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Biochemical and Morphological Changes Following Artificially Stimulated Decidualization in the Mouse Uterus

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ABSTRACT

A uterine decidual cell reaction was stimulated in hormone primed ovariectomized mice by intraluminal injection of sesame oil. Growth of the stimulated uterine horn occurs in two distinct phases. The first phase begins at approximately 12 h after stimulation and results in a doubling in weight. The second phase begins at approximately 30 h and continues for three to four days resulting in approximately a 20-fold increase in weight. The first growth phase is accompanied by a doubling in protein content and a slight increase in total DNA. The second growth phase is paralleled by increases in both DNA and protein content.

Growth of the stimulated uterine horn occurs in discrete masses. Histological examination of stimulated horns reveals the presence of decidual cells 39 h after stimulation. The decidual cell reaction begins at the centers of loculi and spreads centrifugally. Decidualization proceeds grossly and histologically in a pattern similar to that recorded after actual blastocyst contact.

INTRODUCTION

The artificially stimulated decidual cell reaction (DCR) has been used extensively in the investigation of uterine responses to implantation in the rodent (Shelesnyak, 1957; De Feo, 1967; Glasser and Clark, 1975). The principal advantages offered by the artificially stimulated DCR are 1) the reaction is more extensive than that occurring in natural implantation, 2) the time course of the reaction is more easily studied, particularly shorter times, and 3) the role of hormones in the reaction can be studied. The stimuli used in producing the DCR vary from trauma (crushing, scratching or piercing the uterus) to intraluminal injection of chemical stimuli (De Feo, 1967). Peanut oil and sesame seed oil have been used as intraluminal stimuli (Miller, 1973; Miller and Emmens, 1969; Finn and Martin, 1972). The DCR can be stimulated in ovariectomized rodents pretreated with steroid hormones (Finn, 1966; Yochim and De Feo, 1963). The extent of the trauma stimulated DCR is optimized by administration of both estrogen and progesterone; however, a DCR can be produced using progesterone alone (Yochim and De Feo, 1962, 1963). The oil stimulated DCR requires pretreatment with both estrogen and progesterone plus a "nidatory surge" of estrogen 4 to 8 h before stimulation (Miller and Emmens, 1969; Finn and Martin, 1972). The hormonal requirements for the oil stimulated DCR thus closely approximate the hormone environment during the estrous cycle. We have investigated the gross chemical and morphological changes in the uterus following stimulation of the DCR by intraluminal injection of sesame oil.

MATERIALS AND METHODS

ICR mice (Flow Laboratories) weighing approximately 25 gm were ovariectomized. One week after ovariectomy, animals were started on a hormone regimen developed by Miller and Emmens (1969). Three daily subcutaneous injections of 0.1 μg 17β-estradiol in 0.1 ml sesame oil were followed by two days of no treatment. Animals were then given daily subcutaneous injections of 6.7 ng 17β-estradiol and 1 mg progesterone in 0.1 ml sesame oil for the remainder of the experiment. Six hours after the third estradiol-progesterone injection, 10 μl of sesame oil was injected into the lumen of the right uterine horn. The time period between the final steroid injection, "nidatory surge," and oil stimulation is critical for producing the maximal DCR. The left uterine horn served as the control in all experiments.

Animals were killed by cervical dislocation; uterine horns were dissected out and weighed. Individual

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horns were homogenized in 2.0 ml of 0.5 N perchloric acid with a motor driven Duall homogenizer. Homogenates were shaken for 15 min at 70°C in a Dubnoff metabolic shaker. The samples were quickly cooled to 4°C and centrifuged at 27,000 *g* for 10 min. The supernatants were filtered through Whatman #1 filter paper. Pellets were resuspended in 1.0 ml of 0.2 N perchloric acid and centrifuged at 27,000 *g* for 10 min. This perchloric acid soluble fraction was used in DNA determinations by the Burton method (1968). The 27,000 *g* pellet was solubilized in 1.0 ml of 0.5 N NaOH and assayed for protein by the method of Lowry et al. (1951).

Tissues were placed in Bouin's or Newcomer's fixative (Armed Forces Institute of Pathology) for 16 to 24 h, processed for paraffin embedding by a routine procedure (Markwald et al., 1971), sectioned at 4 μ and stained with hematoxylin and eosin; images were recorded on Kodak H&W film.

