PLASMA BINDING OF THIOPENTONE IN LATE PREGNANCY

During pregnancy, there is a progressive reduction in the plasma protein binding of salicylic acid, sulphafurazole, phenytoin, diazepam and dexamethasone (Dean *et al.*, 1980; Ruprah *et al.*, 1980; Lovecchio *et al.*, 1981). These drugs are bound in plasma primarily to albumin. The evidence, at least with salicylate (Lovecchio *et al.*, 1981), suggests that the reduction in binding is due to the presence of elevated concentrations of binding inhibitors or to the presence of unique metabolites peculiar to pregnancy. Recently, Ridd *et al.* (1982) found that the free fraction of diazepam in plasma increased during labour or prior to Caesarean section and reached a maximum 1.6 to 3.2-fold increase at delivery or within 4 h post partum. The change in binding was attributed to the parallel increase in total non-esterified fatty acid (NEFA) concentration during labour. We studied the effect of labour on the plasma protein binding of thiopentone because the elevated concentrations of NEFA, or other endogenous displacers, may affect the binding of this drug, which is bound primarily to albumin in plasma (Christensen *et al.*, 1980). We also studied the effect of NEFA, added *in vitro*, on thiopentone plasma binding.

Nine healthy full-term pregnant women, undergoing elective induction of labour, participated in the study. Labour was induced by synthetic oxytocin, which was administered by intravenous infusion until delivery. Informed consent was obtained from each patient and the study had the approval of the Hospital Ethics Committee. Venous blood (30 ml) was collected into lithium heparinised plastic tubes (Johns Professional Products, Victoria) on the day before induction was commenced and approximately 15 min after delivery. The tubes were kept in an ice bath and plasma was separated by centrifugation. The plasma

was spiked with a small volume of aqueous thiopentone sodium solution to a final concentration of 10 µg/ml thiopentone. Thiopentone binding in plasma at 37°C was determined by ultrafiltration (Morgan *et al.*, 1981a). The concentration of NEFA in plasma was determined by the colorimetric method of Lauwerys (1969). The coefficient of variation of the method at 0.615 mM was 6.7% ($n = 5$). The concentration of albumin in plasma was determined by radial immunodiffusion (M-Partigen, Behringwerke, AG). In another set of experiments, fresh plasma collected from several Blood Bank donors (10 ml from each, collected directly into lithium heparinised tubes), was pooled and divided into three portions which were then spiked with thiopentone to concentrations of 1, 10 and 100 µg/ml respectively. Aliquots of each portion were spiked with a methanolic solution of six fatty acids (myristic, palmitoleic, palmitic, linoleic, oleic and stearic acids, in the same ratios as reported in human plasma (Diem & Lentner, 1971)) to produce a range of total NEFA/albumin ratios. The spiking of plasma was achieved by adding the appropriate volume of methanolic fatty acid solution to an empty glass tube, evaporating the methanol with a stream of nitrogen and adding the plasma. Dissolution was facilitated by ultrasonication for 45 s. Plasma protein binding, NEFA and albumin concentrations were determined as before. The statistical analysis of data was performed using two-tailed paired *t*-test, analysis of variance and linear regression analysis.

The thiopentone binding data and NEFA and plasma albumin concentrations in the nine pregnant women before labour and at delivery, are shown in Table 1. There was a small decrease in thiopentone per cent free from before labour to delivery, but the

Table 1 Thiopentone plasma binding and NEFA and albumin plasma concentrations in pregnant women at term

Subject	Thiopentone (% free)		NEFA plasma concentration (mM)		Albumin plasma concentration (mM)
	Before labour induction	Delivery	Before labour induction	Delivery	
1	25.0	19.0	0.361	0.806	0.410
2	26.6	20.7	0.657	1.44	0.505
3	20.7	18.4	0.452	1.14	0.369
4	25.2	21.4	0.851	1.17	0.426
5	20.1	18.3	0.387	0.560	0.379
6	18.7	16.5	0.479	1.51	0.427
7	21.0	20.3	0.642	1.40	0.361
8	23.0	25.1	0.469	0.568	0.418
9	19.8	21.5	0.542	0.849	0.444
Mean	22.2	20.1	0.538	1.05	0.415
s.d.	2.8	2.5	0.16	0.37	0.042

change was of borderline significance ($P = 0.061$). Plasma NEFA concentration at delivery was nearly double pre-induction values ($P = 0.0013$). The mean NEFA/albumin ratio at delivery was 2.53. There were no correlations among thiopentone binding ratio or percentage free and plasma albumin or NEFA concentration ($P > 0.05$). *In vitro* addition of NEFA to Blood Bank plasma had no effect on thiopentone binding ($P > 0.05$, Table 2).

