A PHYSIOLOGICAL METHOD FOR INDUCING EXPERIMENTAL DECIDUALIZATION OF THE RAT UTERUS: STANDARDIZATION AND EVALUATION

M. C. SHELESNYAK AND P. F. KRAICER

Endocrine and Reproduction Physiology, Weizmann Institute of Science, Rehovoth, Israel

(Received 18th April 1961)

Summary. A method is described for inducing decidualization of the uterus of the pseudopregnant rat, which is based on the utilization of systemically released histamine. Although per os, subcutaneous, intraperitoneal, intravenous and intracardiac routes can be used, the most generally effective is intraperitoneal. A single injection of pyrathiazine hydrochloride (Pyrrolazote, Upjohn) as 1 ml of 0.06 m aqueous solution (2% w/v), administered at 1000 hr on the 4th day of dioestrus of pseudopregnancy, was effective in inducing extensive decidualization of the endometrium.

INTRODUCTION

In 1907, Leo Loeb observed that injury to the uterus of the guinea-pig evoked a massive, transient growth of decidual cells—the deciduoma. Since this initial report, observations were extended to many other species (the rabbit, mouse, rat, dog, cat, monkey and man—see Shelesnyak, 1957, for references). Exploration of this remarkable phenomenon has revealed at least two essential requirements for successful induction and maintenance of decidualization. These are: (1) a responsive endometrium achieved by the action of ovarian hormones, oestrogens and progestogens in proper sequence and proportion; and (2) stimulation of the process achieved by histamine, directly or by histamine-releasing agents, or actions, e.g. trauma (Shelesnyak, 1960).

The hormonal requirements for successful deciduoma have been used as a basis for evaluation of progesterone (Astwood, 1939) and for progesterone activity of other natural and synthetic steroids (Pincus, Chang, Zarrow, Hafez & Merrill, 1956). The realization that the artificially induced deciduoma bears striking resemblance to the decidual tissue of normal gestation (Loeb 1909) has made the deciduoma a useful model system for the study of ovum-uterine interactions associated with nidation or ovum-implantation (Mossman, 1937; Wislocki & Streeter, 1938; Shelesnyak, 1952, 1957, 1960; Shelesnyak & Kraicer, 1960).

However, when trauma is an essential part of the stimulation of the responsive uterus, the early stages of decidualization cannot be analysed, structurally or biochemically. The presence of damaged tissue, debris and various elements...
of tissue response to injury complicate the morphological and biochemical examinations and interpretations. Kreibiel (1937, 1941) attempted to overcome the gross tissue damage by passing electrical current transversely across the intact uterus, but some damage was associated with this technique.

The basis for the development of the ‘physiological’ method for inducing decidualization is the accumulated evidence for the role of histamine as the metabolite associated with the induction of the transformation of the stroma to decidual cells (Shelesnyak, 1952, 1960; Shelesnyak & Kraicer, 1960; Chambon & Lefrein, 1952; Chambon, 1958). By effecting a release of systemic histamine, which reaches the uterine receptors directly or via the vascular system, it has been possible to induce decidualization without trauma or surgical intervention. Employment of pyrathiazine which is antihistaminic, but which in its initial action releases histamine (Shelesnyak & Kraicer, 1959), serves to deliver histamine to the uterus. Therefore, while the liberated histamine is blocked in general systemic action by the histamine-antagonist, it can be active in the uterus, since antihistamines when administered by systemic route do not reach the uterus in doses adequate to interfere with decidual development (Shelesnyak & Davics, 1955).

