The influence of actinomycin D on the protein composition of mouse uterine flushings

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The termination of delayed implantation in the fur seal (Daniel, 1971, 1974), mouse (Aitken, 1977), rat (Surani, 1975) and roe deer (Aitken, 1974, 1975) is thought to be associated with the oestrogen-induced release of a protein-rich endometrial secretion into the uterine lumen. Finn (1974), however, observed that the intraperitoneal injection of actinomycin D, an inhibitor of protein synthesis, terminated delayed implantation in the mouse within 48–72 h. It was suggested that actinomycin D released the blastocysts from their state of diapause by inhibiting the transcription of a proteinaceous inhibitor of blastocyst attachment (Finn, 1974). Actinomycin D may, however, have increased the protein content of the uterine lumen either through the 'superinduction' of uterine proteins (Tomkins *et al.*, 1969) or, possibly, through the stress-induced release of steroids from the adrenal glands. A study was therefore designed to determine the influence of actinomycin D on uterine secretory activity in the mouse.

The surgical and biochemical procedures used in this study were identical to those described by Aitken (1977). Uterine flushings were collected at 3, 6, 12, 24, 40, 48, 64 and 84 h after the intraperitoneal administration of 15 µg actinomycin D, and were analysed quantitatively and qualitatively for protein using the techniques of Lowry, Rosebrough, Farr & Randall (1951) and Davis (1964) respectively. The presence of implantation sites was confirmed by observation of the uterus after an intravenous injection of pontamine blue 15 min before autopsy.

Discrete implantation sites were observed 48–64 h after the administration of actinomycin D, confirming the results obtained by Finn (1974). This implantation response differed in several ways from that obtained with oestradiol however. In a separate series of mice injected with 50 ng oestradiol-17β during diapause pontamine blue-positive areas were observed within 24 h, at least 24 h earlier than in the actinomycin D-treated animals. The response to oestradiol was also more efficient in terms of both the number of animals possessing implantation sites (70% and 100%) and the number

Table 1. Changes (mean ± S.D.) in the protein content of mouse uterine flushings after administration of 15 µg actinomycin D or 50 ng oestradiol-17β during delayed implantation

Time after treatment (h)	Oestradiol-17β*		Actinomycin D	
	No. of mice (5 mice/sample)	Conc. protein (µg/mouse)	No. of mice (5 mice/sample)	Conc. protein (µg/mouse)
0	40	6.9 ± 1.2	40	6.9 ± 1.2
3	10	5.3 ± 0.2	10	3.5 ± 0.1
6	10	5.4 ± 0.2	5	3.6
12	10	10.9 ± 1.1	10	3.0 ± 0.1
24	5	4.3	10	3.7 ± 1.5
40	10	10.1 ± 4.3	10	3.4 ± 1.2
48	10	13.3 ± 7.5	10	2.7 ± 0.3
64	—	—	10	4.9 ± 0.1
84	—	—	10	3.8 ± 1.3

* Data taken from Aitken (1977).

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Text-fig. 1. Polyacrylamide gel electrophoresis of mouse uterine flushings after administration of 15 μ g actinomycin D during experimental delayed implantation. ED = Day 5 of experimental delay; 3, 6, 12, 24, 40, 48, 64 and 84 = h after actinomycin D administration; at 6 h, closed arrows = major non-serum proteins at Ra 0.06, 0.10, 0.25, 0.32, 0.34, 0.83, 0.88, 1.19 and 1.32, open arrows = serum slow α -globulins at Ra 0.16 and 0.20; at 40 h, closed arrows = non-serum fast α -globulins at Ra 0.83, 0.86, 0.90 and 0.94. l = major serum lipoprotein band; t = serum transferrin; h = haemoglobin bands; a = albumin. Proteins identified by closed circles are presumed to be of serum origin.

of implantation sites per mouse (means of 4.1 and 11.3) for mice injected with actinomycin D and oestradiol respectively (see Table 1). These observations are in full agreement with those reported by Finn & Downie (1975).

Considerable disparity was also observed in the uterine secretory response to actinomycin D and oestradiol. In contrast to the biphasic secretory response induced by oestradiol-17 β (Aitken, 1977), the induction of implantation with actinomycin D was associated with a highly significant fall in the protein content of the uterine lumen ($P < 0.001$; Table 1). Despite this decline in total protein concentration, certain serum and non-serum proteins increased in intensity after actinomycin D treatment (Text-fig. 1). The most significant changes were observed at 6 h when 2 serum and 9 non-serum proteins increased in intensity and a new band was observed at Ra 1.06, and at 40 h when a transient increase in 4 fast α -globulins was detected. In untreated animals the protein pattern was found to remain substantially unchanged for up to 1 week after the induction of delayed implantation (Aitken, 1977).

The results obtained in this study indicate that the highly significant increase in luminal protein concentration observed at the time of implantation during normal pregnancy or following the administration of oestradiol-17 β (Aitken, 1977) is not an absolute requirement for the induction of blastocyst attachment: implantation was initiated when luminal protein levels were severely depressed by treatment with actinomycin D. It is possible that some of the minor qualitative changes observed following administration of actinomycin D were sufficient to activate the blastocysts, however, or that implantation depends on a change in the luminal epithelium rather than the luminal fluids (Pollard, Finn & Martin, 1976).

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