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ARRAY GEOMETRY OF STROMAL PROGESTERONE RESPONSE

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ABSTRACT: The stereological parameters Vv (volume fraction), L3 (mean feature intercept), and λ (mean inter-feature free path) were used to describe the array geometry of stromal responses to progesterone in the rat uterus. Stromal cells in the ovariectomized rat form a topologically continuous matrix, having a uniform internal geometry. The response to progesterone is non- uniform across the array. In the stroma immediately underlying the luminal epithelium, the cell enlargement, which is significant at 3 hours, is not associated with extracellular changes. In deep stroma near muscularis, cell enlargement is delayed until 7 hours, and is then accompanied by a considerable expansion in the extracellular space. The non-uniformity of these responses suggests that cell activities are constrained by local factors to do with the geometry of the array, which may originate from other cell classes, or may be intrinsic to the array.

Although stereological parameters permit one to characterize efficiently numerous geometric properties of cell populations, very

little is understood of the biological mechanisms which maintain multicellular systems in stable, non-random structural conformations (Garrod and Nicol,1981). Tissue culture experiments, using both embryological (Cunha,1976; Lehtonen,1976) and adult (Fentiman et al.,1976) tissues, suggest that normal cells influence one another locally, and that malignant cells are less susceptible to these influences (Weinstein et al.,1976). We have been studying structural homeostasis in vivo, using the rat uterus as a simple model system, where structure can be reproducibly modulated by exposure to estrogen and progesterone, and where there is evidence of interaction between different cell classes (Williams and Rogers,1980; John and Rogers,1972).

In the present study, the aggregate geometry of uterine stroma was examined stereologically during the progesterone response. It was shown in an earlier report (Wischik and Rogers, 1982) that in the resting ovariectomized state, the stroma could be visualized as a topologically continuous three-dimensional matrix of cells, having a relatively uniform internal geometry, and presenting surfaces with distinctive characteristics at the interfaces with other cell populations in the uterus.

MATERIALS AND METHODS

Adult female Wistar rats were ovariectomised, under tribromoethyl alcohol ("Avertin", Bayer) anaesthesia, under semi-sterile conditions, by a mid-line incision, 20 to 22 days before the experiment. Experimental animals were given a single s.c. injection of 2.5mg progesterone; control animals received an equal volume of arachis oil alone. Animals were divided into three groups of four. One rat in each group served as control, the other three receiving progesterone. Groups were killed 3, 7 and 25 hours after progesterone.

Both uterine horns were quickly dissected out, and short lengths of uterus were fixed in 2.5% Sabatini-Barnett glutaraldehyde in 0.085M sodium cacodylate buffer (pH 7.4). After post fixation in Zetterkvists's isotonic osmium tetroxide, the pieces were embedded in araldite. Two blocks per animal were used, one from each uterine horn. Several sections at 0.5um were cut, and stained with 1% toluidine blue in 1% borax. Point counting was done on one section per block, without knowledge of experimental treatment.

Sections prepared in this way were viewed through a 100X magnification oil immersion lens and a 10X eye piece, through a Leitz Ortholux microscope fitted with a prismatic viewing arm, in order to project a line and point grid system onto the stromal image. A systematic sampling procedure was used, so that observations of stromal cells were derived from three regions:
E - cells immediately adjacent to luminal epithelium;
G - cells in the mid-zone of stroma;
M - cells near the muscularis.

Three principal stereological parameters were used:
Vv - volume fraction of stroma occupied by cell.
L3 - mean feature intercept given by

$$L3 = 2Vv / Pl$$

where Pl denotes the number of cell boundary intercepts per unit length of test line.
λ - mean inter-feature free path, given by

