

AN EXPERIMENTAL HISTOLOGICAL INVESTIGATION OF THE EPITHELIUM OF THE CERVICAL CANAL OF THE UTERUS IN HUMANS

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The epithelium of the cervical canal of the human uterus and its glands are derived from the Müllerian ducts. Although distinguished by great uniformity in physiological conditions, the epithelium of the cervical canal shows a wide range of variation in pathological processes in humans (proliferation [6], pregnancy and hormonal changes [9, 16, 17, 18], and tumors [3, 4, 21]) and also in animals under experimental conditions [5, 10, 11]. In this situation in particular may be formed structures composed of many rows or layers whose origin and nature are the subjects of animated discussion at the present time.

This paper is devoted to a study of the changes in the structures of the cervical epithelium of the human uterus by the explantation method.

EXPERIMENTAL METHOD

As material for implantation we used particles of the mucous membrane of the cervical canal, obtained from women undergoing operation for various diseases of the body of the uterus. The patients were aged from 28 to 48 years. Altogether 14 series of experiments were carried out. Cultivation was done in flasks. The nutrient medium used was plasma and embryonic extract of chicks, human group AB serum and Tyrode solution. To the nutrient medium was added a mixture of penicillin and streptomycin in a dose of 0.1 μ g per flask. The maximum period of cultivation was 35 days. Altogether 225 cultures were studied, living and in fixed preparations, in both total and serial sections. The fixing agents were 15% formalin and Zenker-formol. Stains: Carazzi's hematoxylin, Heidenhain's iron-hematoxylin, mucicarmine, hematoxylin-eosin, azure P-eosin, van Gieson or Mallory. In order to demonstrate the cell margins the preparations were treated with silver nitrate.

EXPERIMENTAL RESULTS

Cultivation of the cervical epithelium is associated with great difficulty on account of the pronounced fibrinolysis. The inoculated fragments as a rule floated freely after only 24 hours within a cavity of liquefaction. Repeated daily passage of the floating fragments into new flasks was required before it was possible to obtain extensive growth of epithelium. This is a characteristic property of explantates of epithelia derived from the Müllerian ducts [1, 2, 7, 8]. Fibrinolysis was more pronounced the greater the formation of mucus. It falls considerably on the 5th-10th day of cultivation. Extensive growth in the form of membranes is observed relatively rarely, and usually epithelialization of the central fragment occurs. Growth in the cultures is brought about by the epithelium lining the canal and the epithelium of the glands. Only a few hours after inoculation characteristic outgrowths of epithelium appear at the margin of the explantate in the form of folds and of rounded, spherical or elongated formations, freely projecting into the surrounding nutrient medium. Initially these outgrowths have a compact structure. Later, as a result of disintegration of their central part

