Ultrastructure of Human Umbilical Arteries
Studies on Arteries from Newborn Children Delivered by Nonsmoking, White Group D, Diabetic Mothers

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SUMMARY The effect of diabetic metabolism on the human vascular wall was studied, using the fetal cardiovascular system at birth as an experimental model. The ultrastructure of umbilical arteries from nine newborn children of nonsmoking diabetic mothers (White group D) was compared with that of 30 healthy nonsmokers. Intimal cushions, thickening of the basement membrane often with a multilaminal appearance, and glycogen accumulations, both in the cells of the intima and the media, were found. The cells of the intima were very rich in fibrillas, identical to the underlying media myocytes. Endothelial cell death with formation of a pseudoendothelium due to migrating myocytes might be the explanation.


THE fetal cardiovascular system at birth has been used previously in an attempt to obtain an experimental model in atherosclerosis research in humans. Tobacco smoking has been studied using this model to look at biopsies from the umbilical artery, the umbilical vein, and the fetal capillaries in the placenta (Asmussen and Kjeldsen, 1975; Asmussen, 1977, 1979, 1980). As these studies revealed that smoking was damaging to the human vascular wall, we decided to investigate the effect of diabetic metabolism using the same experimental model. There are no data on the effect of diabetes mellitus on umbilical cord vessels. A few studies have been done on the fetal capillaries in the placenta. Okudaira et al. (1966) demonstrated basement membrane material thickening around the fetal capillaries in diabetic placentas, using the electron microscope. Similar observations have been made in placentas using the light microscope (Okudaira et al., 1966; Driscoll, 1965; Burstein et al., 1957; Fox, 1969). Also, in biopsies from diabetic nephropathy and in skeletal muscle (Vracko, 1970; Vracko and Strandness, 1967), thickening of the capillary basement membrane material has been demonstrated. Precise data on the various clinical aspects of the disease seem to be lacking in all these studies. With regard to pregnancy, no correlation has been made to the White classification (White, 1965; Pedersen, 1977). Moreover, as smoking is considered a risk factor for the development of vascular disease, it seems unfortunate that no information has been gathered on tobacco consumption in any of these studies.

The previous studies of the fetal cardiovascular system at term (Asmussen and Kjeldsen, 1975; Asmussen, 1977, 1979, 1980) revealed that tobacco smoking produced basement membrane thickening, both in capillaries and in larger vessels, and it therefore seemed important to elucidate as to whether the previously described changes in diabetics actually were caused by tobacco smoking or by diabetic metabolism.

Therefore, the aim of this investigation has been to determine whether or not diabetes mellitus has a damaging effect on the vascular wall in humans. Biopsies were taken from the umbilical artery from nine white group D, nonsmoking women at delivery, and the arteries were studied with the electron microscope. The morphology was compared with that of 30 healthy nondiabetic nonsmokers.

Material
Nine diabetic women with insulin-requiring diabetes mellitus since childhood, classified as White group D during pregnancy, were selected for the study. All subjects had blood sugar (capillary) measured 3–4 times daily (3 times postprandial), and the aim of the compensation was to keep the mean blood glucose below 100 mg/100 ml. The patients were given insulin injections at least twice daily using a combination of intermediate and fast-acting insulin simultaneously. All diabetics were nonsmokers all through pregnancy. All of the women delivered their children at the maternity ward YB at Rigshospitalet, Copenhagen, and all had Cesarean section approximately 3 weeks prior to estimated term (Pedersen, 1977).

A control group of 30 healthy, nondiabetic, nonsmoking women were used. All these women gave birth at full term to healthy children.

The diabetic placentas were macroscopically easy to separate from those of the controls, being larger, softer, richly vascularized, and containing a
large residual blood volume. Variations in morphology were found, such as villenous insertion of the funiculus umbilicalis and circumvalat placenta. No single arteries or infarctions were found. A general feeling was that the diabetic placentas and cords were more edematous, the funiculus with huge gelatinous accumulations. Among the children of the diabetics, one was born with multiple malformations. The child died when 2 days old.

**Methods**

Immediately after birth of the placenta, perfusion fixation was performed on the umbilical artery using Karnowsky's fixation solution 70% at pH 7.2. The tissue then was cut into 1-mm³ blocks for further fixation at room temperature in the same solution for 2-4 hours. Postfixation was performed in 1% osmium tetroxide for 3 hours at room temperature. The tissue then was washed in phosphate buffer and dehydrated in graded ethanols, cleared in propylene oxide, and embedded in Vestopal W. Ultrathin sections were cut on glass knives with an LKB Ultratome, mounted on copper grids, and contrasted with uranyl acetate and lead citrate. These sections were examined and photographed in a Zeiss EM-9-S2 electron microscope. The study could not be performed blindly due to huge accumulation of glycogen present in diabetics only.

