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Bilirubin-Displacing Effect of Ampicillin, Indomethacin, Chlorpromazine, Gentamicin, and Parabens *In Vitro* and in Newborn Infants

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Abstract □ Displacement of bilirubin bound to human serum albumin by ampicillin, indomethacin, chlorpromazine, gentamicin, methylparaben, and propylparaben was investigated quantitatively. Two methods were used *in vitro*: measurement of bilirubin displacement by studying the rate of bilirubin oxidation with hydrogen peroxide and peroxidase and determination of the albumin reserve for binding of bilirubin by observation of the dialysis rate of an added trace amount of a deputy ligand monoacetyldapsone (*p*-acetamido-*p'*-aminodiphenyl sulfone). The latter method was also used for the determination of the albumin reserve in sera from treated newborn infants. The following doses were given: ampicillin, 100 mg/kg iv; indomethacin, 0.2 mg/kg iv; chlorpromazine hydrochloride, 0.7 mg/kg im; gentamicin sulfate, 2.5 mg/kg im. The parabens were present in injectable preparations of chlorpromazine and gentamicin and were therefore given in the following doses: methylparaben, 0.35 mg/kg, and propylparaben, 0.05 mg/kg. All drugs were given in a single dose. A few additional additives and metabolites were studied *in vitro*. Ampicillin, given to 19 infants, produced a small, significant decrease in plasma albumin reserve, to 82% of the pretreatment level and, thus, had a slight bilirubin-displacing effect, quantitatively consistent with a weak displacing effect measured *in vitro*. None of the other substances showed any measurable displacement *in vivo*, likewise in agreement with the results from *in vitro* studies.

Keyphrases □ Bilirubin—displacing effect of ampicillin, indomethacin, chloropromazine, gentamicin, and parabens, *in vitro* and in newborn infants □ Ampicillin—bilirubin-displacing effects of indomethacin, chlorpromazine, gentamicin, and parabens, *in vitro* and in newborn infants □ Indomethacin—bilirubin-displacing effects of ampicillin, chlorpromazine, gentamicin, and parabens, *in vitro* and in newborn infants □ Chlorpromazine—bilirubin-displacing effects of ampicillin, indomethacin, gentamicin, and parabens, *in vitro* and in newborn infants □ Gentamicin—bilirubin-displacing effects of ampicillin, indomethacin, chlorpromazine, and parabens, *in vitro* and in newborn infants □ Parabens—bilirubin-displacing effects of ampicillin, indomethacin, chlorpromazine, and gentamicin, *in vitro* and in newborn infants

A few drugs, notably sulfonamides, are capable of occupying the bilirubin-binding capacity of albumin in plasma, thereby increasing the risk of kernicterus in icteric human neonates (1, 2) as well as in experimental animals (3). Studies of such binding interactions *in vitro* have indicated displacing effects of ampicillin (4), indomethacin (4), some antimicrobial additives (parabens and sodium benzoate) (4–6) and chlorpromazine (7). Gentamicin in itself does not interfere with binding of bilirubin to albumin but is marketed with displacing additives (8). Measurement of the rate of dialysis of an added trace amount of monoacetyldapsone (I) into an otherwise identical plasma sample without this additive has recently been introduced as a technique for quantitative studies of occupation of albumin by drugs (9). Compound I serves as a deputy ligand for bilirubin, since one molecule of I competes selectively with binding of one molecule of bilirubin to human albumin (10). Determinations in undiluted sera at 37° are possible with this technique. Drug effects can thus be studied quantitatively *in vivo* as well as *in vitro*.

Previous work (11, 12) has shown that the concentration of free bilirubin in plasma may return quickly to the pretreatment level after administration of a bilirubin-displacing drug. Since there are no suitable methods for measuring the free bilirubin concentration in undiluted samples of infant serum (often hemolytic) at body temperature, it was decided to base the present *in vivo* studies on a combination of three measurements: determinations of albumin, bilirubin (bound), and albumin reserve in serum samples obtained before giving a single dose of the drug and at one point of time thereafter. The theoretical basis for this principle will be discussed, and the drugs mentioned will be tested accordingly.

EXPERIMENTAL

Human serum albumin[1] was obtained in the lyophilized state with its natural content of fatty acids and was used as a standard in albumin and

[1] AB Kabi, Stockholm, Sweden.

