sity according to Marmur and Doty (*8*) and Schildkraut *et al.* (*6*) are also presented. There is close agreement for the base composition obtained by these three methods. A striking difference between the nuclear DNA and the chloroplast DNA is in the absence of 5-methylcytosine in the latter. To confirm this observation, 200 μg of chloroplast DNA was hydrolyzed and subjected to paper chromatography; the area on the paper corresponding to the 5-methylcytosine position, as ascertained from the corresponding nuclear DNA analysis, was cut out and eluted. Material absorbing at 260 mμ was not present.

Thus on the basis of density, T_m, renaturation, and base composition, chloroplast DNA represents a specific chloroplast entity. It resembles mitochondrial DNA at least in its renaturation behavior (*9*). Analyses indicate a molecular weight for tobacco chloroplast DNA that is close to 4×10^7 daltons. We have found that purified chloroplasts contain a DNA-dependent, RNA polymerase which has different properties from an RNA polymerase contained in nuclei. Whether chloroplast DNA functions as a template for RNA synthesis, in chloroplasts, as well as whether homologies exist between nuclear and chloroplast DNA's, is unknown (*10*).

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Contractile Cells in Human Seminiferous Tubules

Abstract. *Electron microscopic study of the peritubular connective tissue in human testis reveals the presence of "contractile-type" cells rather than of typical fibrocytes. Their cytoplasm has numerous fine filaments and other components characteristic of smooth muscle cells. The rough-surfaced endoplasmic reticulum, however, is relatively prominent. In some instances, the nuclear surface appears scalloped or folded, and the cell surface presents an irregular profile, similar to that of contracted cells.*

Since Stieve's (*1*) accounts of the structure of the human testis, the boundary tissue (tunica propria) of the seminiferous tubules has been regarded as a typical connective tissue, consisting primarily of fibrocytes, collagen, and elastic fibers. Recently, a contractile cell has been demonstrated by means of electron microscopy in the testis of the rat (*2*) and of the mouse (*3*). These cells form a distinct structure, similar to a sheath, in the connective tissue surrounding the tubular epithelium. When these cells are observed in routinely prepared specimens stained with hematoxylin and eosin, however, their nature is practically indistinguishable from that of the more peripheral layers of fibrocytes.

The presence of a contractile cell in the testis is consistent with observations by other investigators of rhythmic contractions in the testes of various laboratory animals (*4*). Only a few studies of the fine structure of the peritubular connective tissue in humans have been reported. These, however, have failed to identify a similar or comparable contractile component in the human testis (*5*). We now report the presence of a cell within the tunica propria of the seminiferous tubules which has most, if not all, of the morphological characteristics of a contracile cell.

We studied specimens of seminiferous tubules obtained by biopsy from six healthy individuals (22 to 39 years old). The tissues were obtained at operation and immediately fixed in a 3 percent glutaraldehyde solution buffered with phosphate. The specimens were further fixed in 1 percent osmium tetroxide (in phosphate buffer), dehydrated in ethanol, and embedded in Epon. Thin sections were stained with lead citrate (*6*) and were then examined and photographed with an RCA-EMU 2e electron microscope.

Examination of the cells within the peritubular connective tissue reveals that they are uniform in appearance and represent a single cell type. In most instances, there are three to four layers of cells surrounding the tubule epithelium, each layer being separated from the adjacent layer by varying amounts of collagen (Figs. 1 and 2). The shape of the peritubular cells is similar to fibrocytes seen in other tissues; the cytoplasm, however, is more abundant. The nucleus is elongate in shape, but in some specimens its surface is scalloped, as in Fig. 1, or marked by deep folds, an appearance which is characteristic of contracted smooth muscle cells. In those cells with folded nuclear surfaces, the surface contour of the cell is also irregular in profile (Fig. 1).

When viewed at higher magnifications much of the cytoplasm is seen to contain abundant, closely packed, fine filaments. The filaments, which are in the order of 50 to 60 Å in diameter, are arranged in a parallel array (Fig. 3).

