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Effect of Steroid Hormones on the Decidual Reaction in Ovariectomized Rat Uterus

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Summary

The present experiment was undertaken to obtain information on the relationship between the time of a single dose of estrone treatment in experimentally-induced progestational stage and the uterine sensitivity to deciduoma formation in the rat uterus.

1. Faradic stimulation of cervix uteri at proestrus and estrus did not affect the deciduoma formation when the ratio of the right horn to the left horn of uterine weight was used as an index.

2. When traumatization of the uterus was performed at various stages of experimentally-induced pseudopregnancy in ovariectomized rats which had received 4 mg of progesterone daily, the deciduoma formation was induced on the 4, 5 and 6th day of pseudopregnancy.

3. When a single dose of estrone was administered on the 5th day of experimentally-induced pseudopregnancy, the sensitivity for deciduoma formation occurred 6 to 24 hours after estrone administration. On the other hand, when a single dose of estrone was given on the 8th day of experimentally-induced pseudopregnancy, the sensitivity of uterus for deciduoma formation occurred 12 to 24 hours after the estrone injection. However, the responsibility for deciduoma formation had almost disappeared by 48 hours after estrone injection in both cases.

4. When a single dose of estrone was administered 12 hours before traumatization of the uterus on the 5th day of experimentally-induced pseudopregnancy, the deciduoma formation induced was the same as those observed in normal pseudopregnancy.

It is well known that the synergic action of estrogen and progesterone is necessary for the decidual cell reaction in the uterine endometrium and that the implantation of blastocyst is induced by the synergic action of both these hormones (1-3). Shelesnyak et al (4) have reported that the animals ovariectomized at noon on day 4 of pseudopregnancy failed to respond to the decidual inducing stimulus and that when the ovariectomy was performed 12 hours later the uterus responded normally. They concluded that there is a surge or discharge of estrogen from the ovary, which is a prerequisite for uterine decidualization and this surge of

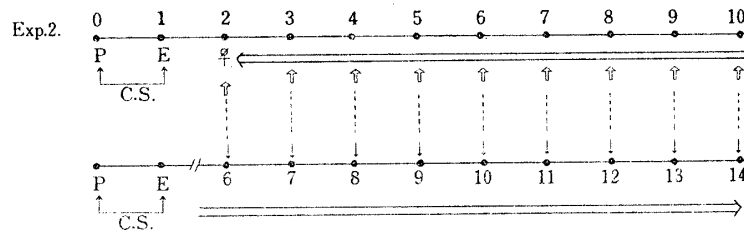
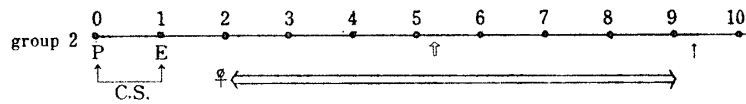
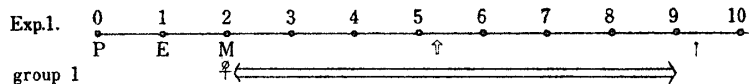
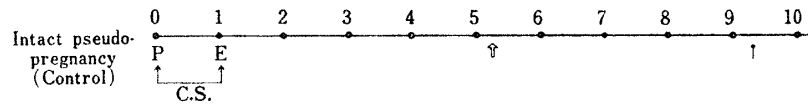
estrogen takes place during the latter half of day 4 of pseudopregnancy and probably also during pregnancy. However, little is known about the pattern of estrogen secretion during the progestational stage. The investigations reported here were undertaken to obtain information on the relationship between the time of a single dose of estrone in experimentally-induced progestational stage and the uterine sensitivity for deciduoma formation in the uterus. Experiments were designed to answer the following four questions;

1. Whether the faradic stimulation of cervix uteri at proestrus and estrus does influence decidualization;
2. Whether the deciduoma is induced by traumatization at various stages of pseudopregnancy in ovariectomized rats which have received only progesterone;
3. When the sensitivity for decidual stimulus reaches maximum after a single dose of estrone on the 5th and 8th days of experimentally-induced pseudopregnancy; and
4. Whether a single administration of estrone in various stages of experimentally-induced pseudopregnancy allows the formation of decidual reaction like those observed in normal pseudopregnancy.

