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# Immunohistochemical Localization of Somatostatin-like Immunoreactivity in the Hypothalamus of the Lizard, *Lacerta sicula*[1]

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Somatostatin-like (SRIF) immunoreactivity was shown in the preoptic area and posterior hypothalamus of lizard (*Lacerta sicula* R.), using both indirect immunofluorescence and PAP-technique. Immunoreactive neurons are seen in the preoptic paraventricular nucleus, in the caudal hypothalamus, and close to the hypophysial recess where they form a massive group of cerebrospinal-fluid-contacting elements. SRIF-immunoreactive fibers are seen also in the outer zone of the median eminence. Employing double-staining methods (double peroxidase–antiperoxidase sequence, according to Vandesande and Dierickx ((1976) *Cell Tissue Res.* 175, 289–296) and PAP-immunofluorescence) the immunoreactivity for SRIF was separated from that for neurophysins.

The occurrence of somatostatin immunoreactive cells and fibres has been demonstrated by immunohistochemical methods in the hypothalamus of two lacertilians (*Lacerta muralis*, Doerr-Schott and Dubois, 1977, 1978; *Ctenosauria pectinata*, Goossens *et al.*, 1980).

In the present report the somatostatin immunopositive pattern in the hypothalamus of *Lacerta sicula Rafinesque* is elucidated by several methods: indirect immunofluorescence, peroxidase unlabeled antiperoxidase (PAP) technique, and double immunohistochemical stainings.

In particular, double immunohistochemical staining procedures were tested in order to check the relations between immunoreactive patterns of somatostatin immunoreactive elements and of neurophysins.

## MATERIAL AND METHODS

Normal adult specimens of both sexes of *L. sicula R.* (about 20 animals) were used.

*Immunohistochemical procedures.* The brains fixed with Gerard, Bouin, Zamboni, or Susa fluids were embedded in paraffin and serially cut (sections 5–10 $\mu$m in thickness) according to transverse, sagittal, or frontal planes. Both as concerns the preservation of the tissue and the intensity of immunoreaction, the best results were achieved using the Bouin and Zamboni fluids. The sections were stained with the unlabeled antibody peroxidase–antiperoxidase complex (PAP) technique, according to Sternberger (1974), or alternatively with the indirect immunofluorescence method (Coons, 1958). In the latter procedure, both fluorescein isothiocyanate (FITC) and tetramethylrhodamine (TRITC) conjugated secondary antisera were used. Moreover, in order to check the localization of the fluorescent materials, after the indirect immunofluorescent technique, a PAP staining was performed (see Gaudino and Fasolo, 1980).

The antibody against somatostatin (SRIF) was routinely employed at the dilution of 1:500, usually at room temperature for 18 hr in a moist chamber. In some instances higher dilutions (1:1000, 1:2000; 1:6000) of the primary antiserum were successfully employed.

In some cases after the immunohistochemical method the sections were counterstained with cresyl-violet.

Since in mammals some cross-reaction was observed between antisomatostatin serum and neurophysins (NPH) (Dierickx and Vandesande, 1979), some adjacent sections to those treated for somatostatin immunoreactivity were checked immunocytochemically for neurophysin (working dilution 1:250, 1:500). To get a better resolution of the reactions pattern, especially in the preoptic area, double-staining methods were also employed, such as (a) the double-staining procedure suggested by Vandesande and Dierickx (1976); (b) a PAP(diaminobenzidine)-indirect immunofluorescence sequence (Gaudino *et*

