in the litters. Canalization of the vagina took place at about the same age as it does with the young stock females of our colony.

Discussion. While a greater number of animals treated with both larger and smaller quantities of these hormones for longer and shorter periods of time is desirable, we believe that our data justify certain conclusions. It seems quite definite that the changes evoked by the administration of theelin, theelol and the luteinizing substance do not permanently affect the normal ovarian function. The fact that those animals receiving the smaller amount of theelol mated immediately upon introduction of the males might indicate that a sterilization was induced in the other 3 groups. However, since we have not determined the average length of time that untreated females remain with the male before fertilization takes place, such a conclusion may be unjustified.

Summary. Prolonged administration of either theelin or theelol to adult female rats does not destroy the reproductive function, as is evidenced by their ability to bear and rear normal litters. The treatment with the luteinizing substance from pregnancy urine produces complete inhibition of estrus together with profound ovarian luteinization but does not permanently impair the reproductive mechanism.

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On the Classification of Cells According to Their Inorganic Structure in vitro.*

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One of the greatest difficulties in carrying out any problem *in vitro* is that of establishing a morphological criterion by which the various cell elements composing the growth can readily be classified. When dealing with pure culture strains of "Fibroblasts" and epithelial cells it is a comparatively simple matter to distinguish between the 2 cell types, as they not only exhibit distinct differences in their morphology, but also in their mode of growth. But when

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attempting to distinguish these 2 different types of cells in a mixed growth colony it becomes more difficult, as the epithelial elements composing the advancing free edge of the new growth invariably isolate themselves from the epithelial sheet and migrate into the culture medium as separate units, assuming a contour similar to fibroblasts (see Fig. 1). But far greater difficulties confront the

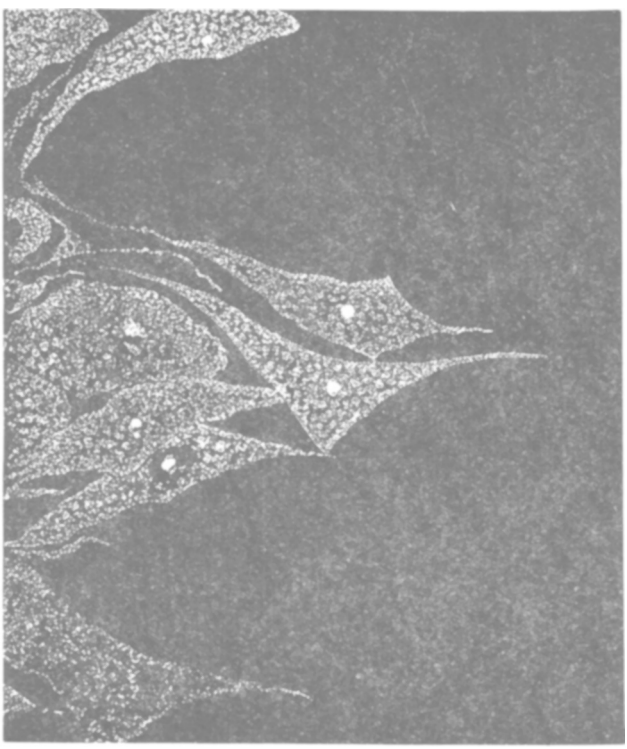

FIG. 1.

Representing inorganic structure of cells in the peripheral region of a 7 days' chick epithelial tissue culture, as seen by dark field illumination. Observe the diffuse distribution of calcium salts, and also the manner in which the cells on the free growing edge of the culture have become mobilized and have assumed a fibroblast-like contour.

investigator when he has to identify cells of the mesenchyme type, such as osteoblasts, chondrioblasts, and heart fibroblasts which, although possessing varying functional activities, exhibit similar morphological values.

Parker and Fischer,[1] realizing this difficulty, successfully demonstrated that mesenchyme elements in tissue cultures, which would be designated morphologically as "Fibroblasts", are found to possess different inherent growth potencies when cultivated under similar

[1] Parker, R. C., and Fischer, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 580.

conditions *in vitro*. On these grounds they rightly contended that "the physiological properties of the cell should constitute the first claims to any definition."