Materials and Methods

Mature virgin female rats of Wistar strain, weighing 227 ± 27 g, were maintained under constant illumination (light on at 7:00 A.M., off at 7:30 P.M.) and temperature ($22 \pm 1^\circ\text{C}$). In the first experiment, intact animals exhibiting regular 4-day cycles served as control. The schedule of experiment is shown in Fig. 1. They were made pseudopregnant by faradic stimulation of the cervix uteri at proestrus and estrus by the method of Sugawara and Takeuchi (5). The day of estrus was noted as day 1 of pseudopregnancy. At 10:00 A.M. on the 5th day of pseudopregnancy, a midventral laparotomy was performed under ether anaesthesia, and the right uterine horn was traumatized by the method of De Feo (6), but the left horn was not traumatized, but used as a control. A blade tipped needle was inserted into the uterine lumen at the bifurcation, gently pressed up to the utero-tubal junction, rotated so that the cutting edge was directed and pressed anti-mesometrially, and then withdrawn. The animals were killed on 4 days post-trauma; the uteri was removed and bifurcated; the mesentery was trimmed off, and each cornu was weighed on a torsion balance. The animals spayed at metestrus or 2nd day of pseudopregnancy were divided into five experiments as follows. In experiment 1, pseudopregnancy was induced by dorsal injection of progesterone. The uterus was traumatized at 10:00 A.M. on the 5th day of pseudopregnancy, and removed for weighing the deciduoma on day 4 of post-trauma. In experiment 2, the animals were made pseudopregnant by faradic stimulation of the cervix uteri at proestrus and estrus, and ovariectomized on day

2 of pseudopregnancy followed by administration of 4 mg progesterone daily to autopsy. Laparotomy was performed under anaesthesia and the right horn was traumatized in various days from the 2nd to the 10th day of pseudopregnancy. These animals were killed on day 4 of post-trauma. In experiment 3, in order to investigate the relationship between the time of estrone injection and the time of traumatization on decidual cell reaction, the animals were injected with a single dose of 1 μ g estrone on the 5th and 8th day of pseudopregnancy. The animals were traumatized at 0, 6, 12, 24 and 48 hours after estrone injection on the 5th day and the 8th day of pseudopregnancy, respectively. In experiment 4, a single dose of estrone was administered to the animals 12 hours before traumatization at 10:00 A.M. on various days i.e. the 3, 5, 7 and 10th day of pseudopregnancy, respectively and autopsy was performed on day 4 of post-trauma. In experiment 5, in order to observe the optimal decidual responsibility in detail, a single dose of 1 μ g estrone was administered at 6 or 12 hour intervals 12 hours before traumatization on 3-5th day of experimentally-induced pseudopregnancy, respectively. Then the animals were killed 4 days after trauma. Hormones were injected subcutaneously in 0.2 ml sesame oil throughout the experiment. The right horn/left horn ratio of uterine weight is the index for decidual reaction.



