Processing Biological Material by the Falck Method and by Its Modification Using the Original Equipment

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The specific nature of the development of physiological and pathological processes in tissues of different organs is determined in many respects by the functional state of the stromal elements, the most important of which is the microcirculatory bed, and of the parenchymal components reacting synchronously to structural and functional changes of the stroma [3,4]. The microcirculatory bed, in its turn, is directly controlled by the neurotrophic apparatus including, in particular, the sympathetic component of innervation. It is not inconceivable that transmitters of the autonomic nervous system directly affect the parenchymal cells, particularly in hormone-dependent organs [7]. One such example is the mammary gland, whose autonomic nerve terminals run in immediate proximity to the epithelial component of the glands and ducts [6].

Difficulties in the qualitative detection of the adrenergic nervous elements and in the objective documentation of the results have so far significantly limited the possibilities of investigators in this field. It is particularly complicated to vizualize the small terminals of the adrenergic fibers and the nervous structures with a low concentration of transmitter.

Among the known histochemical methods, the paraformaldehyde Falck method [8] is considered to be the most sensitive to low concentrations of catecholamines. The majority of investigators do not exploit this advantage, due to the need to use complicated vacuum systems and to the laboriousness of the method. The Falck method in Krokhina's modification [5] does not require vacuum processing of the material but does need cumbersome equipment, and the results obtained are relatively unstable.

The aim of the present study was to automate and simplify the processing of biological material by the paraformaldehyde method in order to vizualize the adrenergic nervous structures qualitatively and to obtain stable results during their study.

MATERIALS AND METHODS

For a study of the optimal regimes at all stages of the processing of biological tissues, operative and biopsy material of dysplasias (15 cases), fibroadenomas (15 cases), and breast cancer (20 cases) in women of reproductive age was used. Rat myocardium and tissues of bronchobioptates and biopsies of the stomach were studied in isolated cases to monitor the uniformity and efficiency of the processing. The material was cryoprocessed with liquid nitrogen no later than 30 min after collection. Parallel cryostat sections were processed after Falck [8] and in Krokhina's modification [5], as well as with hematoxylin-eosin. The staining was con-

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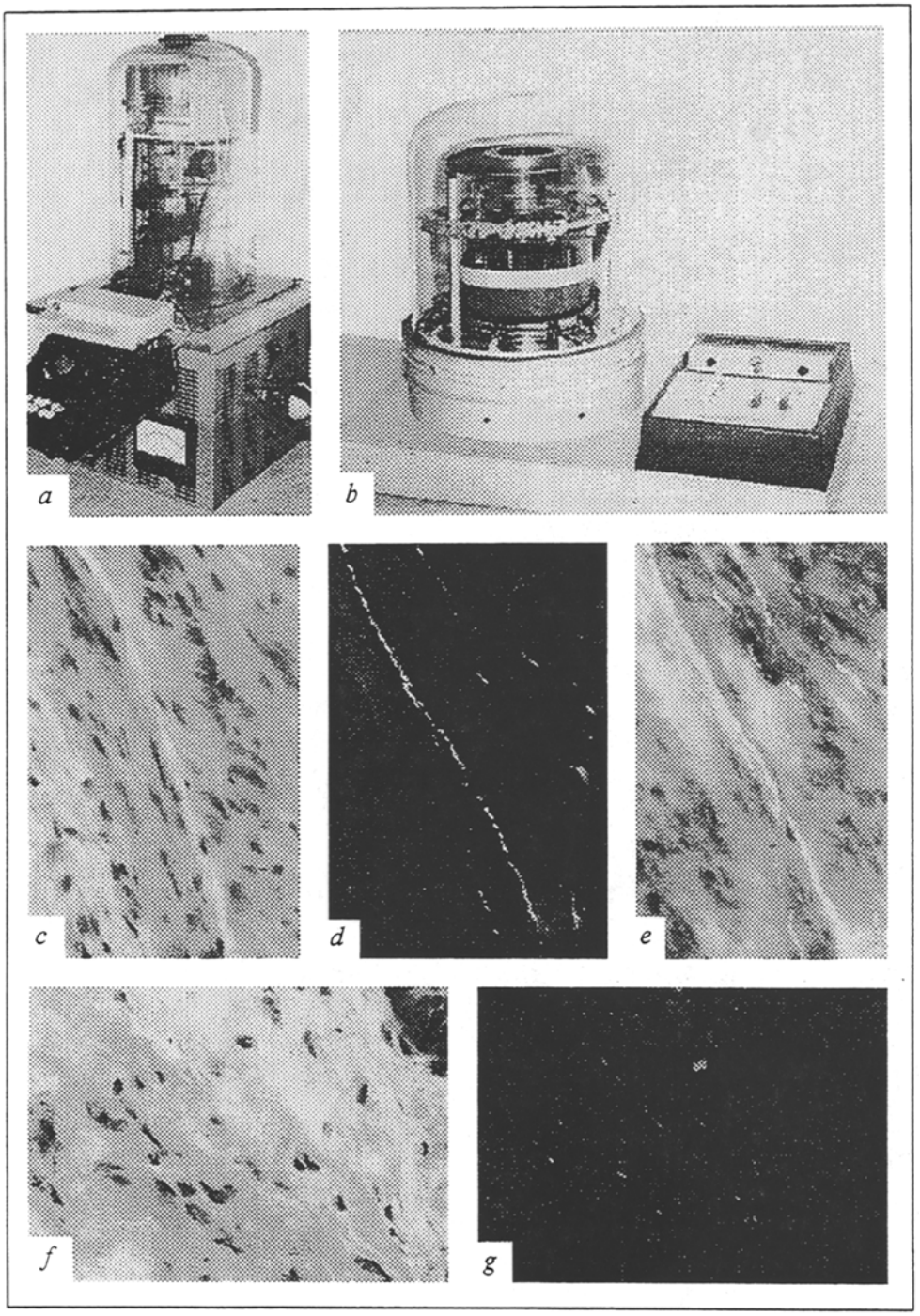