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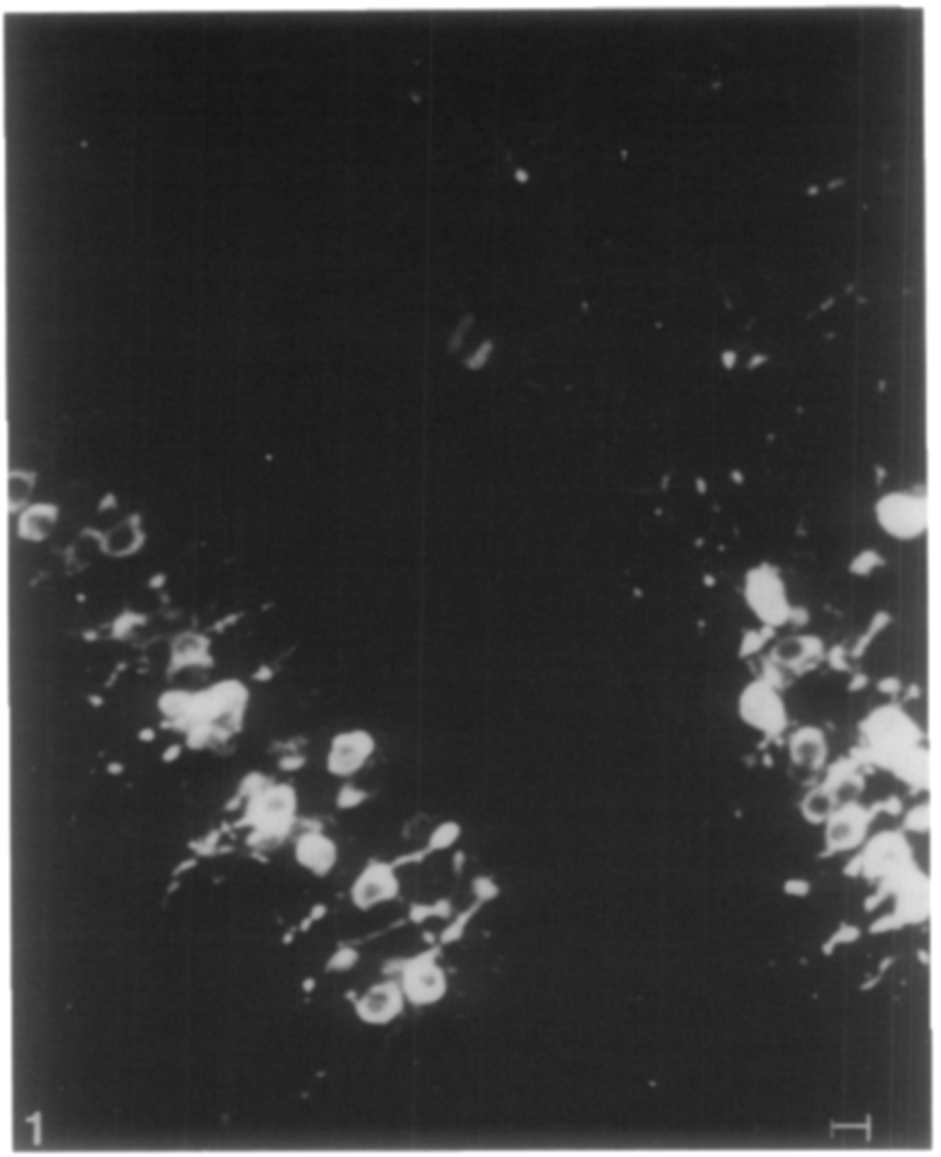


FIG. 1. SRIF-immunopositive neurons in the preoptic paraventricular nucleus of the lizard. Several CSF-contacting neurons are shown. TRITC-indirect immunofluorescence. Cross section. Reference bar 10 μm.

*al.*, 1981) separated by an electrophoretic elution according to Vandesande and Dierickx (1976); or (c) a PAP-indirect immunofluorescence sequence as in (b), followed by a second PAP staining (chloronaphtol) of immunofluorescent material. In brief, the sections were processed for the first antigen according to the classical PAP-technique (revealed by DAB reaction, brown amorphous product), then they were subjected to an electrophoretic elution to remove all the antibodies of the first PAP reaction. The sections were successively processed for the second antigen according to the indirect immunofluorescence schedule (TRITC- or FITC-tagged secondary antisera) and observed both under bright field illumination (for peroxidase reaction) and epillumination fluorescence (for indirect immunofluorescence).

In any of these double methods, if a section has been treated for SRIF (first reaction) and NPH (second reaction), contiguous sections were processed in a vice versa sequence (NPH, first reaction; SRIF, second reaction).

The following specificity controls were performed: (a) some sections contiguous to the positive ones were treated omitting the primary serum; (b) the primary antisomatostatin antiserum was preadsorbed on a solid phase, consisting of Sepharose 4B–CNBr activated (Pharmacia, Uppsala) covalently coupled with synthetic somatostatin (UCB, Brussels); (c) the primary somatostatin antiserum was preadsorbed on a solid phase consisting of Sepharose 4B–CNBr activated and covalently coupled with the neurophysins and alternatively the antineurophysin serum was preadsorbed on the solid phase, coupled with somatostatin, according to Swaab and Pool (1975).

