

THE MYOCARDIAL INTERSTITIUM: ITS STRUCTURE AND ITS ROLE IN IONIC EXCHANGE

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ABSTRACT

The structures present in the rabbit myocardial interstitium have been defined and quantified. Stereological methods were used for the quantification. The extracellular space contains abundant ground substance (23%) distributed in a homogeneous mat throughout the space and within the T tubules. The remainder of the space contains 59% blood vessels, 6% "empty" space, 4.0% collagen, and 7.0% connective tissue cells. The arrangement of the interstitium in relation to the myocardial cells and the capillaries has been described. In addition, the extracellular space was measured using extracellular markers: ^{14}C sucrose (neutrally charged), $^{35}\text{SO}_4$ (negatively charged), and ^{140}La (positively charged). The La^{+++} space differed markedly from the other two ($P \ll 0.001$), indicating extensive binding of La^{+++} to polyanionic extracellular structures. Cetylpyridinium chloride, a cationic detergent specific for polysaccharides, caused precipitation of the ground substance and marked decrease in the La^{+++} space. This study indicates the considerable structural complexity of the interstitium. The effects of an abundant negatively charged protein-polysaccharide within the interstitium has been discussed in terms of cation exchange in arterially perfused tissue.

INTRODUCTION

Many studies have described the cytology of the mammalian heart (6, 13), but little information is available on the ultrastructural aspects of the extracellular space in the myocardium. The region between the myocardial cell surface and the capillary wall has not been studied in detail nor have the structures within this space been quantified.

Kinetic studies on the evaluation of cation exchange in vascularly perfused mammalian myocardium are not consistent with an interstitium composed simply of a vascular transudate (9, 29). Indeed, 10 years ago, Bennett (1) pointed out the abundance of protein polysaccharide

(ground substance) present on the surface and in the vicinity of muscle cells, and he suggested that this material with its fixed negative charges might modify the ionic environment of the plasma membrane and the cell itself. A number of flux and perfusion studies have emphasized the fact that coupling calcium is derived from superficial sites on or near the myocardial cell membrane which are in very rapid equilibrium with the interstitial space (24, 29). Displacement of calcium from these superficial sites and from the interstitium results in a proportional loss of tension, indicating the importance of "extracellular" calcium sources to excitation-contraction coupling (24). Ki-

netically, it is not possible to separate, in the intact myocardium, superficial binding sites of calcium on or near the myocardial cell membrane from sites within the interstitial space. However, Langer and Frank were able to investigate the role of the myocardial basement membrane as a possible binding source of coupling calcium by using myocardial cells grown in tissue culture. In the present study we investigate the interstitial space in intact tissue with respect to its detailed structure and ionic binding characteristics.

The interventricular septum was used, since it is a physiologically perfused preparation and since many functional studies involving the exchange of cationic substances have been studied using this procedure (10). Our findings indicate that, indeed, the interstitium is of considerable structural complexity. Using the morphometric techniques of Weibel (31, 32), we have quantified the majority of the structural components of the extracellular space and have demonstrated that the ground substance occupies, exclusive of the blood vessels, over 58% of the extracellular space. The arrangement of the interstitium in relation to the myocardial cells and the capillaries has been described. In addition, the anionic nature of the abundant ground substance was documented by isotopic extracellular marker studies. Finally, the effect of an interstitium, which contains fixed negative charges, on the exchange of charged and uncharged solutes is discussed.

METHODS

Fixation and Microscopy

Arterially cannulated rabbit interventricular septa (five muscles) were perfused with oxygenated perfusate of the following millimolar composition: NaCl, 133; KCl, 3.6; CaCl₂, 1.0; MgCl₂, 0.3; glucose, 16.0; and buffered with 3 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethane sulfonic acid) (pH 7.4) for 15 min—thus allowing for a complete flushing of vascular space. The perfusion rate was 0.75–0.90 ml/min at hydrostatic pressure between 55 and 66 mm Hg. This flow rate was chosen since it is in the normal physiological range for rabbit myocardium, and the hydrostatic pressures needed to deliver this flow rate were well under the normal rabbit diastolic blood pressure (i.e., ~80 mm Hg). The perfusion medium was switched to 2.0% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4, osmolarity 424 mosmol) and the muscle was perfused with the fixative for 20 min, at which time the septum was quite firm and appeared uniformly dark-yellow throughout its thick-

ness. Another group of muscles (5) were perfused with glutaraldehyde which contained 0.1% cetylpyridinium chloride (CPC). CPC is a cationic detergent which in a water solution forms micelles that act as polyvalent cations with a high charge. Such cationic micelles associate with polyanionic polysaccharides, causing the polysaccharides to precipitate (26, 27).

After 20 min of perfusion, the septum was cut down and minced into small cubes of tissue (< 1 mm per side). Pieces were sampled from the endocardial surfaces and from the middle portion of the septum and placed in the glutaraldehyde fixative for an additional 1½ h. The pieces of septum were rinsed in buffer and then postfixed for 1 h in 1% OsO₄–0.2 M Na-cacodylate buffer solution. After a further rinse the tissue was dehydrated in alcohol and propylene oxide, embedded in Epon 812, and sectioned on Porter-Blum Mt-2 ultramicrotome.

Thin unoriented sections giving a silver interference pattern were stained with lead citrate and with uranyl acetate and examined on a Siemens Elmiskop IA electron microscope. In recording the micrographs, the part of the section in the upper left-hand corner of the squares of the supporting copper grid was photographed in an attempt to obtain an unbiased sample. For calibration, a photograph of a carbon replica cross grating (21,600 lines/cm) was taken with each series of photographs. Measurements were made on 20 × 23 cm prints having a final magnification of 9500.

TERMINOLOGY: We determined the volume percent of the following constituents of the extracellular space:

X_g = extracellular space filled with ground substance.

X_e = extracellular space apparently empty of any structural components.

X_c = collagen fraction.

X_{bv} = the space occupied by blood vessels.

X_f = cells of connective tissue origin, i.e., fibroblast and pericytes.

The data are reported in two ways: (a) All the components of the extracellular space X_g , X_e , X_c , X_f , and X_{bv} are given as a volume percent of the extracellular space (extracellular space = 100%); (b) the extracellular components have been presented as a volume percent of the whole myocardial tissue (myocardial cells and all components of extracellular space = 100%).

All volumes are presented as mean ± standard error. Student's T test was applied to controls and CPC data. Figs. 1 *a* and 1 *b* give examples of what were morphologically characterized as X_g and X_e . Fig. 2 demonstrates examples of components X_c , X_f , and X_{bv} .

MEASUREMENTS: The relative volumes of X_g , X_e , X_c , X_f and X_{bv} were determined using the follow-

ing formula derived from stereologic principles (2, 32): $V_v = P_i/P_T$, the volume fraction V_v of a component contained in unit volume of a material equals the number of points (P_i) falling on profiles of that component divided by the total number of test points (P_T). Point counting was performed on the photographic prints of unoriented sections from the outer and inner segments of the septum. The prints were covered with a clear plastic sheet on which a square grid was imprinted (See Fig. 3). The points for point counting were formed by the intersections of lines on the grid. Since there was considerable variation in size of the volume of the different structures measured (30–1%), the optimal point density (number of points counted) was quite different for the various volumes (5). The squares of the grid used for analysis of large volumes, i.e. (total extracellular space), were 4 cm/side and for the smaller volumes, 2 cm/side and 1 cm/side.

