

ACETYLCHOLINESTERASE STAINING OF PURKINJE FIBERS

Olav M. Sola, Lester R. Sauvage, Byron A. Kakulas, John Mc C. Howell, Qun Shi,
Dorothy L. Mungin, Nadia V. Salama, Valschia Malle, Mary-Ann Nelson, Warren A.
Berry, R. B. Vernon, L. E. Diehl

The Hope Heart Institute
Seattle, WA, USA

University of Washington School of Medicine,
Seattle, WA, USA

The Australian Neuromuscular Research Institute,
Perth, Western Australia

Department of Neuropathology, Royal Perth Hospital
Perth, Western Australia

INTRODUCTION

This is to report an improved method which visualizes Purkinje fibers not shown in the two previous manuscripts published on the web regarding a new method for AChE and H&E staining . These manuscripts can be found at:

<http://www.hopeheart.org/files/Research/sola.html>
<http://www.hopeheart.org/files/Research/sola2.html>

Using the following routine with a copper sulfate solution and adjusting the pH, white cardiac fibers appeared. Longitudinal and cross-sections were taken and photographed, including oil immersion. Although some of these findings have been reported previously, the new staining technique now allows a dimension for visualizing Purkinje fibers and their bursae.

HISTORICAL

In 1853, Jan Purkinje described impulse conducting fibers of the interventricular septum. These fibers showed striations similar to cardiac fibers, except they were much larger. Their function was not understood at the time, but they were referred to as Purkinje fibers from that time forward.

Early reports on the nerve supply of the heart date back to 1893 when Keng showed a large nerve passing from the trunk of the vagus to the superior sympathetic ganglion.

Thus, the relationship of the two autonomic nerves was established. Some fibers descended from the autonomic nerves to the surfaces of the ventricles.

In 1909, Curran detected in a fresh heart, that Purkinje fibers underwent a peristaltic action for a short time. He also noted the presence of bursae containing fluid surrounding the Purkinje fibers. In the same journal edition, L. DeWitt (1909) reported that careful removal of the myocardial tissues and vessels left the Purkinje fibers with bursae or sacs.

Histologists Maximow and Bloom (1942) described the Purkinje fibers as large capsules of glycogen connected by irregular groups of myofibrils, which later became known as myoneural fibers. More recently, Sheets, et al. (1983), carried out the isolation and characterization of single canine cardiac Purkinje fibers. When these fibers were stimulated, action potentials were recorded and vigorous contractions were noted.

A NEW METHOD OF AChE STAINING OF PURKINJE FIBERS

METHODS

The AChE staining solution was prepared by adding in sequence the following chemical mixture to 500 cc of distilled water. The chemicals and their proportions are as follows:

Allow each chemical to dissolve completely before adding the next one.

Na ₂ HPO ₄	3.76 g
NaH ₂ PO ₄	10.15g
Glycine	1.50 g
Acetylthiocholine Iodide	0.73 g
CuSO ₄ •5H ₂ O	1.04 g

It is extremely important to follow the above sequence, then adjust the pH to 5.0 - 5.5 after all the components are dissolved.

The ventricular walls were freed by incisions along the edge of the entire length of both sides of the interventricular septum. The entire fresh or fresh-frozen mammalian heart was suspended in the staining solution with the ventricular walls held laterally and soaked for three to five days. If non-staining areas were present, local injection of the heart tissue with staining solution enhanced the appearance of the nerves. A crystal of Thymol added at this point will preserve the stained specimen for weeks. Subsequently, biopsies of the AChE stained specimen were taken for H&E staining and placed in 10% formalin for three to five days.

Animals studied included rats, guinea pigs, dogs, pigs, monkeys, sheep and cows. All species showed a remarkably similar pattern of cardiac innervation. Images one through nine represent the best examples from the animals studied.

The specimens included cross-sections removed sequentially from the left ventricle epicardial tissue adjacent to the posterior longitudinal descending vessels.

RESULTS

Gross Morphology:

Within minutes after exposure to the staining solution, a fine white sheen appeared on all normal surfaces of the myocardium. Approximately 20 minutes later, a brilliant white stain of the individual epicardial and endocardial nerves was seen. These nerves showed a meandering pattern as they descended irregularly toward the apex. In adult hearts, the greatest concentration of cardiac nerves of the epicardium was on the left ventricle (LV) adjacent to the posterior longitudinal descending (PLD) vessels. In hearts that were only days or weeks old, right ventricular nerves were predominant, especially near the PLD. Freshly incised myocardium showed no reaction to the AChE staining. The endocardial surface stained an even brighter white than the epicardial surface. Again, the vast array of cardiac nerves stained white-on-white, especially structures on the inner surfaces of the endocardium, such as the papillary muscles, chordae tendinae, trabeculae and muscular carnae. The very bright white color was present in all structures except in the numerous recesses of the endocardium of the right ventricle. The bundle of His was readily visualized entering the LV at the anterior ventriculo-septal junction. Large branches passed laterally and inferiorly in remarkably similar patterns in different species. Bundles of nerves tended to “pile up” and bulge into the LV lumen inferior to the bundle of His. Regional patterns were regularly seen, such as the formation of multiple loops on the anterior endocardial wall.

