Morphological and radiochemical evidence for the metabolism of exogenous proteins by the preimplantation sheep blastocyst

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Summary
The ability of the trophoblast of the ovine preimplantation blastocyst to take up and metabolise proteins has been investigated using two experimental approaches, microscopical and radiochemical. The ultrastructure of the expanded blastocyst obtained from 14 and 17 day pregnant ewes was examined. The morphology of tissues maintained in culture for 24 h has been compared with that of fresh tissues. After culture, the cellular morphology of the explants was well preserved. Fresh and 24 h cultured tissues were incubated with horseradish peroxidase and ferritin and these proteins subsequently were found to be localized in coated pits, caveolae and secondary lysosomes of the trophoblast. Comparison of the uptake of [3H]dextran and of 125I-labelled bovine serum albumin indicated that proteins could be taken up by cultured tissue by mechanisms in addition to simple fluid phase endocytosis. During culture of explants of blastocyst with ^I-labelled bovine serum albumin, a large fraction of the radioactivity taken up by the tissue appeared in the TCA-soluble fraction of the culture medium indicating that cultured trophoblast hydrolysed proteins. That amino acids released from captured protein could be used for protein synthesis by the trophoblast was indicated by the labelling of tissue and medium proteins after culturing explants with β-lactamase labelled with [14C]leucine. A major product (Mr approximately 17×10^3) present in the medium was likely to have been ovine trophoblast protein-1. It is concluded that, during the expansion of the ovine blastocyst, the trophoblast has the ability to take up proteins, transport them to lysosomes and degrade them to amino acids which are used for protein synthesis. Thus proteins, as well as free amino acids, present in the histotrophe may be an important source of nitrogen for the sheep conceptus in the critical period just prior to implantation.

Key words: protein metabolism, morphology, blastocyst, sheep.

Introduction
A rapid expansion of the sheep blastocyst occurs between days 12 and 17 of pregnancy (Bindon, 1971). The supply of nutrients to support the embryo at this stage is provided by uterine fluid (the histotrophe), and these nutrients must be taken up by the trophoblast. Since the embryo and extraembryonic membranes must synthesise proteins for growth and development an adequate supply of amino acids is essential. Uterine fluid contains proteins, peptides and amino acids (Wales, 1973) that could contribute to the pool of amino acids used for protein synthesis within the conceptus but the relative contribution that each makes has not been examined. The aim of this study was to determine, by in vitro culture of portions of the blastocyst, excluding the embryoblast, and a combination of microscopical and radiochemical techniques, whether exogenous proteins can be taken up, degraded and used for protein synthesis by the trophoblast. A preliminary report of this work has been presented (Pullar and te Kronnie, 1988).

Materials and methods

Animals
20 Bluefaced Leicester×Swaledale ewes were used in this study. Groups of four ewes were housed indoors with facilities for regulating the lighting cycle. Each ewe was allocated 300 g concentrate per day (575 Supaflock silver pencils; Berks Bucks and Oxon Farmers Ltd, Twyford, Reading, UK). Hay and water were available ad libitum. The ewes were treated with fluorogestone acetate (30 mg) vaginal sponges (Chronogest; Intervet UK Ltd, Cambridge, UK) to control the time of oestrus. After 12 days the sponges were removed and the ewes were injected subcutaneously with serum gonadotrophin (500 i.u.; Folligon; Intervet UK Ltd, Cambridge, UK). Two days later a Suffolk ram wearing a raddle with a crayon
marker was introduced to the ewes. The ewes were checked twice each day and the day following tupping was considered to be the first day of pregnancy. Embryos were removed from ewes 14 or 17 days after tupping and are referred to subsequently as being either day 14 or day 17 conceptuses.

