Duchenne muscular dystrophy (DMD) is a degenerative disorder associated with progressive muscle weakness. Affected children eventually die from respiratory or cardiac failure and rarely survive to adulthood. It is a genetic disease due to a defect at a single locus on the X-chromosome and is therefore transmitted by female carriers to their sons. The disease is relatively common; one in 4,800 males or a total of approximately 20,000 boys in the United States has DMD (239).

The hypothesis has been put forward that DMD is the result of a defective membrane in the mature muscle fiber (240). This was thought to be the cause of the characteristic hundred-fold elevation in serum levels of the muscle enzyme creatine kinase. Despite numerous studies, there is still little solid evidence to support this view (4), although differences between normal and dystrophic sarcoplasmic membranes have been demonstrated by electron microscopy (8).

The reason for the paucity of information regarding this disease is that muscle is a heterogeneous tissue containing adipocytes and connective tissue in addition to muscle cells. This heterogeneity is especially pronounced and problematic in diseased muscle tissue, in which the proportions of these other cell types is often increased and highly variable. Even different biopsies of the same muscle may differ in their cellular composition. Furthermore, upon cultivation of cells from dissociated muscle tissue in vitro, the problem may be further complicated; this is because the fibroblasts present in the cultures frequently outgrow the muscle cells. As a result, establishing consistent differences in morphological and biochemical parameters of
muscle biopsies or the cultured cells derived from them is difficult due to the lack of a solid frame of reference.

To overcome these problems we developed a method for eliminating all other cell types and growing pure populations of human muscle cells in tissue culture. This permitted us to study the muscle cell itself under controlled conditions in order to determine whether there existed an intrinsic defect in that cell type. Although culture methods had been developed for chicken and rodent muscle, methods for cultivating human muscle cells were limited. We sought to extend the work of Dr. Hauschka (241,242) regarding the isolation and growth of human fetal muscle cells. Our methods were developed for postnatal human muscle tissue because we wanted to work with cells from patients with DMD; the diagnosis for this disease is not definitive until after birth (243). The cells we isolated from the muscle were the satellite cells, first defined by Dr. Mauro (144) as the myoblasts that lie between the sarcolemma and the basement membrane of mature muscle fibers. We established methods for obtaining large numbers of satellite cells (approximately $10^{12}$ cells per cell), freezing and storing them, and thawing them for use in numerous replicate cultures (244). This permitted us to compare systematically one sample with itself at different times, individual clones from the same sample, and different samples with each other.

In addition, we sought culture conditions which could be used to stimulate the muscle cells either to grow and proliferate or to fuse and differentiate. This was achieved by the use of different tissue culture media. As shown in Figure 16, upon exposure to a mitogen-rich medium, the cells actively divided; in a mitogen-poor medium, striated, contractile myotubes formed (244). We could also study the proliferative and differentiative properties either of a single myoblast and its progeny, or clone, or of numerous myoblasts derived from a number of pure muscle clones grown at high density.

Our initial investigations were aimed at demonstrating the phenotype of the disease in differentiated myotubes obtained from pure muscle cultures isolated from the tissues of DMD patients. As show in Figure 17, the normal and dystrophic myotubes that formed in culture were morphologically indistinguishable. Upon fixation and staining, the typical striations characteristic of a mature muscle fiber were apparent and active contractions were observed in both types of muscle cultures (Figure 16).