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# ON THE VARIED APPEARANCES OF THE HUMAN PLACENTAL VILLOUS SURFACE VISUALISED BY SCANNING ELECTRON MICROSCOPY

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## Abstract

Scanning electron microscopy enables extensive areas of the human placental villous tree to be viewed at high resolution in a relatively quick and easy manner. It is therefore an invaluable aid to the study of normal placental structure, and may have a potential role to play in pathological diagnosis. However, with increasing experience an awareness of the possible artefacts that may be introduced during fixation and tissue preparation is gradually developing. Some of these artefacts are common to other organs and applications of scanning electron microscopy, whereas others result from the unique structure and three-dimensional configuration of the placenta. Examples of the second category are poorly documented, and yet their occurrence may also significantly influence the appearance of the tissue as seen by light or transmission electron microscopy. This paper illustrates examples of this problem, and emphasises some of the factors that must be considered when interpreting both scanning and transmission electron micrographs of the villous surface.

Key Words: Scanning electron microscopy, human placenta, syncytiotrophoblast, placental villi, villous surface.

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## Introduction

Within the human placenta it is the syncytiotrophoblast layer covering the fetal villous tree which represents the interface with the maternal circulation. This remarkable tissue appears to form a complete syncytium over the entire villous surface (Gaunt and Ockleford, 1986), and thus all placental exchange must take place across its surface. Equally it is responsible for the synthesis of many key hormones, and so the well-being of the syncytiotrophoblast is clearly of crucial importance to the normal development and growth of the fetus.

Not surprisingly, the surface appearances of the placental villi have been described by many authors using scanning electron microscopy (eg., 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; Clint et al., 1979; Demir, 1979; Sheppard and Bonnar, 1979; Kaufmann, 1982; Burton, 1987; Ockleford et al., 1989). These studies have led to a greater understanding of the three-dimensional configuration of the villous tree, and have provided a useful compliment to the examination of traditional sectioned material at either the light or electron microscope level. In particular, the use of scanning electron microscopy has helped to develop a general realisation that the appearances of villous profiles can be significantly influenced by the plane of sectioning. Some of the artefactual appearances that may be generated in this way have been illustrated by Kustermann (1981), Burton (1986a,b) and Cantle et al. (1987).

However, many of the studies listed above were conducted one to two decades ago, and in the interim there have been considerable advances in instrumentation and in tissue processing techniques. As a result it is becoming increasingly apparent that some of the early findings might well be interpreted as artefacts today. Many of these are relatively non-specific and are common to other tissues and applications of scanning electron microscopy. For example, the problems of surface depositions through inadequate washing prior to fixation, precipitation of buffer salts, or tissue distortion resulting from osmotic forces or dehydration procedures.

Other artefacts are, however, more specific and are related to the unique structure and three-dimensional configuration of the placental villous tree.

The present paper illustrates examples of the latter group, and considers some of the factors that must be borne in mind when interpreting electron micrographs of the all-important villous surface.

## Materials and Methods

Placental material from early gestation (8 - 12 weeks) was obtained from therapeutic terminations, whereas full-term material (38 - 40 weeks) was taken from uncomplicated spontaneous deliveries which resulted in the birth of a live singleton baby. All tissue was fixed by immersion in 2% glutaraldehyde, 2% paraformaldehyde in 0.1M Pipes buffer made isotonic with sucrose (pH 7.2, vehicle osmolality 310 $mOsm.kg^{-1}$, total osmolality 1150 $mOsm.kg^{-1}$) for 4 hours at room temperature. Following postfixation in 1% osmium tetroxide for 1.5 hours the tissue was prepared for either scanning or transmission electron microscopy.

For scanning electron microscopy, blocks were dehydrated in ascending concentrations of acetone, critically-point dried and coated with a 30 nm layer of gold in a Polaron Cool Sputter Coater for viewing in a Jeol JSM 35 CF microscope.

For transmission electron microscopy, the specimens were dehydrated in ascending concentrations of ethanol, transferred briefly to acetone and then embedded in Taab resin. Thick sections (1 μm) were stained with methylene blue/Azure II for light microscopy, whereas thin sections were stained with uranyl acetate/lead citrate for electron microscopy. The latter were viewed in a Phillips EM 300.

