

M. C. SHELESNYAK AND P. F. KRAICER

## *The Role of Estrogen in Nidation*

**T**HE OBJECT of this presentation will be to define the role of estrogen in the sequence of events during the onset of gestation that is called "nidation." Much of this report will consist of presenting and interpreting information that signalizes the intrusion of a surge of estrogen secretion during progestation, that interval between fertilization and the fixation of the fertilized ovum in the endometrium either by invasion of the nidus or by engulfment by the nidus. Attention will be directed chiefly to the effects of estrogen on the uterus, particularly to the role of estrogen in the transformation of endometrial cells into the decidua. This transformation of endometrial cells, by growth and differentiation, into decidua is defined as "decidualization."

The general plan of this discussion will consist of a descriptive definition of the process of nidation. Functional and temporal aspects of the estrogen role on the uterus will be correlated with data on histamine and progesterone actions. This interrelationship was explored by using experimentally induced decidualization and natural ovum-associated *déciduome* formation and also by seeking manifestations of estrogen activity during progestation. To search for these manifestations of estrogenic activity, morphological, physiological, and biochemical parameters were examined. The specific problem was approached by investigating natural circumstances or by designed experiment, as the situation dictated.

The late Vilhjalmur Stefansson, thinker and man of deeds, said in his own favorite of his many books that "the accepted facts of a few years ago become the error and folklore of today. You standardize knowledge and while you are on the job, the knowledge changes" (1936). Since our information of many of the episodes and conditions of early gestation is incomplete, permit us to present the following definition of "nidation" as a working definition—for convenience, perhaps even for clarity. We

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propose to define "nidation" as *the sequence of biological events that occur in the female of the species ending with the imbedding of the fertilized ovum in the uterus, the major episodes of nidation being, first, nidus formation, or preparing the uterine zone into which the blastocyst imbeds, and, second, nidus invasion, the active penetration of the ovum into the nidus or passive engulfment of the ovum by the nidus.*

Nidus formation is the primary subject of this discussion, with, of course, the role of estrogen in this process. Nidus formation is a prerequisite for ovum implantation in natural pregnancy; it can be provoked under artificial conditions by experimental means. When the nidus is produced under experimental conditions, it is called a "deciduoma," as distinct from the natural ovum-associated nidus, the "decidua." Extensive use of experimental induction of decidualization was made in exploring the mechanism of nidation, and it is pertinent to stress certain points of technique.

A fundamental element of the work to be reported is the induction of decidualization of the endometrium by systemic administration of a histamine releaser. This technique is based on the results of our early work, which established a role for histamine in the induction of decidualization and the formation of deciduoma (Shelesnyak 1957a, 1957b, 1960). Much evidence has been adduced by our group implicating histamine as an essential stimulatory metabolite, which acts upon the properly prepared or sensitized endometrial stromal cells to induce decidualization. Our method for inducing decidualization of the pregestational endometrium is to inject, via systemic route, a large amount of a histamine liberator. The most efficient material tested to date is pyrathiazine hydrochloride (Pyrrolazote, Upjohn). The most effective time to inject the pyrathiazine is on the morning of the fourth day of the luteal phase of pseudopregnancy (or pregnancy) (Shelesnyak *et al.*, 1961). The systemic administration of pyrathiazine results in the release of histamine from various sources in the body (Marcus *et al.*, 1963). The liberated histamine passes into the blood stream and can reach the endometrium via the vascular network.

This technique for the induction of decidualization is potent and reliable; it is the method of choice in our laboratory. It can be exercised with ease and with precision for studies involving time as a parameter. It requires no surgery or the concomitant physiological stress of surgery. But the greatest advantage derived from the use of the systemic method is that there are none of the complications inherent in the classical method of traumatizing the uterus. Trauma invariably and inevitably produces debris and uterine-tissue reactions to injury, including tissue wounding and regeneration, which are unrelated to decidualization but confound the decidual picture. Freedom from the primary and secondary

effects of tissue injury means that the systemic methods permit unambiguous examination of histogenic and biochemical processes that occur in the very earliest stages of decidualization (Shelesnyak, 1962).

It is convenient at this point to introduce some definitions and some standard terminology that will be used. *Induction* of decidualization, as used here, means *the stimulation of a sensitized or sensitive endometrium to begin to transform certain stromal cells to decidual cells*. When conditions are favorable, decidualization begins and the transformation carries on to production of decidual mass or decidioma. Thus, a "sensitized" endometrium has the potential to decidualize when stimulated by a decidualization inducer.