Table 2 The effect of NEFA added *in vitro* on binding of thiopentone in Blood Bank plasma*

NEFA/Albumin ratio	Thiopentone (% free)		
	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
1.11	23.6	21.8	24.6
2.62	18.3	19.8	21.9
4.60	23.9	20.8	19.8
6.47	22.3	19.2	24.9

* Plasma albumin concentration 0.522 mM

Although oxytocin can cause the NEFA concentration to increase (Burt *et al.*, 1963), an increase of similar or greater magnitude occurs during labour in patients not receiving oxytocin (Whaley *et al.*, 1967; Ogburn *et al.*, 1980). Unlike diazepam, there was little, if any, change in thiopentone plasma binding in pregnant women during labour in spite of a doubling of the plasma NEFA concentration. Furthermore, the binding of thiopentone was similar to that determined previously in healthy non-pregnant subjects by ultrafiltration (20.4% free, Morgan *et al.*, 1981a) and by equilibrium dialysis (17% free, Christensen *et al.*, 1980; 20% free, Jung *et al.*, 1982), showing that pregnancy does not affect thiopentone plasma binding. Recent studies (Morgan *et al.*, 1981b) showed that thiopentone plasma binding in samples collected during the first hour following thiopentone administration in patients undergoing gynaecological surgery or Caesarean section was similar to that in non-pregnant subjects even though plasma NEFA concentration might have been expected to be elevated (Cardon & Gordon, 1959). The present *in vitro*

experiments also indicated a lack of effect of NEFA on thiopentone plasma binding, up to a NEFA/albumin ratio of 6.47. Rudman *et al.* (1971) concluded that NEFA concentrations below 2 mM should not affect thiopentone plasma binding, but found that binding was significantly reduced at a NEFA/albumin ratio of 7. The discrepancy between the two studies may lie in the fact that Rudman *et al.* (1971) used palmitic acid only and bovine serum albumin at low concentration (0.058 mM).

Two high affinity binding sites for anionic drugs on human serum albumin have been proposed (Sudlow *et al.*, 1976). Free fatty acid appears to enhance the binding of drugs at site I and diminish the binding of drugs at site II (the tryptophan or benzodiazepine site (Birkett *et al.*, 1977)). Data on diazepam binding during labour obtained by Ridd *et al.* (1982) are consistent with this theory and led these authors to suggest that the plasma binding of site II drugs generally may be reduced at parturition. They also speculated that a complementary enhanced plasma binding of site I drugs, such as warfarin, will occur during labour and delivery. Although the binding site of thiopentone on albumin is not known with certainty, and Kragh-Hansen (1981) has questioned whether in fact warfarin is a site I drug, it was interesting to note that we observed a slight, though clinically insignificant rise in thiopentone binding during labour in seven of the nine patients (Table 1). This supports the proposal of Ridd *et al.* (1982) that the plasma protein binding during labour of anionic drugs may either decrease or increase under the influence of the elevated NEFA concentration, depending on the site on the protein of drug binding.

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THE DOUBLE-BLIND ASSESSMENT OF THE EFFECT OF DIHYDRO ERGOCRYPTINE ON MUSCLE BLOOD FLOW IN NORMAL HUMAN VOLUNTEER SUBJECTS

Many α -adrenoceptor blocking drugs, direct acting vasodilators and drugs with mixed actions are promoted for the treatment of inadequacies of blood flow, particularly to the skin and muscles of the extremities. There is, however, little convincing evidence that any agent or procedure can increase skeletal muscle blood flow beyond the level produced by exercise to the limits of tolerance (Weiner, 1980). On the other hand, it is also known that locally administered noradrenaline can attenuate the rise in muscle blood flow due to exercise (Berstein & White, 1974). The purpose of the present study was to evaluate the effect of the α -adrenoceptor blocking drug dihydroergocryptine mesylate on resting blood flow and reactive hyperaemia in normal volunteers. Dihydroergocryptine is one of the components of dihydroergotoxine, the others being dihydroergocornine and dihydroergocristine (Rutschmann & Stadler, 1978). Whilst all three dihydrogenated ergot derivatives have α -adrenoceptor blocking activity there is some evidence (reviewed by Muller-Schweinitzer & Weidmann, 1978) to suggest that the most powerful is dihydroergocryptine. Dihydroergocryptine was administered orally in this investigation as the compound preparation Vasobral which also contains caffeine. Caffeine increases the completeness and rate of absorption of many of the relatively insoluble

dihydrogenated alkaloids including dihydroergocryptine by a process of intermolecular complexation (Zoglio & Maudling, 1970). The standard dose employed on the continent for peripheral vascular disease was used in this study.

The subjects were 10 healthy members of the staff of the Royal Free Hospital, London. Their ages ranged between 21 and 43 years. Consent was obtained in writing after a full explanation of the procedures involved. Approval of the local Hospital Ethics Committee was obtained.

Muscle flow was determined according to the method of Lassen *et al.* (1964), by the clearance of xenon¹³³.

Approximately 200 μ Ci xenon¹³³ dissolved in 0.2 ml of 0.9% saline was injected via a small needle (0.25 mm in external diameter and 15 mm in length) into the upper part of the left tibialis anterior muscle and the clearance of radioactivity was followed by means of a scintillation counter with a thallium activated sodium iodide crystal. The crystal was placed at a distance of 6 cm from the skin and was shielded by a lead collimator with a cylindrical bore of 22 mm diameter. By this means an initial count rate of over 3,000 counts/s was achieved. A rate meter time constant of 3 s was employed. Blood flow measurements were recorded on each occasion after the subject had