In addition to the stereology measurements, 16 cells were selected from micrographs from five muscles whose profiles were approximately cross-sectional. Two measurements were made in these cells: (a) the total perimeter of the cell was determined; (b) the fraction of the cell perimeter within 2000 Å of a capillary was measured. These data were used to determine the proportion of the myocardial cellular surface that was within 2000 Å of a capillary (Fig. 4).

Isotopic Measurements of the Extracellular Space

The "5 min" isotopic spaces were measured after 30 min of arterial perfusion of the interventricular septum. After a 30 min equilibration period the septum was labeled for 5 min with either [^{14}C]sucrose, $^{35}\text{SO}_4$, or ^{140}La . The isotopic spaces were then measured according to the method of Page and Page (18). After weighing, the wet muscle was finely minced and placed in a closed polyethylene bottle and extracted for 72 h with 50 ml of 0.1 N nitric acid. For [^{14}C]sucrose and $^{35}\text{SO}_4$, aliquots were taken and analyzed by standard techniques of liquid scintillation counting. For ^{140}La , the bottle containing the minced muscle in nitric acid was counted in a gamma counter, and the counts were compared to matched external standards. Two muscles perfused for 200 min with ^{140}La were counted in the same manner.

The effect of CPC on the isotopic spaces was evaluated using each muscle as its own control. Muscles were perfused for 25 min to achieve stability, followed by 10 min of isotopic labeling. The septum was then washed out with isotope-free perfusate for 30 min, by which time the ^{140}La activity in the venous effluent had declined to approximately 1%

of its activity as compared to its value at initiation of washout. [^{14}C]Sucrose and $^{35}\text{SO}_4$ declined to less than 0.1% of initial activity after 30 min of washout. After initial washout, the septum was perfused for 5 min with standard perfusate to which 0.025% CPC was added. It was then relabeled for 10 min with the appropriate isotopically labeled marker (no CPC in the labeling solution) and again washed out for 30 min, and the venous effluent was collected. The control and post-CPC washout curves were plotted (from 0 to 30 min) on linear paper. The areas under each curve were cut out and weighed in order to integrate each of the washouts. Comparison of the post-CPC integration with the control integration gave a measure of relative change in the magnitude of the spaces induced by 5 min of perfusion with CPC.

Since ^{140}La has a high affinity for the tissue, it was necessary to demonstrate that two successive 10-min isotopic labelings without CPC perfusion before the second labeling produced washout curves which were not significantly different. This was found to be the case, indicating that the "10 min La space" remains stable between the 35th and 80th min of perfusion, and that interventions which induce changes in this space can be accurately evaluated by integration of the washout curves. It is clear that greater quantities of ^{140}La (as compared to [^{14}C]sucrose and $^{35}\text{SO}_4$) remain in the tissue after 30 min, but this in no way influences the comparison of the 10 min space values before and after CPC perfusion as measured from washout curves.

In addition, it should be noted that total tissue water essentially remained stable from 35 to 85 min of perfusion. As Table II indicates, there was only a 1.3% increase in total tissue water and, if all of this is ascribed to the sucrose-measured extracellular space, the total extracellular space increases over the total perfusion time by 3.1%.

RESULTS

Structural Space

COMPONENTS X_g AND X_e : As the data in Table I indicate, a considerable part of the extracellular space (23.1%) is filled with ground substance. When the vascular bed is excluded, the interstitial matrix (ground substance) occupies the largest volume of the extracellular space. This ground substance fills the space not occupied by connective tissue cells or collagen fibers and is in very close association with the muscle cell membranes. Indeed, it is often difficult to delineate between ground substance and basement membrane (see Fig. 8). In routinely fixed and stained tissue (controls) this material has an amorphous

TABLE I
Stereological Measurements of Extracellular Space

Components measured	Controls	CPC	No. of measurements*
A. As % of extracellular space	Vol % mean \pm SE of mean	Vol % mean \pm SE of mean	
X_{ρ}	23.1 \pm 0.54	22.7 \pm 0.99	5, 20, 483
X_e †	5.9 \pm 0.44	13.0 \pm 1.72	5, 20, 483
X_c	3.9 \pm 0.34	5.1 \pm 1.02	5, 20, 483
X_f	6.6 \pm 0.87	5.4 \pm 0.80	5, 20, 483
X_{bv}	59.1 \pm 0.45	53.0 \pm 0.31	5, 20, 96
B. As % of total tissue	Vol %	Vol %	
Extracellular space†	24.6 \pm 0.60	31.2 \pm 1.75	5, 20, 30
Vascular space (bv)	14.0 \pm 0.81	15.3 \pm 1.45	5, 20, 96
bv Lumen	10.5 \pm 0.84	10.9 \pm 1.07	5, 20, 96
bv Endothelial cells	3.6 \pm 0.07	4.3 \pm 0.50	5, 20, 96
X_{ρ}	5.7 \pm 0.12	6.9 \pm 0.37	5, 20, 483
X_e	1.4 \pm 0.11	3.9 \pm 0.47	5, 20, 483
X_c	0.88 \pm 0.11	1.8 \pm 0.21	5, 20, 483
X_f	1.5 \pm 0.16	1.5 \pm 0.24	5, 20, 483

* The numbers are respectively: number of muscles, number of prints per muscle, number of point counts per print.

† Space in control and CPC-treated muscles significantly different, $P < 0.05$, by Student's T test.

appearance and was found to be continuous throughout the extracellular space and the transverse tubules (Figs. 1 *b* and 6). Small pockets of extracellular space ($< 6\%$) appeared empty of any stainable material (Fig. 1 *a*). These "empty" areas were nonuniformly distributed and not associated with any particular structure, and their presence most likely represents an artifact of the fixation procedures. Protein polysaccharides are water soluble and as such are very labile during the fixation. It is more likely that the empty areas in the extracellular space are fluid-filled spaces created during the various steps in tissue preparation.

It is important to note that included within components X_{ρ} and X_e there may be a small number of elastic fibers. These fibers are known to consist of two distinct components, the outer component which consists of microfibrils 150–120 Å in diameter, and the inner "amorphous" core which is exceedingly hard to stain for electron microscopy (23). With the low magnification and routine staining techniques used in this study, it was impossible to identify with certainty the contribution of elastic fibers to components X_{ρ} and X_e .

COMPONENT X_{bv} : The percentage of the total tissue occupied by the vascular bed determined by stereological techniques is 14.0%. This volume includes the volume of the lumen of the vessels as well as the volume occupied by the endothelial cells forming the vessels. The luminal volume determined morphometrically is 10.5% and is in satisfactory agreement with myocardial vascular space of 7% determined by flux studies using labeled albumin (11).

The endothelial cells forming the capillary network occupy 3.6% of the total tissue volume. A considerable portion of the extracellular space is occupied by these cells. We found the endothelial cell volume of the extracellular space to be 14.2%.