A standard terminology for designating the days of pseudopregnancy and pregnancy will be used. Since both conditions are characterized by a persistence of the luteal phase and of leucocytic configuration of the vaginal smear, we refer to these days as  $L_1$ ,  $L_2$ , . . .  $L_n$ ,  $L_1$  being the first day of appearance of leucocytic vaginal smear after the preceding Co, or cornified cell smear of estrus. According to this convention, the day of discovery of sperm in the vagina is day 0 of pregnancy (which is a metestrous day of vaginal cornification). This terminology has the advantage of making the days of pregnancy and of pseudopregnancy strictly comparable. It also overcomes the difficulty presented by certain animals that show a persistence of the cornified vaginal smear for two consecutive days at the beginning of pseudopregnancy. It has been shown that, in animals with an additional day of cornified cell smear,  $L_4$  is identical, with respect to decidual reactions and biochemical parameters that were measured, to  $L_4$  of an animal with only one day of cornified cells (Shelesnyak *et al.*, 1961).

Let us focus our attention on the estrogen secretion during nidation. The estrogen of estrus, which is considered to be a primer of the endometrium for progesterone action, will not be subjected to examination. We are concerned with that estrogen secretion which takes place during progestation when the uterus is under progesterone dominance. The first hints that the estrogen secretion during progestation was a surge of relatively short duration were provided by correlating certain morphological observations with physiological and biochemical ones.

The usual pattern of cells from the vaginal smears of impregnated rats is a persistent leucocytic configuration. However, Nelson (1929) and, later, Mirskaya and Crewe (1930) noted some cases in which estrous-type vaginal smears of cornified cells appeared during  $L_4$  or  $L_5$  of pregnancies otherwise normal and uneventful. Swezy and Evans (1930), studying the pregnant rat (and, later, Bloch [1958] the pregnant lactating mouse), reported that histological examinations of the ovaries revealed a surge of follicular development four days after the estrus in which

the animals had been mated and impregnated. Vokaer's (1952) analysis of athrocytosis in the rat uterus revealed evidence suggestive of estrogenic activity on L<sub>4</sub> of pregnancy. These morphological findings suggested an estrogen surge, but no functional significance in nidation could be assigned to an estrogen surge on the basis of these data.

Westin (1955) reported that mast cells of the uterus were dissipated by estrogen, and Shelesnyak (1959c) observed that during the twenty-four hours prior to the time of expected implantation (nidus invasion) the population of mast cells in the uterus of the pregnant rat fell to almost zero. Since a reduction in mast cells can be correlated with the release of histamine (Riley, 1959), these observations suggested a role of estrogen via histamine release in the uterus. However, the case for a histamine-related functional role of estrogen surge in nidation needed a great deal more evidence; some of the necessary data will be presented later in this discussion.

Turning from structural to biochemical aspects, we find that of many enzyme systems, which vary in activity with the amount of available estrogen, two have been studied in the progestational uterus. These are betaglucuronidase (Prahlad, 1962) and glycylglycine dipeptidase (Albers *et al.*, 1961). Changes in uterine concentration of these enzymes resembling those characteristic of estrogen activity have been noted between L<sub>3</sub> and L<sub>5</sub> of pseudopregnancy and pregnancy. But here again the functional significance of estrogen surge in nidation cannot be inferred from the information.

It was evident that the elucidation of the role of estrogen in nidation required rigid experimental approaches. The first step we considered was the elucidation of the role of estrogen in the induction of decidualization. Accepting estrogen as a requirement for decidualization, we reasoned that it was important to delineate the period in the course of progestation during which the decidual reaction could be induced in the non-traumatized uterus. The systemic method for inducing decidualization permitted this investigation. By administering the systemic inducer at different but closely spaced times, we found that the period of uterine sensitivity to decidual induction was very brief (Shelesnyak *et al.*, 1961). The brevity and transience of the uterine sensitivity suggested that there was an earlier event that was also transient and that it sensitized the uterus to respond to the decidual-inducing stimulus.

It was now necessary to identify this event with the estrogen surge. Experiments were performed to study the role of the ovaries in the sensitization of the endometrium. By removing the ovaries of pseudo-pregnant (and pregnant) rats at different times during progestation, we showed that there is indeed a critical period of estrogen secretion from

the ovary that is indispensable for the sensitization of the uterus (Shelesnyak *et al.*, 1963).

