a transfer of bilirubin to extravascular sites as observed previously (6).

Administration of salicylate by slow infusion caused a much more gradual decrease of total bilirubin concentrations in plasma. More important, the maximum average decrease of the total bilirubin concentration in plasma (52%) was somewhat less pronounced than that after salicylate injection and occurred at the end of the experiment when the salicylate concentration had increased to about 20 mg/100 ml. When the same salicylate concentration was approached from "above," *i.e.*, by rapid injection followed by a maintenance infusion, the total bilirubin concentration in plasma was temporarily decreased by a maximum of 65% on the average. This difference should be even more pronounced with bilirubin-displacing drugs that have a larger distribution phase (as reflected by the difference between the concentration of drug in plasma immediately after rapid injection and the intercept on the concentration axis at zero time obtained by back-extrapolation of the terminal semilogarithmic concentration decay curve).

Administration of salicylate to neonatal guinea pigs while they were infused with bilirubin at a constant rate decreased total bilirubin concentrations in serum and increased bilirubin in the brain (6). In view of this reciprocal relationship, it is possible (but has not yet been demonstrated experimentally) that the magnitude of redistribution of bilirubin from plasma to the brain caused by displacement of bilirubin from plasma protein binding sites by a drug can be reduced by administering that drug slowly so as to prevent the predistributive drug concentration peaks associated with rapid intravenous injection. As stated previously (21) in a different context: "Since the equilibrium of bilirubin between tissue and albumin occurs very rapidly, kernicterus may develop from bilirubin displaced during transient high serum concentrations of a competing drug attained by rapid infusion. As the drug is cleared, binding will revert to normal (the perfect crime)."

The results of this investigation are in agreement with the predictions based on the theoretical pharmacokinetic considerations presented in the introductory paragraphs. A decrease in plasma protein binding of bilirubin caused by administration of a displacing agent decreased the plasma concentration of total bilirubin but had no effect on the steady-state concentration of free bilirubin. The redistribution of the pigment following salicylate injection was so rapid that no increase in the concentration of free bilirubin in plasma was apparent 5 min after injection. However, such temporary elevations of free bilirubin concentrations in plasma were observed shortly after rapid injection of another displacing agent, sulfisoxazole (22).

Since the concentration of bilirubin in extravascular tissues, including the brain, is apparently a function of the free concentration in plasma, a decrease in plasma protein binding *per se* (*i.e.*, without concomitant change in tissue binding) should have no effect on the *steady-state* concentration of total bilirubin in the tissues. However, the return to tissue steady-state conditions may be quite slow. This investigation showed that elevated bilirubin concentrations in erythrocytes persisted for at least 2 hr (Table II). Bilirubin concentrations in the brain of Gunn rats were elevated 2 hr after sulfadimethoxine administration (23). This persistence of bilirubin in tissues may be due, at least in part, to precipitation of the pigment and slow dissolution of the crystalline precipitate. Crystals of bilirubin were observed in the brain, kidneys, bone marrow, cerebrospinal fluid, and blood cells of hyperbilirubinemic infants (24, 25).

While the steady-state concentration of free bilirubin in plasma is not affected by protein binding, it should be increased if the rate of formation of bilirubin increases and/or if the intrinsic metabolic clearance of bilirubin decreases (Eq. 3). Such an increase in the plasma concentration of free bilirubin was observed in three animals during this investigation and was associated with typical signs and symptoms of acute bilirubin intoxication. Thus, in one rat that received bilirubin infusion but no salicylate, the concentration of free bilirubin rose after 3 hr from about 5 to about 19 μg/100 ml (Fig. 7). In addition, the protein binding of bilirubin decreased significantly (Fig. 8) relative to that in plasma of healthy animals. Both of these perturbations are consistent with impairment of hepatic function. The rise in the free bilirubin concentration could have been the result of decreased intrinsic metabolic clearance of the pigment while the diminished protein binding may have been due to an increase in the concentration of endogenous substances in plasma that are capable of displacing bilirubin from protein binding sites and are rapidly cleared by the normal liver.

Another case of acute bilirubin intoxication was observed in a rat subsequently found to be hypoalbuminemic. Had the intrinsic bilirubin clearance by the liver been normal, then the steady-state plasma concentration of total bilirubin in this animal would have been below normal but the concentration of free bilirubin in plasma should have been in the normal range. In fact, the total plasma bilirubin concentration was not decreased and the free bilirubin concentration in plasma was substantially above normal throughout the experiment, suggesting that this animal had impaired liver function from the beginning of the experiment. The hypoalbuminemia is consistent with this conclusion.