In order to obtain the embryos, ewes were sedated with petharbocrine sodium (20 mg kg⁻¹ body weight) and prepared for surgery. Just before the abdomen was opened a lethal dose of barbiturate was administered. The intact uterus was removed after ligation of the major blood vessels using full aseptic precautions. The uterus was transferred to a laminar flow cabinet, the uterine horns opened longitudinally and the conceptuses placed aseptically in Petri dishes containing Minimum Essential Medium with Hanks salts (Gibco Europe Ltd, Uxbridge, UK).

Culture of tissue
One or two explants (total length approximately 1 cm) of elongated blastocyst tissue were cut and placed in 55 mm vented Petri dishes containing 4 ml culture medium (Minimum Essential Medium with Earls salts and glutamine, supplemented with non-essential amino acid solution, penicillin and streptomycin; Gibco Europe Ltd, Uxbridge, UK). The tissue was cultured in a cabinet (Labmark; Boro Labs Ltd, Aldermaston, UK) at 37°C in an atmosphere of air:CO₂ (19:1 v/v).

Microscopy
Fresh and 24 h cultured tissue and tissue incubated with either horse radish peroxidase (HRP, M, 40×10⁵) or ferritin (M, 500×10⁵) was fixed and examined by light microscopy (LM) and by transmission electron microscopy (TEM). Tissues were immersed in a fixative solution (3 % (w/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) with 1 % (w/v) glucose) for 1–1.5 h at room temperature, and postfixed in 1 % (w/v) OsO₄ in the same buffer for 1 h (0°C). The tissue was embedded in epoxy resin. Semithin (1 μm) and ultrathin sections were cut on a Reichert ultratome IV microtome. For light microscopy semithin sections were stained with 1 % (w/v) toluidin blue. For TEM ultrathin sections were stained with uranyl acetate and lead citrate. Micrographs were made using a Philips EM 400 electron microscope.

In the studies of peroxidase and ferritin uptake, explants were incubated for 1 h in culture medium containing 5 mg ml⁻¹ HRP (Type II, Sigma Chemical Co. Ltd, St Louis, USA) or 1 % (w/v) ferritin (Merck, Darmstadt, West Germany). After incubation with ferritin, tissue was treated for routine TEM. Tissue incubated with HRP was treated between fixation and postfixation with diaminobenzidine (DAB) medium (5 mg DAB and 50 μl H₂O₂ (30 % w/v) in 10 ml 0.1 M Tris–HCl buffer pH 7.2; essentially according to Graham and Karnovsky, 1966) for 30 min. This gives rise to an electron-dense precipitate at the site of peroxidase activity. To test for endogenous peroxidase activity, explants were treated with DAB medium without prior incubation with HRP.

These methods have been used by Stroband et al. (1984) to demonstrate the uptake of proteins by the pig blastocyst.

Radiochemical methods
The amount of conceptus tissue available from 14 day pregnant sheep was limited and these experiments were carried out in cultured tissue from day 17 conceptuses when the expansion of the blastocyst enabled sufficient tissue to be available for not less than three replicate analyses to be undertaken for each animal. Tissue obtained from any single ewe bearing more than one conceptus was pooled. Explants of expanded blastocyst were cultured for 8 and 24 h in the presence of (i) [³H]dextran (M, 70×10⁵) in order to determine the rate of uptake of an inert macromolecule by the tissue; (ii) ¹²⁵I-labelled bovine serum albumin (BSA, M, 65×10⁵) to determine the rate of uptake and hydrolysis of a protein or (iii) denatured β-lactamase (M, 30×10⁵) labelled with [¹⁴C]leucine to determine if [¹⁴C]leucine derived from the uptake and degradation of a protein was incorporated into blastocyst proteins.

These methods are similar to those used by Freeman et al. (1981) to demonstrate the role of the visceral yolk sac in protein utilization by rat embryos.