$$\lambda = 2(1-Vv)/Pl$$

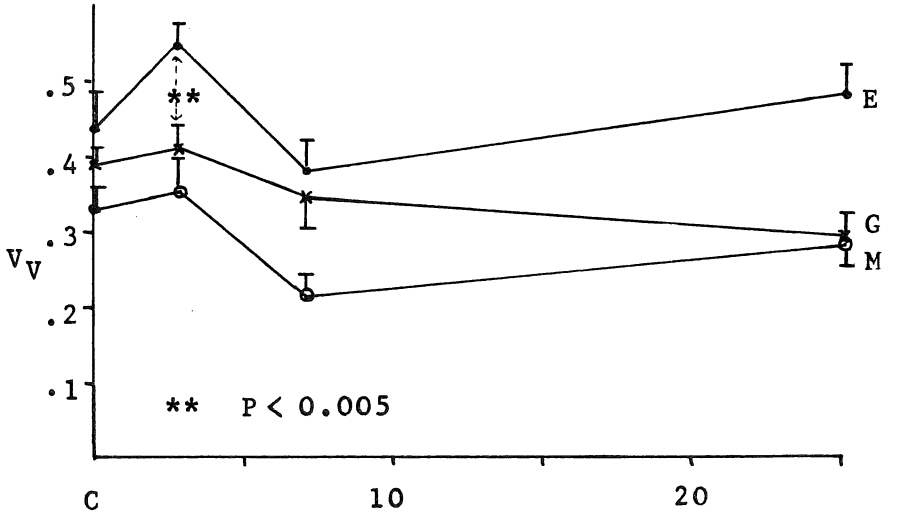
Each parameter was analysed separately in a two factor analysis of variance framework, the factors being hormonal status and stromal zone.

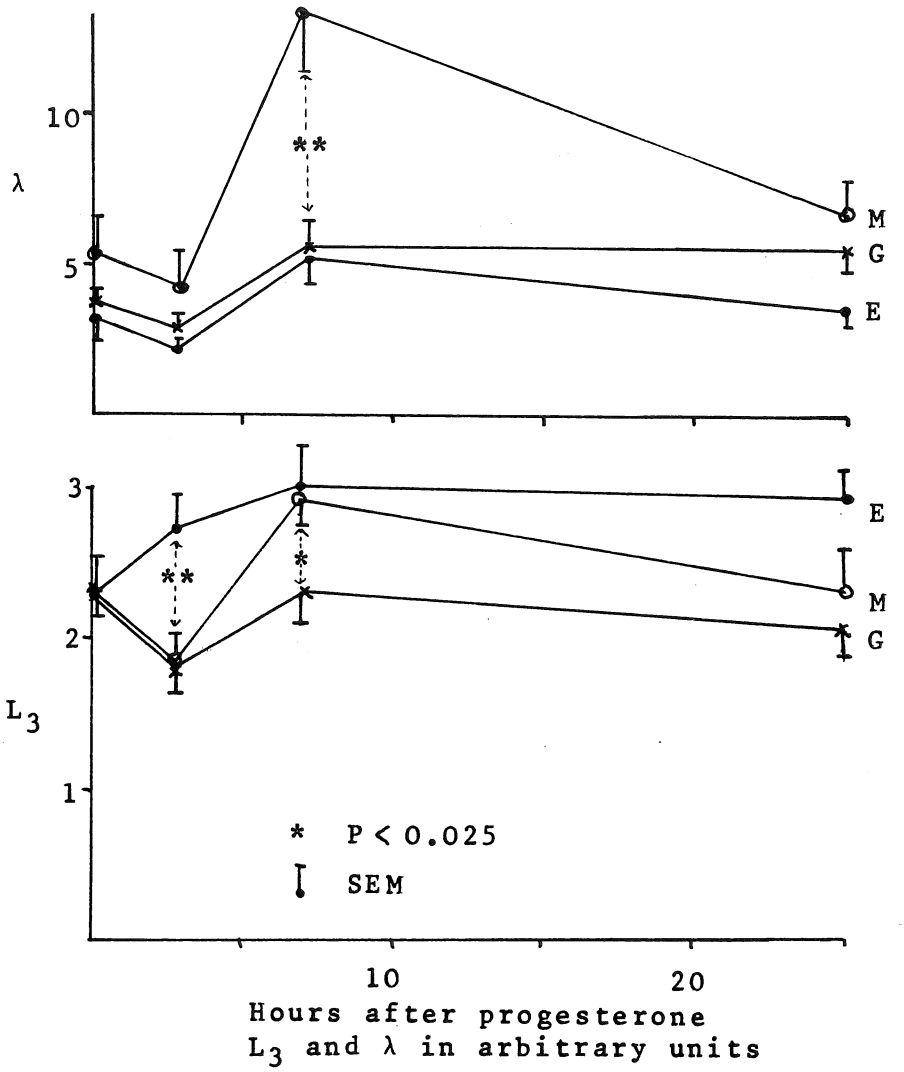
RESULTS

The changes in array geometry, as measured by volume fraction, mean feature intercept, and mean inter-feature free path, during the first 25 hours after an injection of progesterone, are presented in the accompanying figures.