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Table I—Pretreatment Data of the Patients [a]

	Number of Patients	Birth Weight, g	Gestational Age, days	Age at Injection, days	Plasma Concentrations, μM		
					Unconjugated Bilirubin	Reserve Albumin for Binding of I	Albumin
Ampicillin	19	2100 (1300–3680)	241 (218–286)	5 (1–27)	94 (12–160)	67 (23–141)	472 (365–531)
Indomethacin	6	1490 (810–2130)	222 (198–237)	15 (11–21)	72 (14–178)	44 (13–103)	509 (437–638)
Chlorpromazine, Methylparaben	6	3230 (2630–3620)	287 (274–307)	2 (1–28)	64 (11–130)	149 (88–307)	546 (495–604)
Gentamicin, Methylparaben, Propylparaben	14	2240 (1250–3350)	243 (199–291)	5 (1–23)	74 (14–172)	128 (34–278)	515 (399–592)

[a] Median; number in parentheses is the range.

albumin reserve determinations. Bilirubin[2] was purified according to a previous study (13). The drugs for *in vitro* studies were obtained commercially. The following solutions were used for injections: ampicillin[3], 1.0 g of the sodium salt was dissolved in 5 ml of sterile water. Indomethacin[4]; 20 mg dissolved in 2 ml of sterile water. Chlorpromazine[5] was obtained as an injectable solution containing 2.0 mg of chlorpromazine hydrochloride and 1.0 mg/ml of methylparaben. Gentamicin[6] injectable solution contained 10 mg of gentamicin sulfate, 1.3 mg of methylparaben, 0.2 mg of propylparaben, and 3.2 mg of sodium pyrosulfite/ml.

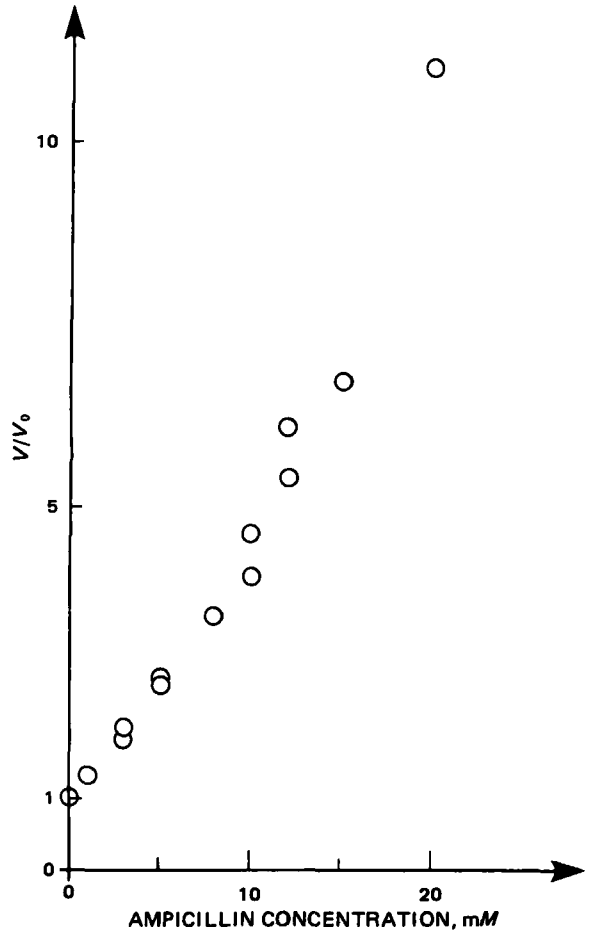

Figure 1—*Rate of oxidation of bilirubin with hydrogen peroxide and horseradish peroxidase in a solution containing 30 μM of human serum albumin and 15 μM of bilirubin as a function of varying concentrations of ampicillin (abscissa). The ordinates are oxidation rates relative to the rate without ampicillin. Sodium phosphate buffer, 66 mM, pH 7.4, 37°.*

[2] Sigma Chemical Co., St. Louis, Mo.
[3] Anhypen, Gist-Brocades, Delft, Netherlands.
[4] Indometacin, Dumex Ltd., Copenhagen, Denmark.
[5] Klorpromazin, DAK Laboratories, Copenhagen, Denmark.
[6] Garamycin, Schering Corp., Kenilworth, N.J.

Bilirubin-displacing effects of the drugs were studied *in vitro* by the following two techniques. The increase of free bilirubin dianion concentration in pure solutions of albumin and bilirubin with the addition of varying concentrations of the drugs was measured by the rate of bilirubin oxidation with hydrogen peroxide and horseradish peroxidase, as previously described (14) at pH 7.4, 37°. None of the drugs studied inhibited the peroxidase process in the absence of albumin. The amount of available albumin after addition of the drug was measured by the rate of dialysis of I, added in low concentration, into an identical volume of the same solution without added I (9). This method was used in its micromodification (10) with ^{14}C-labeled I and with 20-μl compartment volumes of the dialysis chambers. One volume of 0.53 *M* sodium phosphate buffer was added to 15 volumes of the sample to obtain a pH close to 7.4. The temperature was 37°, dialysis time was 10 min. The latter technique was used for studies with pure solutions of albumin as well as with infant serum, in both cases with drugs added in varying concentrations.