COMPONENTS X_c AND X_f : Collagen and connective tissue cells together occupy over 10% of the total volume of the extracellular space. Collagen appears in fibers, most often in bundles that are completely surrounded by and intimately associated with the ground substance (Figs. 1 *b* and 2). This pattern is similar to the collagen distribution in other tissues and has been widely studied (19, 17). Our component X_c is actually representative of collagenous bundles

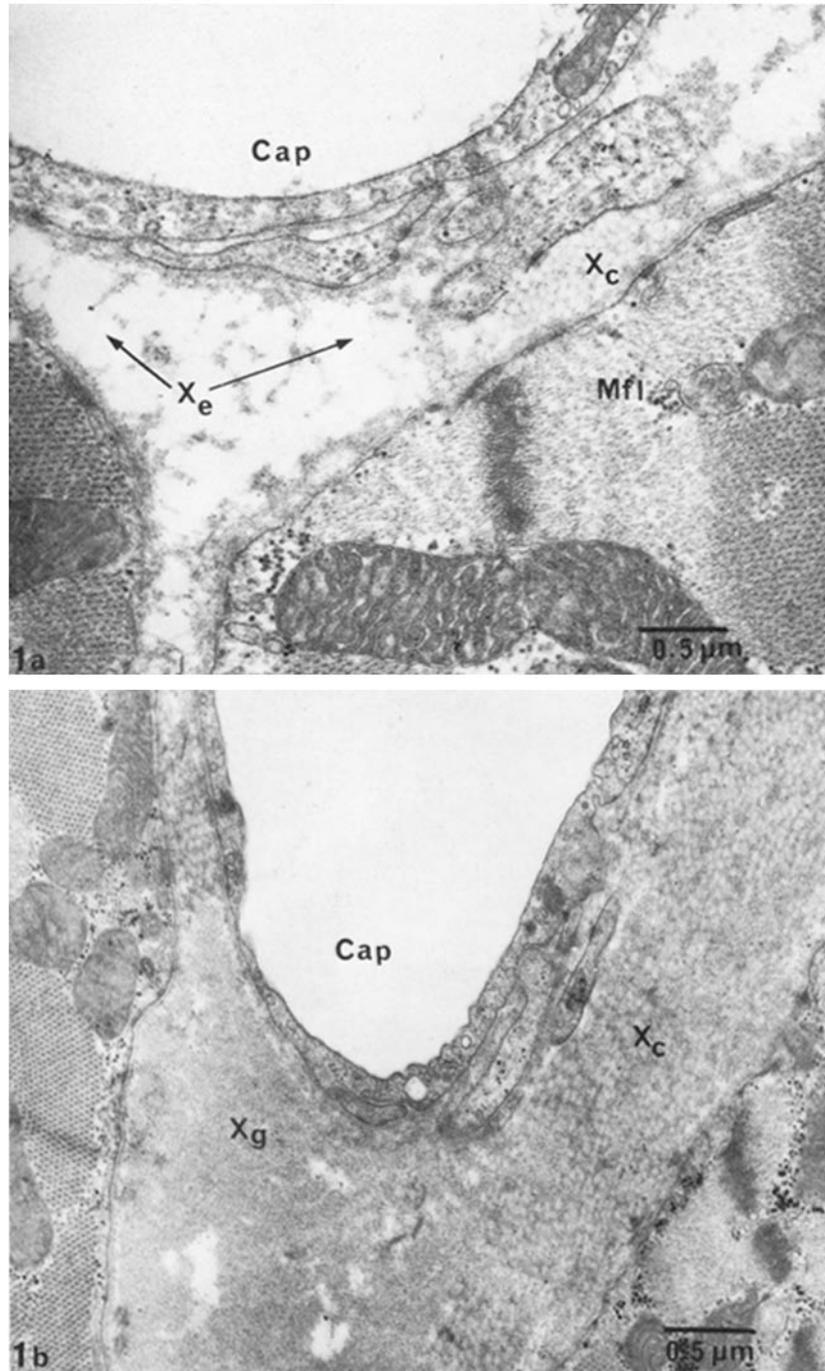


FIGURE 1 *a* Micrograph illustrates portion of extracellular space devoid of structure (X_e). Portions of cardiac cell and capillary are evident. *Mfl*, myofilaments; X_c , collagen; *Cap*, capillary. $\times 32,000$.

FIGURE 1 *b* Micrograph of portions of two cardiac muscle cells. The interstitial space between the cells is filled with ground substance (X_g). The rest of the interstitium is occupied by a capillary (*Cap*) and collagen (X_c). $\times 22,000$.

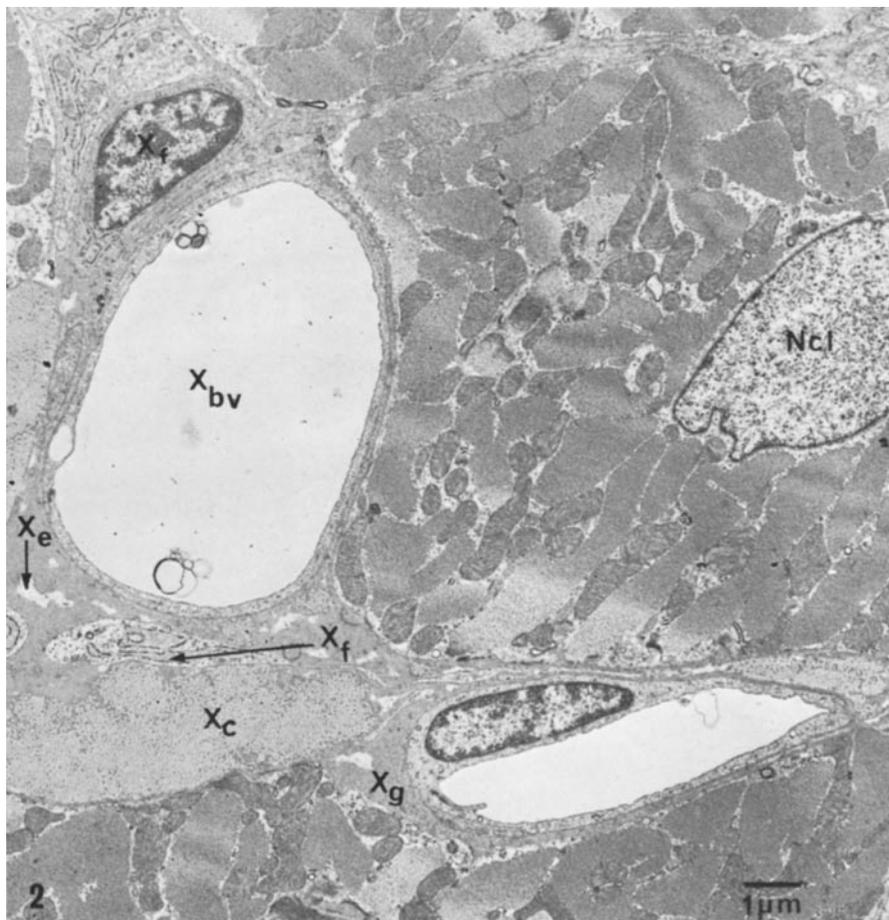


FIGURE 2 Transverse section of cardiac muscle illustrating all the components of the extracellular space: collagen (X_c) embedded in ground substance (X_g), connective tissue cells (X_f), empty space (X_e), and capillaries (X_{bv}). *Ncl*, nucleus. $\times 7500$.

which will include, in addition to collagen fibrils, mucopolysaccharide material in the interfibrillar spaces. At the magnifications used for our measurements, separation of these two components of collagen bundles could not be made. The cells in the extracellular space counted under the heading X_f include true fibroblasts, other cells of connective tissue origin, and the pericytes (6, 13) which are found closely associated with cardiac muscle cell capillaries (Fig. 2). The capillary endothelial cells are included in the component X_{bv} .

