Br. J. Nutr. (1978), **39**, 347

The effect of litter size on placental blood flow and placental calcium transfer in the multifoetate guinea-pig

By H. W. SYMONDS,* R. H. BUBAR, W. CRACKEL AND A. R. TWARDOCK

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Illinois at Urbana, Urbana, Illinois, USA

1. Placental blood flow rate and calcium transfer rate were measured at 61 d of pregnancy in guinea-pigs carrying between one and eight foetuses.

2. Placental blood flow rate was significantly correlated with foetal weight. Ca transfer rate was related to placental size. Irrespective of litter size the mean amount of Ca transferred across a placenta was between 0·22 and 0·34 mg/h per g placental tissue.

3. It was concluded that there was a limit to the rate of transfer which was produced by a combination of limitations in placental blood flow rate, maternal plasma Ca concentration and placental tissue transfer capacity.

Litter size has a limiting effect on the growth rate of foetuses in utero. The larger the litter size the smaller is the average foetal birth weight. This relationship is of economic importance in farm animals where attempts to increase production by increasing litter size above the normal value for the species produces smaller, less viable offspring. The restriction on foetal growth is associated with the inability of the placenta to transfer sufficient nutrient in late pregnancy. For example, piglets delivered by caesarian section 8 d before the expected birth date and reared artificially grow faster than comparable piglets remaining in utero until normal parturition occurs (Ulberg & Lecce, 1971).

In sheep the lower growth rate of foetuses in large litters is due also to a limit to placental transfer capacity which is reached during the last third of pregnancy. Once the maximum transfer capacity has been reached foetal growth rate remains constant and individual foetuses in the litter cannot attain, in the same period, the size reached by the singleton at birth (Twardock, Symonds, Sansom & Rowlands, 1973).

The limit to placental transfer must arise as a result of a limit to one or more of three processes, placental blood flow, availability of nutrients and minerals in the maternal plasma and the ability of the placental tissue to transfer metabolites and minerals. This report describes experiments using the pregnant guinea-pig as a model because of its capacity for carrying large litters and producing new-born young with well mineralized skeletons. It examines the effect of litter size upon the placental transfer of Ca and placental blood flow rate to try to determine which of these three factors may be rate limiting. The results show that placental size is the principal factor, affecting both blood flow rate and the area available for transfer of Ca. Irrespective of litter size Ca was transferred at 0·2–0·3 mg/h per g placenta.

MATERIALS AND METHODS

Animals

Guinea-pigs carrying litters of one to eight foetuses were used on day 61 (±2 d) of their second gestation (term 68 d). The number of foetuses carried was determined by x-ray of the dams. The dams consumed an estimated 30 g/d of Purina guinea-pig chow (Ralston

* Present address: Department of Functional Pathology, Institute for Research on Animal Diseases, Compton, Newbury, Berks, RG16 0NN.

Purina Co., St Louis, Mo. 63188, USA) containing 1·1% calcium and 0·7% phosphorus. Maternal body-weight, less foetal tissue, was approximately 1·0 kg.

Surgery

One jugular vein and the left ventricle were catheterized under general anaesthesia induced and maintained by inhalation of methoxyflurane (Metofane, Pitman-Moore Inc., Washington, NJ 08560). Local anaesthetic was applied topically to the tissues to reduce the depth of general anaesthesia required. Vinyl tubing (i.d. 0·030 in, o.d. 0·048 in, manufacturer's size PE60, Clay-Adams Inc., New York) was used for catheterization. Silastic tubing (Dow Corning Corporation, Midland, Mich. 48640, USA) with a similar internal diameter was attached to the catheters where they emerged from the vessels because its greater flexibility allowed movement of the guinea-pig during recovery without the tips of the vinyl catheters becoming misplaced from their correct positions. Catheters were filled with 200 IU heparin/ml saline solution (9 g sodium chloride/l). The surgical preparation, including anaesthesia, took approximately 50 min to complete.

Experimental Procedure

The guinea-pigs were placed in a box for 1·5–2 h to recover. The ends of the two silastic cannulas were then brought out through a corner of the box to allow blood collection and dosing while the guinea-pig remained quiet in the semi-darkness of the box. Measurements were made of Ca transfer to the foetuses, cardiac output and the distribution of the cardiac output in the following order: Ca transfer to the foetuses was measured during a period of 45 min, the guinea-pig being killed at the end of the period; the cardiac output was determined immediately before, and also 20 and 40 min after the start of the 45 min period, and the distribution of cardiac output (%) was measured immediately after the first and third of these determinations of cardiac output. Packed cell volume was determined on blood samples taken before each measurement of cardiac output.