At various sites within the filamentous areas of the cell, there are other, more dense structures (arrows, Figs. 2 and 4). These localized cytoplasmic densities are identical in appearance to the "dense bodies" (*7*) or "attachment devices" (*8*) seen in smooth muscle cells.

The nonfilamentous regions of the cytoplasm contain the usual cell organelles. The Golgi apparatus and centrioles are perinuclear in position, while mitochondria, free ribosomes, and elements of both the smooth- and rough-surfaced endoplasmic reticulum are more widely distributed throughout the cytoplasm.

The rough-surfaced endoplasmic reticulum consists of single profiles of variable length which are orientated along the breadth of the cell (see Fig. 2). The smooth endoplasmic reticulum consists largely of tubular membraneous elements. In addition, small intensely staining particles, slightly larger in size than ribosomes, are present. Their size and intensity of staining with lead indicate that these particles are glycogen granules.

The plasma membrane is studded with numerous vesicular invaginations of the type generally referred to as

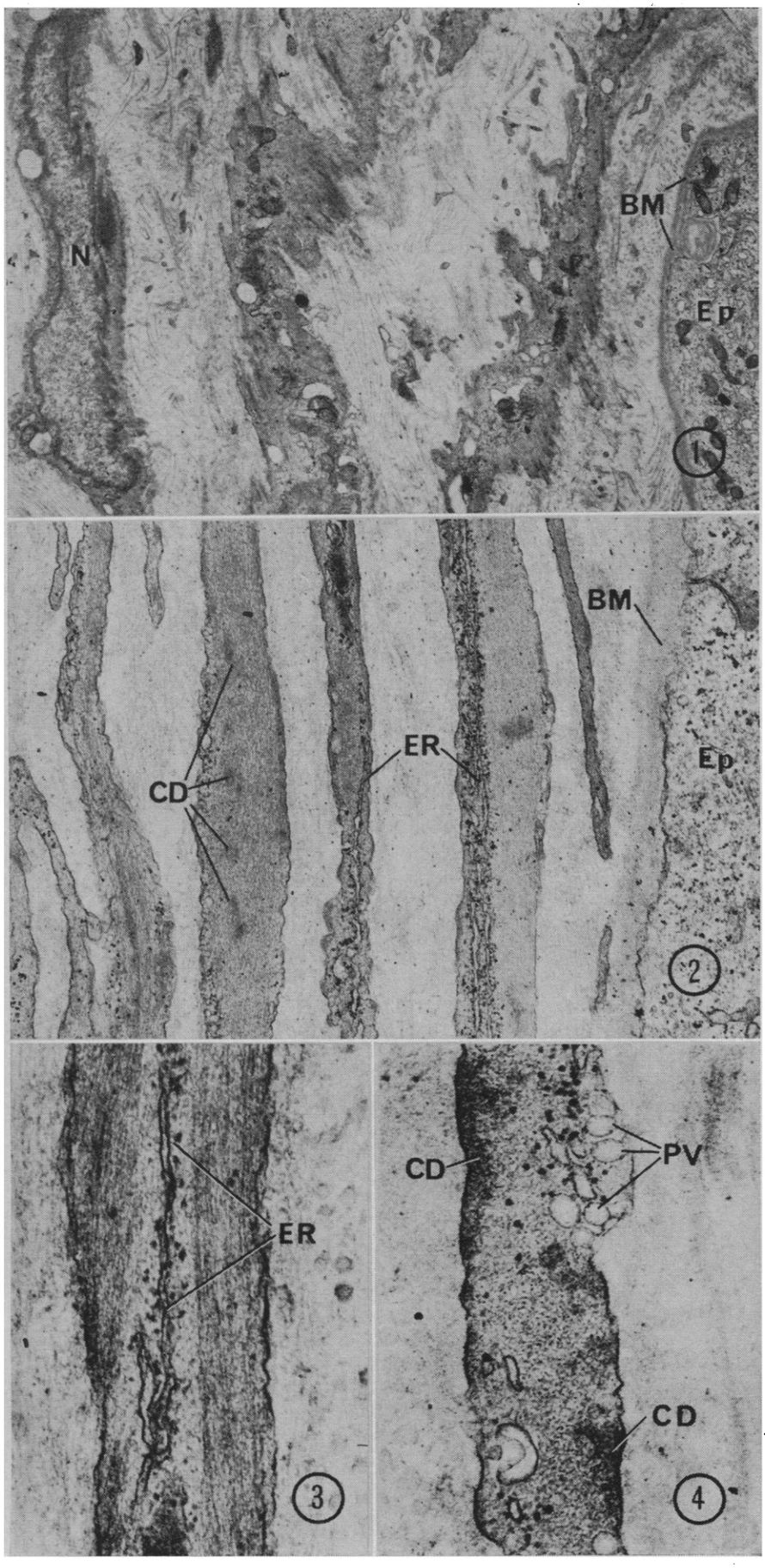

Fig. 1. Low-power electron micrograph of peritubular connective tissue with germinal epithelium (*Ep*) and basement membrane (*BM*) at right. The cell surfaces are irregular, and nucleus (*N*) has a scalloped profile suggesting a contracted state (× 5250).

Fig. 2. Peritubular connective tissue (same orientation as Fig. 1). The cells have smooth surface contours, and there is a suggestion of the fibrillar nature of the cytoplasm. A few localized cytoplasmic densities (*CD*) are evident here and in Fig. 4. Profiles of the granular endoplasmic reticulum (*ER*) are seen in two of the cells (× 22,750).

Fig. 3. A portion of a peritubular cell showing the cytoplasmic filaments in longitudinal array. The nonfilamentous, central region of the cell is occupied by a single profile of the endoplasmic reticulum (× 48,000).

Fig. 4. A peritubular cell revealing the filaments in transverse section. Several cytoplasmic densities (*CD*), as well as the pinocytotic vesicles (*PV*), are evident along the cell surface (× 48,000).

micropynocytotic vesicles. The vesicles are characteristic of smooth muscle cells (*9*), though they are found in other types of cells, such as the endothelial cells of blood vessels and, in fewer numbers, in fibrocytes.

The cytological features of the peritubular cells are more consistent with those of smooth muscle cells than with those of fibrocytes. Indeed, when comparison is made between the smooth muscle cells of blood vessels sometimes encountered within the same section and the peritubular cells, the cytoplasms of the two are found to be essentially the same in appearance. However, there are some characteristics which these particular cells share with fibrocytes.