(i) Explants were cultured with [³H]dextran (Radiochemical Centre, Amersham, UK) at a concentration of 1 μCi ml⁻¹ culture medium. Dextran was added to the culture medium 24 h, 8 h and 0 h before the tissue was removed from the culture medium. Tissue was then thoroughly washed in fresh medium without dextran and solubilised by homogenisation in 1 ml 0.25 M NaOH with incubation at 37°C for 1 h. This solution was neutralised with 1 ml 0.25 M nitric acid. Aliquots of this extract were added to scintillation fluid (Cocktail T; BDH Ltd, Poole, UK) and counted in a Packard Tricarb liquid scintillation spectrometer. Duplicate aliquots of media containing the labelled dextran were also counted. Counts were corrected for background and efficiency. The protein content of the neutralised tissue extract was determined (Bradford, 1976). The uptake of dextran was expressed as an endocytotic index, this being the volume of media whose contained dextran had been captured by the trophoblast (μl mg⁻¹ tissue protein).

(ii) Acid-denatured [¹²⁵I]BSA was prepared by the method described by Williams et al. (1971). Explants were cultured for 24 h and aliquots of [¹²⁵I]BSA were added to the culture medium to give 50 μCi ml⁻¹ 24 h, 8 h and 0 h before the tissue was removed from the culture medium. The explants were homogenised and solubilised as described above and aliquots of the tissue extract and the medium were counted in a gamma counter (MultiGamma 1261; Pharmacia LKB, Milton Keynes, UK) to give the total amount of radioactivity present in each fraction. After counting, 200 μl of carrier protein solution (0.5 % w/v BSA in 0.154 M NaCl) was added to 500 μl of the extract or culture medium followed by 300 μl ice-cold 1.2 M trichloroacetic acid (TCA) to precipitate protein. After centrifugation, 500 μl of the supernatant was counted to give the amount of radioactivity present in the TCA-soluble fraction. The protein precipitates were washed with ice-cold 0.6 M TCA and then counted. The protein content of the explants was determined as above. In order to correct for any proteolysis of BSA that might have been due to enzymes leached from explants into the medium during culture and not resulting from uptake and lysosomal degradation by the intact tissue, [¹²⁵I]labelled BSA was added to culture medium after the explants had been removed and the medium was incubated for a further period. The uptake of [¹²⁵I]BSA was expressed as an endocytotic index, this being equivalent to the volume of media whose contained BSA had been captured by the trophoblast. This was calculated from the sum of radioactivity retained within the tissue and that in the TCA-soluble fraction of the culture medium.

(iii) [¹⁴C]labelled bacterial β-lactamase was produced by radiolabelling the proteins coded for by the plasmid pUC9 which had been transformed into the Escherichia coli maxicell strain CSR003 (recA1 uvrA6 phr-1). The method used was that described by Savva and Butler (1983) except that the protein was labelled with [¹⁴C]leucine instead of [³H]methionine. After electrophoresis of the proteins on 8 % (w/v) polyacrylamide–SDS gels, the β-lactamase was extracted
from the appropriate section of the gel by grinding it in the minimum volume of buffer (10 mM Tris–HCl, pH 7.5 containing 1 mM EDTA disodium salt) and incubating at 4°C overnight. After centrifugation (15 000 g, 4°C) to remove traces of polyacrylamide, aliquots of the supernatant containing the radiolabelled protein were stored at −20°C.

Explants were cultured for 24 h and aliquots of labelled β-lactamase were added to the culture medium 24 h, 8 h and 0 h before the tissue was removed. After incubation, tissue was rinsed in fresh culture medium. The protein was precipitated from the neutral solubilised tissue with TCA as described above. The precipitate was thoroughly washed with TCA and then dried with acetone before dissolving in sample buffer (0.0625 M Tris–HCl buffer pH 6.8 containing 2% (w/v) sodium dodecyl sulphate, 0.001% (w/v) bromophenol blue, 10% (w/v) sucrose and 5% (v/v) β-mercaptoethanol). The culture medium was dialysed against four changes of 10 mM Tris–HCl buffer pH 8.2 and then lyophilised. The dry material was taken up in sample buffer. The proteins from both tissue and culture medium were then separated by electrophoresis on 11% (w/v) polyacrylamide–SDS gels. After electrophoresis the gel was sliced mechanically into 2 mm portions, dissolved in 25% (v/v) periodic acid and the radioactivity present in each slice was determined by scintillation counting as described above. The approximate $M_r$ of the separated labelled proteins was obtained by reference to the position of $M_r$ marker proteins (MW-SDS-70 kit; Sigma Chemical Co. Ltd, St Louis, USA) on the gels.