METHODS

GENERAL

These studies were carried out on young adult female albino rats (180 to 210 g), originally Wistar stock, but colony-bred at the Weizmann Institute since 1951. The rats are housed in air-conditioned quarters, fed Purina lab-chow and supplied with water ad libitum. Rooms are windowed and normal diurnal light cycle obtains. Daily vaginal smear examinations are made both before experimental procedures are instituted to establish cycle rhythm, and during the course of the experiment. For the achievement of a responsive type uterus, one which has been subjected to the required oestrogen-progesterone, pseudopregnancy was employed. After at least two normal 4-day oestrous cycles had been experienced, the rats were subjected to faradic stimulation of the cervical regions on days of pro-oestrus and oestrus (Long & Evans, Stages 1 and 3, see Shelesnyak, 1931). In our colony, this method is successful in >95% of females.

In our colony, between 5 to 10% of cycling rats show two consecutive days of oestrous-metoeestrous smears (Long & Evans, Stage 3, 1922). No electrical stimulation is applied on the 3rd day. Persistence of leucocytic vaginal smears for 8 to 18 days after vaginal cornification indicates a state of pseudopregnancy. The 5 to 10% of animals experiencing a 3-day period of oestrous type smears, i.e. proestrus, oestrus and oestrus-metoestrous, were culled for a special study series related to the question of time of corpora luteal functional activity. It is discussed separately under timing studies.

GRADING OF RESPONSE

Grading was by visual examination of the intact uterus on a scale of from 0 to 4. The criterion, upon which the grading is based, is the proportion of the length of uterine horn which contains decidual tissue. Readings are made 4 days
(96±2 hr) after the induction procedure. Although the grading is estimated by eye (actual measurement of uterine length and deciduoma length is not done) after a little practice, good agreement is found among independent observers, and results in duplicate experiments are reproducible. The scoring is as follows; each horn is graded:

0 = no deciduoma;
1 = one-quarter or less of horn contains deciduoma;
2 = more than one-quarter but less than three-quarters of the horn contains deciduoma;
3 = three-quarters of the uterine length is decidua containing;
4 = entire uterine horn contains decidual tissue. Most of the reactions in this grade fill the horn almost uniformly and give a 'sausage' appearance.

The mean of the scores of the horns of an experimental series or group is referred to as the DIS (decidualization induction score) of that group. The cross-sectional dimension of the decidual growth does not enter in this assessment beyond the fact that the length of the uterine segment which is considered has a greater diameter than non-responsive uterine segment.

The scoring is to measure induction, not decidual growth or development, and is based on the observation (Shelesnyak, 1957) that the decidualization in the rat is focal in origin. Thus the essential criterion is a function of the number of nodes of origin. However, since all animals in this study were pseudopregnant and thus had fairly standard hormonal status, it could be expected that the decidual growth and development parallel the induction score. Four days after induction, the DIS and the weight of the uterus should show good correlation.

DOSAGE AND ROUTE OF ADMINISTRATION

(a) Intravascular injections were administered to ten females under ether anaesthesia. Five received intracardiac punctures, the others intravenous (right dorsal pedal vein) injections of 5 mg pyrathiazine hydrochloride in 0.1 ml of isotonic aqueous sodium-chloride solution. This dose was the maximum tolerated via the intravascular routes. All inducing treatments were made on the morning of the 4th day of the dioestrous phase (leucocyte smear) of pseudopregnancy (see Table 1.)

(b) Intraperitoneal injections of pyrathiazine hydrochloride (aqueous solution) were given to thirty-seven pseudopregnant females on the morning of the 4th day of leucocyte smears. Eight rats received 4 mg of pyrathiazine; eight received 8.9 mg; eight received 20 mg; and five were given 40 mg of pyrathiazine. The pyrathiazine was always delivered in 1 ml of vehicle. The 40-mg dose was lethal to all five rats when administered as a single injection. Eight other rats were given 40 mg in equally divided doses, 20 mg followed an hour later by the second 20-mg injection. Each injection was in 1 ml of vehicle.

(c) Subcutaneous injections were given to nine rats in their 4th day of dioestrous phase of pseudopregnancy. Forty-five mg of pyrathiazine hydrochloride in 1 ml aqueous solution was the only dose used in the evaluation of this route of administration.

