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Comparison of Weigert Stained Sections with Unfixed, Unstained Sections for Study of Myelin Sheaths

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ABSTRACT **Myelinated fibers can be demonstrated in thin unstained brain sections of freshly killed uninjected animals. With this method myelinated fibers stand out as clearly as in stained Weigert sections from formalin-fixed brains. Unstained unfixed sections can be viewed under ordinary light within minutes after death of the animal and immediately photographed for permanent records. Such sections have value for neuroanatomical studies, and for rapid localization of various types of experimental lesions.**

A variety of methods are used for staining myelin sheaths of central and peripheral axons. These techniques include the Weigert, Woelcke, and Weil methods, all of which depend on the use of fixed stained tissue and necessitate a processing time of several weeks.

For many years we have used a simple and rapid method for showing myelin in experimental material—chiefly for quick localization of experimental lesions but also for neuroanatomical studies. This technique uses frozen sections of brains of freshly killed uninjected rats. The sections are viewed at once under a microscope and photographed. This method depends on use of a very quick embedding medium (Ames OCT Compound), a cryostat, and a combined microscope and camera (Zeiss Ultra Phot II) which makes possible immediate viewing and photographing of the sections. Such sections compare very favorably with those obtained with any of the methods in common use.

MATERIAL AND METHODS

The brain of a freshly killed rat is cut into blocks of 10 mm or less—the maximum width of tissue that can be cut in the cryostat. Each block is embedded in Ames OCT compound and frozen in the cryostat at $-18°$ for thinner sections (5–6 μm) and at $-10°$ for thicker sections (40 μm). Thickness of sections can thus range from 2 to 48 μm. The cut sections are mounted on clean dry slides that have been kept at room temperature rather than in the cryostat where moisture condenses on them and interferes with the refractile properties of the sections. Slides with the mounted sections are stored in racks under dry-ice until ready to be viewed under the microscope and photographed. A great many sections can thus be prepared within a short time. The slides are defrosted by holding them against fingers or the wrist for a few seconds.

The Zeiss Ultra Phot II makes it possible to inspect the section under the microscope at various magnifications, and to project the image onto a screen (8.0 × 11.5 cm) for rapid, accurate focusing. Myelinated fibers can be clearly seen without specialized lighting. The optics of the microscope allow for excellent photographs at low and high power. A built-in condensor makes possible the rapid taking of high quality photographs. With the luminar attachment we obtain excellent photographs of slides under high magnification. The photographs can be obtained rapidly by inserting a Polaroid film holder over the viewing screen and adjusting the source of light. If the photographs are not satisfactory they can be repeated at once.

After defrosting the sections do not deteriorate (dry out) for 10 min or more, giving ample time for thorough microscopic inspections and making of a permanent photographic record.

Fixed Unstained Sections. For many years we have also used unstained sections of 10% formalin-perfused brains. These also give excellent resolution of myelin sheaths. They last at least 10 min longer than unfixed sections, allowing more time for microscopic inspections. However, they have several disadvantages. There is much more distortion, particularly of ventricles, than is seen in unfixed sections; also they cannot be quickly embedded. Without embedding, the surface fragments of tissue may become lost, or delicate areas around lesions may be torn. And finally, they cannot be mounted until the brains have become well-hardened in formalin, for at least 24 hr.

RESULTS

Fig. 1*A* shows a photograph of a sagittal section of a rat's brain prepared by the original Weigert method (1). Fig. 1*B* shows a photograph of an unstained sagittal section of a freshly killed uninjected rat. Both sections are 25 μm in thickness, and both were photographed with the same procedures on Polaroid film. Inspection of these sections shows little difference in the demonstration of myelinated fibers. Fig. 2*A* shows a photograph of the section adjoining the one shown in Fig. 1*B* but made with a regular film (Kodak Plus-X Professional Film). The myelinated fibers are shown in greater detail than those seen in Fig. 1*B*. Fig. 2*B* shows the area enclosed in the oblong in Fig. 2*A* magnified 6.4 × (or 105× from the original section). The degree of resolution permits us to visulaize the finest myelinated fibers. It will be noted that the sections are free from shrinkage and distortion of ventricles produced by the ordinary methods.

DISCUSSION

In 1843, Benedict Stilling of Cassel, Germany, pioneer in the histological study of the nervous system, cut sections of tartaric-acid-hardened pieces of brain tissue by hand with a razor. In a great understatement he said that it requires

