DIFFERENTIATED REGIONS OF HUMAN PLACENTAL CELL SURFACE ASSOCIATED WITH EXCHANGE OF MATERIALS BETWEEN MATERNAL AND FOETAL BLOOD: COATED VESICLES

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SUMMARY

Coated vesicles may be an important component of the micropinocytic system of the human placenta. Regions of very dense reaction with glycocalyx stains are restricted to membranes within forming and fully formed coated vesicles. This is interpreted as evidence against permanently grouped specific binding sites having a role in the selective uptake of materials by micropinocytosis, and as support for theories of coated-vesicle formation which take into account the dynamic nature of membrane components. The pyroantimonate precipitation technique which was employed in an attempt to localize cations in placental tissue at term resulted in the deposition of electron-dense material in coated vesicles and basement membrane.

Examination of the distribution of coated vesicles in placental tissue explants at 8–13 weeks of gestation revealed a restricted distribution of these organelles. Probably more than 89% of coated vesicles lie within the largest vesicles' diameter from the cell surface.

Placental coated vesicles were isolated and examined using negative staining. A polygonally patterned structure was apparent on their surfaces. Analysis of the isolated fraction of coated vesicles using sodium dodecyl sulphate polyacrylamide gel electrophoresis shows the presence of a major protein of molecular weight 180000. This is the same molecular weight that has been given for clathrin, the major protein of the raised polygonally patterned structure on the cytoplasmic surface of coated vesicles from other sources.

INTRODUCTION

Much is known of the physiology of transport of materials into the human placenta (Miller & Berndt, 1975) but there is a lack of data on the organization of the dynamic structures involved in uptake. Two major questions arise from the physiological work. Firstly, the processes of uptake are unexplained. Secondly, the placenta is able to collect certain materials from their relatively dilute state in maternal blood and maintain them at a higher concentration in the foetal circulation. Materials taken up and concentrated in this way include proteins, polysaccharides, fats, certain vitamins and inorganic ions (Brambell, 1970; Llewellyn-Jones, 1969).

Allison & Davies (1974) have gathered a consensus of support for their view that endocytic organelles are separable by their different structural natures. These differences in structure may correlate with the physiologically distinct classes of endocytic activity. For example, there are drugs which differentially suppress certain classes of
activity (Wills, Davies, Allison & Haswell, 1972). In addition, different forms of endocytosis have differing energy requirements (Simpson & Spicer, 1973). Micro-pinocytosis by coated vesicle formation represents an area where understanding of the underlying motility is developing (Kanaseki & Kadota, 1969; Ockleford, 1976).

One approach to the problems of the motile processes of uptake in the placenta and the phenomenon of selection is to study particular endocytic organelles. Coated vesicles are a common constituent of cells and are found in the placenta. They occur with great abundance in cells which selectively absorb protein (Lloyd & Beck, 1974). Since the human placenta takes up proteins selectively, there is a comparative cellular anatomical argument for making coated vesicles the organelles of choice in this study.

This research has been undertaken at a favourable moment in the development of techniques for the study of these organelles. Pearse (1975) has recently developed a method for the isolation of porcine brain coated vesicles. This has proved adaptable to the placenta and has enabled us to complement our fine-structural data from sectioned material with structural data from negatively stained, isolated vesicles and with data from polyacrylamide gel electrophoresis. Using this range of techniques we have sought information that could be used to gain an understanding of the dynamic events involved in selective uptake by coated-vesicle formation in the transport processes which support foetal development.

**Materials and Methods**

**Trophoblast tissue**

Portions of human trophoblast tissue were recovered shortly after therapeutic terminations of pregnancy performed in the 8–12th week. Groups of about 20 chorionic villi were isolated by dissection and washed 3 times in Hanks' Balanced Salts Solution (BSS) (Flow Laboratories, Irvine, Scotland). Unless stated specifically to the contrary, this was the tissue used. Term trophoblast was obtained post-partum and was treated similarly. Trophoblast cells were cultured as described by Loke & Borland (1970).

**Transmission electron microscopy**

The methods routinely used for fixation and embedding and for ultrathin sectioning are described fully in a previous publication (Ockleford, 1975).

Samples containing coated vesicles were negatively stained using 1 % uranyl acetate (Huxley, 1963). They were supported on carbon-coated copper grids.

