through the secondary messengers cAMP and cGMP [2, 3, 7], gives rise to a cascade of biochemical reactions, which are connected, ultimately with the methylation of DNA cytosine [4, 6] and manifest themselves in chromatin rearrangement.

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An Ultrastructural Study of Colon Cancer Explants in a Long-Term Organ Culture

A. G. Perevoshchikov and N. N. Petrovichev

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The organ culture (OC) is one of the important and at the same time unique methods permitting an *in vitro* study of the laws governing the formation of a human tumor heterogenous cell population. OC of colon cancer (CC) [4-6, 9] as well as of the normal colon mucosa has been described in several reports [2, 3, 7, 8]. The analysis of these makes it clear that organ-specific structures develop in the OC that are characteristic of the normal colon mucosa and malignant neoplasms derived from it. These as a rule have the histological structure of adenocarcinomas. Earlier [1] we have showed the capacity of tumor cells in OC to synthesize carcinoembryonic antigen (CEA), and presented a comprehensive light-optic characterization of the peculiarities of explant growth depending on the period of incubation.

The goal of this work was a detailed electron-microscopic study of the ultrastructure of CC in OC in order to define the potential of OC as a model for the investigation of the histogenesis or, more precisely, the cytogenesis of colon epithelial tumors.

Laboratory of Histochemistry and Electron Microscopy, and Laboratory of Clinical Cytology, Cancer Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by Yu. N. Solov'ev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Specimens of tumor tissue obtained immediately after operation on the colon were used for the establishment of OC. Histologically, all the tumors belonged to highly to moderately differentiated adenocarcinomas. The tumor tissue was cut into pieces of a size less than or equal to 1 mm^3 and placed on filters with pores of 40 μ diameter (Synpor, Czechoslovakia). The incubation medium consisted of RPMI 1640 supplemented with 20% bovine serum, 10% embryonic extract, 0.06 mg/ml glucose, 0.03 mg/ml ascorbic acid, 5 mg/ml hydrocortisone, 50 μg/ml cantomycin and 50 μg/ml gentamycin. The mincing of the tumor tissue was carried out in incubation medium supplemented with a double quantity of cantomycin and gentamycin under ultraviolet illumination. The OC was grown in a Bruveau incubator at 36.0-36.5°C, in an atmosphere of 100%

