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ATTEMPTED DECIDUALIZATION IN THE HAMSTER AND RAT WITH PYRATHIAZINE

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Summary. Ninety-one hamsters were injected with pyrathiazine intraperitoneally at varying times during the first 5 days of pseudopregnancy with doses of 1, 2, 5, 10, 15 or 20 mg. They were autopsied on the 7th day of pseudopregnancy. Six animals showed limited decidual reactions; two of these had received 10 mg and the remaining four, 15 mg. All positive results were from injections on the 4th day of pseudopregnancy, i.e. in the period corresponding to pre-implantation in a pregnant hamster. These six positive results were equivalent to 14·6% of the total of forty-one animals receiving equivalent injections on the same day. Of fifty-one control animals traumatized for decidualization by threads, injection of histamine, air, saline solution, or insertion of a needle, forty-three or 84·3% stimulated on the 4th day of pseudopregnancy showed decidualization.

Of the nineteen rats receiving 20 mg or more of pyrathiazine intraperitoneally at 10 a.m. on the 4th day of pseudopregnancy, one showed decidualization.

The above findings suggest that intraperitoneal injections of pyrathiazine are not an effective means of decidualization of the hamster uterus. The findings in the rat do not substantiate those reported by Shelesnyak & Kraicer (1961); strain differences are suggested as an explanation.

INTRODUCTION

In a series of papers, Shelesnyak & Kraicer report decidualization of the uterus of the pseudopregnant rat by intraperitoneal injections of pyrathiazine. In their most recent account (1961), they present a standardization and evaluation of the method, reporting 100% success. Any such technique eliminating the necessity for uterine trauma and presenting such reliability would be of extreme value as a tool in analysis of factors involved in decidualization. The present paper presents observations obtained in a study to determine whether similar results could be obtained in the hamster, *Mesocricetus auratus* Waterhouse; coupled with attempts to repeat in a small series of rats the findings of Shelesnyak & Kraicer.

MATERIALS AND METHODS

Developmental age of the ovum is the hypothetical age of the fertilized ovum. When mating occurs prior to ovulation, developmental age is calculated from the time of ovulation or the ovulation age; when mating occurs after ovulation, developmental age is calculated from the time of observed copulation (Ward, 1948). Although all matings in this present paper were with vasectomized males and the ova therefore non-fertile, the hypothetical developmental age is still recorded for conditions in these pseudopregnant females in order to facilitate comparison with events in the pregnant animal. In this paper all matings occurred *prior* to ovulation, so developmental age was calculated from the period when ovulation occurs.

HAMSTERS

The hamsters used were from two colonies maintained in air-conditioned rooms. In the colony kept under normal lighting conditions (N), mating occurs in the late afternoon and night prior to the postoestrous discharge, which marks Day 2 of the hamster oestrous cycle (Deanesly, 1938). The peak of ovulatory activity occurs approximately at 1 a.m. of the morning in which the postoestrous discharge is present. Developmental age of the ova and, in this paper, of pseudopregnancy, is dated from that hour. Implantation in this colony normally begins at approximately 4 days and 2 to 6 hr developmental age.

The second colony (LD) is maintained under reverse lighting, with light from 10 p.m. to 10 a.m. In this colony matings occur from 9 a.m. on, ovulation occurring by 5 p.m. of the same day, and developmental age for pseudopregnancy and pregnancy is reckoned from that hour. Again, implantation (in this colony) occurs at approximately 4 days and 2 to 6 hr developmental age.

Both colonies display definite vaginal phenomena typical of the oestrous cycle, pregnancy and pseudopregnancy. Pseudopregnancy normally lasts 9 days, and is characterized by weight changes and vaginal phenomena similar to pregnancy, but differing from pregnancy by a marked loss of weight on the 7th to 8th day just prior to recurrence of oestrus (Orsini, 1961).

For this study adult virgin females that had displayed at least two normal oestrous cycles and were over 80 g in weight were used. All animals were made pseudopregnant by observed matings with vasectomized males. Pseudopregnancy was confirmed by daily observations of the vaginal phenomena (failure of recurrence of oestrus, etc.), and by reaction to the male, i.e. failure to display lordosis and by definite aggressiveness towards the male.