Extracellular Space

The percentage of the total tissue occupied by the extracellular space is 24.6% (this includes

X_{bv} , X_g , X_e , X_f , X_c ; see Table I, B). This value does not include the transverse (T) tubules, which add about 3% additional volume to extracellular space. The fact that this volume was obtained from fixed tissue makes difficult any direct comparisons with extracellular volumes determined in living tissue by radioactive tracer measurements. The difference between morphologically and chemically measured spaces (27.6% vs. 35.7%) is probably due to a number of factors. However, it does not appear to be due to a gain in tissue water over an 85 min period of perfusion with physiological saline, since the total tissue water increased by only 1.3% (see Table II). It is clear from studies of Page and Page (18) and others (7) that chemical labels of extracellular

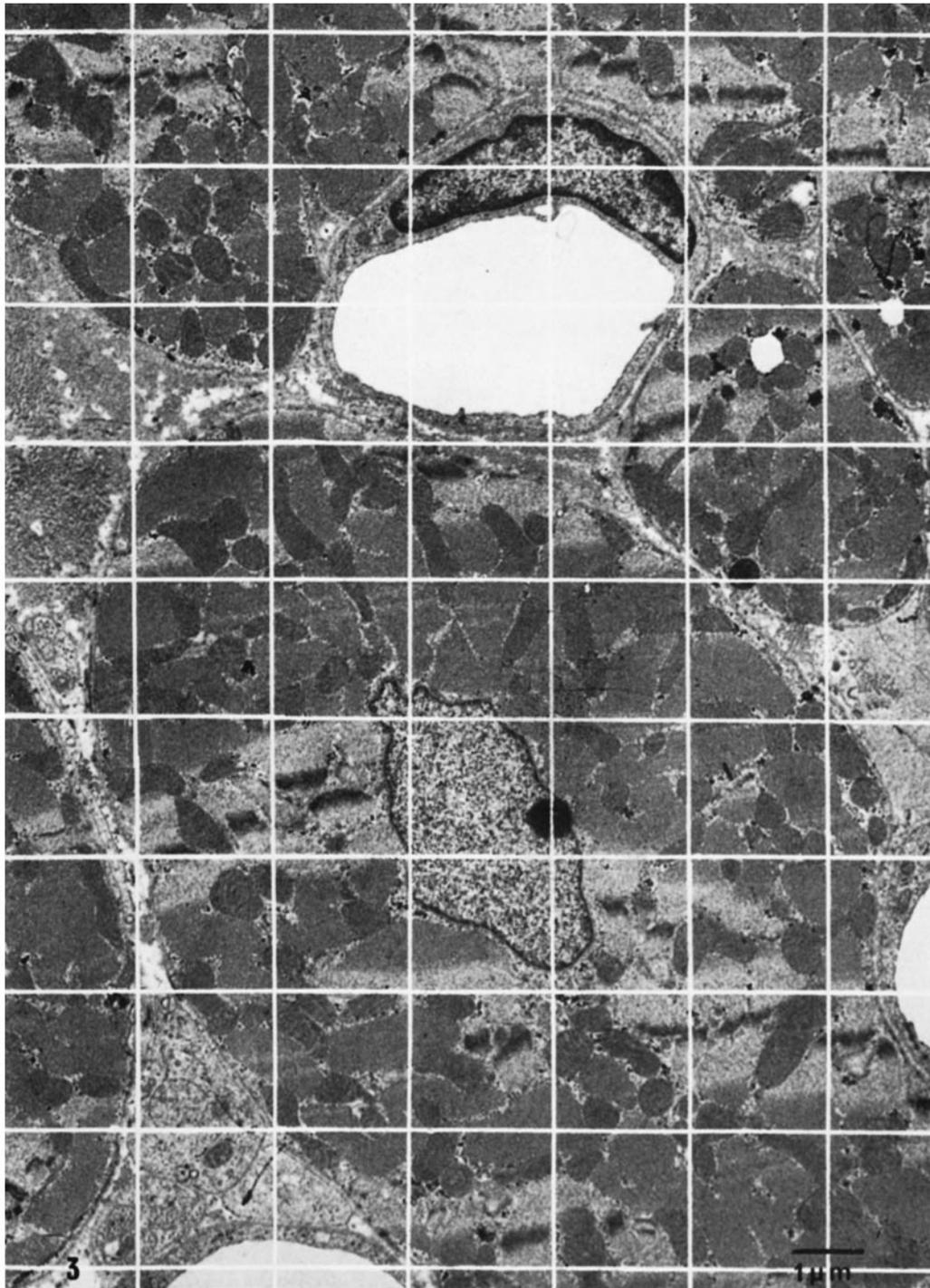


FIGURE 3 Electron micrograph of typical section of the myocardium used for stereological measurements with square grid superimposed. $\times 9500$.

TABLE II
Isotopic Measurements of the Extracellular Space

	5 min isotopic space (% wet wt)	Space change (%) (after 5 min 0.025% CPC)	Tissue water (%)	
			Pre-CPC	Post-CPC
[¹⁴ C]Sucrose	35.7 ± 1.5 (10)*	+13.8 ± 2.1 (10)		
³⁵ SO ₄	36.2 ± 2.5 (10)	+12.7 ± 2.7 (10)	84.6 ± 0.30	85.7 ± 0.29
¹⁴⁰ La	194.0 ± 30.3 (10)	-17.0 ± 3.1 (10)	(10)	(10)

± 1 SE.

* Number of muscles in each series.

space, such as sucrose and sulfate, in mammalian heart continue to gain access with time to areas within the tissue that are not morphologically measurable and that contribute greatly to the measured chemical space. The morphologically measured extracellular space is, on the other hand, subject to artifacts inherent in fixation procedures. In our case the overall tissue shrinkage was less than 10% but this type of measurement is hard to determine exactly because one does not know if all tissue elements shrink to the same extent. However, the rapid equilibration of the primary fixative (< 30 s) with extracellular space suggested predominant shrinkage of the myocardial cells. In addition, our morphologically and chemically measured extracellular spaces compare quite well with Johnson and Simond's study, where those authors specifically compared histological and chemical extracellular spaces in rabbit ventricle (7). However, the important point to be considered from our measurements is not the absolute size of the extracellular space, since this is difficult to measure accurately, but rather that so much of the space can be accounted for by the structured components we have described, namely X_g , X_{bv} , X_f and X_c . Moreover, there was no difference in organization or volumes of extracellular components in the sub-endocardial and mid portions of the septum.

CPC Studies

Five muscles were perfused with glutaraldehyde containing 0.1% CPC. CPC is a polyvalent cation that specifically associates with negatively charged sites on mucopolysaccharides, and, in so doing, causes their precipitation (26, 27). As the data in Table I indicate, the fractional volumes of X_g , X_c , X_f obtained from morphometrics on CPC

muscles are not significantly different from the controls. The exception to this is X_e or that portion of the space seemingly devoid of polysaccharide. There was a significant increase ($P < 0.05$) in volume of the empty portion of extracellular space in the CPC muscles, which appears to be due to interstitial edema. The percentage of the total tissue occupied by the extracellular space increased significantly ($P < 0.05$) in CPC muscles while other structural components of extracellular space (i.e., X_g , X_c , X_f , X_{bv}) did not exhibit any significant difference from the controls. This increase in the empty portion of extracellular space in CPC muscles can be compared to its effect on the size of the sucrose and sulfate spaces when CPC was used in these isotopic studies, keeping in mind that in the morphometric determinations CPC concentration was four times (0.1% vs. 0.025%) that used in the isotopic studies (see Table II).