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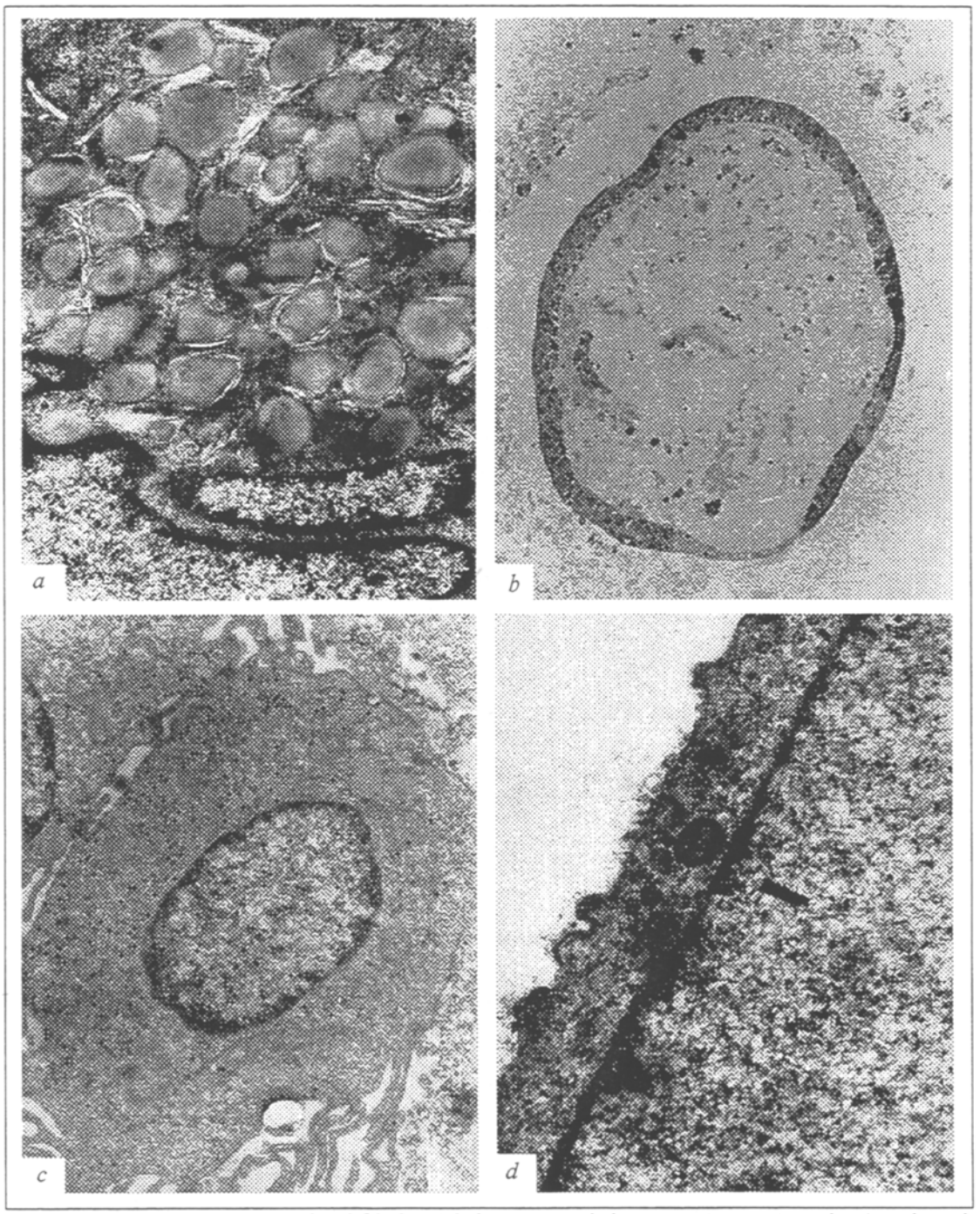

Fig. 1. Organ culture of colon cancer on the 2nd and 5th day. A light–optic and electron–microscopic study. *a*) explant ultrastructure on 2nd day of culture: a cancer cell with signs of dystrophic alterations in the cytoplasm (a large number of fat droplets with features of myelinization); ×10,000. *b*) tangential semithin section of explant on 5th day of culture: the piece has the shape of a hollow tube consisting of a single– to bilayer epithelium; ×40. *c*) explant ultrastructure on 5th day of culture: an undifferentiated tumor cells containing a round nucleus with diffuse chromatin, multiple free ribosomes, single mitochondria, and developed intercellular finger–shaped cytoplasmic processes; ×10,000. *d*) explant ultrastructure on 5th day of culture: a tumor cell with the first signs of the development of single apical microvilli coated with a scarcely visible layer of glycocalyx–like substance; ×15,000. The sections are contrasted with uranyl acetate and lead citrate (*a*, *c*, *d*) or stained with toluidine blue (*b*).

humidity and approximately 10% CO_2. The medium was changed twice a week. The patterns of explant growth were studied by an electron-microscopic examination of the explants on the 2nd, 5th, 7th, 9th, 12th, 15th, 17th, 20th, 24th, and 27th day of OC. The explants were fixed in 2.5% glutaraldehyde and 1% OsO_4 solution, and embedded in EPON-812 routinely. Ultrathin slices contrasted with uranyl acetate solution and lead citrate were examined under a JEM-1200 EX electron microscope (Japan).