Chorionic villi were stained with ruthenium red by the method of Luft (1965); by adding to the tissue a mixture of 1.8 ml of 10 % ruthenium red together with 23 ml of a mixture of the solutions A and B described earlier (Ockleford, 1975); and by preincubating the tissue for 10 min in 1.8 ml of 10 % ruthenium red and 23 ml of Hanks' BSS prior to fixation.

Chorionic villi were stained with Alcian blue by using a 1 % solution of the dye (Behnke & Zelander, 1970; Rothman, 1970).

The tissue was stained with colloidal iron using the method first used for isolated membrane by Marx, Graf & Wesemann (1973). *Vibrio comma* (cholerae) neuraminidase (Behringwerke A.G. Marburg Lahm) at activities of 50 and 500 U/ml was used to predigest one group of tissue samples. Of these, some samples were esterified using 0.1 N HCl dissolved in methanol and others were esterified and later saponified using a 1 % solution of KOH in 80 % ethanol as these authors have recommended (Marx et al. 1973).

For tannic acid fixation tissue-cultured trophoblast cells were fixed using 8 % tannic acid and
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A 1% solution of the penetration agent digitonin (Tilney et al. 1973). The cells were embedded using Spurr's (1969) low viscosity resin.

Cation precipitation was accomplished using the method of Tandler, Libanati & Sanchis (1970). To increase the insolubility of the cationic precipitate the tissue was heated for 5 min at 95 °C in a half-saturated solution of potassium pyroantimonate and then rinsed twice in ice-cold distilled water as these authors recommended. Term placental tissue was used for cation precipitation.

Distribution of coated vesicles

The surface of the syncytiotrophoblast where caveolae occur is highly convoluted. Therefore, it is not obvious whether some membrane-bounded electron-lucent areas represent true vacuoles within the cell or transverse sections through invaginating membrane pockets which are still continuous with the cell surface. This complicates an analysis of the distribution of coated vesicles based upon measured distance from the cell surface. Consequently the data have been treated in 2 ways. Firstly, measurements have been made of the distance of coated vesicles from the nearest definitely recognizable portion of the cell surface. This type of treatment gives an overestimate of the real average distance from the cell surface but gives a firm upper limit to the value. The second more uncertain method used any available indicator (for example faint traces of contents or vesicle shape and size) to designate some 'apparent vesicles' as sections through invaginations of the cell surface membrane. Then, if coated vesicle profiles were closer to these than they were to the definitely recognizable cell surface, this new lower value was substituted in an attempt to achieve a more accurate estimate of the average distance from the cell surface. Only sections that were approximately perpendicular to the long axis of the villi were used. Any variation from the vertical in the section plane would be likely to increase the apparent average distance of coated vesicles from the cell surface obtained using either method. Both potential errors lead to overestimation of the average distance of coated vesicles from the cell surface.

A comparison of distribution and number of coated vesicles in cultured trophoblast cells with and without 0.02% ethylenediamine tetra-acetic acid (EDTA) added to the culture medium was undertaken. The cells were incubated in the presence of EDTA at 37 °C for 15 min; they were removed from the culture flasks by shaking or by means of a rubber policeman, fixed as described earlier and embedded in low-viscosity resin (Spurr, 1969).

Isolation of coated vesicles

Coated vesicles were isolated using the technique devised by Pearse (1975) with the following modifications. Tissue was disrupted (3 x 5-s periods) using an MSE homogenizer operated at half speed. Samples were examined electron microscopically at all stages of purification. Coated vesicles were collected after the first sucrose density gradient step (5-60%) for subsequent examination by electron microscopy and polyacrylamide gel electrophoresis.

