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DRUG-PROTEIN BINDING IN YOUNG CHILDREN

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INTRODUCTION

In many instances, the young infant and developing child have been found to differ from the adult in their response to drugs. This information was initially obtained from clinical experiences in which unexpected adverse (and sometimes fatal) reactions occurred when drugs were administered to this population for therapeutic purposes. Recognition of these differences in pharmacologic response has led to clinical investigations which have shown considerable alterations (usually a diminution) in the processes of biotransformation and renal excretion of drugs.

The investigations of drug-protein binding in the infant and child is of special interest because of the role which drug protein interaction plays in drug distribution. The various body compartments change throughout life and have absolute and relevant size differences from one age period to another. These differences are most marked in the newborn infant where total body water is much greater than in the adult, and varies from as much as 85 percent of body weight in the small premature infant, to 75 percent in the full term infant, until an average value of about 60 percent of body weight is reached at one year of age. Extracellular volume is approximately 40 to 45 percent of total body weight in the newborn in comparison to 27 percent in the one-year-old infant and 15 to 20 percent in the adult. This change mainly reflects interstitial volume alterations since the plasma compartment is relatively constant (4 to 5 percent of body weight) throughout life. Intracellular water increases from 34 percent of body weight at birth to 43 percent at about three months of age and decreases to a value again equal to that of the newborn.

Between years one and three, all of these compartments increase and then gradually decline to adult values. Fat content is lower in the premature infant (1 percent) than in the normal term infant where it is 15 percent of body weight. Skeletal muscle mass is also reduced in the young infant to 25 percent of body weight. Of importance is the fact that organ relationships are also quite different in the young infant, with the brain and liver representing a greater proportion of body weight than in the older organism. In addition, organ composition may also be different, and this may affect drug distribution. For example, myelin content is low in the young infant and cerebral blood flow higher than in the adult. With these significant variations in body composition throughout the pediatric age group, it is not surprising that changes in drug distribution can be anticipated. Of particular importance is the fact that development of the blood-brain barrier in the young organism is incomplete and, as a consequence, there is increased permeability of lipid-soluble drug substances into the brain.

In addition to the above-mentioned factors which will modify drug distribution in the young infant and child, another important developmental variable which will affect this process is the rate and extent of binding to protein. The first suggestion that there might be differences in drug interaction in the neonate came from the studies of Silverman and colleagues more than 25 years ago (Silverman et al. 1956). These investigators carried out a randomized controlled clinical trial of two antibacterial regimens prescribed in order to reduce infections commonly seen in low birth weight infants. A primary purpose of the study was to evaluate the safety and efficacy of a new broad spectrum antibiotic. The control group received penicillin and a sulfonamide. The mortality was significantly greater in the control group and the cause of death as determined at autopsy was due to bilirubin neurotoxicity. Surprisingly, the concentrations of bilirubin determined in the serum were lower than in the group receiving the broad spectrum antibiotic. The mechanisms for the lower serum concentration of bilirubin observed in the affected infants were subsequently shown by Odell to be due to an actual displacement of bilirubin from its binding sites on plasma proteins. Thus, the neurotoxicity or kernicterus was due to an increase in the volume of distribution of bilirubin in the young infant who is physiologically compromised because of its inability to metabolize bilirubin (Odell 1959). Despite the considerable stimulus given to the investigation of drug-protein interaction from these observations, significant gaps still exist in our knowledge of this phenomenon in the pediatric population. In the following sections, four different examples of drug protein interaction in the young child will be presented in order to

emphasize the variations which occur in this phenomenon at different developmental stages:

1. Drug-protein binding in the neonate
2. Drug-protein interaction in the infant (beyond the neonatal period)
3. Bilirubin-protein binding
4. Drug-binding during pregnancy

DRUG-PROTEIN BINDING IN THE NEONATE

Drug protein interaction in infants and children has been most frequently investigated in neonates. This is probably due to the ready availability of plasma or serum from umbilical cord blood. Most published reports have indicated that the degree of binding is lower in newborn plasma or serum than in the adult. We have confirmed these findings in our own studies but have shown that there are certain exceptions to this rule. Some of our findings are summarized in the paragraphs below.