After slaughter of the mother, the viability of each foetus was assessed by checking that it could perform respiratory movements or that its heart was beating.

Determinations of cardiac output

Cardiac output was determined by measuring the dilution in the left ventricle of 10–15 μCi of ^{131}I-labelled human serum albumin (^{131}I RISA; Mallinckrodt Nuclear, St Louis, Mo., USA) given via the jugular catheter in 0·2–0·4 ml Ringers solution. Blood was collected continuously for approximately 15 s as it dripped from the ventricular catheter into aluminium planchets attached to a revolving Plexiglas disc. Each planchet collected blood for 0·014 min. To ensure that approximately equal amounts of blood dropped into each planchet, water was run down the outside of the silastic catheter at a rate of three to four drops per planchet. To determine the volume of blood in each planchet the guinea-pig's systemic blood was labelled with ^{125}I RISA 10–15 min before the first measurement of cardiac output. Planchets containing the blood samples were transferred to counting vials and 5 ml water added. A blood sample (100 μl) collected before each cardiac output measurement was treated similarly and the amount of ^{125}I activity/μl blood determined for all samples.

Determinations of Distribution of Cardiac Output

Blood distribution to placentas, uterus, femur, heart, lungs, kidneys, spleen and brain was measured. The last five tissues were selected to check the evenness of the distribution of blood flow and the physiological state of the animal during the course of the experiment. ^{85}Sr and ^{169}Yb-labelled microspheres (25 $\pm$ 5 μm diameter, 3M Company, St Paul, Minnesota

55101, USA) were used for the first and second determinations respectively. A suspension (0·5 ml) made in a solution of dextran (100 g/l), which included a wetting agent, was injected into the ventricle in a 1 min period with repeated rinsing of the syringe with blood. Vials containing the microspheres were ultrasonicated and shaken thoroughly before withdrawal of the dose and the dose was injected before any settling of microspheres occurred.

Determinations of placental Ca transfer and Ca uptake by placenta, uterus and femur

Approximately 10 μCi of $^{45}CaCl_2$ (ICN Chemical & Radioisotope Division, Irvine, California 92715, USA) in Ringers solution was injected into the jugular vein. Mean plasma Ca specific activity during the 45 min period was calculated from the radioactivity of five (1·0 ml) arterial blood samples taken 2, 5, 15, 30 and 45 min after dosing and related to the ^{45}Ca content of each foetus, placenta, uterus and femur.

Analytical procedures

Tissues were weighed, dry ashed at 600° for 24 h and the ash dissolved in dilute hydrochloric acid. Blood plasma was separated after centrifugation. 'Stable' Ca in plasma and ash solutions of uterus, femurs, placentas and foetuses was determined by atomic absorption spectrophotometry of dilutions of the acid ash solution.

Radionuclide procedures

Plasma samples contained ^{125}I, ^{131}I and ^{45}Ca. Ash solutions contained ^{85}Sr, ^{169}Yb, ^{45}Ca and some ^{125}I and ^{131}I which survived the dry ashing procedure. The ^{125}I and ^{131}I content of whole blood for cardiac output determinations was measured using two channels of a γ spectrometer with a well-type sodium iodide crystal (Nuclear-Chicago, Des Plaines, Ill. 60018, USA) and applying corrections for cross-over. ^{131}I in plasma and ash solutions was then removed by storing samples for at least 80 d (ten half-lives) before further analysis. ^{85}Sr and ^{169}Yb in the ash solutions were separated from each other and any residual ^{125}I by using three channels of the same spectrometer and applying corrections for cross-over.

