Br. J. Pharmac. (1976), **57**, 311–317

THE EFFECTS OF PENTOBARBITONE AND PETHIDINE ON FOETAL BREATHING MOVEMENTS IN SHEEP

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1 Small doses of pentobarbitone (4 mg/kg i.v.) administered to sheep in the last third of pregnancy had little overt effect on the mothers. In the foetus they caused arrest of breathing movements, an alteration in the character of the electrocorticogram and cardiovascular changes which varied with gestational age.

2 In contrast, relatively large doses of pethidine (100–200 mg) administered to the mother had no consistent effect on normal foetal breathing movements, though they abolished the foetal response to hypercapnia.

3 The results are discussed in relation to foetal sleep state.

Introduction

Episodic breathing movements are normally present 40% of the time in the undisturbed foetal lamb *in utero* (Merlet, Hoerter, Devilleneuve & Tchobroutsky, 1970; Dawes, Fox, Leduc, Liggins & Richards, 1970, 1972). This observation differs from the conclusion by Windle (1941) and Barcroft (1946) that the foetus is apnoeic. It is now known that rapid irregular foetal breathing movements in sheep are associated with rapid-eye-movement sleep and are particularly liable to arrest by adverse conditions such as hypoxaemia. Also moderate doses of chloralose (15 mg/kg, i.v.) or pentobarbitone (15 mg/kg, i.v.) given to the ewe abolish foetal breathing movements (Dawes *et al.*, 1972).

In the present experiments the effects of a lower dose of pentobarbitone (4 mg/kg) on the foetus are described, and are compared with those of pethidine in large doses. A brief account of some of these observations has been given elsewhere (Dawes, 1973a).

Methods

Foetuses of known gestational age carried by ewes of mixed breeds were prepared for chronic experiments under maternal epidural and local anaesthesia (Dawes *et al.*, 1972). Catheters were implanted in a maternal carotid artery and external jugular vein. A low midline incision was made using aseptic and antiseptic precautions; the uterus was opened, the foetal neck was exposed and a carotid artery and the trachea were catheterized. The tracheal catheter was advanced until its tip lay within the thoracic cavity. To record the foetal electrocorticogram biparietal stainless steel screw electrodes were placed in contact with the dura mater 1.8 cm apart on either side of the midline; they were isolated electrically by application of cold cure dental acrylic, and the scalp was closed. A soft vinyl catheter was placed in the amniotic cavity. The uterus was closed, the catheters and leads being brought to the exterior through an incision in the maternal flank. In one ewe both twins were catheterized; otherwise only one of twins, where present, was operated on. A continuous slow infusion (5 ml/day) of heparin (400 units/ml) was used to maintain the patency of the foetal carotid artery catheters. Antibiotics, streptomycin 1 G and benzyl penicillin 1 million units, were given intramuscularly once daily for the first 2 days. The ewes were housed in metabolism cages and fed hay and concentrate.

Continuous records of foetal tracheal, carotid and amniotic pressures were made night-and-day on a Schwarzer polygraph. The foetal heart rate was obtained from the carotid pulse with an instantaneous rate-meter (Devices). The foetal arterial pressure was recorded as the difference between carotid and amniotic pressure using an electronic subtraction circuit. Blood samples for gas analysis were drawn

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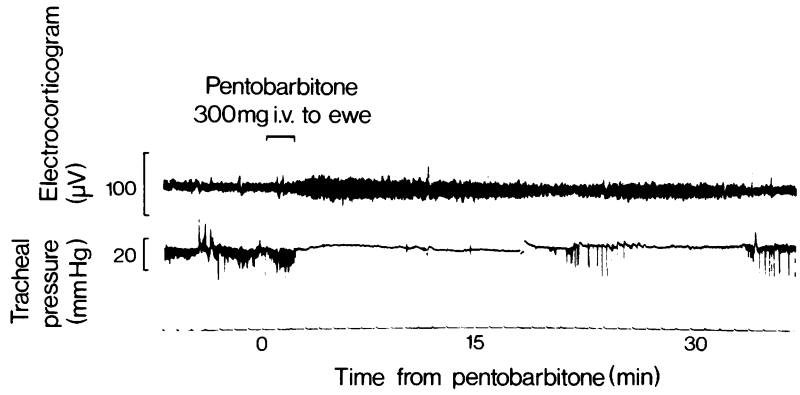

Figure 1 Administration of pentobarbitone (4 mg/kg, i.v. over 2 min) to a pregnant sheep near term caused a change in foetal electrocortical activity (upper record), and arrest of foetal breathing movements.

