This is to certify that the

thesis entitled

INTERNAL SURFACE STRUCTURE OF HUMAN PLACENTAL VILLI USING THE SCANNING ELECTRON MICROSCOPE

presented by

JUSSARA MARIA CAETANO

has been accepted towards fulfillment
of the requirements for

M.S. degree in PATHOLOGY

Charles K. Whitehair
Major professor

Date 9-18-79

INTERNAL SURFACE STRUCTURE OF HUMAN PLACENTAL VILLI USING THE SCANNING ELECTRON MICROSCOPE

By

Jussara Maria Caetano

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1979

ABSTRACT

INTERNAL SURFACE STRUCTURE OF HUMAN PLACENTAL VILLI USING THE SCANNING ELECTRON MICROSCOPE

By

Jussara Maria Caetano

The internal surface structure of human placental villi was determined in nine specimens of third trimester placental tissue using scanning electron microscopy. The tissues studied were from three normal and six abnormal placentas and were obtained from the Michigan Placental Tissue Registry. The abnormal placentas included four with hemorrhagic endovasculitis and hemorrhagic villitis, one with maternal sickle cell disease, and one with massive perivillous fibrin deposition. Blocks were either cut on a cryostat or prepared by freeze-fracture technique, which was followed by dehydration and critical point drying to remove the water. The tissues were coated with gold to prevent charging from the electron beam and examined on an ISI S-III scanning electron microscope operated at 15 KV accelerating voltage, using a magnification of 200 to 5000 times.

The micrographs of the villi from the three third trimester placental tissues from normal placentas illustrated the normal terminal villous branches with their internal surface structure, the fetal vessels, mesenchymal stroma and a thin trophoblastic layer. With the freeze-fracture technique, the vessels and trophoblastic

layer were clearly evident in different planes. An abnormal fetal vascular tree was present in all four placental specimens previously diagnosed as hemorrhagic endovasculitis and hemorrhagic villitis. The vessel walls were hyperplastic with narrowing or obliteration of the lumen. Intravascular thrombi were frequently found in the fetal vessels. Fragmented red blood cells appeared to be within one vessel wall. In some terminal villi there were areas of homogeneous, amorphous material, which was considered to be fibrinoid degeneration. In the tissues of the specimen diagnosed as massive perivillous fibrin deposition, there were fibrin strands forming bridges within the intervillous spaces, compact villous stroma, and red blood cell clots. In the tissues from the case of maternal sickle cell disease, sickled red blood cells were present in the intervillous spaces and on the surface of the villus.

To my parents, Abimael and Aurora,

my husband, Adriano,

and my daughter, Adriana Paula

ACKNOWLEDGEMENTS

To Dr. Charles Whitehair, academic advisor and chairman of my committee, I wish to express my sincere appreciation for help in this research.

I wish to express my gratitude to Dr. Charles Sander, MD, for suggesting this research and supplying suitable specimens, and to Dr. John Dunkel, MD, for encouragement, counseling, and friendly advice.

To Shirley Howard for cooperation in preparing the specimens, Dr. Stanley Flegler for operating the scanning electron microscope, Dr. James Spaulding for guidance in taking and interpreting the micrographs, and all other members of the Department of Pathology for help, cooperation, and assistance, I wish to express my gratitude.

My thanks goes to CAPES (Coordination for the Upgrading of University Graduation Level Personnel) in Brazil for financial assistance.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Considerable research on the morphology of the human placenta surface using the scanning electron microscope (SEM) has been done since 1968. However, the internal architecture of human placental villi has been mentioned only briefly by Ludwig (1971, 1974). In his studies on the surface of the placental structures using the SEM, he referred to the internal aspect of the villus as containing a trophoblastic layer and connective tissue surrounding the fetal blood vessels. The use of specimens fixed in formalin as a routine fixative in surgical and autopsy pathology would be of great value for additional research on the human placenta. In transmission electron microscopy, formalin-fixed tissues have been reported to produce good ultrastructural results and to be economical (Ashworth, 1964; Carson et al., 1973). The present research utilized the scanning electron microscope to determine the feasibility of using placental tissue previously fixed in 10% formalin in research on the internal structure of human placenta tissue.

OBJECTIVES

The objectives of this research were:

1. To determine the value of formalin-fixed placental tissue for scanning electron microscopy studies.

2. To determine and describe the morphological characteristics of the internal surface structure of third trimester human placental villi from three normal and six abnormal human placentas.

REVIEW OF LITERATURE

Development and Anatomy of the Placenta

The placenta is an endocrine organ of short life span, although of vital importance in human reproduction. It is responsible for the transfer of O_2 and nutrients from mother to the fetus and CO_2 and wastes from the fetus to mother. It could react as an immunological barrier to infection. The development of the placenta after the ovum is fertilized in the fallopian tube was described in detail by Boyd and Hamilton (1970).

The fetal placenta is formed by subunits called lobules, each of which arises as a primary stem villus. The distal end of the primary villi divides into secondary and tertiary villi. The terminal villous network is formed by branches of tertiary villi within the intervillous spaces. The terminal villi are the functional units of the placenta and are responsible for the exchange of substances between mother and fetus.

Fetal Circulation

The placenta is supplied by 2 arteries which spiral around the umbilical vein. On the fetal surface of the placenta, the arteries divide in short branches to supply the primary stem villi and become the cotyledonary arteries. The division of the cotyledonary arteries follows the pattern of the villus, branching to the tertiary villi. The terminal villi are supplied by an extensive network of

capillaries (Crawford, 1961, 1962). Dixon and Robertson (1969) described the blood supply during fetal development.

