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The binding of bupivacaine to maternal and foetal plasma proteins

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The binding of bupivacaine to maternal and foetal plasma proteins has been investigated using a three-compartment dialysis apparatus, in which maternal and foetal plasma could be examined simultaneously. Maternal protein bound approximately twice as much bupivacaine as foetal protein per gram of protein at drug concentrations in the range 0·05 to 5·0 μg/ml. Over the same range of concentrations, 92–78% of bupivacaine was bound to maternal plasma protein but only 35–31% was bound to human albumin indicating that other proteins are involved in the binding of the drug to plasma proteins.

We have investigated the maternal and foetal plasma concentrations at delivery of a number of drugs, local anaesthetic agents such as lignocaine and bupivacaine having been of particular interest (Thomas, Climie & Mather, 1968; Thomas, Climie & Mather, 1969; Thomas, Climie & others, 1969). A feature of these results is that, while there is some patient to patient variation, the average ratio of foetal : maternal plasma concentrations is invariably less than 1. For example in a series of 42 patients in labour, who received lignocaine epidurally, the average foetal : maternal plasma concentration ratio at delivery was 0·51 (range 0·33–0·89) (Thomas & others, 1969). Investigations with the local anaesthetic bupivacaine [1-butyl-2-(2,6-xylylcarbamoyl)-piperidine] have shown that the average foetal : maternal plasma concentration ratio at delivery is in the range 0·3–0·5 (Thomas & others, 1969; Reynolds & Taylor, 1970; Hollmen, Nummi & Ojala, 1970; Taylor, Reynolds & others, 1970). The data from these various investigations show that there is no relation between the time from administration of the drug to blood sampling (i.e. delivery) and the foetal : maternal plasma concentration ratio. This suggests that the rate of passage of these drugs across the placental barriers is relatively rapid. If this is so, in relation to the interval between administration of drug and delivery (possibly hours), it might be anticipated that the foetal : maternal plasma concentration ratio would be 1. The plasma concentrations which have been reported in all the investigations mentioned are total plasma concentrations. No attempt has been made to evaluate the proportion which is bound to plasma proteins. Since differential binding to maternal and foetal plasma proteins could be an important factor in controlling the foetal and maternal plasma concentrations of drugs, we have now investigated plasma protein binding of bupivacaine to both foetal and maternal plasma proteins. Reynolds & Taylor (1970) have stated that bupivacaine was bound to plasma proteins to the extent of 90–95% at concentrations occurring clinically. However, they presented no evidence to support this.

MATERIALS AND METHODS

Dialysis

Two types of all-glass dialysis apparatus were used. A two-compartment one when binding to one type of plasma was being investigated and a three-compartment one when binding to maternal and foetal plasma proteins were being examined simultaneously (Fig. 1). Each compartment had a capacity of 5 ml. The dialysis

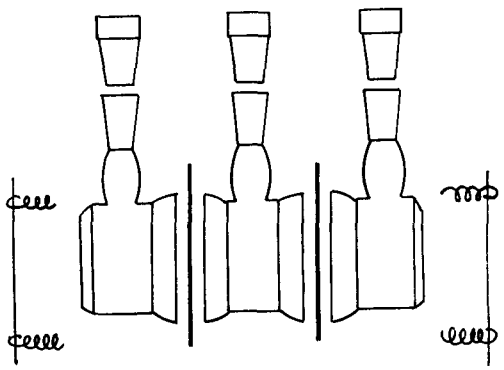

FIG. 1. Three-compartment dialysis apparatus used for simultaneous dialysis of maternal and foetal plasma against bupivacaine solutions. The capacity of each compartment is 5 ml. Construction is of glass with ground faces to support Visking membranes. Stainless steel springs hold the compartments together.

membrane was Visking dialysis cellulose which had been washed four times in distilled water at 80–90° and stored in flat sections at 4° until used. The apparatus was assembled and sterilized by autoclaving at 121° for 20 min. The compartments were filled aseptically under a laminar air flow hood. Dialysis was carried out at 37° for 42 h with constant shaking. One outer compartment of the dialysis apparatus was filled with foetal plasma, the other outer compartment with maternal plasma, while the centre compartment contained a solution of bupivacaine in buffer. Samples from the buffer compartment were checked regularly for protein using salicylsulphonic acid solution (20%).