**Results**

**General morphology**

Samples of blastocyst tissue obtained from 14 day pregnant ewes were characteristically bilaminar (Fig. 1). The outer layer, trophoblast or trophoderm (te) was composed of cuboidal cells with a rounded apical side covered by microvilli (mv). The hypoblast (h) was a very thin cell layer, apart from the nuclear bulge, and was rather loosely associated with the trophoderm. Fig. 3 shows that the most conspicuous cell organelles in the trophoderm were an abundant rough endoplasmic reticulum (rer), small mitochondria (m) at the basal side of the cells, lipid droplets (l), lysosome-like bodies containing randomly orientated needle- and rod-shaped crystals, membrane structures and cell remnants, and junctional complexes between adjacent cells. In the cytoplasm of hypoblast cells no secondary lysosomes were found. The rough endoplasmic reticulum was abundant with a characteristic dense content in distended cisternae. A thin basement membrane (bm) was present between the two cell layers just under the trophoderm.

By day 17 (Fig. 2), mesoderm had migrated from the embryoblast as indicated by the presence of collagen fibres, derivatives of the mesoderm, between the trophoderm basal membrane and the hypoblast. The trophoderm was thicker and was composed of columnar cells which no longer formed a single layer. The hypoblast was more tightly aligned to the basement membrane under the trophoderm. The cell organelles in the trophoderm (Fig. 4) were essentially the same as those at day 14, crystalline inclusions in the secondary lysosomes were more abundant. In the hypoblast cells the cisternae of the rough endoplasmic reticulum with the characteristic dense content were less distended.

**Morphology after 24 h in culture**

After 24 h in culture the tissue explants from day 14 conceptuses formed multiple vesicles, termed trophospheres. These were found in small groups attached to a central mass of cells from which other spheres appeared to start to form (Fig. 7A). Trophospheres varied in diameter from 1 to 5 mm. In cultured explants from 3 animals, the trophospheres were unilaminar. The cells of this layer resembled freshly fixed trophoderm cells in ultrastructure having microvilli on the outer surface, junctional complexes between adjacent cells, pinocytotic activity, large lysosomal bodies and lipid droplets (Fig. 8). In two other animals, the 'trophospheres' were essentially bilaminar, the two cell layers being only loosely associated (Fig. 7B). In some cases 'trophospheres' were found in which the second cell layer was not continuous and cellular debris was found inside the spheres. The cells of the outer layer of the 'trophospheres' the trophoderm, varied from cuboidal in shape to flattened cellular sheets.

Tissue from day 17 conceptuses did not form trophospheres after 24 h in culture. The morphology of the tissue layers as well as the ultrastructure of trophoderm and hypoblast cells were similar to that of freshly fixed tissue.

**Uptake of macromolecules**

The results of both microscopical and radiochemical studies indicated that the trophoderm of both fresh and cultured tissue was able to take up and degrade exogenous proteins. Incubation of fresh explants from day 14 conceptuses with HRP resulted in HRP being localised in caveolae and coated pits (ca) between the microvilli and in small vesicles under the apical membrane of the trophoderm cells (Fig. 5A). Fig. 5B shows that large deposits of HRP were present in lysosome-like bodies in virtually all trophoderm cells. Signs of HRP uptake were occasionally seen at the basal side of the hypoblast cells. No peroxidase activity was observed in explants that were treated with DAB medium without prior incubation with HRP. Ferritin was also shown to be internalised in the trophoderm cells and was most frequently found in multivesicular bodies (mb, Fig. 5C). Endocytosis of HRP by trophoderm from day 17 conceptuses was evident (Fig. 6). Ferritin was also internalised by these trophoderm cells.