RESULTS

Histologic sections from oil stimulated and control uterine horns are shown in Figs. 1–5. Figure 1 is a 39 h control animal and is representative of controls at all time periods. Glandular epithelium was well developed while the endometrial stroma exhibited no evidence of a decidual cell reaction. Mitotic figures were primarily restricted to epithelial tissues. Stromal mitotic activity was not seen. Locular size was relatively constant.

Conversely, in oil stimulated horns locular enlargement was pronounced by 39 h (Fig. 2). Glandular epithelium was restricted primarily to the periphery of the loculus in the 39 h sample, while absent from the loculi of all older groups. An unmistakeable decidual cell reaction was evident as early as 39 h as indicated by central foci of eosinophilic cells (Fig. 2B). Between 48 and 72 h most stromal cells had undergone cytoplasmic enlargement and showed increased eosinophilia. The stromal cell deciduate response developed centrifugally, i.e., beginning centrally and spreading peripherally. Mitotic figures in stromal cells were observed in all experimental groups, but particularly in tissues collected after 72 h of treatment (Fig. 3). Characteristics of the decidual cell reaction at 100 h are shown in Figs. 4 and 5. Hyperemia of the lamina propria with a rearrangement of stromal cells into anastomotic cords is illustrated in Fig. 4. Vacuolization reaches a maximum at 100 h. Glycogen-rich cells characteristic of the decidual cell reaction are apparent particularly near the center of the loculus as shown in Fig. 5.

Growth of uterine horns as a function of time after oil stimulation is shown in Fig. 6. A significant increase in uterine weight can be detected by 12 h after intraluminal injection of sesame oil. Horn weight rapidly increases to approximately three times the control at 24 h after stimulation. The growth rate then slows until sometime prior to 48 h after stimulation when a second rapid growth phase begins. This second phase continues for about 60 h and represents a 20-fold increase in horn weight. Horn weights in excess of 1 g have been observed 144 h after stimulation. The large variation in uterine horn weight at later time points is due to individual differences in the decay of decidualization. Injection of saline in place of oil produces only a slight DCR at the site of injection due to trauma.

The DNA content of uterine horns following oil stimulation is shown in Fig. 7. The DNA content of the stimulated horns does not increase significantly before 24 h after oil stimulation. Rapid DNA replication begins approximately 40 h after oil stimulation. This increase in uterine DNA parallels the second

FIG. 1. A 39 h control animal. The uterus illustrated is representative of all controls. Note the presence of well-developed epithelium. 200X. L (Lamina propria) M (Myometrium). Inset (Fig. 1B) is a higher magnification of the lamina propria. Note the glandular epithelium and nonedematous stroma. There is no evidence of deciduated stromal cells, hyperemia or extensive mitotic activity. 400X.

FIG. 2. A 39 h experimental loculus. Compare loculus size with that of Fig. 1. Note centrifugal displacement of glandular epithelium (arrow). 200X. Inset (Fig. 2B) shows the stromal cells in higher magnification. Note the central focus of hypertrophied eosinophilic cells. Mitotic figures were uncommonly seen at this time. 600X.