Histology of the Placental Villi

In early pregnancy, the villus has an edematous, loose stroma in which numerous Hofbauer cells are present. These cells are macrophages and have a vacuolated cytoplasm. The villi are covered by 2 layers of trophoblasts; the inner layer consists of cytotrophoblast or Langhans' cells and the outer layer of syncytiotrophoblast. Fetal capillaries appear at the end of the second month as small, centrally located vessels. As pregnancy proceeds and the placenta grows, the villous stroma becomes condensed and the fetal capillaries dilate. The syncytial nuclei in mature villi often aggregate and form syncytial knots (Fox, 1973).

There is a variability in the villous pattern in the normal term placenta. Fox (1964) studied 30 placentas from normal women who had delivered normal, healthy babies. He observed a variation in the villous appearance in different areas of the placenta.

Thliveris and Speroff (1977) described the ultrastructure of the placental villi, chorion leave and decidua parietalis in normal and hypertensive pregnant women. They observed that in hypertensive women there was an increase in syncytial degeneration and fibrinoid deposition.

Investigations of human placenta using SEM have been reported since 1968. Ludwig, in Germany, has reported using SEM frequently as a diagnostic aid in placental pathology. In 1971 he described the surface structures of human term placentas after normal and toxemic pregnancies. He also described the uterine wall postpartum. He

observed the anatomical relations between maternal erythrocytes and the syncytiotrophoblastic surface, the brush-like microvilli of the villous surface, and the dense fibrin network in areas of infarction.

Bergstron (1971) investigated the fetal membranes in early human pregnancy and observed the differentiation of the amniotic epithelium. In 1974, Ludwig described the surface structures of the terminal villus and amnion in the mature human placenta, combining the ultrastructure findings with functional parameters of the fetal-utero-placental unit.

The use of SEM to determine the surface ultrastructure of human placenta in normal, prolonged pregnancies and in pregnancies complicated by pre-eclamptic toxemia was described by Fox and Agrafojo (1974). Sheppard and Bonnar (1974) examined the spiral arteries supplying the human placenta at term. They demonstrated that lining the internal surface of the spiral arteries there were cells of two distinctly different sizes which were identified as endothelium and cytotrophoblast cells. King and Menton (1975) observed the slender filiform appearance of the microvilli covering the syncytial surface from early and late gestation. They observed that in early gestation the microvilli appear to be larger in diameter than in mature placenta and transversally furrows are also a common feature in mature placentas.

Thiriot and Panigel (1977) used SEM and TEM to compare the changes in cell structure resulting from physiological activity of the trophoblast during the maturation of the placenta.

All of these studies using SEM indicate that the surface of syncytiotrophoblasts of placental villi is covered by brush-like microvilli which vary in size and shape during various stages of

maturation. Modifications, such as diminished, absent, or coarse slumpy appearance of the microvilli of the villous surface, may occur in abnormal pregnancies.

The Scanning Electron Microscope

History

The SEM is an instrument with broad application in physical and biomedical sciences, engineering and industry. Early development on the construction of the SEM was done in Germany between 1930 and 1938 by Von Ardenne using demagnifying lenses. At this same time, workers in Great Britain, France and the USA were also doing experimental studies on the SEM. World War II delayed the actual engineering of the instrument until 1948. In 1965, the Cambridge University Engineering Department devised an instrument which led to the first commercial SEM (Gray, 1973; Hayat, 1978).

Mechanical Principle

A dehydrated and metal coated specimen mounted on a metal stub is placed in a vacuum chamber. An electron gun at one end of the vacuum column produces a narrow beam of electrons which pass toward the anode. Two or more condenser lenses reduce the beam to less than 100 Å. Deflector coils and objective lenses focus on a small spot on the surface of the specimen, placed in the vacuum chamber at the other end of the column. The lenses act as a probe and the probe bombards primary electrons of high energy at the surface of the specimen. The specimen emits low energy secondary electrons which are gathered by a collector. In the collector the electrons impinge on an electrode made of aluminized scintillator material. The electrons produce a flash of light which is amplified by a photomultiplier.

The signal from this photomultiplier is proportional to the number of secondary electrons emitted and is displayed on a cathode ray tube (CRT).

The generator that operates the deflector coils is also connected to the deflector plate of the CRT. The voltage signal is employed and modulates the brightness of the spot on the CRT, where the picture forms in radar screen fashion as the beam is scanned across the specimen. The number of secondary electrons emitted depends on the topography of the sample and the atomic number of the surface.

Most SEM can operate with voltages varying from low to high, as in a transmission electron microscope (TEM) equipped with scanning attachment. For the study of biological preparation, it is best to use low voltages, because they are poor conductors and high voltage penetration results in greater charging.

A camera is attached to the instrument for making micrographs of the image (Hayat, 1972; Agar, 1974). The facility to rotate or tilt the specimen can give rapid serial pictures at variable magnification as they appear in the field. The best choice of photographic material is indicated by phosphor characteristics on the CRT. The most widely used is Polaroid 55 (P/N) film, because it gives a negative for reproduction as well as rapidly providing a positive print (Revel, 1975; Meek, 1977).

Specimen Preparation

An overview of specimen preparation for SEM was presented by Boyde (1972). The preparation of soft animal tissue presented problems because of the large water content, which must be removed.