The following patients were used for investigations *in vivo*. Nineteen newborn infants suspected of having sepsis were given ampicillin, 100 mg iv/kg of body weight. Six newborn, preterm infants with patent ductus arteriosus received indomethacin, 0.2 mg/kg iv. Six newborns with narcotic withdrawal syndrome were given chlorpromazine hydrochloride, 0.7 mg/kg im. Fourteen newborn infants suspected of having sepsis were

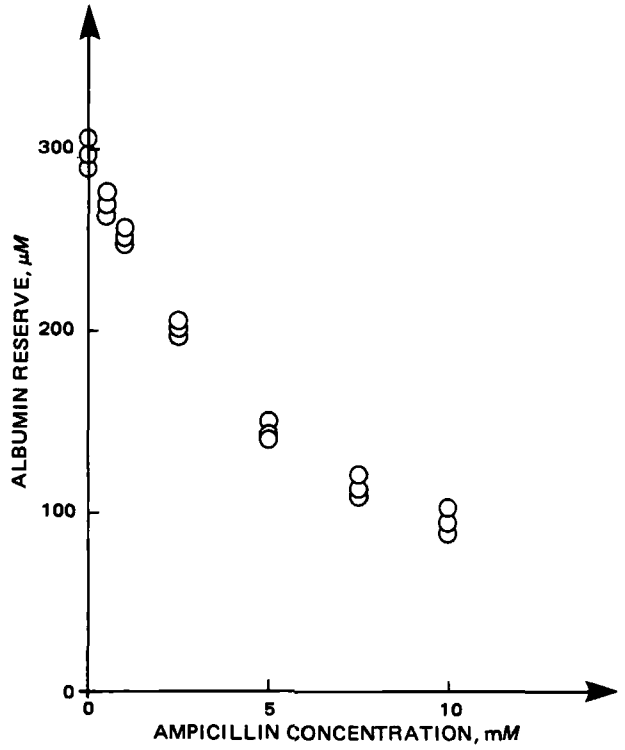

Figure 2—*Reserve albumin concentration for binding of I (ordinates), determined by measuring the rate of dialysis of an added trace amount of [^{14}C]I through a membrane into another compartment containing the same sample without added I. The sample was a solution of human serum albumin, 300 μM, varying concentrations of ampicillin (abscissa). Buffer and temperature as in Fig. 1.*

Table II—Displacing Effects *In Vitro*

	Displacing Drug Concentration					
	Peroxidase Method[a]		Monoacetyldapsone (I) Method[b]		Maximum Drug Concentration in Plasma[c]	
	μM	mg/liter	μM	mg/liter	μM	mg/liter
Ampicillin	3100	1100	4800	1700	5600	1950
Indomethacin	30	11	350	125	11	4
Chlorpromazine	5	1.6	1200	380	39	12
Gentamicin	>5000	>2000	>5000	>2000	111	50
Methyl *p*-hydroxybenzoate	18	2.7	1200	180	44	7
Propyl *p*-hydroxybenzoate	19	3.4	350	65	5	0.9
Sodium *p*-hydroxybenzoate	13	2.1	>2000	>320	44	7
Benzyl alcohol	4000	432	20,000			
Sodium benzoate	600	90	2200			
Sodium hippurate	500	100	750			
Sulfisoxazole	52	14	450			

[a] Concentration doubling rate of oxidation of bilirubin with H_2O_2 and peroxidase (test system contains human serum albumin, 30 μM, and bilirubin, 15 μM). [b] Drug concentration doubling rate of dialysis of I (test system contains human serum albumin, 300 μM, and I, 3 μM). [c] Drug concentration if dose were distributed in infant's plasma volume.

given gentamicin sulfate, 2.5 mg/kg im. Pretreatment data of the patients are summarized in Table I.

Capillary blood samples were obtained by heel prick. One sample was taken immediately before the administration of a single dose of the drug. A second sample was obtained 15 min after giving the intravenously injected drugs and 30 min after those given intramuscularly.

Total unconjugated bilirubin was determined in serum by a modification (15) of a previous method (16). This method was chosen under the assumption that it is more specific than the diazo method. Fifty microliters of serum and 1250 μl of acetone were mixed and centrifuged, thereafter the absorbance of the supernatant was measured at absorption maximum 454 nm[7]. Purified bilirubin (13), dissolved in 0.1*M* potassium cyanide in formamide (17) and added to human serum, was used for the standardization. The coefficient of variation of the analysis was 2% by duplicate determinations.

Albumin was determined in serum by an electrophoretic method (18). The coefficient of variation of the analysis was 3% by duplicate determinations.

The concentration of reserve albumin for binding of I was determined in serum samples from the patients as mentioned above. By triplicate determinations, the coefficient of variation of the analysis was 5–6%, the intraday coefficient of variation being 4–5%.

