Scanning Microscopy

Volume 1 | Number 4 Article 29

6-15-1987

The Fine Structure of the Human Placental Villus as Revealed by Scanning Electron Microscopy

G. J. Burton
University of Cambridge

Scanning Microscopy, Vol. 1, No. 4, 1987 (Pages 1811-1828)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/87$3.00+.00

THE FINE STRUCTURE OF THE HUMAN PLACENTAL VILLUS AS REVEALED BY SCANNING ELECTRON MICROSCOPY

G J Burton

Department of Anatomy, and Department of Obstetrics and Gynaecology,
University of Cambridge
UK

(Received for publication February 27, 1987, and in revised form June 15, 1987)

Abstract

Scanning electron microscopy of the placenta has a history of only twenty years. During that time, however, there have been dramatic advances in instrument technology coupled with the refinement of preparative techniques designed to reduce fixation artifacts to a minimum. As a result many of the early claims must be amended or suitably qualified, and this is one aim of the present review.

Much new data on the internal structure of the placental villus is also presented. By means of the partial digestion technique it is now possible to describe the three dimensional configuration of the various components of the villous tree. This review will consider these sequentially, starting externally with the gross morphology of the villi and ending with replicas of the fetal vasculature produced by corrosion casting.

Key Words: Scanning electron microscopy, placental villi, human placenta, trophoblast.

Address For Correspondence:
G J Burton
Department of Anatomy
University of Cambridge
Downing Street
Cambridge
CB2 3DY
UK Phone No. 0223-333798

Introduction and Historical Perspectives

Scanning electron microscopy has had a relatively short history in placental research, a subject which dates back to the days of the ancient Egyptians (Boyd and Hamilton, 1970). By the time the technique was developed the villous nature of the placenta had been established for almost 150 years, and much of the fine structure was already known. The application of scanning electron microscopy to this organ was nonetheless a major advance, for it immediately enabled the complex three-dimensional configuration of the placenta to be fully comprehended. Superior resolution combined with a large depth of field allowed researchers to identify surface features with ease, and in combination with traditional light and transmission electron microscopy the technique has proved a powerful research tool.

The placenta is not ideally suited for scanning electron microscopy, however, for its peculiar villous nature presents considerable problems with regard to electrical conductance. This complex configuration, compounded by the presence of a microvillous surface that covers the entire villous tree, commonly results in specimen charging, and clearly caused difficulties in the early pioneering studies (e.g., Multier et al., 1968; Herbst et al., 1968; Ludwig, 1971; Ludwig et al., 1971; Okudaira et al., 1972). Since that time improvements in instrument technology have taken place at a rapid rate, whilst on the biological side there has also been much progress in the fields of fixation and specimen drying. The importance of the correct concentration, pH and osmolality of buffer and fixative solutions cannot be overstressed, and has been discussed thoroughly elsewhere by Hayat (1981). An awareness of the ease with which artifactual appearances may be created during tissue preparation is an absolute prerequisite for anyone involved in interpreting scanning electron micrographs. The introduction of critical-point drying was a major advance in reducing such artifacts, and so it is perhaps timely to review the literature pertaining to scanning electron microscopy of the human placenta with these points in mind.

The Developing Placenta

The combination of a large depth of field and the ability to view relatively extensive areas of tissue provides for an easy appreciation of the changing morphology of the placental villi as gestation advances. At present the earliest material available for study results from spontaneous abortions, and although specimen preservation is inevitably not optimal several studies have made use of this source of tissue from the 6-8th week of gestation onwards (eg. Dempsey and Luse, 1971; Bergstrom, 1971; Fox and Agrafojo-Blanco, 1974; King and Menton, 1975; Clint et al., 1979).

At this stage of pregnancy the main villous stems appear large and bulky, being up to 500 μm in diameter, and their surface is covered with a profusion of offshoots (Fig. 1). Over the years there has been much confusion in the literature concerning the nomenclature of surface protrusions from villi, and so it is intended to use the classification of Hamilton and Boyd (1966) and refer to those depicted in Fig. 1 as syncytial sprouts. They may take a variety of forms, which undoubtedly reflect the various stages in the development of a new terminal villus.

The earliest sprouts are cylindrical structures, 10-20 μm in diameter, which may arise singly or in clusters and project almost perpendicularly from the parent villus (Fig. 2). Their surface is covered with microvilli, identical to those over the remainder of the villous tree, and both histological and phase-contrast studies have revealed that internally these early sprouts consist almost entirely of densely-packed syncytial nuclei (Hamilton and Boyd, 1966; Alvarez, 1964; Aladjem, 1967a).

More elaborate than these conical protrusions are elongated paddle-shaped structures. These often show an abrupt reduction in diameter along their length before they expand into a disc-like distal end (Fig. 1). During development the early sprouts are invaded first by cytotrophoblast and then by mesenchymal cells which bring with them vascular elements converting the sprout into a functioning villus. It is tempting to assume the variation in diameter of these early villi reflects the limit of invasion by these tissues, with the original syncytial nuclei filling the expanded tip. As yet, however, there is no definite correlative evidence to support this claim.

For some reason, as yet unknown, a proportion of the early syncytial sprouts are not invaded by cytotrophoblast, and instead their attachment to the villous surface becomes progressively attenuated. The initial stages were illustrated by Fox and Agrafojo-Blanco (1974), and eventually the former sprout is tethered by nothing more than a delicate thread-like structure (Fig. 3). Microvilli persist over the surface of the sprout although they gradually disappear over the neck region as this is narrowed into a stalk. If the stalk breaks then the sprout floats free in the intervillous space and may be deported throughout the maternal circulation, often becoming lodged in the pulmonary vascular bed (Douglas et. al., 1959). Scanning electron micrographs have thus confirmed previous observations made on this immunologically fascinating process by phase-contrast (Aladjem, 1967a) and light and transmission electron microscopy (Hamilton and Boyd, 1966).

The Mature Placenta

According to Boyd and Hamilton (1970) the first convincing description and illustration of chorionic villi was provided by Weber (1832). Since then there have been innumerable accounts based on light and phase-contrast microscopy, but undoubtedly the advent of the scanning electron microscope has allowed for a much clearer understanding of the complexity of the villous tree.

The pattern of villous branching

The powerful combination of light and scanning electron microscopy of equivalent resin-embedded tissue enabled Kaufmann et al.,

Fig. 1 8 weeks gestation. At this stage of pregnancy the main villous stems (V) are covered with a profusion of developing villi. The early sprouts are cylindrical (a), but these later develop expanded distal ends (b).

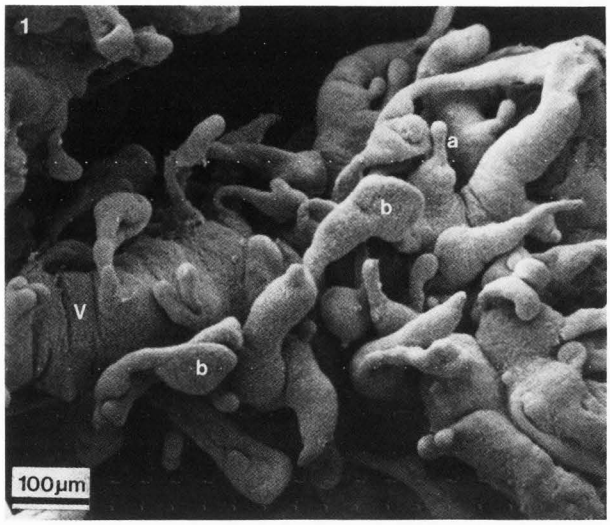
Fig. 2 8 weeks gestation. The earliest syncytial sprouts are small conical elevations (a) rising perpendicularly from the parent villus. These gradually lengthen as at (b) to form the cylindrical structures illustrated at (c). These sprouts, which consist internally of densely-packed syncytial nuclei, may arise in close proximity to each other as at (c). Note the microvillous covering over the entire villous tree.

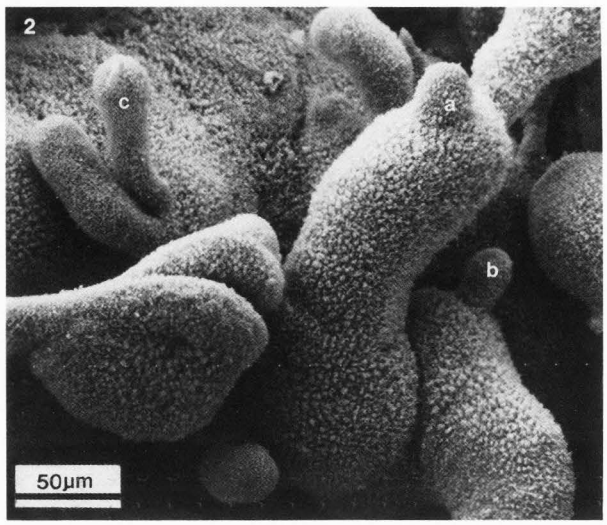
Fig. 3 8 weeks gestation. Some syncytial sprouts fail to develop, and instead their attachment to the parent villus becomes attenuated as shown here. If the delicate connection should break then the sprout floats freely in the maternal bloodstream as a syncytial globule and may be deported throughout the body.

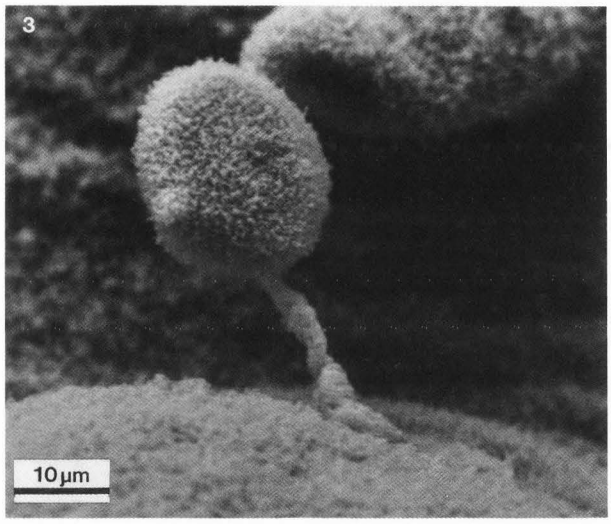
Fig. 4 A mature intermediate villus from a normal pregnancy illustrating the typical branching pattern of terminal villi. (From Kaufmann (1982) by courtesy of the author and Karger AG).

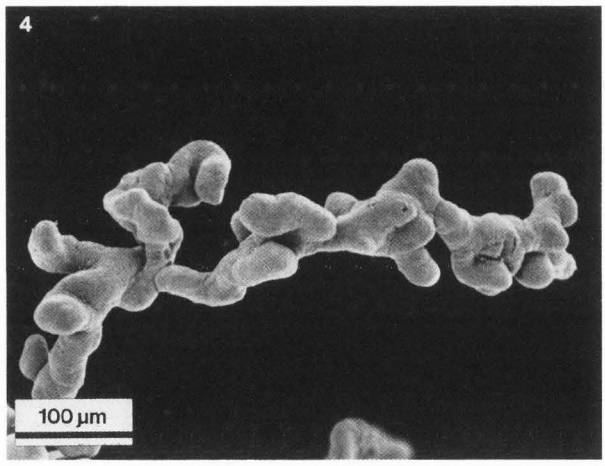
Fig. 5 An intermediate villus from a case of severe rhesus incompatibility showing the delayed maturation and reduced branching of terminal villi that is associated with this condition. (From Kaufmann (1982) by courtesy of the author and Karger AG).