(d) Oral route of administration was tested on forty animals. On the 4th day
Decidualization of the rat uterus

of dioestrous phase of pseudopregnancy, twelve rats received 20 mg of pyrathiazine in aqueous solution via stomach tube; eight received 40 mg and twenty rats received 100 mg of pyrathiazine.

TIME OF ADMINISTRATION

The search for optimal time for induction of decidualization (Kraicer & Shelesnyak, 1959) was carried out on two series: the first consisted of 286 pseudopregnant rats divided into seventeen different time groups from midnight of the 2nd day of dioestrous phase of pseudopregnancy (Day 2/2400 hr)

Table 1

RESPONSE OF UTERI OF PSEUDOPREGNANT RATS TO VARIOUS DOSES OF PYRATHIAZINE ADMINISTERED VIA VARIOUS ROUTES ON THE MORNING OF THE 4TH DAY OF DIOESTROUS PHASE OF PSEU¬

PREGNANCY

<table>
<thead>
<tr>
<th>Treatment Route of administration</th>
<th>Dose (mg/female)</th>
<th>No. females</th>
<th>Decidual reaction. Females</th>
<th>No. females died</th>
<th>Score DIS*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. with</td>
<td>No. without</td>
<td></td>
</tr>
<tr>
<td>Intracardiac</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intravenous</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Intrapерitoneal</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>0-2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>0-6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20 + 20†</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>2-0</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>45</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>1-9</td>
</tr>
<tr>
<td>Oral</td>
<td>20</td>
<td>12</td>
<td>2</td>
<td>10</td>
<td>0-1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>0-1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20</td>
<td>11</td>
<td>3</td>
<td>2-0</td>
</tr>
</tbody>
</table>

* DIS = Decidualization inducing score (see text).
† Two injections, 1 hr apart.

to morning of the 6th day (Day 6/0900 hr). Details are presented in Table 2. Pyrathiazine was administered at these various times in dose and by route found most effective, namely 20 mg (1 ml of 2% w/v aqueous solution) intraperitoneally. Pseudopregnancy was induced by standard electrical stimulation of the cervix. As noted above, in our colony 90 to 95% of the responding animals show a vaginal smear pattern of 2 days pro-oestrus, oestrus and metoestrus, while the remaining 5 to 10% have a 3-day period of pro-oestrus, oestrus and metoestrus. In this series of 286 females, all animals had 2-day oestrous configurations.

The second series in the time-relationship study was composed of thirty-eight females with 3-day oestrous configurations. These animals exhibit an apparent
shift of 24 hr in the onset of the luteal phase of pseudopregnancy and were used to check the temporal requirements for maximal induction of decidualization. The thirty-eight rats were divided into one group of twenty and one group of eighteen. The first group was given a single intraperitoneal injection of 20 mg pyrathiazine on the 3rd day of leucocyte smears. If one counted pseudopregnancy length beginning with pro-oestrus, then Day 3 in this series would match Day 4 of leucocyte smear in the ordinary 2-day oestrous configuration (cf. normal 2-day oestrous configuration: OE OE Leuc. 1, Leuc. 2, Leuc. 3, Leuc. 4, Leuc. 5; cf. normal 3-day oestrous configuration: OE OE OE Leuc. 1, Leuc. 2, Leuc. 3, Leuc. 4; OE means pro-oestrous or oestrous vaginal smear; Leuc means dioestrous vaginal smear).

The remaining eighteen females were given 20 mg pyrathiazine hydrochloride on the 4th day of leucocytic vaginal smear. All animals were killed 4 days (96 ± 2 hr) after injection of pyrathiazine. Uteri were graded and scored.