Ovaries were removed from rats, and daily injections of progesterone were begun on L<sub>4</sub>, which is the day when the uterus is in a state of maximal sensitivity to decidual induction. Administration of pyrathiazine to the animals (on L<sub>4</sub>) to induce decidioma resulted in decidual reactions. The same response occurred if the ovaries were removed at midnight on L<sub>3</sub>. Removal of ovaries during the afternoon or evening of L<sub>3</sub> resulted in partial or complete block of decidual response to pyrathiazine given on L<sub>4</sub>. If the ovaries were removed at noon on L<sub>3</sub>, no decidioma could be induced by pyrathiazine, despite adequate quantities of exogenous progesterone.

The role of estrogen was then examined. Exogenous estradiol, given during the afternoon of L<sub>3</sub> to rats ovariectomized before noon on L<sub>3</sub>, restored the capability of the endometrium to respond to systemic induction of decidualization. From these results it was clear that an ovarian estrogen surge occurred during the afternoon of L<sub>3</sub> that was essential for the sensitization of the pregestational uterus.

The accumulated physiological evidence established (*a*) that there is an ovary-derived estrogen surge and (*b*) that this estrogen secretion sensitizes the uterus to decidual induction. What the nature of the estrogen action was, especially at the tissue and cellular level, remained to be explored.

The disappearance of mast cells from the uterus of the rat prior to ovum implantation invited attention to several other relevant data correlating with the estrogen surge. The relationships of estrogen to mast cells (Westin) and of mast cells to histamine (Riley) have been noted. Spaziani and Szego (1958) and Shelesnyak (1959b) then demonstrated that estrogens (Spaziani and Szego, estradiol; Shelesnyak, estradiol, estrone, and estriol) have a histamine-releasing action on the rat uterus. That this phenomenon occurs in the uterus of the impregnated rat, in synchrony with the estrogen surge prior to ovum implantation, was established by estimating histamine content of the uterus during pregestation (Shelesnyak, 1959a). The estimations were made by bioassay of the histamine extracted from L<sub>2</sub>, L<sub>3</sub>, and L<sub>4</sub> uteri of pregnant rats. We (Marcus and Shelesnyak) have extended our early findings, using chromatographic separation of the histamine and fluorometric determinations, to the uteri of normal estrous cycle, of pregnancy through day L<sub>6</sub>, and of pseudopregnancy. Fluctuation of histamine content and concentration followed the estrogen fluctuation of the normal estrus, and the estrogen surge of pregnancy. The picture of uterine histamine during pseudopregnancy is being analyzed.

The information available at this stage of our experiments clearly

establishes an ovarian-derived estrogen surge during progestation, and the biochemical data presented so far relate the estrogen to histamine and the induction phase of decidualization. However, we have yet to demonstrate a role of the estrogen as activating the pregestational uterus to become sensitive to the histamine stimulus.

Psychoyos (1960) showed that one of the very first reactions to stimuli that provoke decidualization, provided that estrogen is available, is a marked increase in capillary permeability of the uterus. Attempting to link the increased capillary permeability that Psychoyos reported at this stage of progestation and the established permeability activity of estrogen (Hechter *et al.*, 1941) with the massive leucocytic infiltration of the uterus prior to decidualization, and the estrogen surge, suggested a search for an estrogen-dependent leucotoxic substance similar to that reported by Spector and Storey (1958). A plasma kinin would fit their characterization of their unknown substance; plasma kinins are responsible not only for leucotaxis but also for vasodilation. Preliminary results of experiments carried out in our laboratory (by Shelesnyak and Lappé) suggest estrogen-associated changes of kinin-like activity in the uterus. No precise role has been assigned to this substance at this early stage of our investigations, but the existence of a consistent and conforming pattern is real.

One can predict with a degree of confidence that, of the vast array of reputed estrogen-dependent and estrogen-related phenomena that have been observed, many could be detected in association with the estrogen surge. However, again, when we sought to fix a role for estrogen in decidualization, we found it necessary to focus our attention on specific activities. We therefore turned to the relation of estrogen to tissue growth.

We have analyzed the composition of uteri at serial stages of pseudopregnancy, of decidualization, and of the normal estrous cycle (Shelesnyak and Tic, 1963b). Estimations were made of the weight, protein, RNA, and DNA.

Cyclic variations in the weight and in the content of RNA, DNA, and protein occurred during the estrous cycle. All four components achieved maxima during the proestrous phase, and minima at diestrus. Since this pattern of biochemical changes parallels the cyclic appearance of cornification, it is reasonable to suspect that both are manifestations of the same hormonal influences.

