Schwann Cells of the Bone Marrow

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Nerves containing numerous Schwann cells can be found in bone marrow with routine histological methods. Thin nerve bundles and single nerve fibers containing Schwann cells can be identified histologically with help of methods which demonstrate nerve fibers and myelin. Smears of marrow stained with the May-Grünwald-Giemsa method are not adequate for demonstration of nerve fibers, but their satellite Schwann cells appear well stained. Histological, cytological and ultrastructural characteristics of these cells are described in detail. Blood-forming cells and nerve fibers with their Schwann cells lie in close proximity throughout the marrow. This circumstance suggests that interaction may take place between both elements.

The peripheral nerves contain a neurilemmal sheath of cells satellite to the nerve fibers. These cells were first described by Theodor Schwann in 1839; since then they have been known as cells of Schwann (Sch.) The presence of such cells in the bone marrow was first mentioned by Variot and Remy (1880) who described the nerves of the bone marrow as containing oval nuclei that were intensively stained red by carmine. The nuclei of the Sch. cells were again mentioned by Ottolenghi as present in the nerves of the marrow. References to these nerves in more recent literature were based on the study of silver impregnations and do not describe the structure of the Sch. cells. The relation between these cells and the nerve fibers (myelinated and nonmyelinated) have been described by Bauer in studies using the electron microscope. Other ultrastructural studies of the bone marrow mention the presence of the Sch. cells but do not give information about their characteristics. Clinical hematological studies based in bone marrow smears never mention these cells. A renewed interest in the innervation of the bone marrow justifies a detailed morphological study of the Sch. cells in the blood-forming organs.

Materials and Methods

Bone marrow of the femur of young male and female Wistar rats was used for both light and electron microscopy. The animals were sacrificed by decapitation and the marrow cylinders were immediately fixed in cold glutaraldehyde at 3.5 per cent in phosphate buffer,
cut into small fragments and left in 1 per cent osmium tetroxide buffered with the Millonig solution\textsuperscript{10} for one hour at 4° C. Dehydration was carried out in an ascending series of acetone and the tissue was embedded in Araldite. Sections were cut with an LKB Ultratome or with a Reichert Ultramicrotome at approximately 500 Å. The sections were mounted on copper grids coated with Formvar film and treated with uranyl acetate and lead citrate following the method of Venable and Coggeshall.\textsuperscript{11} The sections were examined and photographed with a Siemens Elmiskop IA, using 80 kv. Other marrow cylinders were fixed in 10 per cent formalin, embedded in paraffin and cut at 4 μ thick. The sections were stained with hematoxylin-eosin or with Masson's trichrome for demonstrating the nerves of the marrow with routine histological methods. The method of Gomori was used for the staining of connective tissue fibers. Smears of bone marrow of other femurs were stained with May-Grünwald-Giemsa (pH 5.75). These smears were intended for cytological studies using customary hematological techniques. The material for the smears was taken from the upper third of the femur where the nerves are more abundant. Smears of brachial plexus nerves of the rat were stained with May-Grünwald-Giemsa for comparison with the nerves of the marrow.

Special stains for the demonstration of the nerve fibers and their satellite cells in paraffin sections were the silver impregnation of Rogers and the luxol fast blue-PAS-hematoxylin. The reduced silver nitrate of Cajal, as modified by Castro\textsuperscript{12} for tissues containing bone, was used in a number of cases, impregnating the marrow in the block before embedding the tissue in paraffin. The femur, with its marrow inside, was cut in serial sections.

RESULTS

Histological Studies. In sections of bone marrow stained with hematoxylin-eosin, the nerve bundles appear as straight or slightly curved strings of light eosinophilic fibers with sparsely distributed nuclei (Fig. 1). These are nuclei of the Sch. cells. The fibers are slender, uniform in thickness and almost parallel to each other. The nuclei are fusiform and appear pale in comparison with those of the blood-forming cells. They form rows with their long axes parallel to the length of the nerve and lie between the fibers. The chromat against of the Sch. cells. The fibers are slender, uniform in thickness and almost parallel to each other. The nuclei are fusiform and appear pale in comparison with those of the blood-forming cells. They form rows with their long axes parallel to the length of the nerve and lie between the fibers. The chromatin is finely granular and even distributed. They are quite uniform in size, measuring 2–3 μ across per 12–15 μ in length. The largest nerve bundles are to be found in the vicinity of the nutrient foramen of the femur and close to the main branches of the nutrient artery. In longitudinal sections, the Sch. cell nuclei of the nerve look like a school of fish surrounded by the densely packed blood-forming cells. In a cross section, the nerve appears as a disc containing round nuclei limited by a thin ring of connective tissue fibers. The diameter of the nerve bundles varies between 10 and 30 μ in the vicinity of the nutrient foramen of the femur and diminishes gradually with distance from this area. When a nerve is impregnated with osmic acid, the myelin sheath of the nerve fibers appears stained black (Fig. 2). Isolated nerve fibers are very difficult to distinguish with the hematoxylin-eosin method, while specific silver impregnations, such as reduced silver nitrate of Cajal and the method of Rogers, demonstrate these fibers very clearly (Fig. 3).