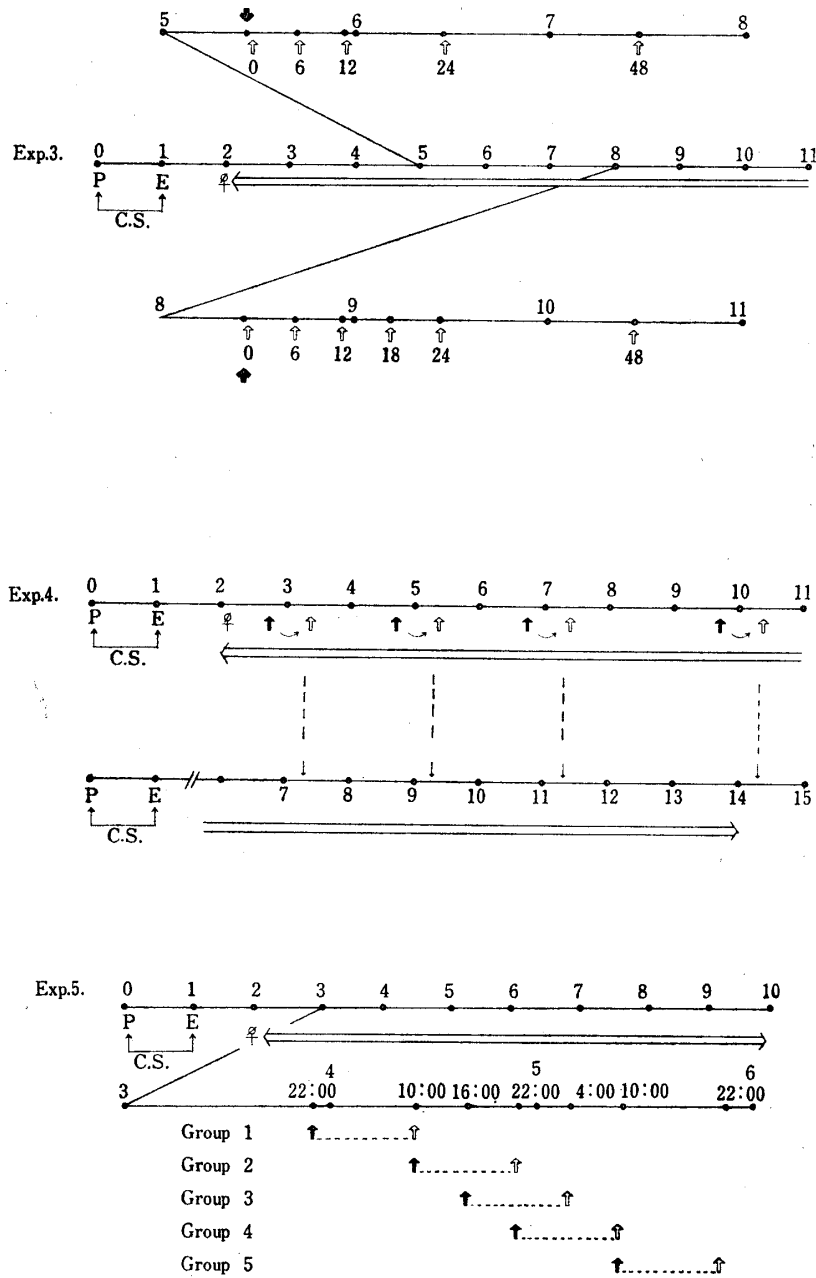


FIG. 1. Design of experiment

- ↔; Progesterone (4 mg/day) treatment
- C.S.; Cervical stimulation
- ♀; Ovariectomy
- ↑; Traumatization
- ↑; 1 μg estrone injection
- P; Proestrus
- E; Estrus
- M; Metestrus
- ↑; Autopsy

The indication of the date of pregnancy and pseudopregnancy is different among many investigators, so that the day of estrus in cited literatures is arranged for the first day of pregnancy and pseudopregnancy in the present report.

Results

The results are summarized in Table 1-5 and Fig. 2-5. As shown in Table 1, the effect of the faradic stimulation of cervix uteri at proestrus and estrus on the decidual formation is shown. There was a difference between the no-cervical stimulation group (Group 1) and the cervical stimulation group (Group 2) in weight of traumatized uterus ($p < 0.05$). However, the ratio of right horn to left horn between the two groups was not significant ($p > 0.05$). The results in Table 2 and Fig. 2 show the deciduoma formation in ovariectomized rat which have received only progesterone treatment following traumatization on various days of

TABLE 1. *The effect of the faradic stimulation of cervix uteri at proestrus and estrus on the deciduoma formation*

Group	1	2
No. of Rats	7	10
Wt. of Right horn (mg)	340±123*	495±202
Wt. of Left horn (mg)	121±32	155±50
Ratio of Right horn to Left horn	3.03±1.40	3.19±0.69

* Standard deviation

TABLE 2. *The effects of traumatization at various days of experimentally-induced pseudo-*

Day of pseudopregnancy	2	3	4
No. of Rats	4	4	4
Wt. of Right horn (mg)	158±21	176±35	666±230
Wt. of Left horn (mg)	129±35	143±27	169±24
Ratio of Right horn to Left horn	1.13±0.11	1.24±0.12	3.86±0.90

TABLE 3. *The uterine sensitivity for decidual stimulus at various times after estrone injection on the 5th and 8th day of experimentally-induced pseudopregnancy.*