During pseudopregnancy, the composition of the uterus undergoes revealing changes. On  $L_1$ ,  $L_2$ , and  $L_3$  the values are like those obtained from the diestrous uterus. On  $L_4$  and  $L_5$  the uterus undergoes a change closely resembling the growth seen in proestrus. This transient surge of growth passes, and the uterus returns to a diestrous composition. It

will be seen presently that this surge represents synthesis of uterine components essential for decidualization. Both the time of these syntheses and the changes in composition observed support the contention that estrogen secretion is the stimulus. The enhancement of protein and nucleic acid synthesis in the uterus by estrogen has been demonstrated by a number of workers (Jeener, 1948; Mueller, 1957; Mueller *et al.*, 1958; Brody *et al.*, 1961).

When decidualization is induced by the systemic technique on L<sub>4</sub>, the uterus grows exponentially for at least four days. However, not all the

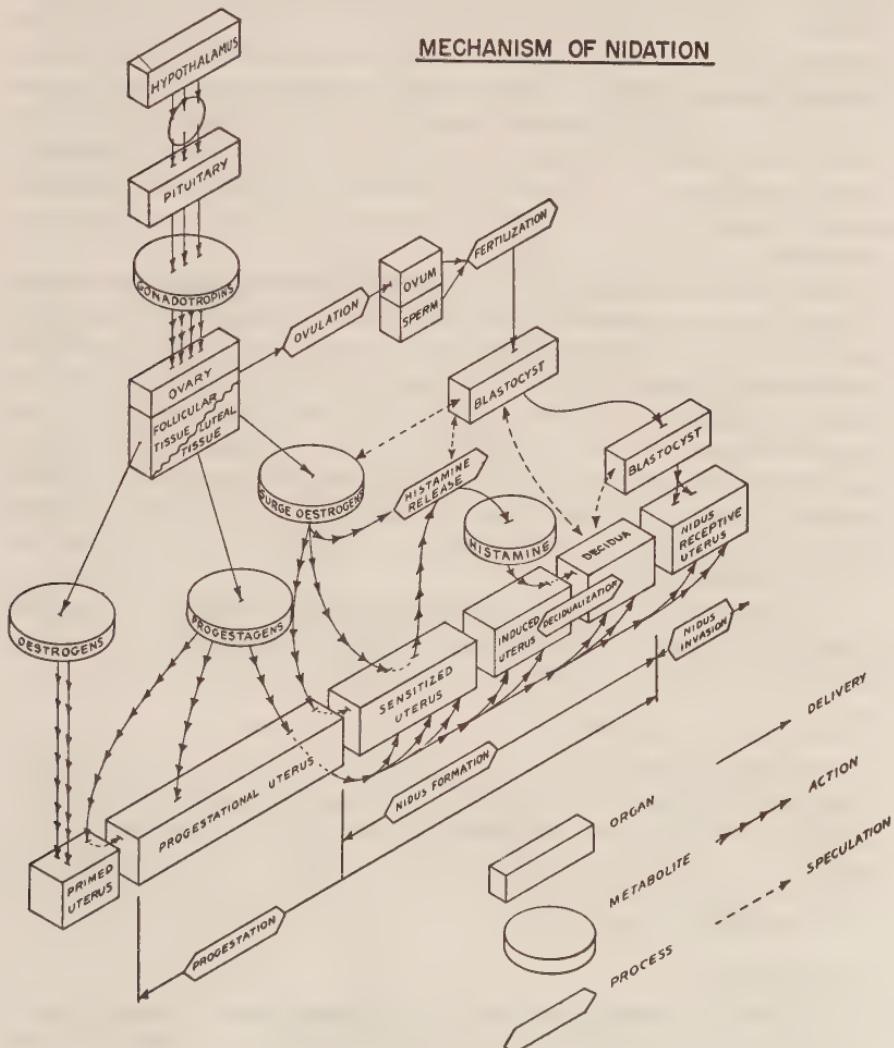


FIGURE 1

components estimated begin to increase from the time of decidual induction. Mathematical analysis indicates that growth begins on  $L_3$ , one day before induction, in terms of weight and RNA increment, and 12–24 hours after induction in terms of protein and DNA. This is, of course, the expected sequence. The growth on  $L_3-L_4$  is the response to estrogen already noted and is not growth of decidual tissue. It is, however, considered to be a predecidual growth of the uterus.