SDS polyacrylamide gel electrophoresis

Electrophoresis of the proteins of the coated vesicle fraction was undertaken using 7.5% polyacrylamide gels without stacking gels (Laemmli, 1970). Runs were initiated at 1 mA per gel. Once the dye front had migrated 1 cm into the gel the current was increased to 2 mA per gel. Gels were fixed for 30 min using 10% glacial acetic acid in 50% aqueous methanol. Protein-containing bands were stained for 30 min using a 1-25% solution of Coomassie brilliant blue in the same solvent. Gels were destained at 60 °C using several changes of 7% acetic acid in 10% aqueous methanol. Mobilities of the major protein bands were expressed according to the formula of Weber & Osborn (1969). Molecular weights of proteins from the coated-vesicle fraction were assessed from a calibration graph. This was plotted using the mobilities of proteins of known molecular weight determined from gels run simultaneously with those containing coated-vesicle protein. The proteins used for calibration were pepsin (Sigma), mol. wt. 35000; bovine serum albumin fraction V (Sigma), mol. wt. 68000; and β-galactosidase (Sigma grade 111), mol. wt of oligomer, 130000. Gels were scanned using a Pye Unicam SP1800 ultraviolet recording spectrophotometer operated at a wavelength of 540 nm and a slit height of 2 mm.
RESULTS

Transmission electron microscopy of sectioned material

Coated vesicles occur at or near the cell surface of the syncytiotrophoblast of the human placenta. They are frequently observed near microvillous areas of the cell surface (Figs. 1, 2) but are rare enough to have escaped detection in areas underlying cell surface which is devoid of microvilli. The microvillous border of the cell is populated with profiles of uncoated vesicles of similar size to the profiles of coated vesicles (Fig. 2). The microvilli and the surface region of the cell contain actin-like microfilaments with a diameter of about 9 nm. The rather disordered nature of these microvilli compared for example with those of intestine is reflected at the macro-molecular level (Fig. 2). The microfilaments in placental microvilli are not packed in parallel in the regular hexagonal arrays seen in microvilli of intestine. Immediately below the cell surface of the syncytiotrophoblast, which \textit{in vivo} is in contact with maternal blood, are a number of microtubules about 24 nm in diameter. Data on the distribution of coated vesicles relative to the nearest cell surface are presented in Table 1 and in Figs. 3-5. These illustrate the fact that a very large proportion (at least 89 \%) of coated vesicles lie less than 540 nm from the cell surface. The distance 540 nm was chosen because it is the longest axis across the largest coated vesicle yet measured (Ockleford, 1976). The fact that a higher proportion of vesicles are in direct contact with the cell surface than are present in the cytoplasm immediately below the surface might suggest that the process of vesicle formation is slow compared with the rate of their subsequent movement away from the cell surface.

The image of the polygonally patterned structure on the surface of coated vesicles obtained using tannic acid-fixed material was less informative than that obtained using conventional fixation techniques. The electron-dense product surrounding the vesicles was much thicker than the height of the ridges of the polygonally patterned lattice.

The cell surface glycoalyx stains all reacted positively with the maternal surface of the syncytiotrophoblast (Figs. 1, 6, 7). However, the electron-dense regions were considerably wider in the lumen of vesicles and in the caveolae of forming vesicles than elsewhere on the surface when both ruthenium red (Fig. 6) and Alcian blue (Fig. 7) were used as stains. Within vesicles and caveolae this layer was up to about 40 nm in width and sometimes completely occluded the lumen of the vesicle. On other areas of the cell surface the layer of stain was usually less than 10 nm wide. Staining with colloidal iron hydroxide at low pH was positive and remained so after neuraminidase digestion, after esterification and after esterification followed by saponification.

The potassium pyroantimonate cation-precipitation technique produced a rather
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general distribution of an electron-dense precipitate in the syncytiotrophoblast (Fig. 8). Marked local increases in the concentration of precipitate, possibly indicating locally increased densities of cations at these sites in vivo, were observed in several positions. Heaviest precipitation occurred in the basement membrane. Granules in the basement membrane (see Ockleford & Menon, 1977, fig. 2) were also covered by precipitate. Irregularly shaped areas within syncytiotrophoblast nuclei were much more heavily covered with pyroantimonate than was the remainder of the nucleoplasm. Several very large cytoplasmic vacuoles were unlabelled. Precipitate was frequently observed in coated vesicles where there was an apparent tendency for it to be localized underlying the luminal surface of the vesicle wall (Fig. 8).

The distribution and apparent number of coated vesicles were not substantially altered by treatment of tissue-cultured trophoblast cells with the divalent cation chelator EDTA at a concentration of 0.02 %. However, the surface morphology of the majority of cells was altered. These became more convoluted in outline, forming a microvillous pattern similar to that of the syncytiotrophoblast cell surface.

