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Early studies of placental ultrastructure by electron microscopy

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ABSTRACT

Background: Transmission electron microscopy (TEM) was first applied to study placental ultrastructure in the 1950's. We review those early studies and mention the scientists that employed or encouraged the use of TEM.
Findings: Among the pioneers Edward W. Dempsey was a key figure who attracted many other scientists to Washington University in St. Louis. Work on human placental ultrastructure was initiated at Cambridge and Kyoto whilst domestic animals were initially studied by Björkman in Stockholm and electron micrographs of bat placenta were published by Wimsatt of Cornell University.
Conclusions: Prior to the introduction of better fixation techniques, TEM images were of modest technical quality. Nevertheless they gave important insights into placental ultrastructure, particularly the nature of the maternal-fetal interface.

1. Introduction

Although electron microscopes were first built in the early 1930s, their application to biological materials awaited the development of techniques for fixation, embedding in hard plastic, and sectioning with glass knives. Methacrylate embedding provided a substrate hard enough to cut sections thin enough to penetrate with an electron beam. However, transmission electron microscopy (TEM) of biological tissues was not really possible before Palade [1] showed that fixation with buffered osmium tetroxide prevented a wave of acidity that caused protein precipitation in advance of fixation. The first studies on placenta appeared in the decade that followed. Palade received the Nobel Prize, as did Ruska who designed the electron microscopes used in these early studies.

Among the pioneers was Edward W. Dempsey (Fig. 1). His 1953 paper on the guinea pig yolk sac [2] was followed by others with George B. Wislocki on placentas of rat [3], human [4], cat [5] and pig [6]. This work was done at the Anatomy Department of Washington University in St. Louis, where Dempsey also facilitated studies on placentation in the nine-banded armadillo [7] and the guinea pig [8].

Credit for the first ultrastructural study of human placenta goes to J. Dixon Boyd and Arthur F. W. Hughes of the Anatomy School at Cambridge University [9]. Other early studies on human placenta were by Wislocki and Dempsey [4] and Sawasaki et al. at Kyoto Medical College in Japan [10].

Given the influence of the Grosser classification, especially after the contemporary publication of E. C. Amoroso's treatise [11], it is unsurprising to see veterinarians at the forefront of ultrastructural studies. Amoroso himself participated in early studies of placentation in the sow and mare [6,12]. Meanwhile, in Stockholm, Nils Björkman wrote on placentation in the cow [13]. This was to be the first of a long series of papers on domesticated species by Björkman and his students.

William A. Wimsatt of Cornell University (Fig. 2) was also convinced of the utility of TEM. He published the first images of the interhaemal area in the placenta of the little brown bat (made by Harold Parks at Rochester and Duncan Chiquoine at Princeton) [14]. Later he collaborated with Enders [15] and Björkman [16]. The literature on placental ultrastructure in Chiroptera is now extensive due in part to Wimsatt's former students such as Mark Cukierski [17] and John J. Rasweiler IV [18].

Palade's [1] buffered osmium was a good membrane fixative, but not good at preserving protein structure or at penetrating into tissues. It was not until the introduction of initial fixation with aldehyde fixatives followed by osmium [19] that placental structures could properly be examined by TEM. Epoxy resin embedding also helped since it did not undergo the shrinkage during polymerization that methacrylates did. It was therefore not until the 1960s that TEM began to yield top quality images. Nevertheless, the

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Fig. 1. Edward W. Dempsey (1911–1975) pioneered ultrastructural studies of the placenta. Here seated at an early electron microscope (RCA EMU2) at Washington University in St. Louis in November 1958. Looking on are (left to right) Jack Davies, E. C. Amoroso and J. Dixon Boyd.

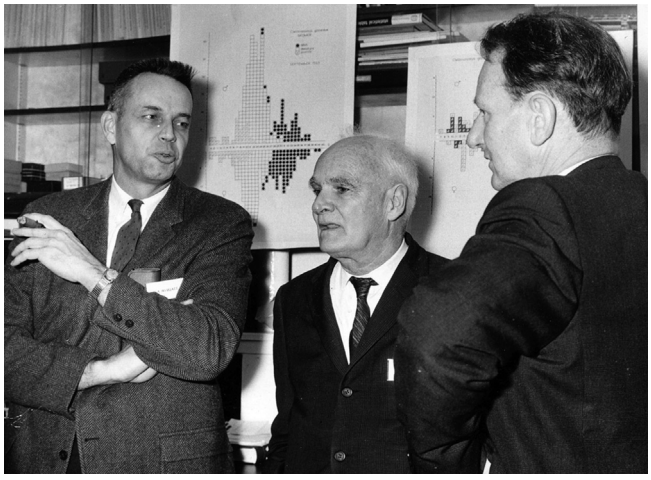

Fig. 2. William A. Wimsatt (1917–1985) was a zoologist who encouraged the study of placental ultrastructure in bats. He is seen here (left) with S. A. Asdell and I. W. Rowlands at the International Symposium on Comparative Biology of Reproduction in Mammals at the Zoological Society of London in November 1964.