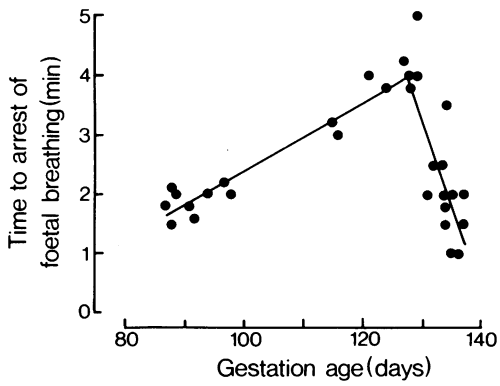

Figure 2 The time to arrest of foetal breathing, after administration of pentobarbitone (4 mg/kg, i.v.) to the mother, varied with gestational age.

into heparinized glass syringes and measured immediately (Radiometer); the results were corrected for the difference between electrode and body temperature, where present. The sheep were allowed to recover and no experiment was performed within 48 h of the operation; thereafter one experiment only was carried out each day. The ewes were weighed before operation and the dose of sodium pentobarbitone (Nembutal, Abbot) was calculated as 4 mg/kg. The pentobarbitone was given slowly to the ewe via the external jugular vein over a 2 min period, during a period of rapid foetal breathing unless stated otherwise, and the catheter was flushed with 10 ml heparinized 0.9% w/v NaCl solution (saline). The start of the injection was taken as time zero. Control measurements were made for the 6 h before the injection. The experiments were performed in the morning or early afternoon to minimize the effects of the diurnal variations in both foetal breathing movements (Boddy, Dawes & Robinson, 1973) and in the time of onset and duration of pentobarbitone action (as in the adult rat; Friedman & Walker, 1968).

Results

Pentobarbitone

The ewe was only lightly sedated by injection of pentobarbitone (4 mg/kg). If standing at the start of the injection she normally remained so; occasionally she continued to eat hay or concentrate. No measurements were made of maternal blood pressure or breathing. On no occasion did the ewe appear to be excited by the dose of pentobarbitone.

Foetal breathing movements. Two types of episodic breathing movements are observed in the sheep foetus, rapid shallow irregular breathing movements at 1–3 Hz (associated with rapid-eye-movement sleep) and slow augmented breaths, described as 'sighs or gasps' (Dawes *et al.*, 1972). When the pentobarbitone was injected during an episode of foetal breathing all breathing movements stopped within 1.0 to 5.0 min (e.g. Figure 1). The time to arrest of foetal breathing was positively correlated with gestational age between 87 and 130 days ($r=0.955$, $P<0.01$) but negatively correlated between 125 and 137 days ($r=-0.69$, $P<0.01$; Figure 2).

After a period of apnoea ranging from 8–31 min foetal breathing movements returned as a series of single inspiratory movements with a rate of 2–3 per minute. The time to the onset of this slow foetal breathing was positively correlated with gestational

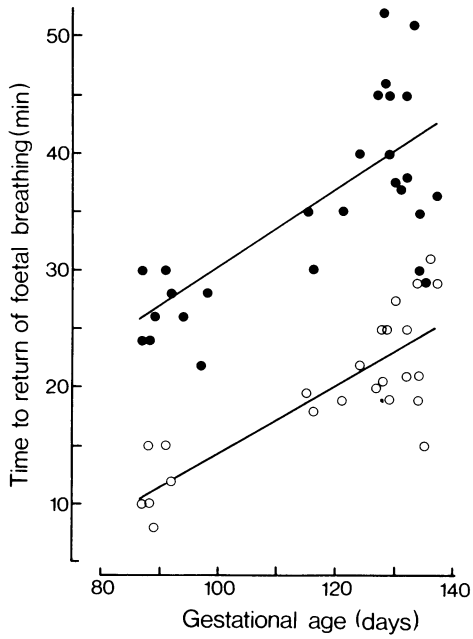

Figure 3 The time to return of slow (○) and of rapid (●) foetal breathing, after maternal administration of pentobarbitone (4 mg/kg, i.v.), varied with gestational age.