This is done by using a dehydration fluid (ethanol or acetone) followed by drying for best resolution and contrast (Humphreys, 1975; Boyde, 1973).

To avoid surface tension forces developing during evaporative drying or crystal formation with the freeze drying technique, the best approach is to use the critical point drying (CPD) procedure, using Freon 13 or CO_2 (Nenamic, 1973).

After drying, it is necessary to coat the specimen with a thin layer of metallic film to increase the thermal and electrical conductivity. The coating material is usually composed of layers of silver, aluminum, palladium, gold or carbon, used singly or in combination. The thickness of the coating layer varies between 2 to 15 nm depending on the surface in the study, the operating conditions of the SEM, and the coating procedure adopted (De Nee, 1975; Hayat, 1978).

It is possible to observe the internal surface of specimens with the SEM. Humphreys (1974) used the method of CPD cryofracture, ehtanol infiltrated tissues which substituted the water of the tissue by ethanol before cryofracture and observed the advantage that ice-crystal damage occurring in freeze-drying was avoided. Flood (1975) used dry-fracture techniques to observe soft internal biological tissues and noted the possibility of more pronounced plastic deformation of the tissue elements than that which occurred with freeze-fractured tissues.

MATERIALS AND METHODS

Placentas

Nine specimens of placental tissue fixed in 10% formalin were obtained from the Michigan Placental Tissue Registry. These specimens had been stored in formalin from 3 to 25 months prior to preparation for scanning electron microscopy. These specimens were sectioned using the cryostat and the freeze-fracture techniques for SEM study of the internal surface structure of the human placental villi.

The following placentas were prepared. Three placentas were from liveborn babies whose placentas had the histopathologic diagnosis of normal third trimester placenta. Four placentas were from stillborn babies with the histopathologic diagnosis of third trimester placenta with hemorrhagic endovasculitis and hemorrhagic villitis (Sander, 1979). Two third trimester placentas were from liveborn babies, one with the histopathologic diagnosis of massive perivillous fibrin deposition and the other with a diagnosis of maternal sickle cell disease (all above are Placental Tissue Registry reports). Four sections measuring 1 cm x 1 cm x 5 mm were prepared from each specimen; 2 prepared by cryostat and 2 by freeze-fracture.

Cryostat Technique

Blocks were cut from the central area between fetal and maternal surface of formalin-fixed placental tissue in blocks of .5 cm x .5 cm x 2 mm and washed in 0.1 M cacodylate buffer containing 5% sucrose

and the pH adjusted to 7.2 with hydrochloric acid. The initial wash was discarded and more buffer added. The tissue was agitated gently for 30 minutes in a shaker instrument. The tissue blocks were fixed onto corks with "OCT" (Ames Company, Indiana), frozen, and trimmed on the cryostat to obtain a smooth surface. The tissue blocks fixed onto corks were thawed in buffer. With the trimmed side up, all OCT was removed from the specimen with buffer. The specimen was then stored in buffer at 4°C.

The tissues were mounted onto aluminum discs with 10% gelatin and dehydrated by placing them through a series of 50, 75, 95 and 100% ethanol for 10 minutes in each, followed by a final 100% ethanol for 12 hours.

Freeze-Fracture Technique

The freeze-fracture procedure used a modification of the procedure of Humphreys et al. (1974). The modification was that formalin rather than glutaraldehyde was used as the fixative.

Formalin-fixed tissue was cut into strips measuring 1 mm x 4 mm and washed for 30 minutes in 0.1 M cacodylate buffer containing 5% sucrose and the pH adjusted to 7.2 with hydrochloric acid. Strips were then dehydrated in an ethanol series of 70, 95, and 100% for 15 minutes in each, followed by a second rinse in 100%. In the final rinse of ethanol, the strips of tissue were inserted into small cylinders of parafilm, which were made by rolling 2 cm strips of parafilm around an applicator stick of about 2 mm in diameter. The cylinders were filled with ethanol by submerging them in the final rinse of ethanol. The ends of the cylinders were then crimped shut. The cylinders containing the tissues were seized with forceps and put under liquid nitrogen until frozen. The cylinders were then

placed on a flat metal block (precooled in liquid nitrogen) and, with a single-edge razor (precooled), held in a hemostat. The fractures were made through the cylinders and tissues.

Critical Point Drying

Tissue blocks prepared by both techniques were dried in a CPD apparatus following the procedure of Anderson (1951), with the exception that the alcohol was not replaced by amylacetate and each block was wrapped in lens paper to prevent foreign material in the CO_2 tank from being deposited upon the tissue. The fractured blocks were then placed in basket containers for CPD of small specimens. Carbon dioxide at not less than 850 psi was released for 15 minutes into the chilled bomb containing the tissues; this procedure was repeated four times. Then the temperature of the bomb and specimens was raised to volatilize the CO_2 at its critical point and the pressure in the bomb was slowly released.

The dried blocks were mounted on aluminum discs which were glued (Television Tube Koat, GE Electronics Division of Hydrometals, Inc., Illinois) to aluminum stubs. The fractured pieces were glued directly to aluminum stubs with the fractured surface up. The blocks were then stored under vacuum in a desiccator containing anhydrous calcium sulfate until SEM examination.