Microscopic:

The pattern of Purkinje nerves was one of meandering. Fibers nearly one millimeter wide appeared from under the fatty layer of the atrio-ventricular sulcus and spread across the epicardium. The nerves (70-80 ums) meandered inferiorly and narrowed from the original width towards the apex.

Purkinje fibers on both endocardial and epicardial surfaces were distinguished readily by their white appearance after the AChE stain. Each Purkinje fiber was surrounded by a bursa which contained clear fluid. After H&E staining, the fibers were prominent for their wide sausage-like appearance and multicolors of red, blue and purple areas. In most longitudinal sections, striations similar to cardiac fibers were present, although larger. Differences between Purkinje fibers and cardiac muscle fibers were noted in the following table and images . The presence of fluid, connective tissue and fat all serve to protect and isolate Purkinje fibers from injury or impulses.

SUGGESTED READING

I

Keng, L. B. On the nervous system of the heart. The Journal of Physiology 1893, pgs

462-482 (6 figures) Vol. XIV. Vagus was seen passing a large nerve to the superior sympathetic ganglion.

II

Curran, E. J. A constant bursa in relation with bundle of His with studies of the auricular connections of the bundle. *Anatomical Record* III, 9, 1909, pgs 618-631 (8 illustrations) Established in several species the presence of space or bursa containing fluid around Purkinje fibers.

III

DeWitt, L. M. Observations on the sino-ventricular connecting systems of the mammalian heart. *The Anatomical Record* III, 9, 1909, pgs 475-497. Removed all fibers except Purkinje.

IV

Sheets, M. W., January, C. T., and Fozzard, W. A. Isolation and characterization of single Purkinje cells. *Circ Res* 53, 4, 1983, pgs 544-548. Showed stimulation and relaxation of isolated Purkinje cells.

V

Berne, A. M., Levy, M. N. *Cardiovascular Physiology*, Sixth Edition, Mosby Yearbook, 1997, pgs 34-35.

VI

Richardson, H. W.,(ed) *Handbook of copper compounds and applications*, Marcel Dekker, Inc., 1997, pgs 56-57.

VII

Maximow A.A. and Bloom, W., *Text Book of History*, Saunders 4th ed., 1942, pg 175

COMPARISON OF MYOCARDIAL CELLS, PURKINJE FIBERS, VAGOSYMPATHETIC FIBERS, AND NARROW FIBERS AFTER AChE AND H&E STAINING

	MYOCARDIAL CELLS	PURKINJE FIBERS	VAGOSYMPATHETIC FIBERS (WIDE)*	NARROW FIBERS*
Location	Myocardial wall	Epicardium and endocardium	Epicardium and endocardium	Very superficial epicardium and endocardium
Width	10-15 ums	70-80 ums	1.0 mm	0.10-0.15 mm

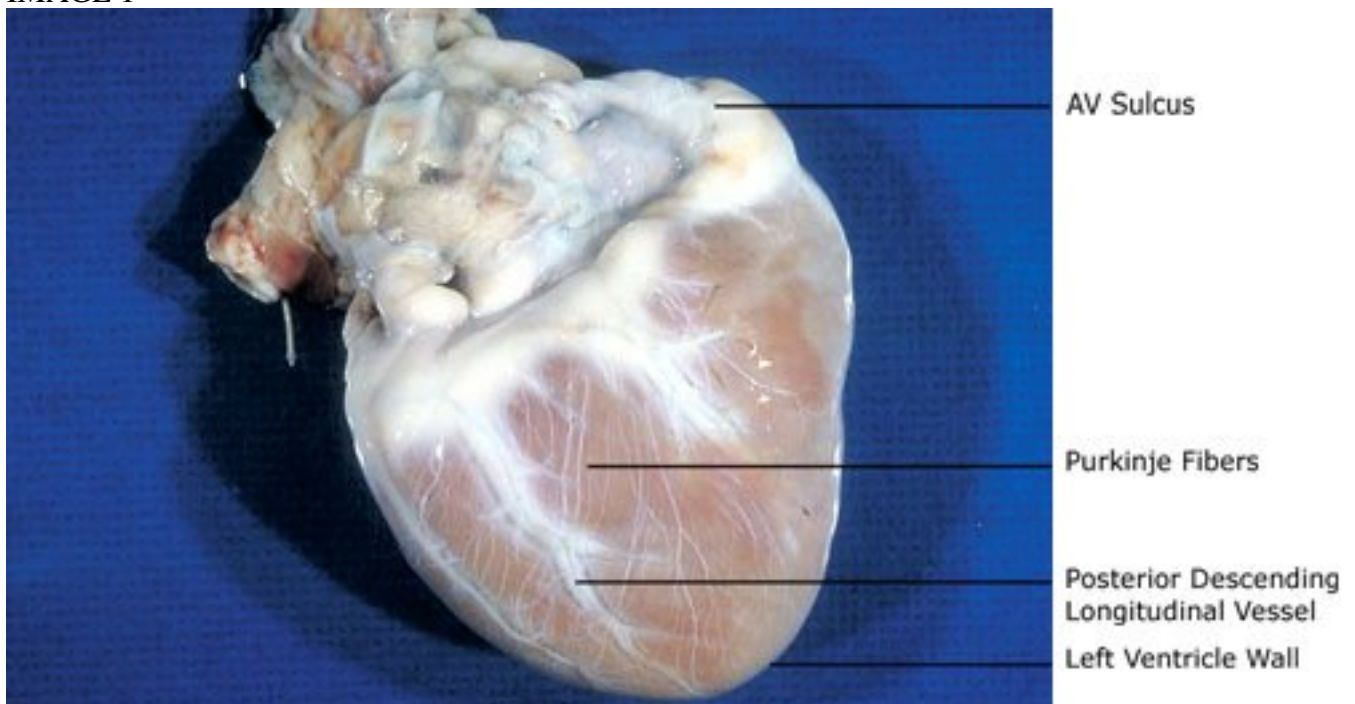
Color	Red, Brown	Red, blue, purple	White	White
Branching	Form nexus	Meandering	Arborization	Nexus
Striations	4+ Regular	3+	Absent	Absent
Sarcoplasm	No special features	Black inclusions	No special features	No special features
Nuclei	2-3+	2+	None	None
Bursae	None	Present	None	None
Blood Vessels	Present	Absent	Terminal branches only	Absent
Glycogen	1+	4+ cells	2+	None