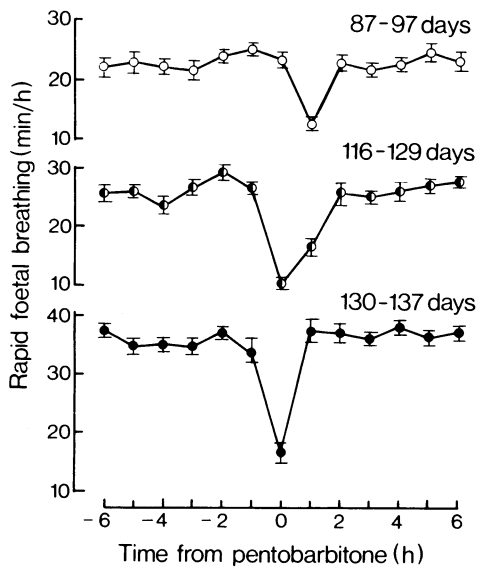

Figure 4 Variations with gestational age in the effects of maternal administration of pentobarbitone (4 mg/kg, i.v.) on the proportion of time during which foetal breathing was present. Note that the proportion varied with gestation before drug administration.

age (Figure 3, open circles). There was then a further period of apnoea before the return of rapid foetal breathing movements. The time to return increased with gestational age (Figure 3, closed circles).

The change in foetal breathing in the 2 h after administration of pentobarbitone varied with gestational age. At 87–97 days (11 experiments, 4 foetuses) the number of minutes of foetal breathing in the first hour after pentobarbitone injection did not differ significantly from that in the 6 h control period (Figure 4). In the second hour it was reduced significantly by 44%. At 116–129 days (7 experiments, 3 foetuses) foetal breathing was reduced significantly from the control values by 60% in the first hour and by 38% in the second hour. In the oldest lambs (130–137 days; 12 experiments, 6 foetuses) foetal breathing movements were only reduced in the first hour following pentobarbitone, by 53%.

Six experiments were done on 3 foetuses of 117–138 days gestation in which pentobarbitone was given to the ewe during foetal apnoea. The breathing movements were reduced from 28.6 ± 0.7 min/h in the 6 h control period to 6 ± 2.0 min in the hour after injection. They were not significantly reduced in the second hour.

Foetal electrocorticogram. The foetal electrocorticogram was recorded in 6 experiments on 4 foetuses between 115 and 134 days. In each lamb soon after the start of a maternal injection of pentobarbitone the electrocortical activity changed gradually from low to high voltage. The rate of change was slower than that observed spontaneously in the transition from rapid-eye-movement to quiet sleep. In the first hour after pentobarbitone administration the incidence of electrocortical low voltage activity was reduced (Figure 5). The number of changes from low to high voltage per hour increased by more than 80% in the 2 h after giving pentobarbitone.

Foetal cardiovascular changes. The effect of pentobarbitone on the foetal heart rate and arterial pressure varied with gestational age. The heart rate in the control period was greater in the younger than in the older foetuses. At 87–97 days there was a small fall in heart rate (Figure 6) after injection of pentobarbitone, which gradually returned towards its initial value.

At 116–129 days the foetal heart rate increased from 158 ± 2 to 181 ± 4 at 10 min (Figure 6); it remained elevated for 45 min and then slowly fell to control values. In the oldest lambs, at 130–137 days, the heart rate rose from 149 ± 3 to 166 ± 5 beats/min for 15 min and then declined.

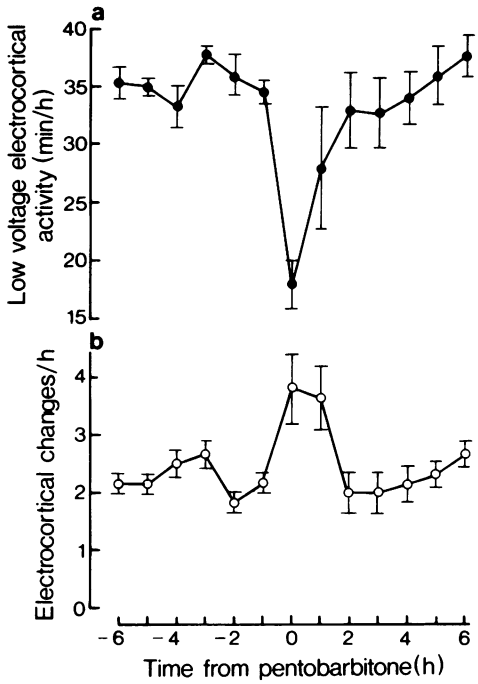

Figure 5 (a) Shows the effect of maternal administration of pentobarbitone (4 mg/kg, i.v.) on the proportion of time during which low voltage foetal electrocortical activity was present. (b) Shows that the number of changes from low to high voltage activity increased as a result of pentobarbitone administration.