The unbound fraction of phenytoin measured in cord plasma from 13 normal infants was 10.6 ± 1.4 percent (Rane et al. 1971). The corresponding value in the adult plasma is 7.4 ± .7 percent. The range of values in individual samples was much greater in the newborn plasma than in adult. There was no difference in the binding capacity for phenytoin at the concentration of 16 μg/ml between cord plasma and plasma obtained by venipuncture from two normal newborn infants. The albumin concentrations in these 13 infants ranged from 3.0 to 4.2 g/100 ml, and a significant relationship was found between the binding capacity and the plasma albumin concentration. As expected, there was less binding at 37°C than at room temperature, with an approximate 50 percent increase in the unbound fraction at the higher temperature. We also investigated the binding of phenytoin in 20 hyperbilirubinemic infants with a concentration of total bilirubin (mainly unconjugated) ranging from 4.5 to 24.5 mg/100 ml of serum. There was a significant correlation between the amount of the unbound fraction of phenytoin and the total concentration of bilirubin. At concentrations of bilirubin greater than 20 mg/100 ml, the unbound fraction of phenytoin was twice as high as in plasma from non-hyperbilirubinemic infants. The correlation became even greater when the albumin concentration was taken into consideration. These results furnish additional support for the contention that the principal protein which binds phenytoin is albumin. While at first glance these findings might suggest that phenytoin and bilirubin compete for the

same binding site on the albumin molecule, subsequent investigations revealed that phenytoin did not affect bilirubin binding to albumin. It is also possible, therefore, that bilirubin interferes with phenytoin binding either by altering the conformation of the albumin molecule, or that variations in binding strength exist between the two substrates.

Nafcillin binding was studied with equilibrium dialysis (Krasner and Yaffe 1975). The unbound nafcillin concentration in the dialysate was measured by microbiological assay. The percent of bound nafcillin decreased from 64 percent at low antibiotic concentrations (5 μg/ml), to as low as 28 percent bound at concentrations of 200 μg/ml. Compared to adult values reported for this antibiotic of 86 percent bound, this result represents a significant difference in binding properties between human adult and newborn cord sera.

Equilibrium dialysis was also used to investigate the binding of diazepam to serum proteins. In this case, the low solubility of diazepam in an aqueous system was an obstacle. An approach somewhat like that used by the manufacturer was employed to dissolve diazepam with 1.8 percent propylene glycol and 0.45 percent ethanol. These concentrations of organic solvents did not appear to affect the binding. Approximately one mole of diazepam is bound per mole of albumin. The apparent association constant derived from Scatchard plots is approximately $4 \times 10^5\ M^{-1}$. This same binding constant was obtained with cord serum, indicating no difference in binding properties between cord and adult serum proteins. Despite the similarity in the association of binding, protein-binding studied at a single diazepam concentration could result in a bound difference between cord serum and adult serum due to the lower concentrations of protein found in the newborn infant.

Salicylate binding to cord serum was also studied with equilibrium dialysis. One mole of salicylate was bound per mole of albumin at the primary site. The association constant found for cord serum was $2.9 \times 10^5\ M^{-1}$. This differs slightly from the value found for adult serum ($4 \times 10^5\ M^{-1}$). When albumin fractionated from blood cord serum was investigated, an association constant of $1.7 \times 10^5\ M^{-1}$ was found. When cord serum was dialyzed for 16 hours against large volumes of buffer at 4°C, prior to instituting the binding study, the differences that existed between cord serum and adult serum disappeared. This tends to indicate that a water-soluble, easily dialyzable factor is present in cord serum which diminishes the affinity of serum proteins for salicylate.

The data available in the literature, and which have been presented in the preceding paragraphs, clearly demonstrate that for most drugs the binding capacity of protein differs in the newborn infant from that seen in the adult. Should these findings be operative in the intact organism, they would explain, in part, the frequent

association of side effects when drugs are administered to the newborn even though the dose employed takes into consideration the smaller size of the patient. The changes in binding may be due to lower concentrations of plasma proteins (particularly albumin). However, the methods employed in the formulation of the Scatchard plots normalize for the amount of protein present. Thus, the binding characteristics observed may be due to variations in the protein structure themselves. In addition, endogenous substances present during the first few days of life, especially hormones transferred across the placenta, may occupy binding sites and reduce binding capacity.