It was shown, by using an anti-estrogen, that, if this predecidual growth is suppressed, then decidualization cannot be evoked. We have injected MER-25 into rats on  $L_3$  of pseudopregnancy or pregnancy (Shelesnyak *et al.*, 1963). Induction of decidualization or nidation was prevented. Determination of the uterine composition and weight showed that the response to the estrogen surge was suppressed and that diestrous values were obtained (Shelesnyak and Tic, 1963a). Administration of the anti-estrogen on  $L_4$ , after the estrogen surge, did not affect decidualization or nidation. The specificity in time of the action of MER-25 serves to emphasize the brevity of the period of essential estrogenic stimulation.

There is ample evidence from observations during normal progestation and from our experiments that an estrogen surge exists during the postovulatory progestational phase of the cycle, essentially a period of progesterone predominance. This estrogen sensitizes the uterus and makes it capable of responding to decidual-inducing stimuli. In the absence of the estrogen surge, or in the event that it is suppressed, decidual-inducing stimuli are ineffective. These findings fit into the theory of the mechanism of nidation and permit a further elaboration of the theory that was first postulated by one of us (M. C. S.) in 1956 and extended in 1960 (Shelesnyak).

This discussion can thus be summarized by presenting the current and expanded presentation of our theory as shown in Figure 1. There are still many opaque areas, but, to us, this is the challenge.

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#### REFERENCES

- ALBERS, H. J., J. M. BEDFORD, and M. C. CHANG. 1961. Uterine peptidase activity in the rat and rabbit during pseudopregnancy. *Am. J. Physiol.*, **201**:554–56.

- BLOCH, S. 1958. Experimentelle Untersuchungen über die hormonalen Grundlagen der Implantation des Säugerkeimes. *Experientia*, **14**:447-50.
- BRODY, S., and N. WIQVIST. 1961. Ovarian hormones and uterine growth: Effects of estradiol, progesterone and relaxin on cell growth and cell division in the rat uterus. *Endocrinology*, **68**:971-77.
- CREWE, F. A. E., and L. MIRSKAIA. 1930. Mating during pregnancy in the mouse. *Nature*, **125**:569.
- HECHTER, O., L. KROHN, and J. HARRIS. 1941. The effect of estrogen on the permeability of the uterine capillaries. *Endocrinology*, **29**:386-92.
- JEENER, R. 1948. Acides nucléiques et phosphatasées au cours des phénomènes de croissance provoqués par l'œstradiol et la prolactine. *Biochim. Biophys. Acta*, **2**:439-53.
- MARCUS, G. J., P. F. KRAICER, and M. C. SHELESNYAK. 1963. Studies of the mechanism of decidualization. II. The histamine-releasing action of pyra-thiazine. *J. Reprod. & Fertil.* (In press.)
- MUELLER, G. C. 1957. A discussion of the mechanism of action of steroid hormones. *Cancer Res.*, **17**:490-506.
- MUELLER, G. C., A. M. HERRANEN, and K. F. JERVELL. 1958. Studies on the mechanism of action of estrogens. *Recent Prog. Hormone Res.*, **14**:95-129.
- NELSON, W. O. 1929. Oestrus during pregnancy. *Science*, **70**:453-54.
- PRAHLAD, K. V. 1962. A study of the rat uterine  $\beta$ -glucuronidase prior to the implantation of the ovum. *Acta Endocrin.*, **39**:407-10.
- PSYCHOYOS, A. 1960. La réaction déciduale est précédé de modifications précoce de la perméabilité capillaire de l'utérus. *C.R. Soc. Biol.*, **154**:1384-87.
- RILEY, J. F. 1959. The Mast Cells. Edinburgh and London: Livingstone.
- SHELESNYAK, M. C. 1957a. Some experimental studies on the mechanism of ova-implantation in the rat. *Recent Prog. Hormone Res.*, **13**:269-317.
- \_\_\_\_\_. 1957b. Experimental studies on the role of histamine in implantation of the fertilized ovum. *Bull. Soc. Roy. Belg. Gynéc. & Obstét.*, **27**:521-37.
- \_\_\_\_\_. 1959a. Fall in uterine histamine associated with ovum implantation in the pregnant rat. *Proc. Soc. Exp. Biol. & Med.*, **100**:380-81.
- \_\_\_\_\_. 1959b. Histamine releasing activity of natural estrogens. *Ibid.*, pp. 713-23.
- \_\_\_\_\_. 1959c. Histamine and the nidation of the ovum. *Mem. Soc. Endocrin.*, **6**:84-88.
- \_\_\_\_\_. 1960. Nidation of the fertilized ovum. *Endeavour*, **19**:81-86.
- \_\_\_\_\_. 1962. Decidualization: The decidua and the decidioma. *Perspect. Biol. & Med.*, **5**:503-18.
- SHELESNYAK, M. C., and P. F. KRAICER. 1961. A physiological method for inducing experimental decidualization of the rat uterus: Standardization and evaluation. *J. Reprod. & Fertil.*, **2**:438-46.
- SHELESNYAK, M. C., P. F. KRAICER, and G. H. ZEILMAKER. 1963. Studies on the mechanism of decidualization. I. The oestrogen surge of pseudopregnancy and progravidity and its role in the process of decidualization. *Acta Endocrin.*, **42**:225-32.