Material form late gestation was also prepared using perfusion fixation. A branch of the umbilical artery on the chorionic plate was cannulated and similar concentration fixative was delivered at a pressure of 50 mm Hg (Burton et al., 1987). Tissue was subsequently processed for scanning and transmission electron microscopy and light microscopy as before. Using maternal erythrocyte diameter as an internal marker it has been estimated previously that this technique is associated with a shrinkage rate of approximately 2% (Burton and Palmer, 1988).

## Results

### The Influence of the Mode of Fixation

The mode of fixation exerts a considerable influence on the appearance of the villous surface. Villi processed by the traditional immersion-fixation technique display numerous surface furrows and wrinkles (Figure 1). These range from shallow linear depressions running in all directions over all parts of the villous surface, to deeper more extensive clefts which generally run transversely across the base of a villus at a branching point.

Similar features can also be observed in sectioned material at the light microscope level (Figure 2). They are present most frequently on villi which display partially collapsed capillaries, as evidenced by the irregular endothelial margins. It is noticeable that the course of the trophoblastic basement membrane is often acutely sinuous beneath these syncytial surface features, indicating that they may be caused by a folding of the villous surface around a non-distended capillary network.

By contrast villi that have been fixed by perfusion at physiological pressure display a more uniform villous surface (Figure 3). The terminal villi appear as gently rounded protruberances covered by a smooth layer of syncytium with no surface furrows. Some slight depressions may be visible in the vicinity of branching points, but they are far fewer and less clearly defined than in the immersion-fixed material.

Sections of perfusion-fixed tissue show that the distended capillary profiles form a large proportion of the villous core (Figure 4). Superficial to them the trophoblastic basement membrane displays only gentle undulations, and the syncytiotrophoblast is distributed smoothly over the villous surface.

Figure 1. Terminal villi from a mature placenta prepared by immersion-fixation. Note the rather shrunken appearance of the villi, and the numerous furrows and wrinkles running in all directions over the villous surface. The microvillous surface has been disturbed by adjacent villi coming into contact at the points arrowed.

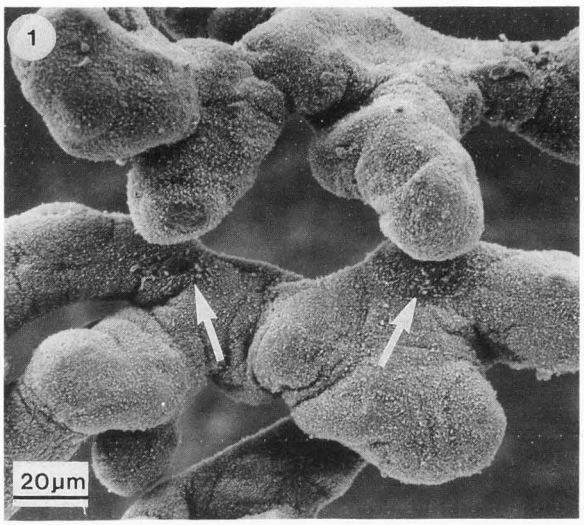
Figure 2. Sections of immersion-fixed villi display partially collapsed fetal capillaries as evidenced by their irregular luminal margins. In such material the trophoblastic basement membrane is markedly folded, as is the overlying syncytiotrophoblast. This creates furrows on the villous surface (arrowed).

Figure 3. By contrast the surface of villi fixed by perfusion at physiological pressure is remarkably smooth and has a velvety appearance. Surface furrows and wrinkles are virtually absent. The few maternal erythrocytes trapped on the villous surface display the typical biconcave shape and so indicate the satisfactory effective osmolality of the fixative.

Figure 4. Sections of perfusion-fixed villi display distended fetal capillaries with evenly rounded luminal margins. Again the villous surface is smooth, and gives the impression of being stretched taut over the capillary network.

Figure 5. During early pregnancy (8 weeks gestation) the villous surface may display a "hummocky" arrangement, with shallow grooves separating individual mounds.