Fig. 7 illustrates CPC effects on the ultrastructural configuration of the protein polysaccharide. The precipitate formed from CPC's association with the protein polysaccharide matrix forms floccular aggregates in contrast to the homogeneous mat of X_e in the controls (compare Fig. 7 to Fig. 1 b).

Myocardial Cell—Capillary Orientation

16 cells were selected from micrographs whose profiles were approximately cross-sectional. In addition, the profiles of the cells selected for this measurement were approximately through the center of the myofiber. Our measurements show that 36.3% ± 0.15 of the circumference of the myocardial cell is within 2000 Å of a capillary. This close association of myocardial cell to capillaries is illustrated in Figs. 4 and 5.

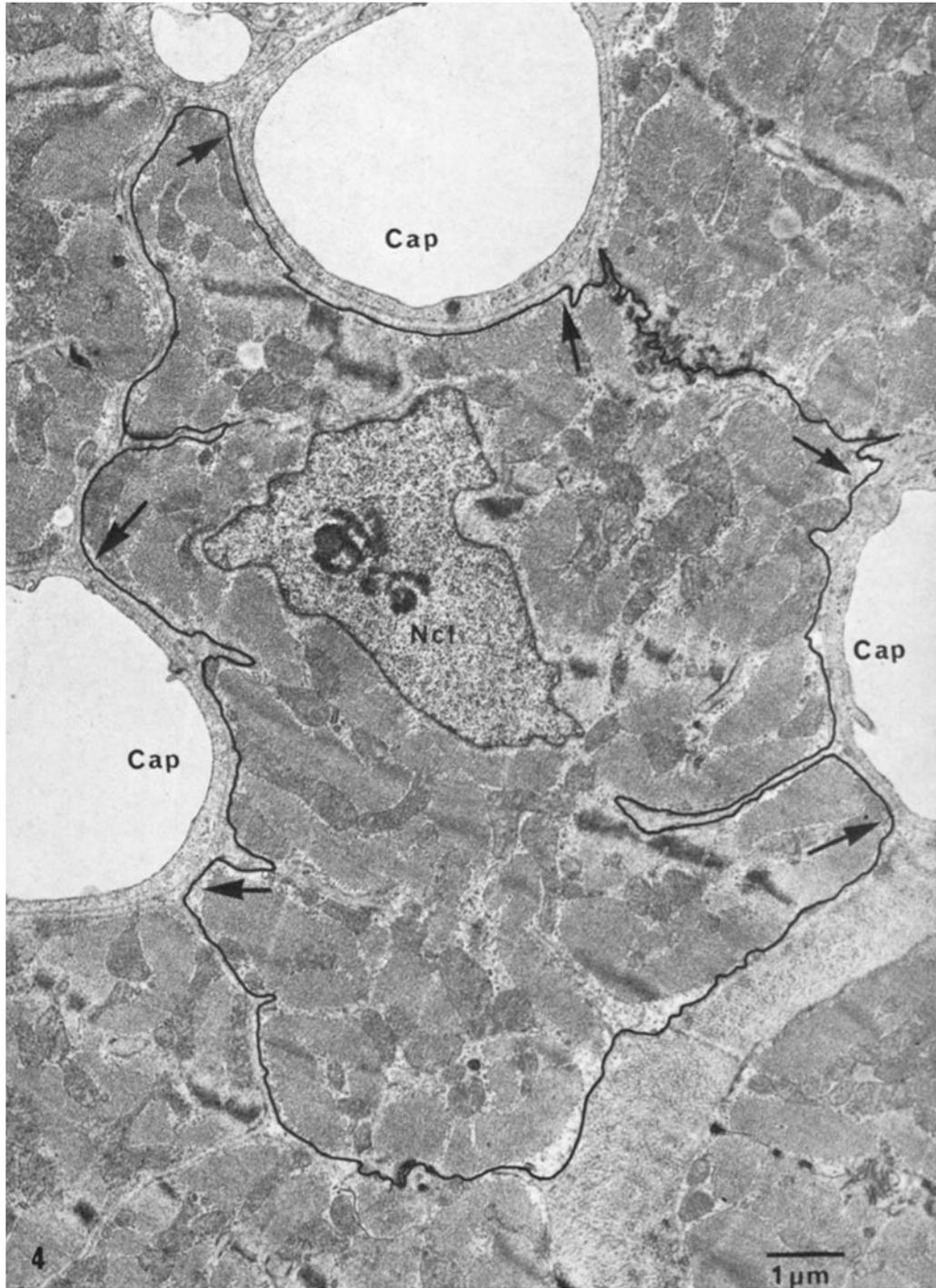


FIGURE 4 Electron micrograph of a transverse section showing a typical cell (cell boundaries outlined in ink) used for the measurement of the association of myocardial cell to capillary. Approximately 36% of the myocardial cell circumference is within 2000 Å of a capillary (*Cap*). The portion of the cell surface which is facing a capillary and between the indicated arrows is representative of this close association of cell and capillary. *Ncl*, nucleus. $\times 10,000$.