Before SEM examination the blocks were coated with 200 to 300 Å of gold in a Film-Vac Sputter Coater, to prevent charging from the electron beam. Specimens were examined in an ISI S-III scanning electron microscope, operated at 15 KV accelerating voltage with magnification varying from X200 to X5000. Black and white micrographs were made of the representative sections with a Polaroid camera attached to the scanning electron microscope using 55 P/N film.

RESULTS AND DISCUSSION

The internal surface structure of human placental villi was determined in 9 specimens of third trimester placental tissue using the scannine electron microscope.

Normal Third Trimester Placental Villi

The normal term human placenta as observed by SEM showed an arborization of the villous stems. Major villous branches divided into minor villi. The last division was often a simple bifurcation (Figure 1). Terminal chorionic villi measured from 25 to 55 μ. This compares well with the characteristic branching demonstrated in glutaraldehyde fixed placentas (Ludwig), where the size of terminal villi was reported to vary from 25 to 40 μ. The space between single terminal villi measured approximately 2 to 2.5 μ (Figure 1). The minimum space between single terminal villi reported by Ludwig was between 1 to 3 μ. Circumferentially oriented furrows were present along the villous tree (Figure 2), as demonstrated by King and Menton (1975), Ludwig (1976) and Fox (1978).

The external surface of the syncytiotrophoblast covering the villous tree appeared velvety. The preservation of the microvilli of the syncytiotrophoblast by formalin fixation was comparable to that demonstrated by Fox and Agrafojo (1974) using glutaraldehyde. The surface of formalin fixed placentas contained more amorphous material. This probably represents coagulated proteins which were fixed in

Figure 1. Scanning electron micrograph of villous branches in normal third trimester placenta. Terminal branches measure 20-55 μ. Intervillous space between terminal villi (white arrows) measure 2 to 2.5 μ. The cut surface of the terminal villi is apparent. Cryostat section, X400.

Figure 2. A terminal villous branch with furrows (arrow) at the surface and fracture of the bifurcated terminal villus. Freeze-fracture section, X400.

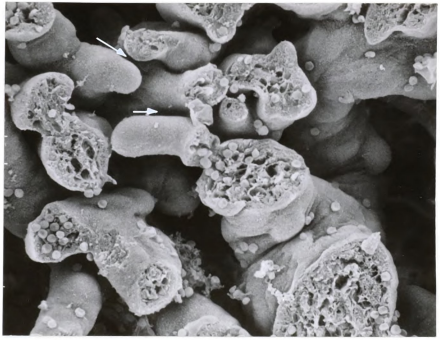

Figure 1

Figure 2

place, since the placentas sampled were placed in formalin without any prior washing or special treatment.

On the surface of the villi and intervillous space, only a few red cells and no platelet aggregates were observed (Figures 1 and 2). Strands of material presumed to be fibrin were occasionally seen in these locations in normal placentas.

Both the freeze-fractured and cryostat sectioned villi revealed an internal organization which consisted of a loose fibrillar stroma in which branches of the vascular tree were supported (Figures 3 and 4). Various types of vessels with a cross sectional diameter of 15 to 25 μ (Figure 4) contained elongated ridges of endothelial cells arranged in parallel array along the long axis of the vessel (Figure 5). Sheppard and Bonnar (1974) described elongated arrangement of endothelium spiral arteries. It can therefore be assumed that these vessels represent arterioles. A second type of vessel was identified in which the endothelium appeared as a smooth sheet-like lining without distinct cellular outlines (Figures 3, 4 and 6). In general these vessels appear to be slightly smaller than the arterioles present in the same villous branch (Figures 3 and 4). One such vessel cut tangentially (Figure 6) demonstrated quite clearly the smooth characteristic of the endothelial lining. These vessels with smooth, non-corrugated endothelium were interpreted to represent venules, as their size and relationship to arterioles were consistent with this assumption. Some vessels could not be clearly placed into one of these two categories, as the endothelium showed both smooth and corrugated areas (Figures 6 and 7). It was noted that in normal placentas processed by both freeze-fracture and cryostat sectioning, the vascular structures were essentially free of circulating blood cells.

Figure 3. Cross section of a villus showing the fetal vessels, thin trophoblastic layer (T) and fibrous network. Notice the velvety surface of the villus and an erythrocyte on top of the internal villous surface. Freeze-fracture section, X2000.

Figure 4. The villous internal surface formed by layers of connective tissue intermixed with fetal vessels. Notice the endothelial cells of the fetal vessels (arrow). Freeze-fracture section, X1000.

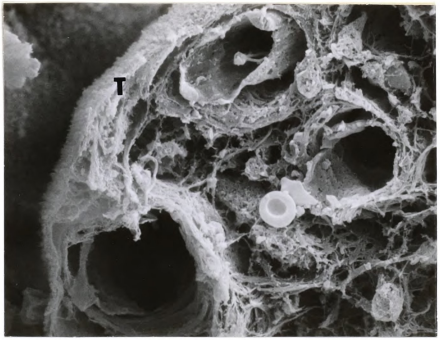

Figure 3

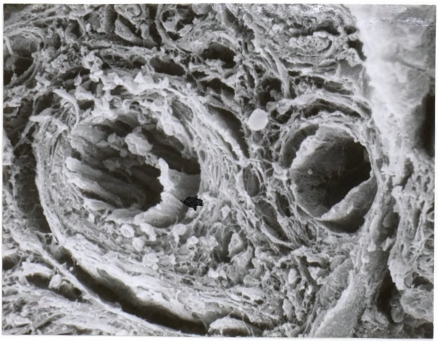

Figure 4

Figure 5. Detail of the internal surface of the endothelial layer of a fetal vessel. Freeze-fracture section, X2000.

