PROCEEDINGS OF THE NUTRITION SOCIETY

The Four Hundred and Forty-fourth meeting of the Nutrition Society was held at the Royal Society of Medicine, Wimpole Street, London, on 16 February 1987, when a guest lecture was given by the 1985 recipient of the Annual Research Award of the Fish Meal Manufacturers' Association, Professor A. D. Care.

Placental transfer of calcium to the ovine fetus and its regulation

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It is generally believed that homeostasis in the fetus is under fetal control, the role of the mother being to provide an adequate supply of nutrients to maintain the growth of the fetus. Inadequate placentation can be achieved experimentally in the ewe by previous removal of most of her uterine caruncles before conception takes place; this leads to fetuses that are under-weight (Robinson *et al.* 1979). Inadequate nutrition of the ewe, especially of protein, also leads to under-sized fetuses but if only the intake of the major minerals is deficient, it is the skeleton of the ewe that becomes depleted in order to ensure an adequate supply of calcium and phosphate to the fetus to satisfy the demands of its growing skeleton (Sykes *et al.* 1973).

Quantification of the transplacental transfer of Ca

By serially slaughtering pregnant Romney ewes of known dates of conception, Grace *et al.* (1986) were able to measure the rates of accumulation of Ca and other elements in the fetuses over the period 62–143 d of gestation (full-term is 145 d). They showed that for both single and twin lambs the rate of accumulation of Ca by the fetus increased sharply at 90 d of gestation and at 143 d reached a maximum rate of 2·61 g/d for a single fetus of mean weight 5·3 kg and 2·80 g/d for twin fetuses of total weight 8·2 kg. That is, the rate of accumulation of total fetal Ca near to term was approximately the same whether or not single or twin fetuses were present (Fig. 1). McDonald *et al.* (1979) reported a similar finding. This probably reflects the fact that the chorio-allantois associated with a single fetus extends into the unoccupied uterine horn. Presumably this maximum rate of accumulation of Ca by the fetus is related to placental size and development of the cotyledons with consequent effect on maternal to fetal transfer of Ca since it is known that placental Ca transport in the sheep is essentially a uni-directional process.
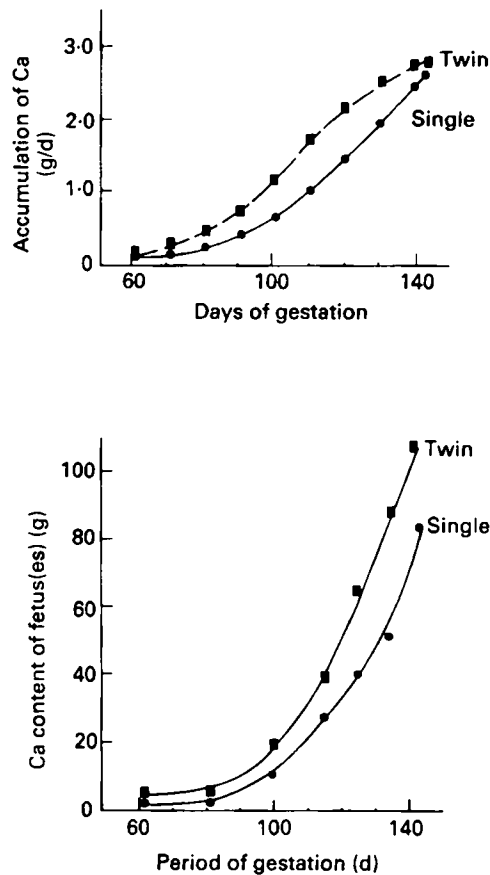
Fig. 1. The rates of accumulation of fetal calcium and the Ca content of the fetus(es) at different gestational ages. Values obtained by serial slaughter of Romney ewes (Grace *et al.* 1986).

