Isolation of Trophoblast Basement Membrane from Full-Term Human Placenta (36423)

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(Introduced by J. L. Brandt)

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Under the electron microscope the villous trophoblast basement membrane is a clearly demarcated structure and appears as an amorphous undulating electron-dense substance in which fine filamentous elements are embedded (1–4). Occasionally the trophoblast basement membrane may be fused with the outer fibrillar elements of the fetal capillary wall, presenting a lamellar appearance. The average thickness of the basement membrane varies from 1000 to 3000 Å. Osmophilic granules embedded in the basement membrane have also been observed (1, 2).

In recent years it became accepted by most investigators that the trophoblast basement membrane increases in thickness in the last few weeks of pregnancy, a finding that has been correlated with a decrease in the permeability of the placental barrier. Marked thickening of the basement membrane has been also observed in patients with diabetes and with toxemia (1, 2, 5–7). Detailed knowledge of the structure of the basement membrane at the chemical level is essential for an understanding of its role as a filtration barrier in normal, and especially in abnormal pregnancies. However, due to the lack of a method for the isolation of the basement membrane no such data are yet available.

The purpose of the present study was, therefore, to develop a reproducible method for (1) the isolation of the terminal chorionic villi, and (2) a "pure" preparation of villous basement membrane.

Materials and Methods. Placentas, at 40 weeks of gestation, were obtained at delivery from subjects with no known clinical history of disease, and were immediately placed on ice. They were used fresh for the isolation of the chorionic villi (CVI).

Lipids were extracted with chloroform: methanol (2:1) and lipid phosphorus was determined (8). DNA was determined by the diphenylamine reaction (9), RNA was extracted with phenol (10) and was determined by absorbance at 260 mμ.

For light microscopy, fresh placental tissue and CVI pellets were fixed in 10% formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin, and PAS. For electron microscopy placental tissue, CVI pellets, and isolated basement membrane were fixed in 3% glutaraldehyde in cacodylate buffer (pH 7.45) for 2 hr in the cold. The pellets were then washed with cold buffer solution and treated with 2% osmium tetroxide in cacodylate buffer (pH 7.45). After washing the specimens were dehydrated in graded alcohols, treated with propylene oxide, and embedded in Epon. Thin sections were cut on an LKB Ultratome III Ultramicrotome with diamond knives, placed on uncoated copper grids (mesh size 200), and after staining with uranyl acetate were examined in a Hitachi 12 electron microscope. Photomicrographs were taken at primary magnifications from ×3,000 to ×36,000 and photographically enlarged, as desired.

Procedures and Results. 1. *Preparation of chorionic villi.* The placenta was placed on ice and the decidual end chorionic plates were removed. The middle zone of the central region, containing only terminal chorionic villi (Fig. 1), was then cut into small fragments (about 3 cm^3) with a knife. The terminal CVI still attached to the minor vil-

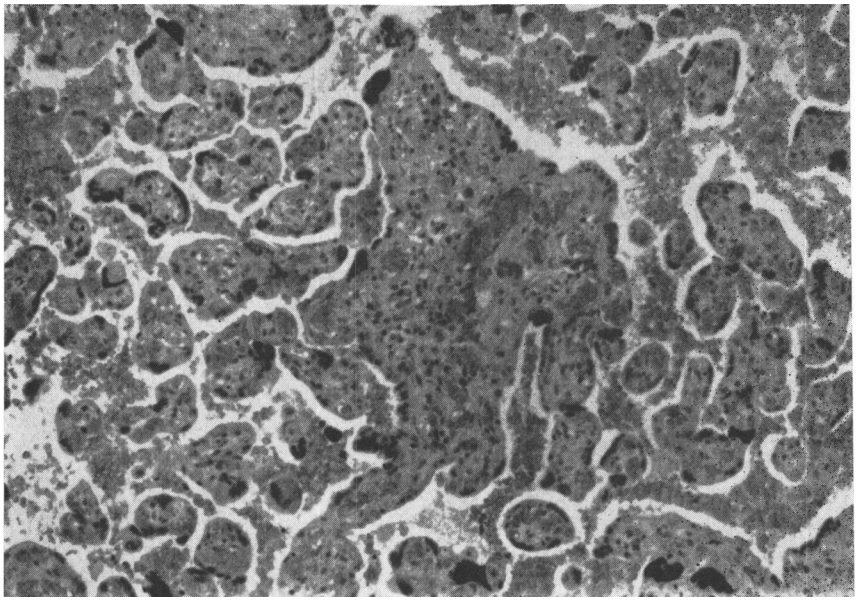

FIG. 1. Photomicrograph of the placenta after removal of decidual and chorionic plates. Note area contains only terminal chorionic villi. Hematoxylin and eosin. ×300.