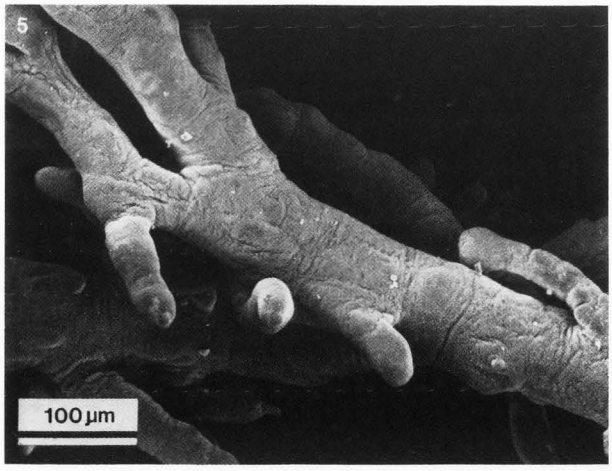
Fig. 6 A short intermediate villus from a case of pre-eclampsia displaying a profusion of terminal villi, suggesting that premature maturation of the villous tree occurs in these pregnancies. (From Kaufmann (1982) by courtesy of the author and Karger AG).

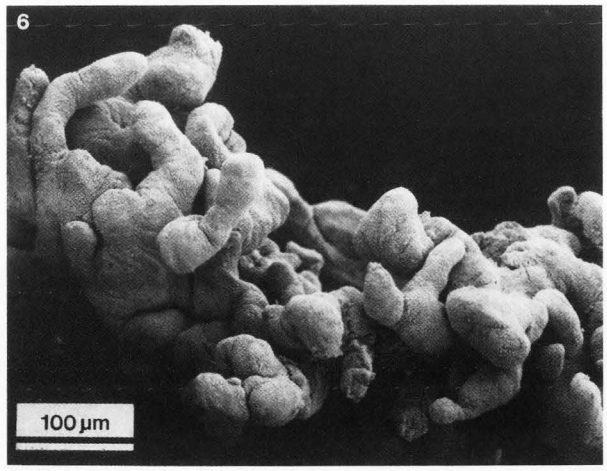

1
a
b
V
b
100µm


2
c
a
b
50µm


3
10µm


4
100µm


5
100µm


6
100µm

(1979) to clarify the pattern of branching of the villous tree and to produce a revised classification of villi. In this system the peripheral villous tree is divided into stem, intermediate and terminal villi. Whereas the stem villi play a supportive role, the terminal villi represent the functional elements, being the site of most placental exchange and hormone synthesis.

Terminal villi may arise from the lateral aspects of stem villi, but the majority (95%) branch from intermediate villi, of which there are two types. In the term placenta mature intermediate villi predominate. These are relatively long, 40-80 μm in diameter and follow a zig-zag course with terminal villi branching off at each change in direction (Fig 4). By contrast the immature form of intermediate villi are the principal villous type in early gestation. Of larger diameter, 60-200 μm, these are covered with syncytial sprouts in early pregnancy, but when they occur in the mature placenta they bear only a few scanty terminal villi.

Attempts have been made using a variety of techniques to correlate the pattern of villous branching with the occurrence of specific antenatal conditions. On the basis of scanning electron micrographs Sandstedt (1979) claimed there was a failure of villous maturation in the placentae of small for gestational age infants, with a consequent reduction in the incidence of terminal villi. Similar findings have been reported from cases of rhesus incompatibility (Fig. 5), whereas the converse of premature villous maturation, with associated proliferation of terminal villi, is found in pre-eclampsia (Fig. 6)(Kaufmann, 1982). These results have thus supported and extended the previous descriptions of several authors (e.g., Aladjem, 1968a; Fox, 1978), but as yet quantification from scanning electron micrographs has not been attempted. Indeed it is difficult to see how this can be reliably achieved, a problem which must limit such an approach to the correlation of clinical and structural findings. In addition given the significant intra-lobular variations in villous morphology that occur in the normal placenta (Fox, 1967; Sandstedt, 1979; Schuhmann, 1982), strict attention must be paid to the sampling regime if meaningful results are to be obtained. Sadly this has not always been taken into consideration in the past, and so many of the published claims must be qualified accordingly.

The morphology of terminal villi

Since terminal villi are functionally the most important division of the villous tree, they have attracted considerable attention and their appearance has been illustrated by many authors with increasing quality as specimen preparation techniques have improved.

Typically, terminal villi are short stubby branches 40-80 μm in diameter, arising from the stems of intermediate villi. Branching occurs in all directions so that the villous tree has a highly complex three-dimensional configuration (Fig. 7).

Fox and Agrafojo-Blanco (1974) first drew attention to the presence of "dome-shaped, blister-like swellings protruding from the villous surface" (Fig. 7). Most frequently located at the tips of terminal villi, they may also occur along the sides of intermediate villi, but are, however, absent from the neck region connecting the terminal villus to the parent stem. The authors concluded correctly that these swellings were the surface features of vasculosyncytial membranes; localised areas of the villous membrane specialised for gas exchange. These are formed when a dilated fetal capillary comes into intimate contact with the overlying syncytium, the diffusion barrier being reduced to as little as 2 μm at these points (Fox, 1967). It is thus of considerable interest that Fox and Agrafojo-Blanco reported an almost complete absence of these surface protrusions in placentae from prolonged pregnancies, a condition associated with a profound regression of the fetal vasculature.

Changes in the fetal blood vessels clearly have a considerable influence on the surface topography of terminal villi, which is understandable since the vessels occupy nearly 40% of the villous volume (Burton et al., 1987). During delivery there is a significant shift of blood from the extra-corporeal placental circulation back to the baby, the so called placental transfusion (Sisson, 1978). As a result the fetal capillaries are in a partially collapsed state post-natally, and remain so if traditional immersion fixation is employed. Several authors have remarked on the numerous circumferential creases and wrinkles present on the villous surface, which King and Menton (1975) felt may represent flexure lines brought about by bending of the villous tree. Such creases are, however, far less prominent if the tissue is fixed via perfusion fixation (compare Fig. 7 with Fig. 8). This technique redilates the fetal vessels to their in vivo state, and morphometric data reveals that in consequence the mean villous diameter is slightly greater following perfusion than immersion fixation. (Burton et al., 1987). To date all scanning electron microscopic studies have been performed on immersion fixed material and as a result it seems probable that many of the surface furrows and creases observed are artifactual. Most likely they are caused by redistribution of the syncytium in response to a contraction in villous volume, which itself is secondary to the partial collapse of the fetal vasculature.

Syncytial sprouts

Although most commonly observed in the phase of rapid placental growth early in gestation syncytial sprouts can still be seen in the placenta at term, confirming that villous growth continues throughout pregnancy. The capacity for new growth seems to lie predominately with villi in the central regions of the lobule, and indeed it was in this region that Sandstedt (1979) found the highest incidence of sprouts. Neither Sandstedt nor any other scanning electron microscopist has attempted to quantify this aspect of placental structure, but phase-contrast studies have suggested the incidence of sprouts may be a useful index of syncytial growth and well-being (Aladjem, 1967b, 1968a,b,; Aladjem et al., 1972).

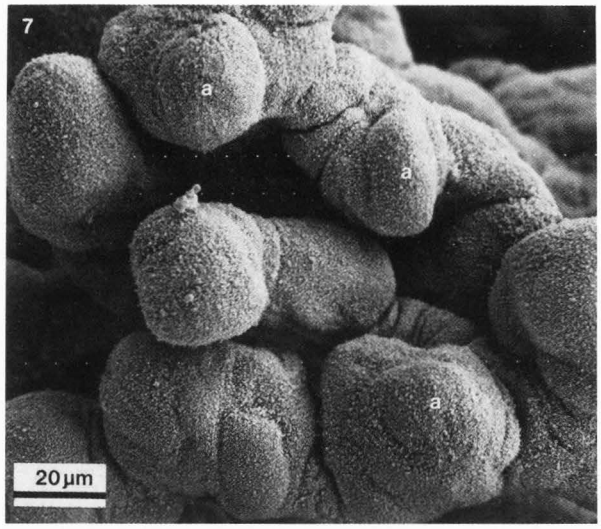
The intervillous space

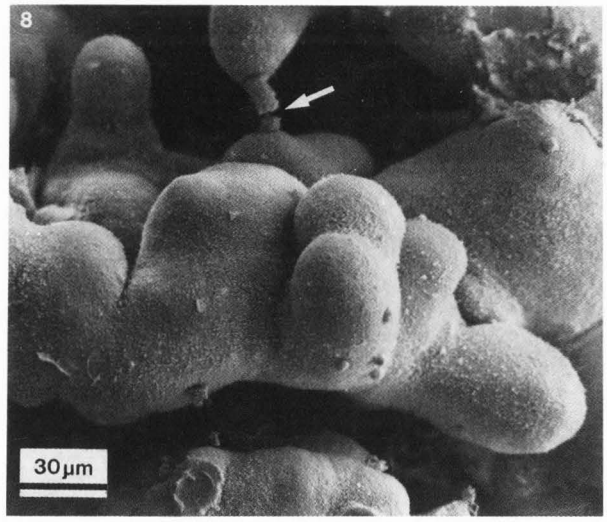
Whilst the intervillous space is not directly related to placental structure, clearly its configuration will have important physiological implications in connection with the pattern of maternal blood flow. Use of the scanning electron microscope has provided for a much clearer appreciation of the complexity of this space, and has shown that many of the tortuous channels between the villi are of capillary dimensions only (Fig. 7)(Ludwig, 1971). Maternal blood flow through the space must consequently be little more than a slow seepage, and the abnormally profuse branching of the villous tree seen in pre-eclampsia must further impede this flow. Hence reduced perfusion of the intervillous space may contribute to the fetal growth retardation that accompanies this condition.

Circulation throughout the intervillous space is particularly sluggish near the chorionic plate and it is a common finding to see large deposits of fibrin emeshing the villi in this region (Fig. 9). Such villi are effectively excluded from participating further in placental exchange, but fortunately it appears that these deposits must be exceptionally extensive before they contribute to placental insufficiency (Fox, 1978).

Syncytial fusion

In view of the complexity of the villous tree and of the small dimensions of the intervillous space it is not unreasonable to suppose that surface contact occurs between adjacent villi. At first this may cause no more than a slight disruption in the normally uniform microvillous covering (Fig. 10) but eventually syncytial fusion can and does occur. This may take two forms, either planar areas of simple apposition and fusion 10-15 μm in diameter, or elongated bridges which span the intervillous





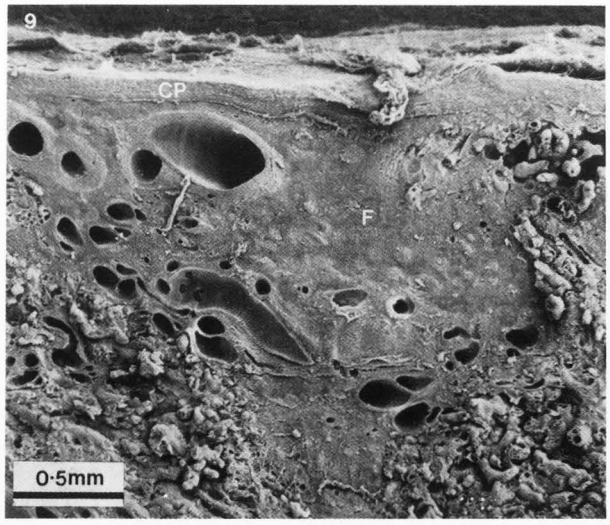


Fig. 7 Mature placenta, immersion fixation. At term the villous tree is highly-branched with terminal villi passing in all directions. The surface of the villi is characterised by the presence of blister-like swellings (a) which represent the sites of vasculo-syncytial membranes. Also visible are numerous circumferential furrows or folds, which are largely artifactual and caused by the partial collapse of the fetal vessels which accompanies immersion fixation. Note that the intervillous space separating the villi is of capillary dimensions only.