Diamond and Schmid (6), in their classic paper on experimental bilirubin encephalopathy, stated that the most significant limitation of the study was their inability to estimate directly the unbound plasma bilirubin fraction available for transfer into the brain. With the use of recently developed experimental methodology for determining free bilirubin concentrations in undiluted plasma (17), this limitation was overcome. The results emphasize the need for monitoring free rather than total unconjugated bilirubin concentrations in the plasma of newborn infants since the total bilirubin concentration may be decreased by endogenous or exogenous (drug or drug metabolite) displacing agents or by hypoalbuminemia.

A potentially serious problem may be the "hit-and-run" nature of bilirubin-displacing agents. For example, administration of a single dose of a bilirubin-displacing drug to a jaundiced patient may cause a pronounced shift of the heme pigment from plasma to extravascular sites, including the brain. If such a drug has a very short half-life or if it is injected intravenously and has a pronounced distribution phase, blood samples obtained even a relatively short time after drug administration may show normal rather than elevated bilirubin free fraction values in plasma. However, this "perfect crime" does leave a clue, namely, the more persistent elevation of bilirubin concentrations in erythrocytes. It will be important to determine how long these elevations persist, if they occur following administration of drugs other than salicylate, and if monitoring of bilirubin concentrations not only in plasma but also in erythrocytes can be of clinical diagnostic value.

REFERENCES

(1) W. A. Silverman, D. H. Anderson, W. A. Blanc, and D. N. Crozier, *Pediatrics*, **18**, 614 (1956).

(2) R. C. Harris, J. F. Lucey, and J. R. MacLean, *ibid.*, **20**, 875 (1958).

(3) L. Johnson, M. L. Garcia, E. Figueroa, and F. Sarmiento, *Am. J. Dis. Child.*, **101**, 322 (1961).

(4) D. Watson, *Clin. Sci.*, **22**, 435 (1962).

(5) R. Schmid, I. Diamond, L. Hammaker, and C. B. Gundersen, *Nature*, **206**, 1041 (1965).

(6) I. Diamond and R. Schmid, *J. Clin. Invest.*, **45**, 678 (1966).
(7) J. C. Topham and R. D. Board, *Biochem. Pharmacol.*, **20**, 718 (1971).
(8) R. A. Yeary and D. R. Davis, *Toxicol. Appl. Pharmacol.*, **28**, 269 (1974).
(9) M. J. Maisels, *Pediatr. Clin. North Am.*, **19**, 447 (1972).
(10) G. Levy and A. Yacobi, *J. Pharm. Sci.*, **63**, 805 (1974).
(11) G. Levy, in "The Effect of Disease States on Drug Pharmacokinetics," L. Z. Benet, Ed., American Pharmaceutical Association, Washington, D.C., 1976, chap. 9.
(12) S. Øie and G. Levy, *J. Pharm. Sci.*, **64**, 1433 (1975).
(13) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).
(14) C.-M. Lai and G. Levy, *J. Pharm. Sci.*, **66**, 1739 (1977).
(15) J. R. Weeks and J. D. Davis, *J. Appl. Physiol.*, **19**, 540 (1964).
(16) J. J. Ashley and G. Levy, *Res. Commun. Chem. Pathol. Pharmacol.*, **4**, 297 (1972).
(17) J. Jacobsen and O. Fedders, *Scand. J. Clin. Lab. Invest.*, **26**, 237 (1970).
(18) R. Brodersen and I. Vind, *ibid.*, **15**, 107 (1963).
(19) B. B. Brodie, S. Udenfriend, and A. F. Coburn, *J. Pharmacol. Exp. Ther.*, **80**, 114 (1944).
(20) A. Yacobi and G. Levy, *J. Pharm. Sci.*, **64**, 1660 (1975).
(21) R. P. Wennberg and L. F. Rasmussen, *J. Pediatr.*, **87**, 1007 (i975).
(22) S. Øie and G. Levy, *J. Pharm. Sci.*, **68**, 6 (1979).
(23) D. R. Davis and R. A. Yeary, *Pediatr. Res.*, **9**, 846 (1975).
(24) T. J. Vietti, in "Kernicterus," A. Sass-Kortsák, Ed., University of Toronto Press, Toronto, Canada, 1960, p. 153.
(25) L. M. Gartner, R. N. Snyder, R. S. Chabon, and J. Bernstein, *Pediatrics*, **45**, 906 (1970).