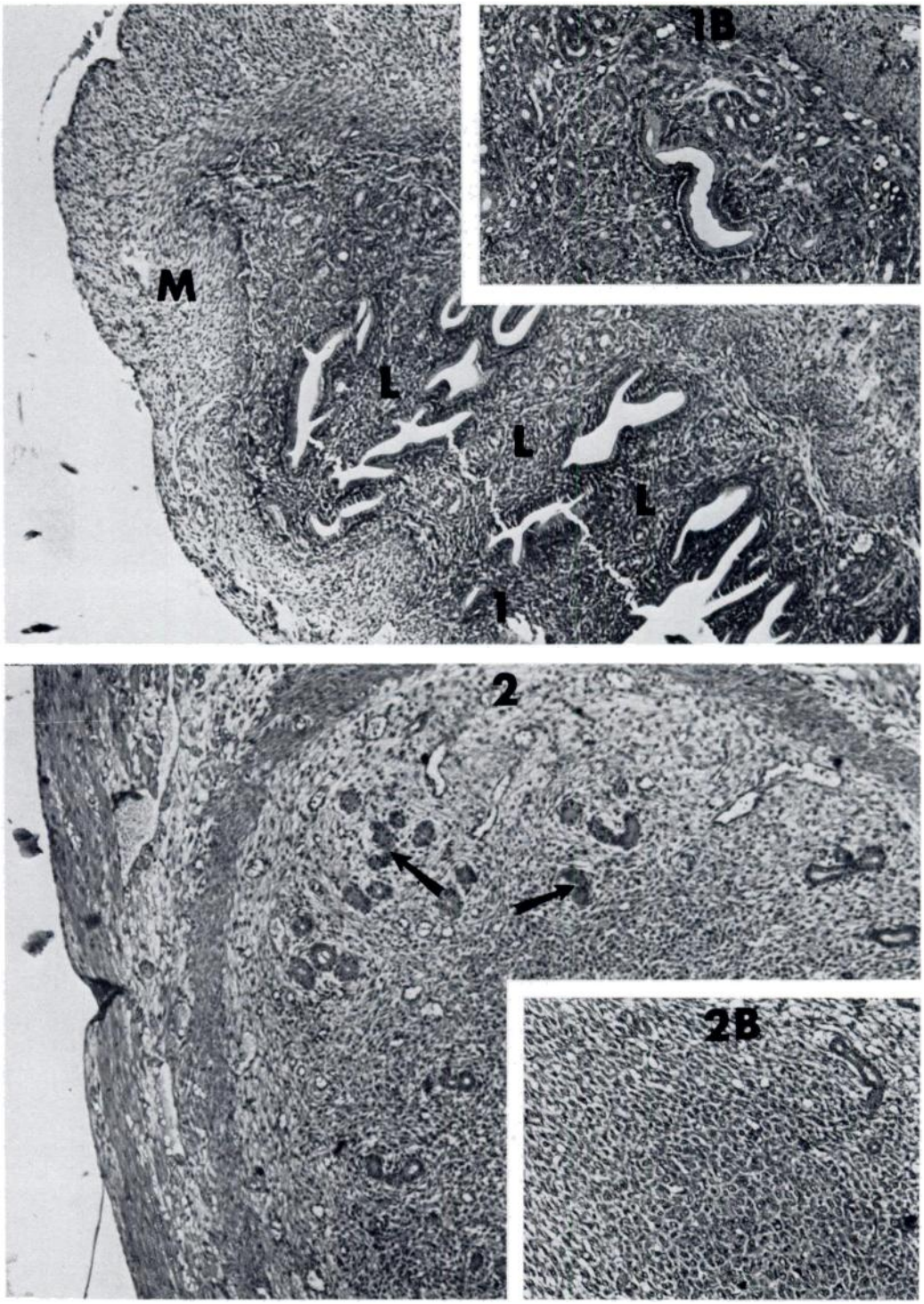
FIG. 3. Lamina propria of a 72 h experimental animal. A distinct deciduated stromal reaction is evident. Note the presence of dilated capillaries indicative of the extensive vascularization present at this stage. 500X. The inset (Fig. 3B) shows typical hypertrophied stromal cells at 100 h. Note the numerous mitotic figures (arrows). 600X.