Figure 6. Cut surface of villus showing nuclei of various cell types such as syncytiotrophoblast (ST) and mesenchyme (M). Notice a spherocyte (s) and an erythrocyte (e) with an aggregate of platelets. Cryostat section, X2000.

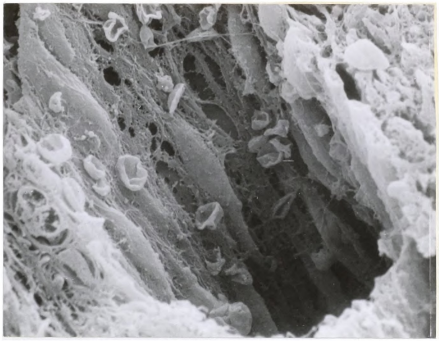

Figure 5

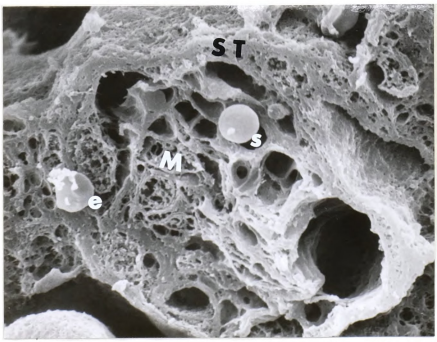

Figure 6

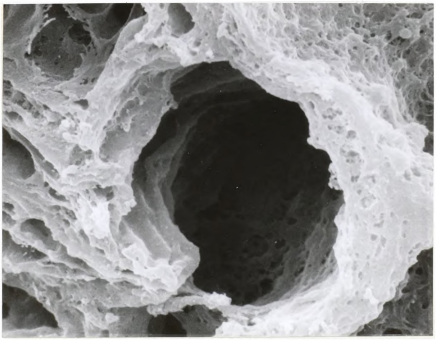

Figure 7. Higher magnification of Figure 6, showing the fetal vessel and its lumen. Cryostat section, X5000.

Figure 8. Villous branch showing an intense fibrosis of the internal surface structure of the villus. Notice the large number of erythrocytes (e) and fibrin (f) deposition along the intervillous space. Cryostat section, X1000.

Figure 9. Scanning electron micrograph of a thrombus in a large vessel. Cryostat section, X200.

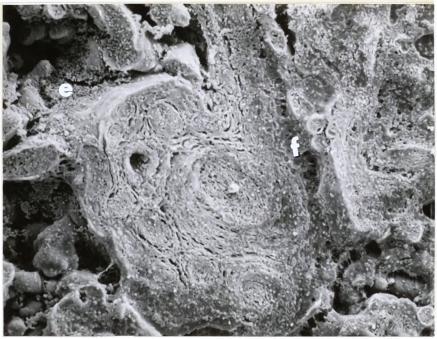

Figure 8

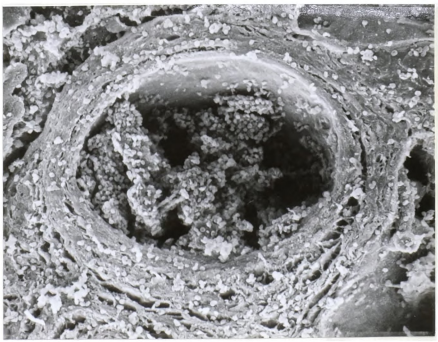

Figure 9

Figure 10. A cut surface of a villous branch showing a thrombus in a large vessel. Cryostat section, X200.

Figure 11. A fractured surface of a villus showing dense fibrous network surrounding a vessel. Freeze-fracture section, X400.

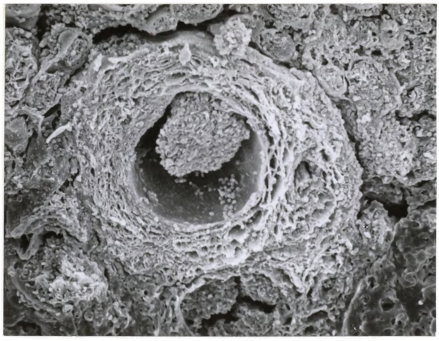

Figure 10

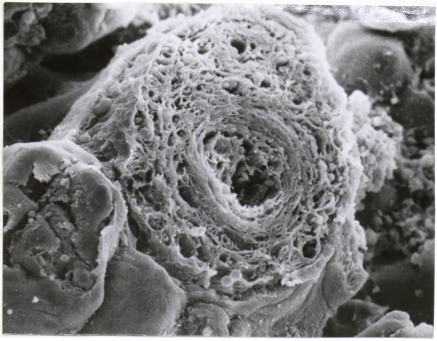

Figure 11

Figure 12. A fractured surface of a villus showing partial obliteration of the vessel. Freeze-fracture section, X1000.

Figure 13. Red blood cells within the vessel wall (arrow). Cryostat section, X1000.