* See manuscript #2 Image 8. Please note the vagosympathetic (wide) and narrow fibers (function unknown of narrow fibers) appear at pH 6 but not at pH 5.0 to 5.5.

Acknowledgements

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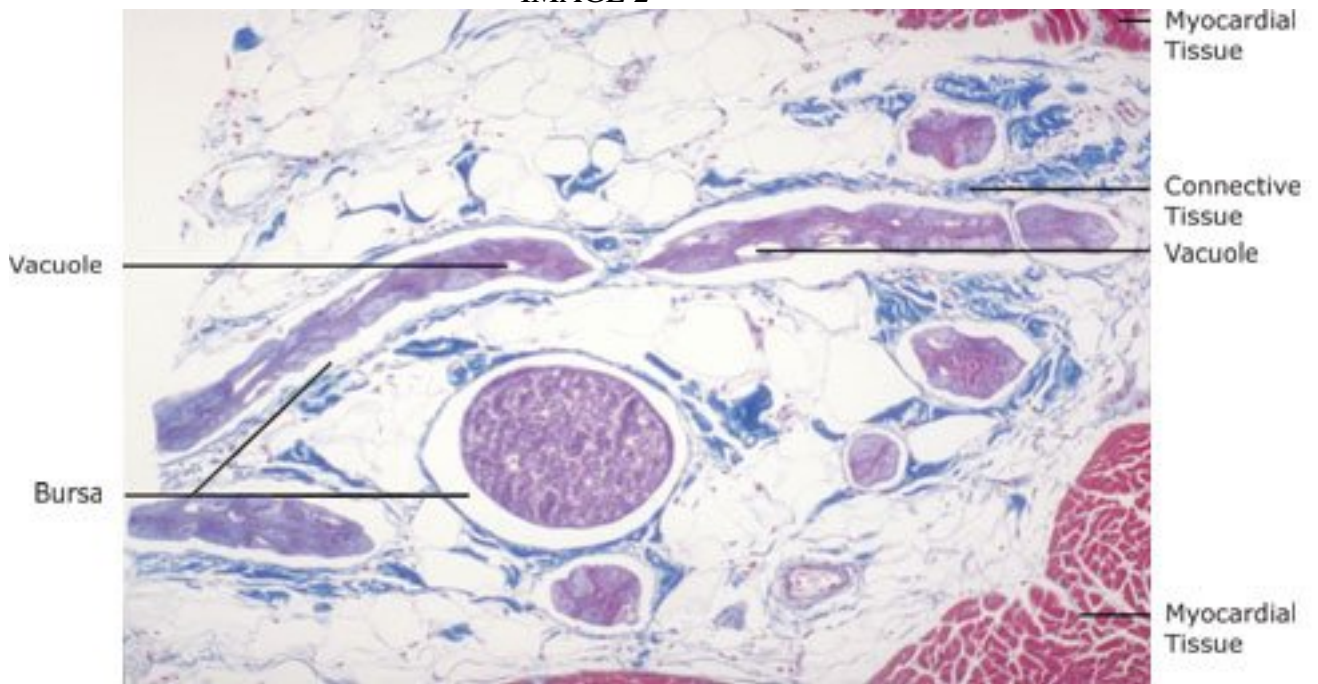
IMAGE 1



POSTERIOR SURFACE OF A COW HEART STAINED FOR ACETYLCHOLINESTERASE (AChE)

1. Large vertically descending vessels stained brightest white.
2. Oblique white epicardial nerves arose from coronary sulcus.
3. White Purkinje fibers were present.