Trophospheres formed during 24 h culture of explants from day 14 conceptuses showed an endocytotic uptake of HRP by the trophoderm which was comparable to that observed in fresh tissue (Fig. 9). Ferritin was also taken up by cultured trophospheres (Fig. 10).

$[3H]$dextran and $[^{125}]$BSA were both taken up by explants of day 17 conceptuses (Fig. 11) and the uptake appeared to be related to time over the 24 h culture period. The rate of uptake of radioactivity expressed as
the endocytotic index per hour was three times greater for BSA than for dextran. The rates of uptake of these two macromolecules were compared directly in tissue obtained from 5 ewes and the mean rates (±S.E.M.) were 1.46±0.70 μg mg⁻¹ tissue protein per hour in the case of dextran and 4.38±1.11 μg mg⁻¹ tissue protein per hour for BSA. These mean values were significantly different (P<0.02; Student's paired t-test). In the case of BSA, less than 5% of the radioactivity taken up was retained in the tissue, virtually all of it being recovered in the TCA-soluble fraction of the media.

When explants were cultured in the presence of ¹⁴C-labelled β-lactamase, radioactivity was found to be present not only in the gel slices containing proteins of
this size ($M_r \approx 3 \times 10^3$) but also in two proteins of approximately $M_r \approx 60 \times 10^3$ and in one protein with $M_r$ of approximately $17 \times 10^3$ (Fig. 12). It was evident that in the medium a labelled protein of $M_r \approx 3 \times 10^3$) but also in two proteins of $M_r \approx 60 \times 10^3$ and in one protein with $M_r$ of approximately $17 \times 10^3$ accumulated during the culture period.

**Discussion**

In this investigation, microscopical and radiochemical studies were performed simultaneously on the same tissues. This combined study offered the opportunity to compare radiochemical data on protein metabolism with morphological characteristics of cellular components of the same tissue both before and after in vitro incubation.

The general morphology of the trophoblast and hypoblast of sheep blastocysts from 14 and 17 day pregnant ewes corresponds to the description of Winterberger-Torrés and Fléchon (1974) for the day 12–16 and day 18 sheep trophoblast, respectively. Crystalline inclusions in lysosome-like bodies in the trophoblast were suggested to represent a storage function (Winterberger-Torrés and Fléchon, 1974). These crystalline inclusions are observed not only in the conceptus trophoblast but also in the uterine epithelium of both cycling non-pregnant and pregnant sheep (personal communication, Stroband). It seems not unlikely that these inclusions are released into the uterine fluid by the uterine epithelium and phagocytosed by trophoblast. After 24 h in culture, the cellular morphology of the day 14 and day 17 explants is well preserved. All organelles found in freshly fixed tissue were also found after culture, while no signs of degeneration were seen. This indicates that the normal physiological functions of the cells are preserved during culture. The formation of trophospheres in the day 14 explants and not in day 17 explants may be due to a difference in the elongation capacity of the trophoblast. By 17 days after fertilization the elongation of the blastocyst is almost complete. The composition of the trophospheres formed by day 14 explants in vitro was either uni- or bi-laminar. Since the age of the individual conceptuses from different animals of each age group may vary by up to 24 h, differences in developmental characteristics among embryonic tissues of each group are likely to be observed. Unilaminar trophospheres are likely to be formed from trophoblasts in which the connection between trophoblast and hypoblast is not yet established. From trophoblast in which this connection is more developed, trophospheres composed of both trophoblast and hypoblast are likely to be formed. This difference in the composition of the trophospheres may influence the function of the trophoblast. However, no influence was detected in this study.