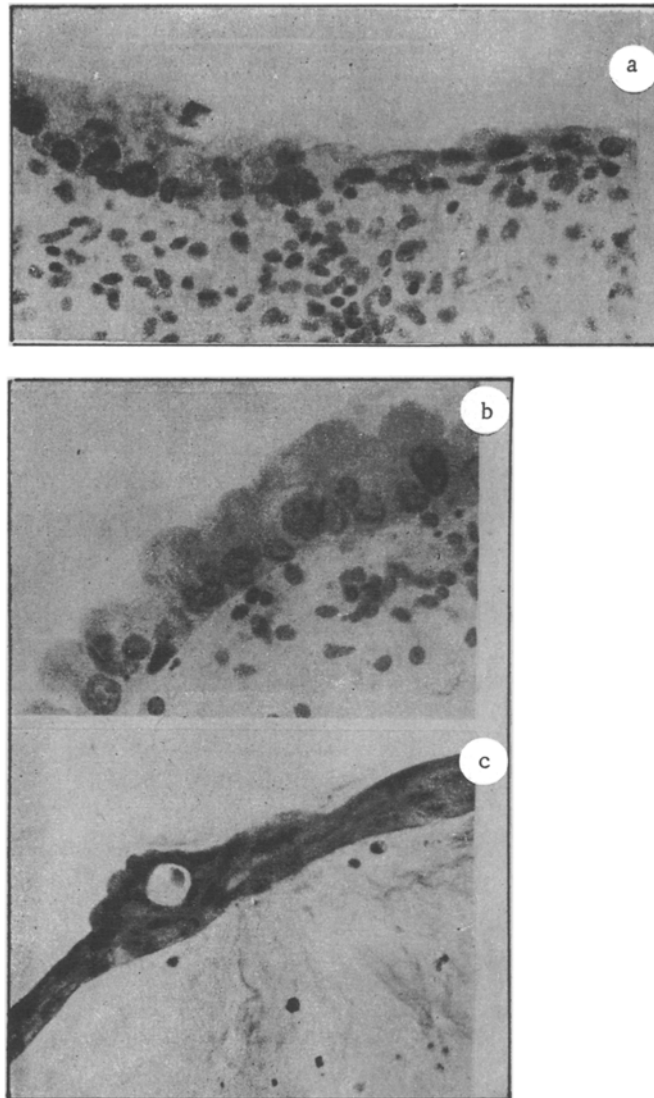


Fig. 1. Integumentary epithelial cell layers. a) Cells of differing height in the composition of the layer; celloidin section, 15 day culture, Zenker formol, hematoxylin - eosin; b) single-layered high prismatic integumentary epithelium, dome-shaped apical surfaces of the cells; celloidin section, 15 day culture, Zenker formol, hematoxylin - mucicarmine; c) multi-layered epithelializing cell lamina, cells cylindrical in shape with dome-shaped apical surfaces bound a cavity; celloidin section, 12-day culture, Zenker - formol, iron - hematoxylin. Magnification 400 x.

and of a general increase in their dimensions, they are converted into vesicles and tubes with a single-layered epithelial wall.

The cervical epithelium rapidly epithelializes the surface of the explantate. Complete epithelialization takes place on the 2nd - 3rd day. The integumentary layer, as a rule, has a single-layered and single column structure. Differentiation begins relatively early in this layer and takes place not simultaneously throughout the layer, but in the form of islets. As a result of this the fragment appears to be covered with epithelium in which there is alternation of areas consisting of condensed isoprismatic and high prismatic cells

(Fig. 1, a). Sometimes the whole covering epithelium of the explantate consists of squamous or cubical cells among which are found only small groups of a few high prismatic cells.

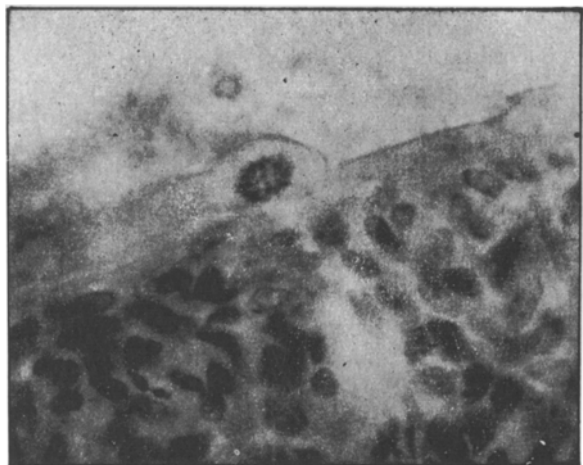


Fig. 2. Growth of connective tissue within a fragment, mitosis in single-layered integumentary epithelium, celloidin section, 16 day culture, Zenker formol, hematoxylin - eosin. Magnification 600 x.

stained nucleus, containing fine chromatin granules. In our material we were unable to see whether these cells were arranged in groups or in a continuous layer. They were usually dotted in one by one. It is difficult to say whether they correspond to the "subepithelial" or "reserve" cells which are described in the cervical epithelium of the uterus [6, 19, 20], although such an impression seems probable.

In the period of most active proliferation of the epithelium areas consisting of many columns or layers are formed in addition to the single-layered integumentary cells. The number of layers here may reach 5 or more. These areas are distinguished from the multi-layered integumentary cells in cultures of epidermal epithelia by the absence of vertical anisomorphism in all stages of cultivation, and consist of solitary round or slightly flattened cells. Mitoses are distributed at all levels of the multi-layered cells. The cells of the superficial layer are often prismatic in shape, with a characteristically convex apical surface and a nucleus situated basally. Sometimes these cells bound a cavity formed as a result of mucin formation and degeneration of cells (Fig. 1, a). Over the whole surface of the multi-layered area there lies a thin film of mucus. The characteristic form of the cells and the presence of mucous secretion indicates that these multilayered structures are derivatives of the cervical epithelium of the uterus.

The multilayered structures formed during cultivation of epithelium from the cervical canal cannot be regarded as an accidental formation or as the result of accumulation of cells [1, 2, 15].