Fig. 8 Mature placenta, perfusion fixation. Note that the surface of the villi is smooth and does not display the furrows or folds seen in immersion fixation. A broken intervillous bridge (arrowed) can be seen in the background.

Fig. 9 Mature placenta. Immediately beneath the chorionic plate (CP) is an area of sluggish maternal blood flow and consequently fibrin (F) deposition may occur. The fibrin emeshes the terminal villi which are then excluded from participating further in materno-fetal exchange.

space (Burton, 1986a). The relationship between these two types of syncytial fusion is not clear, for it is not known whether the elongated bridges develop from areas of intitial apposition and fusion or whether they have a separate mode of formation, possibly from the fusion of syncytial knots.

Areas of villous apposition and fusion are difficult to identify with certainty in the scanning electron microscope, for it is often impossible to view deep into the crevice between closely apposed villi and confirm syncytial continuity. Sandstedt (1979) managed to illustrate an example, however, but they are generally easier to identify using the cryo-fracturing technique since this gives a sectional view.

Conversely the elongated intervillous bridges are highly conspicuous, and so it is all the more surprising that although they have been observed in histological sections for many years (see Hamilton and Hamilton, 1977 for review), it is only recently that their scanning electron microscopic appearances have been described (Burton, 1986b). The bridges fall into two clearly distinguishable categories, and the lack of any intermediate forms suggests these may have different modes of formation.

The first type is remarkably uniform in size, being approximately 10-15 μm in diameter and up to 40-50 μm in length (Fig. 11). Dense microvilli cover the bridge and these are identical to and continuous with the microvilli over the remainder of the villous surface. Histologically such bridges are composed of closely- packed syncytial nuclei (Jones and Fox, 1977). More delicate and thread-like are examples of the second type of bridge, only 2 μm in diameter, and again these are covered with microvilli (Fig 12). They consist of syncytio-plasm only, and not surprisingly many broken examples are encountered in the delivered placenta. Both types of bridges most commonly pass between adjacent terminal villi, although terminal and intermediate or even two inter-mediate villi may be linked in this manner.

The functional significance of intervillous bridges is uncertain. It has been suggested they have a mechanical function, bracing the villous tree and providing support, yet their structure would not appear ideally suited to this role and it is not clear whether such support is actually necessary (Burton, 1986b). It seems most probable that, like areas of simple apposition and fusion, they arise fortuitously, two villous surfaces coming into close contact and syncytial fusion taking place. Jones and Fox (1977) were of the opinion that bridges arise through the fusion of syncytial knots, but this may have a purely physical explanation since these aggregat-ions of nuclei cause elevations on the villous surface and are thus likely points of contact between adjacent villi. It is of interest that Sandstedt (1979) commented on the relatively low incidence of syncytial fusion in the placentae of small for gestational-age babies, in which the reduced branching of the villous tree results in wider intervillous spaces. Chance contact between villi is presumably less likely to occur in these cases than in normal placentae.

Rupture of these bridges is common during the rigours of a vaginal delivery (Stieve, 1940; Peter, 1943), and this may explain why Kustermann (1981) was unable to observe any examples in his scanning electron microscopic study. This severance of the interconnections causes two artifactual appearances, which because they are so common, might justifiably be considered normal features of the delivered placenta. Firstly, stalk-like processes with disrupted distal ends, sometimes carrying a flange of syncytium avulsed from the neighbouring villus may be observed (Fig. 13). These stalks represent the main part of a bridge, for when they shear it is most commonly at their point of attachment to the villous surface (Fig. 14). Almost identical structures were illustrated by Clint et al.,

---

Fig. 10 Mature placenta. Due to the close juxtaposition of villi at term surface contact may occur between adjacent villi. At first this may cause only disruption of the normally uniform microvillous covering, as illustrated here, but ultimately it may lead to syncytial fusion. Note that the affected area (arrowed) is approximately 10 μm in diameter.

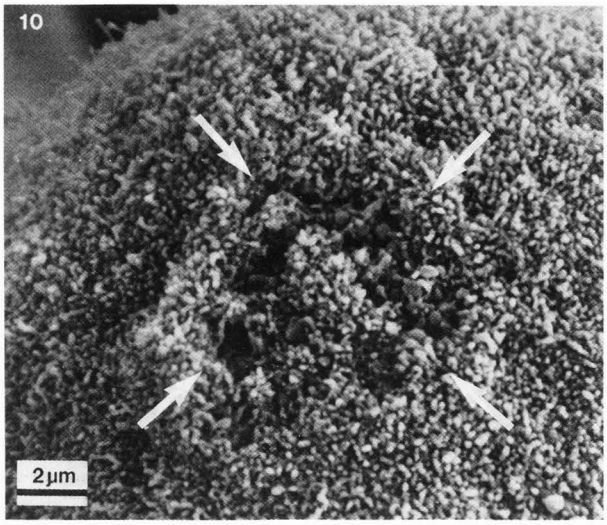
Fig. 11 An example of an intervillous bridge (arrowed) passing between two adjacent terminal villi. Note the uniform covering of microvilli over the bridge.

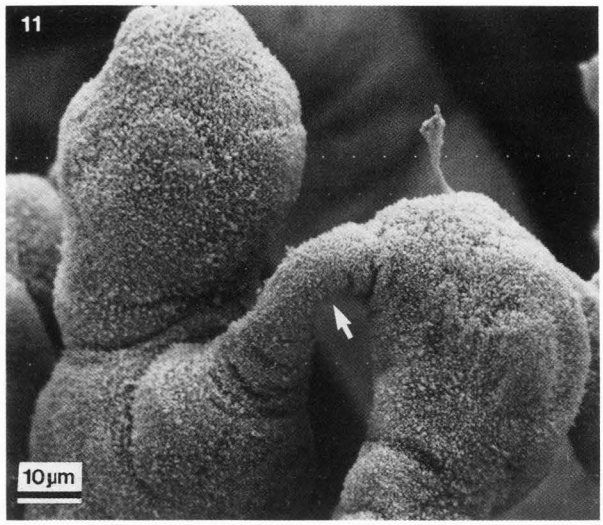
Fig. 12 The second type of intervillous bridge is of a more delicate nature, and few intact examples are seen in the delivered placenta. The frequent occurrence of snapped ends suggests these interconnections are common in vivo, however. (From Burton (1986b) by kind permission of Cambridge University Press).

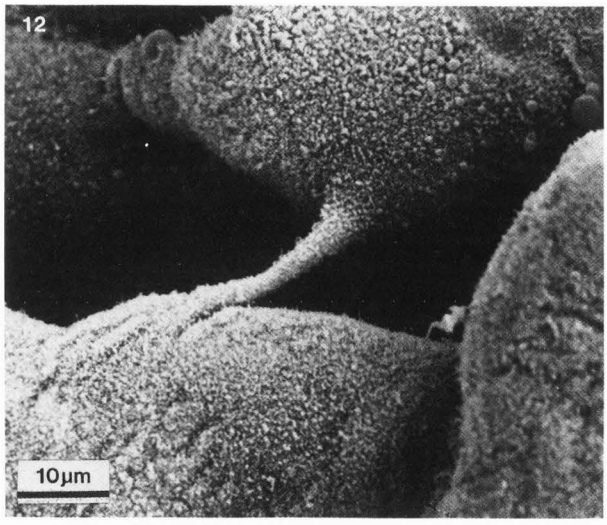
Fig. 13 Numerous stalk-like processes are seen arising from the villous surface, and these are believed to be intervillous bridges that have sheared either during delivery of the placenta or during subsequent tissue handling. Some may carry a flange of syncytium at their distal end, as illustrated here, avulsed from the surface of one of the villi. (From Burton (1986b) by kind permission of Cambridge University Press).

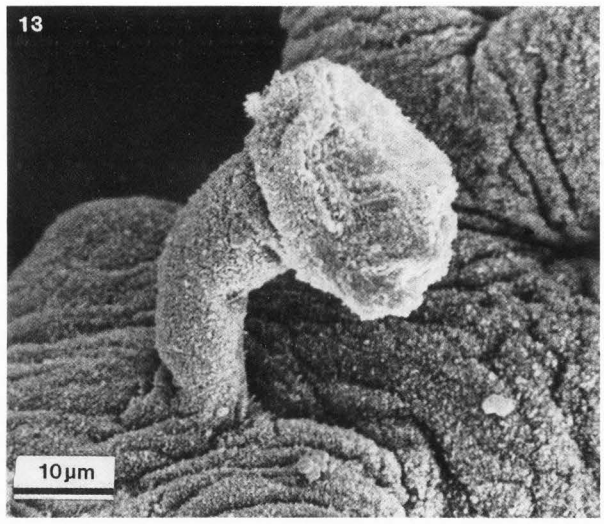
Fig. 14 Mature placenta. When intervillous bridges (b) break it is often at their point of attachment to one of the villous surfaces. This creates a stalk-like artifact, and a localised area of syncytial damage (arrowed). Note that the microvilli in the area (a) have been flattened during tissue processing. (From Burton (1986b) by kind permission of Cambridge University Press).

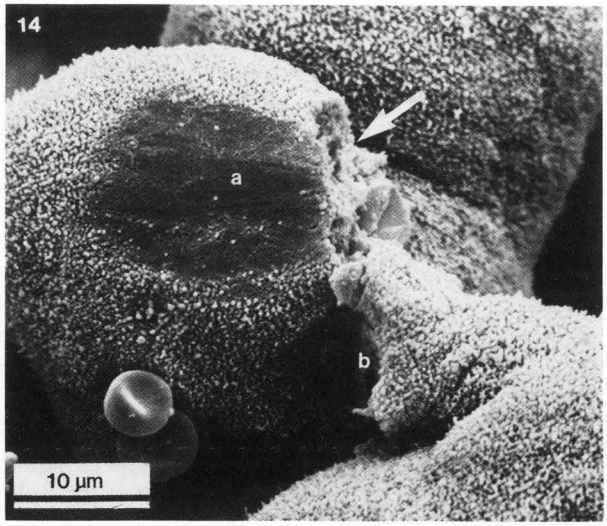
Fig. 15 Circular areas of syncytial damage are not infrequently observed on the villous surface. Because of their size and well-defined margins these are believed to be caused by either avulsion of an intervillous bridge, or by separation of an area of apposition and fusion.