- SHELESNYAK, M. C., and L. TIC. 1963a. Studies of the mechanism of decidualization. IV. Synthetic processes in the decidualizing uterus. *Acta Endocrin.*, **42**:465.
- . 1963b. Studies on the mechanism of decidualization. V. Suppression of synthetic processes of the uterus (DNA, RNA and protein) following inhibition of decidualization by an anti-oestrogen, ethanoxytriphetol (MER-25). *Ibid.* (In press.)
- SPAZIANI, E., and C. M. SZEGO. 1958. The influence of estradiol and cortisol on uterine histamine of the ovariectomized rat. *Endocrinology*, **64**:713-23.
- SPECTOR, W. G., and E. STOREY. 1958. A factor in the oestrogen-treated uterus responsible for leucocyte emigration. *J. Path. & Bact.*, **75**:387-98.
- STEFANSSON, V. 1936. Adventures in Error. New York: Robert M. McBride & Co.
- SWEZY, O., and H. M. EVANS. 1930. Ovarian changes during pregnancy in the rat. *Science*, **71**:46.
- VOKAER, R. 1952. Recherches histophysiologiques sur l'endomètre du rat en particulier sur le conditionnement de ses propriétés athrocytaires. *Arch. Biol.*, **63**:1-84.
- WESTIN, B. 1955. The influence of some ovarian hormones on the occurrence of mast cells in the mouse uterus. *Acta Path. Microb. Scand.*, **36**:337-42.

### DISCUSSION (Chairman: M. N. RUNNER)

SHELESNYAK: Before beginning the discussion I should like to add some information from our current work. Our basic theory of nidation involves at least three physiological phases required for decidualization, induction, and maintenance. There is an interplay of estrogen and progesterone and histamine. By blocking surge estrogen or blocking histamine, we prevent decidual induction. If progesterone is blocked, decidual tissue does not develop, even though the process of decidualization may be induced. We have accumulated much evidence in the rat that ergocornine prevents the development of decidual tissue by interference with progesterone. We have just completed a study in women to determine whether ergocornine is effective in women (Shelesnyak, Lunenfeld, and Höning, 1963, *Life Sciences*, p. 73). The study can be summarized as follows: When a single tablet of 2 mg. of ergocornine methanesulphonate is administered to women who are in the postovulatory phase of their menstrual cycle, there is a sharp drop in the levels of urinary pregnanediol and estrogens. These results, indicating a depression of progesterone in women after ingestion of a single dose of ergocornine, are consistent with observations on the rat.

NOYES: Do you really want to interfere with progesterone metabolism in women?

SHELESNYAK: The indications at the moment are that it is a very transient interference. In the study so far, based on estimation of the

urinary steroids, there is recovery in 24–48 hours. If interference is necessary, I prefer 24–48 hours to 20 days.

NOYES: What is the stage of the investigation? Do you want to lower estrogen and elevate 17-ketosteroids in a population that may be hypoestrogenic and hirsute enough already?

SHELESNYAK: Give us about three years; if we are lucky, then, with diligence, industry, and support, we should have a picture of where it works, how it works, and when it works. When we have this picture in a controlled sample, I shall be prepared to discuss the problem of population control.

GREENWALD: If an estrogen surge exists in rats and if you block it, you should be able to delay implantation. Have you tried using nembutal or other blocking agents that might impair the release of LH?

SHELESNYAK: Implantation is delayed in rats after the removal of the ovary prior to the estrogen surge. We have not attempted to block estrogen surge with nembutal. In our studies (unpublished) nembutal administered after the estrogen surge did not prevent decidualization.

GREENWALD: Is there any variation in the time of implantation depending on the length of previous estrous cycles, that is, a difference between animals having a 4- or 5-day cycle?

SHELESNYAK: If our theory is correct, there should be none. In fact, the estrogen surge is independent of estrus. The estrogen surge occurs during progestation and before the phase of sensitivity in the animals in our colony. These include females with 4-day and with 5-day estrous cycles.