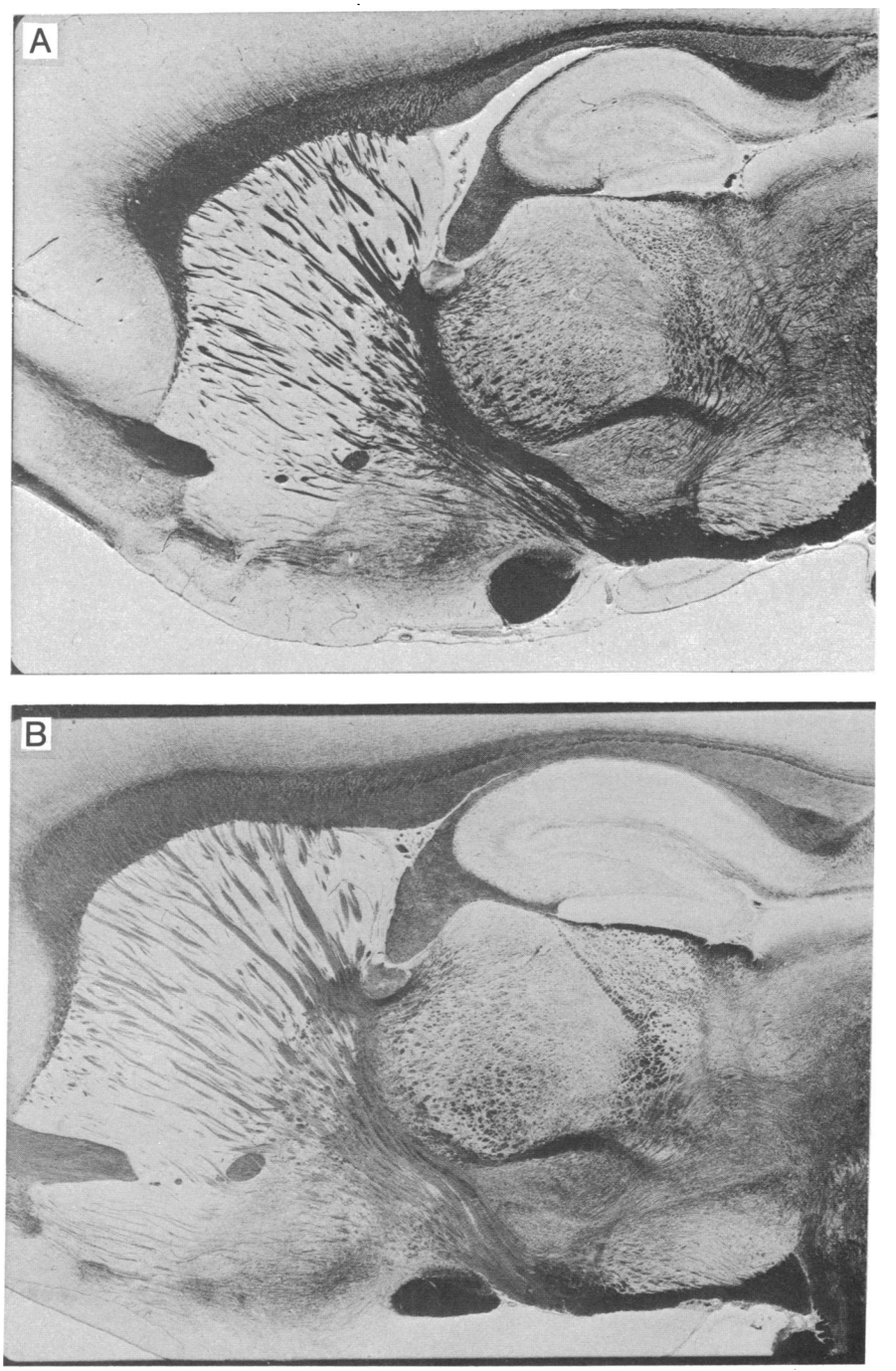

FIG. 1. (*A*) A Polaroid photograph of a 25-μm sagittal section of a rat's brain prepared by the original Weigert method. $\times 12$. (*B*) A Polaroid photograph of a 25-μm unstained sagittal section of a freshly killed uninjected rat. $\times 12$.

considerable skill to cut fine sections by hand. He viewed his sections under a primitive microscope and without any photographic technique had to make drawings of all of the sections (2).

Much later other workers also used unstained sections but viewed and photographed them with various forms of light—phase contrast, polarization, and x-ray defraction (3, 4).

Guzman *et al.* (5) and Powell (6) both used unstained but formalin-fixed sections for the quick localization of implanted stimulating electrodes. Such sections gave good resolution but had certain disadvantages that were described above.

Hutchinson and Renfrew (7) also used unstained fixed sections for gross localization of electrode tracks and reported being able to see outlines of nuclear structures in their slides. We have also seen outlines of some of the nuclei, particularly the suprachiasmatic and supraoptic nuclei, in our fixed sections.

Advantages of Use of the Unstained Unfixed Sections As Compared to Weigert Stained Fixed Sections. Within minutes unstained unfixed sections can be inspected and photographed for a permanent record. This method avoids the time-consuming steps in other staining procedures used in the preparation of permanent sections. The need for technical help is greatly reduced. Sections may also be examined for myelinated fibers and then subsequently used in other staining procedures.