lous stem were then scraped from the rami chorii with a razor blade. This crude preparation of CVI was then gently forced through an 80-mesh stainless steel wire cloth. The material remaining on the top of the sieve consisting of the ramuli chorii or minor villous stem was discarded. The CVI mash emerging from the bottom of the sieve was collected with an angular spatula, and transferred into 40 ml of ice-cold physiological saline. This suspension contained CVI, connective tissue contaminants, cells, and cellular debris (Fig. 2). After thorough mixing, the suspension was washed through the same sieve. The suspension was then poured onto a 325-mesh wire cloth, and was washed with an excess of ice-cold saline. As illustrated in Fig. 3 the material retained on the top of the 325-mesh sieve contained well preserved CVI. The preparation was free from cells and cellular debris, because these contaminants readily pass through the sieve. The CVI were then collected with the aid of a Pasteur pipet and transferred in 12-ml conical centrifuge tubes. Each sample was diluted to 10 ml with cold saline, and centrifuged at 1,060*g* for 15 min. The supernatant was removed by suction and discarded. To ensure a uniform-size collection of CVI, the residue pellet was gently buttered through a 200-mesh sieve resting on a 25 ml-beaker placed in an ice bath. The sieve was then washed with 10 ml ice-cold saline solution. The CVI, so treated, were then washed three times with ice-cold isotonic saline and five times with ice-cold distilled water. They were then collected by centrifugation at 1,060*g* for 15 min after each washing and finally lyophilized. The dry weight of the CVI obtained from one placenta ranged between 200–300 mg. The dry pellet was kept in a dessicator at 0° until used.

2. *Preparation of trophoblast basement membrane.* Trophoblast basement membrane (TBM) was prepared from CVI pellets by ultrasonic disruption. Ten milligrams of dry CVI pellet was suspended in about 15 ml ice-cold 1 *M* saline solution, placed in a loosely fitting Elvehjem–Potter hand homogenizer and subjected to three up-and-down runs of the pestle to obtain a uniform suspension of CVI. The CVI suspension was then poured into a 50-ml beaker resting in an ice bath, and the volume was made up to 30 ml with 1 *M* saline solution. A Bronwill Biosonic III sonifier was used with a 1-in. probe terminating about 0.5 cm below the liquid

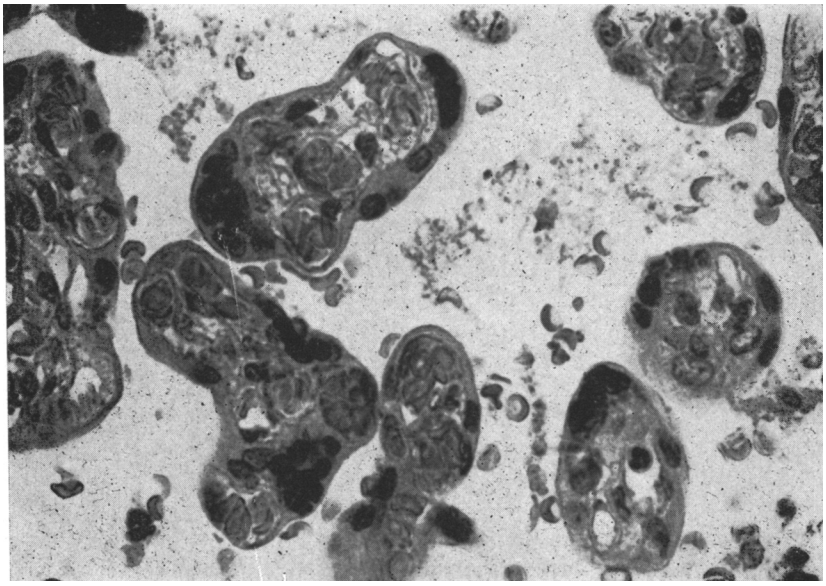

FIG. 2. Photomicrograph of isolated chorionic villi. Hematoxylin and eosin. ×600.

surface. Sonic disruption was conducted at 60% of maximum power output for a total of 7 min. At 1-min intervals the suspension was allowed to cool for 3 min. The sonicate was then transferred into 12-ml conical centrifuge tubes and centrifuged at 250*g* for 2 min. The supernatant was removed, and the residue, containing fibrinoid and unsonicated contaminants, was discarded. The supernatant was then recentrifuged at 1,500*g* for 10 min and the resulting membrane residue washed five times with ice-cold distilled water, and lyophilized. On a dry weight basis the recovery of the TBM from CVI ranged from 5–10%. On electron microscopy (Fig. 4) the TBM preparation showed areas of con-