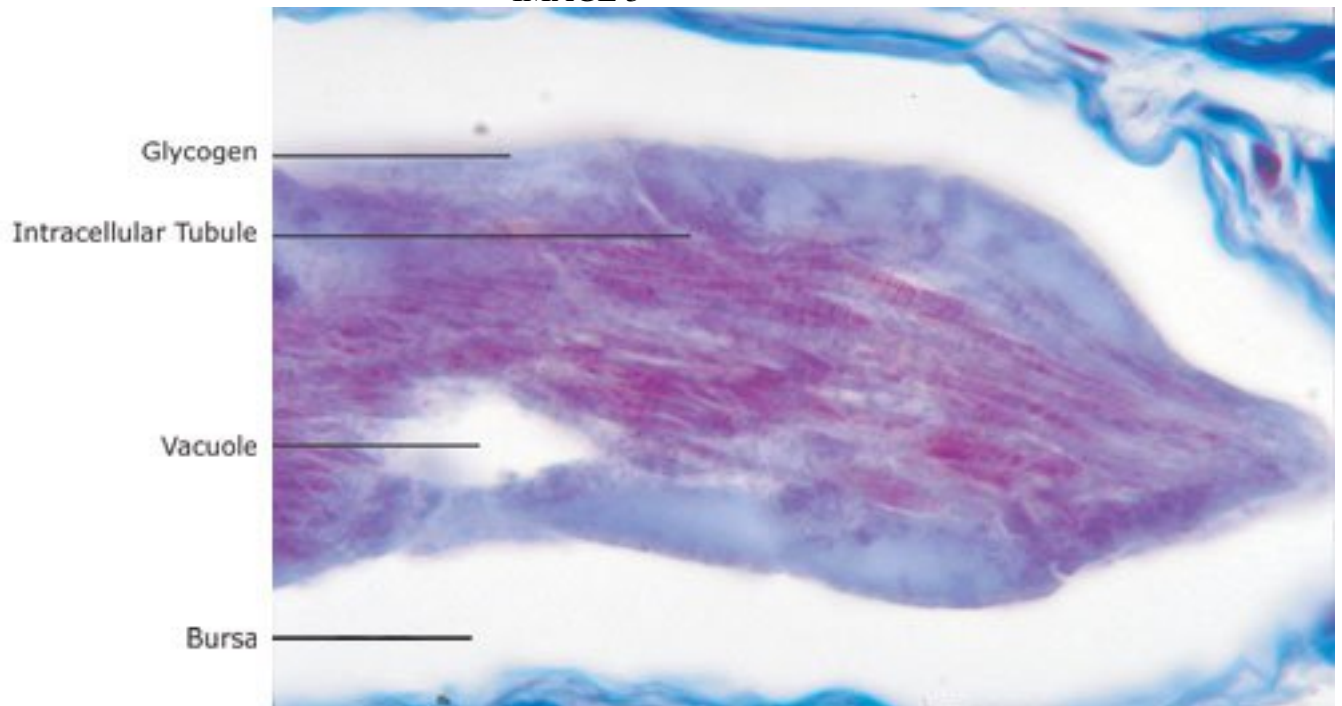
IMAGE 2



MICROSCOPIC CROSS SECTION OF EPICARDIUM (10X)

1. Image revealed heart muscle and epicardial fat plus longitudinal and cross-sections of AChE and H&E stained Purkinje fibers.
2. Wide clear areas formed a sac or bursa around Purkinje fibers which in turn, were surrounded by a border of blue-green connective tissue.
3. Longitudinally sectioned fibers showed clear areas or vacuoles containing fluid. Purkinje fibers stained red-brown; glycogen cells stained purple.
4. For comparison, myocardial tissue was included in two corners.

