decide what would be a safe dose where an injection was indicated. The study did not attempt to compare the effectiveness of parenteral versus IPPB administration of salbutamol in severe asthma. However, there is an implication in the discussion that intravenous salbutamol would be superior. As yet, no definitive study has examined this problem. We should have, strictly speaking, concluded from our data that *if it were decided* to use intravenous salbutamol in the treatment of an acute attack, then it would be reasonable to give an initial bolus of 250 μg.

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FACTORS AFFECTING DRUG-PROTEIN BINDING IN THE PLASMA OF NEWBORN INFANTS

The interaction of a drug with plasma proteins can be a major factor in determining drug distribution. The investigation of drug binding in the newborn infant is of particular interest, since the neonate's body composition differs from that of the adult, with greater susceptibility to the action of drugs and chemical agents (Yaffe, 1966; Mirkin, 1970).

Several investigators (Ganshorn & Kurz, 1968; Rane, Lunde, Jalling, Yaffe & Sjöqvist, 1971) have demonstrated quantitative differences in the plasma protein binding of certain drugs in newborn children and adults. The observed reduction in binding in neonates may be inversely related to total serum bilirubin levels (Chignell, Vesell, Starkweather & Berlin, 1971; Rane *et al.*, 1971), although a decreased binding affinity for some drugs has been shown even in normal infants with hyperbilirubinaemia (Ehrnebo, Agurell, Jalling & Boréus, 1971; Krasner, Giacoia & Yaffe, 1973; Windorfer, Kuenzer & Urbanek, 1974).

Newborn infants have reduced levels of plasma albumin, and this may in part explain the observed decrease in protein binding. However, there is evidence that in neonates a true reduction in the binding capacity of plasma albumin may occur. The present study investigates this possibility.

Blood from neonates was collected from the maternal end of the umbilical cord immediately after birth. Control blood samples were collected from healthy volunteers, aged 19-33 years, by venepuncture. Samples of plasma (1 ml) were incubated for 30 min at 22°C with salicylic acid (salicylate, 400 μg/ml) or sulphadiazine (500 μg/ml). Ultrafiltration was carried out using the Amicon Multi-Micro Concentrator (MMC) with nitrogen gas pressure of 30 psi. Salicylate was measured in the original plasma-drug solution and ultrafiltrate by the method of Trinder (1954) and sulphadiazine by the method of Bratton & Marshall (1939). Bilirubin (Koch-Light) dissolved in 10^{-4}M sodium carbonate, pH 9, was added to adult plasma to give a final concentration of 80 μmol/litre and drug binding measured as described. A crossover of protein and ultrafiltrate from pooled adult and neonatal plasma was performed by the method of Campion (1973). The association constants and the number of binding sites per albumin molecule were calculated by the method of Scatchard (1949) using pooled plasma and the continuous ultrafiltration technique of Campion & Olsen (1974).

Table 1 shows plasma protein and bilirubin levels with percentage binding values for salicylate and sulphadiazine in adults and neonates. Total protein and albumin concentrations were significantly reduced in the neonate group ($P < 0.001$) and bilirubin levels significantly higher than in adults ($P < 0.001$). Binding of both drugs was

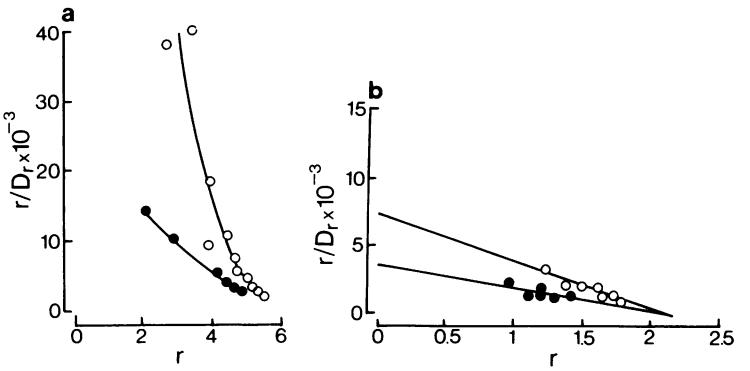

Figure 1 Scatchard plots for salicylate (a) and (b) sulphadiazine binding in adult (○) and cord (●) plasma.

reduced in neonates, and there was a significant correlation ($P < 0.05$) between salicylate binding and albumin levels.