There is no doubt that the epithelia developing from the Müllerian ducts can under certain conditions (chemical irritation, avitaminosis B, hormonal changes, pathological proliferation, explantation) form multi-layered systems of cells, which are not transitional structures but which are preserved in explantates for 30 days or more. In previous work devoted to the celonephrodermal group of tissues [1, 2, 7, 8, 12, 13, 15], insufficient attention was directed to these structures and they were not correctly interpreted. There is now no doubt that they must be regarded as a manifestation of specific histoblastic forerunners of epithelia, derived from the Müllerian ducts. However, these multilayered structures, as may be seen from a comparison of our findings in work on the cervical and vaginal epithelium, are by no means identical with each other, and during explantation they firmly retain their qualitative differences. The multilayered structures of the cervical epithelium do not differentiate according to the epidermal type and in contrast to the vaginal epithelium they form mucus.

The cells and nuclei of the integumentary epithelium in early stages of cultivation are distinguished by the diversity of their shapes and sizes. With increasing age of the cultures the integumentary epithelium acquires a more uniform and differentiated character, often with well marked interlocking zones. In some cases the explantates are entirely covered by a single layer of high prismatic cells, which lie in close contact with each other and have a convex, dome-shaped free surface (Fig. 1, b) and one or several oval nuclei situated at the base. In the epithelialized areas a large mitosis is seen, distributed apically. The integumentary epithelium retains this character until the terminal periods of cultivation. Similar structures in the integumentary epithelium are observed in cultures from the mucous membrane of the uterus [2] and oviducts [1] of the rabbit, and in cultures from endometriosis [7], the epithelium of the tubes and certain ovarian cysts in humans [8].

In isolated cases, under the layer of high prismatic cells in the integumentary epithelial layers are found cells which are round in shape and with a lightly

During full epithelialization of the central fragment with a single layer of cells the growth of connective tissue cells inside the explantate does not cease, as takes place in explantates which are epithelialized by epidermal cell systems. On the contrary its cells proliferate and multiply by mitosis. The connective tissue acquires a "cytogenic" character and stays viable for 4-5 weeks (see Fig. 2). Presumably the epithelium of the cervical canal in contrast to epithelium of the epidermal type, and in particular vaginal, does not prevent to any appreciable extent the interchange of materials between the central part of the explantate and the nutrient medium.

Extensive growth of the epithelium in the form of membranes is seen less often than epithelialization and it begins considerably later, when fibrinolysis has become weaker. Very slender, always single-layered and extensively growing membranes are easily crumbled and soon die. For this reason they rarely attain a considerable size. Membranes of cervical epithelium differ sharply in the shape and arrangement of their cells from membranes of all other human epithelia and from the epithelia of the uterus and oviducts in the rabbit [8]. In them can be clearly distinguished alternating and dissimilar areas of varying extent, consisting of elongated dark and wide, longitudinally splitting lightly staining cells. The mutual arrangement of the cells, their arrangement and distribution in the different planes create a peculiar wave-like pattern which is accentuated by the uneven staining of the cytoplasm (Fig. 3, a). Membranes of this pattern are encountered only in cultures of epithelia derived from the Müllerian ducts in humans, and are regarded as an expression of species specificity. V. P. Mikhailov [8] calls this peculiar pattern of these membranes in explantates of the epithelium of the Fallopian tube and of certain ovarian cysts "watered silk" and considers that this sign may be used as a decisive proof of the origin of a cultivated tissue from the Müllerian ducts.

