weeks of observation [5]. Neonatal androgenization or injection of exogenous estrogens thus has a promotor and (or) cocarcinogenic effect on the development of carcinogen-induced sarcomas of the uterus in mice.

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IMMUNOMORPHOLOGICAL IDENTIFICATION OF MYOEPITHELIAL CELLS IN MIXED MAMMARY GLAND TUMORS IN DOGS

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The histogenesis of the mixed mammary gland tumor is a problem that is still unsolved despite data on its epithelial origin [4, 8]. Discussion of the role and importance of myoepithelial cells (MEC) in the origin and development of the tumor is traditional. There are difficulties in the identification of myoepithelium (ME) at light- and electron-microscopic levels of investigation, due to the morphological and functional variation of the components of the hormonal-dependent mammary gland tissue [2]. With the discovery of contractile proteins (actin, myosin) of smooth-muscle nature in MEC, the way was open for the use of specific immunochemical methods to detect these cells [5, 7]. Because of the structural and functional characteristics of the dog mammary gland, from the technical point of view it affords a convenient object with which to study the difficult problems of histogenesis of mixed tumors of this organ [1].

The object of this investigation was to identify MEC with the aid of monospecific antiserum against smooth-muscle myosin and to study the character of distribution of these cells in mixed tumors of the canine mammary gland.

EXPERIMENTAL METHOD

Materal removed during operations on five dogs, under observation at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, for spontaneous mammary gland tumors was studied. The tumors were: mixed tumor (n = 3), anaplastic carcinoma (n = 1), and adenocarcinoma (n =

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1), developing against the background of a mixed tumor. From each animal four or five pieces of tumor were taken from different places, frozen in liquid nitrogen, and cut into sections on a cryostat. Serial sections 4 μ thick were incubated with monospecific antiserum against human smooth-muscle myosin by the indirect Coons' method. Immune sera were obtained, fluorescent antibodies prepared, and controls of their immunologic specificity were set up in the Laboratory of Immunomorphology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Control sections were treated with nonimmune serum. In parallel tests the material was fixed in 10% neutral formalin and this was followed by staining with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Immunochemical identification of MEC revealed fluorescence of varied intensity and a nonhomogeneous distribution of ME in the mammary gland tumor tissue. As a rule fluorescent cells were found in lobular structures: In the alveoli they had the appearance of "basket" cells, whereas in the ducts they were fusiform in shape. MEC lined the walls of the cystically dilated ducts, in the neighborhood of which islet-like concentrations of MEC were often found, with a palely fluorescent rim of cytoplasm (Fig. 1e), or diffuse foci of proliferation of MEC were observed in the intralobular stroma. Side by side with them there were ducts with papular structures of proliferating epithelium and MEC, in the peripheral zones of which bright, diffuse fluorescence of the cytoplasm and of hypertorphied outgrowths of MEC was found (Fig. 1a, b, d). During migration of ME toward the stroma or lumen of the ducts the intensity and character of distribution of specific fluorescence in these cells changed. The MEC lost their cytoplasmic outgrowths, became more circular in shape, and exhibited weak fluorescence only of the peripheral layers of their cytoplasm (Fig. 1c). Regions of tumor proliferation, which were gland-like in structure (with tubular or duct-like structures) or forming concentrations of fusiform cells in the stroma, did not exhibit specific fluorescence with antiserum against smooth-muscle myosin. MEC were absent in areas of mysomatous growth and in the chondroid and osteocartilaginous zones of the tumor. Fluorescent cells in these zones had the characteristic orange-green fluorescence in both experiment and control, and this was evidently due to accumulation of products of their synthetic activity or metabolism in these cells. The study of anaplastic carcinoma and adenocarcinoma developing against the background of a mixed mammary gland tumor did not reveal the presence of ME in groups of cancer cells.

The character of distribution of MEC in the mixed tumors thus has much in common with the types and forms of hyperplasia of ME which the writers studied previously in dyshormonal dysplasias and benign tumors of the mammary gland in man and the dog [3]. Meanwhile variation of specific fluorescence of MEC in the mixed mammary gland tumors is evidence of different levels of differentiation of these cells. What are the histogenetic mechanisms responsible for the appearance of these different cell forms of MEC in these tumors?

We know that MEC are cells of the epithelium of the terminal ducts and alveoli, whose differentiation is based on synthesis of smooth-muscle proteins that determine their characteristic structure and specialized functions [8, 9]. According to Dardiou et al. [8], the presence of immature forms of ME in mixed tumors is a result of the transformation of undifferentiated epithelial cells, as a result of which previously blocked genes of specific protein synthesis in these cells are derepressed. However, this explanation appears rather oversimplified, for it fails to take into account the concrete mechanisms of intercellular relations that play the "triggering" role in MEC differentiation, i.e., factors leading to expression of the corresponding genes in immature epithelial cells. At the same time, it has been shown that during normal development differentiation of ME does not begin until the cellular components in the epithelial layer have reached a certain density and the basement membrane has been formed [9, 10, 12]. According to data in the literature [11], prolonged passage of mammary gland tumor cells *in vitro* may end with their differentiation into ME if the cells of the peripheral zone of the proliferating tumor form intercellular junctions and a basement membrane, i.e., if they lose their features of malignant transformation partially or completely.