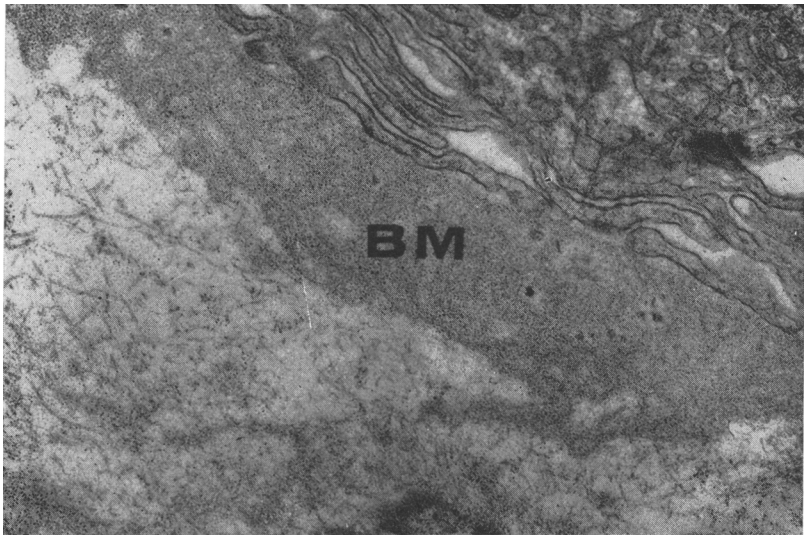

FIG. 3. Electronmicrograph of an isolated villous with the trophoblast and trophoblast basement membrane. Note preservation of cellular components. BM; basement membrane. Uranyl acetate ×42,000.

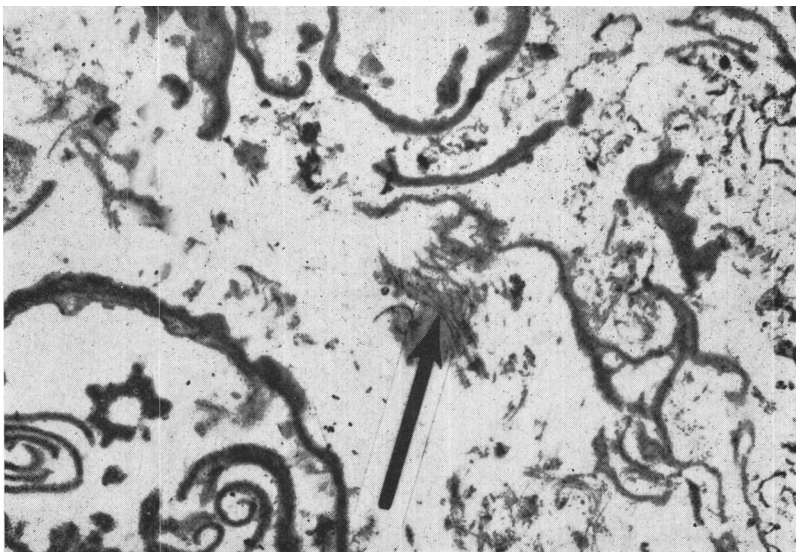

FIG. 4. Electron micrograph of isolated trophoblast basement membrane preparation after 7 min of sonication. Note connective tissue contaminants (arrow). Uranyl acetate ×16,200.

nective tissue contaminants. Furthermore, the TBM contained deoxyribose and ribose approximately 10% of that of the CVI preparation. As shown in Fig. 5 the lipid-phosphorus content remained constant after 4 min of sonication, and accounted for 0.1% of TBM. This was taken to indicate that by 4 min all of the phospholipids of cellular origin had been removed, and the remaining lipid-phosphorus was an integral part of the membrane.

Interstitial collagen was removed from the TBM preparation by extraction with 0.3 *M* acetic acid with gentle stirring at 4° for 24 hr. Following extraction, the suspension was centrifuged at 1,500*g* for 20 min. These steps were repeated three times. The insoluble residue was then washed with distilled water to remove excess salt, and lyophilized. Pooled lyophilized material (about 100 mg) was then incubated in 50 ml 0.1 *M* sodium acetate buffer (pH 5.0) containing 1.0 mg deoxyribonuclease and 1.0 mg ribonuclease for 16 hr with slow stirring at room temperature. After incubation the suspension was centrifuged at 5,000*g* for 30 min, and the residue pellet was washed with distilled water. These procedures eliminated any contaminating deoxyribo- and ribonucleic acids. Electron microscopic studies of the final residue revealed an amorphous basement membrane free from collagen fibers and plasma membrane (Fig. 6).