A series of sections was made for electron microscopy following the complete procedure as described above, the only difference being that every second grid was treated with α-amylase before examination in the electron microscope (α-amylase test performed by J.U. Prause, M.D., Institute of Ophthalmal Pathology, University of Copenhagen).

**Results**

The ultrastructure of the umbilical arteries from the 30 controls revealed a monolayer intima with endothelial cells held closely together by tight junctions and gap junctions. The intimal cells were rich in the usual cell organelles, the rough endoplasmic reticulum, mitochondria, and Golgi apparatus. At the base of the cells, small bundles of fine filaments could be demonstrated. The monolayer intima was separated from the media by a thin, noncontinuous basement membrane of an average width of 0.1 μm. Occasionally, mononuclear white blood cells were found in the subintimal space. The media myocytes were rich in rough endoplasmic reticulum, suggesting that they belonged to the metabolic type of muscle cells. The ultrastructure of the controls is described in more detail elsewhere (Asmussen and Kjeldsen, 1975; Asmussen, 1979).

In the diabetics, monolayer intima with intimal cells resembling the underlying smooth muscle cells was found (Figs. 1 and 2). The intimal cells were held together loosely by few gap junctions and tight junctions. A characteristic feature was the great amount of glycogen granules and rosettes scattered around in the cytoplasm, even forming huge accumulations (Figs. 1 and 3). These easily recognized accumulations of glycogen found in the diabetics made a blind study impossible to perform. The intimal cells—pseudoendothelial or myoendothelial—possessed on their luminal plasma membrane richly branching folds almost forming a network at the intercellular junctional site (Fig. 4). A characteristic feature was the darkly stained thickened luminal plasma membrane of the endothelial lining, suggesting an increased amount of glycogoprotein coat on the intimal cell surface (Figs. 1, 3, and 4). In areas with great accumulation of glycogen rosettes, pinocytotic activity seemed intense (Fig. 1). In the intimal cells, which practically consisted of only fibrillae and filaments, the usual cell organelles occasionally could be demonstrated: rough endoplasmic reticulum, mitochondria, and Golgi apparatus. Often the mitochondria were found in close connection to glycogen accumulations.

In the same specimens from the arteries, two types of intimal lining could be demonstrated. Usually the intimal lining was of monolayer type separated from the media by a broad basement membrane (Fig. 2), but in some areas, the intima seemed to be thickened, consisting of several layers of myoendothelial cells (Fig. 3). It appeared that these intimal cushions of myoendothelial cells were held rather closely together by few tight junctions and gap junctions in the luminal areas, whereas the underlying cells, of identical appearance, just seemed to be placed close to each other without any intercellular junctions. In some areas, five or more layers of cells could be present, forming cushions. In these areas, the basement membrane material was found underneath the cushion.

The basement membrane (Fig. 2) was very thick
and often multilaminal, containing fine filaments at the media surface, the fine filaments having no fine structure as collagen or fibrin. This widened basement membrane was found in areas with monolayer intima. In areas with an intimal cushion, rudimentary basement membranes were found underneath the cushion (Fig. 6).

The media, and also the cells in the cushions of the intima, seemed to possess cytoplasmic protrusions almost forming “coral” protrusions like the intimal myoendothelial cells. It seemed as if the media cells formed a complete network, the media myocytes just touching each other and thus possibly being able to transform messages or energy from one cell to another (Fig. 7).

The media myocytes had exactly the same appearance as that of the cells forming the intima. Also, these cells contained large amounts of glycogen granules and glycogen rosettes either scattered uniformly in the cytoplasm or forming large accumulations (Figs. 5 and 6). A characteristic finding was the electron translucent media, suggesting edema and the collagen fibers (Figs. 6 and 7).

In one subject, a-amylose digestion was performed on every second thin section from the artery. This revealed that, in areas where glycogen rosettes were accumulated on control sections, the treated sections demonstrated empty spots, thus being diagnostic for previous accumulations of glycogen.

Discussion

During pregnancy, maternal diabetes mellitus is reflected in the fetal blood, causing elevated blood glucose. Glucose is the principal source of energy for the fetus and is easily transferred by the placenta in both directions according to the gradient of concentration. Normally, the net transfer is in the direction of the fetus (Holmberg et al., 1956; Folkart et al., 1960). The placental transport system will be saturated at a concentration of about 180–200 mg of glucose per 100 ml, and in normal individuals, the fetal concentration of glucose will rise no further. In the guinea pig, it is found (Krauer et al., 1973) that glucose transfer is highly dependent on placental blood flow, besides the limitation of transfer by the placenta. No human studies focusing on the transfer ability of the placental membrane exist, either in normal individuals or in diabetics. However, the elevated blood glucose in the cord vessels and glucose in the amniotic fluid clearly reflect the maternal diabetic metabolism.
With regard to insulin, the placenta may be considered impermeable to insulin in most species, including normal human beings. On the other hand, antibodies against insulin present in the maternal blood are transferred through the placenta in diabetic women. Labeled insulin has been measured in four diabetic and four nondiabetic patients (the only study in humans), and it was concluded that the placental transfer of insulin was hardly measurable and that the presence of insulin antibodies did not induce insulin transfer (Kalhan et al., 1975).