Determination of bupivacaine

The gas chromatographic method of Thomas & others (1969) was used with one modification. A glass column, 3% OV-17 on 100–120 mesh Gas Chrom Q ¼ inch o.d., 6 ft long replaced that previously described. Nitrogen was the carrier gas at 37 ml/min. Under these conditions the retention times of bupivacaine and cyproheptadine (internal standard) were 9·0 min and 13·0 min respectively. A calibration curve of peak height ratio to weight of bupivacaine was constructed over the range 0·1–10·0 μg bupivacaine. The curve was a straight line and passed through the origin. Sample sizes from the dialysis solutions were selected to contain amounts of bupivacaine within this range.

Solutions

Bupivacaine hydrochloride was dissolved in Sørensen phosphate buffer M/15 adjusted to pH 7·35 at 25° (7·39 at 37°). The concentrations of the solutions ranged from 0·1 to 1000 μg/ml. These solutions were autoclaved at 121° for 20 min in 300 ml

bottles closed with a silicone elastomer multidose closure unit. No change in pH occurred during autoclaving.

A solution of human serum albumin (Commonwealth Serum Laboratories) in sterile buffer was prepared under aseptic conditions. Autoanalysis showed that the solution contained 4·8 g/100 ml total protein and 4·8 g/100 ml albumin. Cellulose acetate electrophoresis revealed the presence of $<1\%$ globulins.

Plasma

Blood was collected from 10 labour ward patients via the antecubital vein. Eight patients had received no medication during the previous 24 h, two had been given bupivacaine. Sodium citrate dihydrate was added to the collecting bottles to give a final concentration of approximately 5 mg/ml in the blood and the bottles were then sterilized. The blood was centrifuged at 2500 rev/min for 10 min and the plasma collected was pooled and sterilized by filtration through a Millipore filter (0·22 μm) with a fibreglass prefilter using positive pressure. After the patients had been delivered, foetal blood was collected from the placentae. Each placenta was supported in a large short stem funnel with the maternal surface uppermost and the cord protruding through the stem. A Braunula was inserted into the umbilical vein. The blood was removed from the placenta by connecting the Braunula to a sterile evacuated blood bottle containing sodium citrate dihydrate. Approximately 60 ml of blood could be collected in this manner. Plasma was separated from the whole blood by the same method as described for maternal plasma. The foetal plasma from the 10 patients was pooled and sterilized by filtration.

Plasma was also obtained from six volunteers each 36–38 weeks pregnant and kept separate. The albumin/total protein concentrations of these six plasma samples were normal and ranged (100 ml) from 3·4/6·4 g to 4·0/7·3 g (by autoanalysis).

Autoanalysis and cellulose acetate electrophoresis of the maternal and foetal plasma gave the results in Table 1.

Table 1. *Constituents of pooled maternal plasma and foetal plasma as determined by autoanalysis and electrophoresis.*

Constituent	Foetal g/100 ml	Maternal g/100 ml
Total protein (autoanalysis) ..	4·9	5·9
Albumin (,,) ..	3·0	3·1
Albumin (electrophoresis) ..	2·70	2·85
α1 Globulin (,,) ..	0·23	0·40
α2 Globulin (,,) ..	0·40	0·99
β-Globulin (,,) ..	0·70	0·76
γ-Globulin (,,) ..	0·87	0·92

RESULTS

The degree of binding of bupivacaine to plasma proteins from six patients varied from 71·3 to 91·6% over a total drug concentration range of 0·83 to 3·74 μg/ml. The degree of binding was related to the plasma concentration of drug (Table 2). Results from the three compartment apparatus are given in Table 3 from which it can be seen that bupivacaine is bound to a greater extent in maternal plasma than in foetal plasma. Also in Table 3 is the amount of bupivacaine bound per g of both maternal

Table 2. *Bupivacaine binding to diluted whole plasma.* Equilibrium dialysis used. Bupivacaine dissolved in Sørensen phosphate buffer M/15 pH 7·39 at 37°. Dialysis carried out at 37° for 42 h.