Isolated coated vesicles

Coated vesicles were isolated successfully as judged by electron microscopy (Fig. 9 A, B). The polygonal pattern visualized in these negatively stained preparations corresponds to that predicted from a prior 3-dimensional reconstruction based on micrographs of sectioned material (Ockleford, 1976). As has been pointed out for negatively stained coated vesicles isolated from other sources (Crowther, Finch & Pearse, 1976), the vertices of the polygons (Fig. 9A, inset) are particularly prominent. Occasionally the walls of the polygons have a beaded appearance (Fig. 9A, lowest vesicle in inset). The centre-to-centre spacing of the swollen regions is about 3.0 nm.

The overall size of these vesicles varies. The range from smallest to largest shown in Fig. 9A is 70–120 nm across their longest axis. The shape of the vesicles is also variable (Fig. 9B). Both these features are described in detail elsewhere (Ockleford, Whyte & Bowyer, 1977). The membranous portion is apparently absent from many of the polygonally patterned structures. It appears in a collapsed form within others. In Fig. 9A the lattice nearest the centre of the field contains a large electron-lucent structure which is probably the collapsed phospholipid bilayer.

Fig. 2. Electron micrograph of section through the microvillous surface of the syncytiotrophoblast stained with lead citrate and uranyl acetate. These are similar to other microvilli (mv) in that they apparently contain microfilaments (mf) of the same diameter as that usually given for actin microfilaments (9 nm). The microvilli are less uniform in size and shape and less regularly ordered than in other microvillous cell surfaces. Microtubules (mt) about 24 nm in diameter are preserved using this method of fixation (see Materials and methods of Ockleford & Menon, 1977). Coated vesicles (cv), caveolae (cav) and smooth-surfaced vesicles (v) are commonly found components in this region. × 38 800.
Table 1. Numbers and percentages of coated vesicles at various distances from cell surface and the percentage of the total number of vesicles nearer to the cell surface than the largest recorded diameter of a coated vesicle

<table>
<thead>
<tr>
<th>Area investigated</th>
<th>No. of coated vesicles at specified distances from cell surface, nm</th>
<th>% of coated vesicles at specified distances from cell surface, nm</th>
<th>% of total no. of vesicles nearer to cell surface than largest recorded diam. of a coated vesicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal surface of syncytiotrophoblast</td>
<td>82 17 22 17 14 6 10 2</td>
<td>47.6 10.1 13.1 10.1 8.3 3.6 5.9 1.2</td>
<td>89.2</td>
</tr>
<tr>
<td>Maternal surface of syncytiotrophoblast, corrected data</td>
<td>98 20 25 15 8 2 — —</td>
<td>58.3 11.9 14.9 8.9 4.8 1.2 — —</td>
<td>98.8</td>
</tr>
<tr>
<td>Cytotrophoblast basal lamina surface</td>
<td>28 17 6 8 9 9 4 5</td>
<td>32.5 19.8 7.0 9.3 10.5 10.5 4.6 5.8</td>
<td>79.0</td>
</tr>
<tr>
<td>Cytotrophoblast surface adjacent syncytiotrophoblast</td>
<td>3 2 5 2 1 — — —</td>
<td>23.1 15.4 38.5 15.4 7.7 — — —</td>
<td>— —</td>
</tr>
</tbody>
</table>

Data relate to 10-12-week-old human placenta. The term 'corrected data' is explained in the Materials and methods section.

* The longest axis of the largest coated vesicle in cells of placental origin so far observed is 540 nm (Fig. 5; Ockleford, 1976). When the number of vesicles closer to the cell surface than 540 nm is expressed as a percentage of the total number of vesicles, the qualitative impression that their distribution is restricted to a narrow cell surface band is reinforced. At the time of fixation a majority of the coated vesicles lay extremely close to the cell surface.
Fig. 3. Histogram of information included in Table 1. It illustrates the 'corrected data' (Materials and methods) referring to the distribution of coated vesicles at and below the syncytiotrophoblast cell surface. The distribution is restricted to a narrow band of cytoplasm immediately internal to the surface of the syncytium bordering maternal blood space in vivo.