Other factors of importance for the development of vascular disease are lipids and free fatty acids (FFA). FFA concentration is increased in most normal pregnancies and is still higher in diabetic pregnancies. However, FFA are low in the fetus, and human placental diffusion experiments (Dancis, 1975) have indicated that the rate of transfer of FFA is too low and insufficient to make FFA probable precursors for lipid depositions during the last trimester.

During pregnancy, growth hormone (HGH) is found in the human fetus at high concentrations from an early stage of gestation, the concentration of HGH in cord blood being much higher than that of the mother (Kaplan and Grumbach, 1965; Yen et al., 1967; Kaplan et al., 1972; Spellacy et al., 1973). Thus, the fetal cardiovascular system in both diabetics and nondiabetics is highly influenced by growth hormone. In cell cultures of muscle cells, it...
has been shown that growth is markedly increased when adding diabetic serum or HGH (Ledet, 1976, 1977) to the culture, and it is concluded that HGH may play an important role in the development of vascular disease in diabetics. However, a recent discovery has been made (T. Koschinsky, personal communication, 1980) of a still unidentified substance present in diabetic serum. This substance is responsible for the acceleration of growth in cell cultures induced by adding diabetic serum to the media. This substance has a far greater influence on growth than does growth hormone in “supernormal” values on identical cell cultures. The unknown substance is different from FFA, insulin, HGH, and glucose, and can be extracted from diabetic serum by dialysis.

The cells in both the intima and the media of the vessel wall contained large amounts of glycogen. It could be postulated that these accumulations of glycogen were due to the patients being neglectors or to incomplete insulin treatment during pregnancy. Unfortunately, measurements of glycocylated hemoglobin were not available for routine screening of these patients (McDonald and Davis, 1979; Bunn et al., 1978). However, pregnant diabetics usually must be regarded as the most carefully controlled diabetics and, also, the fact that the pregnant women are admitted to hospital several weeks prior to birth tends to eliminate the likelihood of insufficient treatment of the diabetic metabolism. Moreover, the fetus in diabetics from lunar month four already has developed a hypertrophy of the islet of the pancreas with an insulin production capable of lowering the fetal blood glucose. Also, it has to be borne in mind that presence of insulin is not required for incorporation of glycogen into endothelial cells (Gabbay, 1975; Rasio, 1975).

The metabolism of the cells in diabetics is changed. The glucose supply to the cell is increased and, thus, an increased use of glucose-6-phosphatase may induce alternative metabolic pathways with formation of fructose and sorbitol. Accumulation of sorbitol in the cells may take place since sorbitol passes the cell membranes very slowly and leads to an increase in intracellular osmotic pressure. Also, the elevated blood glucose results in increased glycocylated hemoglobin. This tends to lower the oxygen transportation capacity of the blood and may thus influence the cellular metabolism within the arterial wall in diabetics (McDonald and Davis, 1979; Bunn et al., 1978).

The thickening of the basement membrane previously has been described in the fetal capillaries in the placenta from diabetic pregnancies (Okudaira et al., 1966; Driscoll, 1965; Burstein et al., 1957; Fox, 1969). Whether these diabetic women were smokers cannot be determined from the papers. However,
fibrous degenerations within the villi and around the capillaries have been determined by both light microscope (Driscoll, 1965; Burstein et al., 1957; Fox, 1969) and electron microscope (Okudaira et al., 1966).

Vracko (1970; Vracko and Strandness, 1967), in studies of skeletal muscle capillaries in diabetics has demonstrated thickening of the basement membrane, and so has Siperstein et al. (1968, 1971, 1973), Kilo et al. (1972), and Williamson and Kilo (1977), among others. Vracko and Benditt (1970) have discussed the relationship of endothelial cell death and basement membrane thickening. The theory of repeated intimal cell death and subsequent replacement with myoendothelial cells, leaving a multilaminar basement membrane, seems a valid explanation for the findings in the diabetics in the present study.

The glycogen accumulation in the vessel wall in diabetics corresponds well to similar findings. In the placenta, Villee (1975) has demonstrated that diabetics have, on average, 63% higher glycogen content than comparable controls.