Discussion. In principle, TBM was prepared from full-term normal human placentas in two steps. Chorionic villi were first isolated and purified, from which the basement membrane was then obtained. Isolation

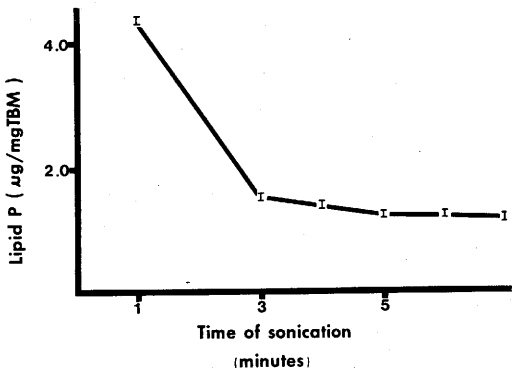

FIG. 5. Effect of sonication on the lipid-phosphorus content of the trophoblast basement membrane. Each point on the curve represents the mean of ten determinations. Vertical line through each point indicates the standard error of the mean. P: Phosphorus, TBM: trophoblast basement membrane.

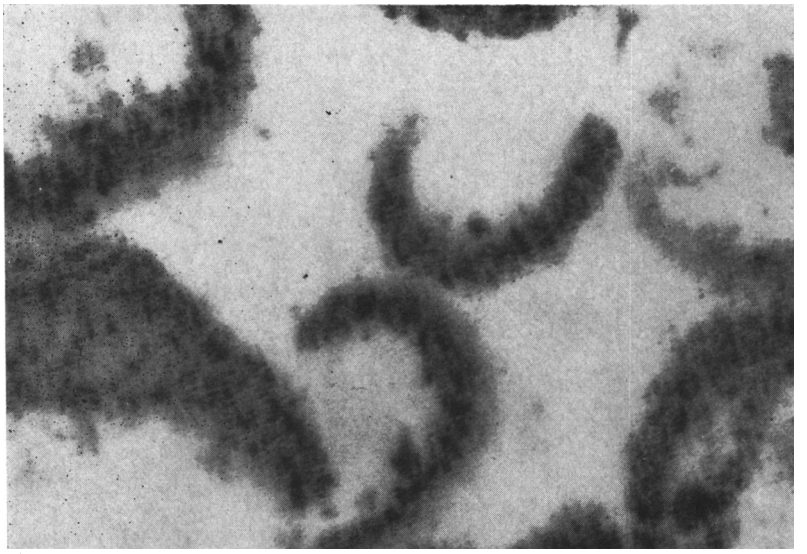

FIG. 6. Electron micrograph of trophoblast basement membrane preparation after extraction with 0.3 *M* acetic acid and treatment with deoxyribo- and ribonuclease. Note amorphous basement membrane; absence of connective tissue contaminants and plasma membrane fragments. Uranyl acetate ×60,000.

of chorionic villi could be easily achieved by scraping them off from the rami chorii, and by passing them through a 150-mesh, and a 325-mesh stainless steel wire cloth. The preparation of "pure" TBM from the CVI by physical means was not possible. It was due to the failure of sonication to remove connective tissue, and cellular contaminants. Judging from measurements of nucleic acids, and from electron microscopy the sonicated TBM preparation contained at least 10% contaminants. Extraction of the sonicated TBM preparation with 0.3 *M* acetic acid, followed by treatment with deoxyribo- and ribonuclease, however, successfully removed these tissue components. On the other hand sonic disruption seemed to be sufficient to remove cell membranes from the TBM, since the lipid-phosphorus content of the membrane remained constant after 4 min of sonication.

Although several reports are available on the chemical composition of the whole placenta, or parts of it (11–13), to the best of our knowledge no attempts have yet been made to isolate trophoblast basement membrane.

Summary. Terminal chorionic villi (CVI) were prepared from the rami chorii of the middle zone of the placenta, and were purified by forcing them through an 80-mesh followed by washing through a 325-mesh wire cloth. Trophoblast basement membrane (TBM) was obtained from CVI pellets by sonication in 1 *M* saline followed by extraction with 0.3 *M* acetic acid, and treatment with deoxyribo- and ribonuclease. The final preparation, on electron microscopy, was free of connective tissue contaminants and of plasma membranes.

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