Varying doses of pyrathiazine hydrochloride (i.e. 10-[2-(1-Pyrroliody) ethyl] phenathiazine hydrochloride) were administered at varying times to the pseudopregnant females; details of dose level at time of administration are given in the tables summarizing the observations. Since, to the best of the investigator's knowledge, the length of activity of any single suspension of this drug is unknown, eleven separate dilutions of the drug were used; most animals were injected on the day when the suspension was prepared, or at the most within 3 days after preparation with the suspension refrigerated

until used. Varying concentrations, 1, 2, 5, 10, 15 and 20 mg, were used to offset possible species differencies between the hamster and rat.

Animals were observed and weighed daily. All were autopsied during the 7th day of pseudopregnancy at 6 days and 12 to 17 hr developmental age. At this time decidual cell response (DCR), if present, is usually obvious.

At autopsy the reproductive tracts were not weighed, but were removed with ovaries and mesenteries intact; they were examined for gross swellings and the ovaries were checked for corpora lutea. They were then fixed in AFA, (30 ml 95% alcohol, 10 ml commercial formalin, 10 ml glacial acetic acid and 50 ml water), bleached, dehydrated through ascending alcohols and cleared through benzol to benzyl benzoate. Here they were re-examined in oblique light for white opacities which are associated with normal nidation and DCR as obtained in this laboratory by trauma, such as thread insertion or injection of histamine or air. Large deciduomata may appear as yellowish-white opacities. Such opacities on sectioning do show decidualization (Orsini, 1962a, b; Orsini & Mossman, 1961; Prasad, Orsini & Meyer, 1960; Orsini & Meyer, 1962). This technique has the advantage of 'pin-pointing' decidual areas which could otherwise only be observed by serial sections, and differentiates between inflammatory swelling and DCR which can easily be confused in macroscopic fresh or fixed specimens. It is currently being used for studies of the life history and development of the DCR, the relative effectiveness of various traumatic stimuli to the uterus at varying periods in pseudopregnancy, and the optimal times in pseudopregnancy for induction of deciduomata. These studies serve also as a control for the present paper.

RATS

Three groups of rats were used. Group 1, comprised of eight Sprague-Dawley rats purchased in pro-oestrus (the condition of the rat being guaranteed by the vendor, whose technician has had much more experience with rats than this investigator). The cervices of these rats were immediately stimulated with a glass rod. Four days later, at 10 a.m., these animals were injected intraperitoneally. The first four rats received 56, 44, 52 and 44 mg of pyrathiazine, respectively, or 20 mg/100 g body weight. These four rats went into shock within minutes, so the dose was halved for the second four of this group, which received 24, 25, 24 and 22 mg of pyrathiazine, respectively. (These eight rats were injected prior to issue of Shelesnyak & Kraicer's 1961 paper on standardization).

The second group of eight rats were from the colony of Dr Arthur Chapman, of the Department of Genetics. This colony is from Sprague-Dawley stock but has been selectively inbred for the past 20 years. The cycles of these rats were checked daily and they were stimulated cervically by a glass rod when in pro-oestrus and oestrus, and injected with 20 mg in 1 ml of fluid at 10 a.m. of the 4th day of dioestrus. Vaginal smears were taken daily throughout the experiment.

The third group comprised six rats purchased in pro-oestrus like those of the first group, but they were treated like those of the second group. Autopsy procedure for all the rats was like that for the hamsters, but all rats were killed

96 hr after the injection of pyrathiazine. In every instance, the presence of large corpora lutea confirmed the state of pseudopregnancy.

OBSERVATIONS

The hamster observations are summarized in Table 1. No differences in effect were noted between the eleven separate dilutions used or between the normal versus the LD colony, and all the data are pooled in the table.

Table 2 presents in tabular form a detailed description of the six animals which revealed DCR on autopsy.