Figure 6. Correlative transmission electron microscopy of the same specimen confirms that this appearance is caused by the microvilli arising in clumps from the syncytial surface. Note that the gold has only coated the most superficial tips of the microvilli. L, dense lipid vacuole.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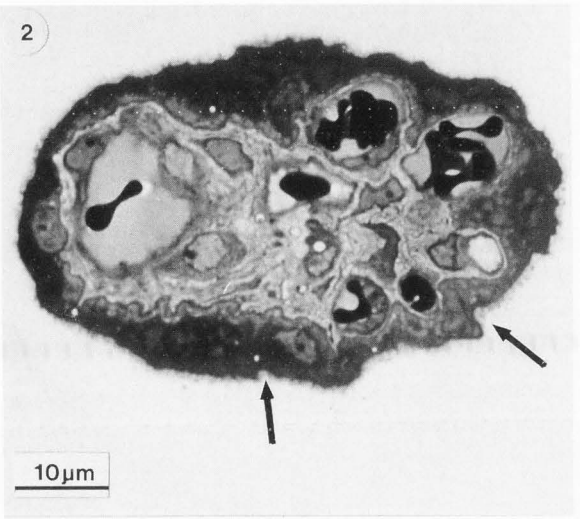
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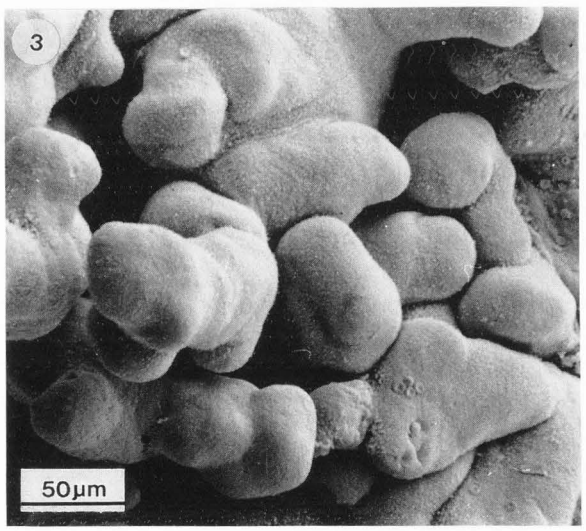
