Histological and Ultrastructure of the Peripheral Nerve in Cadaveric Embalmed Specimens and in Fresh Cadavers: the Efficacy of Several Fixatives

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ABSTRACT

Objective: The study is to observe the histological structure and the ultrastructure of the peripheral nerve from cadaveric embalmed specimens and from fresh specimens by light microscope and by transmission electron microscope. Also to study the efficacy of the embalmed fixative to the tissue.

Methods: The peripheral nerves were dissected from the arms of five cadavers of the Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, and from fresh cadavers. Each specimen was bisected, one put into 10% formaldehyde which was prepared for routine H&E staining and study by light microscope. The other was put into 2.5% glutaraldehyde, the best preserved specimen was then chosen to prepare for the TEM study.

Results: There is no significant difference between the peripheral nerves of the cadaveric embalmed and the fresh specimens when viewed with the light microscope. On the other hand when viewed by transmission electron microscope, the lipid part of the myelin sheath of the peripheral nerves from the cadaveric embalmed specimens are totally degenerated while the protein part is still intact. While in the fresh specimens which are fixed by 2.5% glutaraldyhyde, there is a complete preservation of the lipid and protein part of the myelin sheath.

Conclusion: The cadavers were fixed by excess formalin injection into the femoral artery and embalmed in formalin for at least 1 year, this could not preserve the lipid part of the myelin sheath. However, in the fresh peripheral nerves fixed in 2.5% glutaraldehyde it could preserve the lipid and proetin parts of the myelin sheath perfectly.

Keywords: Cadaveric embalmed specimen, peripheral nerve, TEM

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Peripheral nerves are composed of numerous nerve fibers collected into several fascicles or bundles. These bundles except for a few very thin nerves made up of unmyelinated fibers, nerves have a whitish, homogenous, glistening appearance because of their myelin and collagen content. The nerve bundles possess a thick connective tissue sheath, the epineurium which is the outermost layer of the three connective tissue investments covering nerves. It is composed of dense, irregular, collagenous connective tissue containing some thick elastic fibers that completely ensheath the nerve.[1-4] Collagen fibers within the sheath are aligned and oriented to prevent damage by over stretching of the nerve bundle. The epineurium becomes progressively thinner as the nerves branch into smaller nerve components, eventually disappearing. Each fasicle within the epineurium is surrounded by a perineurium, the middle layer of connective tissue investment, consisting of an outer dense connective tissue layer which is thinner than the epineurium, and an inner layer of flattened epitheloid cells joined by zonulae occludentes or tight junctions, making the perineurium an effective barrier to penetration of macromolecules and is surrounded by a basal lamina that isolates the neural environment. Between the layers of epitheloid cells are sparse collagen fibers oriented longitudinally and intertwined with a few elastic fibers. The thickness of the perineurium is progressively reduced to a sheet of flattened cells. Each nerve fiber and associated Schwann cell has its own slender connective tissue sheath, the endonurrium, the innermost layer of the three connective tissue investments of nerves, which surrounds individual nerve fibers. A loose connective tissue composed of a thin

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layer of reticular fibers which are produced by the underlying Schwann cells, scatter fibroblasts, macrophage, capillaries and perivascular mast cells in extracellular fluid. The endoneurium is in contact with the basal lamina of the Schwann cells. Near the distal terminus of the axon, the endoneurium is reduced to a few reticular fibers surrounding the bassal lamina of the Schwann cells of the axon.

The nerves of the PNS consist of varying numbers of myelinated and unmyelinated axons originating from neurons located in the brain, spinal cord, or ganglia. The myelin sheath is a highly refractile layer that invests the axon. The lipids make up the bulk of this layer which are cholesterol, phospholipid and glycolipids which are extracted in specimen preparation for light microscopy, leaving behind a delicate network of material called neurokeratin. Myelin is preserved by fixing in osmium tetroxide for electron microscopy.[5-7] It appears as a dense layer of varying thickness, made up of concentric dense and less-dense lines.

The highly specialized cells that envelope all axons in the peripheral nerve are called Schwann cells which provide both structural and metabolic support. The non-myelinated nerve fibers are axons simply enveloped by the cytoplasm of Schwann cells. The myelinated nerve fibers are wrapped by a variable number of concentric layers of the Schwann cell plasma membrane forming a myelin sheath. The single segment of myelin produced by each Schwann cell is termed an internode; which ensheaths the axon between one node of Ranvier and the next.