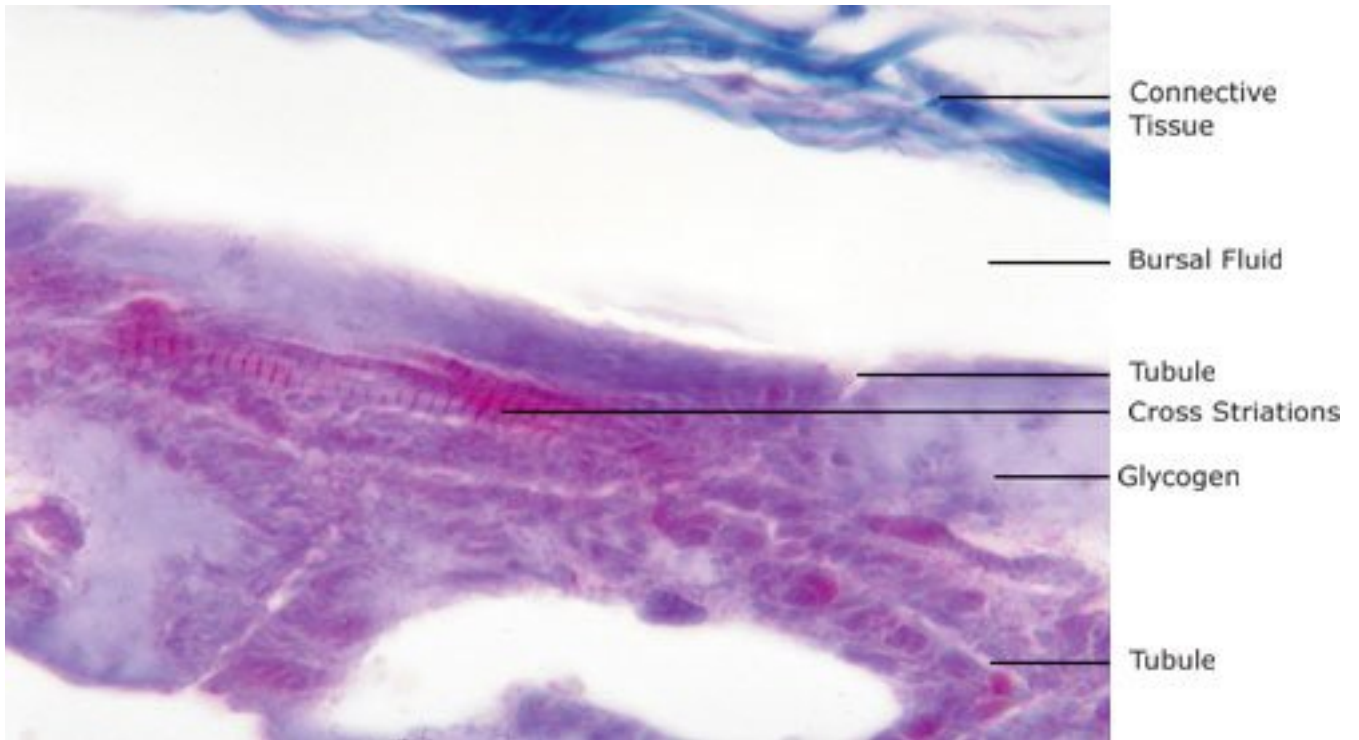
IMAGE 3



HIGH MAGNIFICATION (400X) FROM CENTER OF LONGITUDINAL PORTION OF PRUKINJE FIBER FROM PREVIOUS PAGE

1. Red fibers showing striations dominated the central part of nerve fibers. The purple peripheral areas contained glycogen.
2. Intracellular channels or tubules ran longitudinally. They appeared to divide and connect from the vacuoles to the extracellular space.
3. Wide clear bursal spaces were present between fibers and blue-green connective tissue.