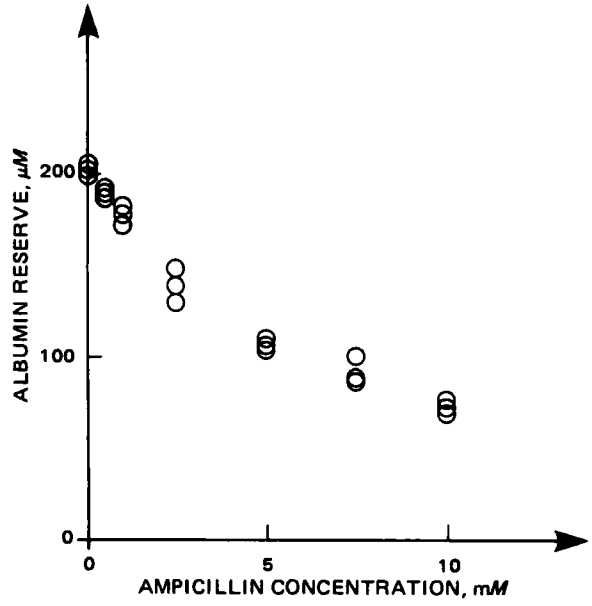

Figure 3—*Reserve albumin concentration for binding of I, determined as in Fig. 2 but in pooled infant (umbilical cord) sera with varying concentrations of added ampicillin, pH 7.4, 37°. Albumin concentration in the infant serum was 500 μM, nonesterified fatty acids, 460 μM. Note that the albumin reserve for binding of I, and thereby for binding of bilirubin, is only 40% of the total albumin concentration and in agreement with previous findings (19).*

[7] Zeiss PM2D spectrophotometer.

Statistical analyses were performed using the *t* test for paired observations. Ninety-five percent confidence limits for the true change in the plasma concentrations of unconjugated bilirubin, reserve albumin, and albumin, have been calculated from the formula: Confidence limits = $\bar{x} \pm t_{0.975} \times SD/\sqrt{n}$, where $t_{0.975}$ is the 97.5 percentile of the *t* distribution with $n - 1$ degrees of freedom.

Informed consent was obtained from the mothers of all infants included in the study.

RESULTS

***In Vitro* Studies**—All drugs and additives except gentamicin, showed some degree of bilirubin displacement (Table II, Figs. 1–3). Drug concentrations giving a twofold increase of oxidation rate in the peroxidase method, and a twofold increase of dialysis rate in the I method, are reported. In both techniques this ideally should be the concentration of drug needed to establish a binding equilibrium in which half of the vacant albumin capacity for binding of bilirubin is occupied by bound drug, increasing the free bilirubin concentration twofold. Major discrepancies are seen between the two methods and will be discussed later.

***In vivo* Studies**—Table III shows the observed changes in plasma concentrations of unconjugated bilirubin, reserve albumin for binding of I, and albumin, caused by a single injection of the four preparations studied.

Ampicillin, injected intravenously in a dose of 100 mg/kg, resulted in a significant decrease of average reserve albumin for binding of I by 18% in samples taken 15 min after the injection. The plasma albumin concentration remained unchanged, indicating that the decrease of reserve albumin was not caused by dilution of the infants' blood. It is noteworthy that the concentration of unconjugated bilirubin in the plasma was not influenced by injection of ampicillin. Individual values of reserve albumin concentrations for binding of I, obtained from plasma samples before and 15 min after giving ampicillin to 19 infants, are plotted in Fig. 4. The albumin reserve after treatment ranged from 62 to 100% of pretreatment levels. Unconjugated bilirubin and albumin concentrations remained unchanged.

The other preparations examined, indomethacin, chlorpromazine with methylparaben, and gentamicin with methylparaben and propylparaben, did not cause any change of the measured parameters.

DISCUSSION

Choice of Analytical Methods—Methods for the study of bilirubin-displacing effects of drugs *in vitro* and *in vivo* should measure either the free bilirubin dianion concentration or the reserve albumin for binding of bilirubin. Results should be obtained in quantitative terms, measurements should be performed in undiluted serum or plasma and at a pH and temperature the same as that of the patient. Techniques should be avoided in which the parameter to be measured is shifted by the addition of large amounts of binding dyes, by removal of bilirubin to agarose, or by attempted titration of albumin binding capacity with bilirubin, as previously discussed (20). The peroxidase method, whereby changes of free bilirubin dianion concentration are estimated by variations of the rate of oxidation with hydrogen peroxide and peroxidase (14), can be used for *in vitro* studies in pure solutions of albumin in low concentrations. False indications of displacement are, however, obtained

Table III—Changes in the Plasma Concentrations of Unconjugated Bilirubin, Reserve Albumin for Binding of I, and Albumin After a Single Dose of Ampicillin, Indomethacin, Gentamicin with Additives, and Chlorpromazine with Additive