The determination of ^{45}Ca in plasma and ash samples of femur, uterus, placentas and foetuses was more difficult because ^{125}I, ^{85}Sr, and ^{169}Yb present interfered with the measurement of the ^{45}Ca present. In order to determine the proportion of the count rate in the liquid scintillation system attributable to ^{45}Ca the following procedure was adopted. Plasma samples were prepared by taking 100 μl and adding 1 ml of NCS and 10 ml scintillator cocktail (Amersham/Searle Corporation, Arlington Heights, Ill. 60005, USA); ash solutions were prepared by mixing 1 ml with 10 ml of PCS. For all samples radioactivity was measured first in a liquid scintillation spectrometer (Isocap 300, Searle Analytic Inc., Des Plaines, Ill. 60018, USA) in the channel set for the ^{45}Ca band of the spectrum and then, in the same order, in the γ spectrometer in the three channels set for ^{125}I, ^{85}Sr and ^{169}Yb. Standards of each pure nuclide were prepared and determined similarly and the relative counting efficiency of each γ emitter in the different types of samples was determined. The proportion of the total count rate due to ^{45}Ca could then be calculated. Quenching of each of the four radioisotopes was similar and was corrected for by using an external standard. All samples were analysed in duplicate.

To determine the amounts of radionuclides injected, aqueous dilutions were made of the $^{45}CaCl_2$ and ^{125}I and ^{131}I RISA solutions used for dosing. The doses of ^{85}Sr and ^{169}Yb-labelled microspheres were estimated either by adding measured volumes of the dose suspensions to non-radioactive foetuses and dry ashing them or by adding measured volumes to liver homogenates. Samples of the latter were either analysed direct, because settling of microspheres was very slow in the homogenate, or after they had been dry ashed. No significant loss of activity occurred during the dry ashing procedure.

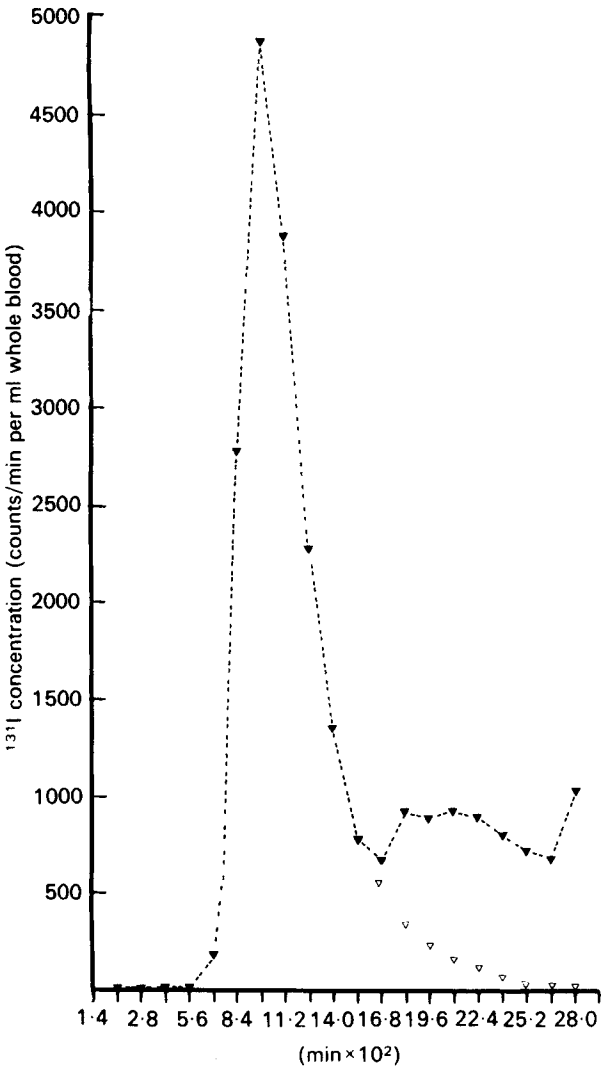

Fig. 1. ^{131}I concentration changes (counts/min per ml whole blood) in guinea-pig arterial blood with interval of time (min) after a single intrajugular injection of ^{131}I-radioiodinated human serum albumin. (▽) Extrapolation of dilution curve.

Calculations

Cardiac output. The ^{131}I content of each planchet blood sample corrected to counts/min per ml whole blood was plotted *v.* interval after administration of dose (see Fig. 1). Recirculation of ^{131}I was corrected for by extrapolating the 'post-peak' exponential decrease to 1 % peak height. The area under the corrected curve was obtained gravimetrically and the mean ^{131}I concentration of whole blood obtained. Cardiac output (ml/min) was calculated as:

$$\frac{\text{dose of } ^{131}\text{I injected (counts/min)}}{\text{mean whole blood radioactivity (counts/min per ml)}}.$$

Tissue blood flow rate. Total ^{85}Sr and ^{169}Yb radioactivity in a tissue was calculated as a

proportion of the dose injected. Since this value also represented the proportion of cardiac output reaching the tissue concerned, blood flow (ml/min) in the tissue was calculated as: proportion of dose of radioactivity injected present in tissue × cardiac output (ml/min).