The connective tissue fibers of the nerve bundles (perineurium and endoneurium) can be well demonstrated with Masson's trichrome or with methods using silver salts, such as the method of Gomori. These methods show a sheath of connective tissue fibers (perineurium) separating the nerves from
Fig. 1.—Bone marrow from the upper third of a femur of an adult rat. A nerve (N) with numerous Schwann cells is shown stained with the hematoxylin-eosin method (× 550).

Fig. 2.—Bone marrow from the upper third of a femur of an adult rat. A nerve (N) is shown with myelin stained by osmium tetroxide. In the center of the nerve the nucleus (Nu) of a Schwann cell is visible. The fat is also stained in black (× 800).

See opposite page for legends to Figs. 3–5.
the adjacent blood-forming cells. Thin connective tissue fibers separate bundles of nerve fibers and Sch. cells within the nerve (endoneurium).

**Cytological Studies.** Smears of bone marrow show nerves and arteries poorly stained with the May-Grunwald-Giemsa method. Both are compact structures and can not be spread as easily as the blood-forming cells. For this reason, they are too thick to allow this particular method to stain in a satisfactory manner. Capillaries, single nerve fibers or very thin nerve bundles, on the other hand, are soft enough to be spread together with blood-forming cells and have good staining conditions. The Sch. cells are well demonstrated but the axon and myelin of the nerve fibers appear almost unstained with the above-mentioned method. One of the main characteristics of the Sch. cells is that they are satellite to the nerve fibers. The cytological characteristics of the Sch. cells of the bone marrow (Fig. 4) can be compared with the same cell type in peripheral nerves elsewhere (Fig. 5). The May-Grunwald-Giemsa method shows the Sch. cells as very elongated elements attached to the axons of the nerves. Their cytoplasm is lightly basophilic (pale blue) and extends for a long distance, forming a very thin sheath around the nerve fibers. It is very homogeneous and agranular in most of the cell. Only one or two small azurophilic granules exist in some cells, very close to the nucleus. These are the so-called "pi" granules. The nuclei are fusiform and very long, with the main axis parallel to the length of the nerve fibers. The chromatin is finely granular and evenly distributed.

**Electron Microscopy.**

The cross-section of a nerve of the bone marrow shows the Sch. cells surrounded by collagen fibers, forming the perineurium and the endoneurium. In the thinnest nerve bundles, the Sch. cells are almost in contact with the blood-forming cells. Only a few connective tissue fibers appear interposed (Fig. 6). The relations between the nerve fibers and the cells of the marrow parenchyma are demonstrated in Figs. 6 and 7. The Sch. cell is a polarized element. On one side, lies the nucleus and a small amount of cytoplasm; on the opposite side, lies most of the cytoplasm with the organelles. This area of the cytoplasm is related to the nerve fibers. The nucleus is roundish in cross-section. A double membrane limits a perinuclear space, mostly regular in width but also showing occasional widening. One can find ribosomes (Figs. 8 and 9) attached to the external layer of this membrane. There are
Fig. 6.—Schwann cell (Sch) in relation with numerous nonmyelinated axons (Ax). Around the Schwann cell a lymphocyte (L), a monocyte (M) and a neutrophilic leukocyte (N) of the bone marrow can be seen. In the lower right part of the figure, there is a portion of the wall of an arteriole (A) (× 22,000).

Fig. 7.—Bundle of nonmyelinated axons surrounded by blood-forming cells of the marrow. Axons of this type are shown in cross-section in Fig. 6. Reduced silver nitrate of Cajal-Castro (× 800).
Fig. 8.—Schwann cell satellite to a myelinated axon (MAx). The myelin sheath of this nerve fiber is composed of 18 dense layers. The arrow shows the membrane of the Sch. cell in continuation with the myelin structure. Other axons (Ax) are related to Schwann cells whose nuclei are not visible in this figure (× 28,600).