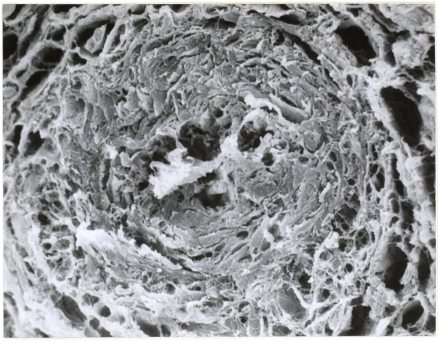

Figure 12

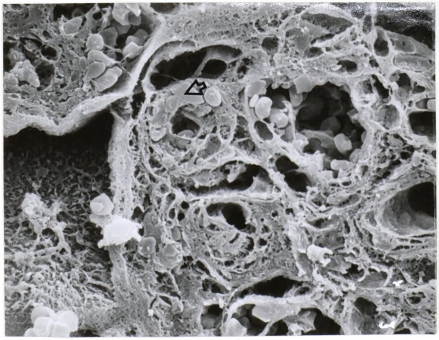

Figure 13

Figure 14. Red blood cells filling most of the fetal vessels of this villus. Cryostat section, X1000.

Figure 15. Fibrinoid degeneration resulting in obliteration of villous connective tissue. Observe the same change in the intervillous space (arrow). Cryostat section, X1000.

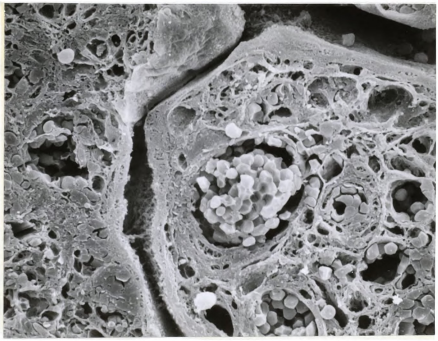

Figure 14

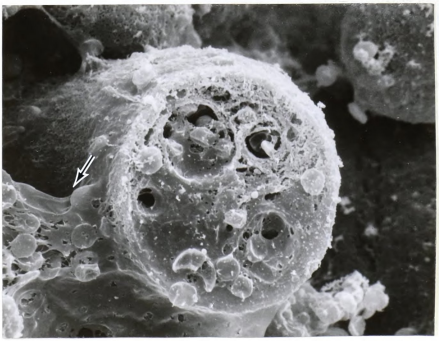

Figure 15

was considered to be fibrinoid deposition. There was the formation of bridges between villi by deposition of fibrillar material, presumed to be fibrin. Entrapped red cells were identified within this fibrillar network.

These findings correlate with some features of hemorrhagic endovasculitis and hemorrhagic villitis described by light microscopy.

Massive Perivillous Deposition

This lesion was present in one abnormal placenta. The lesion was characterized by an accumulation of fibrillar strands which filled and obliterated the intervillous space (Figures 16 and 17). By light microscopy this fibrillar material was demonstrated to be fibrin (Fox, 1967, 1978). The amount of fibrin deposition was in considerable excess of that demonstrated in the intervillous space in placentas from full-term pregnancies. The stroma appeared more compact than normal and showed an unusual condensation in the trophoblastic layer (Figure 16). The tissues in this area had a homogeneous, almost waxy appearance. This may represent a degenerative change. Erythrocytes were identified entrapped within the fibrin network as well as adherent to the cut or fractured surface of the villus.

The results observed in this research using the SEM correlate with those findings reported using light microscopy, such as villi embedded in fibrin and widely separated by fibrin deposition obliterating the intervillous space.

Sickle Cell Disease

A placenta from a case of maternal sickle cell disease had sickle-shaped red blood cells in the intervillous space and over the

Figure 16. Massive perivillous fibrin deposition. The fibrin strands fill the intervillous space (arrow). Cryostat section, X700.

Figure 17. Detailed fibrin strand in the intervillous space and covering the structures of the internal surface of the villus. Cryostat section, X1000.

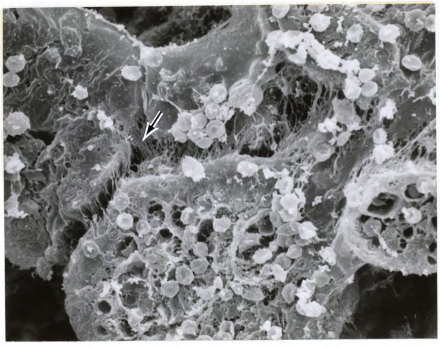

Figure 16

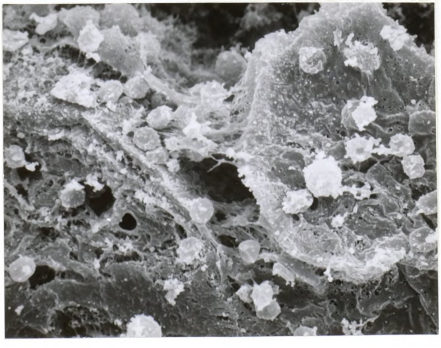

Figure 17

cut villous surface (Figures 18 and 19). Some nonstructural elements were observed.

Maternal sickle cell disease is a serious problem in pregnant women, as complications such as urinary tract infection, toxemia and heart failure occur (Anderson et al., 1960; Benirschke and Driscoll, 1967).

In conclusion, SEM has promise as a useful technique to evaluate the internal structure of the formalin fixed placenta by cryostat frozen section or freeze-fracture techniques.

Figure 18. Sickle-shaped red blood cells within the intervillous space and on the surface of a villus. Cryostat section, X1000.

Figure 19. A cut surface of a villous branch with sickle red cells. Cryostat section, X2000.