Because ^{169}Yb-labelled microspheres were only available for half the guinea-pigs used, blood flow rates were calculated from the distribution of the microspheres injected at the start of the measurement period (in all but two instances ^{85}Sr-labelled microspheres) and the average of the three cardiac output determinations.

Ca transfer to foetus. The specific activity (counts/min per mg) of ^{45}Ca in each plasma sample was plotted graphically and the mean specific activity during the sampling period measured gravimetrically from the area under the curve. 'Stable' Ca transferred to a foetus (mg/h) or taken up by placentas, uterus and femurs was given by:

$$\frac{^{45}\text{Ca in tissue (counts/min)} \times 60}{\text{mean plasma specific activity (counts/min per mg)} \times 45}. \quad (1)$$

Ca available to a placenta for transfer (mg/min). The Ca available for transfer across a placenta depended on the concentration of Ca in the plasma passing through the placenta. The amount available (mg/min) was calculated as:

$$\text{Placental plasma flow rate (ml/min)} \times \text{plasma Ca concentration (mg/ml)}. \quad (2)$$

Ca extraction (%). A limit to the ability of the placenta to extract Ca from plasma is a possible contributory cause of a maximum transfer rate of Ca to the foetus. The amount of Ca extracted (%) is given by:

$$\frac{\text{equation no. 1} \times 100}{\text{equation no. 2}}\ \%.$$

Statistical analysis of data

For each variate the average response of the foetuses within a litter was calculated. These data were then subjected to one way analysis of variance testing both for differences among the groups and for a linear trend with increasing litter size. In addition, correlation coefficients were calculated between all pairs of variates within each group.

RESULTS

Tables 1 and 2 give the mean trends occurring with litter size after the results were analysed statistically to accommodate any wide variation between or within groups. As litter size increased, mean foetal body-weight, foetal Ca content, mean placental weight, maternal plasma Ca concentration and maternal femur Ca content decreased significantly, whilst uterine blood flow, Ca content of the uterus and the amount of Ca accreted to uterine tissue increased significantly. The other trends occurring with changes in litter size were as follows.

(1) Placental blood flow rate: the blood flow per unit wet weight of placental tissue was relatively constant throughout the range of litter sizes indicating that on average the smaller placentas of the larger litters received less blood.

(2) Placental transfer of Ca: though not statistically significant the average amount of Ca transferred by a placenta to a foetus (mg/h) decreased as litter size increased from one to three foetuses. Transfer was greatest in litters of one foetus. When more than two foetuses were present the transfer rate per placenta changed little with increasing litter size, being between 1·3 and 1·5 mg Ca/h. Placental size was the controlling factor because irrespective of litter size the amount of Ca transferred ranged between 0·22 and 0·34 mg/h per g placental tissue.

Table 1. *Effect of litter size on foetal and placental weight, maternal plasma calcium content, and femur Ca content in guinea pigs*

(Values in parentheses indicate numbers of guinea-pigs/group)

	Litter size								Coefficient of variation	Regression coefficient on litter size ± SE
	1 (3)	2 (5)	3 (2)	4 (4)	5 (4)	6 (1)	7 (3)	8 (1)		
Foetal wt (g)	102·9	100·3	87·7	82·2	83·7	82·0	74·6	66·7	17	$-4·9 \pm 1·5^{**}$
Foetal total Ca (g)	0·79	0·76	0·61	0·65	0·65	0·58	0·57	0·48	20	$-0·038 \pm 0·013^{*}$
Placental wt (g)	6·4	6·1	7·1	4·8	5·0	4·9	4·8	3·9	16	$-0·39 \pm 0·08^{***}$
Maternal plasma Ca (mg/l)	89	85	80	86	72	61	65	82	12	$-3·3 \pm 0·9^{**}$
Maternal femur Ca content (mg)	809	739	546	655	691	650	620	572	12	$-25 \pm 8^{**}$
Ca content of uterus (mg)	1·3	2·3	1·3	1·7	3·6	2·0	2·0	4·4	45	$0·22 \pm 0·10^{*}$