**SDS polyacrylamide gel electrophoresis**

The gels of the isolated coated vesicle fraction show major protein bands with apparent molecular weights of 180,000, 100,000 and 42,000 Daltons. In addition there is some material present which has an apparent molecular weight in excess of 200,000 Daltons (Fig. 10). In all, 17 bands were resolved. The 180,000 band corresponds in molecular weight with clathrin, the protein of the polygonal lattice in porcine brain (Pearse, 1975) and within the limits of error of the technique the 42,000 band may correspond with actin (mol. wt usually given as 46,000 Daltons).

**DISCUSSION**

*Models of coated vesicle motility*

The finding that coated vesicles of the human placenta contain glycocalyx material which stains densely with Alcian blue and ruthenium red is consistent with the results of Jollie & Triche (1971), showing similar staining with ruthenium red in rat visceral yolk sac placenta. It supports suggestions that selection in uptake involves recognition at receptor sites composed of molecules with saccharide portions (Allen, Cook & Poole, 1971). As others have suggested, selection and concentration in uptake may operate by contact of randomly moving molecules in the external medium with...
receptor sites lying on the external surface of the membrane (Lloyd & Beck, 1974). It is easy to see how such a system may be compatible with Kanaseki & Kadota’s (1969) model of vesicle motility. Their hypothesis depends upon the transformation of particular hexagons existing in an extended hexagonal lattice under the cytoplasmic surface of the membrane into pentagons with the same length of side; an effect which is presumed to generate curvature in the membrane, ultimately causing a vesicle to pinch off. Selective uptake would simply require coextensiveness of the internal polygonal lattice and the external glycomolecule receptor region.

Because the number of coated vesicles at the surface of cells does not diminish with time, and because they are continually being internalized, it is necessary to assume that they or their components are being recycled into the membrane or that the membrane is continually repopulated with newly synthesized vesicles or components. Initially when vesicle components enter cell surface membranes, and prior to the transformation of the first hexagon to a pentagon according to Kanaseki & Kadota’s (1969) model, there should be a flat hexagonal lattice underlying the membrane and a dense but flat thickened area of glyocalyx on the exterior surface. No observations of flat or convex regions of membrane with thickened glyocalyces similar to those found in caveolae and fully formed vesicles have been made in this study. Unless the flattened stage is extremely short-lived, these data detract from confidence in the fixed differentiated area model. On the other hand, the observations are consistent with a later view (Ockleford, 1976) that suggests movement together in a manner analogous...
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Fig. 5. Histogram representing the distribution of coated vesicles in the cytoplasm of cytotrophoblast cells immediately adjacent to the basement membrane (dark bars). The height of these bars has been raised (diagonal-striped bars), maintaining their existing height ratios, to the same total number of observations as in Fig. 4. This makes visual comparison of the distribution with that in Fig. 4 easier. The distribution in the 2 cell types is similarly skewed with the incidence of coated vesicles falling rapidly with increasing distance from the cell surface.

To 'patching' (de Petris & Raff, 1973) of receptors connected to initially separate components of the polygonal lattice within the membrane. Crowding of the components of the membrane complex would generate curvature when complexes which include hexagons and pentagons were formed. Such a model predicts that thickened regions of glycocalyx would be noted only where concave membrane curvature was starting to develop.

A study of the polygonal lattice of negatively stained coated vesicles isolated from porcine brain and bullock adrenal medulla (Crowther et al. 1976) indicates that some portion of the protein molecule of the lattice may project into the interior of the vesicle. It is conceivable that this arm of the lattice molecule, or a molecule attached to the arm, spans the phospholipid bilayer of the vesicle providing the postulated connexion to the external receptor molecule (Ockleford, 1976). Orci & Perrelet (1973) have published micrographs of freeze-fractured micropinocytic caveolae of intestinal smooth muscle cells in culture. These clearly demonstrate 8.5-nm diameter particles in the fracture plane between the phospholipid bilayers in caveolae. Presumably these particles represent proteins which in vivo may have spanned the membrane. Evidence for lateral mobility of particles in the plane of the membrane in micropinocytosis by coated or uncoated vesicle formation is also provided by Orci & Perrelet's (1973)
observation that particles similar in size to those close-packed in caveolae occur in the surrounding membrane. In the syncytiotrophoblast the distribution of coated vesicles at the cell surface appears to be linked with the distribution of microvilli. The functions of the 2 organelles may therefore be interdependent. Usually the suggested selective advantage of an irregular or microvillous border is that the increase in surface provides a larger area for exchange of materials with the extracellular environment. If it is assumed that because of their common distribution the increased cell surface area created by microvilli is important to the selective uptake function of coated vesicles, then it seems probable that the microvillous surface provides an increased area of receptor sites. Thus the observation that coated vesicles form only at and between the bases of microvilli is significant, because it suggests loaded receptors move downwards to the bases of microvilli before they and their adherent extracellular material are enclosed in vesicles. This correlation may therefore be taken as evidence for the lateral mobility of receptors for selective uptake.