Plasma concentration (%) in protein compartment	% Bound	Bupivacaine concentration (μg/ml) Total	Free
100	81·2	3·2	0·6
50	66·0	3·1	1·0
25	47·8	2·7	1·4
12·5	36·4	2·5	1·6
6·3	17·7	2·3	1·9
3·1	7·1	2·1	1·9

Table 3. *The binding of bupivacaine to human maternal plasma proteins and foetal plasma proteins.* Equilibrium dialysis in a three compartment apparatus was used. Bupivacaine was dissolved in Sørensen phosphate buffer M/15 pH 7·39. Dialysis carried out at 37° for 42 h.

No. of exp.	Bupivacaine mean concn In plasma (μg/ml) Maternal	Foetal	In buffer μg/ml	Ratio maternal: foetal	Bupivacaine bound to plasma proteins Mean and range (%) Maternal	Foetal	Mean (μg/g) Maternal	Foetal	Ratio maternal: foetal
4	0·13	0·05	0·01	2·40	92·1 (88·9–93·7)	81·1 (76·0–84·0)	1·98	0·87	2·30
3	0·15	0·08	0·02	1·85	85·5 (82·5–88·2)	73·2 (70·6–78·3)	2·23	1·23	1·81
3	0·26	0·11	0·04	2·31	83·5 (80·0–85·2)	61·9 (53·7–68·0)	3·69	1·42	2·59
2	0·49	0·25	0·09	1·93	80·7 (80·0–81·4)	63·2 (63·0–63·3)	6·69	3·27	2·04
3	4·47	2·30	1·00	1·94	77·6 (72·8–80·6)	56·5 (54·2–54·3)	58·8	26·5	2·21
3	35·1	14·0	9·2	2·51	73·8 (67·2–79·9)	34·3 (25·5–46·6)	439·0	97·9	4·48
2	81·4	30·4	21·0	2·68	74·2 (72·3–76·0)	30·9 (30·4–31·5)	1040	191·8	5·42
2	172·5	105·5	66·5	1·64	61·5 (60·7–62·3)	37·0 (35·5–38·6)	1796	796	2·25
3	1493	798	541	1·87	63·7	32·2	16135	5245	3·07

Table 4. *The binding of bupivacaine to human albumin as determined by equilibrium dialysis.* Bupivacaine dissolved in Sørensen phosphate buffer M/15 pH 7·39 at 37°. Albumin solution 4·8 g/100 ml. Dialysis at 37° for 42 h.

Bupivacaine Mean total concn (μg/ml)	Mean free concn (μg/ml)	Bound (% ± s.e.)	No. of determinations
0·037	0·024	35·1 ± 1·4	4
0·110	0·073	34·2 ± 1·4	8
0·234	0·156	33·5 ± 2·3	6
0·452	0·301	33·0 ± 2·1	5
4·52	3·11	31·0 ± 1·8	5
44·64	31·82	28·7 ± 1·2	5
224	165	26·3 ± 1·5	5
441	349	20·8 ± 0·9	6
787	658	16·3 ± 1·8	5

and foetal plasma proteins. These data show that maternal plasma proteins bind more bupivacaine than foetal plasma proteins over the range of concentrations of drug examined. Table 4 shows that while purified human albumin binds bupivacaine it does not do so to the same extent as total plasma proteins.

