Fat-Containing Uterine Smooth Muscle Cells in "Toxemia": Possible Relevance to Atherosclerosis?

Abstract. *Uterine smooth muscle cells in "toxemia of pregnancy" contain varying amounts of fat—a feature to date believed to characterize only the arterial smooth muscle cells in atherosclerotic lesions. Thus, the smooth muscle cells at these two sites do not differ essentially in their reactivity to certain forms of injury; hypoxia may represent an injurious factor common to both "toxemia" and atherosclerosis. These observations imply that the view that the arterial smooth muscle cells are biologically different than are those elsewhere may no longer be tenable.*

Fat-containing smooth muscle cells have been observed to date solely in atherosclerotic lesions, but even this is only relatively recent general knowledge. Whereas the phenomenon was reported first at the turn of the century and later described in a few scattered communications, the fat-filled cells in atherosclerotic arteries were considered to be either histiocytes or fibroblasts (*1*) until the early 1960's when the initial electron microscopic observations (*2*) that the majority of these fat-containing cells were in fact smooth muscle cells were confirmed by subsequent studies (*3*).

The fatty metamorphosis of the smooth muscle cells, apparently confined to the atherosclerotic lesions, has been a puzzling phenomenon. It was argued that these intimal or arterial smooth muscle cells had to be metabolically different from morphologically similar cells at other sites, and as late as in 1973 one of us (*4*) stated: "No matter what the details of the mechanisms of the intracellular lipid accumulation may be, no such mechanisms operate anywhere else in the body."

In the course of our ongoing studies into the nature of the hypertensive disorders of pregnancy, fat-containing smooth muscle cells were observed in the uterine wall of patients with "toxemia of pregnancy." We considered these features and their implications sufficiently significant to warrant a special report. The study is based on 18 uterine biopsy specimens obtained immediately postpartum from the placental bed in two normal and 16 "toxemic" pregnancies. The tissues were processed by established techniques for light and electron microscopy. Changes affecting the smooth muscle cells were observed in the uterine tissues of all "toxemic," but not in those of normal, pregnancies.

The presence of fat in typical smooth muscle cells could be appreciated on light microscopic examination of 1-μm-thick sections of plastic-embedded tissues for electron microscopy (Fig. 1). In these preparations the fat droplets were in part extracted, whereas in the paraffin-embedded tissues the extraction by organic solvents was complete with resultant empty vacuoles. The droplets were preserved in frozen-cut (fixed and unfixed) tissues and gave a positive reaction for neutral fats with the Oil Red O stain. Occasionally, these fat-containing cells were related spatially to the thin-walled vascular channels of the myometrium, but more commonly no such relation was apparent. Electron microscopic examination confirmed that these fat-containing cellular elements were smooth muscle cells (Fig. 2). They were easily recognizable by their elongated shape, numerous pinocytotic vesicles, marginal densities (not always triangular), and the enveloping basal lamina. In addition to the fat droplets such cells displayed other altered features. Often there were cytoplasmic membranous whorls, and paucity of myofilaments and rough-surfaced endoplasmic reticulum; profiles of smooth-surfaced endoplasmic reticulum were on the other hand numerous and large (Fig. 2). Cells containing minute amounts of fat retained their myofilaments and often failed to show any of the other unusual features. The number of fat droplets varied considerably from one cell to another; the cytoplasm of some cells was almost entirely occupied by fat, whereas in other cells only a few droplets were present. The fat-containing smooth muscle cells alternated in any given area with cells devoid entirely of fat droplets (Fig. 2).