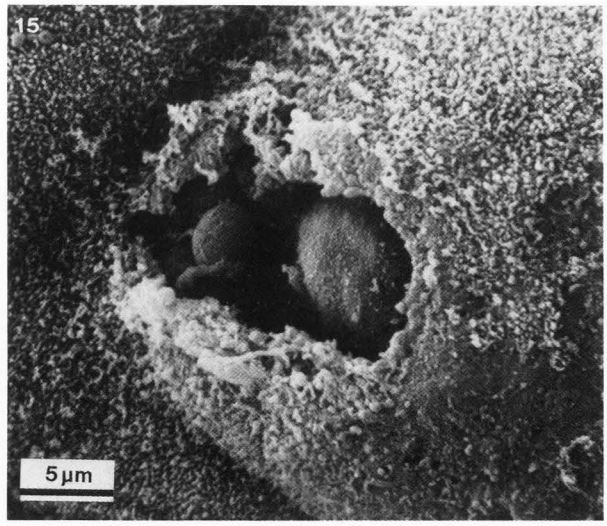

10
2µm


11
10µm


12
10µm


13
10µm


14
a
b
10 µm


15
5µm

(1979), who considered them to be holdfasts tethering the villous tree to the basal plate. Whilst some bridges may indeed perform this function it is clear from the intact examples that many pass between adjacent villi.

Conversely, when the attachment of a bridge does shear a localised area of syncytial damage results. Such areas are remarkably consistent in size, being roughly circular and 10-15 μm in diameter, and these dimensions are of course equivalent to those of intact bridges. The areas of damage are well circumscribed, often with raised margins, and depending on the depth of the lesions the underlying stroma may be exposed (Fig. 15). Similar artifacts would also be created if areas of villous apposition and fusion were forcibly separated.

Such areas of syncytial damage are typical of the fixed delivered placenta in which there has been no possibility of healing or repair. Interestingly Clint et al., (1979) described isolated masses of cellular tissue associated with what appeared to be areas of syncytial damage. The authors offered the possible explanation that these cells represented a proliferation of cytotrophoblast elements in an attempt at syncytial repair. It is apparent from these scanning electron microscopic studies that in vivo rupture of intervillous bridges or areas of syncytial fusion might trigger such a response.

The Microvillous Surface

General appearances

Not surprisingly the microvilli covering the villous surface have attracted the attention of many investigators, and their appearances during the various stages of gestation have been described in great detail (e.g., Dempsey and Luse, 1971; Bergstrom, 1971; Ferenczy and Richart, 1972; Fox and Agrafojo-Blanco, 1974; King and Menton, 1975; Thiriot-Herbert and Panigel, 1977; Sandstedt, 1979). There is general agreement between the descriptions provided by these authors.

In early pregnancy the microvilli appear to be arranged in an irregular honeycomb pattern (Fig. 2) and this undoubtedly reflects the fact that in the immature placenta microvilli originate in tufts, rather than being evenly distributed over the villous surface. Indeed on the basis of sections cut tangential to the syncytial surface Boyd et al., (1968) suggested these tufts may on occasions be elongated into ridges of considerable length (see their Fig. 4). In confirmation of this Dempsey and Luse (1971), Bergstrom (1971), and King and Menton (1975) all described ridge-like elevations in the microvillous covering of villi during early pregnancy. These have not been reported in the mature placenta, in which the distribution of microvilli over the syncytial surface is more even, Sandstedt (1979) estimating the density to be 10-12 x 10^6 per mm^2.

Individual microvilli may present several forms in the scanning electron microscope. Approximately 2-4 μm in length and 0.2-0.3 μm in diameter the majority are cylindrical in shape, whereas others are more spatulate or possess expanded bulbous tips. Microvilli are, however, notoriously vulnerable to both hypoxic changes (Illsley et al., 1985) and to fixation artifacts (Hayat, 1981). Their configuration depends to a considerable extent on the osmolality, ionic concentration and temperature of the fixative employed, and so great significance should not be attached to these various descriptions.

Nonetheless it is difficult to understand how branching of the microvilli may be influenced in this way and several investigators have commented that bifid or multiply-branched microvilli are especially common in early gestation (Bergstrom, 1971; Ferenczy and Richart, 1972; Fox and Agrafojo-Blanco, 1974). This may represent a genuine change in the structure of the microvilli during gestation, or simply reflect the fact that when microvilli arise in tufts, as in early pregnancy, the bases of individual microvilli are hard to define. Particularly complex branching of the microvilli was observed in cases of molar transformation by Ferenczy and Richart (1972).

Microvillous-free areas

Few facets of placental structure have created such controversy within the literature as the presence or absence of microvillous-free areas on the villous surface. Several accounts describe a gradual transition between the normal extensive microvillous covered areas and regions where the microvilli are greatly reduced in number or absent (Fox and Agrafojo-Blanco, 1974; Leibl et al., 1975a, 1975b; Clint et al., 1979; Demir, 1979).

Frequently such regions are seen on the tips of terminal villi or over the dome-shaped protruberances thought to be vasculo-syncytial membranes. As has been stated, however, microvilli are particularly susceptible to hypoxia and to fixation artifacts, and it is worthy of note that most of the studies quoted above employed air-drying in their preparative techniques. The surface tension effects associated with air-drying may cause microvillous loss (Bergstrom, 1971), and more recent studies using the superior critical-point drying technique have found no evidence of microvillous-free areas (Ferenczy and Richart, 1972; King and Menton, 1975).

Specimen drying is only one complicating factor, and other steps in tissue processing may also lead to aberrations. For example there is the problem of protein deposition from the maternal plasma onto the villous surface, causing agglutination of the microvilli. Adequate washing during preparation or ideally fixation via the dual perfusion technique minimises but cannot completely prevent this deposition.

Fig. 19 During tissue handling areas of the syncytium (s) may be lifted away exposing the smooth featureless basement membrane (bm).

Fig. 20 In other areas where the syncytium (s) has been torn away cytotrophoblast cells (c) may be seen lying on the basement membrane (bm).

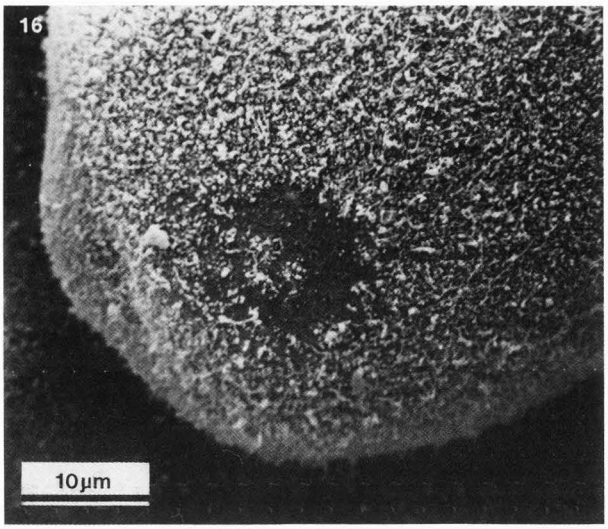
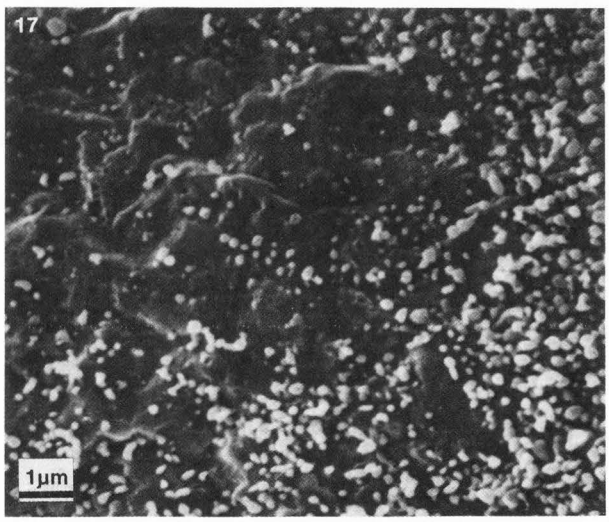
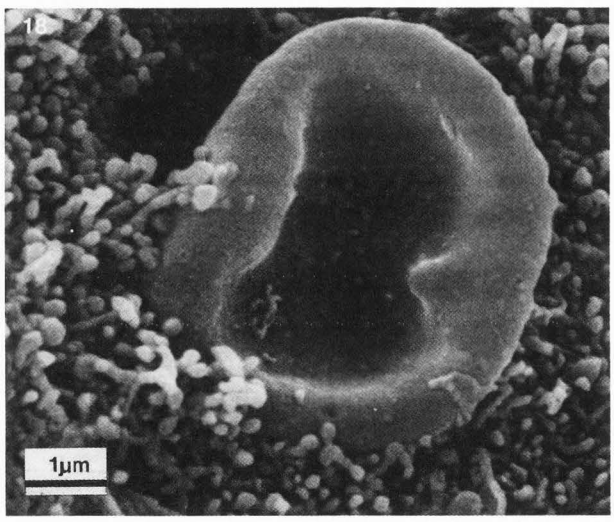


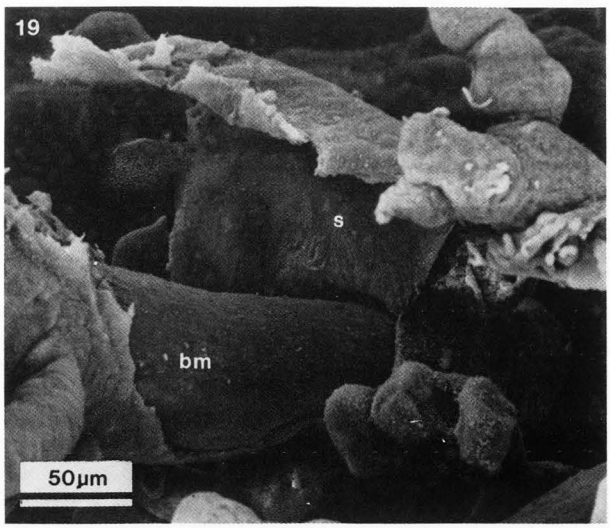
Fig. 16 Areas of true microvillous loss are only rarely observed, and since they are always approximately 10 µm in diameter it might be assumed they are caused by contact between villi, perhaps representing a stage in the process of syncytial fusion intermediate between those shown in Figs 10 and 11.

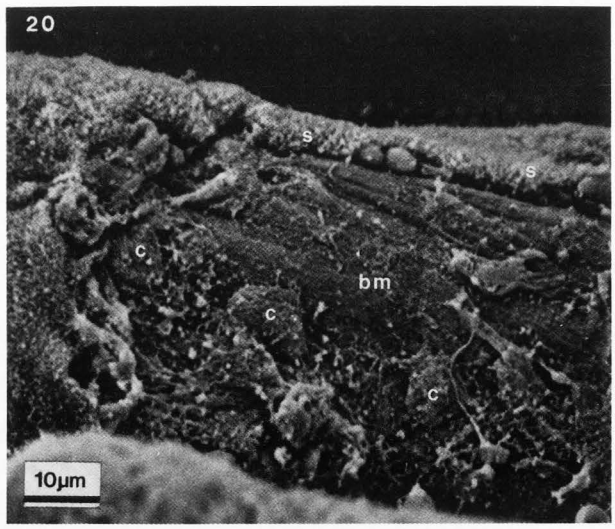
Fig. 17 Examination at higher power confirms microvillous loss in this instance. The normal microvilli shown on the right abruptly give way to the smooth undulating surface of the syncytium.