TABLE 1

DECIDUALIZATION INDUCED BY INTRAPERITONEAL INJECTIONS OF PYRATHIAZINE IN THE HAMSTER

Day injected and developmental age	*No. animals*	*Dose of pyrathiazine*	*No. with* DCR	*No. without* DCR
Day 1				
14 hr	1	20 mg		1
Day 2				
1 day and 17 hr	2	1 mg		2
1 day and 9 to 17 hr	3	2 mg		3
1 day and 23 hr	3	20 mg		3
Day 3				
2 days and 17 hr	2	1 mg		2
2 days and 17 hr	1	2 mg		1
2 days and 11 hr	1	5 mg		1
2 days and 11 to 19 hr	4	10 mg		4
2 days and 2 to 23 hr	4	20 mg		4
Day 4				
3 days and 17 hr	2	1 mg		2
3 days and 9 to 17 hr	2	2 mg		2
3 days and 11 to 20 hr	10	5 mg		10
3 days and 11 to 18 hr	15	10 mg	2	13
3 days and 15 to 23 hr	26	15 mg in split dose	4	22
3 days and 18 to 23 hr	5	20 mg single dose		5
3 days and 18 to 23 hr	2	20 mg in split dose		2
Day 5				
4 days and 8 hr	1	2 mg		1
4 days and 7 to 10 hr	6	15 mg in split dose		6
Total	91		6	85

The trauma of the injection was reflected in the animal weights, which in the hamsters dropped immediately after injection of 5 mg or more of pyrathiazine; diarrhoea sometimes accompanied this weight loss, which was usually recovered prior to autopsy. A few showed localized necrosis at the injection site. This was found to be avoidable by care in sterilizing the injection region. Rats showed only the drop in weight.

Among the three groups of rats, a total of twenty-two, nineteen survived injection, but only one from the first group which had received an actual dose of

25 mg showed by gross examination one DCR in each horn on autopsy (Table 3). Clearing revealed that this specimen had two in the left and three in the right

TABLE 2

DETAILED DESCRIPTIVE TABULATION OF SIX HAMSTERS SHOWING DCR

Individual animal	*Colony source*	*Weight of animal (g)*	*Time of injection and dose of of pyrathiazine*	DCR *right horn*	DCR *left horn*
E 10	LD	100	10 mg in 0·3 ml at 3 days and 17½ hr was also given prolactin daily subsequent to pyrathiazine injection	0	1
K 5	LD	97	10 mg in 0·5 ml at 3 days and 17 hr	0	1
F 3	LD	89	15 mg in split dose of 0·175 ml per dose at 3 days and 17½ hr and 3 days and 18½ hr	0	2
G 4	N	100	15 mg in split dose of 0·175 ml at 3 days and 15 hr and 3 days and 16 hr	2	0
H 4	N	103	15 mg in split dose of 0·175 ml at 3 days and 8½ hr and 3 days and 9½ hr	1	1
H 6	N	100	15 mg in split dose of 0·175 ml at 3 days and 8½ hr and 3 days and 9½ hr	1	3

uterine horn. At autopsy one from the second group showed a dilated left uterine horn, which, after clearing, was observed to be filled with a white

TABLE 3

RESULTS OF INTRAPERITONEAL INJECTION OF PYRATHIAZINE INTO PSEUDO-PREGNANT RATS

Group	*Body weight (g)*	*Strain*	*Dose*	*Died*	DCR *positive*	DCR *negative*
Group 1						
No. 1	280		56 mg			+
No. 2	220		44 mg	+		
No. 3	270		52 mg	+		
No. 4	225	All	44 mg	+		
No. 5	240	Sprague-Dawley	24 mg		+	
No. 6	255		25 mg			+
No. 7	260		25 mg			+
No. 8	225		22 mg			+
Group 2 (eight rats)	160 to 202	Chapman	20 mg in 1 ml			+ (all eight)
Group 3 (six rats)	214 to 250	Sprague-Dawley	20 mg in 1 ml			+ (all six)

Animals autopsied at 10 a.m. of 4th day of dioestrus, 96 hr after injection.

substance within the uterine cavity. This horn was sectioned but no decidual tissue was present.