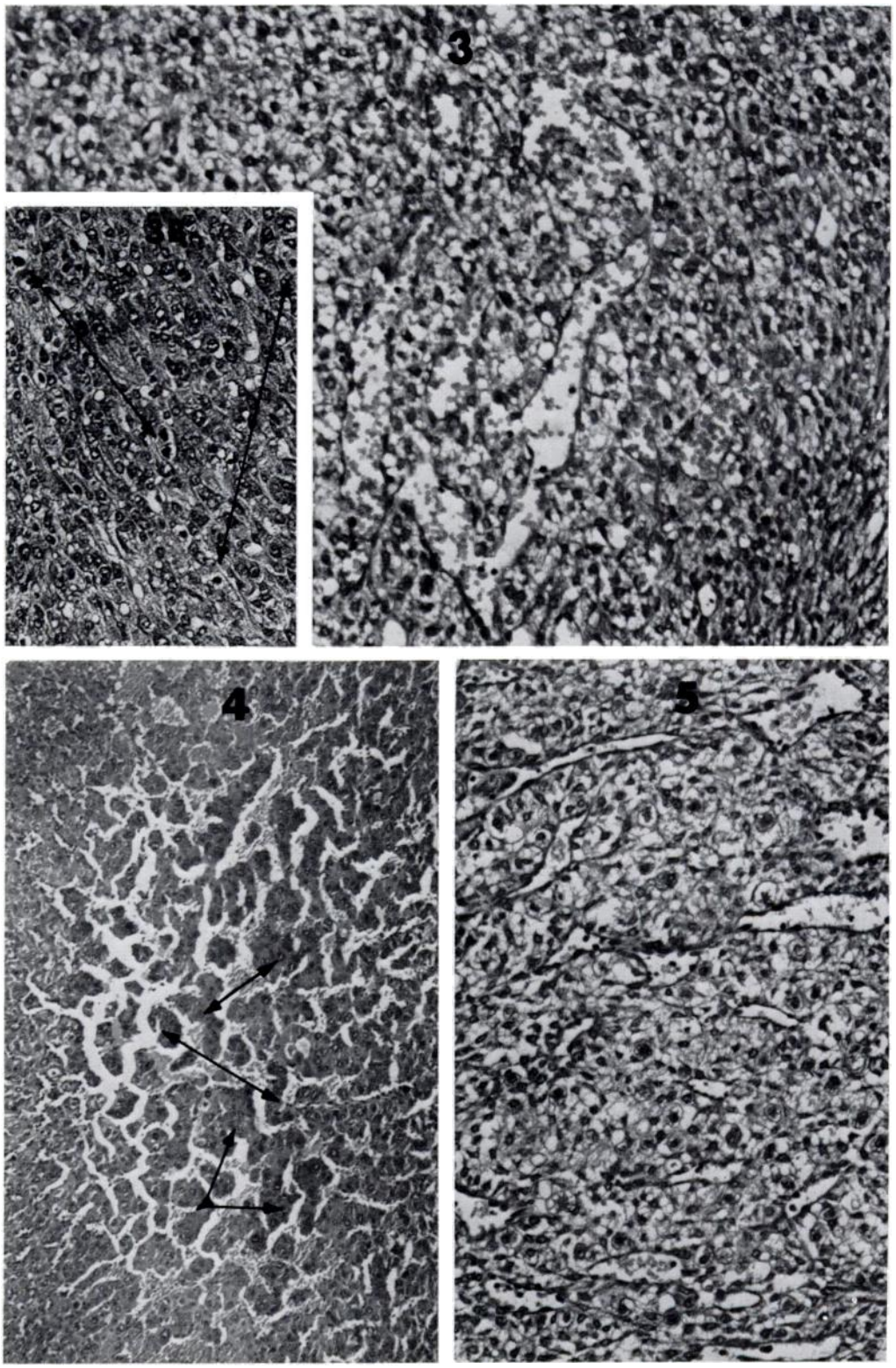
FIG. 4. The 100 h experimental animal. Note the hyperemia of the deciduated lamina propia. Many of the stromal cells have become arranged as anastomotic cords separated by vascular channels (arrows). 400X.

FIG. 5. A 100 h experimental animal. Vacuolization of the deciduated cells was maximal at this period, particularly in cells located at the center of the loculus. Such vacuolization is typical of glycogen-rich cells. 1000X.

growth phase of the stimulated uterus. The protein content of uterine horns is also shown in Fig. 7. The protein content of stimulated horns parallels the increase in horn weight, exhibiting two distinct phases of increase.

The gross appearance of uterine horns taken





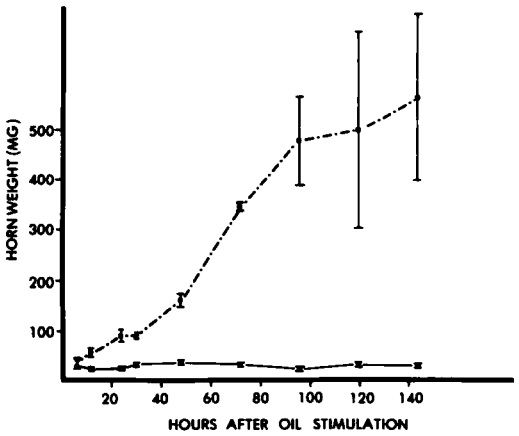


FIG. 6. Growth of uterine horns following oil stimulation. The right horn in each animal was stimulated by intraluminal injection of 10 μl of sesame oil. The left horn served as the unstimulated control. Right horn (———), left horn (—). Bars represent the standard errors of the means.