EVALUATION OF EFFECTIVENESS OF INDUCER

The systemic induction of decidualization by intraperitoneal pyrathiazine has been used in this laboratory as a standard procedure for the past 2 years (Kraicer & Shelesnyak, 1958; 1959). During this period, 199 rats have been used as controls to various experiments on the regulation of decidualization. These controls received intraperitoneal injections of 20 mg pyrathiazine hydrochloride on the 4th day of dioestrous phase of pseudopregnancy, between 0900 and 1100 hr. They were killed 4 days later. It is possible, therefore, to examine the pooled responses of these 199 animals to evaluate the effectiveness of this method of induction of decidualization.

RESULTS

ROUTES OF ADMINISTRATION AND DOSAGE

Pyrathiazine hydrochloride was effective in inducing decidualization in pseudopregnant rats, administered in any manner: per os, subcutaneous, intraperitoneal or intravascular (intravenous and intracardiac). Effective dose varied with route of administration. The highest degree of response per milligramme of injected pyrathiazine was achieved by intracardiac route. A 5-mg dose was effective in 4/5 animals. However, it is not possible to increase this response, since amounts greater than 5 mg pyrathiazine are lethal when administered intracardially.

Intraperitoneal route was the next in effectiveness: 20 mg induced decidualization in 8/8 animals in this series. Details of effectiveness with respect to dose and route are shown in Table 1.

TIME OF ADMINISTRATION OF INDUCER

In Table 2 and Text-fig. 1, the effect of varying the time of decidual induction is presented. Both the uterine weight and the decidual score reflect critical
Decidualization of the rat uterus

443

time dependence of uterine sensitivity to decidual induction. Both values make it clear that the optimal time for decidual induction is 1000 hr on the 4th day of the dioestrous (leucocyte) vaginal smear of pseudopregnancy.

Regression analysis of the mean uterine weights and the dis shows that the

**Table 2**

**RESPONSE OF UTERI OF PSEUDOPREGNANT RATS TO 20 MG OF PYRATHIAZINE ADMINISTERED INTRAPERITONEALLY AT VARIOUS TIMES DURING PSEUDOPREGNANCY**

<table>
<thead>
<tr>
<th>Time of injection</th>
<th>No. females</th>
<th>Females with deciduoma</th>
<th>Uterine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Day 2; 2400 hr</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3; 1200</td>
<td>12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3; 1800</td>
<td>19</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>3; 2000</td>
<td>20</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>3; 2200</td>
<td>17</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>3; 2400</td>
<td>17</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>4; 0200</td>
<td>20</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>4; 0400</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>4; 0600</td>
<td>19</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>4; 0800</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>4; 1000</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>4; 1200</td>
<td>20</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>4; 1400</td>
<td>20</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>4; 1600</td>
<td>20</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>4; 2400</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>5; 0900</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>6; 0900</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>

**Text-fig. 1.** The chronology of sensitivity of the uterus to induction of decidualization during pseudopregnancy. The abscissa shows the time of decidual induction with reference to the time of maximum sensitivity; the ordinate shows the response elicited as dis (see text). Maximum response: injection on Day 4/1000 hr.
two are correlated (coeff. of correlation = 0.965) as would be expected, and that an increase of one point in the DIS corresponds, under these conditions, to a 500-mg increase in the mean uterine weight.

The study of the thirty-eight females which had 3 days of oestrous type vaginal smears (in contrast to usual 2 days) revealed that administration of the pyrathiazine on Day 4 of leucocyte smears resulted in decidualization in 17/18 animals with a DIS of 3.0. Injection of the 20 mg of pyrathiazine on Day 3 resulted in a DIS of 0.3 and effected decidual reactions in only 4/20 cases. These findings show that the reactivity of the uterus is related to the period of luteal activity of the ovary.

EVALUATION OF EFFECTIVENESS OF THE INDUCER

A single intraperitoneal injection of 20 mg pyrathiazine hydrochloride in 1 ml aqueous solution administered between 0900 and 1000 hr on the 4th day of dioestrus of pseudopregnancy was effective in inducing decidualization in 163 of the 199 rats (82%). The frequency distribution with respect to DIS was:

DIS 4, 47%; 3, 14%; 2, 10%; 1, 17%; 0, 12%. The mean DIS was 2.65.

