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CALCIUM AND PHOSPHATE FLUXES ACROSS THE FETAL MEMBRANES OF THE GUINEA PIG: IN VITRO MEASUREMENT

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Bidirectional calcium (^{45}Ca) and phosphate (^{32}P) fluxes across the amnion and visceral yolk sac of the guinea pig were measured in vitro in modified Ussing chambers. The net flux of these ions across both fetal membranes was in the maternal-to-fetal direction. The net flux of ^{45}Ca and ^{32}P across the yolk sac was significantly greater than that across the amnion. This difference was due to a greater maternal-to-fetal flux across the yolk sac. In addition, net ^{32}P flux was greater than net ^{45}Ca flux across the yolk sac, while in the amnion, there was no significant difference in the net flux of the two ions. It is suggested that the fetal membranes, especially the visceral yolk sac, contribute significantly to fetal acquisition of calcium and phosphate in mammals possessing a functional yolk sac placenta.

Studies of maternal-to-fetal nutrient transfer have focused primarily on the chorioallantoic placenta (1-4). However, it has been suggested that fetal membranes may also be important in nutrient transport (5-8). Adequate delivery of calcium and phosphate is essential for proper fetal growth. Therefore, the present study was designed to investigate the transfer of these ions across the amnion and visceral yolk sac of the guinea pig.

MATERIALS AND METHODS

Pregnant guinea pigs at 55-60 days gestation were anaesthetized with 30 $mg \cdot kg^{-1}$ sodium pentobarbital. A fetus was delivered by cesarian section via a midline incision and 4 x 4 cm sections of the fetal membranes to be studied were removed. The membranes were mounted in modified Ussing chambers (9) and bathed on each side with 13.0 mL of Krebs-Ringer bicarbonate buffer (KRBB) maintained at 37°C and bubbled with 95% O_2-5% CO_2. The KRBB (pH 7.4) contained 140 mM Na, 10.0 mM K, 1.10 mM Mg, 1.25 mM Ca, 127.7 mM Cl, 25.0 mM HCO_3, and 2.00 mM H_2PO_4. The added amounts of Ca and PO_4 represented the ionic concentrations of these elements found in normal plasma. In addition, 2.0 $mg \cdot mL^{-1}$ (11.0 mM) glucose was added to provide a metabolic substrate.

Maternal-to-fetal (J_{MF}) and fetal-to-maternal (J_{FM}) fluxes of ^{45}Ca and ^{32}P were determined on paired sections of tissue from the visceral yolk sac and amnion. The difference between these two fluxes was the net flux (J_{NET}).

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^{45}Ca (carrier free as $^{45}CaCl_2$) and ^{32}P (carrier free as orthophosphate) were obtained from Amersham Corporation, Oakville, Ontario. Five microcuries (1 Ci = 37 GBq) of each isotope were added to the maternal side of one tissue and to the fetal side of the other paired tissue. After a 20 min. equilibration period, samples were taken from the unlabelled sides of the tissues at 10 min intervals for 50 min to determine basal fluxes. Each volume (1.0 mL) removed was replaced with "cold" KRBB to maintain a constant volume and hydrostatic pressure on the tissue. Aliquots were taken from the initially labelled side of the tissue to determine the exact concentration of tracers (disintegrations per minute per millilitre buffer).

Each 1.0 mL sample was added to 10.0 mL Aquasol 2 (New England Nuclear, Lachine, Quebec) and counted in a Beckman LS-355 liquid scintillation counter for 10 min or 2.0% error. The counting efficiency for ^{45}Ca was 40-45% and for ^{32}P 55-60%. Appropriate corrections were made for the spillover of ^{32}P counts into the ^{45}Ca counting channel. This spillover (5-7%) was determined for each sample and subtracted from the ^{45}Ca count before the fluxes were calculated.

RESULTS

The ^{32}P and ^{45}Ca fluxes across the visceral yolk sac and amnion are shown in Fig. 1 and summarized in Table 1.

In the visceral yolk sac, the ^{45}Ca and ^{32}P J_{MF} fluxes were 18-19 times greater than the corresponding J_{FM} fluxes. The result is a net flux of these ions in the maternal-to-fetal direction. The net ^{32}P flux (3738 ± 1311 nmol$\cdot$cm$^{-2}\cdot$h^{-1}) was approximately 1.6 times greater than the net ^{45}Ca flux (2357 ± 837 nmol$\cdot$cm$^{-2}\cdot$h^{-1}) ($p < 0.025$).

In the amnion, no significant differences were observed between the net ^{45}Ca and ^{32}P fluxes. The net fluxes of both ^{45}Ca (891 ± 511 nmol$\cdot$cm$^{-2}\cdot$h^{-1}) and ^{32}P (1327 ± 761 nmol$\cdot$cm$^{-2}\cdot$h^{-1}) were 2.7 times less than the net fluxes of these ions in the visceral yolk sac. Further, the ^{45}Ca and ^{32}P J_{MF} fluxes, in the amnion, were only 5-8 times greater than the corresponding J_{FM} fluxes.