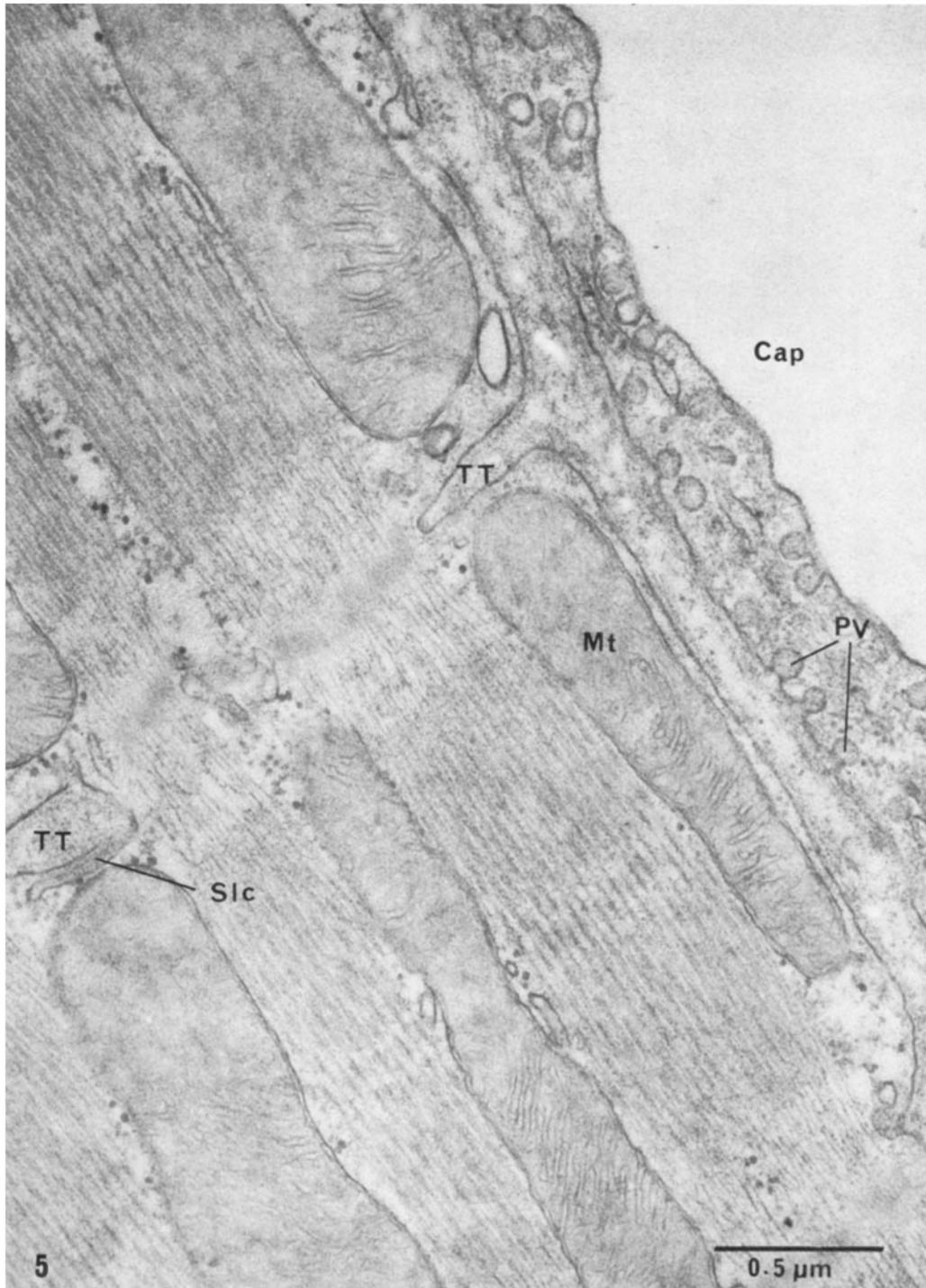


FIGURE 5 Higher magnification micrograph showing close association of muscle cell with a capillary (*Cap*). Note that the protein polysaccharide coating on the surface of the muscle and the capillary endothelial cell fills the interstitial space. The T tubules (*TT*) are also filled with similar material, as are the numerous pinocytotic vesicles (*PV*) of the endothelial cell. *Slc*, flattened saccules or cisternae of the sarcoplasmic reticulum; *Mt*, mitochondria. $\times 48,000$.

Isotopic Measurements of Extracellular Space

The extracellular space of the perfused septum was measured using three extracellular markers—sucrose, sulfate, and lanthanum (Table II). These are neutral, negatively charged, and positively charged, respectively. The control spaces were measured after a 5 min period of perfusion with the isotopically labeled marker, and the space was calculated on the assumption that the marker was distributed through the muscle in free solution. The values of 35.7% and 36.2% for sucrose and sulfate are not significantly different ($P > 0.80$). The La space differs markedly from the other two ($P \ll 0.001$). If La were distributed in free solution it would occupy 194.0% of the total tissue water. It is well known that La is confined to the extracellular space, and the high value of its space indicates extensive binding to extracellular structures (4).

Two septa were perfused with 0.5 mM concentrations of La (labeled with ^{140}La) for 200 min. The La contents at this time were 3.84 and 3.83 mmol/kg wet tissue. If La were distributed only in free solution in the 5 min sucrose or sulfate space a total content of 0.18 mmole/kg wet tissue would be expected. The values after 200 min indicate a marked binding of the trivalent cation.

The effect of the cation detergent, CPC, at a concentration of 0.025%, on the spaces was quite different, depending upon the charge on the marker. The space as measured by the neutral and negatively charged markers *increased* by 12–13%, whereas the La space *decreased* by 17%. This difference is highly significant ($P \ll 0.001$). The effect of CPC on the sucrose and sulfate spaces was not significantly different ($P > 0.70$). It is to be noted that 0.025% CPC produced an increase in tissue water (Table II) ($P = 0.01$). The structural studies indicated that this was largely attributable to an increase in the extracellular empty space (X_e), and is consistent with the production of interstitial edema by the CPC. This increase in “free” water space is consistent with the increase in extracellular space as measured by neutral and negatively charged markers. Despite this increase in space sizes, the La space was significantly decreased by a low concentration of the cation detergent and adds further support to the presence of a large number of extracellular fixed negative charges.

DISCUSSION

It is clear from Table I that the myocardial interstitium contains little, if any, empty space (X_e) under in vivo conditions. The value of 5.9% for X_e (Table I, A) is maximal since aggregation and dissolution of interstitial material during fixation will tend to produce empty areas.

The structured interstitium contains ground substance (X_g) and connective tissue (X_c and X_f) material. The ground substance is precipitated by the cationic detergent CPC (Fig. 7), indicating that it contains anionic components. Many studies (1, 25, 3, 22) have indicated that protein polysaccharide is abundant in the interstitium of many tissues, and it is well known that this material contains a large number of negatively charged groups, i.e., they are immobile polyanions. The ground substance (X_g), which amounts to 23% (Table I, A) of the stereometrically measured total extracellular space and which is distributed quite homogeneously throughout the myocardial interstitium, is likely to be the locus of abundant fixed, negatively charged sites. If blood vessels are excluded, the ground substance occupies over 58% of the extracellular space.

The anionic nature of this substance is supported by the isotopic extracellular marker studies. The extracellular space determinations as defined by the neutral (^{14}C]sucrose) and anionic ($^{35}\text{SO}_4$) markers were not significantly different at 36% wet weight (Table II). Perfusion with CPC, thereby neutralizing some of the negative charge, *increased* these spaces. This is consistent with the edema induced by CPC (as documented by the increase in tissue water [Table II] and in the ultrastructural measurements) and indicates the absence of extensive binding of these neutral and negatively charged markers. The measurements with a cationic marker ($^{140}\text{La}^{+++}$) were very different. The 5 min ^{140}La space would occupy 194% of the tissue water if it were distributed in free solution (Table II). This suggests a high degree of binding to extracellular negatively charged sites. This is confirmed by the action of CPC on the ^{140}La space. Despite the increment in edema, perfusion with 0.025% CPC *reduced* this space by 17%—a highly significant reduction from control and an even more significant change when compared to the effect of CPC on the sucrose and sulfate spaces.

^{140}La binding after 200 min of perfusion indi-

cates that there are extracellular sites capable of binding over 10 meq/kg tissue. This is not a maximum value since, after 200 min, ^{140}La uptake had not reached asymptote. Nor should the value be taken as a fixed quantity since it will certainly vary, depending upon the species of cation.

The extracellular stereometrics did not include the transverse (T) tubular system. It is known

that, in heart muscle, this system is lined with basement membrane (6, 30) which is largely protein polysaccharide and would, therefore, contribute negatively charged sites (28, 12). In addition, the remainder of the T tubular lumen is filled with material which appears identical to the interstitial ground substance (X_g , Fig. 6). T system lumina, then, represent invaginations of the