Twardock *et al.* (1973) also found that the maximum rate of transfer of Ca from mother to fetus near to term was approximately 2·8 g/d and that this rate was almost unaffected by the number of fetuses between two and five. However, the maximum rate of transfer for singletons was only 1·5 g/d. Part of this difference for single fetuses between these results and the results of Grace *et al.* (1986) may stem from the fact that the fetal lambs used by Twardock *et al.* (1973) were approximately 5 d younger than those used by Grace *et al.* (1986) and this is during a period in which the rate of transport of Ca is increasing rapidly (Fig. 1). Field & Suttle (1967) also found a much larger total rate of accumulation of Ca by twin and triplet fetuses compared with a single fetus. Relative differences in placental size, as reflected by the comparatively larger relative differences in total fetal weight may account for much of the differences found between the various research groups. Thus, Twardock *et al.* (1973) found that twin through to quintuplet fetuses were each growing at a slower rate than singletons by 136–140 d

Table 1. *Daily net uptake of calcium by the fetus(es) of single and multiparous ewes (mg Ca/d per kg fetal weight)*

Reference	Gestational age (d)	One fetus	Two fetuses	Three fetuses
Braithwaite *et al.* (1970)	139	360	400	—
Field & Suttle (1967)	140	330	398	458
Twardock *et al.* (1973)	138	269	267	197
Grace *et al.* (1986)	143	492	391	—
McDonald *et al.* (1979)	140	—	243	230
Mean	—	363	340	333

of gestation and contained less Ca per fetus. They concluded that this stemmed from a limitation on the maximum rate at which Ca could be transported across the placenta. However, from the work of Sykes *et al.* (1973) it would appear that the placental capacity to transport protein for bone matrix formation, rather than to transport Ca, is likely to be the major factor which limits total fetal weight. When the rate of accumulation of Ca is expressed relative to total weight of the fetuses it is found that the mean rate is similar (345 mg/d per kg fetus) during the last week of gestation whether there are one, two or three fetuses (Table 1). Also, Braithwaite *et al.* (1972) found that the accretion rate of Ca by the fetal skeleton, expressed in terms of fetal body-weight, was approximately constant (452–489 mg Ca/d per kg fetal weight), irrespective of gestational age over the range 120–140 d and the presence of single or twin fetuses. These results suggest that the growth of the fetus and its consequent demands for homeostasis play the major role in defining the rate of placental transfer of Ca from the mother.

By the use of deconvolution analysis of the relation between the specific activities of the fetal and maternal plasma with time after injection of $^{45}CaCl_2$ into the fetal umbilical vein and $^{47}CaCl_2$ into the jugular vein of the mother, it was demonstrated that the rate of transport of Ca from ewe to fetus was 215 mg/d per kg fetal weight whereas the rate of transport in the opposite direction was only 12 mg/d per kg fetal weight (Ramberg *et al.* 1973). This striking result has also been shown indirectly by Braithwaite *et al.* (1972) when they used the Aubert & Milhaud (1960) method of compartmental analysis following the injection of $^{45}CaCl_2$ into a fetal umbilical vein. They found that the rate of net bone resorption in the fetus was negligible and that the rate of bone accretion approximated to the rate of net transport of Ca from the ewe to the fetus (360–490 mg/d per kg fetal weight). That is, in the ovine fetus almost all Ca that passes to it from the mother is used by the developing skeleton. This is in sharp contrast to the monkey in which the bidirectional rates of Ca transport across the placenta are comparatively similar to one another (Ramberg *et al.* 1973).

Control of Ca transport across the placenta

As in all mammals studied the plasma concentration of ionized Ca in the ovine fetus exceeds that in its mother (Care *et al.* 1981). There is no significant