ability to examine placentas at an ultrastructural level represented a paradigm shift. Despite the modest technical quality of these first images, early attempts at TEM gave some fascinating insights into the biology of trophoblast. As often is the case in a new field, there were also some interesting errors in interpretation.

2. The microvillous membrane

Boyd and Hughes [9] examined a single human specimen from early in the first trimester. They described the microvilli on the maternal-facing surface of the syncytiotrophoblast and pinocytotic vesicles beneath them. Wislocki and Dempsey examined two first trimester and two term placentas [4]. In addition to the microvilli and pinocytotic vesicles, they described the endoplasmic reticulum of the syncytiotrophoblast. They recognized its equivalence to the cytoplasmic basophilia, representing ribonucleoprotein, previously seen in the light microscope. It must be remembered that the translational machinery of the cell had yet to be worked out. Crick's Central Dogma – DNA makes RNA makes protein – was not published until three years later [20]. With regard to villous cytotrophoblast, Wislocki and Dempsey determined that residual, flattened Langhans cells were present at term, a point until then contested. In addition this paper characterized the extravillous trophoblast of the cell columns.

3. The interhaemal barrier and visceral yolk sac of rat, rabbit and guinea pig

Mossman had argued that trophoblast disappeared from the interhaemal region in the term placentas of rodents and lagomorphs, proposing the term haemoendothelial for this condition. Wislocki and Dempsey were able to refute this by TEM in one rabbit (*Oryctolagus cuniculus*) and four rat (*Rattus norvegicus*) placentas [3]. Indeed they showed there were three layers of trophoblast in the rat and two in the rabbit. They thought incorrectly that all three layers in the rat were cytotrophoblast; the precise details were worked out only later and the terms mono-, di- and trihaemochorial introduced [21].

Following initial observations in the guinea pig (*Cavia porcellus*) [2], close attention was paid to the villous region of the inverted yolk sac of the rat [3]. The endothelial endoderm cells were shown to be clothed with microvilli. There were tubular invaginations at the apical surface and small vesicles that appeared to be derived from them. In short these cells were specialized for absorption from the uterine cavity. Much later this was confirmed by functional studies [22].

4. Endotheliochorial and epitheliochorial placentas

Dempsey and Wislocki [23] used TEM to explore the endotheliochorial placenta of the cat (*Felix catus*). They were the first to describe discontinuities in the interstitial membrane through which maternal endothelial cells came into contact with syncytiotrophoblast. Now this is known to be a common feature of endotheliochorial placentas [24]. They also examined the decidual giant cells and the brown border (paraplacental allantochorion). For the latter they found evidence that, in addition to phagocytosing maternal erythrocytes, the columnar trophoblast cells absorbed uterine secretions by a mechanism similar to that described for rodent yolk sac.

Dempsey, Wislocki and Amoroso [6] examined the epitheliochorial placenta of the pig (*Sus scrofa*). Their paper is chiefly remarkable for demonstrating interdigitation between the microvilli of uterine epithelial and trophoblast cells. This close contact at the maternal-fetal interface implied that uptake of uterine gland secretions necessarily must be confined to the areolae. The ultrastructure of the uterine gland epithelium was comparable to that of other secretory cells such as those in the pancreatic acini.

Björkman and Bloom [13] gave the first description of the maternal-fetal junction in the placenta of the cow (*Bos taurus*). The paper is notable for proving that the bovine placenta was epitheliochorial rather than syndesmochorial, as previously thought [11], since the uterine epithelium remained intact (Fig. 3). There was interdigitation between the microvilli of the uterine epithelial and trophoblast cells both within the placentomes and in the interplacentomal areas. A description was given of the binucleate trophoblast cells. In addition these authors recognized giant cells in the epithelium that were regarded as binucleate. These no doubt were the trinucleate cells now known to result from fusion of a binucleate trophoblast cell with a uterine epithelial cell [25]. A comprehensive study of placentation in Cervidae [26] also included TEM showing interlocking villi in the placentome of the fallow deer (*Dama dama*).