5th day of pseudopregnancy						
Traumatize period after estrone injection (Hrs).	0	6	12	24	48	
No. of Rats	7	4	3	3	3	
Wt. of Right horn (mg)	495±202	785±31	712±182	604±137	195±18	
Wt. of Left horn (mg)	155±50	187±13	169±4	177±39	151±9	
Ratio of Right horn to Left horn	3.19±0.69	4.22±1.30	4.20±0.97	3.72±1.40	1.30±0.15	
8th day of pseudopregnancy						
Traumatize period after estrone injection (Hrs).	0	6	12	18	24	48
No. of Rats	8	6	7	4	6	6
Wt. of Right horn (mg)	178±52	292±95	638±157	370±120	477±180	205±67
Wt. of Left horn (mg)	125±20	127±25	154±29	135±10	137±32	148±30
Ratio of Right horn to Left horn	1.40±0.18	2.20±1.16	4.18±1.35	2.76±0.62	3.55±1.27	1.35±0.22

pseudopregnancy. The deciduoma formation was not observed on the 2nd and 3rd day of pseudopregnancy in the traumatized uterus (right horn), but was observed on the 4, 5 and 6th day. There are not significant difference between the subgroups of the 4, 5 and 6th days. The significant reduction of response for the decidual stimulus was observed from the 7th day of pseudopregnancy.

In experiment 3, the uterine sensitivity for decidual stimulus was progressively evident at each time of traumatization after a single dose of estrone administration on the 5th and 8th day of pseudopregnancy. As shown in Table 3 and Fig. 3, the sensitivity for deciduoma formation increased at 6, 12 and 24 hours in comparison with that of 0 hour ($p < 0.05$), but decreased significantly at 48 hours ($p < 0.01$). On the other hand, when a single dose of estrone was administered on the 8th day of pseudopregnancy, the sensitivity for the deciduoma formation increased at 6 hours as compared to that of 0 hour ($p < 0.01$) and reached the maximum at 12 hours ($p < 0.01$). Although the reduction of sensitivity was observed at 18 hours ($p < 0.01$), there was no difference between groups of 12 hours and 24 hours after estrone administration. However, the response for deciduoma formation was not seen at 48 hours after estrone injection as in the case of estrone administration on the 5th day of pseudopregnancy.

pregnancy on deciduoma formation in ovariectomized rats treated only with progesterone.

5	6	7	8	9	10
10	5	6	8	3	9
495±202	478±72	211±34	178±29	147±24	153±40
155±50	159±29	155±36	129±23	112±34	119±23
3.19±0.69	3.21±0.94	1.42±0.35	1.40±0.18	1.37±0.17	1.30±0.31

TABLE 4. *The effect of a single dose of estrone administration 12 hours before traumatization on 3, 5, 7 and 10th day of experimentally-induced pseudopregnancy on deciduoma-formation in the rat uterus.*

	Day of traumatization				Normal pseudo-pregnancy
	3	5	7	10	5
No. of Rats	4	4	3	4	5
Wt. of Right horn (mg)	190±61	779±204	520±135	432±41	1071±214
Wt. of Left horn (mg)	161±51	133±47	156±29	126±26	193±53
Ratio of Right horn to Left horn	1.18±0.03	6.28±1.36	3.58±1.37	3.55±0.53	5.81±1.24

The results obtained in experiment 4 are shown in Table 4 and Fig. 4. When a single dose of estrone was administered at 12 hours before traumatization on the 3rd day of pseudopregnancy, the deciduoma formation was not induced, similar to that on the 3rd day of pseudopregnancy in experiment 2. On 5, 7 and

TABLE 5. The effect of a single dose of estrone administration 12 hours before traumatization at various stages on 3rd to 5th day of experimentally-induced pseudopregnancy

Group	1	2	3	4	5	Intact Control
No. of rat	5	5	5	4	3	5
Wt. of right horn (mg)	510±307*	445±105**	514±104**	779±204	712±182*	1071±214
Wt. of left horn (mg)	136±40	129±30	113±47*	133±47	169±4	193±53
Ratio of right horn to left horn	3.58±1.17*	3.63±1.09*	4.55±0.40	6.28±1.36	4.20±0.97	5.81±1.24

* $0.01 < P < 0.05$ Compared with intact control. ** $P < 0.01$ Compared with intact control.

10th day of pseudopregnancy, however, the deciduoma formation was induced greatly in comparison with those in experiment 2. ($p < 0.01$). Especially, when a single dose of estrone was administered 12 hours before traumatization on the 5th day, the deciduoma formation was induced the same as those observed in normal pseudopregnancy. The sensitivity for deciduoma formation decreased on 7th and 10th day in comparison with 5th day ($p < 0.05$).