Statistical significance of linear relationship with litter size: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Table 2. *Effect of litter size on blood flow through placenta and uterus; calcium transferred to foetus, femur, placenta and uterus and average amount of Ca (mg/h) passing through a placenta in plasma and the proportion of this Ca transferred to the foetus in guinea-pigs*

	Litter size								Coefficient of variation	Regression coefficient on litter size ± SE
	1 (3)	2 (5)	3 (2)	4 (4)	5 (4)	6 (1)	7 (3)	8 (1)		
Placental blood flow (ml/min)	9·2	7·8	8·4	7·4	5·4	6·2	7·7	7·2	57	
Placental blood flow (ml/min per g placenta)	1·4	1·2	1·1	1·6	1·1	1·3	1·5	1·8	51	
Cardiac output (ml/min)	253	210	289	312	264	474	270	321	31	
Ca transferred to foetus (mg/h)	2·2	1·9	1·5	1·5	1·6	1·3	1·3	1·4	40	
Ca transferred to foetus (mg/h per g)	0·34	0·28	0·22	0·31	0·32	0·26	0·28	0·26	36	
Ca accreted to placenta (mg/h)	0·18	0·21	0·15	0·21	0·26	0·14	0·14	0·11	49	
Ca accreted to maternal femur (mg/h)	0·56	0·55	0·33	0·81	1·15	0·65	0·80	0·83	49	
Ca accreted to uterus (mg/h)	0·52	0·46	0·42	0·88	0·96	0·78	0·78	1·24	37	$0·086 \pm 0·027^{**}$
Uterine blood flow (ml/min)	2·2	1·8	2·2	2·8	2·3	4·0	3·4	3·7	31	$0·25 \pm 0·08^{**}$
Ca passing through placenta (mg/h)	31·6	26·8	26·0	24·1	15·0	15·1	26·3	22·7	59	
Amount of Ca passing through placenta transferred to foetus (Ca extraction %)†	8·8	5·3	6·9	6·4	11·9	8·3	4·7	6·1	46	

Statistical significance of linear relationship with litter size: ** $P < 0·01$. All other relationships non-significant.

† For details see p. 351.

Table 3. *Correlations of statistical significance between variables measured*

Variables		*r*
Placental blood flow (ml/min/g placenta)	*v.* foetal wt	0·51
	placental Ca	0·57
	foetal Ca content	0·49
	uterine Ca	0·56
	placental blood flow (ml/min)	0·96
	uterine blood flow	0·60
Cardiac output (ml/min)	*v.* placental blood flow (ml/min)	0·54
	placental blood flow (ml/min/g placenta)	0·58
	uterine blood flow	0·68
Ca accreted to:		
Placenta	*v.* Ca transferred to foetus	0·78
Maternal femurs	*v.* Ca transferred to foetus	0·51
	Ca accreted to placenta	0·53
Uterus	*v.* plasma Ca	0·51
	Ca transferred to foetus	0·54
	Ca accreted to placenta	0·52
	Ca accreted to maternal femur	0·61
Total foetal Ca	*v.* foetal weight	0·96
Placental calcium	*v.* foetal weight	0·62
	placental blood flow (ml/min)	0·63
	foetal calcium	0·64

Statistical significance of correlations: 0·50–0·60, $P < 0·05$; 0·63–0·74, $P < 0·01$; $>0·74$, $P < 0·001$.

(3) The amounts of Ca passing through a placenta in plasma: the amount of Ca passing through a placenta and therefore available for transfer showed no definite trend with litter size. The average percentage extraction of this Ca from plasma did not exceed 12% and for all litter sizes except group 5 was between 5 and 9%.

Table 3 gives the statistically significant correlations between the variables measured. Some correlation between cardiac output and tissue blood flow rate is to be expected because of the method used to determine the latter. A dependence of foetal growth rate upon blood flow rate was indicated by the significant correlation of 0·51 between placental blood flow rate and foetal weight and a correlation of 0·49 between placental blood flow rate and foetal Ca content.