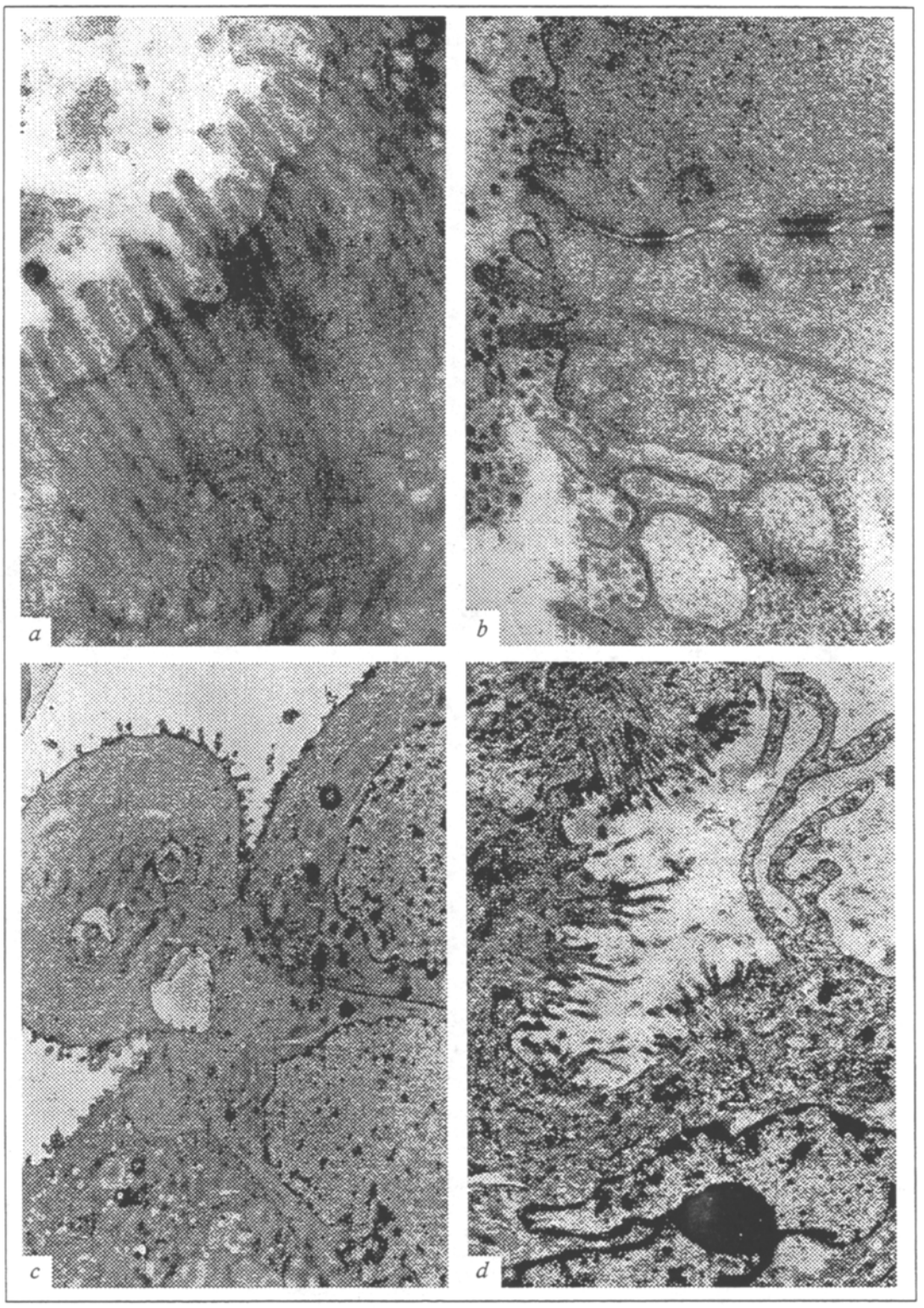

Fig. 2. Ultrastructure of colon cancer explants on 7th—9th day of organ culture. *a*) 7th day of organ culture. A fragment of tumor cell with developed apical microvilli forming brush—border—like structure. Dense roots of tonofibrils lie at the bottom of the microvilli; ×15,000. *b*) 7th day of organ culture. Ultrastructure of tumor cells with features of brush—border enterocyte. Note the typical cell junctions in th form of zonulae occludenses, zonulae adherenses, and desmosomes, apical microvilli, and glycocalyx bodies. Solitary mucin—type secretory granules appear; ×25,000. *c*) 7th day of organ culture. At the end of explant process a budding—off of the tumor cell can be seen; ×3000. *d*) 9th day of culture. A process of glandular structure formation in the complex of ultrastructurally differentiated tumor cells having lost the connections with the main explant process; ×5000. Sections are contrasted with uranyl acetate and lead citrate.