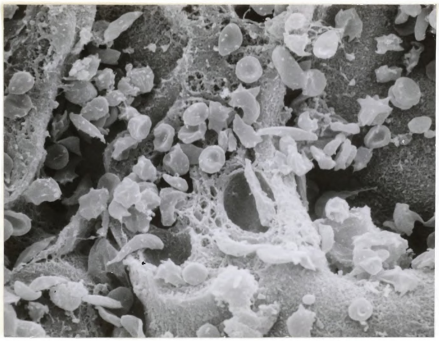

Figure 18

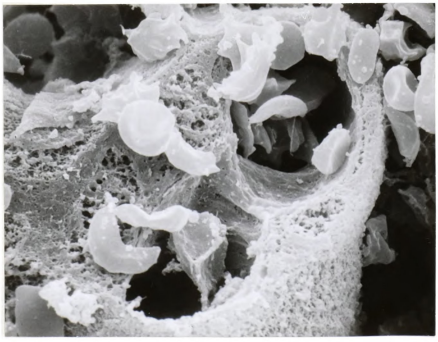

Figure 19

SUMMARY

The internal surface structure of human placental villi was determined in nine specimens of third trimester placental tissue using scanning electron microscopy. The tissues studied were from three normal and six abnormal placentas and were obtained from the Michigan Placental Tissue Registry. The abnormal placentas included four with hemorrhagic endovasculitis and hemorrhagic villitis, one with maternal sickle cell disease, and one with massive perivillous fibrin deposition. Blocks were either cut on a cryostat or prepared by freeze-fracture technique, which was followed by dehydration and critical point drying to remove the water. The tissues were coated with gold to prevent charging from the electron beam and examined on an ISI S-III scanning electron microscope operated at 15 KV acclerating voltage, using a magnification of 200 to 5000 times.

The micrographs of the villi from the three third trimester placental tissues from normal placentas illustrated the normal terminal villous branches with their internal surface structure, the fetal vessels, mesenchymal stroma and a thin trophoblastic layer. With the freeze-fracture technique, the vessels and trophoblastic layer were clearly evident in different planes. An abnormal fetal vascular tree was present in all four placental specimens previously diagnosed as hemorrhagic endovasculitis and hemorrhagic villitis. The vessel walls were hyperplastic with narrowing or obliteration

of the lumen. Intravascular thrombi were frequently found in the fetal vessels. Fragmented red blood cells appeared to be within one vessel wall. In some terminal villi there were areas of homogeneous, amorphous material, which was considered to be fibrinoid degeneration. In the tissues of the specimen diagnosed as massive perivillous fibrin deposition, there were fibrin strands forming bridges within the intervillous spaces, compact villous stroma, and red blood cell clots. In the tissues from the case of maternal sickle cell disease, sickled red blood cells were present in the intervillous spaces and on the surface of the villus.

BIBLIOGRAPHY

BIBLIOGRAPHY

Agar, A. W., Alderson, R. H., and Chescoe, D.: *Principles and Practice of Electron Microscope Operation*, Vol. 2 of Glauert, A. (ed.), *Practial Methods in Electron Microscopy*. North-Holland-Elsevier, Amsterdam and New York, (1974).

Anderson, M., Went, L. N., MacIver, J. E., and Dixon, H. G.: Sickle cell disease in pregnancy. Lancet, ii, (1960): 516-521.

Anderson, T.: Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N.Y. Acad. Sci., Ser. II(13), (1951): 130-134.

Ashworth, C. T., and Stembridge, V. A.: Utility of formalin fixed surgical and autopsy specimens for electron microscopy. Am. J. Clin. Pathol., 42, (1964): 466-480.

Benirschke, K., and Driscoll, S. G.: *The Pathology of the Human Placenta*. Springer-Verlag, New York, (1967).

Bergstron, S.: Surface ultrastructure of human ammion and chorion in early pregnancy. A scanning electron microscopic study. Obstet. Gynec., 38(4), (Oct., 1971): 513-524.

Boyde, A.: Biological specimen preparation for the SEM, an overview. Proc. Ann. SEM Symposium IIT Res. Inst. Scanning Electron Microscopy/1972, IIT Res. Inst., (1972): 257-264.

Boyde, A.: Freeze and freeze drying - A preparative technique for SEM. IITRI SEM/1973: 759-766.

Boyde, J. D., and Hamilton, W. J.: *The Human Placenta*. Cambridge University Press, (1970).

Carson, F. L., Martin, J. H., and Lynn, J.: Formalin fixation for electron microscopy. Am. J. Clin. Pathol., 59, (1973): 365-373.

Crawford, J. M.: A study of the human placental capillary. J. Obstet. Gynecol. of Brit. Commonwealth, 68, (1961): 378-381.

Crawford, J. M.: Vascular anatomy of the human placenta. Am. J. Obstet. Gynecol., 84, (1962): 1543-1567.

De Nee, P. B., and Walker, E. R.: Specimen coating technique for the SEM - A comparative study. Proc. 8th Ann. SEM Symposium IIT Res. Inst. Scanning Electron Microscopy/1975(Part I), IIT Res. Inst., Chicago, IL, (1975): 225-232.

Dixon, H. G., and Robertson, W. B.: *The Growth of the Conceptus and Its Blood Supply in Foetus and Placenta*, ed. Arnold Klopper and Egon Diezfalusy. Blackwell Scientific Publications, Oxford and Edinburgh, (1969).