The observations and photographs were performed with a Zeiss photomicroscope or a Leitz Ortholux II microscope equipped both for transmitted light bright field and for incident fluorescence (equipped with filters for TRITC and FITC fluorescences). Some photographs were done with the Nomarski interferential contrast optics (Zeiss Universal microscope).

*The antisera.* The antisomatostatin antiserum was a gift from Dr. Vandesande, Gent, Belgium (see Vandesande and Dierickx, 1980). The antineurophysins primary serum, reacting with both neurophysin I and II, was a gift from Dr. Sofroniew, Munich, West Germany (Sofroniew *et al.*, 1979).

## RESULTS

### *Specificity*

The SRIF-immunopositive reaction was abolished by preadsorbing the anti-SRIF serum on a solid phase covalently coupled with synthetic somatostatin. The reaction pattern was unaffected after preadsorption


FIG. 2. Some bipolar or multipolar neurons in the lateral areas of the ventral hypothalamus. SRIF PAP-immunostaining shown by means of Nomarski optics. Reference bar 10 $\mu$m.

of the anti-SRIF serum on a solid phase coupled to neurophysins.

*SRIF-Immunopositive System*

(*a*) *Preoptic area.* SRIF-immunopositive perikarya form a cluster in the preoptic paraventricular nucleus. The cells, of medium–small size, dispersed in 1 to 2 rows, were often of the cerebrospinal fluid (CSF) contacting type, with a dendrite making contact with the CSF and at the opposite pole, usually a single dendritic stump, from which 2–3 dendrites and the axon arise (Fig. 1). Moreover, also some hypendymal elements were immunopositive. Some scattered SRIF-immunopositive cells were also observed in the lateral portions of the preoptic area.

(*b*) *Posterior hypothalamus.* Immunopositive perikarya form a loose system of multipolar cells both in the ventral periventricular gray and in the lateral areas of the region. Caudally the immunopositive cells are grouped in the tuberal hypothalamus forming a massive cluster of CSF-contacting elements just in front of the median eminence. These latter cells were usually bipolar in shape (Figs. 2–4).