DE FEO: We have tried to block the "surge" in pseudopregnant rats by administering nembutal in a dose known to be effective in proestrous rats. This was done not only at the critical 2:00 P.M. period but also at 11:00 A.M., daily from day 1 through day 4. Maximal uterine sensitivity still appeared on day 4 and was lost by day 5 as in normal animals (1963, *Endocrinology*, 72:305). We concluded that there is no estrogen surge or else that it cannot be blocked by this method.

YOCHEM: By manipulating the amount of estrogen priming in ovariectomized rats, we have been able to vary the timing of uterine sensitivity after treatment with estrone and progesterone. In one group of animals we observed maximal sensitivity on both the third and the fourth day of treatment. Sensitivity was completely lost by the fifth day.

SHELESNYAK: To animals from which the ovaries had been removed weeks earlier, we administered a single dose of estradiol, a dose that was adequate to induce vaginal estrus. We then gave daily progesterone. Sensitivity as on day L<sub>4</sub> of normal pseudopregnancy was not evident on L<sub>4</sub> of this design. It was necessary to give surge estrogen, and then after 14–18 hours the uterus responded to pyrathiazine induction.

DAVIS: You mentioned mast cell histamine. The work of Schayer in this country and of Kahlson in Sweden indicates that important amounts of non-mast cell histamine are present in most tissues of the body, and the release of this material is an important physiological regulatory mechanism. Does your work support this idea, and would non-mast cell histamine be released in the uterus in response to estrogen?

SHELESNYAK: Mast cell histamine does exist in the rat uterus. In attempting to correlate the physiological picture with the histological one, we noted a depletion of mast cells prior to the beginning of decidualization. We assume that the histamine released from the mast cells played the inducing role.

DAVIS: Does this make a difference in our thinking about the site of action of estrogen in the uterus?

SHELESNYAK: It might.

WIMSATT: Do you have any information on recovery time of mast cells after degranulation is induced by injection?

SHELESNYAK: No, but after the deciduoma runs its course and a second pseudopregnancy is provoked, histamine-releaser will again induce decidualization.

WIMSATT: Did you imply that this mast-cell releasing substance is effective on mast cells only at this time, or any time?

SHELESNYAK: The effectiveness of the inducer (histamine-releaser) is very time-specific. Ordinarily, there are very few mast cells in the rat endometrium. When the time is reached and histamine is needed for induction of decidua, the mast-cell population drops. Recovery of this mast-cell population may be by regeneration or infiltration.

WIMSATT: If you could eliminate histamine before you give estrogen, and then give estrogen, would you get deciduoma?

SHELESNYAK: In experimental procedures by which the histamine has been depleted but the estrogen not manipulated, we failed to get decidualization by histamine-releasers. The experiment about which you ask, namely, giving added estrogen (that is, additional to the animal's surge estrogen) has not been carried out.

GLASSER: I was interested in your graph depicting a progesterone-directed increase in nucleic acid, weight, and protein from L<sub>3</sub> onward. Were these determinations done on the whole rat uterus or prospective and actual deciduoma?

Generally, although we have described biochemical changes within a short period of time after trauma, I have not been pleased with the information derived from such studies. How much of the increase in protein that we describe is myometrial? If the increase in protein is non-parallel with respect to the increase in DNA, as you describe, where is the increased protein coming from? Have you corrected your

data for the significant alterations in tissue water that are to be noted? Are your data expressed on a wet weight, dry weight, or milligram nitrogen basis?

SHELESNYAK: Myometrium and decidua tissue are separable only after day L<sub>7</sub>. Earlier stages can be studied only together with the myometrium. Any study of early changes that does not employ "systemic" induction of decidualization is, in my mind, open to serious criticism.

BIGGERS: You showed a picture of a uterus from a rat that had been treated with pyrathiazine, in which swellings occurred in different parts of the uterine horns. How do you account for the fact that the drug, which was administered systemically, acted only in certain parts of the uterus?

SHELESNYAK: Alexandre Psychoyos of Paris (Collège de France) and Dr. Orsini should be complimented here on their work. They have shown quite clearly that the first response to stimulus was localized, according to vascular distribution. This is also true following pyrathiazine.

MEYER: What are we to assume about levels of progesterone in the first days?

SHELESNYAK: Do you mean levels on days L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, and L<sub>4</sub>?

MEYER: If estrogen is at a given level and sensitization is on day 4, could not this represent an increased output of progesterone for your test? You could study this possibility by giving a constant amount of estrogen with varying doses of progesterone. We do not say anything about where progesterone is before ovulation on day 4.

