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## HISTOCHEMICAL OBSERVATIONS OF FLUORESCENT BIOGENIC AMINES IN CRYOSTAT SECTIONS OF PERIPHERAL AND CENTRAL NERVOUS TISSUE

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The histochemical fluorescence method of Falck and Hillarp<sup>7</sup> has proven to be a valuable histological tool to study the monoamine localization in central and peripheral nervous tissue. The method shows an extreme sensitivity when the tissue is embedded in Araldite and sectioned down to 1  $\mu$  of thickness (Hokfelt<sup>9</sup>). A high degree of specificity is demonstrated by the microspectrophotometric measurements of the several fluorescent compounds of catecholamines and serotonin (Corrodi and Jonsson<sup>1</sup>). However, the original procedure cannot be practically adapted as a regular histologic technique. Besides others, the main disadvantages are: 1) the freeze-drying is time-consuming; 2) the enzymatic system (cholinergic and MAO-enzyme) is practically destroyed in a procedure that heats the tissue twice around 80°C.

In our present report we describe a simple histochemical modification of the cryostat technique for demonstrating the catecholamine and serotonin fluorescence in thin sections of peripheral and central nervous tissue.

### MATERIAL AND METHODS

Small pieces of fresh nervous tissue (up to 0.5 cm by side) were obtained from decapitated small animals, from large animals under a light barbiturate anesthesia (cat, rabbit, dog), from material obtained at the slaughter house (pig, cow), and human specimens from the hospital surgical room. The fresh tissue was adequately oriented in a small flask, tightly closed and then quickly frozen by immersion in a mixture of acetone in dry ice. The frozen piece of tissue was

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Abbreviations used: Ache, acetylcholinesterase; MAO, monoamine oxydase.

transferred to a cryostat (Ames Lab Tek) at  $-30^{\circ}\text{C}$ . and the sections were made of 5 to 20 microns thickness. The sections were mounted on pre-cooled slides, and were dried over phosphorous pentoxide in a small evacuated desiccator overnight at room temperature. No difference in the fluorescent results was observed by storing the desiccator (with the sections and phosphorus pentoxide inside) in the refrigerator at  $-4^{\circ}\text{C}$ . or in the freezer at  $-40^{\circ}\text{C}$ ., but in these conditions the sections were left under the drying process for more than three days.

After drying the sections, the phosphorus pentoxide was replaced by 10-15 gms. of paraformaldehyde equilibrated at 60-70% of relative humidity under the conditions suggested by Hamberger et al.<sup>8</sup> The exchange of paraformaldehyde for phosphorus pentoxide was accomplished in a few seconds under a hood. The desiccator was again evacuated and heated in an oven at  $65^{\circ}\text{C}$ . for 45 minutes, and then cooled under the hood at  $25^{\circ}\text{C}$ . for one hour. After that, the sections were removed and directly mounted with Harleco non-fluorescent mountant medium in xylene. Glycerin is useful as a mounting medium, too. Mineral oil, however, is not recommended, as it often gives too much fluorescent artifact. The fluorescent preparations were studied with a Zeiss fluorescent microscope fitted with a primary filter BG 12 and a secondary filter 470  $\mu$ .

Black and white pictures were taken with a polaroid film 3000 speed, being the exposure time of 10 to 45 seconds. Color pictures were taken with the high speed Kodak film Ektachrome EHB, which was developed for 3000 speed.

## R E S U L T S

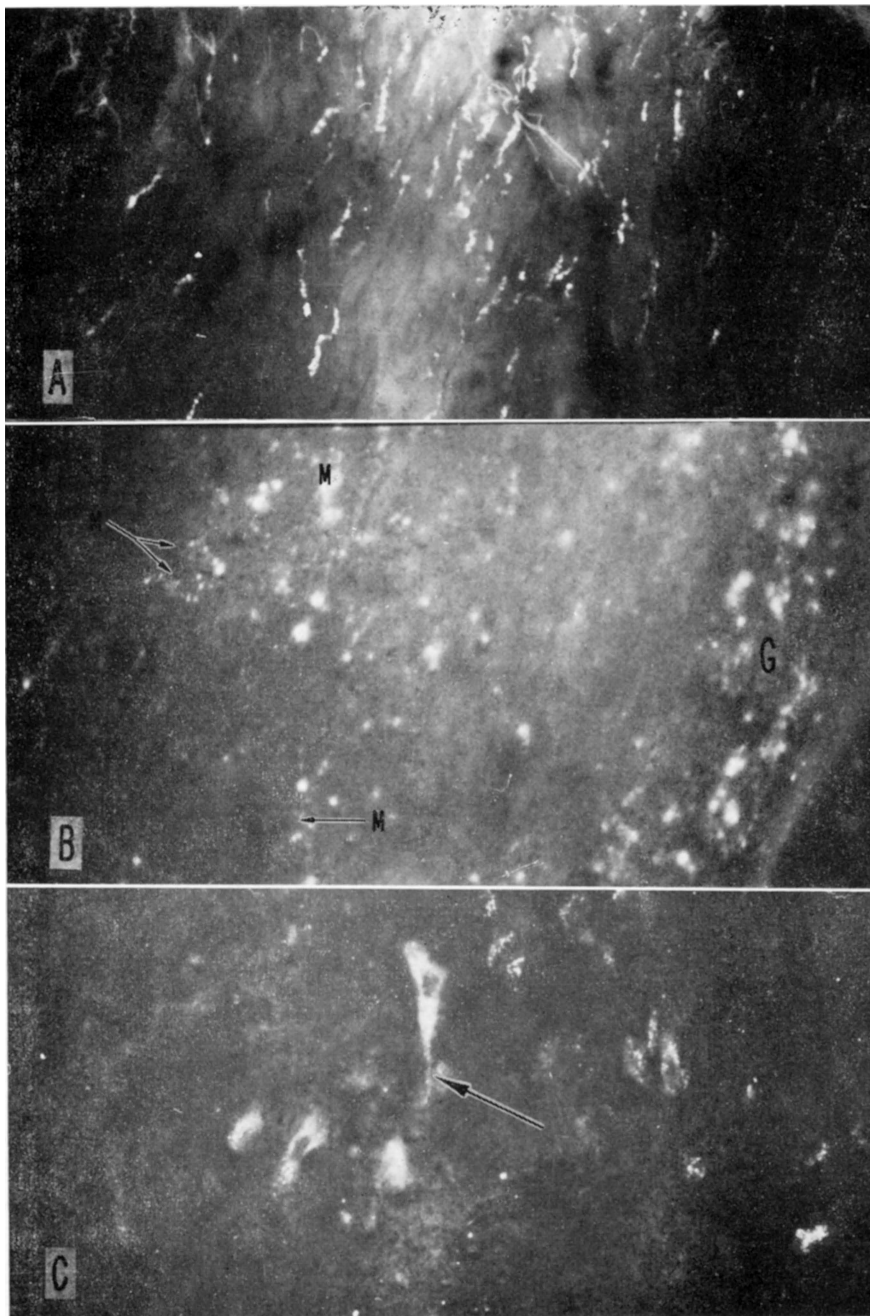
a) In Figure 1a, a bundle of varicose adrenergic fibers can be observed coursing along the longitudinal smooth muscle layer of a human ureter (8  $\mu$  thickness). In other sections, both thick and delicate beaded adrenergic fibers in the adventitia and epithelial layers of human ureter were observed all along the whole ureter, as well as a group of ganglion cells fluorescing in varying degrees of intensity at the lower ureteral segment.