Fig. 18 Occasionally, apparent engulfment of maternal erythrocytes by the syncytium can be observed. Here microvilli are seen migrating over the surface of an erythrocyte possibly drawing it into a phagocytic vacuole.









In addition handling and contact between the specimen and the walls of the container may cause crushing or flattening of the microvilli. Such areas are frequently observed, and whereas at first sight it may appear there is microvillous loss, on closer inspection it is clear the microvilli have only been flattened (Fig. 14). Since the tips of the terminal villi are likely to be most vulnerable in this respect this may explain in part the distribution of these supposed microvillous-free areas.

Personal experience of viewing critically-point dried material supports the view that true microvillus loss is rarely observed in the mature placenta. The only cases that have been identified beyond doubt are areas circular in outline and approximately 10-15 μm in diameter (Figs. 16 & 17). It is the author's belief that these are caused by closely juxtaposed villi coming into contact as described previously, and microvillous loss may represent an early stage in the formation of syncytial adhesions rather than a functional differentiation of the syncytiotrophoblast. It thus represents a slightly later stage than that illustrated in Fig 10. Microvillous-free areas have been reported in transmission electron microscopic studies (Boyd et al., 1968), but in all cases the underlying syncytium appears to be either degenerating or necrotic. Similarly, Sheppard and Bonnar (1979) found that distortion and loss of microvilli was closely associated with degenerative changes within the syncytium in cases of spiral artery occlusion.

It would seem safe to conclude, therefore, that the normal healthy or well-fixed syncytium has a continuous covering of microvilli over its entire surface.

Other surface features

Bathing the syncytial surface is the maternal blood circulating through the intervillous space and, although erythrophagocytosis is not extensive within haemochorial placentae (Burton, 1982) examples of apparent engulfment of maternal red cells are occasionally seen (Fig. 18). The contact between the microvilli and the red cells is certainly more intimate in these instances than when erythrocytes come to rest on the syncytial surface during fixation. Indeed the creeping of microvilli over the surface of the erythrocytes surface closely resembles the process observed in the haemophagous region of the ovine placentome where erythrophagocytosis is known to occur (see Fig. 10, Steven et al., 1981). The surface appearances of this intriguing phenomenon were first described by Clint et al., (1979), but as yet there is no corroborative proof from transmission electron microscopy that erythrocyte engulfment and subsequent breakdown actually takes place.

Possible examples of erythrophagocytosis must be carefully distinguished from the more common artifactual situation in which maternal erythrocytes come to lie on the microvillous surface during fixation and may leave an impression of their shape on that surface. The microvilli do not appear to be entrapping the erythrocytes in these cases, but are simply crushed in a ring-like pattern. Al-Zuhair et al., (1983) mistakenly believed that such intimate contact between erythrocyte and microvilli was of special importance in placental gas transfer, but it is clear that diffusion across the maternal plasma presents little resistance to gas flow (Mayhew et al., 1984). A number of other surface features have been described, but these are also most likely artifactual in origin. For example Kaufmann (1971) described smooth polypoid protrusions extending from the villous surface into the maternal blood space. These protrusions almost certainly resulted from the strongly hypertonic fixative used in his study, for they are found in great numbers on villi following hypertonic saline induced abortion (Stegeman and Treffers, 1980). Extreme caution must be exercised when interpreting surface appearances in the scanning electron microscope, in particular when dealing with crystalline deposits. Al-Zuhair et al., (1984) suggested that small flecks and plaques lying on the microvillous surface represented the initial stages of placental calcification, yet no supporting evidence from microanalysis confirming these deposits did in fact contain calcium was presented. In the absence of such evidence the possibility that they arose from crystallisation of salts in the fixative or buffer solutions cannot be excluded, and indeed it seems clear

Fig. 21 The syncytium and basement membrane may be removed by brief (30 seconds) exposure to hypochlorite solution as demonstrated here. Only the collagen framework of the villi remains but as can be seen the shape of the villous tree is nonetheless preserved.

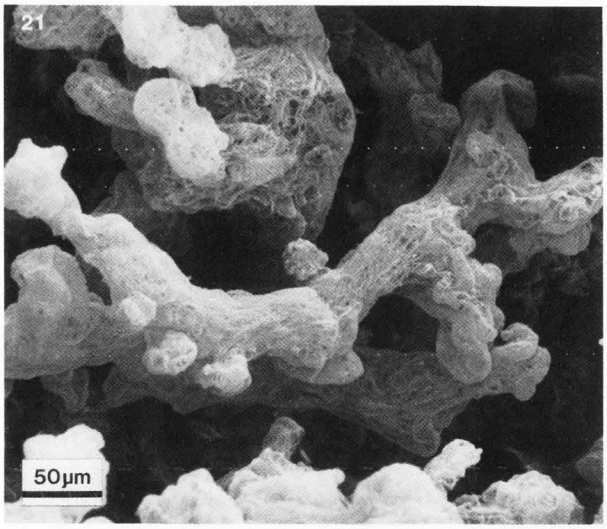
Fig. 22 At higher power the delicate interlacing of the collagen fibres can be observed. Note that they are often arranged circumferentially around the course of the fetal capillaries, and that their density varies along the length of those vessels.

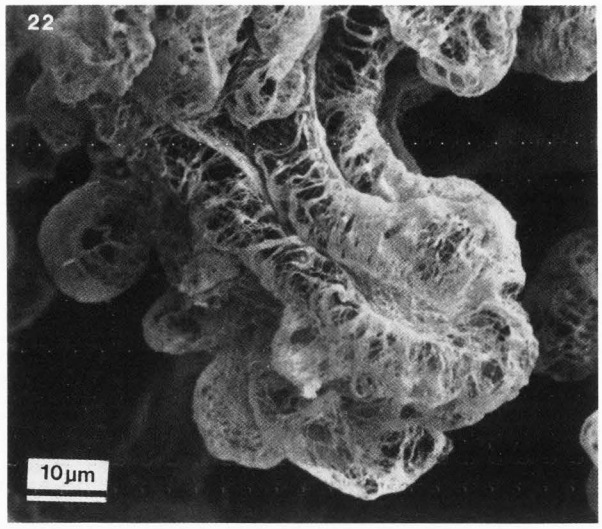
Fig. 23 8 weeks gestation. Cryo-fractured preparation of a mesenchymal villus showing the stromal core consisting of scattered fibroblasts and collagen fibres. Small spaces (a) are beginning to develop within the core and occasional Hofbauer cells (arrowed) may be seen in the walls of these. Blood vessels (b) are sparse at this stage of development.

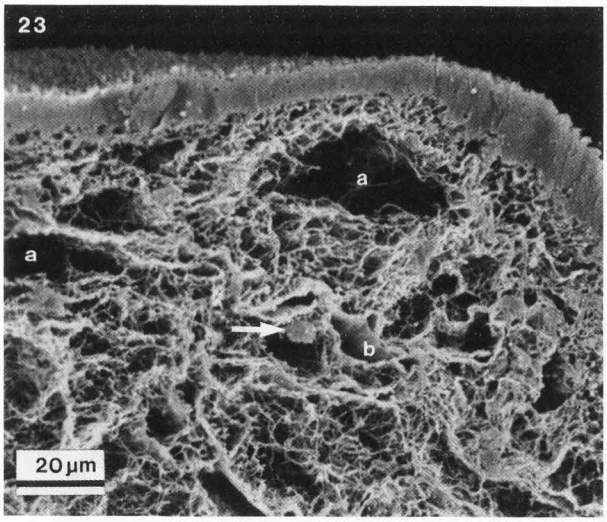
Fig. 24 Mature placenta. An immature intermediate villus showing the presence of numerous fibre-free stromal channels and their contained Hofbauer cells (arrowed). Fetal capillaries (c) can be distinguished around the periphery of the villus.

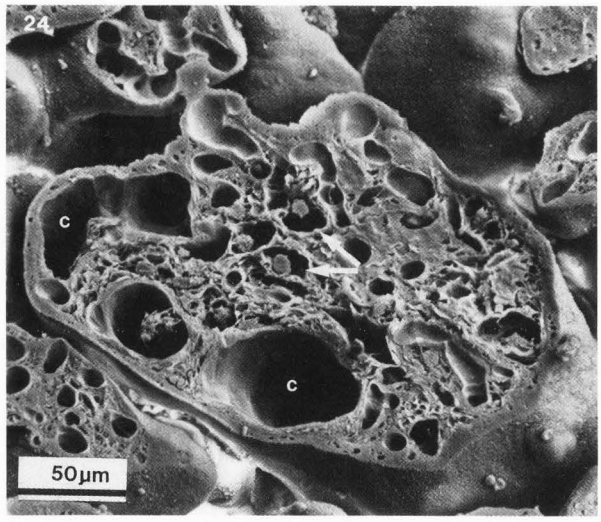
Fig. 25 Within the stromal channels lie Hofbauer cells (h) which may be identified by their characteristic surface projections and numerous cytoplasmic vacuoles.

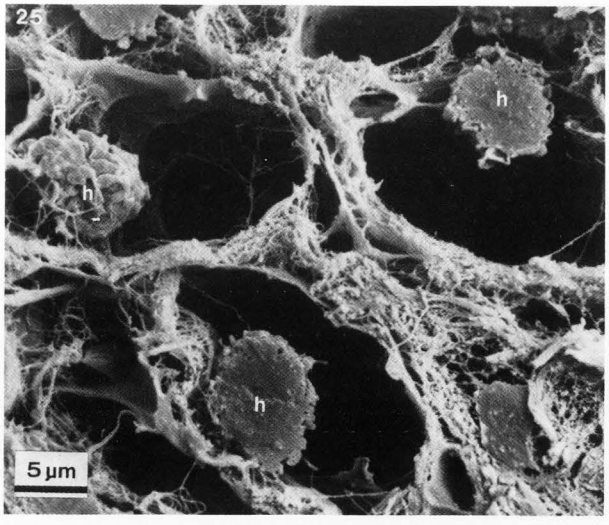
Fig. 26 Mature placenta. A mature intermediate villus showing the dense villous stroma and absence of stromal channels. A point of villous apposition and fusion (arrowed) can be identified in sectional view.

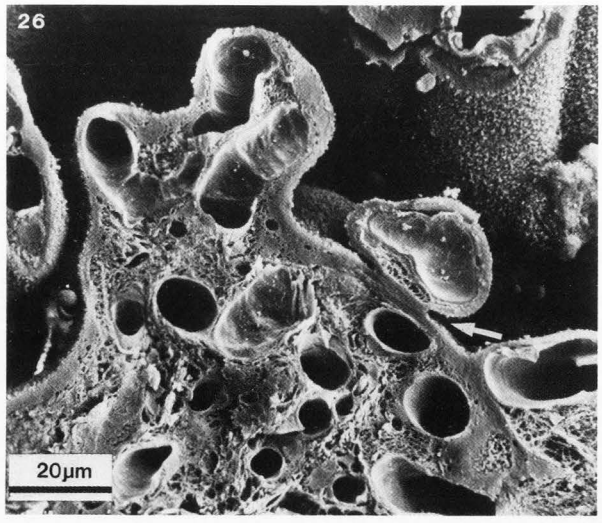

21
50µm


22
10µm


23
a
a
b
20µm


24
c
c
50µm


25
h
h
h
5µm


26
20µm

from the well-controlled study of Varma and Kim (1985) that the earliest mineral deposits are seen along the trophoblastic basement membrane and not on the syncytial surface.