As the myelin components are mainly lipids, it is very important when one has to study about the nerve fibers histologically, because the fixatives used are essential to the micrograph and also the interpretation. We tried to study the nerve fibers in the cadaveric embalmed specimen and compare to the fresh specimen fixed in glutaraldehyde. Light and electron microscopy were used to view the details.

MATERIALS AND METHODS

The peripheral nerves were dissected from the arm of five cadavers of the Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok. The cadavers of the Department were routinely preserved by using an injected fixative formula of formalin, alcohol and carbolic acid mixture which was injected through the femoral artery with slight pumping force. Then the cadavers were kept in the soaked formula fixative of glycerine and carbolic acid mixture for 1 or 2 years before bringing to the gross anatomy dissecting room.

The whitish and glistening appearance of nerves, the good preserved representatives, were chosen for the study. Each nerve from the cadavers was bissected and put into two kinds of fixatives. The first kind was 10% formaldehyde, the nerves which were prepared in this fixative were used for the routine Haematoxylin and Eosin staining for light microscopic study. The other was 2.5% gluteraldehyde, in which the nerves were prepared for the routine transmission electron microscopic study.

From the fresh cadavers, the peripheral nerves were dissected and put into the two kinds of fixatives. The nerves fixed in 10% formaldehyde were prepared for the routine H&E staining for light microscopy and the nerves fixed in 2.5% glutaraldehyde were prepared for routine transmission electron microscopy as was done with nerves from the embalmed cadaver.

RESULTS

Figure 1 is routine formalin preserved fresh peripheral nerve fibers while Figure 2 is the nerve fibers of the cadaveric embalmed cadavers. Both groups were cut longitudinally and stained with hematoxylin and eosin. When viewed by light microscope, they show no significant difference and look similar to the micrographs in several textbooks of histology. Briefly, in routinely fixed and stained preparations (Fig 1), in the nerve fibers of the cadavers (Fig 2), myelin (M) is poorly preserved since it is largely composed of lipid material. Schwann cell cytoplasm is, however, well-preserved and has eosinophilic staining properties. All nerve fibers contain axons of many different types and most of which are myelinated. Heavily myelinated fibers (M) can be identified by the unstained parts covering the centrally located axon (A). Schwann cells (S) are distributed among the nerve fibers and they show oval-shaped nuclei, while the fibroblast nuclei are flattened.

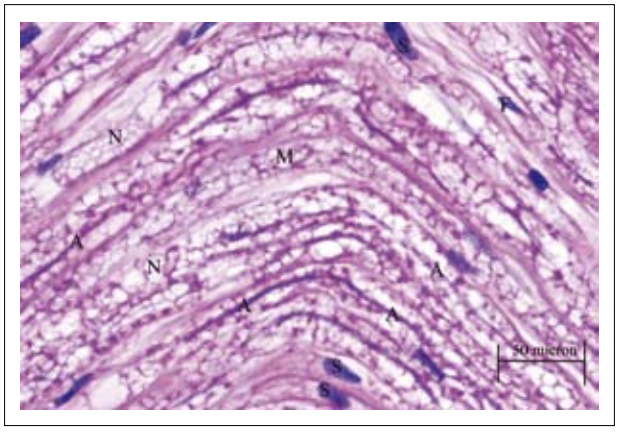


Fig 1. Light micrographs of the freshed nerves fixed in glutaraldehye (paraffin section; HE staining) (x 400).

M = Myelin, A = Axon, S = Schwann cell, N = Neurokeratin network, F = Fibroblast

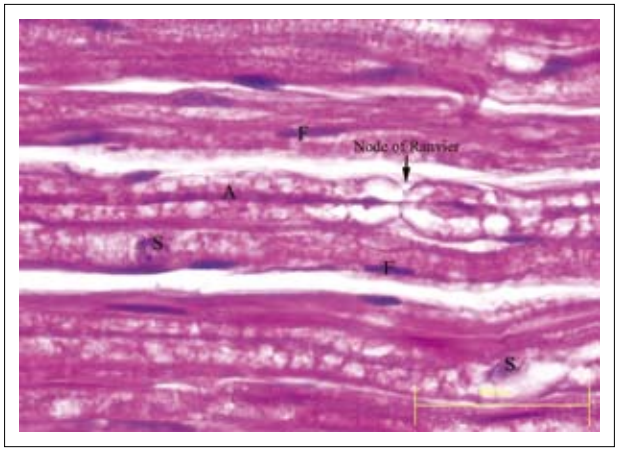


Fig 2. Light micrographs of the cadaveric embalmed peripheral nerves (paraffin section; HE staining) (x 400)

