

COMBINED METHOD OF SILVER IMPREGNATION AND FEULGEN REACTION

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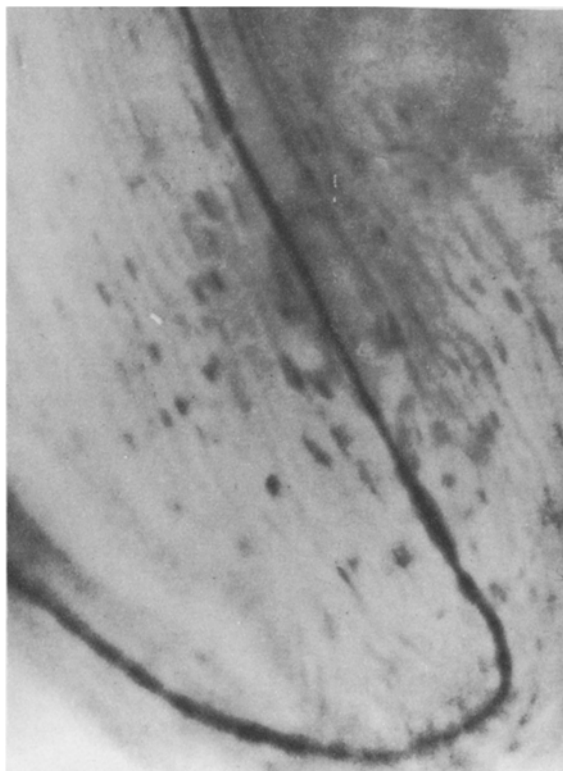
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We have succeeded in combining the method of silver nitrate impregnation of nerve fibers and endings with the Feulgen reaction. This new combined method makes it possible to elicit in the same section nerve fibers and endings and the nuclei of DNA-containing Schwann cells and special cells surrounding them.

The combined method calls for fixing the material in 12% formalin-calcium carbonate for 2-3 weeks; slicing of the frozen objects and washing of the sections in distilled water for 15-30 min; transfer of the sections to 20% silver nitrate for 1 h; washing the sections in 20% acetic on distilled water for 5 min; treatment of the sections in three portions of 20% formalin on distilled water for 2 min; filtering of excess formalin from the sections.



Pacinian corpuscle from the skin of a cat's paw. Silver impregnation-Feulgen reaction. Objective $\times 40$, ocular $\times 7$.

The sections are then impregnated with ammoniacal silver under a microscope until the nerve elements are best elicited. The ammoniacal silver is prepared from silver nitrate with the addition of strong ammonia until the formation and solution of the precipitate. For greater impregnation the additional quantity of ammonia needed is determined empirically. Impregnation is stopped in ammonia water ($\frac{1}{3}$ strong ammonia and $\frac{2}{3}$ distilled water). The sections can be in this for 1-2 h, after which they are washed in distilled water for 3 min, gilded in a diluted solution (0.1%) of chloroauric acid for 15 min, fixed in a 10% solution of hyposulfite, and carefully washed in 4-5 portions of distilled water for 30 min.

Afterward the sections are treated with 1 N solution of cold hydrochloric acid for 3 min, with 1 N solution of hydrochloric acid at a temperature of 60° for 10 min, with a 1 N solution of cold hydrochloric acid for 3 min; then the sections are washed in distilled water for 1 min, treated with fuchsin sulfuric acid for 30 min-1 h, with three portions of sulfur water for 5 min, washed with tap water (three portions) for 15 min, and, finally, the sections are embedded in balsam via 96% alcohol, carbolxylene, and xylene (toluene, benzene) under a cover glass.

With a properly conducted reaction the nerve fibers and their terminations, as well as the nucleoli in the nuclei, are stained black and the nucleoplasm with the DNA, red.