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ALTERED PLASMA ALBUMIN IN THE NEWBORN INFANT

Qualitative differences in the plasma protein binding of certain drugs in newborn infants have been widely reported (Ganshorn & Kurz, 1968; Chignell, Vesell, Starkweather & Berlin, 1971; Ehrnebo, Agurell, Jalling, Yaffe & Boréus, 1971; Pruitt & Dayton, 1971; Rane, Lunde, Jalling, Yaffe & Sjöqvist, 1971; Krasner, Giacoia & Yaffe, 1973; Gorodischer, Krasner & Yaffe, 1974; Windorfer, Kuenzer & Urbanek, 1974). The presence of competing ligands such as bilirubin (Chignell *et al.*, 1971; Rane *et al.*, 1971), free fatty acids and steroids (Krasner *et al.*, 1973), in addition to hypoalbuminaemia, has been suggested as a contributory factor to the observed reduction in binding in neonates, and in a previous study (Wallace, 1976) this possibility was examined. It has also been postulated that altered plasma proteins might be present in neonates (Krasner *et al.* 1973) and that these might be involved in reduced binding. This study investigates the properties of albumin isolated from neonatal plasma.

Blood from neonates and adults was obtained as previously described (Wallace, 1976). Samples of albumin were prepared from adult and neonatal plasma using an affinity chromatography technique based on the methods of Böhme, Kopperschlager, Schultz & Hofmann (1972) and Travis & Pannell (1973). Phosphate buffer (0.05 M) with 2 M NaCl, pH 7.4, was used to elute the albumin

in place of 6 M urea as described by Travis & Pannell (1973).

Samples (100 μ g) of albumin were freeze-dried in vacuum hydrolysis tubes (Pierce) and 1 ml of constant-boiling HCl added to each tube. Samples were hydrolyzed under vacuum at 110°C for 24 h, freeze-dried once more, and analyzed on a Technicon amino acid analyzer.

Immunodiffusion studies of adult and neonatal albumin were carried out using agarose gel plates with trypan blue indicator (Miles). Rabbit anti-serum (20 μ l) to human serum albumin (Miles) was placed in the central well and allowed to diffuse for 30 min before 10 μ l of adult and neonatal albumin solutions of equal concentration were placed alternately into the six surrounding wells. The plate was incubated at 22°C for 24 h and examined for precipitin lines.

Isoelectric focussing of adult and neonatal albumin was carried out using the method detailed in Bio-Rad Bulletin 1030 (1975). The gels were stained in a solution of ethanol, water, acetic acid (50 : 45 : 5) containing 0.2% bromophenol blue. Destaining was carried out in the Canalco Electrophoretic Destainer, loaded with 10% isopropanol and 10% acetic acid, and the gels were then scanned using a Gilford spectrophotometer with Model 2520 gel scanning attachment.

Crystalline HSA was used as a reference stan-

Figure 1 Interaction of albumin isolated from the plasma of an adult (A) and a newborn infant (N) with rabbit antiserum to human serum albumin.

dard in the amino acid analysis of adult and neonatal albumin. The amino acid content of each sample was calculated as g amino acid per 100 g protein, and the amino acids in the test albumins were then expressed as ratios of their percentage distribution to that found for the reference albumin (Denko, Purser & Johnson, 1970). Table 1 shows the distribution of amino acids in the reference albumin compared to data from Peters (1975), similarly expressed. Agreement between the analyses is good, except for glycine. Amino acid analyses of adult and neonatal albumin samples are given in Table 2. The content of threonine, valine and methionine was reduced, while serine, glycine and lysine content was increased in neonatal albumin.

Immunodiffusion studies of neonatal and adult albumins against rabbit antiserum to HSA showed that each produced a single precipitin line with no spurring at the point of intersection, indicative of complete immunological identity between the two (Figure 1).

Analytical scale isoelectric focussing of adult albumin revealed the existence of two albumin bands (Figure 2a), with isoelectric points of 4.8 and 5.0, respectively. Neonatal albumin showed only one band (Figure 2b) with an isoelectric point of 4.8.

These observations suggest that the albumin of neonatal plasma is different to that of the adult. Isoelectric focussing has shown that neonatal albumin contains only one of the components present in the adult protein. That this component is common to both adult and neonatal albumin is shown by the immunodiffusion studies in which both samples appeared identical. The variations in amino acid content of neonatal and adult albumin could then be explained on the basis of varying proportions of the two albumin components. Similar variations have been described for albumin in plasma of renal failure patients (Shoeman & Azarnoff, 1972), but this study provides the first evidence for altered plasma albumin in neonates. It appears that the development of plasma albumin, like haemoglobin, involves both foetal and adult forms, although this could only be confirmed by studying plasma albumin from birth to, say, 6 months.