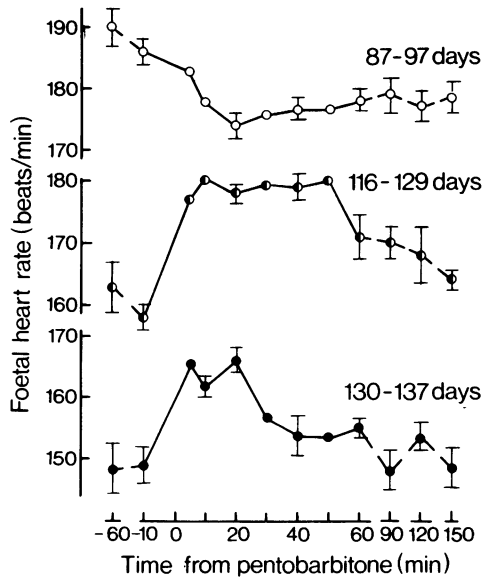

Figure 6 Changes in foetal heart rate, on maternal administration of pentobarbitone (4 mg/kg, i.v.), varied with gestational age. Note the fall in heart rate with age.

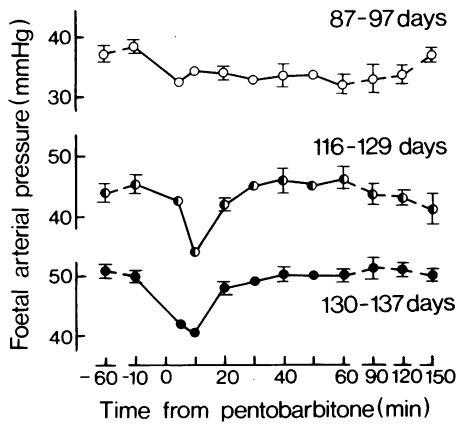

Figure 7 Changes in foetal carotid arterial blood pressure, on maternal administration of pentobarbitone (4 mg/kg, i.v.), varied with gestational age. Note the rise in blood pressure with age.

In the control period the arterial pressure increased with gestational age. In the youngest group of foetuses it fell after pentobarbitone and recovered only very slowly (Figure 7). At 116–129 days, there was a biphasic response, while at 130–137 days there was a brief fall in pressure lasting a bare half hour.

Blood gases and pH. There were barely significant changes in maternal carotid blood gas and pH values after administration of pentobarbitone (4 mg/kg). The foetal carotid Pa_{O_2} (22 ± 1.6 mmHg), Pa_{CO_2} (49.9 ± 2.3 mmHg) and pH (7.33 ± 0.007) in 7 experiments on 4 foetuses of 115–136 days gestation were not changed significantly.

Pethidine

Doses of pethidine hydrochloride 100–200 mg were given intramuscularly, intravenously or retrograde via a carotid artery into the ascending aorta in 25 experiments on 13 ewes during the last third of pregnancy. The arterial injections sometimes caused maternal convulsions and were abandoned. There was no consistent effect on foetal breathing movements, blood pressure or heart rate even though maternal administration of pethidine to sheep gives foetal blood

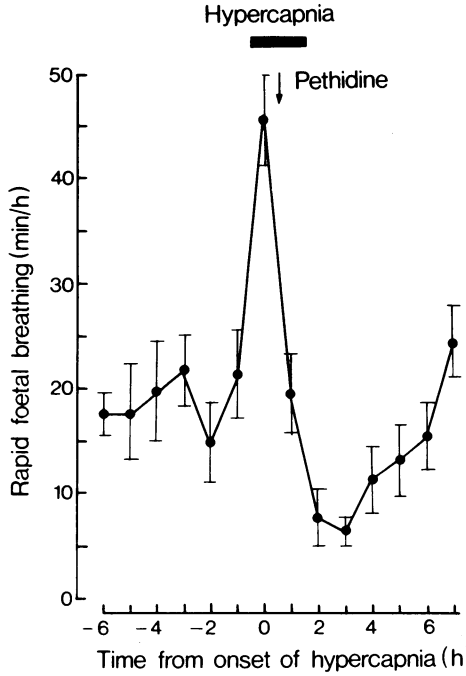

Figure 8 Observations on 5 foetal lambs, showing that maternal administration of pethidine (200 mg, i.v.) abolished the increase in foetal breathing induced by hypercapnia (6% CO_2, 18% O_2 in N_2 given to the ewe).

concentrations which after 10 min are higher than those in the mother (Jenkins, Talbert & Dilts, 1972).