Recently Horning[2] found that tissue culture cells which exhibit the same morphological characteristics and behavior under normal conditions *in vitro*, differ, however, in their reactions to similar pathological conditions, as the rate of cytolysis was in all cases found to be dependent upon the inherent growth energy of the given strain.

To devise a more practicable method by which cells exhibiting different functional values, and expressing a similar morphology could easily be recognized, tissue cultures of such growths, that were isolated from the same embryo and incubated under similar conditions, were subjected to microincineration. The inorganic structure of the incinerated explants was studied in dark field illumination obtained by using a Zeiss cardioid condenser. The cultures were incinerated in an electric quartz oven at 650°C. in the manner described by Scott and Horning,[3] except that when employing tissue cultures it was found advantageous, for optical reasons, to explant the selected tissues upon a small glass slide, and mount the coverglass over the inorganic remains.

An examination of the inorganic properties of the cells forming the zone of new growth in a pure strain, shows no visible gradation in the mineral content between those elements nearer the original implant and those which have migrated the farthest into the liquid medium forming the growing edge of the culture. Similar observations, however, upon cultures of mixed growth, that were obtained from the digit of a 7 days' chick embryo, revealed a striking variation of the inorganic structure of the different types of cells. Clasmatocytes and other amaeboid elements are the most outstanding owing to the enormous concentration of mineral salts, both within the main body of the cell and in their pseudopodia. These elements are rendered most conspicuous by the large amounts of calcium oxide they possess, and stand out in this respect when compared with the other "Fibroblast" structures. The inorganic remains of those cells possessing the fibroblast-like contour reveal a visible difference in the changes and distribution of their mineral ash deposit. The mature and immature blood structures are easily detected, but their nuclei contain no appreciable traces of iron oxide. This also applies to the nuclei of all microincinerated tissue

[2] Horning, E. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, in press.

[3] Scott, Gordon H., and Horning, E. S., *J. Morph. and Physiol.*, in press.

culture cells. When compared with incinerated sections of chick embryos of the same age, whose nuclei are rendered most conspicuous by the concentration of iron oxide especially within the blood elements, the contrast is most apparent. This interesting phenomenon was also a prominent feature in cultures of pure tissue strains. This change of the inorganic salts in tissue cultures, after periods of growth *in vitro* is significant and might possibly be regarded as an interesting intrinsic chemotactic reaction to implantation in a foreign medium.

The ash deposit as seen by dark field illumination in pure cultures of epithelium and heart fibroblasts obtained from 7 days' embryonic chicks shows an interesting but slight variation of the mineral salts. The inorganic structures of the epithelial cultures are represented by a diffuse distribution of calcium oxide and apparently also of sodium. As a whole, the ash within these cells is less concentrated than that observed in sections of whole embryos of the same age. This might be due to the fact that cells *in vitro* lose their compactness as they are enabled to migrate freely and spread out under the surface of the coverglass. Cells on the extreme periphery of the epithelial sheet, that become mobilized, and assume a "Fibroblast-like" contour, show no difference or orientation in their mineral constituents (see Fig. 1). This is extremely interesting as it has been recently demonstrated, experimentally *in vitro,* that this apparent difference is not a true differentiation, but a superficial mechanism due to an effort on the part of the cell to increase its surface area owing to lack of oxygen.[4]

The heart fibroblasts contain considerably greater concentrations of calcium deposit than the epithelial cells and visibly less sodium. In mixed cultures it is possible after careful examinations to discriminate between the 2 cell types according to their inorganic structure. When these cells are compared with the incinerated remains of osteoblast cultures which were obtained from the supraorbital of an 8½ days' chick embryo, it is seen that these osteoblast structures contain such enormous concentrations of calcium salts, together with no visible trace of sodium, that their inorganic individuality becomes apparent.

This investigation is significant inasmuch as it has indicated that cells designated as mesenchymal elements expressing similar morphological values can be classified according to their inorganic properties.

[4] Horning, E. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, in press.