Fig. 1. Adrenergic nerve terminals in breast tissues and equipment for their detection. *a*) original equipment for processing biological material by the Falck vacuum method; *b*) original equipment for accelerated processing of biological material by the Falck method; *c*) stromal component of mammary dysplasia (stained with hematoxylin–eosin); *d*) the same section, moderate–intensity luminescence of the adrenergic nerve fiber (staining after Falck using the mentioned equipment); *e*) the same section, luminescence of the same adrenergic nervous structures in the stromal component (the method of successive staining and photography of biological material); *f*) parenchymal and stromal components of mam-mary fibroadenoma (hematoxylin–eosin staining); *g*) the same section, low–intensity luminescence of the same adrenergic nervous structures (staining after Falck using the mentioned equi-pment); *c–g*) ×360.

trolled by incubation in a 2% solution of glyoxylic acid [9] and by thermoprocessing of the slides without paraformaldehyde. Luminescence of the structures was visualized at wavelength 480 nm using a LYuMAM-IZ luminescence microscope.

RESULTS

A number of pieces of equipment [1,2] for qualitative detection of the adrenergic terminals in tissues of different organs and for determination of the features of their histoarchitectonics in relation to the surrounding structures were devised and tested based on the investigations and experiments performed. The qualitative processing of tissues by the paraformaldehyde method involves certain peculiarities both of cryoprocessing and of further histological treatment.

First, after rapid collection of the samples of biopsy or surgical material, cryoprocessing must be performed not in liquid nitrogen itself, but in its vapors, the degree of cooling varying slightly for

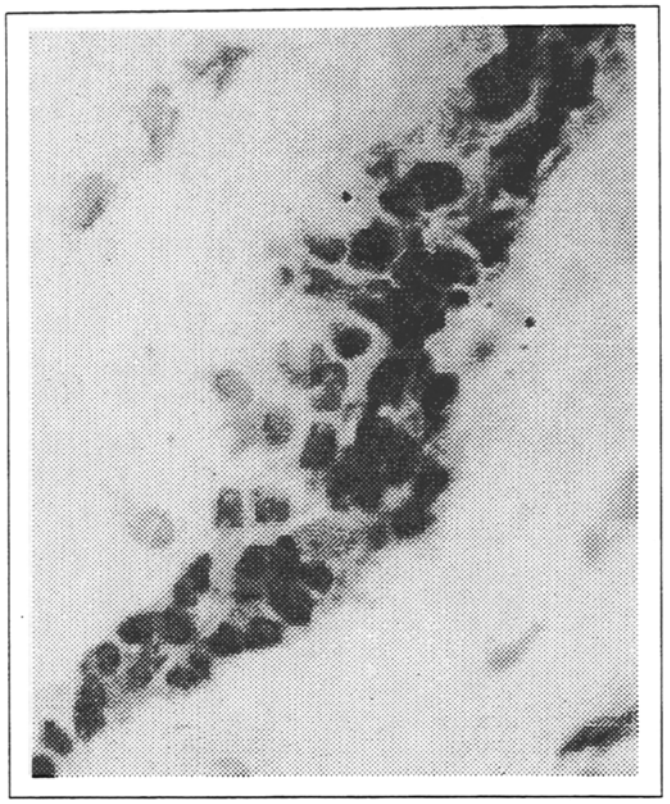

Fig. 2. Fragment of mammary dysplasia tissue with parenchymal and stromal elements. Staining with hematoxylin–eosin, ×360.

different tissues. The specific choice of the temperature regime of cryoprocessing and maintenance of its constancy during transportation to the place of further processing may be achieved using a simple piece of equipment, "the chamber for cryoprocessing of biological material" [1], in which the degree of tissue cooling depends on the height of the sample situated on a float and on the level of cryoagent, while a reserve of cryoagent constantly replenishes its level in the operating compartment according to the principle of communicating vessels, allowing for prolonged transport of the material to the place of further processing.