Because the net charge on the surface of most cells is negative, and because many of the receptors are presumably chemically similar, it is reasonable to suppose that there may be a repulsion between predominantly negatively charged receptor molecules at close range. Multivalent molecules such as immunoglobulins may act as agents which bind receptors into the close-packed membrane complex and overcome the hypothetical tendency for receptors to repel each other. Röblich & Allison (personal communication) have demonstrated the presence of Fc receptors in the caveolae of coated vesicles, and this hypothesis is further supported by the observation of Contractor & Krakauer (1976) showing a stimulatory effect of serum in the uptake of protein by cultured human trophoblast cells. Presumably a divalent cation such as calcium could also perform this 'bridging' role. The deposition of pyroantimonate in placental coated vesicles would seem to indicate the presence of a divalent cation. The observation of a calcium-binding component in isolated brain coated vesicles may consequently be significant (Blitz & Fine, 1976). The fact that EDTA does not obviously alter the occurrence or distribution of coated vesicles in cultured trophoblast cells does not rule out such a potential function.

Clathrin

The successful isolation of human placental coated vesicles by a similar method to that which Pearse (1975, 1976) developed for porcine brain and bullock adrenal medulla suggests that the organelles from the different sources share common properties. The ultrastructural similarity in the polygonal patterns on the cytoplasmic

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Fig. 6. This section through the cell surface of syncytiotrophoblast which in vivo is in contact with maternal blood, has been stained with ruthenium red. The lining of the luminal surfaces of caveolae (arrows) and coated vesicles exhibit increased electron density using this technique for the demonstration of glycosylcays. $\times 101000$.

Fig. 7. Transmission electron micrograph of a section through the cell surface of syncytiotrophoblast stained with Alcian blue. The lining of the luminal surfaces of caveolae and coated vesicles (arrows) stain positively with this cationic dye. $\times 70000$. 

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surface of the vesicles suggests a similarity at the molecular level. The similarity of the SDS gel electrophoresis protein-banding patterns of the isolated placental coated vesicle fractions to those of porcine brain points to the same conclusion. The protein of greatest interest in this respect is the major protein band in the coated-vesicle fraction, which has an apparent molecular weight of 180,000 Daltons. This is identical with the weight of the protein clathrin which Pearse (1975) has shown forms the polygonally patterned network on the cytoplasmic surface of porcine brain coated vesicles.

Coated vesicle function

Various, sometimes apparently contradictory, roles have been proposed as primary functions for coated vesicles. However, it is possible to discern similarities between processes previously classified as separate when more attention was given to the product of coated-vesicle formation than to its mechanism. (a) Coated vesicles are known to take up materials by micropinocytosis. There is evidence for this function from both comparative anatomy and more directly from tracer studies which indicate the uptake of proteins (Rodewald, 1973; King & Enders, 1971; Roth & Porter, 1964; Bowers, 1964; Lagunoff & Curran, 1972). (b) They may also remove membrane from the cell surface, thus facilitating membrane turnover in secretory cells. Because the surface of these cells does not grow larger as a result of incorporating exocytic vesicle membranes during secretion, the cell must possess a method for internalization of membrane at the same approximate rate as incorporation. This may (Henser & Reese, 1973; Fried & Blaustein, 1976) or may not (Nordmann & Morris, 1976; Pearse, 1976) be mainly the result of coated-vesicle action. The main difference between membrane recycling and selective uptake may simply be the absence of functional receptors. (c) Although it was not the case for syncytiotrophoblast, coated vesicles have been described forming in association with Golgi membrane. It has therefore been suggested that some coated vesicles are specialized exocytic vesicles, which discharge materials processed by the Golgi to the exterior (Friend & Farquhar, 1967; Jamieson & Palade, 1971). That coated vesicles may function in both endocytosis and exocytosis is apparently paradoxical. One function of the Golgi is to package and condense cellular products. Crowding of clathrin molecules attached through the Golgi membrane to molecules which may be secreted would concentrate them in much the same way as the process of concentration in selective uptake by micropinocytosis has been