Fig. 9.—Schwann cell satellite to nonmyelinated axons (Ax). Note the large Golgi area (G) between the axons and the nuclei. The ergastoplasmic reticulum (Er) is small and narrow. Below the Golgi apparatus, the cytoplasm contains osmiophilic masses. Collagen fibers (C) can be seen around the Schwann cells in both figures (× 28,600).
pores in the nuclear membrane. The ergastoplasmic reticulum appears as a few short parallel lamellae, close to each other, with ribosomes in contact with their walls. Other ribosomes are free in the cytoplasm, either isolated or forming rosettes.

In the centrosomic area (Fig. 9) the Sch. cell shows a well developed Golgi apparatus. Some irregular osmiophilic masses appear at random in the cytoplasm. Mitochondria are present, but they are small and few in number. Expansions of the cytoplasm of each Sch. cell surround a number of nonmyelinated axons. The number of these axons is variable. We have found Sch. cells related to only one axon but generally they wrap up two to eight axons. As a differentiation of the Sch. cells, a multilayered membrane, the so-called myelin, develops around some of the nerve fibers. During this process, one Sch. cell becomes satellite to only one myelinated fiber per internode. The myelin shows 10–20 dense bands of approximately 25–30 Å in thickness. The spacing between these lines is approximately 120 Å. Between every two dense bands there is a wider band having less electron density and measuring 35–60 Å (Fig. 8). The cross-section of a myelinated nerve fiber shows that the myelin in some cases forms almost perfect rings, while in others it has an oval shape or a wavy profile. A mesaxon can be seen in some sections of myelinated and nonmyelinated fibers. The diameter of the myelin sheath in different fibers varies from 1 to 5 μ. The thickness of the wall of this sheath is 0.1–0.5 μ and is proportional to the number of dense and clear layers. Each Sch. cell is separated from the surrounding elements by a basement membrane. Beyond this membrane, there are collagen fibers (endoneurium) separating the Sch. cells from each other.

**Discussion**

The present studies give some of the much needed morphological information about a cell type that is present in normal marrow and very seldom mentioned in the literature. The Sch. cells would have been more widely known if they were present in larger numbers in smears of aspirated human marrow. The scarcity of these cells in such a material is possibly due to the peculiarity of the tissue and to limitations of the methods routinely used in clinical hematological studies. The very soft consistency of the parenchymal component of the marrow allows its aspiration in the syringe, while the stroma with the largest nerves remain in the organ. These nerves contain numerous Sch. cells. Single nerve fibers or very thin nerve bundles with their satellite Sch. cells can be aspirated, but they are difficult to recognize in smears stained with May-Grunwald-Giemsa because this method does not stain either the myelin or the axons. Without these nervous structures for reference, the Sch. cells are easily mistaken for endothelial cells if the lumen of the vessel is distended or empty. The Sch. cells can be better recognized in sections with the help of special methods which stain myelinated and nonmyelinated nerve fibers also. Histological methods commonly used in the laboratory, such as hematoxylin-eosin and Masson’s trichrome are capable of demonstrating thick nerve bundles only, Luxol fast blue-PAS-hematoxylin and silver impregnations, such as the method of Rogers, also permit recognition of the thinnest nerve
bundles with their satellite Sch. cells. These methods can be performed in sections of tissue fixed in formalin and embedded in paraffin. Such a condition makes them useful in experimental as well as human material.

The light microscopy can be complemented with electron microscopic studies. The ultrastructural characteristics of the Sch. cells of the nerves in the bone marrow correspond to the morphology of these cells in the peripheral nerves, described by Dixon in the trigeminal ganglion of the rat. The structure of the myelin sheath is the same as that described by Sjöstrand in the sciatic nerve of the mouse. The differentiation of this structure begins in the bone marrow of the femur of rats between the end of the second week and the beginning of the third week after birth.

Around this time, the hemopoiesis in the rat begins to show a response to hormonal stimulation and to nephrectomy, starvation and hypoxia. At the end of the first month, the response of these animals is of adult type. This coincidence is important if we consider that the studies of Langworthy show that the nerves become functional at about the time they acquire the myelin, and the myelin is a structural differentiation of the Sch. cells. The above-mentioned facts emphasize the importance of the studies of the innervation of the bone marrow for the understanding of its function.

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