The ability of the trophoblast to take up macromolecules of widely differing $M_r$ from a surrounding fluid is clearly illustrated in the experiments where HRP ($M_r \approx 4 \times 10^3$) or ferritin ($M_r \approx 500 \times 10^3$) were added to the incubation medium. Uptake of these proteins was observed in all fresh and cultured explants and in all trophoblast cells. In similar studies involving incubation of whole pig blastocysts with HRP and ferritin (Stroband et al. 1984), no macromolecules were observed in the intercellular space between trophoblast and hypoblast or in the hypoblast itself. The authors concluded that macromolecules stay in the trophoblast where they are digested in the secondary lysosomes and form a source of amino acids for the proliferating trophoblast. In the present study, tissue explants were used and the blastocoel cavity was no longer sealed off from the exterior. This explains the observation of macromolecules being taken up by the hypoblast, since in this case macromolecules had direct access to the basal side of the hypoblast. However, it seems very likely that in vivo uptake of macromolecules from the uterine fluid will occur only in the cells of the trophoblast.

Macromolecules are taken up by means of endocytosis of caveolae or coated pits. The observation of numerous coated pits to which HRP and ferritin were bound suggests that, at least in part, a receptor-mediated mechanism is involved in the uptake of proteins by the trophoblast of the sheep conceptus. The endocytotic index for an inert macromolecule such as dextran is a measure of capture of the fluid phase by pinocytosis. The fact that the endocytotic index for [\(^{125}\)I]BSA was some three times greater than that for dextran adds further support to the view that the uptake of protein by the trophoblast must be aided by adsorption of the protein to the trophoblast cell surfaces. The mean value for the endocytotic index for dextran observed in trophoblast explants from sheep in this study is of the same order of magnitude as the value reported by Freeman et al. (1981) for the visceral yolk sac of the rat. The difference between the endocytotic
indices for $[^{125}\text{I}]$BSA and for the inert dextran is also in accord with the observations of Freeman et al. (1981).

Uptake of $[^{125}\text{I}]$BSA was estimated by measuring both the accumulation of radioactivity within the tissue and the appearance of radioactivity in the TCA-soluble fraction of the culture medium which resulted from proteolysis within the tissue. Since iodinated tyrosine cannot be reincorporated into protein, the appearance of radioactivity in the TCA-soluble fraction of the culture medium when trophoblast was cultured with
Protein metabolism in the ovine blastocyst

Figs 7–10. LM and TEM micrographs of day 14 trophoblast after 24 h culture. Abbreviations: b, blastocoel; ca, caveola; h, hypoblast; l, lipid droplets; ly, lysosomes; m, mitochondria; mu, microvilli; rer, rough endoplasmic reticulum; t, tubular vesicle; te, trophoderm; tr, trophosphere.

Fig. 7. LM sections through trophospheres. (A) Unilaminar trophosphere with a mass of cells from which new trophospheres develop (arrow). (B) Detail of a bilaminar 'trophosphere'. Note loose associations between te and h. Scale bars 25 μm.

Fig. 8. TEM micrograph of a unilaminar trophosphere. Note well developed junctional complexes between adjacent cells typical for trophoderm. Scale bar 1.25 μm.

Fig. 9. Apical side of a trophoderm cell after incubation with HRP for 1 h. Dark precipitates at position of peroxidase activity are indicated with single arrows. Scale bar 1 μm.

Fig. 10. Apical side of a trophoderm cell after incubation with ferritin for 1 h. Ferritin accumulation in tubular vesicles is indicated with a double arrow. Scale bar 1 μm.

Fig. 11. Uptake of [3H]dextran (■) and of [125I]bovine serum albumin (●) by explants cultured for 24 h. The macromolecules were added at 0 h, 8 h and 24 h before the tissue was harvested. Values are expressed as the volume of culture medium whose substrate content has been captured per mg trophoblast protein (endocytotic index) and are given as means of four replicates at each time point for tissue obtained from a 17 day pregnant ewe. The bars represent the standard deviation. The uptake of the macromolecules appears to be directly related to the time of exposure.