SHELESNYAK: If we assume that it maintains a constant status, we can be satisfied. A drop in progesterone does not induce pregnancy in delayed nidation, and, although Johnson and I were successful with this once, we cannot reproduce this result at will.

MEYER: I am very much concerned with the hypothesis that sensitivity on day 4 may be due to increased amounts of progesterone without necessarily increasing the amount of estrogen. Suzuki studied pseudopregnant and pregnant rats. He found that progesterone is increasing and reaching higher levels around day 4 in blood from the ovarian vein. We have not tested the question whether sensitivity may be due to increased progesterone level in pseudopregnancies as compared to a normal cycle.

SHELESNYAK: How do you fit the role of increased progesterone in inducing nidation and delaying nidation with the fact that injection of doses of estrogen in an already pregnant endometrium is necessary to get pyrathiazine-induced decidioma?

MEYER: I must say that we have not tested this hypothesis, but we

have not said much about progesterone levels. We assume that it goes to a level after ovulation and stops.

SHELESNYAK: There are so many things that favor estrogen. It seems more direct. In order to be certain, we must try to prove that we are wrong.

DE FEO: While we wholeheartedly agree that pyrathiazine—which, incidentally, has not worked well enough for us as yet—may be a good inducer of spontaneous decidiomata by non-traumatic means, we disagree with its being referred to as “physiological” when (a) it is a pharmacologic agent whose entire spectrum of actions is not clearly known and, (b) unlike the rat blastocyst, operates from the peritoneal cavity rather than the uterine lumen.

SHELESNYAK: We believe that it is more “physiological” than any other method. We are convinced that histamine plays a role as inducer; we have proof that pyrathiazine releases histamine, and that leads us to suggest that the histamine reaches the uterus via systemic means and approximates a physiological induction. We do not claim that pyrathiazine is a body metabolite.

Success in inducing decidualization by pyrathiazine has been achieved in thousands of rats in our laboratory and also in the laboratories at Birmingham (Anatomy Department), Bordeaux, Rome, and Paris.

Failure may be due to strain differences (although the strains in the various laboratories mentioned differ). Response to antihistamines can vary greatly in different strains (see Ambrus *et al.*, 1961, Proc. Soc. Exp. Biol. & Med., 108:360).

MAYER: The important point is that pyrathiazine acts as decidual inducer only when estrogen acts in synergy with progesterone. Pyrathiazine does not induce decidioma in ovariectomized rats injected only with progesterone, but it does in a normal pseudopregnancy at day 5, that is to say, after the estrogen release taking place at day 4.

A similar result is observed with the physiological decidual reaction induced by the implantation of the egg. If there is no estrogen (in rats ovariectomized at day 4 and injected with progesterone), the eggs do not implant and do not induce decidual reaction. But they do in rats ovariectomized at day 4 and injected with progesterone and estrogen.

DE FEO: We have studied mast cells in relation to decidioma formation. The rat uterus, particularly in the region of the mesometrial triangle, has a potent source of stored histamine in the mast cells. However, even if these cells are markedly depleted by 48/80 pretreatment, the uterus still responds to intraluminal inducers on day 4 by formation of decidiomata. In addition, complete elimination of stainable mast cells still enables the uterus to develop the characteristic hydration associated with estrogen administration. We wonder whether we are still dealing

with histamine release from the non-stainable mast cells, that is, in a non-storage phase, or else the histamine source may be from cells other than the mast cells.

SHELESNYAK: Have you measured histamine release in mast cell-depletion studies? It is pertinent. If you have removed the mast cells from the uterus and measured histamine release following estrogen, you will be able to correlate whether there is an additional source other than the mast cells.

DE FEO: Do you still believe that the blastocyst induces the decidua via histamine release?

SHELESNYAK: Some speculation exists, but we have yet to rule out the possibility that the estrogen factor is contained in the "stuff" with the blastocyst. That is, the stuff that the blastocyst brings down from the ovary, particularly in the larger blastocysts. We cannot rule out the presence of a certain amount of estrogen in this mass. Very little is necessary to trigger this response.

DICKMANN: In your opinion, what role does the blastocyst play in implantation?

SHELESNYAK: I am greatly interested in the role of the blastocyst in nidation, but I do not know enough about the blastocyst at this time to say. This is, of course, one object of all our studies, in fact of every one who works in this field.

NOYES: Why will not the mature blastocyst stick to decidua?

SHELESNYAK: I really do not know, and you know that I do not know.