ACKNOWLEDGMENTS

Supported in part by Grant GM 19568 from the National Institute of General Medical Sciences, National Institutes of Health, and by an unrestricted grant from an anonymous donor.

Effect of Sulfisoxazole on Pharmacokinetics of Free and Plasma Protein-Bound Bilirubin in Experimental Unconjugated Hyperbilirubinemia

SVEIN ØIE and GERHARD LEVY ˣ

Received April 7, 1978, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication May 26, 1978.

Abstract □ The effect of sulfisoxazole on the time course of free (unbound) bilirubin concentrations in plasma was studied. Normal adult rats were made hyperbilirubinemic by continuous intravenous infusion of bilirubin. Sulfisoxazole was administered by either rapid intravenous injection or slow intravenous infusion, and the plasma concentrations of free and total (free plus bound) unconjugated bilirubin were determined as a function of time. Rapid injection of sulfisoxazole caused a rapid and pronounced decrease of total bilirubin concentrations in plasma but had only a transient effect on the concentration of free bilirubin. Slow infusion of sulfisoxazole caused a gradual and eventually pronounced decrease of total bilirubin concentrations in plasma but had no apparent effect on the concentration of free bilirubin at any time. These results are consistent with recently developed pharmacokinetic theory according to which the plasma clearance of total bilirubin should increase upon administration of a displacing agent while the plasma clearance of free bilirubin should remain unchanged. Bilirubin-induced encephalopathy caused by sulfisoxazole or other displacing agents may be due to very transient elevations of free bilirubin concentrations in plasma of infants with elevated plasma concentrations of total bilirubin and the consequent redistribution of the pigment to extravascular sites, including the brain.

Keyphrases □ Sulfisoxazole—effect on pharmacokinetics of bilirubin in hyperbilirubinemic rats □ Pharmacokinetics—bilirubin in hyperbilirubinemic rats, effect of sulfisoxazole □ Bilirubin—pharmacokinetics in hyperbilirubinemic rats, effect of sulfisoxazole □ Antibacterials—sulfisoxazole, effect on pharmacokinetics of bilirubin in hyperbilirubinemic rats

Administration of sulfisoxazole to premature infants with neonatal jaundice has caused kernicterus (brain damage), often with fatal outcome (1, 2). Typically, this effect has been associated with a decrease of bilirubin concentrations in plasma. The same phenomenon was observed in rats with unconjugated hyperbilirubinemia (3). Sulfisoxazole is a potent displacer of plasma protein-bound bilirubin (4), and some of this displaced bilirubin is redistributed to extravascular sites, including the brain where it exerts its toxic effect (5).

Previous *in vivo* studies of the interaction between sulfisoxazole and bilirubin were limited by the lack of suitable methodology for the determination of free (unbound) unconjugated bilirubin in plasma. The more recently developed reaction rate method (6) permits determination of free bilirubin in undiluted plasma under clinically realistic conditions, *i.e.*, at bilirubin to albumin molar ratios of less than unity (6, 7). Therefore, an investigation was initiated to explore the kinetics of the interaction between sulfisoxazole and bilirubin in rats with experimental unconjugated hyperbilirubinemia, with emphasis on the temporal pattern of free and total (sum of free and protein-bound) unconjugated bilirubin concentrations in plasma and total unconjugated bilirubin concentrations in erythrocytes before and after intravenous injection or infusion of sulfisoxazole.

BACKGROUND

Bilirubin is eliminated almost entirely by conjugation in the liver and subsequent excretion of the conjugates in the bile and urine (8). The total plasma clearance of bilirubin is about 10% of the plasma perfusion rate of the liver (9), and the concentration of total bilirubin in erythrocytes is about 10% of the concentration in plasma of rats with experimental hyperbilirubinemia under otherwise normal physiological conditions (Ref. 10 and results of this study). Therefore, the following pharmacokinetic relationships may be expected to apply (11):

$$TC = f_p k'' \quad \text{(Eq. 1)}$$

$$C_\infty = R^0/TC \quad \text{(Eq. 2)}$$

$$f_p C_\infty = R^0/k'' \quad \text{(Eq. 3)}$$

0022-3549/79/0100-0006$01.00/0