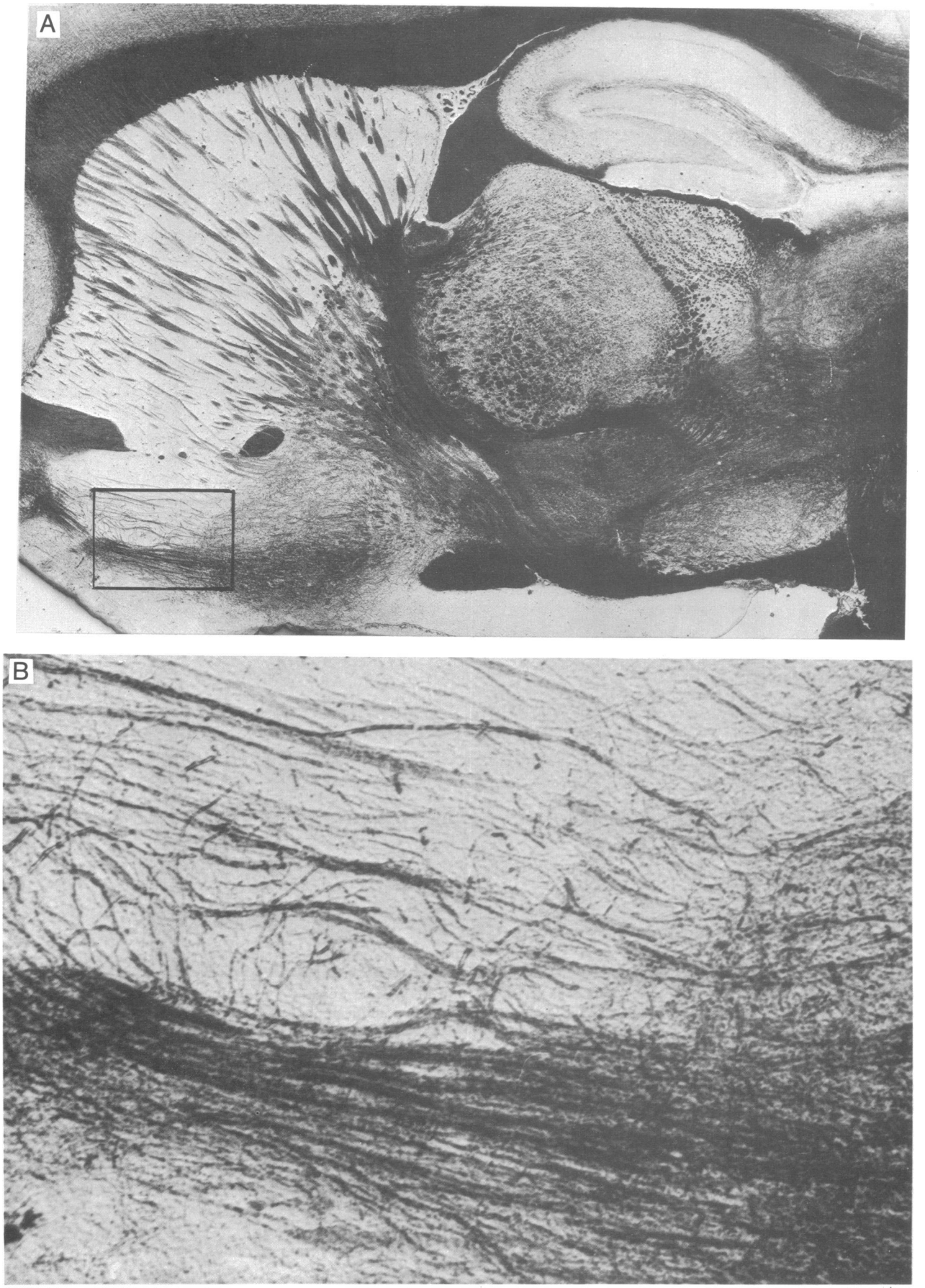

FIG. 2. (*A*) A photograph of the section adjoining the one shown in Fig. 1*B*, but made with a regular film (Kodak Plus-X Professional Film). ×16.4. (*B*) The area enclosed in the *oblong* in (*A*) magnified ×6.4 from Fig. 2*A* (×105 from the original section).

Close examination of unfixed unstained sections reveals that myelinated fibers show with great clarity, even under high magnification. There is no fixative-produced distortion of tissue. Excellent Polaroid photographs can be made of these fibers. Because it is possible to maximize resolution of photographs by varying exposure time, one is not limited by the visual parameters of lightly or darkly stained areas.

Finally it is obvious that the slides must be viewed by the experimenter or neuroanatomist himself. In viewing the slides the experimenter can decide from section to section which ones he wants to photograph and which ones he wants to study under high-power magnification. He need select for the permanent records only those slides that show the lesions or structures of interest.

This technique permits the experimenter to obtain information rapidly and allows him to actively check, develop and pursue his ideas with study of further sections, different magnifications, or additional stains.

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