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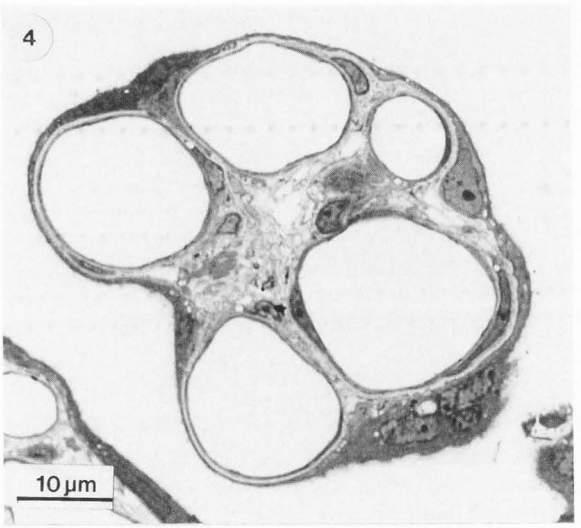
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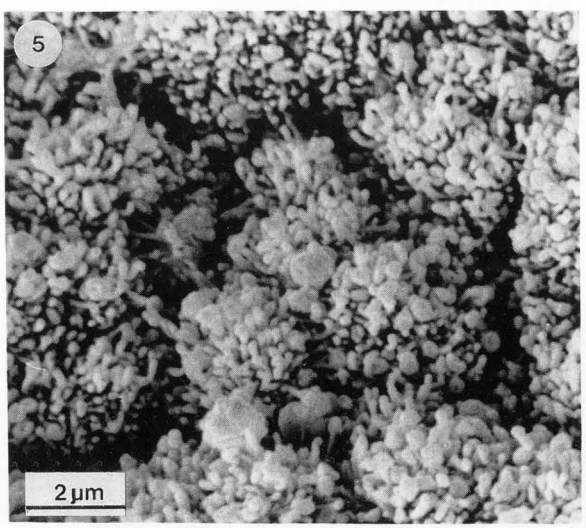
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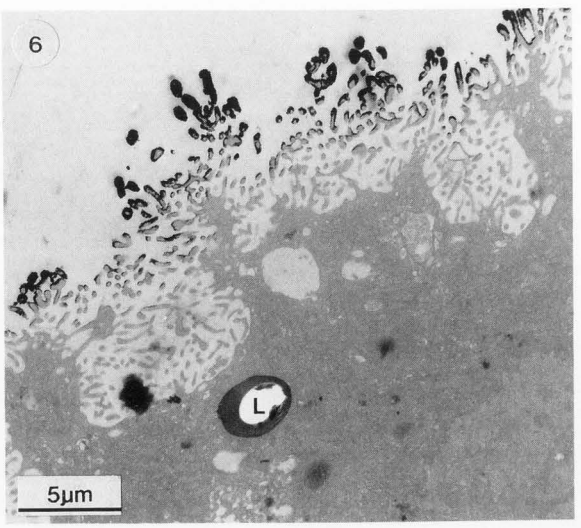
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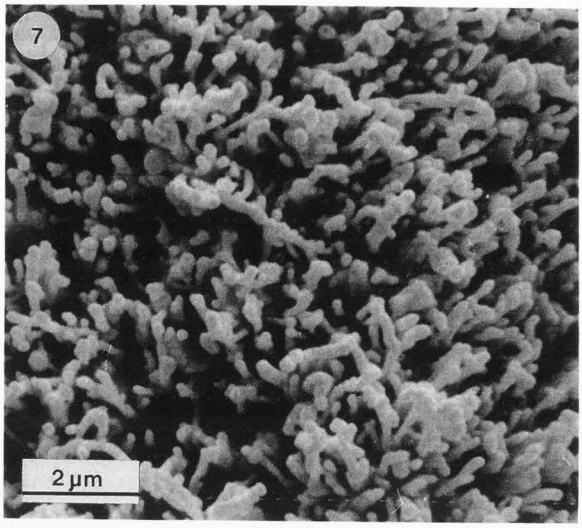
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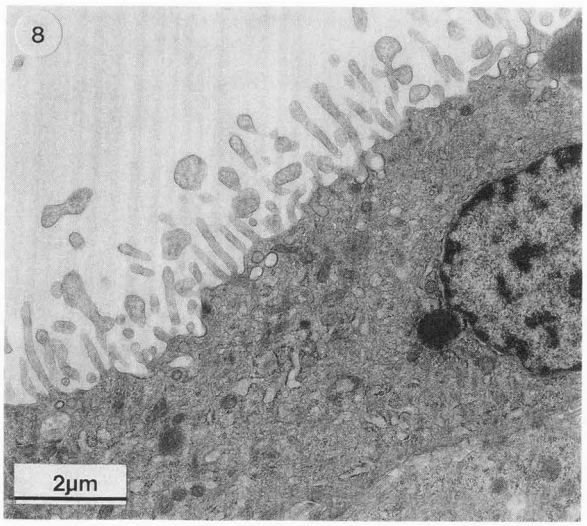
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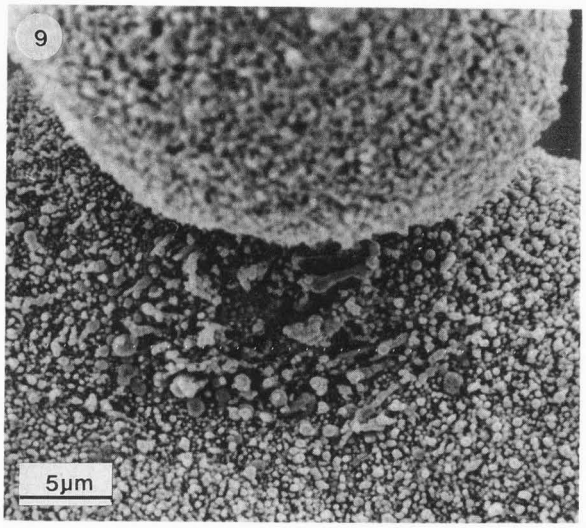