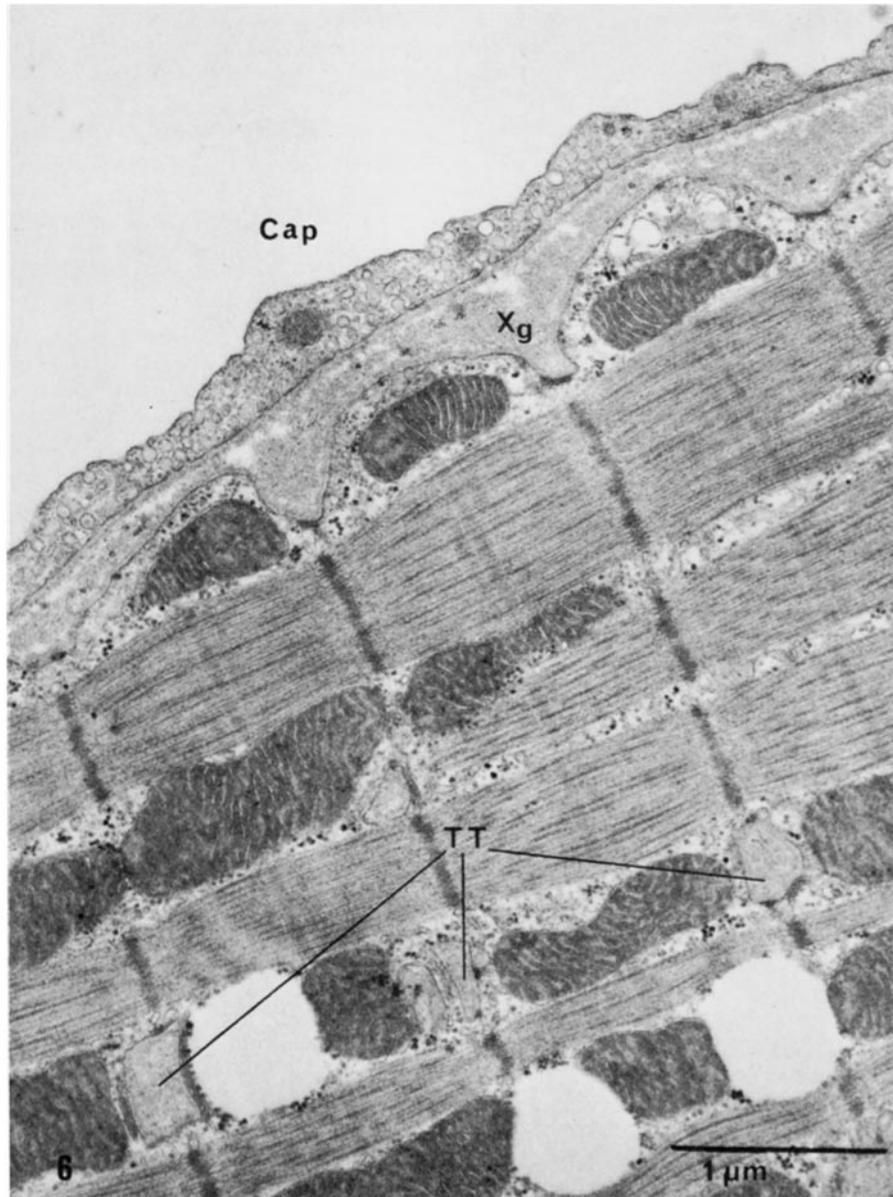


FIGURE 6 Longitudinal section of a portion of muscle cell bordered by a capillary (*Cap*). The ground substance (X_g) within the interstitium appears to completely fill the T tubules (*TT*). $\times 29,791$.

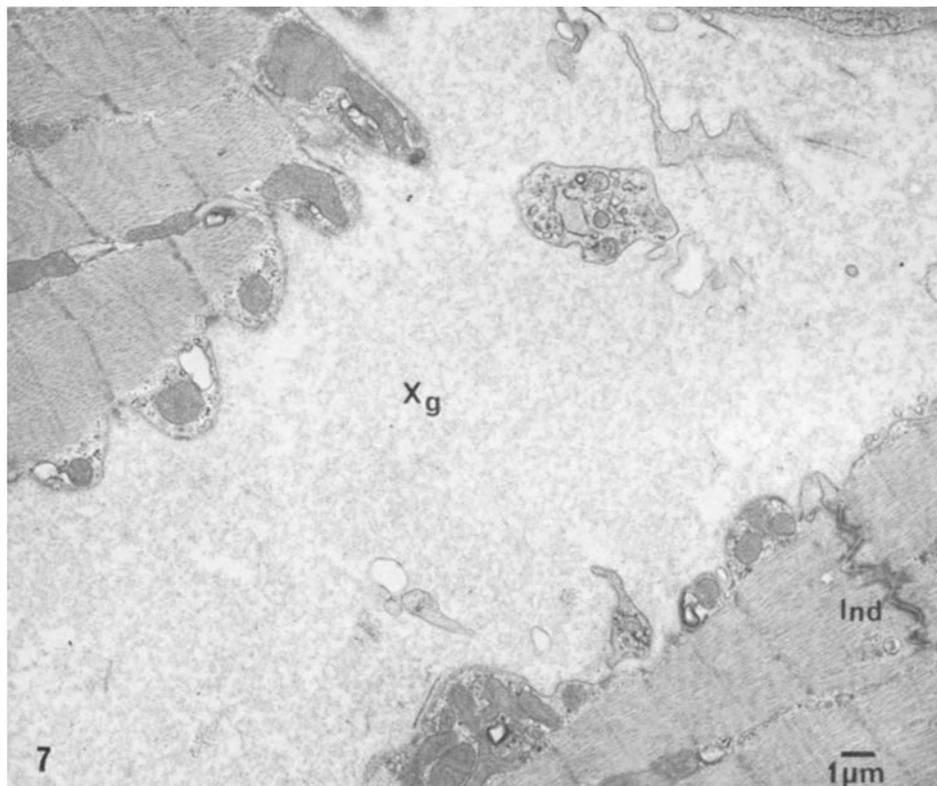


FIGURE 7 Micrograph of muscle treated with 0.1% CPC. The interstitium is filled with aggregated ground substance (X_g). *Ind*, intercalated disk. $\times 5000$.

interstitium and contain basement membrane and ground substance which seem to be similar in composition, at least with respect to their polyanionic nature.

In the study of the ultrastructural changes in mammalian myocardium produced by variations in the ionic composition of the perfusion medium, Legato et al. (15) postulated the presence of mucopolysaccharide in the T tubules. It was suggested that the T tubular swelling produced by perfusion with solutions deficient in chloride resulted from charge-induced changes in the hydration of the mucopolysaccharide gel (16) in the lumina. The study of Rapoport (21) on volume changes in the T system of frog sartorius suggested a model in which the tubules contained fixed negative charges attached to a polyelectrolyte matrix.

The structure and composition of the interstitium (including T tubular lumina) denote a number of physiological considerations:

(a) It is clear that one cannot estimate extra-

cellular content of a cation by measuring the extracellular space with a neutral or negatively charged material and multiplying this value by the concentration of cation in the perfusate. Such a computation ignores the component of cation which is bound.

(b) Protein polysaccharides have a particularly strong affinity for Ca^{++} ion (8). La^{+++} has been shown to be capable of displacing significant quantities of calcium as it uncouples excitation from contraction (24, 12). Much of this displaceable calcium exchanges with characteristics which indicate that it is localized to the interstitium (29). The presence of a calcium-binding polysaccharide matrix in the interstitium may augment the capacity for storage of the extracellular component of calcium which participates in the excitation-contraction coupling process in heart muscle (29, 24).