correlation between maternal and fetal plasma Ca^{2+} concentration in pairs of samples taken from a flock of pregnant ewes (Bawden *et al.* 1965) and short-term maternal hypercalcaemia has no significant effect on fetal calcaemia or on the fetal secretion of calcitonin, a good biological indicator of incipient hypercalcaemia (Garel *et al.* 1974). However, more recently it has been shown that maternal hypercalcaemia maintained over 4 d does lead to a small but significant rise in the fetal plasma Ca^{2+} concentration (Care & Abbas, 1987). Similarly, short-term (1 h) maternal hypocalcaemia leads to no significant changes in either fetal plasma Ca concentration or the fetal plasma concentration of immunoreactive parathyroid hormone (iPTH), which has been shown to be increased during induced fetal hypocalcaemia (Care *et al.* 1975). Longer-term maternal hypocalcaemia produced either spontaneously (Care, 1980) or by maternal thyroparathyroidectomy 24 h beforehand caused no marked change in fetal calcaemia (3·30 *v* 3·14 mmol/l). Thus, relatively short-term changes in maternal calcaemia are without significant effect on fetal Ca^{2+} concentration, presumably because the fetus can react to relatively small changes in the trans-placental rate of transfer of Ca^{2+} in order to preserve Ca homeostasis. In the longer term, however, the influence of changes in maternal calcaemia may be able to exert a small influence on fetal calcaemia. Thus, treatment of ewes with 1α-hydroxycholecalciferol (1αOHCC) for 12 d led to significant increases in both maternal and fetal plasma Ca concentrations (but no increase in the fetal:maternal ratio) and an increased placental transfer of Ca to the fetus (Durand *et al.* 1983). These effects of (1αOHCC) were reproduced by treatment of pregnant ewes with ovine prolactin for 14 d alone or with (1αOHCC) (Barlet, 1985*b*). It was shown that this treatment increased intestinal absorption of Ca in the ewes and consequently their plasma Ca concentration. This in turn led to an increase in the placental transfer of Ca and to consequent increases in the fetal content of Ca and also in fetal calcaemia. It was interesting to note that these treatments did not lead to a significant alteration in the fetal:maternal ratio for plasma Ca concentration. This suggests that the changes in placental transfer of Ca were not the result of stimulation of the putative Ca pump in the placenta but were rather the consequence of the maternal hypercalcaemia. Conversely, one might account for the decrease in the placental transfer of Ca, which has been reported by Barlet (1985*a*) to follow the administration of salmon calcitonin to pregnant ewes, in terms of the potential hypocalcaemic effect of calcitonin.

Direct assessment by placental perfusion of the effect of maternal thyroparathyroidectomy, carried out 3 d beforehand, and with an intravenous infusion of Ca to maintain normocalcaemia, showed a small fall in the rate of Ca transfer to the fetal side of the placenta despite the fact that fetal calcaemia was within the normal range (Weatherley *et al.* 1983). The mechanism of this effect is not understood and may represent a small maternal influence on the placental transfer of Ca to the fetus. Despite the previously mentioned examples of maternal modulation of placental Ca transfer, it is concluded that fetal Ca homeostasis is largely independent of the mother and that the fetus has the major role in the control of Ca^{2+} transport from the mother.