RESULTS

The ultrastructural study of 2-day explants revealed regions with vast structureless masses, containing fragments of dead cells with vague outlines of karyolemma and clumps of decayed chromatin. The cell cytoplasm was weakly contoured and electron dense, without any organelles to be seen, and the cell borders could not be determined. Solitary intact cells of fibroblast nature, small blood vessels, and multiple freely lying macrophage-derived cells with numerous lysosomal bodies in the cytoplasm (from primary lysosomes to autophagosomes) were disposed among the collagen fibers. Single more or less preserved complexes of tumor cells also occurred. The cells exhibited ultrastructural features of necrobiotic and dystrophic alterations, namely the cytoplasmic accumulation of numerous fat droplets with myelinization phenomena (Fig. 1, *a*).

On the 5th day of OC the explant was wholly covered with epithelial cells and had the form of a hollow tube in cross section (Fig. 1, *b*). The epithelial nature of the cells was evidenced by the presence of a basal membrane and of specific junctions presenting as developed desmosomes. On the whole, the ultrastructure of the cells answered the common definition of poorly differentiated cancer cells lacking any sign of organ specificity. They had, as a rule, a large or moderately sized round nucleus with a diffuse distribution of chromatin (Fig. 1, *c*). The apical plasmalemma was smooth, without microvilli, and contained mostly free ribosomes, single mitochondria, profiles of rough endoplasmic reticulum (RER), bundles of tonofibrils, and a varying number of lysosome-related dense bodies. The presence of the latter testified to an active process of intracellular digestion. Areas of immature striated collagen of the embryonic tipe, a small number of elastin fibers surrounded by an amorphus mass of moderately electron-dense substance, and solitary fibroblasts could be seen. Careful scrutiny of the fine structure revealed single undifferentiated tumor cells that had small, short processes of plasmalemma on the apical surface coated with a scarcely visible, smooth layer of "fuzz" resembling glycocalyx (Fig. 1, *d*). Such findings were interpreted as initial signs of the formation of apical microvilli of the brush border belonging to cells differentiating after the type of brush border enterocytes (BE).

The subsequent observation (7th day of OC) proved that the tumor cells had largely acquired the features of ultrastructurally mature cells, with the characteristics of an organ-specific BE-type differentiation. This was expressed in the appearance of developed apical microvilli with well-formed roots of electron-dense tonofibrils at the bottom (Fig. 2, *a*). The cell junctions also had a structure typical of BE and consisted of successive apical sites of zonulae occludenses, zonulae adherenses, and developed desmosomes (Fig. 2, *b*). On the surface of the apical microvilli the typical glycocalyx bodies and a scarcely visible layer of glycocalyx-like substance could be seen. The tumor cells formed numerous thin finger-shaped cytoplasmic processes and a basal membrane in the basal part. At this period of OC, as mentioned earlier [1], multiple processes consisting of ultrastructurally differentiated cells departed from the initial explant. It should be pointed out that at the ends of the processes individual cells were budding off (Fig. 2, *c*). Both in the initial explant and in its processes it was possible to discern tumor cells with different electron density, so-called light and dark cells, and single round granules with floccular contents resembling mucine granules (Fig. 2, *b*).