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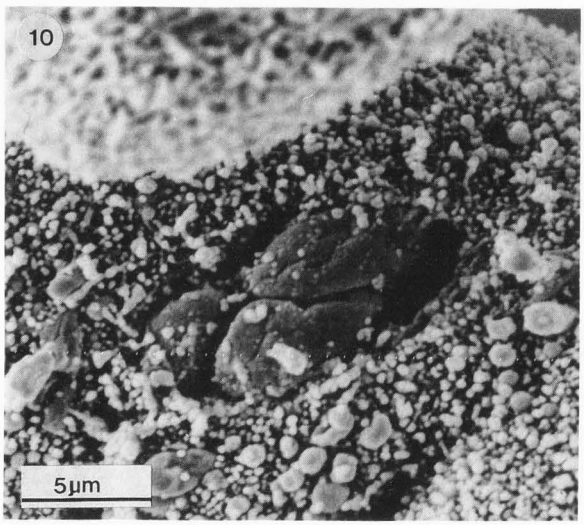
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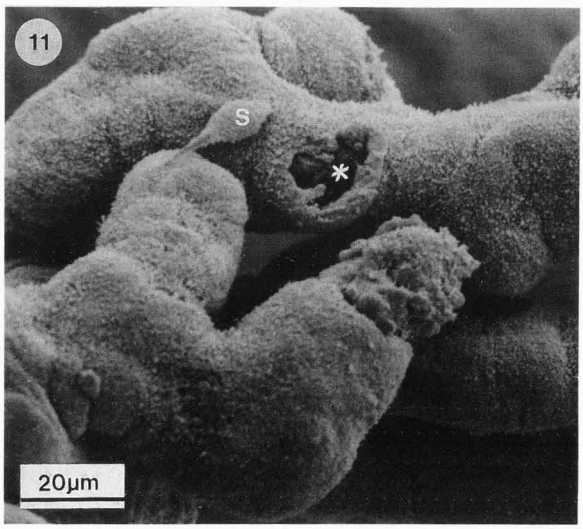
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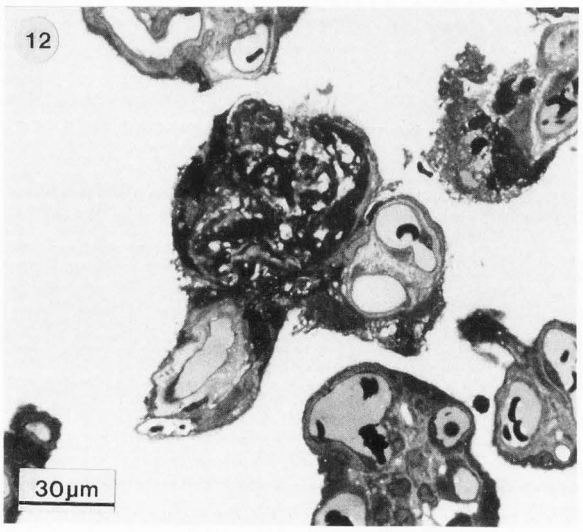
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Figure 7. Microvillous surface of term placenta showing the more uniform distribution of microvilli at this stage of gestation.

