ANS (1-anilo-8-naphthalene sulphonate)[10,11] were unsuccessful. Further studies to estimate the volume increase per link by quantitative determination of the effect of pressure on the successive polymerization constants will be undertaken.

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In vitro Production of Nucleolar Channel System by Progesterone in Human Endometrium

Electron microscope examination of normal human endometrium during the midsecretory phase of the menstrual cycle demonstrates that the nucleolus of the epithelial cell differentiates into an organelle variously termed a nucleolar canalicular system[1], a nucleolar basket[2] or a nucleolar channel system[3]. Such a structure appears if ovulation has occurred. Nucleolar baskets have been seen as early as the sixteenth day of a normal 28 day cycle. After this they may increase in number, reaching a maximum around the twentieth day and then disappear, not being found later in the cycle. They have not been seen in the proliferative phase of the cycle or in early pregnancy.

This system seems to be a unique form of nucleolar differentiation having been observed in human glandular endometrial cells and in no other tissue. It consists of an involved labyrinth of channels coursing through a more dense homogeneous matrix (Fig. 1). Electron opaque 150 Å particles are arranged in a regular fashion around the periphery of the total channel mass but may also surround the individual channels (our unpublished results).

Previous attempts to demonstrate the specific physiological effect of progesterone by means of organ culture were frustrated by the induction of secretory changes in the endometrial cell in the control culture[4]. Electron microscope examination of such organ cultures failed to show nucleolar channel systems[5]. Using organ culture of proliferative human endometrium it has recently been shown[6,7] that progesterone does act directly on the epithelium to convert proliferative to secretory endometrium. Thus the effects observed *in vivo* could be mimicked *in vitro*. This conversion was specifically due to progesterone and was not produced in the control cultures, in cultures with oestrogen alone or with cortisol. We undertook this study to find out whether nucleolar differentiation can be induced by progesterone in organ cultures of proliferative endometrium.

Specimens were obtained aseptically from normal women by endometrial biopsy on the tenth day of the menstrual cycle. A small piece was processed for electron microscopy and another was examined histologically. The remainder of the endometrium was cut into pieces 1–2 mm³. Each of six pieces was placed on 'Millipore' filter paper resting on agar enriched with TC 199 medium to which 10 per cent calf serum and 10 μg/ml. insulin had been added. In addition to control cultures, cultures containing progesterone (1 μg/ml. and 10 μg/ml.), medroxyprogesterone acetate (1 μg/ml. and 10 μg/ml.), oestradiol (10 μg/ml.) or oestradiol (10 μg/ml.) plus progesterone (10 μg/ml.) were prepared. These were incubated at 37° C in a moist atmosphere of 95 per cent oxygen and 5 per cent CO_2. Specimens were examined both histologically and by electron microscopy after 48 and 96 h. For electron microscopy, a small portion of the tissue was fixed in 3 per cent glutaraldehyde in phosphate buffer (*p*H 7·0) at room temperature for 1 or 2 h. After re-fixation for 1 h in 1 per cent veronal buffered osmium tetroxide and subsequent dehydration, the specimens were embedded in 'Epon 812'. Thin sections were double stained with uranyl acetate and lead citrate and examined in an Hitachi HU-11B electron microscope. At least two tissue blocks containing representative samples of endometrium were examined from each specimen.

The histological appearance of the control cultures resembled that of the original proliferative endometrium. Oestrogen produced no significant change. The addition of progesterone produced the changes characteristic of secretory endometrium, first subnuclear deposition of glycogen, then secretion of the glycogen into the lumen of the glands.

Electron microscope examination of the original specimen showed the characteristic appearance of proliferative endometrium (ref. 8 and our unpublished data). No nucleolar channel systems were seen. Extensive search failed to reveal nucleolar channel systems in the control cultures and none were found in the cultures exposed to oestradiol. Characteristic nucleolar channel systems were produced in the cultures containing progesterone (Fig. 2). They were found after 48 and 96 h at both progesterone concentrations but it was not possible to quantitate the results in terms of length of exposure to, or concentration of, progesterone. Although our results suggest that differentiation may be accelerated *in vitro*, such a conclusion is not justified without further study. Nucleolar baskets were also produced in the culture

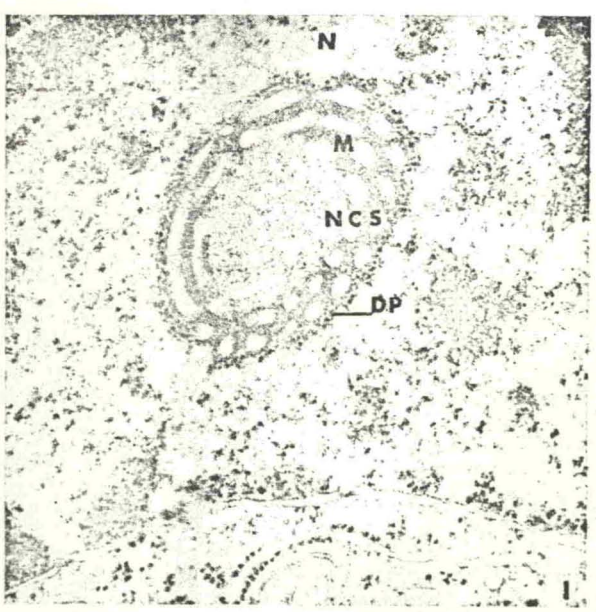

Fig. 1. Electron micrograph of endometrial epithelial cell from a biopsy specimen taken on the eighteenth day of a normal 28 day cycle. A nucleolar channel system (NCS), characteristic of secretory stage endometrium, is seen within the nucleus (N). The channels course through a dense matrix (M). Dense particles (DP) are seen at the periphery of the nucleolus. (×24,375.)