Second, vacuum processing of biological material after Falck in apparatus (Fig. 1, *a*) described as "equipment for processing biological material" [2] allows for processing without interruption of the pressurization of the system from tissue freeze drying to paraffinization, achieves effective subliming of paraformaldehyde, and yields good results in processing pieces of tissue. The quality of detection of catecholamines on sections processed after Falck is similar to that after Krokhina. It should be noted that biological material processed in a vacuum system by paraformaldehyde without additional fixation in formalin stains well with hematoxylin-eosin.

Third, the processing of cryostat sections mounted on slides is performed after Krokhina in apparatus (Fig. 1, *b*) described as "equipment for accelerated processing of biological material after Falck." This apparatus has been registered as an innovation in the Smolensk Medical Institute. The efficiency of processing in this case is achieved by placing the slides with histological specimens in a drum where the slides formed the rotating blades. The necessary temperature regime was achieved by circulating hot air between inner and outer hoods, placed over the drum. Various regimes of rotation of the drum in the closed space of the inner hood permit effective drying of the histological sections, uniform action of the gas medium, and a regulated action of the paraformaldehyde vapors, all this making it possible to detect the adrenergic nervous structures with a low concentration of transmitter (Fig. 1, *f*, *g*).

Fourth, the study of the histoarchitectonics of the adrenergic nervous structures in tissues of different organs calls for exact determination of their location in relation to the surrounding elements of

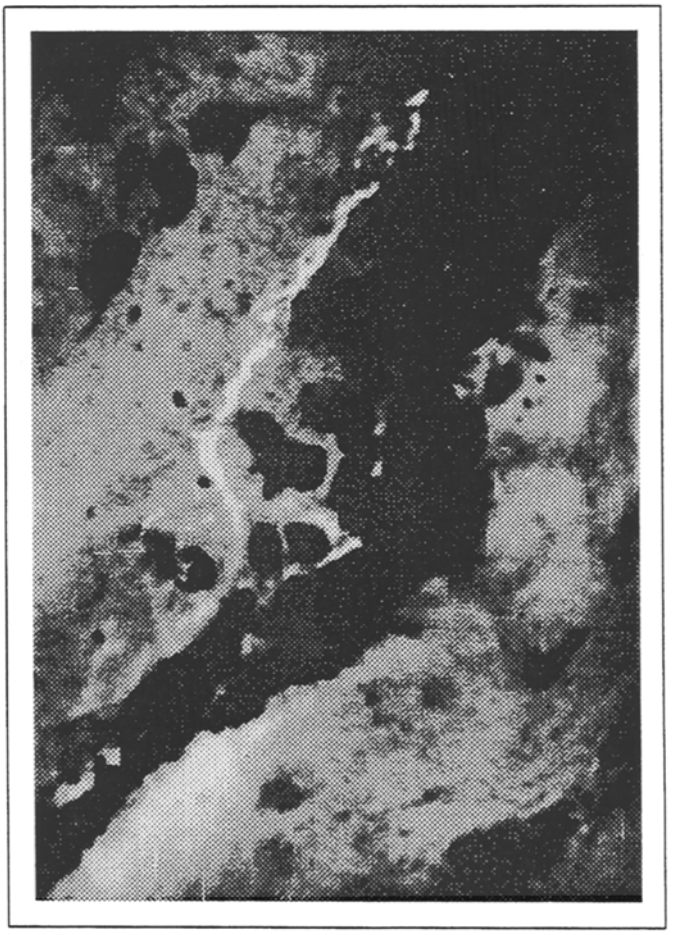

Fig. 3. Adrenergic nerve fibers and their cell miocroenvironment (the same section as on Fig. 2). Method of successive staining and photography of biological material, ×360.

the stroma and parenchyma. The routine orientation using the phase-contrast and dark-field methods is not always sufficiently accurate and does not permit one to document everything detected by the photomethods. The fact that slides processed by paraformaldehyde become well fixed and can be repeatedly stained with hematoxylin-eosin makes it possible to document the location of the adrenergic nervous terminals and their microenvironment using the method of successive staining of biological material processed after Falck (which is registered in the Smolensk Medical Institute as an innovation). Microphotography is performed as follows. The luminescing object found on the histological section is photographed and its accurate location on the slide fixed by the coordinates of the mounting screen. Then the same histological section is stained with hematoxylin-eosin (with additional fixation in formalin solution if necessary) and secondary photography is performed according to preset coordinates of the mounting screen on the same negative. Due to the double projection of the same section stained by different methods on one negative, accurate juxtaposition of the luminescing structures and of parenchymal and stromal components occurs (Figs. 1, *c-e*, 2, 3).

Thus, automatization of the detection of adrenergic nervous structures makes its possible to obtain stable results even in cases of a low concentration of transmitter. The technique of successive staining of biological material and corresponding determination of the localization of the adrenergic fibers allow for detection of the specific architectonics and cell microenvironment.

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