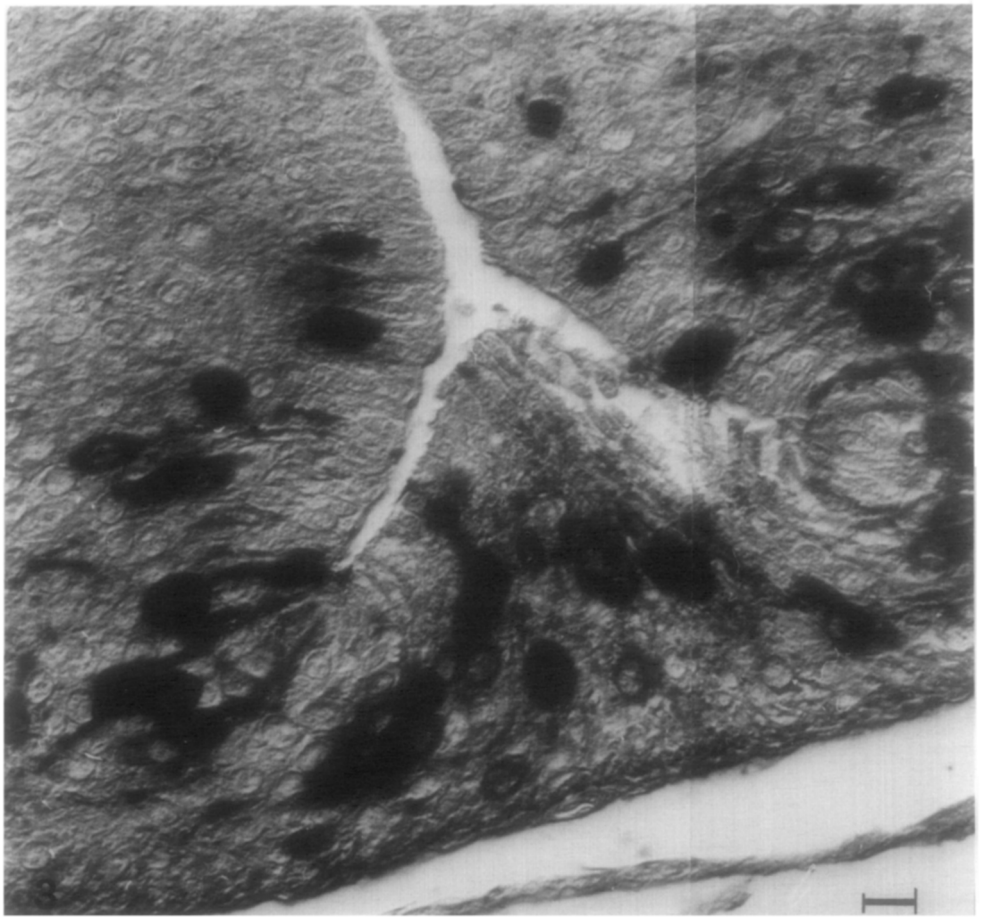

FIG. 3. Cross section of the hypothalamic floor just in front to the median eminence showing many SRIF immunopositive neurons, several of CSF-contacting type. PAP (DAB) immunostaining. Nomarski optics. Composite photograph. Reference bar 10 $\mu$m.

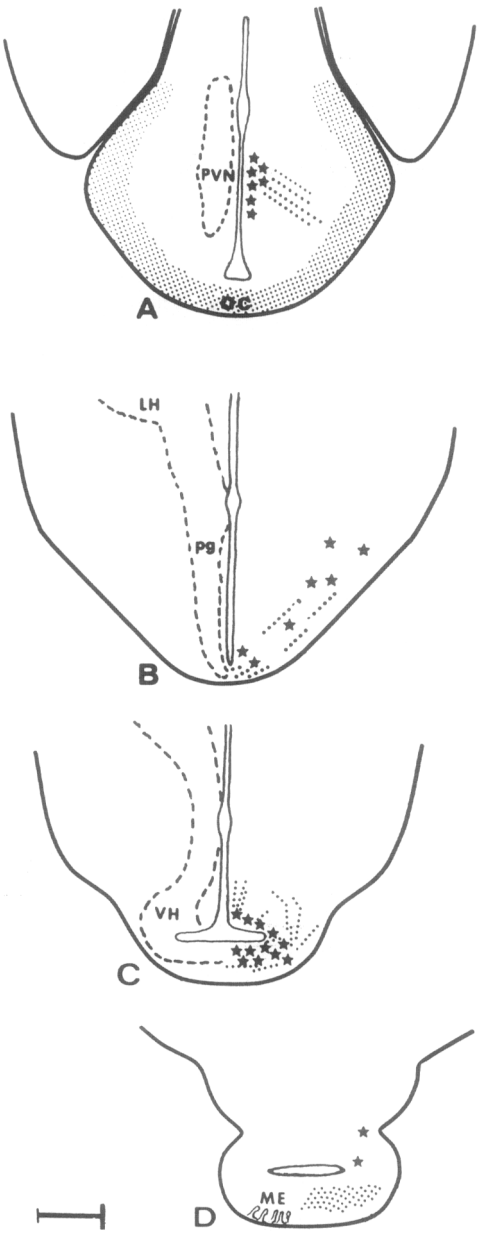


FIG. 4. Diagrammatic representative cross sections of the lizard hypothalamus showing the distribution of SRIF immunopositive cell bodies (★) and fibers (...) at the level of (A) the preoptic area, the posterior hypothalamus (cranial B, caudal C), and (D) the median eminence. LH, lateral hypothalamus; ME, median eminence; OC, optic chiasma; pg, hypothalamic periventricular gray; PVN, preoptic paraventricular nucleus; VH, *n. hypothalamicus ventralis*. Reference bar 500 $\mu$m.

*Immunopositive Nerve Fibers*

These were altogether very abundant (a) in the preoptic area where a diffuse network of fibers is seen. They seemingly connect the preoptic region to the telencephalon, and to the posterior hypothalamus, and (b) in the posterior hypothalamus, where they form a diffuse system in the lateral neuropil, converging in part toward the median eminence. In the neurohemal region of the median eminence the SRIF immunopositive fibers are abundant in the external pericapillary zone (see also Fig. 7). The overall distribution of SRIF-immunopositive material is summarized in Fig. 4.

*Neurophysin-Immunopositive System* (Figs. 5–7)

The pattern of NPH-immunopositive perikarya and fibers is separated, although contiguous in some areas, from that for SRIF-positive material. NPH-immunopositive perikarya make up the classical neurosecretory nuclei (i.e., supraoptic and paraventricular nuclei proper) loosely connected by some sparse small medium-size NPH-positive cells. In particular the NPH-immunopositive paraventricular nucleus results just dorsal to the SRIF-immunopositive cluster of cells and in many cases closely intermingled (Fig. 5). NPH-immunopositive nerve fibers form the preoptic-hypophysial tract, converging medially in front of the median eminence to reach the neurohypophysial complex (Fig. 6).