Kurz attempted to examine the mechanisms responsible for the differences in protein binding through a series of in vitro manipulations (Kurz, Mauser-Ganshorn, and Stickel 1977). Umbilical cord and adult plasma were adjusted to different protein concentrations by ultrafiltration. At equal protein concentrations in cord and adult plasma, there was still less binding to cord than to adult plasma for several drug substrates. To determine whether the factors responsible for the reduced binding to cord plasma were present in the protein fraction of plasma or in the ultrafiltrate, both cord and adult plasma were submitted to ultrafiltration. Then the ultrafiltrate of the cord plasma was added to the residue of the adult plasma and vice versa, in such an amount that the original protein concentration was restored. Under these circumstances, the cord and adult plasma still exhibited different binding properties, and Kurz concluded that the difference in binding properties must be due to intrinsic differences in the residual protein themselves. It should also be kept in mind that the sick newborn infant often receives many drugs for therapeutic purposes, and in the clinical situation, there may be considerable interaction and competition for binding sites. This, of course, does not apply when one examines binding characteristics using umbilical cord blood as a source of binding protein.

DRUG-PROTEIN BINDING IN INFANCY

For many drugs, the therapeutic dose is much smaller in the young infant than in older children and adults. This is true when the dose is expressed either on the basis of body weight or body surface area. This has been usually associated with immaturity of the mechanism of biotransformation and renal excretion, the major pathways for drug elimination. In this respect, digoxin is a unique substance because its therapeutic dosage is appreciably greater in infants than in adults. The recommended and most widely used digitalizing dosage is 60 to 80 μg/kg in the infant, as compared to only 10 to 20 μg/kg in the adults. The pharmacologic reasons for the requirement of such

a relatively large therapeutic dosage of digoxin in infants are not clear. We have shown that relative to their creatinine clearances, infants have an unusually large renal excretion of digoxin. Furthermore, when we examined drug-protein binding in umbilical cord serum, there was no difference between the newborn infant and the adult (Gorodischer, Krasner, and Yaffe 1974). We therefore attempted to look at drug tissue binding and distribution to determine whether differences might exist between infancy and adulthood.

Eleven infants with congenital heart disease and heart failure requiring digoxin therapy were studied (Gorodischer, Jusko, and Yaffe 1976). The infants weighed between 1560 and 6300 gm and were one to five months of age. Samples were obtained both during digitalization and during the course of maintenance therapy. Postmortem samples of myocardium and blood were obtained from six additional infants who had received digoxin therapy prior to death. Following accepted practice at our institution, the total digitalizing dose varied between 30 and 60 μg/kg divided into three intramuscular doses 8 hours apart. The maintenance dose varied between 12 and 20 μg/kg and was administered orally. Digoxin concentrations were measured by radioimmunoassay. Digoxin concentrations and tissue:serum concentration ratios did not seem to differ between samples obtained during the period of digitalization and in the course of maintenance therapy. There was a linear relationship between myocardium and serum digoxin concentrations, and no saturation characteristics were observed over the serum concentration range of 0.5 to 8.6 ng/ml. The mean myocardium concentration of digoxin was considerably higher than that seen in adults. When the myocardium:serum digoxin concentration ratio was determined to take into consideration the higher serum digoxin concentrations in the young infant, there was an appreciably greater ratio in the infant (mean 146 compared to the adult mean 68). Examination of the erythrocyte:plasma concentration ratio of digoxin in blood samples obtained from infants during digitalization and during maintenance therapy also revealed considerable differences with a higher erythrocyte:plasma digoxin concentration ratio during maintenance therapy in the infant than in the adult. During digitalization, the concentration ratio was approximately unity and this was the same as in adults.

The mechanism for the higher tissue uptake of digoxin in infants is unclear. The large apparent volume of distribution is not due to lower plasma protein binding of digoxin in infants since the serum binds digoxin to a limited and similar extent (25 percent) as in adult serum. Also serum concentrations of digoxin in infants are usually greater (and not smaller) than in adults. The finding of a much greater erythrocyte:plasma digoxin concentration ratio during maintenance therapy than during digitalization is intriguing. Since the

same methodology was used for digoxin measurement in erythrocytes and plasma on both occasions, the difference cannot be attributed to technique. Perhaps the erythrocyte in pharmacokinetic terms behaves as a "deep compartment" where equilibrium is reached later than other tissues. In any case, these findings of greater tissue binding of digoxin in infants are consistent with a greater apparent volume of distribution, and may partially explain the unusually large therapeutic dosages needed clinically in infants with congestive heart failure.

BILIRUBIN-PROTEIN BINDING

Of great concern to the pediatrician is the bilirubin-binding capacity of serum albumin. Many drugs have been suspected of displacing bilirubin from its binding to albumin. This may have serious clinical consequences in the neonate whose capacity to metabolize bilirubin is limited and whose blood-brain barrier is not as established as in the adult organism. A prerequisite to investigation of this phenomenon is an understanding of the interaction of bilirubin itself, with albumin.

