DNA ACTIVITY IN THE ENDOMETRIAL STROMA OF THE CYCLING RAT AS MEASURED BY FEULGEN STAINING AND BY [³H]THYMIDINE INCORPORATION

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By using cytophotometry, Fautrez (1966) and his colleagues have shown in a variety of hormonal receptors (i.e. thyroid, adrenals, ovary and vagina) that specific stimulation leads to an increase of Feulgen-measured DNA within the nuclei of target cells. According to these authors, this phenomenon is not necessarily related to an enhanced mitotic rate but would witness to a hormone-dependent metabolic activity.

As similar data were obtained in the uterus of spayed mice (Leroy, Manavian & Hubinont, 1967) and rats (Leroy & Manavian, 1969), the present work was undertaken in order to verify if this concept is applicable to the endometrial stroma during the oestrous cycle.

The oestrous cycles of groups of four animals (Wistar strain) were followed by daily vaginal smears and animals were killed around mid-day at various moments of the cycle (i.e. pro-oestrus, early and late oestrus and early and late di-oestrus). Sections of the uteri were homogeneously Feulgen-stained. The relative Feulgen-DNA content was determined with the Lison histophotometer (Lison, 1950) in about 100 stromal nuclei per uterus. The results obtained in groups of animals were pooled and statistically analysed according to the large samples method described by Snedecor (1956). As the observed values showed a log-normal distribution, the mean ($\log x$) and standard error were calculated on a logarithmic basis. The significance of differences between adjacent groups was estimated by Student's 't' test. These results are shown in Table 1.

Another series of animals was selected according to the same criteria. They received an intraperitoneal pulse injection of [³H]thymidine (10 μ C/g body weight) 15 min before being killed. The uteri were processed for autoradiography. Labelling indices were counted on at least 3000 stromal cells (leucocytes were not taken into account). Numerical results and significance of differences between mean values corresponding to successive experimental groups are given in Table 2.

It was observed that the mean nuclear DNA content, as measured by Feulgen staining, rises sharply (18%) between early and late oestrus. During di-oestrus, this parameter progressively returns to the low values observed in pro-oestrus and early oestrus.

From the end of di-oestrus up to the first day of oestrus, [^3H]thymidine labelling indices were found to be relatively high and did not vary significantly, although there is some indication that the rise from pro-oestrus to oestrus could be meaningful ($P = 0.1$). From the end of oestrus to the first half of di-oestrus, a significant decrease takes place in the number of nuclei which have taken up [^3H]thymidine.

The comparison of results obtained with Feulgen cytophotometry and autoradiography indicates that the peak of Feulgen-measured DNA observed at

TABLE 1
MEAN RELATIVE FEULGEN-DNA CONTENT OF ENDOMETRIAL STROMAL NUCLEI
IN THE CYCLING RAT

Stages	No. of rats	No. of nuclei	$\log \bar{x} \pm S.E.$	P
Pro-oestrus	4	400	2.70030 ± 0.003	N.S.
Oestrus (first half)	4	394	2.69663 ± 0.005	< 0.001
Oestrus (second half)	4	397	2.76378 ± 0.004	< 0.001
Di-oestrus (first half)	4	389	2.73094 ± 0.004	N.S.
Di-oestrus (second half)	4	386	2.72146 ± 0.004	< 0.001

N.S. = not significant.

TABLE 2
[^3H]THYMIDINE LABELLING INDEX IN THE ENDOMETRIAL STROMA OF
THE RAT DURING THE OESTROUS CYCLE

Stages	No. of rats	Labelling index /1000* $\pm S.E.$	P
Pro-oestrus	3	4.4 ± 0.8	N.S.
Oestrus (first half)	3	7.8 ± 1.4	0.05 to 0.02
Oestrus (second half)	3	2.4 ± 1.2	N.S.
Di-oestrus (first half)	4	1.35 ± 0.2	0.01 to 0.001
Di-oestrus (second half)	4	8.4 ± 1.9	N.S.

* Number of labelled endometrial stroma cells per thousand.

N.S. = not significant.

the end of oestrus cannot be accounted for by an accelerated proliferation rate since there is no concomitant increase in the proportion of DNA-synthesizing cells as measured by the uptake of labelled thymidine. An alternative hypothesis would be that, after having passed through the S phase (DNA synthesis) of the mitotic cycle, the endometrial stromal cells remain dormant in the G_2 (pre-mitotic) phase and thus contain a tetraploid amount of DNA, as is the case in certain other tissues (Gelfant, 1963). Such an explanation seems to be precluded by the finding that, in every experimental group, cytophotometrical values were log-normally distributed, without sufficient numbers of tetraploid (G_2) nuclei (never more than 1 to 3%) to justify the observed shift of mean Feulgen-DNA from the 1st to the 2nd day of oestrus.

Thus, it is clear that during the oestrous cycle in the rat the action of ovarian hormones results in two different time-dependent effects on endometrial

stromal nuclei, i.e. increased thymidine incorporation and intensification of Feulgen staining.

In connexion with regulation of genetic transcription by sex steroids, it was recently shown in the normal rat that chromatin isolated from the uterus in oestrus exhibits an increased DNA template capacity, a higher ratio of total proteins to DNA and a smaller proportion of histones than in di-oestrus (Hamilton, 1968). On the other hand, the efficacy of hydrolysis for Feulgen reaction depends on the amount and nature of the nuclear proteins (Walker & Richards, 1959; Böhm & Sandritter, 1966). As a possible working hypothesis, sex steroids might be considered capable of increasing the amount of DNA available for Feulgen hydrolysis by modifying in some way the nucleo-protein—DNA bonds and Feulgen staining intensity might (at least partially) reflect genetic de-repression induced by hormones.

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