DISCUSSION

The results show that at 61 d of gestation there was an upper limit to the placental transfer of Ca which, irrespective of litter size, was 0·22–0·34 mg/h per g of placenta. The two principal factors controlling Ca transfer across the placenta were placental size and maternal plasma Ca concentration. Placental size was a limiting factor in Ca transfer for two reasons. It determined both the area available for allowing movement of Ca from maternal to foetal circulation and the rate at which blood could flow through the placenta. The combined effect of these two factors was shown by the finding that, irrespective of size, each placenta only removed a maximum of 8–12% of the Ca passing through in plasma. Dams with large litters tended to have lower plasma Ca concentrations. Where plasma Ca concentrations were lower maintenance of the same amount of Ca available for transfer per placenta would require a compensatory increase in placental blood flow rate. Instead placental blood flow was less in the larger litters.

Placental blood flow also has a limiting effect upon foetal growth and mineralization. This was shown by the significant correlation between placental blood flow (ml/min per g) and foetal weight and Ca content. Other reports have demonstrated more clearly the depen-

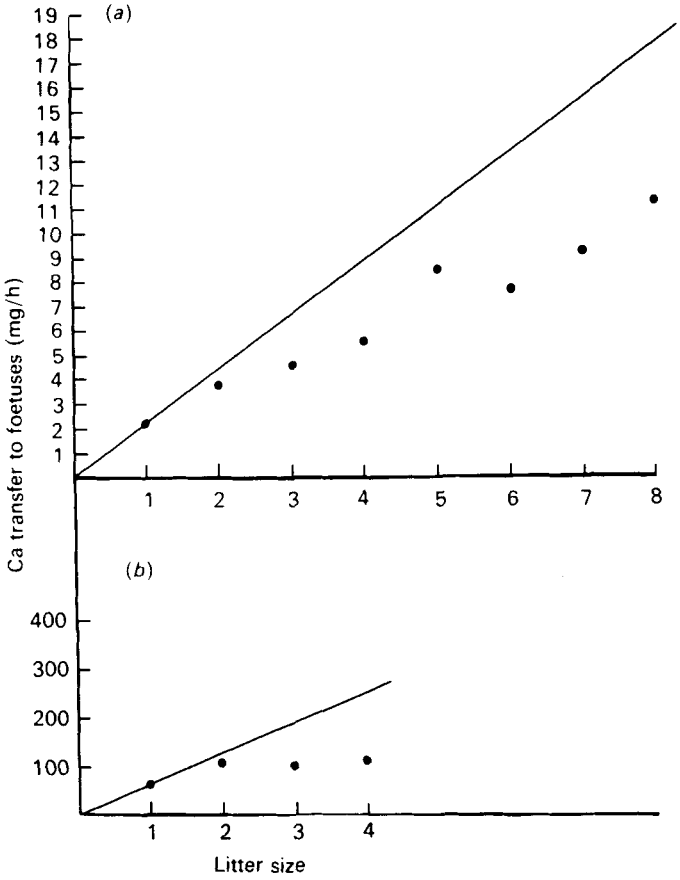

Fig. 2. Effect of litter size on the total amount of calcium (mg/h) transferred across the placental tissue in (*a*) the guinea-pig and (*b*) the sheep in late pregnancy. (——) indicates slope if each foetus in a litter receives Ca at the same rate as the single foetus. Sheep values taken from Twardock *et al.* 1973.

dence of the foetus upon blood flow and, in conjunction, placental size. In the ewe, reduction in blood flow by selective blockage of placental blood vessels with microspheres (Creasey, Barrett, De Swiet, Kahanpää & Rudolph, 1972) and the reduction of placental size by surgical removal of the number of sites for placental cotyledons (Alexander, 1964) result in a retardation of foetal growth. Also Eckstein, McKeown & Record (1955) have produced well-documented evidence of the positive relationship between placental size and foetal weight in the guinea-pig. A statistically significant relationship was not apparent in the present study although placental size and foetal weight both decreased significantly as litter size increased.

The decrease in plasma Ca concentration with increase in litter size is an indicator of the effect that the high rate of Ca transfer across the placenta has upon the maternal Ca homoeostatic mechanism. There was an increased reliance upon skeletal Ca reserves, shown by the decrease in Ca content of maternal femurs in dams with large litters. This is to be expected in view of the fact that in the largest litter the amount of Ca transferred to the eight foetuses in 24 h was approximately sixty-five times the total Ca in the maternal blood plasma. By comparison in the multifoetate ewe with four foetuses the maximum placental transfer of

Ca is approximately eight times its total plasma Ca content and in the cow, approximately three times its plasma Ca content.