The significance of the observed alterations in composition of neonatal albumin is clearly difficult to assess without measuring the drug binding capacities of each albumin component. In addition

Table 1 Amino acid composition of albumin

	Crystalline HSA (A)	Standard (B)	B/A
Aspartic acid	9.74%	9.30%	0.96
Threonine	5.15%	5.15%	1.00
Serine	4.66%	3.80%	0.82
Glutamic acid	14.44%	14.26%	0.99
Proline	5.01%	4.29%	0.86
Glycine	3.23%	2.06%	0.64
Alanine	10.99%	10.82%	0.99
Valine	6.88%	6.70%	0.97
Methionine	1.18%	1.03%	0.87
Isoleucine	1.65%	1.37%	0.83
Leucine	10.71%	10.48%	0.98
Tyrosine	3.25%	3.09%	0.95
Phenylalanine	5.66%	5.15%	0.91
Lysine	10.01%	9.97%	1.00
Histidine	2.94%	2.75%	0.94
Arginine	4.44%	3.95%	0.89

Standard (B) is taken from Peters (1975)

Figure 2 Isoelectric focussing of albumin isolated from (a) adult and (b) neonatal plasma.

to possible differences in drug binding capacity, the two albumin types may also bind inhibitors, such as bilirubin, with different affinities. Krasner *et al.* (1973) have reported that neonatal albumin, although having a reduced affinity for many drugs, has a greater ability to bind bilirubin than has adult albumin. This finding may also reflect altered albumin in neonatal plasma.

The author wishes to thank the staff of the Naval Hospital, Bethesda, for supplying samples of cord blood, Mr Roger Lee, National Heart, Lung, and Blood Institute, for carrying out the amino acid analyses, and Dr Colin F. Chignell, National Heart, Lung, and Blood Institute, for his advice and assistance. The work was supported by a grant from Hoffman-La Roche Incorporated.

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Received July 26, 1976

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Table 2 Amino acid ratios (mean $\pm$ s.d.) derived from albumins from adults and neonates

Amino acid	Adults (n = 7)	Neonates (n = 6)	P
Aspartic acid	1.02 $\pm$ 0.03	1.07 $\pm$ 0.04	NS
Threonine	1.00 $\pm$ 0.05	0.93 $\pm$ 0.05	< 0.025
Serine	0.93 $\pm$ 0.05	1.05 $\pm$ 0.04	< 0.005
Glutamic acid	1.01 $\pm$ 0.04	1.08 $\pm$ 0.01	NS
Proline	1.00 $\pm$ 0.04	0.96 $\pm$ 0.04	NS
Glycine	0.81 $\pm$ 0.05	0.90 $\pm$ 0.06	< 0.02
Alanine	1.10 $\pm$ 0.03	1.11 $\pm$ 0.05	NS
Valine	0.96 $\pm$ 0.11	0.80 $\pm$ 0.11	< 0.05
Methionine	0.98 $\pm$ 0.04	0.81 $\pm$ 0.06	< 0.001
Isoleucine	0.86 $\pm$ 0.11	0.64 $\pm$ 0.09	< 0.005
Leucine	1.01 $\pm$ 0.04	0.99 $\pm$ 0.01	NS
Tyrosine	1.03 $\pm$ 0.04	0.96 $\pm$ 0.04	< 0.025
Phenylalanine	1.02 $\pm$ 0.05	0.98 $\pm$ 0.04	NS
Lysine	0.98 $\pm$ 0.08	1.06 $\pm$ 0.03	< 0.05
Histidine	1.03 $\pm$ 0.04	0.95 $\pm$ 0.03	< 0.05
Arginine	0.99 $\pm$ 0.07	0.96 $\pm$ 0.01	NS

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FOOD INTAKE AND PLASMA BINDING OF DIAZEPAM

Diazepam, a widely used tranquilizer and anticonvulsant, exhibits extensive binding to plasma constituents in different species including man (Kleijn, 1969; Klotz, Antonin & Bieck, 1975). Since the extent of plasma binding determines the elimination of diazepam (Klotz, Antonin & Bieck, 1976), changes in binding may alter its disposition.

Table 1 Influence of food intake on the plasma binding of diazepam in man

Patient	% plasma binding of diazepam		
	Before	1 h after	3 h after lunch
UK	94.8	94.9	94.9
KN	95.8	96.1	95.4
EG	95.4	95.7	95.8
		2 h after	4 h after lunch
BS	95.8	96.6	97.1
CL	95.0	93.4	95.1

Linnoila, Korttila & Mattila (1975) have shown that 1 to 3 h after food intake diazepam serum levels were significantly elevated. We therefore investigated if food intake might influence the plasma levels of diazepam via changes in plasma binding.

Plasma samples were obtained from five healthy volunteers 1 h before and 1 (2) and 3 (4) h after lunch. Plasma binding was measured by equilibrium dialysis (Evans, Nies & Shand, 1973) at a therapeutic concentration of 200 ng diazepam/ml at 37°C. Concentrations of diazepam were assayed after 24 h in the inner medium (5 ml fresh human plasma) and outer medium (10 ml 0.067 M phosphate buffer pH 7.4) of the dialysis bag by a specific and sensitive gaschromatographic procedure (Klotz, Avant, Hoyumpa, Schenker & Wilkinson, 1975). Additionally in serum samples obtained at the same time, free fatty acid (FFA) were measured according to Duncombe (1964).

The percentage of diazepam bound to the different plasma samples is summarized in Table 1. There was no significant change in diazepam bound before and after lunch, suggesting that

Table 2 Free fatty acids (FFA) in man before and after lunch (range, $n = 4$)

	Before lunch	Time after lunch (h)				Normal range
		1	2	3	4	
FFA (mEq/l)	0.24-0.34	0.23-0.42	0.22-0.37	0.23-0.35	0.27-0.41	0.1-0.6