The presently reported observation indicates that the smooth muscle cells of the arterial wall are not as unique in their reaction to injury as had been assumed and that similar cells at other sites may react to adverse stimuli in an identical way. Moreover, it becomes apparent that for a smooth muscle cell to accumulate fat, the presence of lipids derived from the circulating blood, including those of prebeta or beta lipoproteins, is

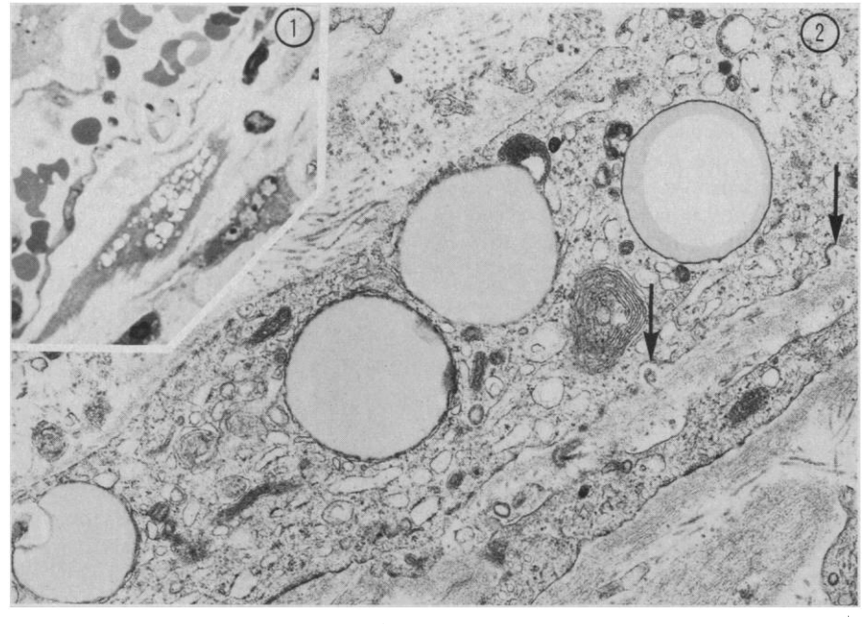

Fig. 1. Uterine smooth muscle cells in the zone between the myometrium and decidua from "toxemia" of pregnancy contain fat droplets that vary from a few (upper left corner) to numerous (elongated cell, center-right). One-micrometer-thick section of plastic embedded tissue; toluidine blue stain (×750). Fig. 2. Electron micrograph of myometrial layers deeper than those in Fig. 1. The cytoplasm of one of the smooth muscle cells contains four distinct lipid droplets of considerable size, a whorled "myelin" figure; numerous dilated profiles of smooth surface reticulum; a small to moderate number of secretory vesicles and caveolae cellulares; a few small accumulations of pigment-like substance associated with the periphery of lipid droplets, and electron-dense, small, membrane-bound structures with lucent areas that are of size approximating that of the "Porter pit" (arrows). It is probable that these electron-dense structures represent cross sections of "tail ends" of altered mitochondria. The cell is still enveloped in basal lamina and there are a few densities along some segments of the peripheral cytoplasm, but myofilaments are not apparent. Thin sections of tissues fixed in glutaraldehyde, postfixed in osmium tetroxide, and stained with uranyl acetate and lead citrate (×17,000).

not a sine qua non as has been advocated by so many investigators in the field of atherosclerosis. Thus, the observation of Zilversmit *et al.* (*5*) that certain lipids (phospholipids) may be synthesized in atherosclerotic lesions assumes a renewed importance.

The hypertensive syndromes of pregnancy may be the outcome of several conditions; all of these may be setting a stage for an injury to the uterine smooth muscle cells comparable with respect to severity and other parameters to that prevailing in the arterial wall at the inception of the atherosclerotic process (*6*). It is believed by most investigators that in hypertensive disorders of pregnancy there is a reduced uteroplacental perfusion (*7*) with resultant hypoxia. Since hypoxia has been also considered to represent a factor in the etiology and pathogenesis of atherosclerotic lesions (*6*), it may play a causative role in fatty metamorphosis of both the uterine and arterial smooth muscle cells.