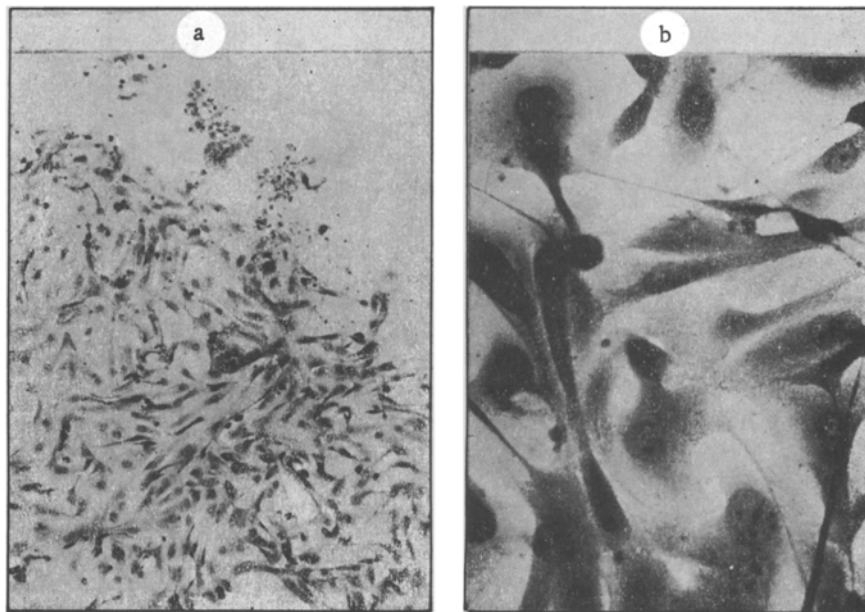


Fig. 3. Epithelial membranes. a) Usual appearance, peculiar wavelike pattern of the membrane, total preparation, 13 day culture, formalin, Carazzi hematoxylin, magnification 80 x; b) detail of the membrane with a wave-like pattern, elongated and longitudinally splitting cells, long narrow cytoplasmic processes situated in different planes; total preparation, 15 day culture, formalin, Carazzi hematoxylin, magnification 400 x.

The cells in the membranes are characterized by marked polymorphism and by considerable variations in their size. The perinuclear part of the cytoplasm stains very intensely but towards the periphery the staining becomes less intense and the cell margin is hardly distinguishable. For this reason the wrong impression may be created that the cells do not lie next to each other but are separated by intervals. The cell margins are not clear in sections stained with hematoxylin and they are well shown only when stained with silver.

The cells are often equipped with long, fine cytoplasmic processes which connect cells far removed from each other (Fig. 3, b). The processes merge with the substance of the membranes, cross each other, are distributed in different planes and form a complex network on the free surface of the membrane. The round nuclei usually contain 1-2 nucleoli and fine chromatin granules. The nuclei are situated in the center of the cell, eccentrically or even in one of its processes.

For this reason their distribution is often highly uneven. The majority of the cells are mononuclear, but occasionally cells with 2-4 nuclei are seen.

Particularly in the early stages of cultivation numerous mitoses are found in the membranes, up to 8-10 in a field of vision of a 40 x objective. Amitoses and fragmentation of nuclei are seen relatively rarely. They are commoner in aging cultures. Rare discoveries, but worthy of attention, include solitary cells with ciliae in the composition of some membranes. In living preparations the waving motion of these living ciliae was observed.

Only in isolated explantates is an extensive mixed growth of epithelium and connective tissue observed. This takes place only in cases where the connective tissues, quantitatively predominant, is able to grow into the fibrin before it begins to liquefy and the fragment epithelializes.

Throughout all periods of cultivation secretion of mucus is observed in explantates of cervical epithelium. In the first few days after inoculation an abundant formation of mucus takes place both by the cells of the living epithelium and by the cervical glands which happen to be present in the fragment. Glands distended with mucus may open out on the surface of the explantate and their contents be liberated. Mucus formation gradually diminishes. Mucus is formed as tiny inclusions and small vacuoles in the cells of the membranes and of the integumentary epithelial layers, both single- and multilayered, and also as a narrow film on their surfaces. In the high prismatic cells the whole of the cytoplasm above the nucleus stains intensely with mucicarmine. In contrast to this there are indications [1] that in a particular case active formation of mucus may take place by the growing cells themselves and not by the preservation of inclusions of secretion already present in the explanted material.

Thus the epithelium of the human cervical canal in culture not only shows certain histological properties typical of and common to tissues of celonephrodermal type [14], but also reveals profoundly distinctive features and species specificity. The epithelium of the cervical canal, normally uniform, shows considerable plasticity in cultural conditions, giving rise to high prismatic cervical epithelium and to undifferentiated uterine epithelium, and forming structures characterized by many layers and columns. The formation of multilayered structures by the cervical epithelium is connected with active proliferation of its cells.

Summing up its histological typical properties as revealed during explanation, the epithelium of the cervical canal may be sharply and firmly distinguished from the epidermal epithelia, including the epithelium of the vagina which we have ourselves studied. This is shown by a difference in the fibrinolytic activity, by the character and the time of growth, the number and situation of mitoses, the secretory function, the structure of membranes and epithelializing layers, by the different trends of their differentiation and by their cytological peculiarities.

SUMMARY

Culturing of normal epithelium of the cervical canal is associated with pronounced fibrinolysis and mucus secretion.

Single-layered, multirowed and multilayered strata are formed in epithelization of various sections. The latter differ from the multi-layer strata of the epidermal type by the absence of the vertical anisomorphism and by the presence of mucus secretion. They should be considered as the manifestation of the specific histoblastic potentiality of epithelia, derived from the Müllerian ducts.

Peculiar membranes with characteristic wave-like "moiré" pattern are formed in extensive growth, which is the manifestation of the species-specificity of the human Müller's epithelium. The cervical canal epithelium shows a great difference from the epithelia of the epidermal type.

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*In Russian.

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