b) In Figure 1b, the fluorescent varicosities in the glomeruli layer of olfactory bulb of a normal cat can be observed, as well as a few smaller cells showing the same type of greenish-yellow fluorescence. The varicosities are observed to be of several sizes: 1) larger and medium size (1-2  $\mu$ ) with a more greenish fluorescence localized around the glomeruli (G) and around the mitral (M) and external granular cells; 2) small size (less than 1  $\mu$ ) of yellowish-green fluorescence localized throughout the three cellular layers, but predominantly around the external and internal granular cells.

c) In Figure 1c, a group of fluorescent neurons from medulla oblongata of a normal cat is shown. Varicosities of several sizes and varying degrees of fluorescence intensity are observed around the cells. Fluorescent cells and fluorescent varicosities were observed in the other structures of pons, mesencephalon and diencephalon.

## D I S C U S S I O N A N D C O N C L U S I O N

The results of the reported procedure are comparable to the previous procedure applied in peripheral nervous tissue (Eränko<sup>6</sup>; Sprigs et al.<sup>12</sup>; Csillik and Kalman<sup>2</sup>), and the attempts of Nelson and Wakefield<sup>10</sup> and Placidi and Masuoka<sup>11</sup> in central nervous tissue. The fluorescence developed in our material is very sensitive to the ultraviolet light irradiation. The fluorescence is quickly abolished by the action of sodium borohydride and it can be redeveloped by a second treatment with the paraformaldehyde



*Fig. 1 — a) Normal human ureter, 8  $\mu$  thickness. The varicose adrenergic fibers are observed coursing along the smooth muscle fibers  $\times 160$ ; b) Olfactory bulb from a normal cat, 8  $\mu$  thickness. Varicosities of different sizes are located at the glomeruli layer (G) and around non-fluorescent mitral cells (M). A few cells (M) develop the adrenergic fluorescence in a low intensity,  $\times 400$ ; c) Medulla oblongata from a normal rat, 8  $\mu$  thickness. It can be observed a group of adrenergic neurons and varicosities of different size around the neuron and along the nerve fiber (arrow)  $\times 400$ .*

gas. As in the preliminary observations of Dahlström and Fuxe<sup>3</sup> the fluorescence from our material treated with reserpine disappears from the central and peripheral nerve fibers, as well as from the central and peripheral nerve cells. An original observation is that in the material treated with one or two doses of amphetamine (Ellinwood and Duarte-Escalante<sup>5</sup>), the fluorescent varicosities are the first to disappear from central and peripheral nerve tissue, while the fluorescence from the cell bodies disappears by the 6th to 8th weeks in the material daily treated with increasing doses of amphetamine, 10-40 mg/kg.

There are some difficulties in the development of the fluorescent technique even with the original freeze-drying procedure. The more critical problems are related to the handling of the tissue during the drying process and to the preparation of paraformaldehyde with the proper percentage of relative humidity. We have found that in our environment the 60-70% of relative humidity is the useful value to develop the biogenic amine fluorescence in a well dried nervous tissue. Furthermore, the heating time at 65°C allows us the preservation of the enzymatic systems to develop subsequently in alternate sections the histochemical reactions for cholinesterases (Duarte-Escalante et al.<sup>4</sup>) and MAO-enzyme (to be published).

#### R E S U M O

*Verificação histoquímica de aminas biogênicas fluorescentes em secções criostáticas de tecido nervoso central e periférico.*

Relato de modificação da técnica criostático-histoquímica para a verificação da fluorescência das catecolaminas e da serotonina em secções de tecido nervoso central e periférico. São discutidas as vantagens desta modificação técnica em relação a outras propostas para a mesma finalidade.

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