Fig. 8. The most electron-dense portions of the image in this transverse section through a term placental chorionic villus represent precipitated cation pyroantimonates. Ultrastructure is generally poorly preserved using this technique, but mitochondria (mi) and coated vesicles (cv) are recognizable. The basement membrane (bm), which is often multilaminous later in pregnancy, is in 2 parts in this section. Both layers are sites of rather heavier precipitation than in other areas. It is important to observe that the precipitate is absent from large parts of electron-lucent vacuoles (lv). The small quantities of precipitate over coated vesicles may not therefore simply be the result of coincidences in distribution between one group of randomly distributed small particles occurring with a high frequency superimposing on a lesser number of larger structures which are also randomly distributed. × 125,000.
suggested to occur (Ockleford, 1976). The functions in both processes to which the clathrin lattice is related may be concentration of selected molecules and invagination of a membrane. (d) It has been suggested that coated vesicles carry their clathrin lattices in several processes purely as a 'fender', which prevents vesicular contact and fusion with lysosomal membranes (Wild, 1976). In this hypothetical manner the contents of the vesicle are protected from degradation by lysosomal enzymes. There are apparently no cases recorded in the literature of vesicles still bearing clathrin coats fusing with lysosomes.

**Loss of coats**

Kanaseki & Kadota (1969) have provided evidence for the loss of clathrin lattices by vesicles. If clathrin loss occurred very shortly after vesicle formation in the syncytiotrophoblast then an obvious explanation for the apparently restricted distribution of coated vesicles in this tissue follows. If coats are not lost then some other explanation of the restricted distribution of coated vesicles at the syncytiotrophoblast cell surface must be sought.

**Intracellular motility of coated vesicles**

The protein of isolated coated vesicles appearing as a band on SDS polyacrylamide gels with an apparent molecular weight of 42 000 may be actin. If this is the case, it is possible that actin is a contaminant of the preparation, reflecting the close cellular association of coated vesicles and microfilaments. Equally, actin may be attached to coated vesicles in a more specific and meaningful way, as it apparently is to chromaffin granules (Burridge & Phillips, 1975), where it is a proposed link in the chain of transport processes causing intracellular movement of the granules.

The physical association of coated vesicles with actin-like microfilaments and microtubules may not be fortuitous. Both these structures have been considered necessary for proper motile function. Either or both may be necessary components for the transport in the syncytiotrophoblast of coated vesicles, vesicles which have lost their clathrin coats, or of empty coats.

The nature of the remaining Coomassie-staining bands on the polyacrylamide gels must remain a matter for speculation, though it is perhaps not without significance that a major component of apparent molecular weight 100 000 Daltons has been found by

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**Fig. 9A.** Electron micrograph of coated vesicles (cv) isolated from human placenta and negatively stained using uranyl acetate. It illustrates the polygonally patterned network of the protein clathrin on the surface of the vesicles. There are some smooth-surfaced membrane structures (s) in the samples. Some of the coated vesicles (arrow) contain an electron-lucent portion thought to represent the membrane of the vesicle retracted into the central region. On occasion (arrowhead in inset) the walls of polygons appear to exhibit beaded substructure. The longest axis across images of vesicles in this figure varies between 70 and 120 nm. × 55 000; inset × 140 000.

**Fig. 9B.** A similar sample to that shown in Fig. 9A which illustrates variability in vesicle shape over and above the variability in size already indicated. One vesicle (arrow) is definitely non-spherical. × 55 000.
Blitz & Fine (1976) in a coated-vesicle preparation isolated from mammalian brain having high $\text{Ca}^{2+}$-activated ATPase activity. In sarcoplasmic reticulum Scales & Inesi (1976) have described a $\text{Ca}^{2+}$-dependent ATPase with a molecular weight of 106000 Daltons. In contrast, however, Blitz & Fine (1976) demonstrated in their preparation a component with a molecular weight of 60000 Daltons which was not apparent on gels of placental coated-vesicle proteins.
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