This negative result was surprising. Therefore additional experiments were conducted on 5 foetal lambs in which breathing activity was increased for 2 h by hypercapnia, induced by administration of 6% CO_2 with 18% O_2 in N_2 to the ewe (Boddy *et al.*, 1974). This caused a rise in foetal $PaCO_2$ from 42.3 ± 1.6 to 56.7 ± 1.5 mmHg, with no significant change in PaO_2. The proportion of time during which breathing movements were present was more than doubled during the first hour's hypercapnia (Figure 8). Administration of pethidine (200 mg, i.v. to the ewe) at the beginning of the second hour abolished this response. There was subsequently a reduction in the proportion of time during which breathing movements were present, after the end of hypercapnia. In control experiments increased breathing movements were sustained during 2 h hypercapnia.

Discussion

Episodic irregular foetal breathing movements are normally present from early gestation, about 0.27 of full term (40 days in sheep, 11 weeks in man; Boddy & Dawes, 1975). These movements can be affected by the consequences of maternal anaesthesia, by drug action on the foetus and also by the experimental procedure. They are present in foetal lambs only during rapid-eye-movement sleep (Dawes *et al.*, 1972). Procedures which disturb this state, such as restraint after delivery or the acute effects of foetal operation, may prevent the appearance of foetal breathing movements for many hours. They are only present for a normal proportion of the time (~40% in lambs) in chronic preparations in sheep (Dawes *et al.*, 1970; Merlet *et al.*, 1970), or using ultrasound to measure the chest wall movements of intact foetuses *in utero* (Boddy & Robinson, 1971) or acutely on delivery of an intact foetal lamb unrestrained into a warm saline bath under maternal epidural or spinal anaesthesia. Only under such conditions can unambiguous information be obtained as to the effects of drugs on normal foetal breathing movements. Even under otherwise favourable circumstances foetal breathing movements are abolished by a reduction in foetal PO_2 (from 24 to 16 mmHg; Boddy, Dawes, Fisher, Pinter & Robinson, 1974) or by hypoglycaemia. The doses of pentobarbitone used in the present experiments were insufficient to cause a significant change in either PaO_2 or blood glucose. Small doses of barbiturate can cause excitement and catecholamine release. Infusion of catecholamines into a foetal lamb has no effect or increases foetal breathing movements (K. Ritchie; unpublished observations), so the arrest of foetal breathing by pentobarbitone cannot be ascribed to catecholamine release. The abolition of foetal breathing is therefore attributed to a direct effect on the foetus.

Pentobarbitone crosses the sheep's placenta rapidly to give foetal carotid concentrations of about 5 μg/ml plasma 20–40 min after injection of 5 mg/kg into the ewe (Mirkin, 1974). The foetal arterial blood concentration reaches a peak in 20–30 min, whereafter it both exceeds that in the mother and slowly declines over more than an hour. This is in agreement with a theoretical analysis of drug equilibration in the foetus (Dawes, 1973b, c). These data are more consistent with the observed duration of action of pentobarbitone, administered maternally, on the foetal electrocorticogram (Figure 5) than on breathing movements (Figure 1).

In newborn animals breathing spontaneously administration of 4–5 mg/kg pentobarbitone does not normally cause respiratory arrest. Larger quantities are usually required to induce general anaesthesia in newborn rabbits, sheep or rhesus monkeys (Downing, 1960; Dawes, Jacobson, Mott & Shelley, 1960; Dawes, 1968). Mature foetal monkeys delivered under maternal pentobarbitone anaesthesia are readily induced to breathe spontaneously, even after prolonged total asphyxia (Cockburn, Daniel, Dawes,

James, Myers, Niemann, Rodriguez de Curet & Ross, 1969). It is therefore evident that irregular episodic foetal breathing movements are unusually susceptible to pentobarbitone, more so than regular maintained breathing after birth. The difference is not explained by the greater susceptibility of very young animals to barbiturates, as measured by sleeping time or the induction of surgical anaesthesia. It is more likely to be due to an effect on the state of sleep. In adult man the dose of pentobarbitone used (4 mg/kg) causes a reduction in rapid-eye-movement sleep activity (Kleitman, 1963).