It should be of interest to extend the reported studies to assess the extent of changes with respect to the entire thickness of the uterus (hysterectomy specimens); to other pathological pregnancies (for example, diabetes mellitus, dysmaturity); and to other organs containing smooth muscle cells (for example, gastrointestinal tract, peribronchial tissues) under a variety of pathological conditions. Notwithstanding the results of any such studies, the observations reported herein imply that the view that the arterial smooth muscle cells are biologically (metabolically) different than are those elsewhere in the body is no longer tenable, at least not with respect to cytoplasmic lipid accumulations.

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Neurophysin Biosynthesis: Conversion of a Putative Precursor During Axonal Transport

Abstract. *[^{35}S]Cysteine injected adjacent to the supraoptic nucleus of the rat is rapidly incorporated into a 20,000-dalton protein that, in time, is converted to a 12,000-dalton labeled protein, neurophysin. This putative precursor of neurophysin appears to be synthesized in the supraoptic nucleus and transformed to neurophysin and related peptides during axonal transport to the neurohypophysis.*

Neurophysins represent a group of cysteine-rich proteins that are synthesized by neurons in the supraoptic and paraventricular nuclei of the hypothalamus and transported to nerve terminals in the posterior pituitary, where they are released together with their associated peptides, oxytocin and vasopressin (*1*). Sachs and colleagues (*2*) have hypothesized that the neurohypophysial peptides (such as vasopressin) and neurophysin derive from the posttranslational cleavage of a common precursor protein. Although indirect evidence supports this hypothesis (*2*, *3*), no biosynthetic evidence identifying this postulated precursor has been reported. In this report, biosynthetic evidence for the existence of a precursor to neurophysin and for its posttranslational modification during axonal transport is presented.

Female rats of the Osborne-Mendel strain (225 to 250 g) were studied. The heads of ether-anesthetized animals were fixed in a stereotaxic instrument (5 deg, nose down) and their brains were exposed. Two 31-gauge stainless steel needles were positioned 7 mm rostral to the interauricular line, 2.5 mm on each side of the midline. The needles were lowered 8.6 mm beneath the dural surface, and 1 μl of a solution containing 10 μc of L-[^{35}S]cysteine (50 c/mmole, New England Nuclear) in 0.9 percent NaCl and 10 m*M* dithiothreitol was injected through each needle over a period of 10 minutes. The tips of the needles were found by histological examination of the brains to have been just above and lateral to the supraoptic nuclei. Following the injection, the needles were left in position for 10 minutes, and were then removed from the brain. The scalp was closed with wound clips, and the animals awoke 10 minutes after surgery. At various times after injection, the animals were killed by decapitation, and their brains and pituitaries were quickly removed and frozen on Dry Ice. Serial frontal sections (300 μm thick) of the brain were cut in a cryostat at −9°C. Representative regions of neuronal perikarya in the supraoptic nucleus and of axons in the median eminence were dissected from these frozen sections by the Palkovits punch technique (*4*), and the posterior pituitary was isolated as a sample of the nerve terminal region. The isolated tissues were homogenized in 0.1*N* HCl in order to destroy degradative enzymes (*5*) and were stored at −70°C. The proteins in the tissues were extracted with 1 percent Triton X-100 in 8*M* urea and separated by acid-urea polyacrylamide gel electrophoresis (*6*). The gels were sliced and radioactivity was counted by conventional techniques.

The time course of appearance of labeled proteins in the various regions of the hypothalamo-neurohypophyseal system following [^{35}S]cysteine injection in the supraoptic nucleus was similar to that in previous reports (*7*). Labeled proteins first appeared at the level of the median eminence after about 1 hour, while in the posterior pituitary labeled proteins appeared about 1.5 hours after injection. These values were consistent with the expected axonal transport rates of the neurohypophyseal proteins (*7*), and intraventricular injection of colchicine completely prevented the appearance of labeled proteins in the median eminence and posterior pituitary with little or no effect on incorporation in the supraoptic nucleus (*8*).

The profiles of the labeled proteins separated on acid-urea gels from three regions of the hypothalamo-neurohypo-