The result in experiment 5 is shown in Table 5 and Fig. 5. When a single dose of $1 \mu\text{g}$ estrone was administered at 10:00 P.M. on the 4th day and traumatization

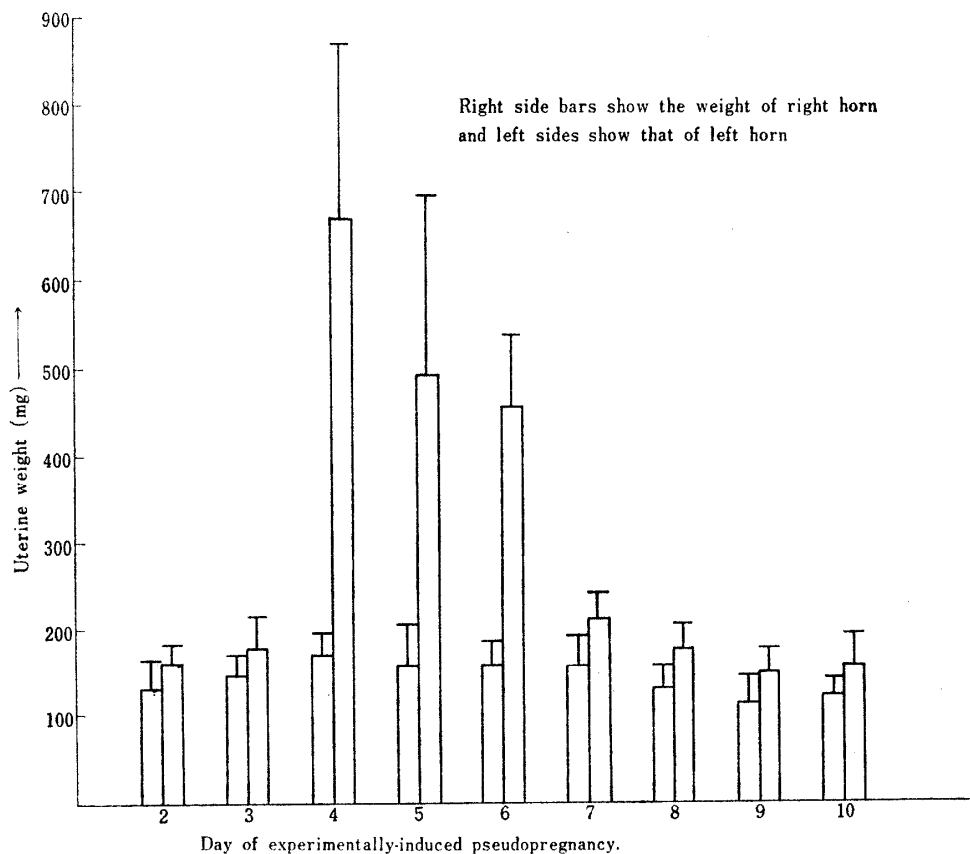


FIG. 2. Deciduoma formation by traumatization on each day of experimentally-induced pseudopregnancy.

was made at 10:00 A.M. on the 5th day of experimentally-induced pseudopregnancy (Group 4), the optimal deciduoma formation was induced and they had no statistical significance in comparison with the intact control (Normal pseudopregnancy) as to the weight of right horn. Moreover, there is no statistical difference between the intact control and groups 3 and 4 as to the ratio of the right horn to the left horn.

Discussion

The faradic stimulation of cervix uteri at proestrus and estrus resulted in an increase of deciduoma weight in comparison with those having no cervical stimulation on those days. However, the ratio of the right horn to the left horn was not significant ($p > 0.05$). Considering those result, the faradic stimulation of cervix uteri applied at proestrus and estrus seems to induce the secretion of some hormones from the ovary on those days. There is need of further experiment on this phenomenon. When the uterine traumatization was made at various stages of pseudopregnancy, the sensitivity for deciduoma formation was observed on the 4, 5 and 6th day, but not on the 2, 3, 7, 8, 9 and 10th day of pseudopregnancy.