Perfusion of the fetal placenta to assess the activity of the placental Ca pump

Because of the buffering effect of the fetal skeleton it is not possible to draw valid conclusions from a change, or lack of change, in fetal plasma Ca^{2+} concentration which apply to supposed changes in the placental rate of transfer of Ca^{2+}. One must therefore study placental transport directly. It is believed that the transport of Ca^{2+} across the ovine placenta is an active process. Evidence for this stems from the observation that replacement of the ovine placenta by a semi-permeable colloidal membrane reversed the fetal–maternal Ca gradient (Delivoria-Papadopoulos *et al.* 1967). Furthermore, fetal placental perfusion with heparinized maternal blood in the absence of the fetus showed net transport of Ca to the fetal side against a Ca concentration gradient (Alexander *et al.* 1973). When this work was repeated using heparinized fetal blood the capacity of the placenta to transport Ca^{2+} was increased (Care, 1980; Weatherley *et al.* 1983). This comparison is clearly shown in Fig. 2 which shows the effect of perfusion of the placenta of one twin fetus with maternal blood and the other twin with autologous fetal blood. It can be seen that the rate of increase in Ca^{2+} concentration on the
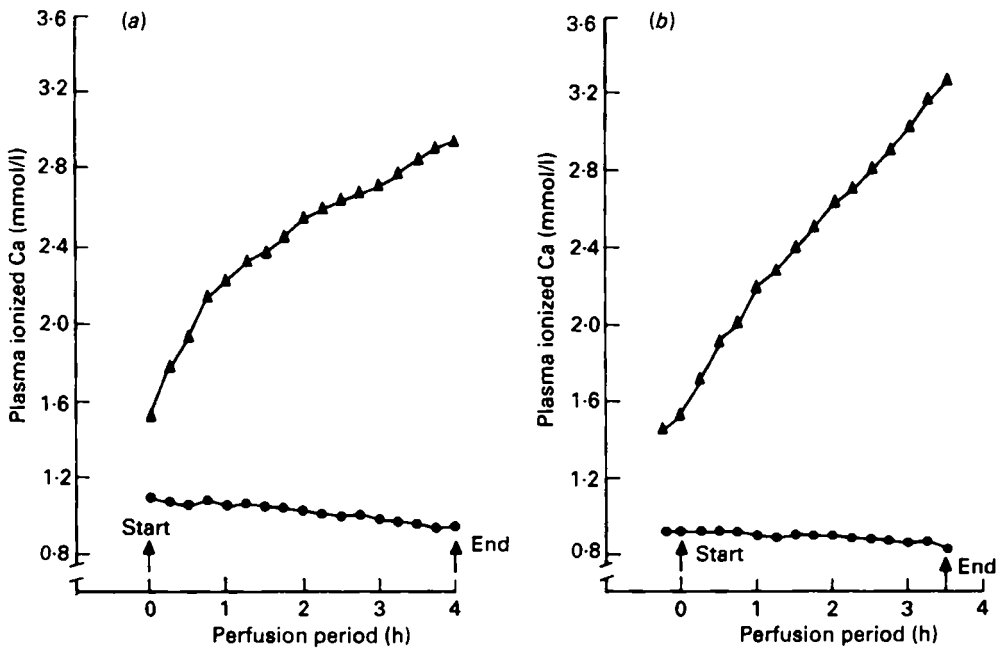

Fig. 2. Changes in plasma ionized calcium concentration during perfusions of the placenta of an intact fetal lamb (*a*) with heparinized maternal blood (▲————▲) and that of its twin lamb (*b*) with heparinized autologous fetal blood (▲————▲) under controlled conditions of flow-rate and perfusion pressure. The corresponding maternal Ca^{2+} concentrations (●————●) are also shown. ↑, The time of commencement of each perfusion.

fetal side of the perfused placenta is significantly greater when autologous fetal blood is used as the perfusion medium.

This study has been taken further by perfusing the fetal placenta with a blood substitute (Fluosol-43; Green Cross Corporation, Osaka, Japan) containing electrolytes, glucose, hetastarch and the oxygen carrier, fluorocarbon. No significant increase in its Ca concentration was observed over the first hour of perfusion whereas in comparable experiments which used autologous fetal blood as the perfusion medium there was an increase of $1 \cdot 1$ mmol Ca/l (Care & Ross, 1984). It was concluded that fetal blood contained a substance that promoted the active transfer of Ca^{2+} to the fetal side of the placenta but at this stage it was not possible to distinguish between the maternal circulation and a pool of Ca in the placenta itself as the source of this increase in Ca on the fetal side of the placenta. This question was answered by raising the maternal Ca concentration during the perfusion of a fetal placenta with fetal blood. There was a close correlation between the Ca^{2+} concentration in the fetal-perfusing plasma and that in the maternal plasma, the fetal:maternal ratio always remaining greater than unity (Care & Ross, 1984). This indicated that, in the absence of the fetal skeleton, maternal hypercalcaemia could increase the Ca^{2+} concentration on the fetal side of the placenta and that it was the maternal circulation rather than the placenta that constituted a rapidly available source of Ca for the fetal circulation.