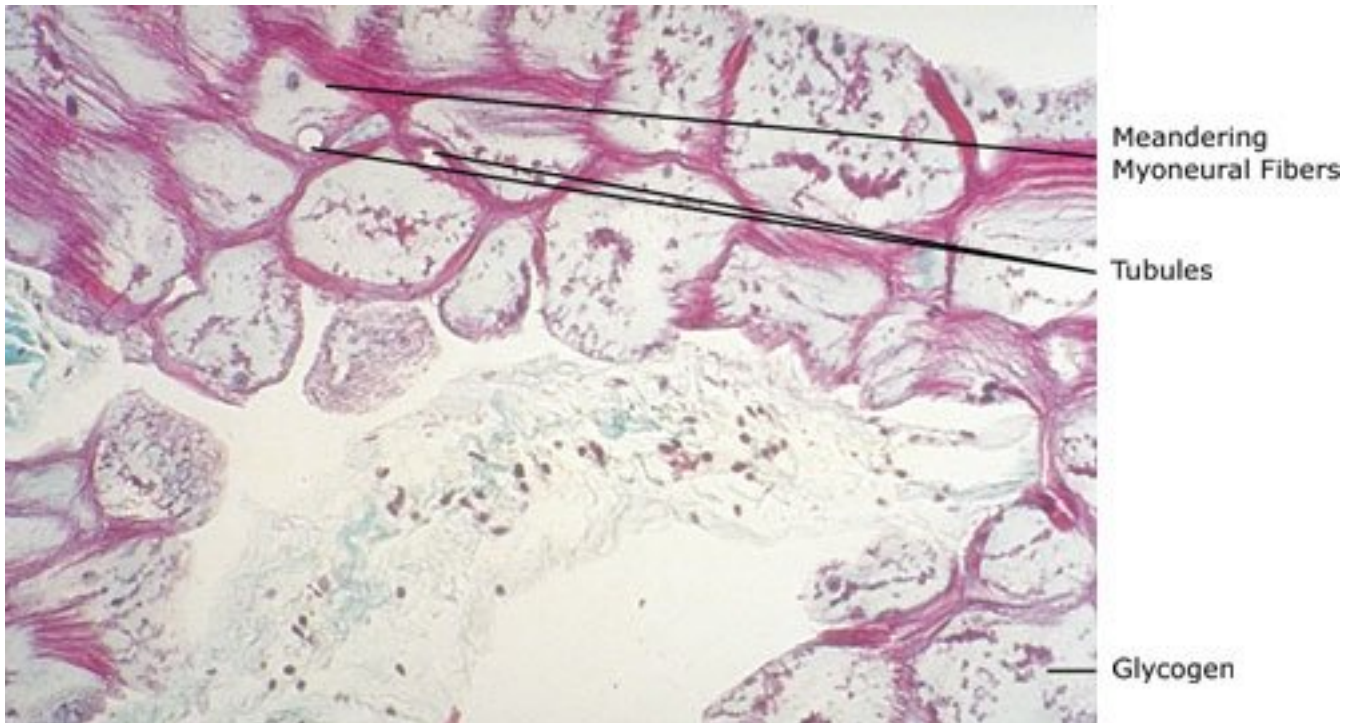
IMAGE 4



LONGITUDINAL SECTION (400X) OF PURKINJE FIBERS WITH STRIATIONS AND TUBULES

1. Cross or vertical striations stained in red-brown represented myoneural fibers.
2. Glycogen granules were present at either end of image.
3. Purkinje fibers were 60-90 ums wide.
4. Narrow straight tubules ran between glycogen and myoneural fibers.

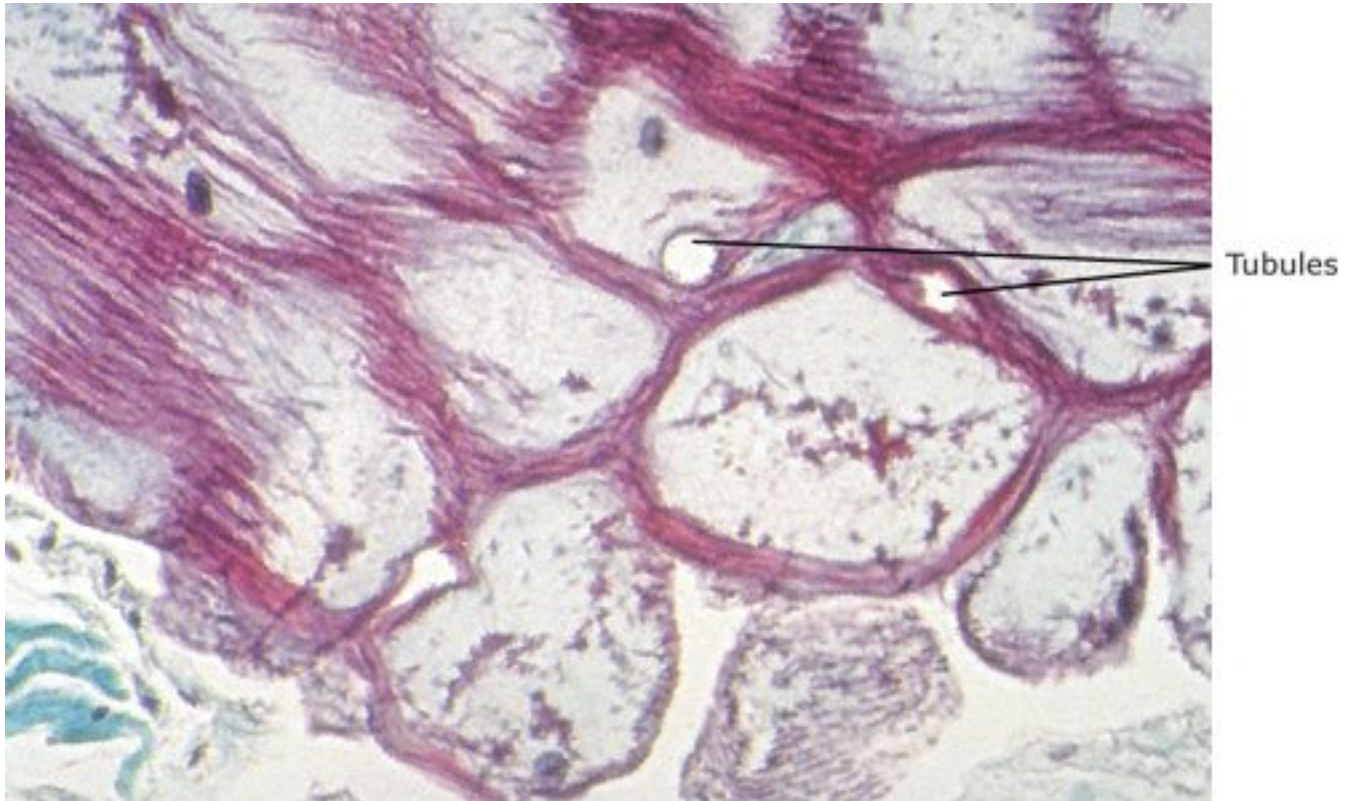
IMAGE 5



LONGITUDINAL SECTION PURKINJE FIBER MAGNIFIED 400X (OIL IMMERSION)

1. Area contained meandering myoneural fibers.
2. Myoneural fibers appeared to form walls around clumps of glycogen. Tubules were visible.