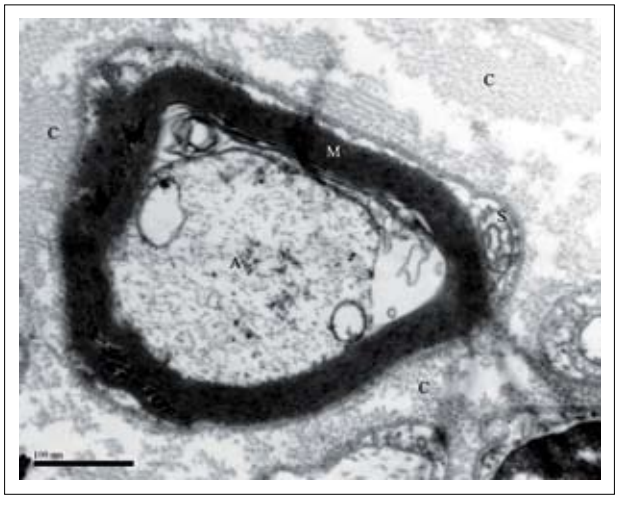
M = Myelin, A = Axon, S = Schwann cell, N = Neurokeratin network, F = Fibroblast

The neurokeratin network (N), the protein part of the myelin sheath distributes as a loose network in the unstained myelin.

When viewed by light microscope, the different fixation shows no significant structural difference, therefore the ultrastructural evaluation by transmission electron microscopy was used to confirm the efficacy of the preservation.

Figure 3 show the fresh nerve which was fixed in glutaraldehyde and viewed by transmission electron microscope. The myeline sheath covers the axon is the cell membrane of the Schwann cells which are packed together to be serveral lamellae. The cytoplasm of the cell is excluded so that the inner leaflets of plasma membrane fuse with each other and the axon becomes surrounded by multiple layers of plasma membrane which together contribute to the myelin sheath. Figure 4 shows that Schwann cell cytoplasm is absent within the compact myelin sheath which consists of many regular layers of membrane.

Figure 5, 6 is the transmission electron micrograph of the nerve fiber of the cadaveric embalmed specimen. The axon (A) is surrounded by the myelin sheath which shows significant difference from the nerve fibers of the fresh cadaver which were previously fixed with glutaraldehyde (Fig 3,4). The axon of the cadaveric embalmed specimen (A) is surrounded by the myelin sheath which shows the electrondense lines radiate from the axon and shows no concentric lamellae of the lipid part of the Schwann cell membrane. However, these electrondense lines which radiate from the axon are also the protein part of the Schwann cell membrane. The surroundings of the nerve fibers, which are composed of the fibroblasts (F) and the collagen fibers (C) are well preserved.



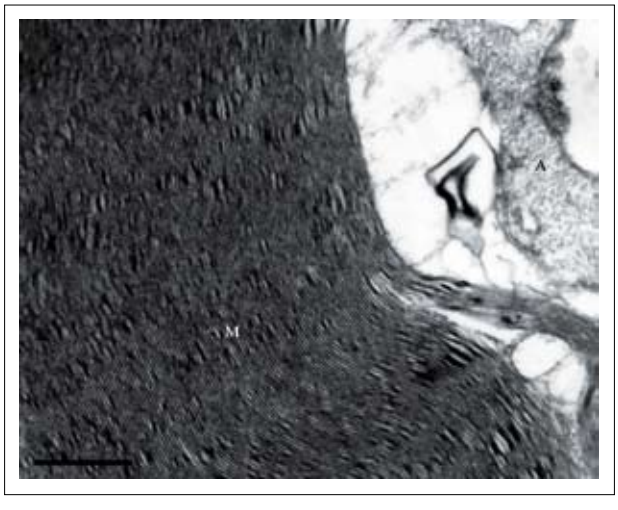
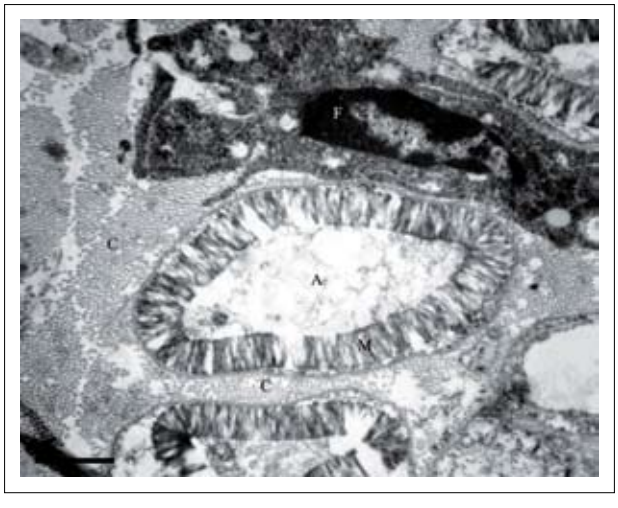


Fig 3,4. Transmission electron micrographs of the fresh peripheral nerves show the lamellated arrange of the myelin sheath.