Role of the fetal parathyroid glands in the placental transport of Ca

In keeping with the elevated level of Ca^{2+} concentration in fetal plasma, the circulating concentration of iPTH is very low and often undetectable (Smith *et al.* 1972; Care *et al.* 1975). However, in contrast, the concentration of bioactive PTH-like material in fetal plasma samples was later shown, using a very sensitive cytochemical bioassay, to be greater than that in maternal samples taken at the same time (Care *et al.* 1987). A similar observation has been made in the human (Allgrove *et al.* 1981) and in the pig (Care *et al.* 1982). From these results it was inferred that either the set-point for stimulation of PTH secretion by hypocalcaemia is altered in the fetus or another substance is present that reacted in the bioassay in a similar manner to PTH. This latter hypothesis has now been closely examined using several different approaches. The cytochemical assay for PTH (Chambers *et al.* 1978) has been used in the presence and absence of an antiserum to bovine (1–84) PTH (the standard PTH preparation used) in order to differentiate between PTH and non-PTH-like bioactivity in samples of plasma or extracts of ovine parathyroid glands obtained at surgery. It was found that whereas the bioactivity of bovine PTH could be completely neutralized by the addition of excess antiserum to bovine PTH, as much as 80% of the bioactivity in an extract of fetal parathyroid glands could not be neutralized in this way (Abbas *et al.* 1987). Similarly, whereas all the bioactivity in a sample of maternal plasma could be neutralized by the addition of excess antiserum to bovine PTH this was not so with fetal plasma.

Further evidence for the presence of a second bioactive substance in fetal

parathyroid glands was provided by Kubota *et al.* (1987). They showed, using an adenyl cyclase system in UMR106 osteoblast-like cells, that an extract of fetal parathyroid glands had adenyl cyclase-stimulating activity only part of which could be neutralized with an excess of PTH antiserum, whereas the bioactivity of the PTH standard could be completely neutralized by the addition of excess PTH antiserum.

It was previously shown that fetal thyroparathyroidectomy (TXPTX), with thyroxine replacement, resulted in a precipitate fall in fetal plasma Ca^{2+} concentration within 24 h and the reversal of the placental Ca gradient (Care & Ross, 1984). This was at first considered to be caused by a decrease in the fetal plasma concentration of 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) but, subsequently, this could not be confirmed (Care *et al.* 1985). This later study confirmed the profound effect of fetal TXPTX on fetal plasma Ca^{2+} concentration and eliminated the loss of calcitonin as a result of the TXPTX as the cause of the hypocalcaemic response. This study also showed that the infusion of supra-physiological amounts of either PTH or 1,25(OH)$_2$D to a hypocalcaemic TXPTX fetus for 2–3 d failed to produce significant increases in plasma Ca^{2+} concentration.

In placental perfusion studies using twin fetuses, it was clearly shown that the final Ca gradient achieved between the fetal blood used to perfuse the fetal side of the placenta and the maternal circulation was significantly less than that with placentas from the intact twins (Care *et al.* 1985, 1986). It was concluded that partial failure of the placental Ca pump associated with the removal of the parathyroid glands could be partially compensated by reversing the placental Ca gradient to facilitate the passive transport of Ca^{2+} to the fetus. Nevertheless, it was found that the skeleton of hypocalcaemic TXPTX fetuses (treated with thyroxine) showed clear signs of rickets, demonstrated by histomorphometric analysis (Aaron *et al.* 1985). Presumably this was the result of the prolonged hypocalcaemia.

The reduced ability of the placenta of a TXPTX fetus to sustain a normal maximum value for the placental Ca^{2+} gradient could not be increased by the addition to the perfusing blood of supra-physiological amounts of PTH (maximum 70 μg PTH). However, the addition of an extract of fetal parathyroid glands (equivalent to only 1 μg bovine PTH bioactivity in the UMR106 cell adenyl cyclase assay) led to a marked increase in the net placental transfer of Ca^{2+} to the fetal side of the placenta (Abbas *et al.* 1987). Similar extracts of ewe parathyroid glands were without effect (S. K. Abbas *et al.* unpublished results).

From the above evidence it is concluded that the ovine fetal parathyroid glands secrete a substance capable of stimulating the placental transfer of Ca^{2+} to the fetus. The results of preliminary experiments suggest that the secretion of this substance is depressed by fetal hypercalcaemia (Care *et al.* 1987). The possible significance of this proposed substance in the aetiology of neonatal hypocalcaemia encountered in man remains to be elucidated.

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Printed in Great Britain