Effect of an Intrauterine Thread on Acid Cathepsin Activity in the Endometrium of the Pseudopregnant Rat

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(Received 15 July 1969)

Since 1955 it has become increasingly clear that many processes of tissue breakdown and regression are brought about by hydrolytic enzymes that have optimum activity at acid pH and that are normally contained within lysosomes (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955). Although the histological appearance of the rat endometrium at the time of egg implantation suggests that breakdown of the connective-tissue matrix is occurring (Enders & Schlafke, 1967), de Duve & Wattiaux (1966) stated that whether lysosomes 'are involved in the nidation phenomenon in mammals is not known'. During post-partum involution of the rat and human uterus loss of collagen is associated with an increase in the activity of the lysosomal enzyme acid cathepsin D (Woessner, 1962; Woessner & Brewer, 1963), and Wood & Psychoyos (1967) found that this enzyme also shows a transient increase in activity in the rat endometrium during early pseudopregnancy.

The ability of the rat uterus to support a normal pregnancy depends initially on its capacity to produce maternal decidual cells, which form an essential part of the placenta. The presence of an intrauterine thread acting as a contraceptive device is thought to inhibit the decidual-cell reaction (and therefore the process of implantation), and the possibility exists that it does so by interfering with the normal pattern of enzyme changes occurring in the rat endometrium. Hence it was decided to

measure the activity of acid cathepsin D in endometrial samples taken from uteri bearing such devices before, during and after the period in which the uterus is sensitive to a decidualizing stimulus.

Methods. Adult female Wistar rats bearing an intrauterine silk thread 3–5 mm. long in the lumen of one uterine horn were made pseudopregnant by mechanical stimulation of the cervix on the day of vaginal cornification (day 0). In the 32 animals used for determination of enzyme activity the thread had been present for approx. 25 days before stimulation. In a group of 17 rats used to study the percentage dry weight of endometrial tissue the device had been in place for approx. 45 days. Animals were killed on days 2, 4, 5 and 7 of pseudopregnancy. Both uterine horns were removed and slit open. Endometrial tissue was scraped from the underlying muscle and weighed. The percentage dry weight was calculated after drying the tissue obtained from the smaller group of animals to constant weight at 110°; in the other group acid cathepsin activity was determined in tissue homogenates by the method of Woessner & Brewer (1963), with a haemoglobin substrate at pH 3.5. Results were expressed as μ moles of tyrosine liberated/hr./g. fresh wt. An analysis of variance was performed on each of the parameters measured (fresh weight, percentage dry weight and enzyme activity), to establish whether they varied significantly during the period chosen for study.

Table 1. *Effect of an intrauterine device on acid cathepsin activity in the endometrium*

Results are expressed as means $\pm$ s.e.m., with the numbers of animals in each group in parentheses.

Day of pseudopregnancy	Endometrium				Acid cathepsin D activity (μ moles of tyrosine liberated/hr./g. fresh wt.)	
	Fresh wt. (mg.)		Dry wt. (%)		Control	Device
	Control	Device	Control	Device		
2	9.6 $\pm$ 0.8 (7)	15.4 $\pm$ 1.4 (7)	19.5 $\pm$ 1.7 (5)	16.6 $\pm$ 1.0 (5)	194 $\pm$ 17 (7)	183 $\pm$ 13 (7)
4	16.5 $\pm$ 1.1 (10)	24.0 $\pm$ 1.8 (10)	20.3 $\pm$ 1.2 (7)	18.0 $\pm$ 0.3 (7)	308 $\pm$ 26 (10)	198 $\pm$ 7 (10)
5	9.0 $\pm$ 1.2 (8)	17.4 $\pm$ 1.9 (8)	22.1 $\pm$ 0.9 (5)	19.5 $\pm$ 1.1 (5)	337 $\pm$ 20 (7)	207 $\pm$ 22 (7)
7	8.4 $\pm$ 0.6 (7)	18.7 $\pm$ 2.1 (7)	19.4 $\pm$ 0.4 (5)	19.6 $\pm$ 0.7 (5)	234 $\pm$ 27 (7)	161 $\pm$ 6 (7)
Analysis of variance	$P < 0.001$	$P < 0.025$	$P > 0.97$	$P < 0.05$	$P < 0.005$	0.05 $< P < 0.1$

Results and discussion. Table 1 shows that the fresh weight of the endometrial tissue that can be recovered from the uterus reaches a maximum value on day 4 of pseudopregnancy in both the control and device sides. The specific activity of acid cathepsin D in the control side shows a highly significant ($P < 0.005$) increase on days 4 and 5 (the period of uterine sensitivity), compared with the activity found on days 2 and 7, when the uterus is not sensitive to decidualizing stimuli. This pattern of changes is similar to that described by Wood & Psychoyos (1967). However, no such increase in enzyme activity is observed in the device side. In addition, a paired comparison test between enzyme activities in the two sides shows that the activity in the control side is higher than that in the side bearing the device on days 4, 5 and 7 ($P < 0.05$). Since the percentage dry weight of endometrial tissue is constant in the control side but increases steadily in the device side, it is clear that a similar result would be obtained if enzyme activities were expressed on the basis of dry weight rather than fresh weight.

It seems likely that the increase in enzyme activity that occurs during the period of uterine

sensitivity is directly associated with the ability of the endometrium to undergo a decidual-cell reaction. If acid cathepsin D is involved in the breakdown of uterine collagen then the increase in activity described here may be associated with a dissolution of the connective-tissue matrix that allows the endometrial cells to swell and become differentiated into true decidual cells. The fact that no such increase in enzyme activity is observed in the device side, where decidual cells are formed far less readily (J. C. Wood & D. R. S. Kirby, unpublished work), supports this hypothesis and gives another clue to the mode of action of such an intrauterine device.

This work was supported by a grant from the Medical Research Council.

- de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, W. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
de Duve, C. & Wattiaux, R. (1966). *Annu. Rev. Physiol.* **28**, 435.
Enders, A. C. & Schlafke, S. (1967). *Amer. J. Anat.* **120**, 185.
Woessner, J. F., jun. (1962). *Biochem. J.* **83**, 304.
Woessner, J. F., jun. & Brewer, T. H. (1963). *Biochem. J.* **89**, 75.
Wood, C. & Psychoyos, A. (1967). *C. R. Acad. Sci., Paris, D*, **265**, 141.