On the 9th day of OC on the ends of some processes complexes of cells differentiated in the BE pathway could be detected which had lost contact with the processes and seemed to be forming glandular structures (Fig. 2, *d*). In other regions of the explants well-formed primitive glands occurred, composed of several cells with developed apical microvilli forming a brush-border (Fig. 3, *a*). Inside the initial explant one could also see an intensive process of gland formation by the invagination of the epithelial lining. The gland-forming tumor cells possessed prominent ultrastructural features of brush-border and, occasionally, goblet-like enterocytes. At this time of OC the peripheral lining of the explant showed gaps (Fig. 3, *b*), an individual tumor cells spread outside the explant. These cells did not form any structures, but lay apart from the initial piece and had an ultrastructure of undifferentiated cells lacking the features of organ-specific differentiation.

On the 12th-27th day of OC a gradual increase in the number of glandular structures was observed both inside the main piece and at the ends of the processes. There was an active pinching-off the glandular structures from the processes consisting of ultrastructurally differentiated cells with the features of BE (Fig. 3, *c*). Inside the explants numerous glandular structures were discerned, composed either entirely of BE-like cells or of a mixture of those and goblet-type cells with multiple intracytoplasmic mucin granules (Fig. 3, *d*). Occasionally mixed-type cells were encountered which combined the ultrastructural features of brush-border and goblet enterocytes. On the whole, in the late stages of OC the changes in the explants were uniform and consisted of an increase in the number of glandular structures both in the explanted piece and at the ends of its processes, the number of which continued to increase appreciably.