The volume fraction and mean free path are largely mirror images of one another, suggesting that, excepting the three-hour point in the subepithelial stroma, the changes in volume fraction are determined predominantly by changes in the extracellular space. These obscure the cellular changes, which are only revealed by L3

The cell enlargement which occurs at three hours is restricted to the subepithelial stroma, is unaccompanied by a change in the extracellular space, and persists to 25 hours. In deep stroma, cell enlargement is delayed until 7 hours, and is associated with a generalized increase in the extracellular space. According to all parameters, no significant changes are seen in the mid-zone stroma throughout the period of observation.





DISCUSSION

For convex non-contiguous structures of one phase, distributed in a second phase, the parameter L3 is generalizable to three dimensions, as the mean diameter of the first or disperse phase particles. However, for a topologically continuous solid with concave elements dispersed in the second phase, particle

diameter does not exist, and this parameter describes only the mean intercept of a random linear probe overlying elements of the disperse phase, and $\bar{\lambda}$ describes the mean free path between such elements (Weibel,1980). Since stroma has the latter topological property, L3 as found in the present study cannot strictly refer to cell size, but rather to the mean linear intercept overlying cellular elements, which will be referred to loosely as cell size. Clearly L3 and $\bar{\lambda}$ provide more information than Vv alone, although the variance attributable to shape and number is not available from the present analysis. Despite these limitations, the simple parameters chosen do characterize a class of perturbations in the array geometry of the stroma which have interesting biological implications.

Changes in patterns of protein synthesis associated with these changes in array geometry have recently been reported (Rogers and Wischik, in press, J Anat). There appears to be good overall agreement between the relative density of newly labelled proteins appearing in the intracellular and extracellular spaces, and L3 and $\bar{\lambda}$ respectively, although vascular factors need also to be taken into account at 7 hours (Ljungkvist,1975). In particular, the early increase in cell size, restricted to the subepithelial stroma at 3 hours, appears to correspond with an increase in protein synthesis, measured autoradiographically, which is likewise restricted.

The most striking feature of the present data is the non- uniformity of response seen in this largely homogeneous array. Cells appear to be constrained by positional factors, both as regards the timing and the character of their response to progesterone. It is improbable that these regional differences are the result of either preferential distribution of hormone or of diffusion delays, given the time scale involved. More likely constraining influences operating locally could be of two kinds:
- exogenous, modulatory substances emanating from

other cell classes;
- intrinsic mechanisms of the array.

Williams and Rogers (1980) have argued that information transfer takes place across the interface between stroma and epithelium during the response of the uterus to progesterone. The present data shows that there is a close temporal correspondence between the onset of protein synthesis in the luminal epithelial cells which can be observed at three hours (Brown-Grant,John & Rogers, 1972), and the early stromal changes restricted to the subepithelial stroma. This further supports stromo-epithelial interaction during the progesterone response, and suggests that the local stroma is under the influence of short-range substances emanating from the luminal epithelium.

Intrinsic mechanisms could be visualized as follows. If signals are transmitted at sites of membrane contact, stromal cells would be subject to unique membrane inputs depending on position. Because cells immediately adjacent to the luminal epithelium are functionally distinct, a second layer stromal cell would differ from a third, by virtue of having more of its surface in contact with first layer cells, and so on. In this way a topologically continuous array, such as the stroma, could intrinsically map out the space it occupies, given the ability to encode and decode the appropriate membrane information.

It is conceivable that non-uniformity of response is a necessary property in this kind of setting (Shymko and Gloss,1976), if structural homeostasis is to be attained, since the maintenance of stable structure in a multicellular system implies that cell activities are locally constrained by the geometry of the array. Structure in biological systems is the morphological counterpart of the biochemical equilibrium, or of homeostasis in physiology, and represents the stabilized equilibrium attained by an underlying dynamic multicellular process. The geometry observed in such systems, characterized

readily by the aggregate parameters, can be used to define this equilibrium.

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