Figure 8. Transmission electron microscopy confirms that in late gestation the microvilli arise individually from the syncytial surface.

Figure 9. Higher power view of the area arrowed on the right of Figure 1 showing slight disturbance of the microvillous surface where the two villi may have come into contact. The microvilli in this area are elongated, distorted and rather swollen compared to their counterparts over the remainder of the villous surface.

Figure 10. Higher power view of the area arrowed on the left of Figure 1 showing more extensive damage to the villous surface. Here the microvilli have been lost and the villous core is exposed.

Figure 11. Villi from a term placenta showing a syncytial sprout (S) attached by only an extremely delicate stalk, and an area of syncytial damage caused by disruption of a point of villous contact and fusion (asterisked). If this occurs in vivo then HLA antigens on the stromal cells may be exposed to the maternal immune system. Also shown is a terminal villus, the tip of which has a very roughened, amorphous surface and may be undergoing fibrinoid necrosis. Note the clean line of demarcation between the normal microvillous surface and the roughened tip.

Figure 12. Fibrinoid necrosis as seen in sectioned material showing the accumulation of fibrinoid material in the villous core and the very roughened villous surface.

Variations in the microvillous arrangement related to gestational age

Another factor that influences the appearance of the villous surface is the stage of gestational development. In early gestation the surface may have a "hummocky" appearance, but there are no clear boundaries between adjacent mounds and certainly no breaks in the microvillous surface (Figure 5). Correlative transmission electron microscopy confirms that these configurations reflect the fact that the microvilli may arise in clumps from the syncytial surface, with relatively microvillous-free areas in between (Figure 6). Such areas are interspersed between others which display a more even appearance on scanning electron microscopy.

Towards the end of gestation the microvillous surface appears uniformly even, with no hummocky areas, and transmission electron microscopy reveals that the majority of microvilli now arise individually and are distributed more regularly over the syncytial surface (Figures 7 and 8).

It is interesting to note from Figure 6 that despite the use of a sputter coater only the tips of the microvilli are covered with gold. Thus when interpreting scanning electron micrographs of the villous surface it must be remembered that only about the apical third of an individual microvillus is likely to be visible. Descriptions of the length, shape and branching pattern of the microvilli must take this fact into consideration, or be corroborated by transmission electron microscopy.

Interactions between adjacent villi

Disruption of the regular arrangement of microvilli can be caused, however, by adjacent villi coming into contact. The intervillous space is generally of capillary dimensions only, and so not surprisingly cases can be observed where adjacent parts of the villous tree appear to have been touching. In Figure 1 for example, two terminal villi arising from one intermediate villus are closely approximated to another intermediate villus, and appear to have disturbed the arrangement of the microvilli on the latter. In the area immediately opposite the terminal villus on the left the microvilli appear to be fewer in number and those remaining are elongated, swollen and several possess bulbous tips (Figure 9). Opposite the second terminal villus the damage appears more severe, with complete loss of the microvillous layer and exposure of the underlying villus core (Figure 10). The margins of the disrupted area are quite distinct, but again the surrounding microvilli are swollen and distorted. Both the affected areas are roughly circular in outline and approximately 10 μm in diameter.

Once recognised numerous other similar areas of microvillous disruption can be identified, though more frequently in the mature than in the immature placenta. The severity of the lesions may vary between the extremes shown in Figures 9 and 10, but the dimensions of the affected areas are remarkably consistent.

More extensive damage to the syncytial surface

Although physical interactions between neighbouring villi may cause very localised disturbances of the microvillous layer, examples are seen where there is more extensive disruption of the villous surface (Figure 11). In this example the microvillus surface comes to an abrupt end and the tip of the terminal villus appears to be composed of an amorphous acellular tissue. Although the general contours of the villous tree are maintained the surface is very roughened and irregular.

It seems likely that such areas correspond to sites of fibrinoid necrosis, for under the light microscope examples of this lesion display many similar features (Figure 12). In particular there is a maintenance of the normal villous dimensions, and a sharp demarcation between the healthy and affected regions. However, correlative microscopy is necessary to confirm this point.

## Discussion

The human placental villous tree is highly branched and displays a complex configuration in three-dimensional space. Although the villous nature of the placenta has been known for at least 150 years (Corner, 1963; Boyd and Hamilton, 1970), the use of the scanning electron micro-

scope has contributed considerably to our understanding of its finer structure. By combining a large depth of field with high resolution, the instrument allows relatively extensive areas of the villous surface to be viewed at high magnification. This has yielded much new information on the surface appearances of the villi, and also on the way in which adjacent parts of the villous tree may physically interact. Such information is impossible to obtain through the study of conventional light or transmission electron microscopy of sectioned material alone.