The most obvious is the shape of the cell, which when seen in the light microscope readily accounts for their designation as fibrocytes. In addition, profiles of granular endoplasmic reticulum are frequently encountered. While this organelle system is a conspicuous component of the fibrocyte, it is generally very sparse in smooth muscle cells. The extent of the rough-surfaced endoplasmic reticulum suggests that the peritubular cells can still subserve the needs of the connective tissue stroma in which they lie. Moreover, the absence of fibrocytes indicates that this is indeed a likelihood. Nevertheless, the overall structure of the peritubular cells indicates that they represent a

cell type which is sufficiently modified to be considered smooth muscle.

With respect to sperm transport, it is generally assumed that the mature, nonmotile spermatozoa are carried along the seminiferous tubules simply by the flow of fluid which is constantly being absorbed into the lumen (*10*). The presence of smooth muscle cells suggests that the movement of the tubule contents may not be simply a passive phenomenon, but may also be, in part, an active process involving the peritubular cells.

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Protein and Nucleic Acid Synthesis In Escherichia Coli: Pressure and Temperature Effects

Abstract. *The incorporation of glycine-C^{14}, leucine-C^{14}, and adenine-C^{14} into the respective protein and nucleic acid fractions of* Escherichia coli *K-12 is markedly affected by application of moderately high hydrostatic pressure. Pressure application may result in either stimulation or inhibition of incorporation depending on the temperature.*

The disruptive but reversible effect of high hydrostatic pressure on the integrity of intracellular gel structures has been well documented (*1*). In bacteria, pressure effects on enzyme activity (*2*), growth (*3, 4*), cell-wall synthesis (*5*), and virus replication (*6, 7*) have been investigated. Little is known, however, about the effects of pressure on protein and nucleic acid synthesis. Zimmerman (*8*) reported that DNA sythesis in sea urchin eggs is not affected at pressures up to 5000 pounds per square inch (psi; 1 psi is equal to 0.068 atm), and Pollard and Weller (*9*) have reported pressure effects on induced enzyme synthesis in *Escherichia coli*. I now report the effect of pressure and temperature variations on the synthesis of protein and nucleic acid in *E. coli*.