[125I]BSA indicates that captured proteins are hydrolysed to free amino acids in the trophoblast. The fact that 95% of the radioactivity taken up by the trophoderm in the form of [125I]BSA appeared in the TCA-soluble fraction of the culture medium indicates that very little of the protein captured was retained in the tissue. It would seem likely that the hydrolysis of protein takes place within lysosomes as these are clearly visible in the trophoderm.

Evidence for protein synthesis from amino acids derived from the hydrolysis of captured proteins has been obtained in the experiments using β-lactamase. This protein consists of a single polypeptide chain (Sutcliffe, 1978) and therefore the [14C]leucine appearing in proteins of lower molecular weight in either the tissue or medium must have been derived from leucine liberated from hydrolysed β-lactamase and not from the dissociation of a polymeric protein into its subunits. The most predominantly labelled protein found in the media after culture had $M_r$ approximately $17 \times 10^3$. This is not surprising as a protein of this size, termed ovine

Fig. 12. Incorporation of radioactivity into protein recovered from (A) tissue and (B) culture media from explants of trophoblast cultured for 24 h. 14C-labelled β-lactamase was added 0 h (●), 8 h (■) and 24 h (▲) before the tissue was harvested. $M_r$ values for marker proteins are indicated. In the media most of the radioactivity, apart from that in β-lactamase $M_r$ $30 \times 10^3$, was detected in a protein of $M_r$ approximately $17 \times 10^3$ likely to be ovine trophoblast protein-1.
trophoblast protein-1, has been shown to be the major protein released from sheep blastocysts at this stage of development (Godkin et al. 1982). Ovine trophoblast protein-1 has been implicated in the mechanisms involved in the maternal recognition of pregnancy (Heap et al. 1986).

It has been established previously that free amino acids present in the culture medium are utilised for protein synthesis by sheep conceptuses (Godkin et al. 1982; Pullar et al. 1985). The results of the present study show clearly that during the period of expansion of the blastocyst the trophoderm of the conceptus has the ability to take up proteins, transport them to lysosomes and degrade them to amino acids which are used for protein synthesis. Thus amino acids required for the growth of the preimplantation blastocyst need not only be in the form of free amino acids in the histotroph but may also be derived from uterine proteins. In sheep conceptuses there is no direct contact between the endodermal cells of the yolk sac and the uterine fluid. It appears therefore that the trophoderm of the sheep conceptus performs a similar function to the inverted yolk sac of the rat during early development (Freeman et al. 1981).

As well as providing the conceptus with a supply of nitrogen for protein synthesis, the uptake of proteins may serve other functions which are also important for the nutrition of the ovine embryo. Proteins derived from the uterus may act as transport proteins. For example, uteroferrin ($M_r 35 \times 10^3$) secreted by the uterus of the pig has been implicated in the transport of iron to the conceptus (Roberts and Bazer, 1988). In this species, there is also histochemical evidence that proteins are taken up by the trophoblast and appear in the lysosomes of blastocysts obtained from sows at 8–11 days of pregnancy (Stroband et al. 1984). It has also been suggested that low molecular weight ($M_r 18 \times 10^3$) acidic uterine proteins may be implicated in the transfer of retinol to the conceptus (Roberts and Bazer, 1988).

The importance of the uptake and degradation of proteins by the yolk sac for the provision of amino acids for the synthesis of proteins in the rat conceptus has been confirmed in studies of rat embryos cultured in the presence of trypsin blue or leupeptin. Inhibition of protein uptake and lysosomal proteolysis by these agents has deleterious effects on the development of the embryo (Lloyd et al. 1985). Defects in the uptake and utilisation of uterine proteins from the histotroph could be one of many factors responsible for embryo mortality in sheep.

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