The physical chemical properties of bilirubin have hampered examination of its binding in aqueous systems. In order to circumvent this problem, we utilized dimethylsulfoxide (DMSO). Bilirubin-albumin interactions were studied in varying concentrations of DMSO and the molar binding ratio to serum albumin was found to be unity. Extrapolation of binding through the various concentrations of DMSO to zero concentration yielded an apparent association constant of $1.5 \times 10^7\ M^{-1}$ for an aqueous system. The same methods of obtaining binding association constants with Scatchard plots in different DMSO concentrations was utilized to determine the association of bilirubin to purified adult and cord blood albumin. Neonatal albumin appears to bind bilirubin with a greater affinity than adult albumin with k values of $5.2 \times 10^7\ M^{-1}$ and $2.4 \times 10^7\ M^{-1}$ respectively (Krasner, Giacoia, and Yaffe 1973).

Because of the many problems encountered in dialysis studies involving bilirubin, a fluorimetric method that measures the direct interaction of bilirubin with albumin in microliter quantities was developed (Krasner and Yaffe 1975). The fluorescent properties of the bilirubin-albumin complex can be measured by titrating a serum sample with an alcoholic bilirubin solution. Fluorescence of the bilirubin when bound to albumin increases until the binding capacity is saturated. Increasing quantities of unbound bilirubin results in quenching when the binding capacity of the primary site has been reached. In order to examine the interaction of drugs with albumin and their capability of displacing bilirubin from binding to albumin, this

fluorescent titration technique was employed. If both the drug and bilirubin competed for the same binding site, saturation of the site should appear at a lower bilirubin concentration than it does in the absence of the drug. With a significant number of drugs that are used in neonatal therapeutics (penicillin, ampicillin, phenobarbital, chloramphenicol, and diazepam), no alteration in bilirubin binding was detected.

Sodium benzoate at a concentration of 144 mg/dl produced a 30 percent reduction in bilirubin binding. Sodium salicylate added at concentrations of 20 and 50 mg/dl had no displacing effect, but a 20 percent decrease in bilirubin binding occurred when the salicylate concentration was 200 mg/dl. The concentrations of salicylate and sodium benzoate which produce decreases in bilirubin-binding capacity are considerably higher than those normally encountered clinically. Most drugs have an association constant of the order of 10^4 to 10^5 M^{-1}, whereas the association concentrations of bilirubin binding to albumin is 100 to 1,000 times greater. Therefore, in order for a drug to displace bilirubin from binding by direct competition, a molar concentration 100 to 1,000 times greater than bilirubin would be required. This great difference in the magnitude of k value makes it difficult to rationalize why one should expect these drugs to be competitive with bilirubin binding to albumin. On the contrary, one might anticipate that bilirubin would prevent, or displace, drugs from binding to albumin. This was indeed found when phenytoin binding was studied, as mentioned previously.

DRUG-PROTEIN BINDING IN PREGNANCY

Normal human pregnancy is associated with considerable physiologic changes which may interact with drug-protein binding. These include changes in serum protein concentrations, hormone concentrations, and serum free fatty acid concentrations. Although limited studies have been carried out up until the present time, those data which are available suggest that plasma protein binding of many (but not all) drugs is decreased during pregnancy with the greatest decrease noted during the last trimester (Perucca and Crema 1982).

Notable examples of drugs whose unbound fraction increases during pregnancy include diazepam, valproic acid, phenytoin, phenobarbital, salicylic acid, pethidine, lignocaine, dexamethasone, and propranolol. These differences in plasma protein binding particularly in late pregnancy may affect the distribution of drugs to the fetus and may also play a role in the decrease of binding noted in umbilical cord plasma and serum. Yoshikawa and co-workers attempted to examine the mechanism for this phenomenon using fluorescent probes

(Yoshikawa et al. 1984). They concluded that serum protein binding of drugs that bind to site I or site II on albumin, decrease mainly because of the reduced serum albumin concentration noted during pregnancy. The binding of drugs that bind to site III change little during pregnancy because the decrease in serum albumin concentration is compensated for by an increase in the binding affinity of drugs to this site. Furthermore, they found that the serum concentration of α_1-acid glycoprotein and serum binding of propranolol did not change in pregnant women. This is in contrast to the findings reported previously by Perucca. Reasons for the discrepancy may be due to differences in methodology employed to assess binding.

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