	Changes in Plasma Concentrations					
	Unconjugated Bilirubin		Reserve Albumin for Binding of I		Albumin	
	Percent	μM[a]	Percent	μM[a]	Percent	μM[a]
Ampicillin	−2	−2 (−7, +2)	−18	−12 (−16, −8)[b]	0	0 (−12, +12)
Indomethacin	0	0 (−7, +7)	0	0 (−4, +3)	+1.0	+5 (−24, +33)
Chlorpromazine Methylparaben	0	0 (−6, +5)	+3	+5 (−12, +23)	−1.5	−8 (−21, +6)
Gentamicin Methylparaben Propylparaben	+1	+1 (−1, +3)	−1	−1 (−6, +4)	−0.6	−3 (−16, +10)

[a] Mean (95% confidence limits). [b] $p < 0.001$; all other changes are insignificant, $p > 0.05$.

with certain drugs, forming free radicals on oxidation, especially with some derivatives of phenol and phenothiazine (5, 7), as exemplified by the parabens and chlorpromazine. Also, due to the presence of hemoglobin, which has a peroxidase effect, the peroxidase method cannot be used in infant sera.

An alternative approach is determination of the concentration of albumin available for binding of bilirubin. In the presence of a displacing drug, the albumin reserve is generally decreased in the same proportion as the free bilirubin concentration is increased. A trace amount of a deputy ligand for bilirubin, I, is added to the sample, and the binding of this ligand is assessed by observing its rate of dialysis into a compartment containing the same sample without added I. The difficulties connected with determination of free bilirubin are obviated. On the other hand, individual drugs may displace I and bilirubin to different degrees due to differences of allosteric effects, a difficulty avoided in the peroxidase method where displacement of bilirubin itself is studied.

Results Obtained *In Vitro*—Table II shows considerable differences between results obtained for displacement of bilirubin with either of the two methods. These are explained by the above-mentioned free-radical effects, by possible differences of allosteric effects upon binding of bilirubin and I, and by the differences of albumin concentrations.

At equilibrium, half of the available albumin molecules will be occupied by a drug at a specific site when the concentration of free drug is equal to the inverse binding constant. The total drug concentration needed to obtain this comprises the concentrations of free drug, of drug bound to the specific site, and of drug bound to other sites on the albumin molecule. A higher albumin concentration was used in the I method than in the peroxidase test, and it was expected that half-saturation of the bilirubin site required a higher concentration of the drug in the former technique. The discrepancy could be avoided if equal concentrations of albumin were employed. This is not practical for routine purposes, since increased concentrations in the peroxidase method cause increased rates of unspecific oxidation reactions (7), while decreased concentrations in the I technique resulted in poor precision due to weak binding of I. Previous experience with sodium benzoate and other substances has shown, however, that identical results may be obtained if equal concentrations of albumin are used in both methods (9).

Ampicillin gave fairly consistent results in the two methods (Table II); the somewhat higher concentration of ampicillin needed to occupy half of the albumin in the I method is explained by the higher concentration of albumin. The dose of ampicillin injected during the *in vivo* studies would, if it were evenly distributed in the plasma volume of the infant, give a slightly higher concentration, as seen in the last column of Table II, and would thus occupy slightly more than half of the total amount of circulating albumin. Due to distribution of the drug into tissues, smaller degrees of occupation of albumin would be expected at 15 min after the injection. Distribution volumes for ampicillin, calculated from literature data (21), range from 8 to 12 times the plasma volume. It is thus theoretically conceivable that ampicillin, in a dose of 100 mg/kg iv, would occupy a fraction of the plasma albumin somewhere between 5 and 50%.

Indomethacin is bound firmly to albumin, and it seems likely that the higher concentration needed to occupy half of the albumin in the I method, when compared with the peroxidase results, can be explained by the higher albumin concentration in the former technique. The I method gave a more reliable basis for an estimate of the displacing effect *in vivo* and showed that larger amounts of indomethacin than given would be needed to occupy an appreciable fraction of the albumin. It was concluded that the dose of indomethacin was too small to cause any considerable bilirubin displacement.

Chlorpromazine gave a false indication of displacement in the peroxidase method, as previously reported (7), and again the I method must be relied upon. The dose given was too small to produce a significant displacement.

Gentamicin, in agreement with previous reports (8), did not occupy bilirubin-binding loci on the albumin molecule in any of the two tests and, therefore, was not expected to cause displacement.

The parabens, and their metabolite, *p*-hydroxybenzoic acid, again gave falsely high displacements in the peroxidase method. The doses given were apparently too low to cause displacement.