(c) The interposition of a negatively charged matrix in the interstitium may produce major effects upon ionic exchange between cell and

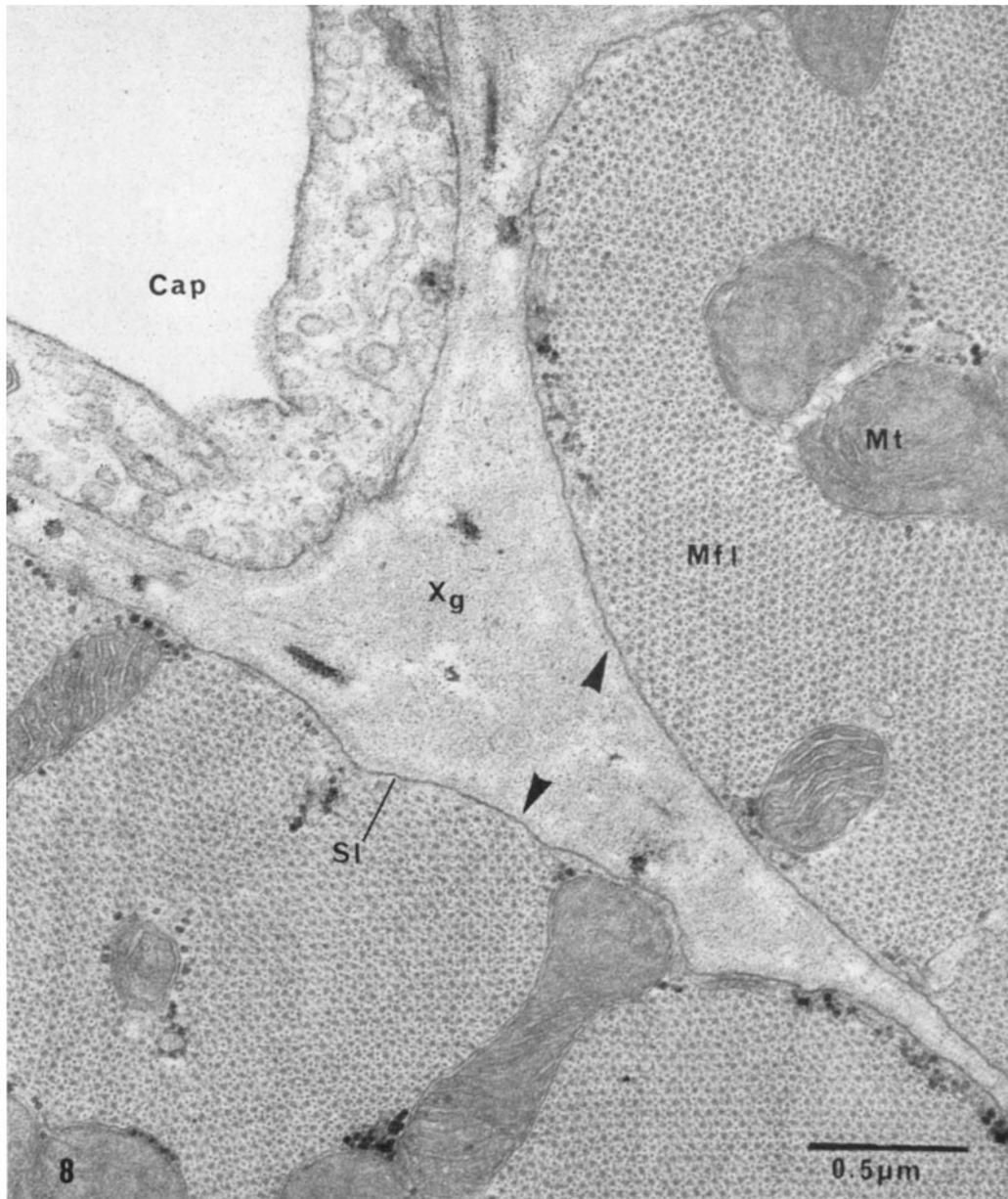


FIGURE 8 Portions of two cardiac muscle cells and a capillary cut in transverse section. The interstitial space between the cell and capillary is filled with ground substance (X_g). Basement membrane (arrowheads) of the myocardial cell and of the capillary (*Cap*) blend with X_g . *Sl*, sarcolemmal membrane; *Mt*, mitochondria; *Mfl*, myofilaments. $\times 43,000$.

capillary (1). The myocardium might be modeled as two chambers representing cell and vascular space separated by a region containing immobile polyanions representing the interstitium. Preston and Snowden (20) have constructed such a sys-

tem in order to study the movement of isotopes of diverse charge across the charged region. The system is in equilibrium with respect to non-labeled solute. Isotopically labeled solute is then added to an upper aqueous chamber, analogous

to an isotopically labeled cell. Its rate of movement is then followed across a membrane, analogous to the interstitium, to the lower aqueous chamber, analogous to the capillary lumen. The system in operation mimics an isotopic washout of arterially perfused heart tissue. The interposed membrane was composed of gelatin-agarose, and diffusion of various isotopically labeled substances was measured under two conditions: without and with negatively charged glycosaminoglycan incorporated into the membrane. As Preston and Snowden (20) point out, under the conditions of this system the isotopic flux, J_{s*} , is described by:

$$J_{s*} = \lambda_{s*}' \bar{D}_{s*} \frac{C_{s*}' - C_{s*}''}{d}, \quad (1)$$

where λ_{s*}' is the molar distribution of the solute, \bar{D}_{s*} is the diffusion coefficient of the solute within the membrane phase, C_{s*}' and C_{s*}'' are the concentrations of the isotopic solute in the upper and lower chambers, respectively, and d is the diffusional path length. In this system, λ_{s*}' is defined by:

$$\lambda_{s*}' = \bar{C}_s / C_{s*}', \quad (2)$$

where \bar{C}_s and C_{s*}' are the molar concentrations of untagged solute in the membrane and in the upper chamber, respectively. \bar{P}_{s*} , the operational permeability coefficient, is defined by:

$$\bar{P}_{s*} = \lambda_{s*}' D_{s*}. \quad (3)$$

Substituting in Eq. 1, the operational flux equation for washout is:

$$J_{s*} = \bar{P}_{s*} \frac{C_{s*}' - C_{s*}''}{d}. \quad (4)$$

In the case of the membrane without added negative charge, diffusion through the membrane for cations, anions, and nonelectrolytes was not significantly different from aqueous diffusion—thus $\bar{P}_{s*} = \bar{D}_{s*}$, indicating $\lambda_{s*}' = 1$. The incorporation of negatively charged proteoglycan in the membrane did not change the permeability coefficient for neutral, low molecular weight solutes. The effect on charged species was markedly different. The incorporation of negatively charged proteoglycan caused a marked increase in the permeability coefficient for Na^+

and a corresponding decrease for Cl^- . At a concentration of 2.5 mM NaCl the Na^+ permeability coefficient was increased 2.3 times in a membrane which contained proteoglycan with a charge density of 22.3 meq/liter. The effect of the negative proteoglycan on the Ca^{++} permeability coefficient at a Ca^{++} concentration of 5 mM was an increase of 1.5 times. This study indicates that the interposition of fixed negative charges between two regions in which substances are relatively freely diffusible creates a Donnan potential which results in an increase of the permeability coefficient for cationic substances. During isotopic washout studies this would augment the movement of tagged cations in the cellular-to-capillary direction through the interstitium according to Eq. 4. This would counteract the tendency for isotopic backflux to the cell.

(d) The measurements show that approximately 36% of the circumference of the myocardial cell at its widest dimension is less than 2000 Å from a capillary (Figs. 4 and 5). The percentage of extracellular space localized between capillary and 36% of the cellular surface is very small—less than 3% of the total extracellular space. In addition, this space is filled with polysaccharide. This arrangement suggests that a significant fraction of cellular-to-capillary exchange shunts the interstitial space. The cellular-capillary orientation and the negatively charged interstitial content with its deterrent effect on cationic backflux to the cell are pertinent considerations in the modeling of isotopic cation exchange in arterial perfused tissue. Both of these factors will operate to favor the selection of a parallel rather than a series model in the analysis of cation isotopic washout studies (9, 29).

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