The ^{45}Ca and ^{32}P J_{FM} fluxes of the visceral yolk sac and amnion do not differ significantly. Thus, the difference in the net ^{45}Ca and ^{32}P fluxes between the two fetal tissues is due to the ion fluxes in the maternal-to-fetal direction.

No measurable potential difference across either of the two fetal membranes was observed.

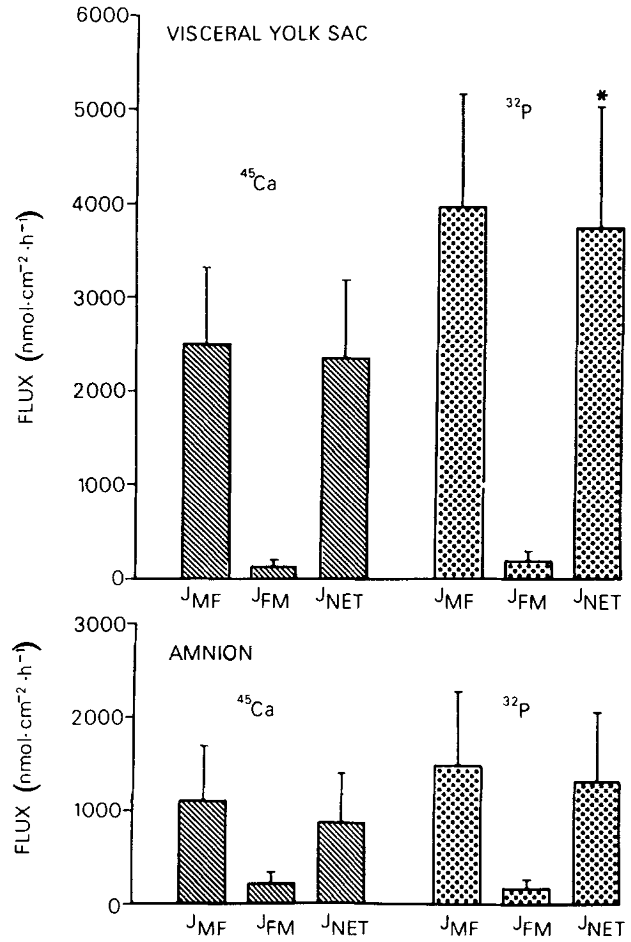

Figure 1. ^{45}Ca and ^{32}P fluxes across the visceral yolk sac and amnion of the guinea pig. The net flux (J_{NET}) is the difference between the maternal-to-fetal flux (J_{MF}) and the fetal-to-maternal flux (J_{FM}). Values represent mean ± SEM, n = 8 for the visceral yolk sac and 4 for the amnion.

*Significantly greater than J_{NET} for ^{45}Ca ($p < 0.025$).

DISCUSSION

The chorioallantoic placenta is the primary site of maternal-to-fetal exchange and therefore is responsible for the bulk of nutrient transport to the fetus. However, it is widely recognized that the visceral yolk sac of the guinea pig is also functionally important in both transport and metabolic processes throughout gestation (5,7,8).

TABLE 1. ^{45}Ca and ^{32}P fluxes across the visceral yolk sac and amnion of the guinea pig. Values are mean ± SEM (n = 8, visceral yolk sac; n = 4, amnion).

	J_{MF} Maternal-to-Fetal $nmol \cdot cm^{-2} \cdot h^{-1}$	J_{FM} Fetal-to-Maternal $nmol \cdot cm^{-2} \cdot h^{-1}$	J_{NET} Net Flux $nmol \cdot cm^{-2} \cdot h^{-1}$
VISCERAL YOLK SAC			
^{45}Ca	2500 ± 836	143 ± 67.5	2357 ± 837
^{32}P	3952 ± 1311	213 ± 111	3738 ± 1311
AMNION			
^{45}Ca	1104 ± 607	213 ± 130	891 ± 511
^{32}P	1501 ± 837	174 ± 103	1327 ± 761

We observed that calcium and phosphate are transported selectively in the maternal-to-fetal direction across both fetal membranes. The vascular nature of the visceral yolk sac membrane may contribute to the greater ability of this tissue to transport calcium and phosphate when compared with the amnion. The net phosphate flux across the visceral yolk sac was significantly greater than the net calcium flux, a phenomenon which was not significant in the amnion. This characteristic of ion transport across the visceral yolk sac is qualitatively similar to that of the chorioallantoic placenta (11).

It has been suggested that transport processes are not of prime importance in the amnion (5). Despite this, we observed that the amnion displayed a directionality of calcium and phosphate transport which suggests a selective permeability in one direction.

The fetal-to-maternal ion flux appears to be unrelated to the magnitude of the maternal-to-fetal flux, since no significant differences in this parameter were observed either between tissues or between ions.

The apparent absence of a potential difference across the fetal membranes would suggest that there is no active sodium transport in these tissues. These findings agree with those of Welsch (10), who did not find evidence for a membrane potential or polarized sodium transport across human fetal membranes.

This study has demonstrated the ability of the guinea pig visceral yolk sac and amnion to transport calcium and phosphate in a highly directional manner (maternal-to-fetal). Thus, these tissues may play an important role throughout gestation in fetal growth and development of the guinea pig, as well as other mammals with yolk sac (vitelline) placentas.

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