Benzyl alcohol and sodium benzoate are sometimes used as antimicrobial additives in injection medicines, and sodium benzoate is present in many foods, naturally or added. These substances and their metabolite, sodium hippurate, must be added in fairly high concentrations in the I technique in order to occupy half of the albumin. Excessive doses would probably be needed to produce a significant displacement. This is in agreement with results published previously (22).

Sulfisoxazole has been tested for comparison. Therapeutic plasma concentrations of this drug are in the range of ~800 μM, considerably in excess of the concentrations needed to occupy half of the albumin in both techniques. Sulfisoxazole should thus be capable of producing displacement *in vivo*, in agreement with clinical experience (1, 2).

Procedure for Testing *In Vivo*—Due to shortcomings in the *in vitro* methods, as discussed above, and due to possible displacing effects of drug metabolites, it may be necessary sometimes to test a drug *in vivo*. Some information can be obtained from experiments in Gunn rats, observing displacement by a fall of total bilirubin concentration in plasma after drug administration (3, 23) or by damage to Purkinje cells after drug-induced displacement of bilirubin into the central nervous system (24). However, species differences of drug metabolism and of binding to albumin prevent final conclusions from animal experiments. The ultimate test must be conducted in human patients.

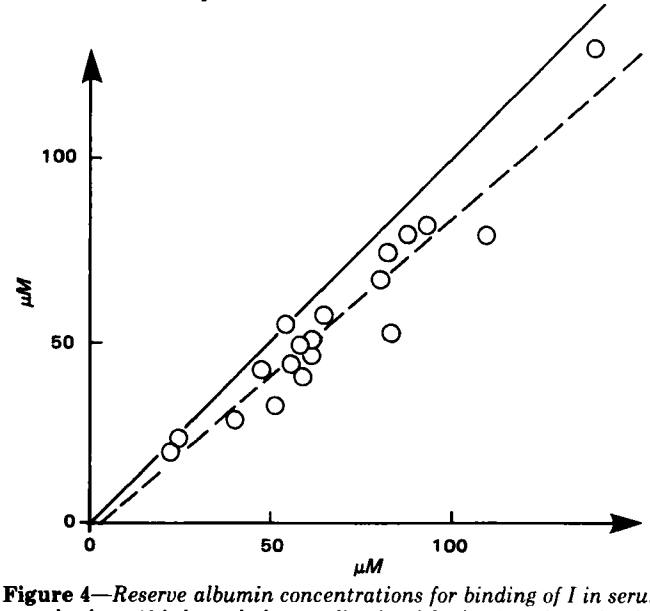

Figure 4—*Reserve albumin concentrations for binding of I in serum samples from 19 infants, before medication (abscissa), and 15 min after giving ampicillin (ordinate) by intravenous injection, dose 100 mg/kg. pH 7.4, 37°. A full line, x = y, is shown. The regression line, stippled, is y = 0.87x − 3 μM. The average decrease of reserve albumin concentration was 18% of the pretreatment level with 95% confidence limits, 12 and 24%. The average decrease was significant, p < 0.001, by paired t test.*

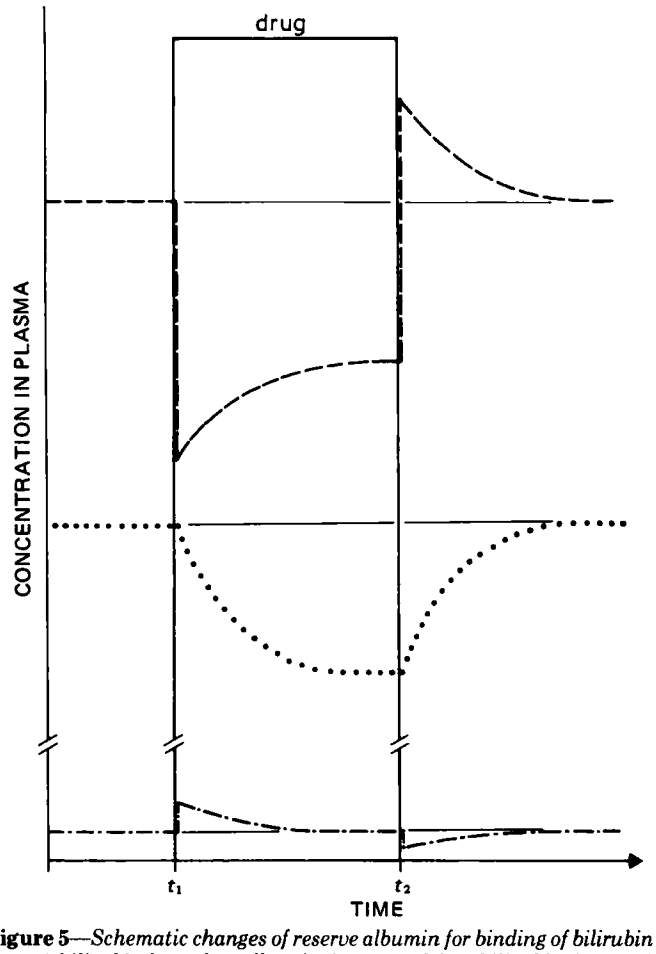

Figure 5—*Schematic changes of reserve albumin for binding of bilirubin (- - -) bilirubin bound to albumin (····), and free bilirubin (- · - · -) resulting from administration of a drug. A constant plasma concentration of the drug is hypothetically established during the interval of time from* t_1 *to* t_2.