Three other rats from the second group which were completely negative to the eye on autopsy, on extremely careful microscopic examination after clearing

showed tiny, minute semi-opaque areas within the uterus and apparently bordering on the uterine cavity. They were by no means the distinct white opacities characterizing well-developed decidual tissue, but they resemble the very early decidua seen less than 24 hr after injection of air into the uterus. They were thought to be either early predecidual areas, degenerating or inflammation areas, or cysts. The largest of these semi-opaque regions was photographed, the region was then cut out and sectioned serially. This semi-opacity was found to be associated with an antimesometrial diverticulum of the uterine cavity filled with necrotic cells resembling material found in the vaginal smear. The uterine epithelium and the surrounding connective tissue was filled with leucocytes, some mitotic cells and enlarged connective tissue cells which are similar to predecidual cells and to those found in early inflammatory reactions. Since these do not correspond to typical deciduomata and since cellular changes in early inflammation do resemble early decidual changes, it is felt that these are probably areas of early inflammatory change.

It is also interesting to note that in no instance among the seven animals (six hamsters and one rat) which showed positive reactions was there any extensive decidualization comparable to that reported by Shelesnyak & Kraicer (1961) for the rats injected at 10 a.m. on Day 4; in all seven positive instances, the decidualization was focal in origin, but limited to something comparable to a 'decidual induction score' of less than one in the horn most involved, i.e. less than one-quarter of the uterine horn contained decidua.

DISCUSSION

It is manifestly unfair to say that only six, or 6·59% of the injected hamsters showed decidualization, since dose level and maximal period of sensitivity must also be considered. The maximal period of sensitivity for the hamster is not yet established. If, however, one considers that positive responses were elicited at the 10- and 15-mg response, and *only* on the 4th day of pseudopregnancy, and then compares the number of positive reactions with the possible number of animals injected under these conditions, one still has a figure of only 14·6% or six positive results of forty-one animals injected. This ignores the 20-mg-dose level, which might be thought to be equally effective.

It is perhaps best to compare the percentage of animals showing positive DCR induced by intraperitoneal injections of pyrathiazine on the 4th day of pseudopregnancy, with the percentage of animals showing positive DCR induced by uterine trauma at this same period in pseudopregnancy. Table 4 shows the number of animals stimulated on this day, the type of trauma used and positive or negative results. This is a summary of material currently being used for an investigation of the effectiveness of various types of trauma. This summary is presented here as a control of uterine sensitivity alone. Table 4 presents data on the 4th day of pseudopregnancy only, and any tract showing any deciduomata is counted as positive; it does not indicate percentage of horn involved or type of swelling. It is sufficient at this time to say that the degree of DCR was much greater than with the pyrathiazine, the swellings were usually focal and

even showed fusion, similar to the apposed conceptual swellings at the comparable stage of development. Moreover, it should be stated that the number showing decidualization from trauma at this time, 3 days and 6 to 23 hr developmental age (the 4th day of pseudopregnancy) was far greater than among animals stimulated on the day prior to or subsequent to this period. By these varying techniques 84·3% showed decidualization, definitely indicating that the hamster uterus is sensitive to DCR induction on the 4th day of pseudopregnancy. It is of interest that this period corresponds to the immediate pre-implantation period in the pregnant hamster.

Most of the work done on deciduomata reaction in the hamster has been performed by Dr George Kent and co-workers (Kent & Lytle, 1960; Turnball & Kent, 1962), who have inserted threads as a stimulation 48 hr after sterile mating. This suggests sensitivity of the hamster uterus at an earlier time, but this traumatic technique probably provides a *continuing* state of stimulation.