Scatchard plots for binding of salicylate and sulphadiazine are shown in Figure 1. These were calculated on the assumption that both drugs bind predominantly to albumin (Andréason, 1973). For sulphadiazine, the association constant for adult albumin is $3.75 \times 10^3\ M^{-1}$ and for neonatal albumin $1.75 \times 10^3\ M^{-1}$. The association constant for the interaction of salicylate with the primary binding site of adult albumin is $13.64 \times 10^3\ M^{-1}$ and with neonatal albumin $4 \times 10^3\ M^{-1}$.

Bilirubin added to adult plasma to a concentration of 80 μmol/litre caused a significant reduction in sulphadiazine binding ($P < 0.05$) but salicylate binding was unaffected.

As some other substance present in neonatal

Table 1 Comparison of plasma protein, bilirubin and drug binding capacity of neonates and adults. The results are expressed as mean ± s.e. mean.

Subject group	*Total protein (g/litre)*	*Albumin (g/litre)*	*Bilirubin (μmol/litre)*	*% bound salicylate*	*% bound sulphadiazine*
Adults (n = 14)	75.0 ± 0.1	44 ± 0.1	12.8 ± 0.1	73 ± 1.6	53 ± 1.7
Neonates (n = 23)	62.4 ± 0.2	38 ± 0.1	32.0 ± 0.2	68 ± 1.3	40 ± 3.0
P value	< 0.001	< 0.001	< 0.001	< 0.025	< 0.005

Table 2 Effect of an ultrafiltrate of plasma from newborns on drug binding capacity of plasma proteins from normal subjects. The results are expressed as mean ± s.e. mean.

Plasma	*Plasma albumin (g/litre)*	*% bound salicylate*	*% bound sulphadiazine*
Normal plasma	42	81 ± 1.2	51 ± 1.8
Neonatal plasma	40	61 ± 0.9*	29 ± 1.9*
Normal plasma protein, Neonatal plasma ultrafiltrate	42	74 ± 1.9**	37 ± 2.2**
Neonatal plasma protein, Normal plasma ultrafiltrate	40	61 ± 1.0†	24 ± 2.7†

* Significantly different from normal plasma, Student's t-test ($P < 0.001$).
** Significantly different from normal plasma, Student's t-test ($P < 0.005$).
† Not significantly different from neonatal plasma.

plasma could interfere with drug binding, a crossover of adult and neonatal proteins and ultrafiltrates was carried out. In this way, adult plasma proteins were exposed to any retained metabolites present in neonatal plasma which might influence protein binding. Results of binding experiments on original and 'crossover' plasmas are given in Table 2. Adult plasma proteins exposed to neonatal plasma ultrafiltrate showed a significant reduction ($P < 0.005$) in binding of both drugs. Binding capacity of neonatal plasma proteins was not significantly altered after exposure to adult plasma ultrafiltrate. Neonatal plasma ultrafiltrate contained negligible amounts of bilirubin.

These observations suggest that although low plasma albumin levels may cause reduced binding of some drugs in neonates, there is also a decrease in binding capacity which is independent of albumin concentration. This is supported by the noted reduction in the affinity constants for the interaction of salicylate and sulphadiazine with neonatal albumin; a similar reduction in affinity constant in neonates was observed by Chignell *et al.* (1971) using sulfaphenazole. When adult plasma proteins are suspended in an ultrafiltrate of neonatal plasma, their binding capacity for both drugs is reduced, suggesting the presence of a competing ligand in neonatal ultrafiltrate. This does not appear to be bilirubin, since bilirubin added to adult plasma had no effect on salicylate binding, and ultrafiltrate contained negligible amounts of bilirubin. Chignell *et al.* (1971) and Windorfer *et al.* (1974) have suggested that non-esterified fatty acids may cause reduced binding in newborn infants, and endogenous substances, in particular hormones transferred across the placenta *in utero*, may occupy binding sites and reduce drug binding capacity. There may also be competiton for binding sites with drugs given to the mother during labour. The clinical significance of the decreased binding capacity of neonatal plasma proteins is difficult to assess, but it may contribute to altered drug distribution and the extreme sensitivity of the newborn infant to certain drugs.

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