The effect of pentobarbitone in causing a reduction in the incidence of predominantly low voltage rapid electrocortical activity in foetal lambs can only be examined after 115 days gestation, when electrocortical activity is sufficiently differentiated into its fast and slow components. Bernhard, Kaiser & Kolmodin (1959) found that the lamb's electroencephalogram becomes continuous at 90–100 days gestation, and different foetal sleep states (arousal, quiet and paradoxical sleep) were identified in late pregnancy (Dawes *et al.*, 1970, 1972; Ruckebusch, 1971). In other species of animals and man studied postnatally the sleep-wakefulness pattern can be recognized by changes in behaviour and functions such as breathing before the characteristic electrocortical patterns can be identified (Ellingson, 1972). It may thus be possible to study the effects of barbiturates on the foetal state of arousal or sleep at an earlier stage of cerebral development. The long term effects of barbiturates on the foetal brain are not known, but the evidence that function is necessary for the proper development of structure (e.g. in the development of the visual cortex of the kitten and the somato-sensory region of the mouse) at certain crucial periods is worth noting.

There are complex age-related changes in the times to onset of foetal apnoea and to return of breathing movements (Figures 2 and 3) after maternal administration of pentobarbitone. Interpretation of these differences must be speculative in the absence of information about the time-related changes in foetal plasma pentobarbitone concentrations with gestational age. Data are available for foetal pentobarbitone pharmacokinetics and protein binding in sheep only at 124–136 days gestation as yet (Mirkin, B.L., personal communication). The results are unlikely to be due to changes in the foetal circulation with age (Dawes, 1968; 1973c; Rudolph & Heymann, 1974). They are more likely to be due to changes in foetal hepatic uptake and detoxification and/or to changes in the susceptibility of cerebral tissues to barbiturates with age (as after birth).

Interpretation of changes in foetal heart rate after pentobarbitone administration is complicated by the fact that there are, independently, an association between heart rate and electrocortical activity (Dawes, 1973a). and an inverse correlation between foetal heart rate variability and foetal breathing movements (Dalton, Dawes & Patrick, 1976). The foetal heart rate changes after maternal pentobarbitone administration are not attributable to changes in foetal blood gas values or pH. The baroreceptor mechanisms are increasingly active in the last third of gestation in sheep (Dawes, 1968; Shinebourne, Vapaavuori, Williams, Heymann & Rudolph, 1972) and could explain the heart rate changes in the lambs of 130–137 days (Figure 5) where there is an inverse relation between heart rate and arterial pressure but not otherwise. The initial fall in arterial pressure at all three gestational ages (Figure 6) appears similar to that observed in animals after birth on barbiturate administration, and attributed to systemic vasodilatation. But in this instance pulmonary vasodilatation could also contribute.

It is noticeable that there is a large prolonged rise in heart rate at 116–129 days gestation (Figure 5), associated with restoration of arterial pressure to or above its initial value (Figure 6). This contrasts with the prolonged fall in both heart rate and blood pressure earlier in gestation (87–97 days), at an age when the autonomic control of the foetal circulation is less well developed (Dawes, 1968).

The susceptibility of the foetal lamb to small doses of pentobarbitone, administered to the mother, is in contrast to the absence of any consistent effect with doses of pethidine which, by analogy with those in normal clinical use, are relatively large. Yet these doses of pethidine did abolish the foetal respiratory response to hypercapnia. It is also evident that acute experiments in which the effects of drugs were examined on the foetal circulation under pentobarbitone anaesthesia will require reconsideration, a conclusion arrived at on other grounds by Assali, Brinkman & Nuwayhid (1974) who reported a gross alteration of maternal uteroplacental vasomotor reactivity by pentobarbitone in anaesthetic doses.

We acknowledge gratefully the help of a grant from the Medical Research Council, and the assistance of A. Stevens. S.P. was a Travelling Fellow of the Hungarian Academy of Sciences.

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(Received December 11, 1975.)