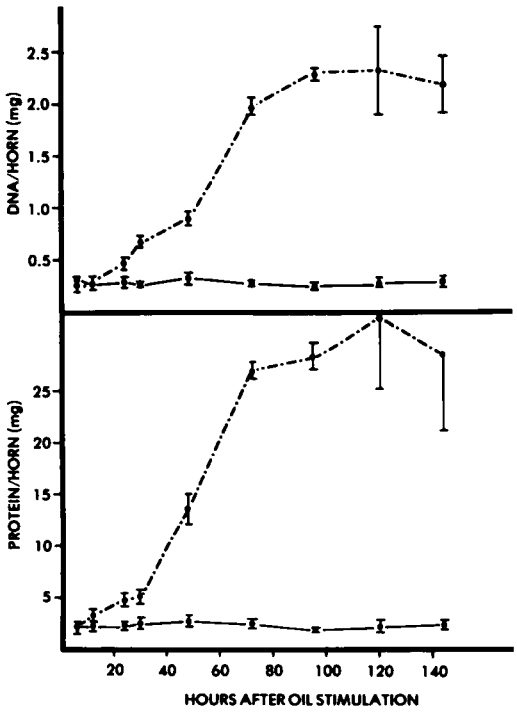


FIG. 7. DNA and protein content of uterine horns following oil stimulation. The right horn in each animal was stimulated by intraluminal injection of 10 μl of sesame oil. The left horn served as the unstimulated control. Right horn (———), left horn (—). Bars represent the standard errors of the means.

from an animal 144 h after oil stimulation is shown in Fig. 8. The stimulated right horn has increased in weight approximately 30-fold. Growth of the horn has occurred as discrete masses. These masses are shown dissected out of the horn. In this case the horn contained 10 loculi. The number has been found to vary from 1 to 11, and occasionally as a continuous mass extending over the length of the horn.

DISCUSSION

Intraluminal injection of sesame oil into the uterus of the hormone primed ovariectomized mouse stimulated the transformation of static stromal cells to rapidly proliferating decidual cells. The nature of the stimulus is presently unknown. The reaction is not stimulated by intraluminal injection of saline. This is unlike the reaction in the rat, which is stimulated by saline (De Feo, 1967); and it indicates that the stimulus is not simply manipulative in nature.

Growth of the uterine horn following oil stimulation occurs in two distinct phases. A similar biphasic growth curve has been reported by Hetherington (1968). The first growth phase begins approximately 12 h after stimulation resulting in a threefold increase in uterine horn weight by 24 h. A second and more sustained growth rate is reflected in the horn content of protein, indicating that both phases represent growth rather than simply edema. DNA content, on the other hand, does not change significantly until approximately the beginning of the second growth phase, suggesting that the first growth phase does not result from cell proliferation. This is consistent with morphologic findings of increased mitotic figures only in 72 h and later experimental groups.

The decidual cell reaction occurs at evenly spaced loculi. As growth progresses these become discrete masses of decidual cells which result in a uterine horn that closely resembles a naturally pregnant horn. The development of discrete masses of decidual cells appears to be due to contraction of the uterus. It can be seen in Fig. 3 that the loculus is defined before there is any extensive proliferation of decidual cells. It cannot yet be determined whether this represents an isolation of sensitive cells or a localization of the stimulus. A similar result has been observed by De Feo (1967) in the rat uterus following trauma stimulation. The development of discrete decidual masses in this case is clearly not the result of a localized stimulus. The decidual cell reaction begins at

FIG. 8. Uterine horns 144 h after oil stimulation of the DCR. The left (untreated) horn is shown at the top. The right (treated) horn is shown in the middle. The discrete masses of decidual cells removed from the right horn are shown at the bottom in the order of their arrangement in the horn.

the center of the loculus and spreads centrifugally. Decidualization is accompanied by the development of large sinusoidal and venous channels. Decidualization following sesame oil treatment proceeds grossly and histologically in a pattern similar to that recorded after actual blastocystic contact (Tachi et al., 1972; Glasser and Clark, 1975). Increased cytoplasmic eosinophilia probably represents an increase in smooth endoplasmic reticulum, an acidophilic organelle, which normally occurs in cells pursuing a natural DCR (Tachi et al., 1972). Vacuolization of cells collected after 100 h probably reflected glycogen accumulation which also occurs in a blastocyst-induced DCR (Tachi et al., 1972).

ACKNOWLEDGMENTS

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RECOMMENDED REVIEWS

De Feo, V. J. (1967). Decidualization. *In* "Cellular Biology of the Uterus" (R. M. Wynn, ed.), pp. 191-290, North Holland Publishing Company, Amsterdam.

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