The effect of albumin concentration on the degree of binding of bupivacaine was investigated. It was found that bupivacaine was bound to the extent of 28·7, 11·8 and 6·0% when the albumin concentration was 4·8, 0·48 and 0·048 g/100 ml and the initial concentration of bupivacaine in the buffer solution was 78 μg/ml.

The effects of ions from the buffer solution on binding of bupivacaine to albumin was examined. In three separate dialysis experiments both the concentration of albumin (4·8 g/100 ml) and the amount of bupivacaine added (78 μg/ml) to the buffer were kept constant while the concentration of the phosphate buffer was varied. The three buffer concentrations used were 1/15, 1/30 and 1/60M and the respective degrees of binding were 28·7, 41·6 and 48·7%.

DISCUSSION

In establishing the procedures to be used a number of issues were considered. The first was the use of drug-free plasma for all the dialysis experiments. The drugs most frequently used in labour are analgesics of which pethidine is particularly popular. Most of these drugs are basic so the various drugs may compete for binding sites on the plasma protein. Hence data obtained for the degree of plasma protein binding of bupivacaine, when blood from patients who have received other drugs is used, could be suspect. To check this possibility, preliminary experiments were made in which the influence of pethidine on the binding of bupivacaine to human albumin was examined. Bupivacaine, 1 μg/ml in buffer, was bound to the extent of 33·0% when dialysed against albumin (4·8 g/100 ml), pethidine (5 μg/ml) added to the buffer reduced the binding to 23·5%. Because of the possibility of displacement of one drug by another from plasma proteins, only blood from the eight patients who had received no medication during the course of labour and the two who had received only bupivacaine was used.

A second point which was considered was the temperature of the dialysis. Dialyses at low temperatures reduce bacterial growth, however the amount of a drug bound to human albumin may be dependent on the temperature of dialysis (e.g. Sellers & Koch-Weser, 1969). While the temperature dependence of the binding of bupivacaine to proteins was not examined, it was considered preferable to dialyse at 37° in an apparatus designed to avoid contamination.

The degree of binding of bupivacaine to human albumin was found to be affected by the concentration of buffer solution. To check whether the buffer ions interfered with the relative binding of bupivacaine to maternal and foetal plasma proteins drug-free maternal plasma was dialysed against plasma from the placenta from the same patient. Bupivacaine was added to the maternal plasma and the system allowed to equilibrate. The ratio of concentrations of bupivacaine in the plasma samples was found to be between 2 and 2·5 (maternal : foetal). The amount of bupivacaine added was such that the plasma concentrations were in the range 0·4 to 3·4 μg/ml. These results obtained with systems in which no buffer was used were essentially the same as those obtained with the three compartment system (Table 3). This indicates that the buffer ions used did not significantly alter the relative binding capacities of maternal or foetal plasma proteins for bupivacaine. It would appear, therefore,

that the three compartment system is a reasonable *in vitro* model of the placenta to study the effects of plasma protein binding on placental transfer.

In the first four experiments the concentrations of bupivacaine in Table 3 were in the range commonly found clinically, in experiment 5 the total plasma concentration of 4·47 μg/ml is about the highest found in practice. The last four experiments were made to obtain information about the capacity of plasma proteins for bupivacaine. The results in Table 3 show that bupivacaine is extensively bound to plasma proteins over a wide range of concentrations. The data in Table 3 also show that the ratio of maternal : foetal plasma concentration of bupivacaine is around 2 over the range examined. The difference in concentration in the two plasmas could be due to differences in the degree of binding to the respective proteins or to different amounts of proteins present.

The ratios of bupivacaine bound to maternal and foetal proteins expressed in terms of μg bupivacaine per g of protein (from Table 1) for the first 5 experiments in Table 3 are approximately 2 which indicates that maternal plasma proteins bind bupivacaine more than foetal plasma proteins. This suggests that differential binding to maternal and foetal plasma protein plays a significant role in determining the transfer of bupivacaine across the placenta.