The Villous Stroma

For viewing the stromal tissues underlying the villous surface one can either attempt to remove the syncytial surface using micro-dissection techniques, or fracture the villi producing a sectional view.

Micro-dissection techniques

In this category can be placed a variety of techiques which range in severity from mild disruption by treatment with ultrasonication and detergents to partial digestion by means of hypochlorite solutions. For example Ockleford et al., (1981) immersed villi in 0.5% Triton X-100 for various periods of time and illustrated a progressive dissolution of the syncytium from loss of microvilli to the emergence of nuclear humps. Little detail of the syncytial organelles can be discerned from their micrographs, however. The more vigorous techniques of Highison and Tibbitts (1986) removed the syncytium completely and exposed the underlying cytotrophoblast layer in early placentae of 12-18 weeks gestational age.

In addition advantage can also be taken of fortuitous rents in the syncytial surface caused during tissue handling. A case is illustrated in Fig. 19 where a relatively large flap of syncytium has been raised revealing a smooth featureless basement membrane. Individual syncytial organelles may often be identified along the torn edge of the villous surface. In other instances a number of flattened pleomorphic cells, approximately 10-15 μm in diameter, are seen lying on the basement membrane (Fig. 20). Microvilli extend from these cells over the membrane, and this characteristic along with their dimensions strongly suggests they are cytotrophoblast cells, a stem cell population which maintain the syncytium (Boyd and Hamilton, 1970).

On occasions the basement membrane may split, exposing the bundles of stromal collagen fibres which provide the framework for the villous tree. These can be viewed en masse if both the syncytium and basement membrane are removed. This may be achieved either by detergent extraction (Highison and Tibbitts, 1986) or more easily by brief immersion in hypochlorite solution prior to fixation. Using the latter technique the complex architecture of the villous tree is preserved (Fig. 21), and at higher power the delicacy of the interlacing bundles of fibres can be readily appreciated. Often the fibres give the impression of having been arranged circumferentially around the fetal vessels as they course within the villi (Fig. 22), their density varying from point to point along the length of those vessels. It is likely their disposition is related to the localised sinusoidal dilations that occur along the fetal capillaries, but whether the pattern of the bundles dictates the sites of these dilations or vice-versa has yet to be determined.

Cryo-fracture techniques

Ever since the earliest scanning electron microscopic studies of the placenta authors have commented upon and illustrated the cross-sectional appearances of villi that had snapped during tissue processing. In general these descriptions made little advance upon previous knowledge of villous structure gained through traditional histological methods, and indeed many of the early illustrations were of inferior quality to those provided by light microscopy.

However the application of cryo-fracturing produced significant progress particularly in the understanding of the villous stromal core. By freezing villi immersed in absolute alcohol in liquid nitrogen and then fracturing the block with a razor blade a very clean surface is achieved. Results are improved if perfusion fixation can be employed since this not only redilates the vessels but also clears their lumina of fetal plasma rendering the endothelium easier to observe. The technique has been principally applied by Castellucci and his co-workers (Castellucci et al., 1980, 1984; Castellucci and Kaufmann, 1982).

Early in gestation the villous core is composed of loosely-packed mesenchymal cells emeshed in a delicate network of collagen fibres (Fig. 23). The fibres are not arranged in any

Fig. 27 Vasculo-syncytial membranes are clearly demonstrated using the cryo-fracturing technique. In this terminal villus the dilated capillary (c) is coming into very close apposition with the syncytium (s). Note that microvilli can be seen over the entire extent of the vasculo-syncytial membrane.

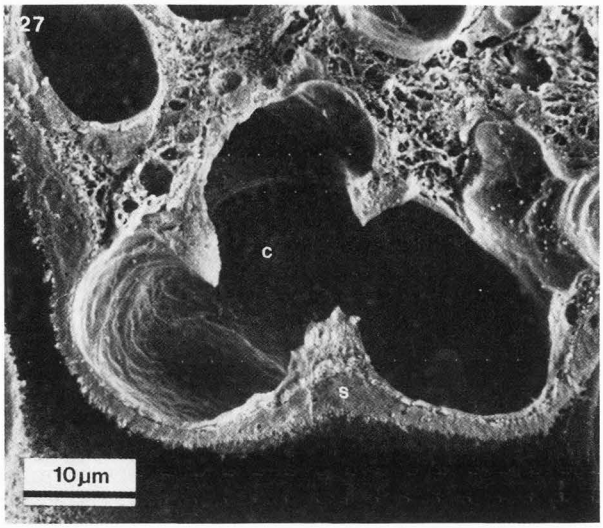
Fig. 28 Occasionally the syncytial covering may be torn from the villus, revealing the underlying fetal capillaries (c). In this instance the capillary is forming a simple loop, but it is noticeably dilated at the apex of the loop.

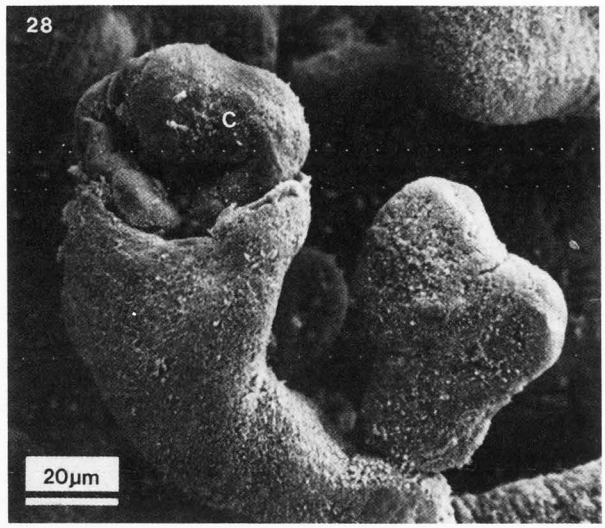
Fig. 29 Methyl methacrylate cast of the vasculature of a mature placenta demonstrating the extensive para-vascular network (pvn) which surrounds many of the larger vessels.

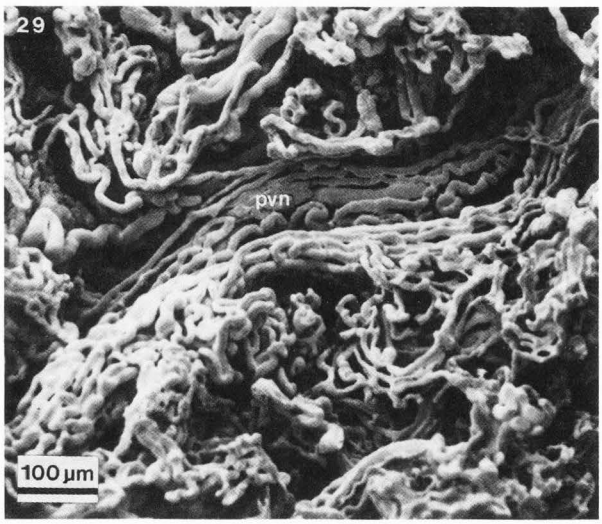
Fig. 30 Higher power view of the para-vascular network showing the high degree of interconnections between the vessels which characterises this plexus. The main vessel (v) can be identified passing through the centre of the network.

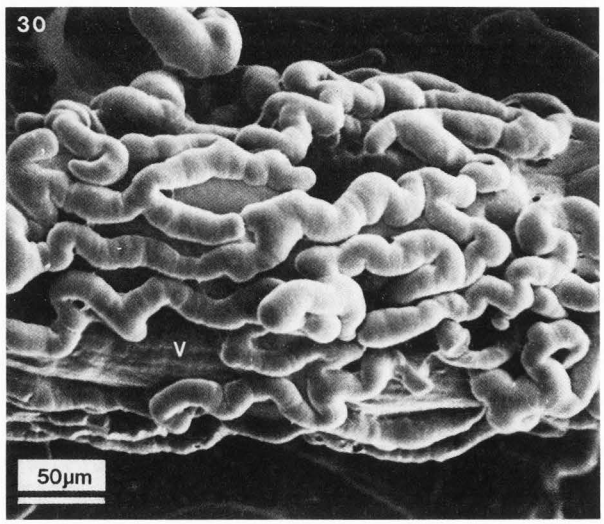
Fig. 31 In some instances the capillaries supplying terminal villi appear to arise from the para-vascular network as shown here. Note the localised dilations (arrowed) which occur along the course of the fetal capillaries. These may be involved in the formation of vasculo-syncytial membranes.

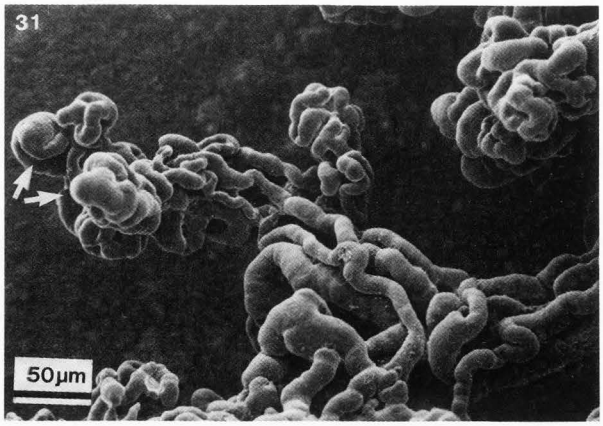
Fig. 32 Methacrylate cast of the vessels within a terminal villus illustrating the way capillaries may intercommunicate, and also the manner in which localised dilations occur along their length, particularly at points of confluence.

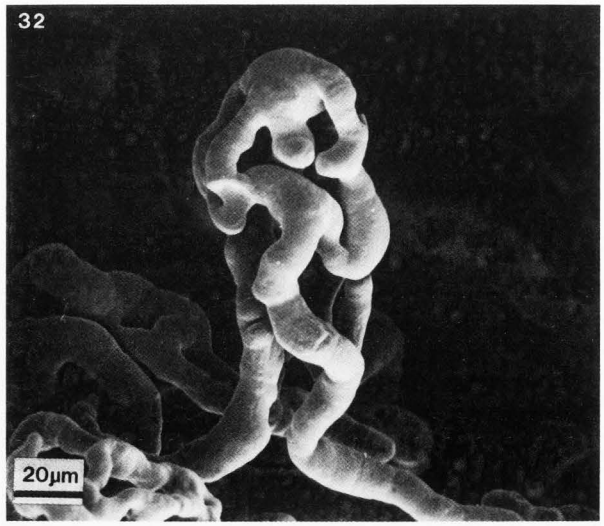

27
c
s
10μm


28
C
20μm


29
pvn
100μm


30
V
50μm


31
50μm


32
20μm

preferred orientation but several ill-defined cavities, free of fibres, are present within the core. On the walls of these cavities, partially emeshed in the collagen network, wandering macrophages known as Hofbauer cells are occasionally seen.