## DISCUSSION

The following points deserve further comment:

1. By immunohistochemical methods an important SRIF-positive system can be reliably shown in the hypothalamus of lizard. This is separated from the NPH-immunopositive system and no cross-reaction between the two antisera occurs, in agreement with the recent observations by Vande-

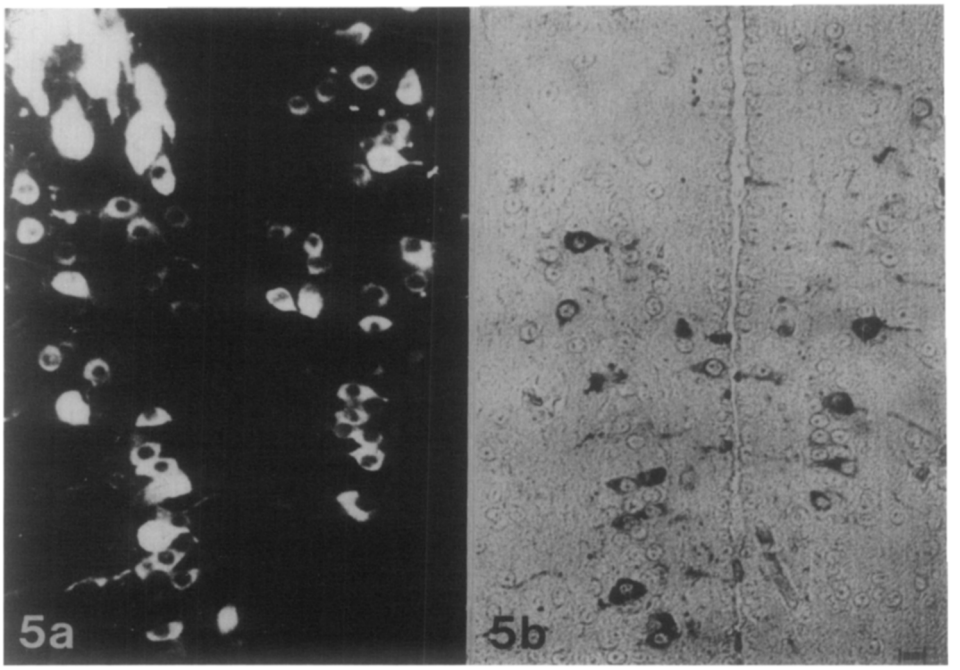


FIG. 5. PAP–IFL double-staining method. The same cross section of the preoptic area of (a) NPH–FITC-immunofluorescent cells (anti-NPH 1:500, preadsorbed on activated Sepharose coupled to SRIF) and of (b) a distinct pattern of SRIF–PAP-immunostained neurons (anti-SRIF 1:500, preadsorbed on activated Sepharose coupled to NPH). Reference bar 10 $\mu$m.

sande and Dierickx (1980) in frog, Goossens *et al.* (1980) in the lizard *Ctenosauria*, and Fasolo and Gaudino (1981) in the crested newt.

The present results support the interest of the double method employing a PAP-indirect immunofluorescence sequence (Gaudino *et al.*, 1981). Observations of the same microscopical area in bright field (for PAP-diaminobenzidine staining), in dark field and epillumination for fluorescence (showing the IFL material of the second reaction), and in mixed transmitted/incident illumination, give unambiguous information on the localization of the antigen, in close but separate cells.

2. In general a close correspondence between the present data and the localization shown by Goossens *et al.* (1980) in thc hypothalamus does exist. The preoptic periventricular SRIF-immunopositive cell cluster is of peculiar interest, since it shows a clear-cut segregation of functionally distinct neurons in the same paraventricular nucleus.

3. As concerns the abundance of CSF-contacting neurons in the preoptic area and in the tuberal hypothalamus, this result strengthens the importance of the relationships of some releasing-hormone-producing neuron systems (and particularly the somatostatin and LHRH ones) to the ventricles of the brain (Knigge *et al.*, 1980).

4. The morphology of the neurons as shown in the immunohistochemical reaction (after PAP method and Nomarski interferential microscope observation) is similar to that shown by classical Golgi method in lizard (Franzoni and Fasolo, 1982; Fasolo and Franzoni, in preparation).