Focal intimal thickening was demonstrated in the vessels influenced by diabetic metabolism. A possible explanation of the intimal cushions that were formed by muscle cells is the theory of clonal selection (Benditt, 1974; Benditt and Benditt, 1973). This theory has been put forward again recently in relation to atherosclerosis by Björkerud (1979). The theory could explain the focal cushions and might perhaps also explain why basement membrane thickening is absent in these areas. This absence could be due to different cellular metabolism within the clone. The clonal selection theory originated from studies of tumors and also has been put forward in connection with leukemias. Thus, injury should favor the survival of special cell clones, and this also might explain the changes in lipid metabolism within the diseased arteries.

Diabetic pregnant women, White group D (White, 1965; Pedersen, 1977) were used in this study, as these women had a well-defined insulin-requiring diabetes mellitus without any verified malignant vascular complications. Thus, for instance, proliferative retinopathy or nephropathy was not present in this group. Looking at birth weight in children born to diabetics, one finds an increase in birth weight in White groups A, B, C, D, whereas White group F gives birth to small children. Thus, clinically, White group F, with verified severe vascular changes in the mothers, clearly has complications exceeding those of the diabetic metabolism. Further, it was an aim of the study to eliminate the previously demonstrated vascular damage produced by tobacco smoking (Asmussen and Kjeldsen, 1975; Asmussen, 1977, 1979, 1980). The wish to study the effect of diabetic metabolism without the influence of tobacco smoking arose from the fact that diabetics who smoke have a 10-fold increase in cardiovascular disease compared with diabetics who do not smoke.

A problem concerning the use of the fetal cardiovascular system at birth as an experimental model in which to study the vascular changes caused by diabetic metabolism in humans is the fact that diabetics deliver their children 3 weeks prior to estimated term. For obvious reasons, a perfect control group cannot be obtained for this study, as healthy women usually do not deliver their children 3 weeks prior to term. The control group, therefore, consisted of healthy nondiabetic women giving birth to children at full term. However, basement membrane thickening, intimal cushions, and glycogen deposits cannot be due to this difference in time. Basement membrane thickening is usually regarded as an irreversible damage to the vessel wall, as seen in studies with streptolycine-treated rats (Ruth Østerby, 1979, personal communication).

The present study has shown that human vessels, when influenced by diabetic metabolism, develop severe changes which, apart from glycogen deposits and intimal cushions, are similar to those previously demonstrated in comparable vascular biopsies from smokers (Asmussen and Kjeldsen, 1975; Asmussen, 1977, 1979, 1980). The vascular response to injury, whether it concerns tobacco smoking or diabetes mellitus, seems to some extent to be nonspecific, consisting of intimal cell death with increased intimal cell turnover. This requires that the intimal cells be replaced by migrating media myocytes (Vracko and Benditt, 1970). The repeated cell death then will be reflected in the vessel wall, leaving a basement membrane thickening, often with a multilaminar appearance, resembling growth rings in trees. In areas with intimal cushions, the basement membrane thickening was almost absent. Rudimentary basement membranes occasionally could be found underneath the cushions. Perhaps the migrating muscle cells or the proliferating muscle cells in the cushions may influence the degradation of the basement membrane material or, rather, inhibit the extracellular polymerization of the collagen synthesized by the intimal cells. It is a question whether or not basement material is synthesized uniformly by intimal cells and/or media myocytes. In studies of umbilical arteries, uniform basement membrane thickening was found in biopsies from smokers as compared with nonsmokers (Asmussen and Kjeldsen, 1975; Asmussen, 1980). In this present study, the focal absence of thickened basement membrane in relation to intimal cushions might be regarded as the morphological sign of focal proliferation of special cell clones with altered metabolism. This seems to point out that in man the vascular wall response to smoking differs in some respects from that of diabetic metabolism.

Probably the media consist of various types of muscle cells, and this could explain the difference in response to injury. According to Björkerud (personal communication, 1979), at least two cell types are present in media: the contractile and the met-
abolic muscle cell. This could be an indication of the media muscle cells playing an important role in vascular wall response to injury.

In conclusion, the present study of the umbilical artery at birth from diabetics compared with a control group of nondiabetics, all biopsies deriving from nonsmokers, has shown that vascular changes follow exposure to diabetic metabolism. The changes consist of glycogen accumulation in both the intima and media, glycogen deposits in such an amount that the normal function of the individual cells may be affected. Basement membrane material thickening similar to that previously described in skeletal muscle capillaries in diabetics (Vracko, 1970; Vracko and Strandness, 1987; Siperstein et al., 1968, 1973; Siperstein, 1971; Kilo et al., 1972; Williamson and Kilo, 1977) and that from diabetic neuropathy was demonstrated. Also, intimal thickening with intimal cushions was demonstrated, a finding that corresponds to the clonal selection theory.

Besides proving that vascular damage occurs in diabetics, the study also demonstrated that vascular damage may occur after 8 months' exposure to diabetic metabolism.

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