Flood, P. R.: Dry-fracturing techniques for the study of soft internal biological tissues in the SEM. Proc. 8th Ann. SEM Symposium, IIT Res. Inst. Scanning Electron Microscopy/1975 (Part I), IIT Res. Inst., Chicago, IL, (1975): 287-294.

Fox, H.: The pattern of villous variability in the normal placenta. J. Obstet. Gynec. Brit. Commonwealth, 71, (1964): 749-758.

Fox, H.: Perivillous fibrin deposition in the human placenta. Am. J. Obstet. Gynecol., 98, (1967): 245-251.

Fox, H., and Langley, F. A.: *Development, Anatomy and Physiology of the Placenta in Postgraduate Obstetrical and Gynecological Pathology*. Pergamon Press, (1973).

Fox, H., and Agrafojo-Blanco, A.: Scanning electron microscopy of the human placenta in normal and abnormal pregnancies. Eur. J. Obstet. Gynecol. Reprod. Biol., 4(2), (1974): 45-50.

Fox, H.: *Pathology of the Placenta*, Vol. VII in the series, *Major Problems in Pathology*. W. B. Saunders Co., Ltd., (1978).

Gray, P.: *Encyclopedia of Microscopy and Microtechnique*. Van Nostrand-Reinhold Co., (1973).

Hayat, M. A.: *Principles and Techniques of Electron Microscope - Biological Application*, Vols. 1 and 2. Van Nostrand-Reinhold Co., (1972).

Hayat, M. A.: *Introduction to Biological SEM*. University Park Press, (1978).

Humphreys, W. J., Spurlock, B. O., and Johnson, J. S.: Critical point drying of ethanol-infiltrated, cryofractured biological specimens for SEM. Proc. 7th Ann. SEM Symposium, IIT Res. Inst. Scanning Electron Microscopy/1974(Part I), IIT Res. Inst., Chicago, IL, (1974): 275-282.

Humphreys, W. J.: Drying soft tissues for SEM. IITRI SEM/1975: 707-714.

King, B., and Menton, D.: Scanning electron microscopy of human placental villi from early and late gestation. Am. J. Obstet. Gynecol., 22(7), (1975): 824-828.

Lewis, E. R., and Nenamic, M. K.: Critical point drying. Techniques. IITRI SEM/1973: 767-774.

Ludwig, H., Junkermann, H., and Klingele, H.: Oberflachenstruturen der merischlichen -lacenta in rasterelektronenmikroskop. Arch. Gynak., 210, (1971): 1-20.

Ludwig, H.: Surface structure of the human term placenta and of the uterine wall post partum in the screen scan electron microscope. Am. J. Obstet. Gynecol., (Oct. 1, 1971): 328-344.

Ludwig, H.: Surface structure of human placenta, in *Placenta*, ed. K. S. Moghissi and E. S. E. Hafez. Arthur H. Thomas, Springfield, (1974).

Ludwig, H., Metzger, H., and Wolf, H.: The placenta (man), in *Scanning Electron Microscopic Atlas of Mammalian Reproduction*, ed. E. S. E. Hafez. Igaku Shoin, Tokyo, (1975).

Ludwig, H., and Metzger, H.: *The Human Female Reproductive Tract - A Scanning Electron Microscopic Atlas*. Springer-Verlag, (1976).

Meek, G. A.: *Practical Electron Microscopy for Biologists*, 2nd Ed. John Wiley and Sons, (1977).

Nenamic, M. K.: Critical point drying, cryofracture and serial sectioning. Proc. of the Workshop on Biological Specimen Preparation Techniques for Scanning Electron Microscopy. Scanning Electron Microscopy/1972(Part II), IIT Res. Inst., Chicago, IL, (1972): 297-304.

Revel, J. P.: Elements of SEM for biologists. IITRI SEM/1975(Part I): 687-695.

Sander, C.: Hemorrhagic endovasculitis and hemorrhagic villitis of the placenta. Arch. Path. Lab. Med. (in press).

Sheppar, B. L., and Bonnar, J.: Scanning electron microscopy of the human placenta and decidual spiral arteries in normal pregnancy. J. Obstet. Gynecol. Brit. Commonwealth, 8, (1974): 20-29.

Thiriot, H. M., and Panigel, M.: Etude en microscopie electronique de la surface des villosites placentaire humaine en debut et la fin de la gestation. Bull. Assoc. Anat. (Nancy), 61, (June, 1977): 260-281.

Thliveris, J. A., and Speroff, L.: Ultrastructure of the placental vill, chorion leave and decidua parietalis in normal and hypertensive women. Am. J. Obstet. Gynecol., 129(5), (1977): 492-498.

VITA

VITA

The author was born in Salvador, Bahia, Brazil, on September 3, 1949. She received her primary and secondary education in Salvador, Bahia. In 1967, she graduated from high school. She continued her education at medical school of the Federal University of Bahia and received the Doctor of Human Medicine Degree in January of 1975.

She participated in the internship program at the Obstetrics and Gynecologic Clinic at the Medical School of the Federal University of Minas Gerais, Belo Horizonte, Brazil.

Following graduation from medical school, the author practiced human medicine in the gynecologic clinic of the Nossa Senhora da Natividade Hospital in Santo Amaro, Bahia, and in a private clinic until July of 1976.

In May 1976 she was approved by a Federal examination to work for the INPS (National Institute of Social Providence). It is planned to return to this position following research training at Michigan State University.

In December of 1972, she married Adriano Caetano, DVM. They have one daughter, Adriana Paula.