The pharmacokinetic consequences of giving a displacing drug should be considered before a final choice of technique for *in vivo* studies is made. Wennberg and Rasmussen (25) have pointed out that "kernicterus may develop from bilirubin displaced during transient high serum concentrations of a competing drug." A theory has been worked out by Levy and Yacobi (11). As a consequence, the following train of events may be envisioned (Fig. 5). A steady state is thought to be present before the drug is given, with constant plasma concentrations of bound bilirubin, free bilirubin, and reserve albumin with a vacant capacity for binding of bilirubin. A constant concentration of the drug is established at the time t_1. The concentration of reserve albumin decreases immediately, since the binding capacity is partially occupied by the drug. This causes an immediate increase of free bilirubin concentration followed more slowly by transfer of bilirubin from plasma into tissues or to red blood cells, whereby the bound bilirubin concentration in plasma decreases. The reserve albumin increases with the decrease of bilirubin until a new steady state is established with the pretreatment level of free bilirubin and with decreased bound bilirubin and reserve albumin.

Such changes of free and bound bilirubin have been experimentally verified (12) after giving sulfisoxazole to rats, in which it was found that the free bilirubin concentration, after a sharp, transient increase, returned to its pretreatment value in spite of continued infusion of the displacing drug. If the drug is now removed instantly (time t_2), the concentration of reserve albumin will increase to above pretreatment level, resulting in a fall of free bilirubin which again will cause back-diffusion of bilirubin from tissues to plasma, leading to re-establishment of the original steady state.

These changes have not been experimentally verified and will be less acute when the drug level recedes gradually. It will, however, be theoretically possible to have normal or increased levels of reserve albumin when the drug concentration is declining. In this case, the bound bilirubin concentration will be below pretreatment value.

In conclusion, if a single blood sample is obtained after giving the drug, as in the present study, it will be possible to observe an unchanged level, either of bound bilirubin or of reserve albumin, even if the drug has caused displacement of bilirubin. However, unchanged concentrations of both bilirubin and reserve albumin cannot occur at the same time if a displacing drug was given. The concentration of bound bilirubin[8] as well as that of reserve albumin should be measured accordingly. In addition to this, determination of albumin should be done, since hemodilution by injection of the drug theoretically may cause low values of bound bilirubin and reserve albumin, imitating displacement of bilirubin. The sample should be obtained while the drug level is high. If all three concentrations (bound bilirubin, albumin, and reserve albumin), under these circumstances are unchanged by the medication, it may be concluded that bilirubin displacement was not observed.

Results Obtained *In Vivo*—After giving ampicillin, a small, statistically significant decrease of reserve albumin was observed (Table III, Fig. 4), indicating that a fraction of the available albumin binding capacity for bilirubin was occupied by this drug. The average occupancy observed, 18%, and the range of individual values is in good agreement with those expected from the *in vitro* tests.

Indomethacin, chlorpromazine, gentamicin, and the parabens did not cause any change of reserve albumin, total unconjugated bilirubin, or albumin concentrations and did not show displacing effects in the doses given (Table III). This again is consistent with expectations from *in vitro* results.

The practical significance of the bilirubin-displacing effect of ampicillin may be evaluated as follows. Effective plasma concentrations of ampicillin are usually considered to be in the range of 120–180 μM (40–60 μg/ml). These concentrations would cause a 3–5% increase of free bilirubin concentration, as measured by the peroxidase method (Fig. 1), using a low albumin concentration of 30 μM. The real displacement of bilirubin in plasma would be less. The I technique, applied to infant sera with varying added concentrations of ampicillin, shows that the reserve albumin concentrations would be decreased by 1.5–2.5% (Fig. 3), and the free bilirubin concentration would be increased by a similar percentage. This latter estimate seems to indicate a very small or insignificant increase of the risk of bilirubin encephalopathy, if weighted against the benefit obtained from the antibacterial effect of the drug. Somewhat higher degrees of displacement were seen in the present study; in one patient as much as 38% of available albumin for binding of I was occupied, presumably indicating an increase of free bilirubin concentration by a percentage of the same order of magnitude. In cases of threatening kernicterus, when such an increase of free bilirubin concentration may be unwanted, ampicillin could be given by slow injection, or by continuous infusion, thus avoiding significant occupation of albumin by the drug (26).