Consequently, differentiation of MEC both in normal epithelial tissue and in proliferating tumors is determined by the borderline position of the closely contacting cells adjacent to the basement membrane. Disturbances in regulation of the number of cells in foci of dyshormonal proliferation and in proliferating tumors of the memmary gland, accompanied by mi-

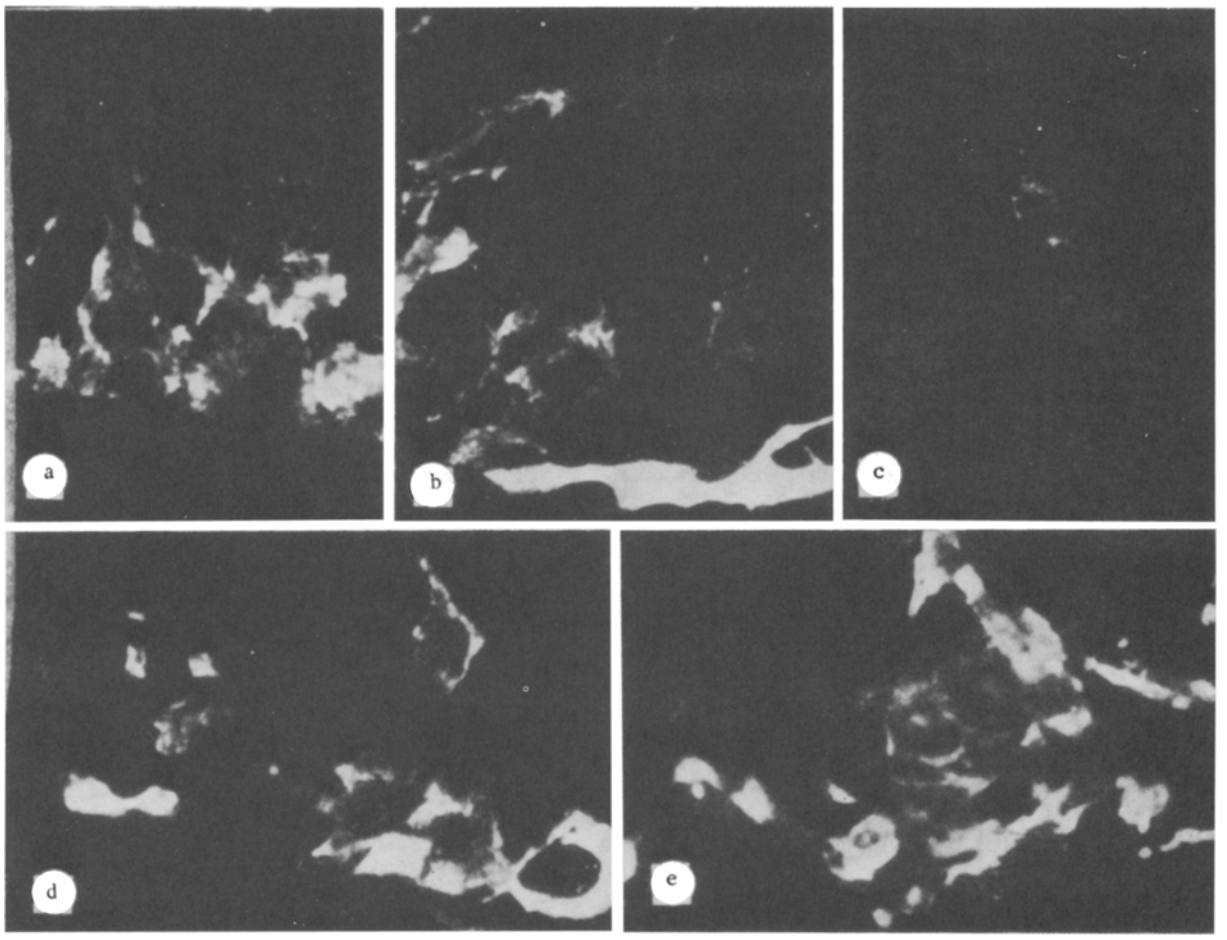

Fig. 1. Intensity and character of distribution of MEC in mixed mammary gland tumors in dogs. a, b, d) Against a background of bright fluorescence of MEC and their cytoplasmic processes, MEC with a palely stained rim of cytoplasm can be seen in the intralobular epimyoepithelial foci of proliferation, migrating toward the lumen of the duct structures (c) or into the stroma, with the formation of islet-like concentrations (e). Frozen sections treated with monospecific antiserum against smooth-muscle myosin by the indirect Coons' method, 280×.

gration of the cells relative to the basement membrane may, evidently, lead to disappearance of the features of myoepithelial differentiation. This hypothesis is in agreement with data on the ability of ME to undergo modulation in the normally functioning mammary gland [2, 6].

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