A score of 3 and above indicates massive (or extensive) decidualization. The combined frequencies of DIS 3 and 4 add up to 61%. Thus, this method induces massive decidualization in 61% of the treated animals (74% of the reacting animals).

DISCUSSION

Demonstration of the role of histamine as the mediator for induction of decidualization provided the theoretical basis for inducing the process without surgical or mechanical trauma (Shelesnyak, 1957). In testing the histamine role, Kraicer & Shelesnyak (1958) showed that delivery of histamine by systemic routes provoked decidualization in the responsive uterus. Because this trauma- and surgery-free method for inducing decidualization and deciduomata is of great potential value as a tool for the study of uterine physiology, we report in detail a refined, standardized technique for inducing massive deciduomata in the pseudopregnant rat by systemic administration of pyrathiazine hydrochloride (Pyrrolazote, Upjohn).

Thus, we have a method for delivering to the uterus a quantity of histamine adequate to induce the process of decidualization, and at the same time make available to the animal an histamine antagonist adequate to protect the animal from the lethal effects of the liberated histamine.

This method approximates the physiological means of induction of decidualization more closely than other methods by being free of trauma and requiring no surgery. The complications of tissue damage, debris and tissue repair are avoided; the altered responses of damaged uterine tissue to hormone action are also avoided by use of this systemic method. Moreover, time-relationship studies, inducing deciduomata in early pregnancy, reveal a synchrony for induction reactions between the natural decidualization of pregnancy and induced decidualization (Shelesnyak & Kraicer, 1960). The time-relationship
for maximal effectiveness fits well with the postulated oestrogen-surge concept of decidualization and nidation (Shelesnyak, 1960).

Although intraperitoneal administration is the most effective means for inducing massive decidualization, the possibility of getting reactions by other routes has both theoretical and practical value. Intraperitoneal injection brings the pyrathiazine into direct contact with the uterine serosa; this pyrathiazine could interfere with in vitro measurements. In such instances, per os administration could be used. Since all routes tested were effective but at different levels, the choice of a route other than intraperitoneal requires adjustment of dose.

It is pertinent to emphasize that this study is of a method to induce decidualization. There is basis for speculation that the process of decidualization, namely, the transformation of endometrial stroma cells into decidual cells and deciduomata, is a triggered reaction requiring a stimulus acting on a uterus capable of reacting (Shelesnyak, 1957, 1960). The inducing stimulus governs the number of foci stimulated and hence the extent of the decidual reaction (Shelesnyak, 1957); but the growth, development and maintenance of the mass of decidual tissue, the deciduoma, requires additional elements for support. Thus, due to the fact that all the animals of the large timing study were in approximately the same physiological status, i.e. pseudopregnancy, the correlation of ‘extent of induction’, the ds with ‘development of the deciduoma’ in the uterine weight, was extremely high (r = 0.965). By varying the factors which control development and maintenance of the deciduoma, chiefly hormonal but not uniquely so, and using the pyrathiazine for induction of the decidual process, it should be possible to separate the essential components involved in the uterine transformations associated with ovum-implantation. The method is also useful to study uterine response to progestagens — both natural and synthetic — in relation to decidualization.

ACKNOWLEDGMENTS

The authors wish to thank the Population Council, Inc., New York, whose grant-in-aid has supported this study. We are indebted to Mr Joseph Shalom for his loyal, devoted and conscientious help. We are grateful to Dr H. Upjohn of the Upjohn Co, Kalamazoo, for the generous supply of pyrathiazine hydrochloride (Pyrrolazote, Upjohn).

REFERENCES


Shelesnyak, M. C. (1952) Inhibition of decidual cell formation in the pseudopregnant rat by histamine antagonists. Amer. J. Physiol. 170, 522.