With further development towards the end of the first trimester sail-like processes extend from the stromal cells, which have been variably termed fibroblasts and myofibroblasts (Feller et al., 1985), establishing contact between neighbouring cells. The surrounding collagen fibres become condensed in the interstices with the result that a series of intercommunicating fluid-filled stromal channels are created. Hofbauer cells, more numerous at this stage of gestation, are frequently observed either free within or adherent to the walls of these channels, and may be identified by their characteristic surface projections and numerous cytoplasmic vacuoles. This stromal configuration characterises the immature intermediate villus as illustrated in Figs. 24 & 25. Although the presence of stromal channels and the relationship of Hofbauer cells to them has been known from light and transmission electron microscopy (Enders and King, 1970; Boyd and Hamilton, 1970), the cryo-fracture studies of Castellucci have done much to clarify and reinforce these earlier descriptions.

By comparison only a few short channels typify mature intermediate villi, and in terminal villi the connective tissue core has become so condensed they are entirely absent (Fig. 26). Dilated fetal capillaries occupy much of the villous core, and these come into close apposition with the overlying syncytiotrophoblast. As can be seen in Fig. 27 the barrier thickness is greatly reduced in the region of vasculo-syncytial membranes, where no stromal tissue intervenes between the capillary endothelium and the trophoblastic basement membrane.

Little detail can be discerned within the trophoblast using the cryo-fracture technique, and at no stage of gestation can a differentiation between cytotrophoblast and syncytiotrophoblast be made. Instances of villous apposition and fusion can often be identified, however, for the technique provides a sectional view revealing syncytial fusion at the point of intervillous contact (Fig. 26).

The Villous Vasculature

One of the principal functions carried out by the placenta is gaseous exchange between mother and fetus, and the effectiveness with which this can be achieved is determined to a considerable extent by the complexity of the villous vasculature. Not surprisingly, therefore, the fetal capillary network has been extensively investigated (see Boyd and Hamilton, 1970; Ramsey and Donner, 1980 for reviews), and recently scanning electron microscopy has made a valuable contribution.

Generally the fetal vessels are shrouded from view by the syncytium and villous stroma, but occasionally these may be fortuitously stripped away during tissue processing. An example of such is illustrated in Fig. 28 where a single capillary loop is exposed within a terminal villus. It is noticeable that the capillary dilates at the apex of the loop, and so is likely to have been taking part in the formation of a vasculo-syncytial membrane at this point.

Microvascular casting allows the fetal circulation to be studied in a more extensive and systematic fashion, and several workers applied this technique at the light microscope level with considerable success (Bøe, 1953; Arts 1961; Freese, 1968). The first published scanning electron micrographs of such vascular casts were contained in the brief report of Thiriot and Panigel (1978), who injected the placenta with a silicone rubber compound. More detailed information was provided, however, by the studies of Habashi et al., (1983) and Goyri O'Neill (1983), and these have been supplemented by the works of Lee and Yeh (1983), Leiser (1985) and Kaufmann et al., (1985).

The existence of a para-vascular network of vessels surrounding the larger arteries and veins contained within the stem villi has been known for many years. It is particularly prominent near the chorionic plate and in the deeper parts of the villous tree that are removed from the intervillous space. The network consists of a series of freely intercommunicating vessels of both arterial and venous origin, which run parallel to and in close proximity with the major vessels (Figs 29 & 30). Various functions have been attributed to the paravascular network, including responsibility for non-gaseous exchange, and for nutrition of the placental parenchyma. Irrespective of its exact function it is clear that many of the capillaries vascularising terminal villi originate from this plexus (Fig. 31) and their configuration is largely determined by the shape of the containing villus. In those cases where the terminal villus possesses a short but relatively narrow neck region before expanding at its distal end, the supplying vessels run straight and parallel before elaborating into a convoluted knot (see Fig. 3 in Habashi et al., 1983). Within this knot the capillaries twist and turn in all directions so that the flow relationships between the fetal and maternal circulations become extremely complex.

A conspicuous feature is the localised dilations which may occur along the course of a capillary, or at the point of junction of several vessels (Fig. 32), but as yet the functional significance of these specialisations is uncertain. Since they are usually involved in the formation of vasculo-syncytial membranes it has in the past been assumed that they serve to slow the rate of blood flow, thus facilitating gas transfer (Arts, 1961). More recently, however, Kaufmann et al., (1985) proposed that in addition they are of key importance in reducing vascular resistance, which is essential considering the long length of capillary pathways. Support for this theory comes from the three-dimensional reconstructions of the vascular networks performed by Kaufmann and his co-

workers. From these studies they concluded that the vasculature of terminal villi consists of highly convoluted capillary loops, which may serve several terminal villi in series and which display minimal branching and interconnection. The capillary pathways are thus long, and within terminal villi the majority were found to be between 3,000 and 5,000 μm in length. The occurrence of the sinusoidal dilations may thus be essential to reduce resistance and also to ensure an equitable distribution of flow. It must be pointed out, however, that the views of Kaufmann and his colleagues based on the reconstruction specimens are contrary to much of the evidence provided by scanning electron micrographs of corrosion casts. These suggest a much higher degree of branching and intercommunication between the capillary loops, with the result that the vascular pathways are both short and direct (Fig. 32). Clearly the superimposition of SEM images can lead to mistaken estimates of the incidence of vessels joining, but it cannot be denied that branching and union between capillaries does occur within terminal villi. The problem may be only one of degree, but at present it has yet to be resolved.

Impressions gained from viewing corrosion casts must certainly be treated with caution for the technique is susceptible to a number of errors including over- or under-distension of the vessels, non-filling of certain areas, breakages during preparation and a number of others. At present therefore, they are not suitable material on which to base quantitative studies. Nonetheless, Lee and Yeh (1986a,b) have applied the technique to examine the placental vasculature in cases of small for gestational age babies and rhesus incompatibility. Similar impressions were gained in both cases, there being a reduction in the degree of branching of the larger arteries and veins but an increase in the number of vascular buds arising from the capillary network. The latter feature was interpreted by the authors as suggesting neovascularisation, and an attempt at compensation by the placenta.

Obviously the corrosion casting technique has much potential for comparative and clinical studies, and if ever accurate quantification could be achieved then it would become a most powerful research tool.

Conclusions

Scanning electron microscopy is undoubtedly a very photogenic technique, and also one that is relatively easy and quick to perform. Results can thus be rapidly obtained, often in the past on a "look and see" basis. There are, however, a number of stages involved in tissue preparation, and at each artifactual appearances may be induced. Furthermore, the placenta is not a homogeneous organ, despite its gross appearances, and so adequate attention must be paid to sampling. One cannot criticise the early pioneering studies for a lack of awareness of these problems, but it behoves anyone currently working in the field to hold them firmly in mind when assessing their own or others' work. It is to be hoped this review has corrected several false impressions that have passed into and been perpetuated within the literature due to such lack of recognition.

It is also to be hoped this review has identified some of the areas of placental research where further scanning electron microscopic studies would be profitable. Quantification remains the major obstacle to comparative studies, and at present there seems to be little progress on this front. Nonetheless it appears that scanning electron microscopy, in conjunction with other techniques such as partial digestion or corrosion casting, can still yield much new data concerning basic placental structure. Equally important, it can assist in the appreciation of the third dimension, allowing us to interpret data acquired by the more traditional sectional methods in a manner that more closely approximates to reality.

Acknowledgements

Grateful thanks are due to Dr K J Dalton, University Lecturer and Consultant Obstetrician, and to the nursing staff of the Delivery Ward, Rosie Maternity Hospital, Cambridge for their help and co-operation in obtaining the placental material. Professor P Kaufmann, Abteilung Anatomie der RWTH, Aachen kindly provided Figs. 4,5,6,; otherwise the illustrations are original.

Within this Department expert technical assistance was provided by Mr J N Skepper, Miss M E Palmer and Miss S C Ingram. Thanks are also due to Mr I Edgar for printing the photographs and Mrs J M Seymour-Shove for typing the manuscript.

Most of the new work presented in this paper was supported by a grant from Action Research for the Crippled Child - The National Fund for Research in Crippling Diseases.

References

Aladjem S (1967a) The syncytial knot: a sign of active syncytial proliferation. Am. J. Obstet. Gynec. 99, 350-358.

Aladjem S (1967b) Correlation of pregnancy wastage and hypoplasia of placental syncytium. Study by phase-contrast microscopy. Obstet. Gynec. 30, 408-413.

Aladjem S (1968a) An index of syncytial growth in normal and complicated pregnancies. Am. J. Obstet. Gynec. 101, 704-708.

Aladjem S (1968b) Phase contrast microscopic observations of the human placenta from six weeks to term. Obstet. Gynec. 32, 28-39.

Aladjem S, Perrin E, Fanaroff A (1972) Placental score and neonatal outcome. Obstet. Gynec. 39, 591-602.

Alvarez H (1964) Morphology and physiopathology of the human placenta. I: Studies of morphology and development of chorionic villi by phase-contrast microscopy. Obstet. Gynec. 23, 813-825.

Al-Zuhair AGH, Ibrahim MEA, Mughal S, Mohammed ME (1983) Scanning electron microscopy of maternal blood cells and their surface relationship with the placenta. Acta Obstet. Gynecol. Scand. 62, 493-498.

Al-Zuhair AGH, Ibrahim MEA, Mughal S (1984) Calcium deposition on the maternal surface of the human placenta: a scanning electron microscopic study. Arch. Gynecol. 234, 167-172.

Arts NFTh (1961) Investigations on the vascular system of the placenta. Part I. General introduction and the fetal vascular system. Am. J. Obstet. Gynec. 82, 147-158.

Bergstrom S (1971) Surface ultrastructure of human amnion and chorion in early pregnancy. A scanning electron microscope study. Obstet. Gynec. 38, 513-524.

Bøe F (1953) Studies on the vascularisation of the human placenta. Acta Obstet. Gynec. Scand. 32, (Suppl 5), 1-92.

Boyd JD, Hamilton WJ, Boyd CAR (1968) The surface of the syncytium of the human chorionic villus. J. Anat. 102, 553-563.

Boyd JD, Hamilton WJ (1970) The Human Placenta. Heffer, Cambridge. pp. 365.

Burton GJ (1982) Placental uptake of maternal erythrocytes: a comparative study. Placenta. 3, 407-434.

Burton GJ (1986a) Intervillous connections in the mature human placenta: instances of syncytial fusion or section artifacts? J. Anat. 145, 13-23.

Burton GJ (1986b) Scanning electron microscopy of intervillous connections in the mature human placenta. J. Anat. 147, 245-254.

Burton GJ, Ingram SC, Palmer ME (1987) The influence of mode of fixation on morphometrical data derived from terminal villi in the human placenta at term: a comparison of immersion and perfusion fixation. Placenta, 8, 37-51.

Castellucci M, Zaccheo D, Pescetto G (1980) A three-dimensional study of the normal human placental villous core. I: The Hofbauer cells. Cell Tiss. Res. 210, 235-247.

Castellucci M, Kaufmann P (1982) A three-dimensional study of the normal human placental villous core. II: Stromal architecture. Placenta. 3, 269-286.

Castellucci M, Schweikhart G, Kaufmann P, Zaccheo D (1984) The stromal architecture of the immature intermediate villus of the human placenta. Functional and clinical implications. Gynecol. Obstet. Invest. 18, 95-99.