Binding of bupivacaine to human albumin

Albumin is considered to play a central role in binding of drugs to plasma proteins. Table 4 shows that while albumin is capable of binding bupivacaine over a wide range of concentrations, it does not bind the drug to the same extent as total plasma proteins. At clinical concentrations of bupivacaine (first five results), albumin binds approximately 30–35% of bupivacaine, while at similar concentrations of drug, whole maternal plasma proteins binds approximately 80–92%. The concentration of albumin in maternal plasma proteins was 3·1 g/100 ml while in the purified albumin system it was 4·8 g/100 ml. This provides further evidence that albumin does not account for the total binding of bupivacaine to plasma proteins. Treatment of the albumin binding data by the Scatchard method indicates that there are 1·16 statistical bupivacaine binding sites per molecule of albumin with an association constant of 552 litre/mol.

As other proteins must be involved in the binding of bupivacaine presumably there are a number of different sites each with different association constants and capacities to be considered when analysing the differential binding between the two kinds of plasma proteins. Plotting the data according to Rosenthal (1967) gives curves for both the maternal plasma and foetal plasma (Fig. 2) which indicates that more than one binding site is involved.

The difference in binding of bupivacaine to maternal and foetal plasma proteins could therefore be due to (i) the absence of a particular protein in the foetal plasma which had a high affinity for bupivacaine, (ii) the association constants of bupivacaine for the maternal and foetal proteins being different, or (iii) the number of sites available for binding being different.

The finding that bupivacaine binds more to maternal plasma protein than to foetal plasma protein is at variance with Goldstein, Aronow & Kalman (1969) who suggest that, in general, binding to maternal and foetal plasma is similar. However, Ganshorn & Kurz (1968) report that many drugs bind to maternal plasma protein more than they do to foetal plasma protein.

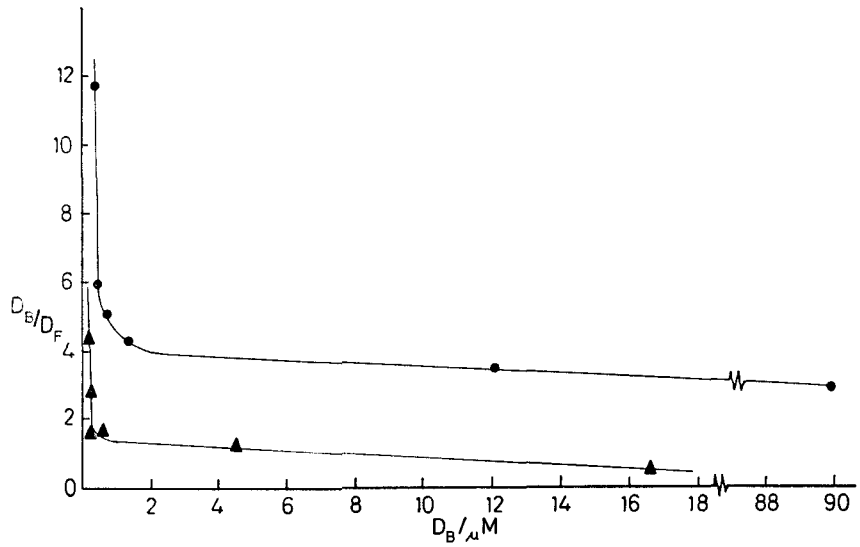

FIG. 2. Scatchard plot as modified by Rosenthal of the binding of bupivacaine to maternal plasma and foetal plasma. Temperature of dialysis 37°. Sørensen phosphate buffer M/15 pH 7·39. ●—● Maternal plasma. ▲—▲ Foetal plasma.

The observation that binding to human albumin accounts for less than half the plasma protein binding is contrary to the commonly held belief that this protein is the dominant one in binding and indicates that care should be taken in using albumin solutions as models for plasma protein binding studies.

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