Cultures of *E. coli* K-12 were grown to a cell density of 2.6×10^8 per milliliter. A portion (approximately 12 ml) of the suspension was transferred to the upper compartment of a mixing-type pressure chamber (*10*). The lower compartment contained an equal volume of nutrient broth containing C^{14}-labeled leucine, glycine, or adenine. The appropriate pressure was applied, and the contents of the two compartments were mixed. The pressure was released after a stipulated period of time and 20 ml of the cell suspension was placed in 2 ml of cold 50 percent trichloroacetic acid. This procedure took about 40 seconds. The protein and nucleic acid fractions were then isolated (*11*). The radioactivity is recorded in counts per minute per identical sample.

Temperature control was maintained by immersing the chambers in a water bath controlled by a direct-reading thermistor. Each pressure sample had its individual atmospheric control.

The radioactive amino acids used were leucine-1-C^{14} and glycine-1-C^{14} (specific activity 100 μc/mg). These were diluted for use in the chamber by addition of sufficient nutrient broth to yield a final radioactivity of 0.4 μc/ml. The adenine-C^{14} (specific activity 0.01 mc/mg) was similarly diluted to 0.2 μc/ml.

Counts were made on quadruplicate plates to test the possible effect of pressure on the viability of the cells. Only application of 10,000 psi (the maximum pressure used) for 25 minutes (the maximum time period) resulted in a change in viable count, and this amounted to a decrease of 5 to 8 percent. Utilizing media other than nutrient broth, other workers (*6*) have reported a greater loss in viability under these conditions of pressure, temperature, and time. The nutrient broth seemed to have a protective influence and was therefore routinely used in these experiments.

The effect of pressure on the incorporation of glycine into protein is shown in Fig. 1. At 6,000 psi and 37°C, incorporation is identical to that of controls at atmospheric pressure; at higher pressures there is an inhibition, and at lower pressure a marked stimulation occurs. The stimulatory effect of 4000 psi, and similarly the inhibitory effect of 8000 psi, occurs within the first 5 to 10 minutes of the application of pressure. After this period, the rates are identical with that of the control. In several experiments, pressure was released after 10 minutes at 4000 psi, and samples were taken at successive 5-minute intervals. The resultant incorporation curve was identical to the one shown where 4000 psi was maintained over the full period of the experiment. At 10,000 psi, after a small initial uptake, almost complete inhibition occurs.

It has been shown that a pressure of 10,000 psi effectively prevents division of *E. coli* and that release of pressure is followed by an extended lag period prior to resumption of the division process (*4*). At this pressure, protein synthesis is almost completely inhibited, and the question arose whether release

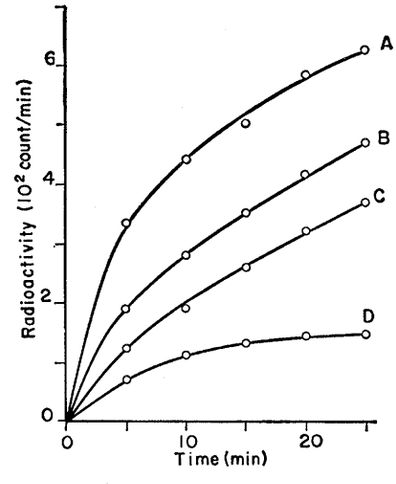

Fig. 1. Pressure effects on glycine-C^{14} incorporation into protein at 37°C. A, 4000 psi; B, 6000 psi and 15 psi; C, 8000 psi; D, 10,000 psi.