Clint JM, Wakely J, Ockleford CD (1979) Differentiated regions of human placental cell surface associated with attachment of chorionic villi, phagocytosis of maternal erythrocytes and syncytiotrophoblastic repair. Proc. R. Soc. Lond. B. 204, 345-353.

Demir R (1979) Scanning electron-microscopic observations on the surfaces of chorionic villi of young and mature placentae. Acta Anat. 105, 226-232.

Dempsey EW, Luse SA (1971) Regional specializations in the syncytial trophoblast of early human placentae. J. Anat. 108, 545-561.

Douglas GW, Thomas L, Carr M, Cullen NM, Morris R (1959) Trophoblast in the circulating blood during pregnancy. Am. J. Obstet. Gynec. 78, 960-969.

Enders AC, King BF (1970) The cytology of Hofbauer cells. Anat. Rec. 167, 231-252.

Feller AC, Schneider H, Schmidt D, Parwaresch MR (1985) Myofibroblast as a major cellular constituent of villous stroma in human placenta. Placenta. 6, 405-415.

Ferenczy A, Richart RM (1972) Scanning electron microscopic study of normal and molar trophoblast. Gynecol. Oncol. 1, 95-110.

Fox H (1967) The incidence and significance of vasculo-syncytial membranes in the human placenta. J. Obstet. Gynaec. Br Commonw. 74, 28-33.

Fox H (1978) Pathology of the Placenta. London, W B Saunders. pp. 491.

Fox H, Agrafojo-Blanco A (1974) Scanning electron microscopy of the human placenta in normal and abnormal pregnancies. Europ. J. Obstet. Gynec. Reprod. Biol. 4, 45-50.

Freese UE (1968) The uteroplacental vascular relationship in the human. Am. J. Obstet. Gynec. 101, 8-16.

Goyri O'Neill JE (1983) Vascularizacao da placenta humana. Dissertacao Universidade Nova de Lisboa, Portugal.

Habashi S, Burton GJ, Steven DH (1983) Morphological study of the foetal vasculature of the human term placenta; Scanning electron microscopy of corrosion casts. Placenta. 4, 41-56.

Hamilton WJ, Boyd JD (1966) Specialisations of the syncytium of the human chorion. Brit. Med. J. i, 1501-1506.

Hamilton WJ, Hamilton DV (1977) Development of the human placenta. In: Scientific Foundations of Obstetrics and Gynaecology, 2nd edition (eds.). EE Philipp, J Barnes, M Newton. pp. 292-358. London, Heinemann.

Hayat MA (1981) Fixation for Electron Microscopy. Academic Press, New York. pp. 501.

Herbst R, Multier AM, Inatsugi Y (1968) Die raumliche Darstellung der menschlichen chorionzotten mit dem JEOL-Raster- Elecktronen-mikroskop. Beitr Electronenmikroskop Direktabb Oberfl. 1, 297-304.

Highison GJ, Tibbitts (1986) Ultrasonic microdissection of immature intermediate human placental villi as studied by scanning electron microscopy. Scanning Electron Microsc. 1986; II: 679-685.

Illsley NP, Fox H, Van der Veen F, Chawner L, Penfold P (1985) Human placental ultrastructure after in vitro dual perfusion. Placenta. 6, 23-32.

Jones CJP, Fox H (1977) Syncytial knots and intervillous bridges in the human placenta: an ultrastructural study. J. Anat. 124, 275-286.

Kaufmann P. (1971). Die Darstellung trophoblastischer Plasmapolypen mit dem Rasterelektronenmikroskop. Arch. Gynaekol. 211, 523-526.

Kaufmann P. (1982). Development and differentiation of the human placental villous tree. Biblthca Anat. 22, 29-39.

Kaufmann P, Sen DK, Schweikhart G (1979) Classification of human placental villi. Cell Tiss. Res. 200, 409-423.

Kaufmann P, Bruns U, Leiser R, Luckhardt M, Winterhager E (1985) The fetal vascularisation of term human placental villi II: Intermediate and terminal villi. Anat. Embryol. 173, 203-214.

King BF, Menton DN (1975) Scanning electron microscopy of human placental villi from

early and late in gestation. Am. J. Obstet. Gynec. 122, 824-828.

Kustermann Von W (1981) Uber "Proliferationsknoten" und "Syncytialbrucken" der menschlichen plazenta. Anat. Anz. 150, 144-157.

Lee MML, Yeh M-N (1983) Fetal circulation of the placenta: a comparative study of human and baboon placenta by scanning electron microscopy of vascular casts. Placenta. 4, 515-526.

Lee MML, Yeh M-N (1986a) Fetal microcirculation of abnormal human placenta I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus. Am. J. Obstet Gynec. 154, 1133-1139.

Lee MML, Yeh M-N. (1986b). Fetal microcirculation of abnormal human placenta II: Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis. Am. J. Obstet. Gynec. 154, 1139-1146.

Leibl W, Kerjaschki D, Horandner H (1975a) Mikrovillusfreie Areale an chorionzotten menschlicher Placenten. Gegenbaurs. morph. Jahrb. 121, 26-28.

Leibl Von W, Kerjaschki D, Rockenschaub A, Horandner, H (1975b) Die Oberflache menschlicher Plazentarzotten Eine rasterelektronenmikioskopische Studie. Wien. Med. Wochenschr, 125, 144-153.

Leiser R (1985) Fetal vasculature of the human placenta scanning electron microscopy of microvascular casts. Contr. Gynec. Obstet. 13, 27-31.

Ludwig H (1971) Surface structure of the human term placenta and of the uterine wall post partum in the screen scan electron microscope. Am. J. Obstet. Gynec. 111, 328-344.

Ludwig H, Junkermann H, Klingele H (1971) Oberflachenstrukturen der menschlichen Placenta im Rasterelektronenmikroskop. Arch. Gynaekol. 210, 1-20.

Mayhew TM, Joy CF, Haas JD (1984) Structure-function correlation in the human placenta: the morphometric diffusing capacity for oxygen at full term. J. Anat. 139, 691-708.

Multier AM, Herbst R, Blaschke R (1968) Face maternelle du placenta et villosites choriales observees au "Scanning Microscope." Bull. Fed. Soc. Gynecol. Obstet. Lang. Fr. 20, 411-414.

Ockleford CD, Wakely J, Badley RA (1981) Morphogenesis of human placental chorionic villi: cytoskeletal, syncytioskeletal and extracellular matrix proteins. Proc. R. Soc. Lond. B. 212, 305-316.

Okudaira Y, Hayakawa K, Hamanaka N, Ueda G, Yoshinare S, Sato Y, Kurachi K (1972) Human placental villi: scanning electron microscopic observations. Acta Obstet. et Gynaec. Jap. 19, 109-117.

Peter K (1943) Placenta-Studien. I: Zotten und Zwischen zottenraume zweier Placenten aus den letzten monaten der Schwangerschaft. Z. mikrosk-anat. Forsch. 53, 142-174.

Ramsey EM, Donner MW (1980) Placental Vasculature and Circulation. Thieme, Stuttgart. pp. 101.

Sandstedt B (1979) The placenta and low birth weight. Curr. Top. Pathol. 66, 1-55.

Schuhmann RA (1982) Placentone structure of the human placenta. Biblthca Anat. 22, 46-57.

Sheppard BL, Bonnar J (1979) The effect of uteroplacental vascular occlusion on the fetal placenta. In: Fetal Medicine (Eds.), B Salvadori, A Merialdi. pp. 43-51. Borla, Rome.

Sisson TRC (1978) Blood Volume. In: Perinatal Physiology (Ed.), U Stave. pp. 181-198. New York, Plenum Medical.

Stegeman JHJ, Treffers PE (1980) Histological appearances of the human placenta observed by electron microscopy after hypertonic saline abortion. Acta Obstet. Gynecol. Scand. 59, 43-53.

Steven DH, Burton GJ, Samuel CA (1981) Histology and electron microscopy of sheep placental membranes. Placenta. (Suppl. 2), 11-34.

Stieve H (1940) Die Entwicklung und der Bau der menschlichen Plazenta. I: Zotten, Trophoblastinseln und Schiedewande in der ersten Halfte der Schwangerschaft. Z. mikrosk-anat. Forsch. 48, 287-358.

Thiriot-Hebert M, Panigel M (1977) Etude en microscopie electronique de la surface des villosites placentaires humaines au debut et a la fin de la gestation. Bull. Assoc. Anat. 61, 269-281.

Thiriot M, Panigel M (1978) La microvascularisation des villosites placentaires humaines. CR. Acad. Sc. Paris Serie. D. 709-711.

Varma VA, Kim KM (1985) Placental calcification: ultrastructural and X-ray microanalytic studies. Scanning Electron Microsc. 1985; IV: 1567-1572.

Weber EH (1832) Handbuch der Anatomie des Menschen (Ed.), Hildebrandt), 4. Eingeweidelehre und Entwicklungsgeschichte des Menschen. (cited by Boyd and Hamilton, 1970).

Discussion with Reviewers

M.M.L. Lee: In Fig. 9 what is your criteria for identifying fibrin in this picture?

B.F. King: In Fig. 9 a large area is described as being composed of fibrin. It is unclear what criteria were used in arriving at this conclusion. What are the large vessels illustrated in Fig. 9? Is it possible that the area illustrated in Fig. 9 is a stem villus containing large caliber vessels and abundant collagen?

Author: Fibrin deposition in this area of the intervillous space has been thoroughly investigated using light microscopy, and is well documented (eg., Fox, 1978). The fibrin clot emeshes the villi, which subsequently lose their syncytiotrophoblastic covering but retain the basement membrane and stromal elements. The fetal vessels remain patent supplying the distal part of the villous tree. In Fig. 9 it is likely that the fibrin clot has indeed been deposited around a stem villus, hence the large vessels, but it is also emeshing terminal villi at its periphery. One can see how some villi have been dislodged during the cracking procedure (particularly in the right hand corners of the

photograph), leaving scallop-shaped niches in the edge of the clot. At higher power a meshwork of fibres and entrapped maternal erythrocytes, characteristic of newly forming clot, could be seen around the margins of the affected area.

M.M.L. Lee: As to Figs. 19 and 20, I do not think that both pictures show the basement membrane. In Fig. 19, the syncytium is peeled away to reveal the basement membrane; however, the basement membrane is not usually revealed so neatly and smoothly. I am not sure what structure is revealed in Fig. 19; I think Fig. 20 has a truer picture of the basement membrane where there is a coarse, irregular appearance.

Author: The syncytium has indeed peeled away very cleanly in Fig. 19, but there is no doubt that it is a syncytial flap that has been raised for it bears microvilli on its outer surface. The extreme thinness of the flap suggests that the basement membrane exposed must be that of the syncytiotrophoblast. It appears smooth at this magnification but at a higher power, similar to that of Fig. 20, surface folds and furrows become apparent. The images presented here closely resemble those of Highison and Tibbitts (1986), illustrating the basement membrane during early pregnancy.

T. Okagaki: How can you conclude that the cells marked (c) in Fig. 20 are cytotrophoblastic cells?

Author: Without embedding and sectioning the block one cannot be absolutely certain. Nonetheless they are definitely cells lying on the trophoblastic side of the basement membrane, and only cytotrophoblast cells occur in this location. They are also very similar in size and morphology to cytotrophoblast cells reported in TEM studies (Boyd and Hamilton, 1970) and SEM studies (Highison and Tibbitts, 1986).