However, as with all tissues, it is important to ensure that the material examined is as representative as possible of the in vivo state. This means that not only must the placenta be sampled in a suitable manner (Mayhew and Burton, 1988), but also that consideration should be given to the means of fixation employed. For example, many authors have commented in the past on the presence of the numerous surface wrinkles and furrows seen by scanning electron microscopy, but it is only recently that researchers have attempted to quantify the dramatic impact that different fixation methods have upon villous dimensions (Bouw et al., 1976; Voigt et al., 1978). In a comparison of tissue fixed by perfusion and immersion techniques, it was found that the percentage of a villus occupied by the fetal capillaries fell from 38.4% in the former to 25.9% in the latter (Burton et al., 1987). It is also clear from these studies that as the fetal capillaries collapse the villous surface area falls and the mean thickness of the villous membrane increases. Folding of the membrane commonly results, and so the majority of these furrows are no more than an artefact caused by the cessation of the fetal circulation through the villous tree. Unfortunately, despite the placenta being such a vascular organ all previous scanning electron microscopic studies have been based on immersion-fixed material.

Another cause of distortion of the villous surface is clearly the interaction between closely packed adjacent villi. The intervillous space is of capillary dimensions only, and so it is likely that juxtaposed villi may come into intermittent or permanent contact in vivo or during tissue processing. This is supported by the observation from serially-sectioned material that neighbouring villi may display localised areas of contact and syncytial fusion over circular areas approximately 10-15 μm in diameter (Burton, 1986a; Cantle et al., 1987). Presumably microvillous interdigitation and interaction takes place prior to syncytial fusion, but whether the changes illustrated in Figure 9 represent an early stage in this process or were caused by tissue handling during fixation remains uncertain. It is also known that such areas of fusion may be disrupted, resulting in localised damage to the villous surface (Burton, 1986b). This might be caused in vivo by uterine contractions, during the rigours of delivery of the placenta or during tissue processing for microscopy. The clearly defined margins of the lesions shown in Figures 10 and 11, and the absence of any attached maternal white cells suggests these particular examples were most likely caused during or subsequent to delivery. However, it is interesting to note that Clint et al. (1979) described small masses of cellular tissue closely associated with what appeared to be localised areas of syncytial damage, and interpreted these as proliferating cytotrophoblast cells involved in the process of syncytial repair.

Whether or not these lesions occur in vivo, it is clear that they are present on material prepared for microscopy, and investigators should be familiar with their existence and not ascribe them pathological significance. For example, localised disturbances of the microvilli and villous surface have been described in a number of maternal conditions, such as diabetes mellitus, pregnancy-induced hypertension and cigarette smoking (eg., Jones and Fox, 1980; Van der Veen and Fox, 1982; Laurini et al., 1987). These areas have generally been taken as evidence of "focal syncytial necrosis", but it is possible that if 10-20 μm in diameter they may have a more straightforward physical basis. It is perhaps relevant that Kaufmann et al. (1987) demonstrated particularly profuse branching of the villous tree in cases of pregnancy-induced hypertension, a condition associated with a significantly increased incidence of "focal syncytial necrosis". Physical contacts are likely to be more frequent between the densely-packed villi in these placentae, and so some disruption of the villous surface must be expected on this basis alone. Correlative transmission electron microscopy would be necessary, however, to determine whether or not areas of "focal syncytial necrosis" and points of physical contact between villi are indeed the same phenomenon.

## Acknowledgments

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## Discussion with Reviewers

G.H. Highison: Is the "hummocky" appearance a consistent feature in all your early material? If so, since early material is so hard to perfuse, could this hummocky appearance as in Figure 5 be an artefact induced by immersion fixation?

Author: The "hummocky" appearance was seen to a varying extent on all the early material examined, with areas of the villous surface displaying this feature being interspersed with others of a more regular nature. This surface appearance was also frequently observed on villous sprouts, and as these are non-vascularised it is difficult to see how it may be an artefact induced by immersion rather than perfusion fixation. Nontheless, it could be the result of a differential shrinkage of tissues that might accompany fixation of early gestation material by any method.

S.V. Nicosia: The author comments on extensive disruption of the villous surface (Figure 11). Which steps, if any, is the author taking to control for technical artefacts which may potentially induce such changes?

Author: One can only ensure that the tissue is handled as gently as possible at all stages of processing, but inevitably some mechanical damage results. Fortunately, areas where the syncytium is torn or villi are broken can easily be identified and excluded from consideration. When one sees a discrete lesion amidst an area of otherwise intact and normal villi, however, then it is likely that this represents a genuine finding rather than a processing artefact.