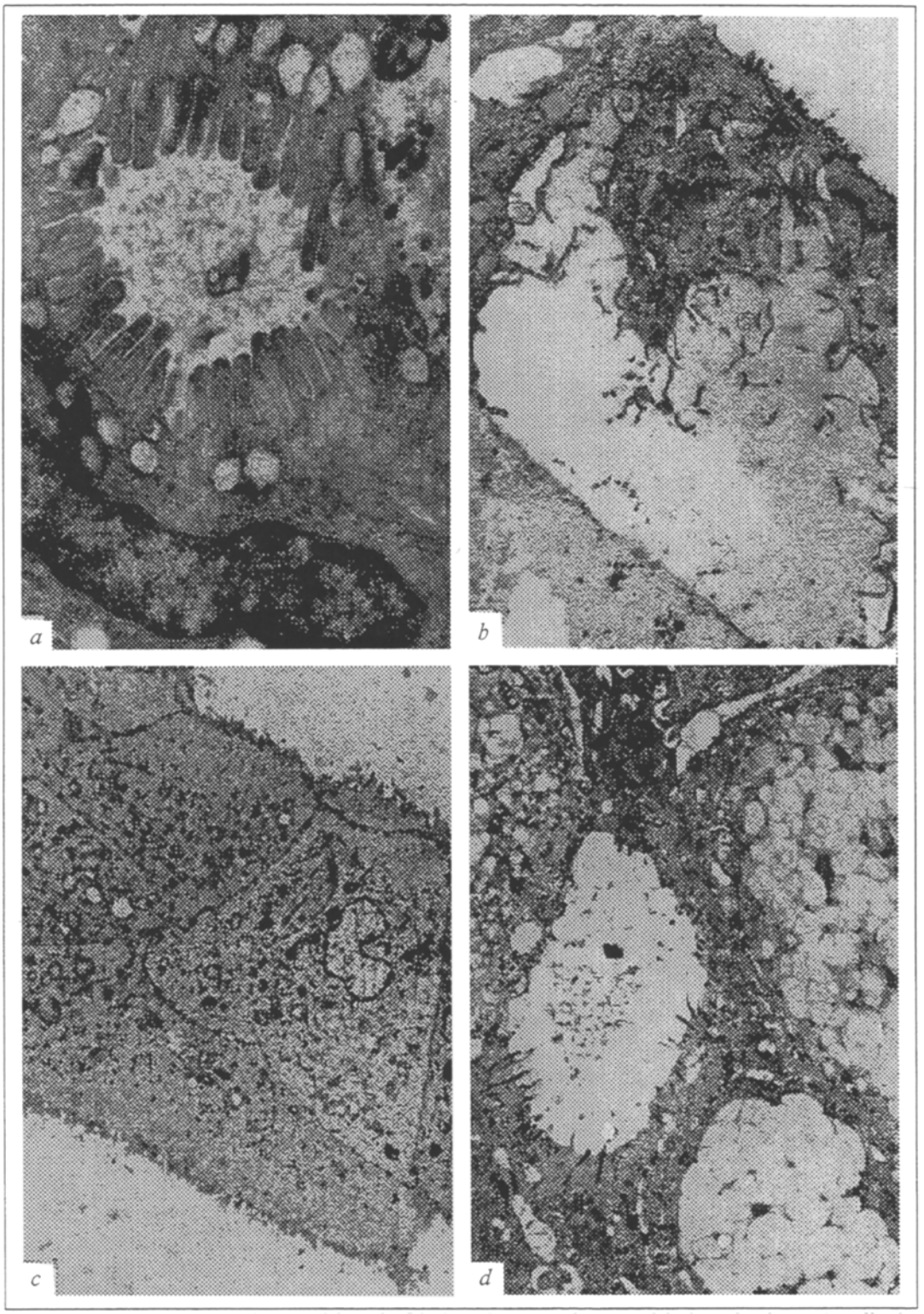

Fig. 3. Ultrastructure of colon cancer explant on 9th and 15th day of organ culture. *a*) 9th day of culture. A well-formed primitive glandular structure disposed near the process and consisting of several tumor cells with developed apical microvilli; ×15,000. *b*) 9th day of culture. A gap is developing in the peripheral lining of the main piece (explant); ×4000. *c*) 15th day of culture. A complex of ultrastructurally differentiated tumor cells (brush-border enterocyte type) pinched off from the process; ×3000. *d*) 15th day of culture. Ultrastructure of gland lying inside the explant and consisting of brush-border enterocytes-like and goblet enterocyte-like cells; ×4000. Sections are contrasted with uranyl acetate and lead citrate.

Thus, the study of CC growth in the OC revealed that the ultrastructurally undifferentiated cells are not only able to proliferate, forming an outer lining of the explant by the 3rd-5th day, but can also undergo

a further organ-specific differentiation, e.g., in the direction of brush-border and goblet-like enterocytes (7th-9th and subsequent days of OC). It is possible to assume on the basis of the data obtained that in the first 3 days a portion of the tumor cells die, as judged from the observed signs of necrosis and dystrophy, this stage being followed by a gradual adaptation of individual surviving tumor cells to the new conditions. Later, on the 3rd-5th day, an intensive proliferation of ultrastructurally undifferentiated cells can be seen. Perhaps some of these cells are represented by cambial elements (stem cells? committed cells? and/or precursor cells?) which on the 5th-7th day acquire, due to the proceeding differentiation, the ultrastructural features of first immature and then mature brush-border enterocytes. These form gland-like structures characteristic for this type of tumor. As for the second direction of the specific differentiation of tumor cells in OC, the first ultrastructural features of goblet-like enterocytes appear on the 7th day and reach their maximum development on the 9th-12th day. The finding of mixed-type cells also provides evidence in favor of the existence of a monoclonal source of development in the CC cell population.

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Ultrastructure of Nuclear Chomatin in Hepatocytes from Regenerating Guinea Pig Liver: Effect of Vitamin B_{12}

R. I. Klyasheva

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Key Words: *hepatocytes, chromatin rearrangement; vitamin B_{12}; nucleoplasmic chromatin; perimembrane chromatin*

The study of the effect of vitamin B_{12} on chromatin ultrastructure (specifically, the area of dense perimembrane chromatin - PmC) in the nuclei of the regenerating liver is very topical. For this reason we carried out an electron-microscopic study of the area of nuclei, nucleoplasmic chromatin (NpC), and PmC in the regenerating liver of guinea pigs which received high doses of vitamin B_{12}.

Department of Pharmacology, Medical Faculty of the I. N. Ul'yanov State University, Chuvash. (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The experiments were carried out on 10 male guinea pigs aged 6 months and weighing 0.5 kg. The experimental animals underwent a partial hepatectomy (resection of the left external lobe - lobus externus sinister) and received vitamin B_{12} in a dose of 0.8 mg

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