DISCUSSION

Myelin is a relatively lipid-rich membrane and contains 70-80% lipid in the PNS and CNS respectively.[8] The major lipid species are cholesterol. The other components are proteins and water. About 30% of adult human myelin is protein. The myelin proteins have been examined by polyacrylamide gel electrophoresis of isolated myelin. At least three proteins have been identified.[2] When lipids are removed from the myelin sheath, as in histological processing without prior fixation of lipid components, a network of proteinaceous neurokeratin is visible by light microscopy. This represents the denatured remains of the major protein component of myelin.

The nerve fibers of the cadaveric embalmed specimens, which were preserved by intrafemoral artery injection of the formaldehyde and later embalmed by



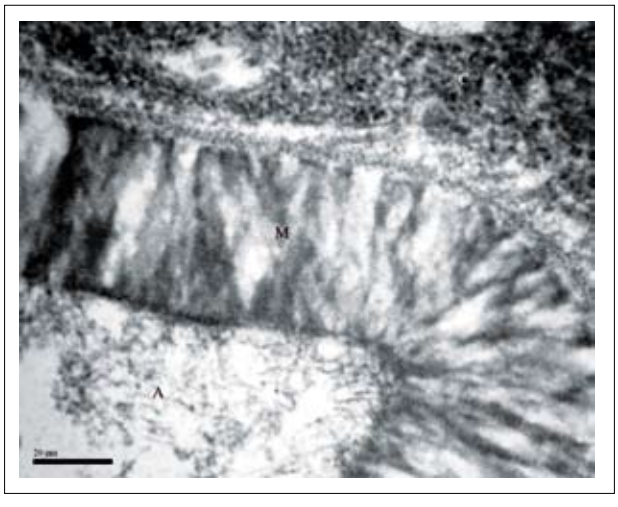


Fig 5,6. Transmission electron micrographs of the cadaveric embalmed peripheral nerves show the protein parts of the myelin sheath radially arranged perpendicular to the axon.

A = Axon, C = Collagen fiber, F = Fibroblast

the same fixative for about at least 1 year, showed clearly the denatured lipid part of the myelin. The remaining proteins are viewed by TEM as the electron-dense lines perpendicular to the surface of the axon or radiating from it. The arrangement of this part of the myelin sheath is perpendicular to the lamellae of the lipid part. The fibroblast and collagen components of the endoneurium are intact. The nerve fibers of the fresh cadavers fixed in 2.5% glutaraldehyde show the contrast feature. The lipid part of the myelin sheath is well preserved and the transmission electron micrographs show lamellated characters of the myelin sheath which is the lipid part of the Schwann cell membranes.

We have tried to study the serveral tissues of the cadaveric embalmed specimens such as the parathyroid gland,[9] and the skin (in print). We found that the parathyroids[9] are well preserved both viewed by light and transmission electron microscopy,[9] but not in the case of peripheral nerves. The cadaveric embalmed nerve fibers are well preserved in the protein parts but not in the lipid parts, as the protein of the myelin itself and the collagen fibers nearby which are proteins are also well preserved. The parathyroid glands of the previous study[9] of the cadaveric embalmed specimens were also well preserved as the transmission electron micrographs show the intact secretory vesicles and all of them are also proteins. We have concluded that the cadaveric embalmed specimens which are preserved by intrafemoral artery injection of the formalin and later embalmed in the same fixation for at least 1 year could preserve the proteins parts of the cell or tissue but not the lipid parts. The myelin sheath has 70% lipid components which are denatured by preservation of this method.

CONCLUSION

Fixatives are very essential for histology. The specimens should also be as fresh as possible. When one want to study the human tissues it is very difficult to obtain such tissues because of the ethical points of views. The cadaveric embalmed specimens are donated for education, so we have to study the efficacy of the fixation. From this study we compared the peripheral nerves of the cadaveric embalmed specimen to the fresh cadavers and found that the lipid parts of the myelin sheath cannot be preserved in the cadaver, but are well preserved in the fresh specimens which are fixed by glutaraldehyde.

The intrafemoral artery injection of formalin can preserve the proteins but cannot preserve lipids.

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