TABLE 4

RESULTS OF TRAUMA TO INDUCE DECIDUALIZATION ON THE 4TH DAY OF PSEUDOPREGNANCY IN THE HAMSTER

Type of trauma	*No. animals traumatized*	*No. with* DCR	*No. without* DCR
Histamine in lumen of one horn, saline solution in other	15	13	2
Histamine in lumen of one horn, air in other	13	10	3
Injection of air into uterine lumen	16	15	1
Injection of saline solution into uterine lumen	3	1	2
Insertion of threads into uterine horn	3	3	
Insertion of needle into uterine lumen	1	1	
Total	51	43	8

A chemical stimulation, such as intraperitoneal pyrathiazine, can be compared with traumatic stimuli as a group. Such a chemical stimulus would probably be of shorter duration than any stimulation involving actual trauma, which in itself might be of prolonged duration.

Differences, too, must be recognized between the traumatic inducers generally used. A thread inserted through the uterus provides a continuing state of stimulation, which would probably be of longer duration than injection of air, saline solution or histamine.

Only one of the nineteen rats surviving injection with 20 mg or more of pyrathiazine at 10 a.m. on the 4th day of pseudopregnancy and autopsied 96 hr later showed decidualization, and this rat had a 'decidual induction score' of less than one. One must recall that Shelesnyak & Kraicer (1961) obtained 100% success with injections at 4, 6, 8 and 10 hr on the 4th day, and averages ranging from 40 to 95% in groups injected at other times on that day. They used young adult females weighing 180 to 210 g. Our rats were young

females, previously virgin, but the weight span was greater (*see* Table 3). It is doubtful if this is a significant difference; since the overlapping group was completely negative. Strain differences may account for this discrepancy. The Shelesnyak–Kraicer colony is of Wistar derivation, but bred at the Weizmann Institute since 1951. Stain differences are well known to play a part in many physiological phenomena, and reaction to this antihistamine may be only one facet of these differences. My choice of rats from two such variable groups was made in an attempt to offset possible strain differences. Environmental differences may also play a part.

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REFERENCES

DEANESLY, R. (1938) The reproductive cycle of the golden hamster (*Cricetus auratus*). *Proc. zool. Soc. Lond.* A, **108,** 31.

DUAGAARD, A. & KENT, G. C. (1962) Failure to produce deciduomata with progesterone in hamsters. *A. S. B. Bull.* **9,** 38.

KENT, G. C. & LYTLE, S. L. (1960) Decidual cell responses following exogenous prolactin in uterine-traumatized hamsters. *Anat. Rec.* **138,** 360.

ORSINI, M. W. (1961) The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation and the anestrous hamster, *Mesocrioetus auratus* (Waterhouse). *Proc. Anim. Care Panel,* **11,** 193.

ORSINI, M. W. (1962a) Technique of preparation, study and photography of benzyl-benzoate cleared material for embryological studies. *J. Reprod. Fertil.* **3,** 283.

ORSINI, M. W. (1962b) A study of ovo-implantation in the hamster, rat, mouse, guinea-pig and rabbit in cleared uterine tracts. *J. Reprod. Fertil.* **3,** 288.

ORSINI, M. W. & MEYER, R. K. (1962) Effect of varying doses of progesterone on implantations in the ovariectomized hamster. *Proc. Soc. exp. Biol., N.Y.* **110,** 713.

ORSINI, M. W. & MOSSMAN, H. W. (1961) Gross visualization of early implantation sites in the hamster, *Mesocricetus auratus* (Waterhouse). *Anat. Rec.* **139,** 331.

PRASAD, M. R. N., ORSINI, M. W. & MEYER, R. K. (1960) Nidation in progesterone-treated, estrogen-deficient hamsters, *Mesocricetus auratus* (Waterhouse). *Proc. Soc. exp. Biol., N.Y.* **104,** 48.

SHELESNYAK, M. C. & KRAICER, P. F. (1961) A physiological method for inducing experimental decidualization of the rat uterus: standardization and evaluation. *J. Reprod. Fertil.* **2,** 438.

TURNBALL, J. G. & KENT, G. C. (1962) Chronological study of deciduomata in hamsters. *A. S. B. Bull.* **9,** 38.

WARD, M. C. (1948) The early development and implantation of the golden hamster, *Cricetus auratus,* and the associated endometrial changes. *Amer. J. Anat.* **82,** 231.