In previous rat experiments (12) the plasma concentration of unconjugated bilirubin dropped acutely to a lower level in <5 min after intravenous administration of a displacing drug. No change of bilirubin concentration was observed in the patients, in a sample taken 15 min after giving ampicillin. It might appear as if this finding could be taken as evidence against a bilirubin-displacing effect of ampicillin in human infants. Considerations of the rate of the expected decline of plasma bilirubin concentration show, however, that a measurable change could not be anticipated within 15 min, even after administration of a drug occupying half of the available albumin. The rats received a continuous infusion of bilirubin, regulated after a larger initial dose to a continuous rate of 100-fold that of normal bilirubin turnover in the rat, resulting in a high, constant concentration of unconjugated bilirubin in the plasma. The rate of infusion of bilirubin was 0.55 μmole/kg/min, the plasma bilirubin pool of the rats in the stationary state was ~7 μmoles/kg, and the turnover time of plasma bilirubin was thus ~13 min. However, in the infants, bilirubin turnover was of the order of 0.005 μmole/kg/min, and the total plasma bilirubin pool in the average patient was ~5 μmoles/kg (total unconjugated plasma bilirubin concentration, 94 μM, and plasma volume, 55 ml/kg), which gives a turnover time of ~1000 min for plasma bilirubin of the infants.

If a drug that occupies half of the available albumin is given to the infant one can expect that the free bilirubin concentration is doubled and that the rate of bilirubin removal from the plasma is doubled. This will result in a decline of the bound (and total) bilirubin concentration to a final value of ~50% of the pretreatment level. This decline would proceed slowly, with a rate constant of 0.001/min. In 15 min, the total bilirubin concentration would thus decline from 100 to 99.2 μM, an immeasurable

[8] The concentration of bound bilirubin for practical purposes is measured as that of total, unconjugated bilirubin, since the concentration of the free pigment is very low compared with the bound.

change. In addition, observation of such a slow change is precluded by the fact that the bound bilirubin concentration in these infants increases by an average of 2 μM/hr when no treatment is given. Observation of total plasma bilirubin concentrations alone, in consequence, cannot be used for estimating bilirubin-displacing effects of drugs, given to human infants. Measurement of the albumin reserve should be the main tool for this purpose. As shown above, plasma bilirubin and albumin concentrations should be measured in parallel. Unchanged values of all three parameters, observed at a point in time when the drug concentration in plasma is high, constitute evidence against a bilirubin-displacing effect.

These results underline the necessity of using quantitative methods for evaluation of bilirubin-displacing effects. Drugs cannot be rated as displacing or nondisplacing; dosage and plasma concentrations should be related to the displacing effect, expressed in quantitative terms. This method using monoacetyldapsone (I) seems feasible for such studies *in vitro* and *in vivo*.

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Elementary Osmotic Pump for Indomethacin

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Abstract □ Based on the principles of an elementary osmotic pump, systems were designed to deliver indomethacin in solution at a constant rate, Z, to contain a total amount of drug, M_t, and to deliver 80% of their content at time t_{80}. To allow selection of the optimal delivery rate into the body, three different prototypes were prepared with respective values for Z, M_t, and t_{80} of: 7 mg/hr, 85 mg, 11 hr; 9 mg/hr, 85 mg, 8 hr; and 12 mg/hr, 85 mg, 6 hr. These systems were found to deliver 70% of each system's contents at zero-order rates. Delivery rates were independent of pH, method of measurement, and stirring rate. In keeping with these results, the systems in the GI tract of dogs delivered at the same rate as *in vitro*, which qualifies the *in vitro* test as a bioanalogous method predictive of the *in vivo* performance of the dosage forms. Preliminary results in normal volunteers yielded similar urinary recoveries, while plasma profiles were different from each other and distinct from those following conventional capsules.

Keyphrases □ Indomethacin—design and preliminary evaluation of an oral osmotic delivery system, zero-order drug delivery □ Osmotic pump—oral, design and preliminary evaluation, indomethacin □ Drug delivery—design and preliminary evaluation of an oral osmotic delivery system containing indomethacin □ Anti-inflammatory agents—indomethacin, design and preliminary evaluation of an oral osmotic delivery system

The concept of continuous drug delivery that maintains the lowest delivery rate and that will elicit a therapeutic effect has much appeal. Intuitively, such a situation should represent the most efficacious use of the drug, while presenting a minimal risk of adverse reactions. Within certain prescribed constraints, theoretical analyses (1, 2) appear to favor dosing patterns that approach a constant *infusion*. However, direct experimental support for this hypothesis is limited (3). In part, this shortcoming may result from a lack of a practical way to deliver a suitable drug chronically and at a constant rate.

The recent development of an oral dosage form, based

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