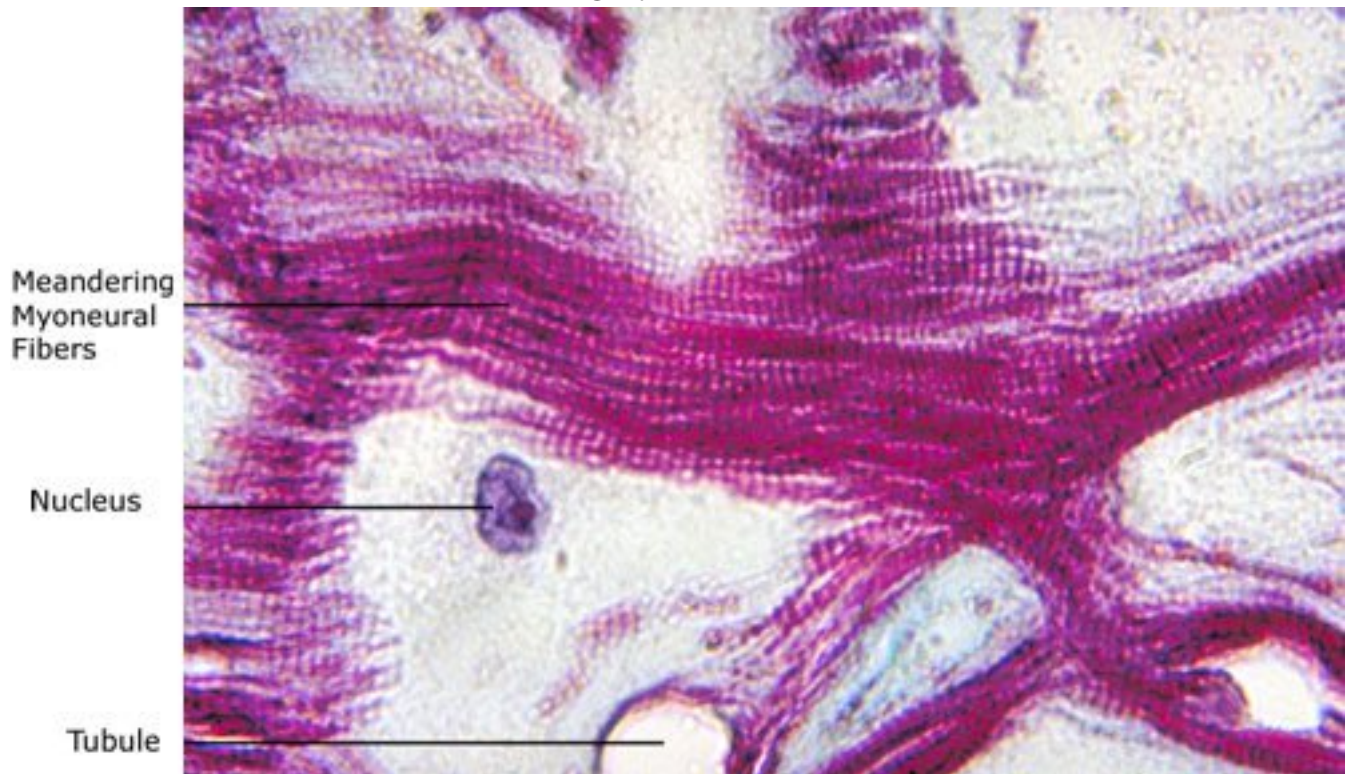
IMAGE 6



**CROSS SECTION OF DISTAL PURKINJE FIBERS
(enlargement of Image 5)**

1. Glycogen areas were predominant.
2. Small empty tubules were present within and between glycogen areas.

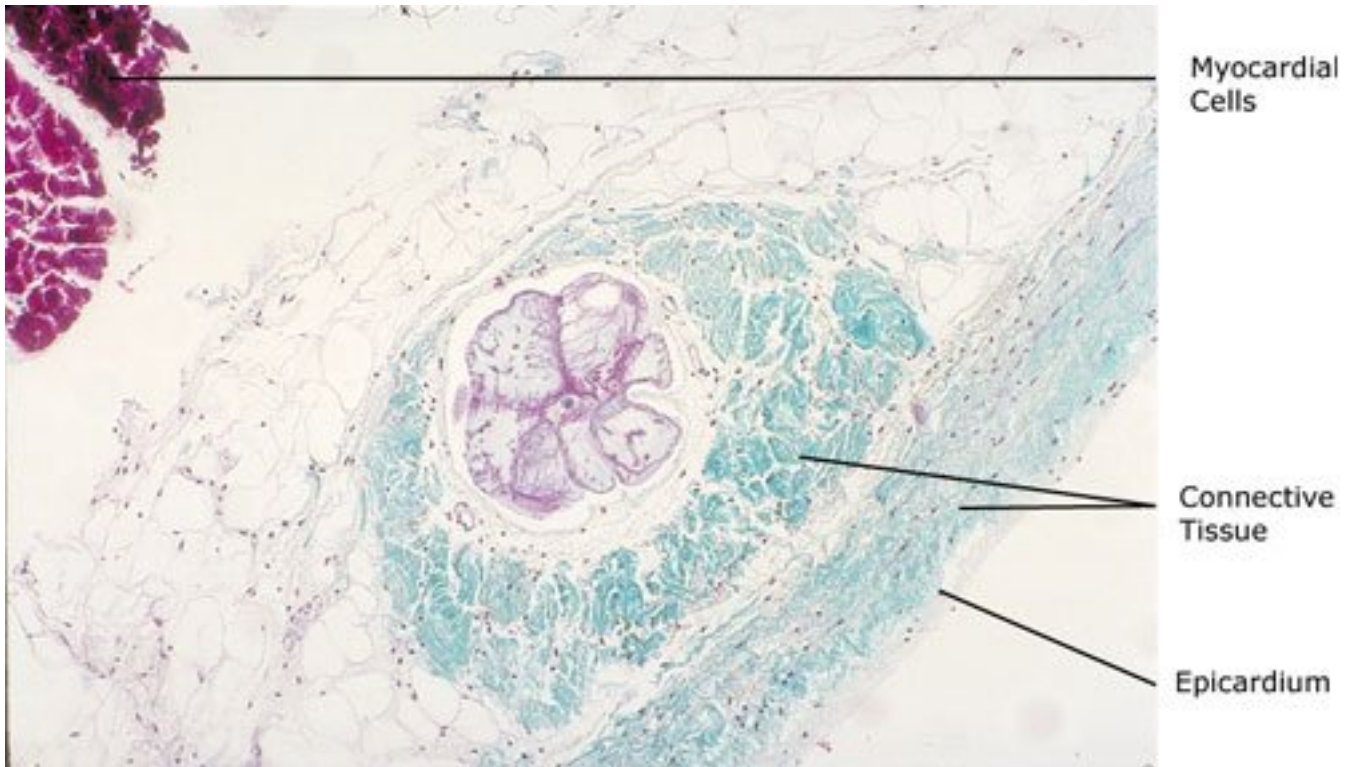
IMAGE 7



**LONGITUDINAL SECTION OF PURKINJE FIBER
(enlargement of Image 6)**

1. Image showed combination of glycogen and meandering myoneural fibers suggesting close interplay.
2. Striations were clearly visible in the myoneural fibers.
3. A single nucleus was present.

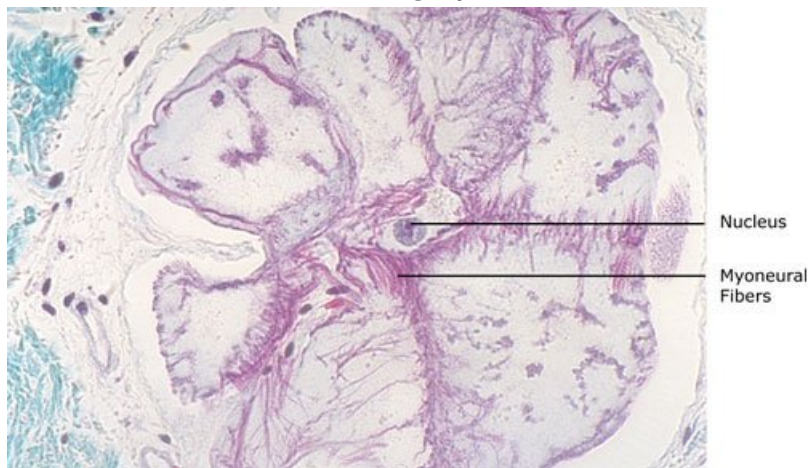
IMAGE 8



CROSS SECTION OF PURKINJE FIBER SHOWING RELATIONSHIP TO HEART AND EPICARDIUM

1. Cardiac cells were included for size comparison.
2. The Purkinje fiber was closer to the epicardial surface than to the cardiac cells.
3. Green connective tissue surrounded the bursa containing the Purkinje fiber.

IMAGE 9



**MAGNIFICATION 400X (OIL IMMERSION) OF
TRANSVERSE SECTION OF PURKINJE FIBER**

1. Area sampled showed predominant glycogen granules with myoneural fibers between the clumps of glycogen.
2. The Purkinje fiber contained invaginations and irregular surfaces.