ORNITHINE DECARBOXYLASE ACTIVITY IN THE UTERUS OF THE RAT DURING EARLY PREGNANCY

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It is well established (Tabor & Tabor, 1972) that tissues undergoing rapid growth and differentiation exhibit increased levels of ornithine decarboxylase [E.C. 4.1.1.17]. In reproductive tissues, ornithine decarboxylase (ODC) activity is elevated following a response to hormones, as in the oestrogen-treated chick oviduct (Cohen, O'Malley & Stasty, 1970), the uterus of the oestrogen-treated castrated or prepubertal rat (Kaye, Ickson & Lindner, 1971; Russel & Taylor, 1971), and in the prostate gland of the testosterone-treated castrated rat (Williams-Ashman, Pegg & Lockwood, 1969).

The enzyme activity in the uterus of the cycling rat has been closely associated with the levels of circulating oestrogens since it has been shown (Kaye *et al.*, 1971; Hernandez, Ballesteros, Mendez & Rosado, 1973) that ODC activity is maximal at pro-oestrus but is very low or absent at oestrus, and remains low until the commencement of the normal cyclical oestrogen release in late dioestrus. Ornithine decarboxylation gives rise to putrescine and to polyamines and it has been suggested that these products are closely involved in the stimulation of RNA and protein synthesis in the growing tissues.

Since the period preceding the onset of decidualization in the pregnant rat is characterized by well-defined changes in growth and division in the epithelial and stromal cells of the uterine lumen in response to a precise hormonal regimen, and by changes in RNA and protein synthesis (Heald, 1973; O'Grady, Moffat & Heald, 1974), it was of interest to examine the patterns of change of ODC in the uterus at this time.

Virgin female rats of a Holtzman-derived Sprague-Dawley strain were mated, and the morning on which spermatozoa were found in the vaginal smear was designated Day 1 of pregnancy. The rats were killed between 10.00 and 11.00 hours on the appropriate day and the uteri were rapidly removed. Weighed segments were minced finely with scissors and homogenized in 5 vols 0.25 M-sucrose, 0.1 mM-EDTA, 100 mM-tris HCl and 10 mM-dithiothreitol at 0 to 5°C for 30 sec, using an Ultra-Turrax homogenizer at full speed. The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant was kept at 0 to 5°C for enzyme assay. Omission of dithiothreitol from the homogenizing medium led to a total loss of activity. The enzyme was assayed essentially as described by Kaye *et al.* (1971) in 0.3 ml containing 0.05 ml enzyme (0.3 to 0.5 mg protein), 440 nmol (0.23 μ g) of L-[¹⁴C]ornithine (sp. act. 58 mCi/mmol) together with

the following at their final concentrations: 100 mM-tris HCl (pH 7.0), 200 μ M-pyridoxal phosphate and 10 mM-dithiothreitol.

The results of determining ODC activity in uterine extracts during early pregnancy are shown in Text-fig. 1. The tissue showed little activity until Day 3 of pregnancy and declined significantly by Day 4, rising again by Day 5. Following implantation late on Day 5, the decidualizing tissue on Days 6 to 9 continued to show high activity whilst that of the inter-implantation tissue showed none on Day 6 and little or none on Days 8 and 9. The absence of

TEXT-FIG. 1. Ornithine decarboxylase activity of rat uterine extracts in early pregnancy. Uterine tissues were obtained from mated rats and, from Day 6 onwards, they were separated into decidualizing and non-decidualizing tissue. Enzyme activity was determined as described in the 'Methods' section: $\blacktriangledown$, whole uterus to Day 5, and ($\square$) decidualizing tissue and ($\blacksquare$) non-decidualizing tissue thereafter. Each point represents the mean of separate determinations of enzyme activity in the uterus of each of four or five animals. Horizontal lines represent the S.E.M. Absence of such lines indicates an S.E. falling within the symbol.

activity on Day 6 was not due to the presence of inhibitors in the tissue since a mixture of extracts of inter-implantation tissue with extracts of implanted tissue did not depress ODC activity in the system by more than 13%.

The absence of activity before Day 3 and also in the non-decidualizing tissue shortly after implantation has not been found with other uterine enzymes studied at this time. The levels of *iso*-citric dehydrogenase, the pentose-shunt enzymes and phosphofructokinase (Surani & Heald, 1971), alkaline phosphatase and β -glucuronidase (Manning, Meli & Steinetz, 1966) and acid cathepsin (Wood, 1969) show no change in the non-decidualizing tissue during Days 6 and 7 of pregnancy. The synthesis of RNA, however, follows a pattern similar to that shown by ODC activity with peaks on Days 3 and 5 and a further increase in the decidualizing tissue on Day 6 with a decrease in the non-decidualizing tissue (Heald & O'Grady, 1970). It has been shown (Tachi, Tachi & Lindner, 1972) that the luminal epithelial cells in the rat show maximal mitotic activity on

Day 2 while the stromal cells show a peak on Day 4. On this basis, it has been proposed (O'Grady *et al.*, 1974) that the changes in RNA synthesis reflect the sequential cell cycles. It is now suggested that the changes found in ODC activity are also similarly related.

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