## ACKNOWLEDGMENTS

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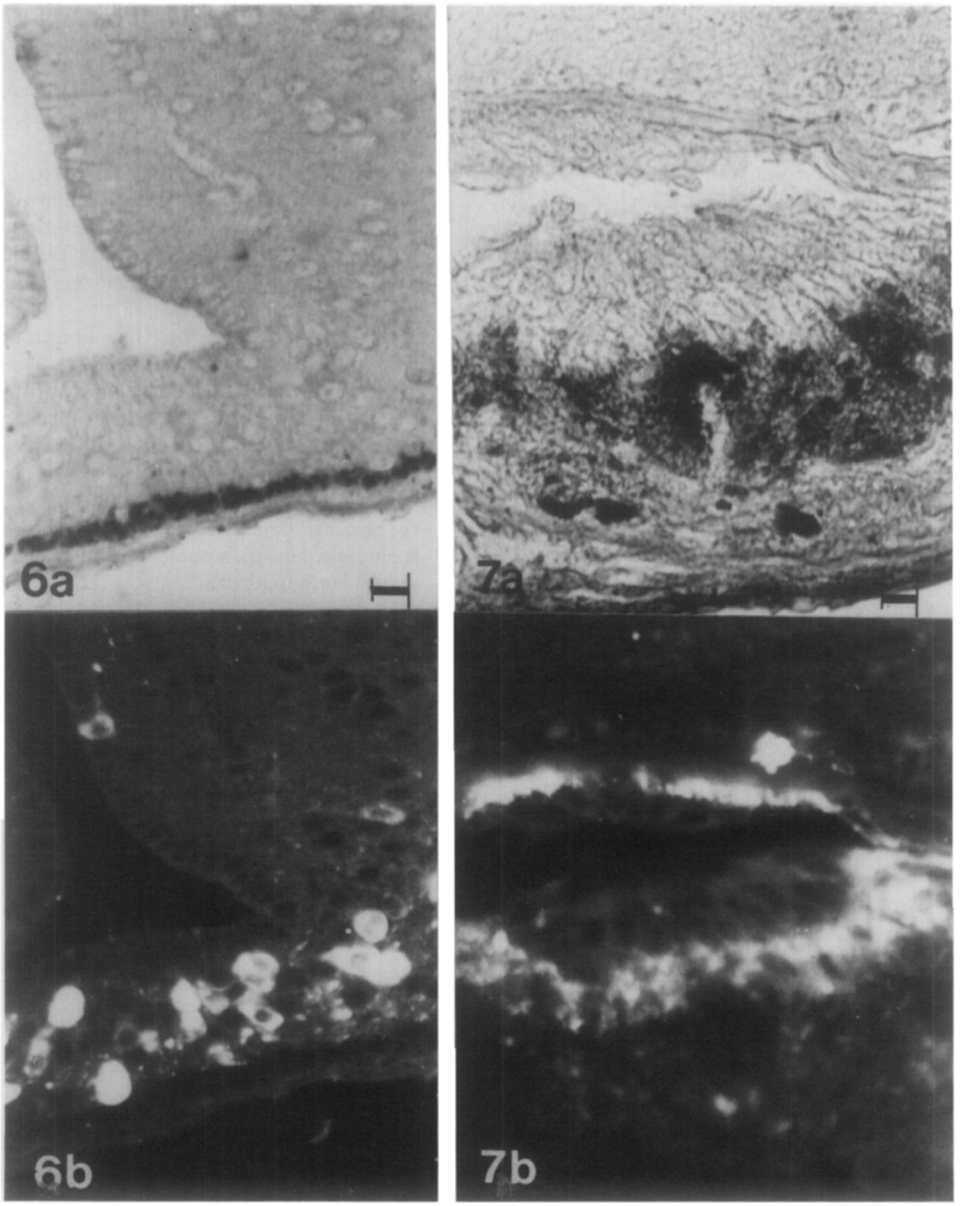


FIG. 6. PAP–IFL double staining method. (a) NPH–PAP-immunostained fibers converging toward the median eminence. (b) SRIF immunopositive neurons shown in the same section after FITC-immunofluorescence. Reference bar 10 $\mu$m.

FIG. 7. PAP–IFL double-staining method. A cross section of the median eminence of (a) SRIF–PAP-immunostained fibers and (b) NPH immunofluorescent fibers. Reference bar 10 $\mu$m.

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