In those guinea-pigs where both ^{85}Sr and ^{169}Yb-labelled microspheres were used to measure the distribution of cardiac output (%) some assessment of the physiological effects of the experimental procedure could be made. During the experimental period there was a tendency for blood flow to be redistributed away from placentas, heart, lungs, brain and spleen. Microspheres were equally distributed between right and left kidneys and the proportions of both ^{85}Sr and ^{169}Yb-labelled microspheres present in the kidneys were similar. It is probable, therefore, that the other changes were physiological and not attributable to analytical variation.

Because the measurements of blood flow involved measurements of cardiac output the values obtained for cardiac output influenced directly the flow rates obtained. No trend in mean cardiac output with litter size occurred although an increase might have been expected in order to supply blood to additional placentas. There are no published values which are directly comparable, but in both sheep (Metcalf & Parer, 1966) and rabbits (Leduc, 1972) cardiac output increases as gestation proceeds and in the sheep the blood flow to the cotyledons increases as foetal weight increases without any significant change in the flow to uterine muscle and endometrium (Makowski, Meschia, Droegemuller & Battaglia, 1969).

There is a marked difference between the effect of litter size on the total amount of Ca transferred across the placentas of the guinea-pig and the ewe in late pregnancy (Fig. 2). If the transfer capacity in both species is related to placental mass the difference between them must be caused largely by anatomical differences in the placentas, a fact which will make successful increase in litter size in the sheep difficult other than by genetic means. In the guinea-pig each additional foetus results in an additional placenta which can transfer approximately 1·5 mg Ca/h from maternal blood. The ewe has 90–100 cotyledonary sites for placental attachment which are shared amongst the foetuses present. Therefore, in the ewe each additional foetus has a much greater effect upon the mineralization of the other foetuses than it does in the guinea-pig. For example, in the Scottish half-bred ewe in late gestation the maximum amount of Ca which the cotyledonary type of placenta can transfer is approximately 2·8 g/d (Twardock *et al.* 1973) irrespective of the number of foetuses present. Fig. 2 demonstrates the difference the effect of litter size has upon the placental transfer of Ca in the two species. The figure indicates the increase in total Ca transfer rate which would occur if each additional foetus received as much Ca as a singleton foetus. In the ewe when litter size is greater than two there is no increase in the total amount of Ca transferred. In the guinea-pig the total Ca transferred is below the projected line for litter sizes of more than two but unlike the ewe continues to increase as the litter size increases.

In summary Ca transfer in the pregnant guinea-pig at 61 d of gestation was maximal at 0·22–0·34 mg/h per g placental tissue. This maximum transfer rate was principally associated with placental size which also affected placental blood flow rate and the amount of Ca extracted from the plasma by placental tissue.

The authors acknowledge the technical assistance of Miss K. Pritchett and Mrs Mary-Anne Lindahl. They also thank Dr Barbara Kitchenham of the Institute for Research on Animal Diseases, Compton, Newbury, Berks for performing the statistical analyses.

REFERENCES

Alexander, G. (1964). *J. Reprod. Fert.* **7**, 307.
Creasey, R. K., Barrett, C. T., De Swiet, M., Kahanpää, K. V. & Rudolph, A. M. (1972). *Am. J. Obstet. Gynecol.* **112**, 566.
Eckstein, P., McKeown, T. & Record, R. G. (1955). *J. Endocr.* **12**, 108.

Leduc, B. (1972). *Am. J. Obstet. Gynecol.* **112**, 374.
Makowski, E. L., Meschia, G., Droegemuller, W. & Battaglia, F. C. (1969). *Am. J. Obstet. Gynecol.* **101**, 409.
Metcalf, J. & Parer, J. T. (1966). *Am. J. Physiol.* **210**, 821.
Twardock, A. R., Symonds, H. W., Sansom, B. F. & Rowlands, G. J. (1973). *Br. J. Nutr.* **29**, 437.
Ulberg, L. C. & Lecce, J. G. (1971). *J. Anim. Sci.* **32**, 395 Abstr.

Printed in Great Britain