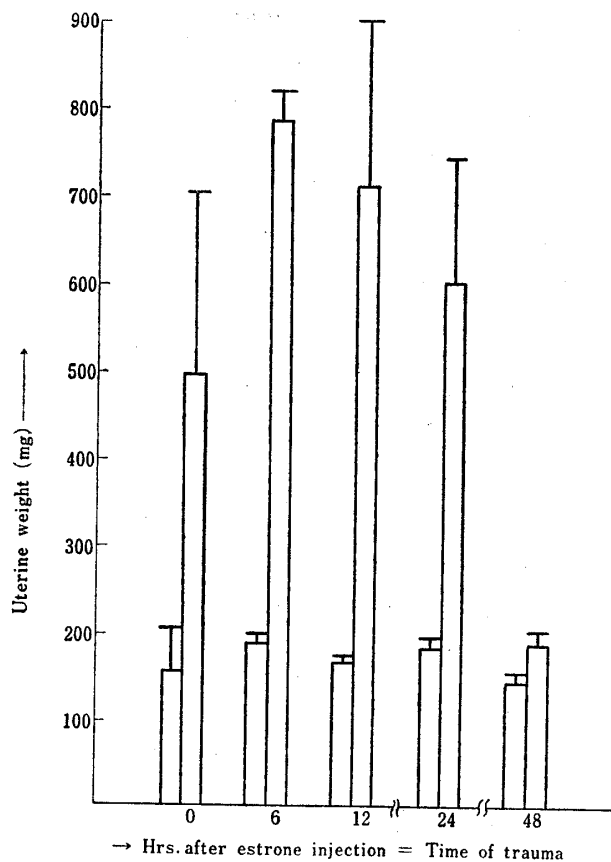


FIG. 3-1. The time of uterine sensitivity for decidual stimulus after a single injection of estrone on the 5th day of experimentally-induced pseudopregnancy. Right side bars show the weight of right horn and left sides show that of left horn.

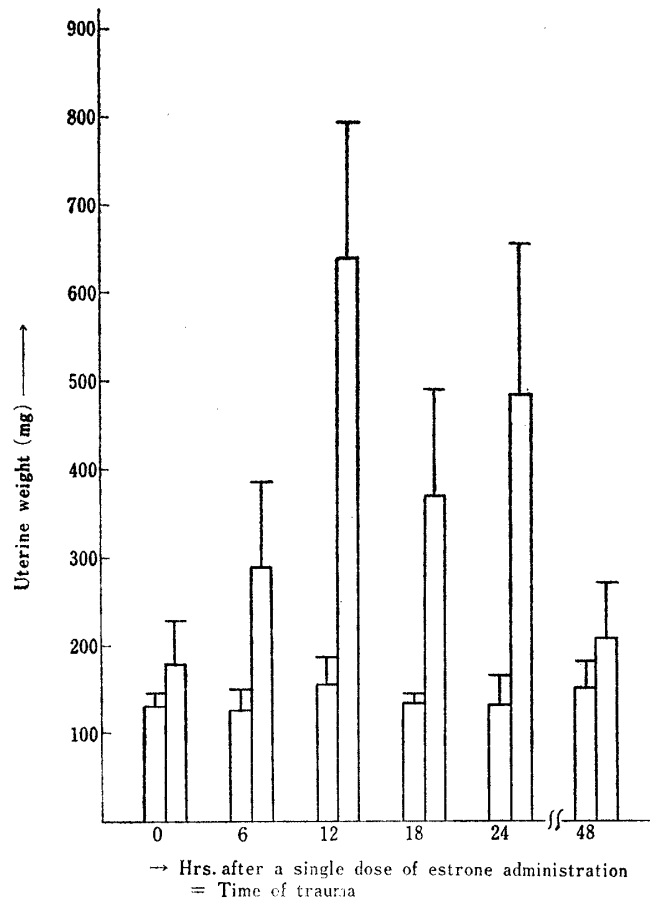


FIG. 3-2. The time of uterine sensitivity for decidual stimulus after a single injection of estrone on the 8th day of experimentally-induced pseudopregnancy. Right side bars show the weight of right horn and left sides show that of left horn.

Although there are many studies indicating that decidual reaction is induced by the synergic action of estrogen and progesterone, the sensitivity for deciduoma formation was observed on the 4, 5 and 6th day of pseudopregnancy in the case of only progesterone administration. As the reason for this phenomenon, it is speculated that the successive administration of progesterone more than 3 days before traumatization is necessary for inducing deciduoma in the rat uterus. But the reason for the reduction of decidual reaction on and after the 7th day of pseudopregnancy is unknown.

In experiment 3, when a single dose of estrone was applied on the 5th day, the sensitivity for deciduoma formation was high 6 to 12 hours after estrone administration. On the other hand, when a single dose of estrone was applied on the 8th day of pseudopregnancy, the sensitivity of uterus for the deciduoma formation was high 12 to 24 hours after estrone injection. However, the sensitivity for deciduoma formation almost disappeared 48 hours after estrone injection in both groups. Present results were in agreement with the report by Sugawara and Takeuchi (1).

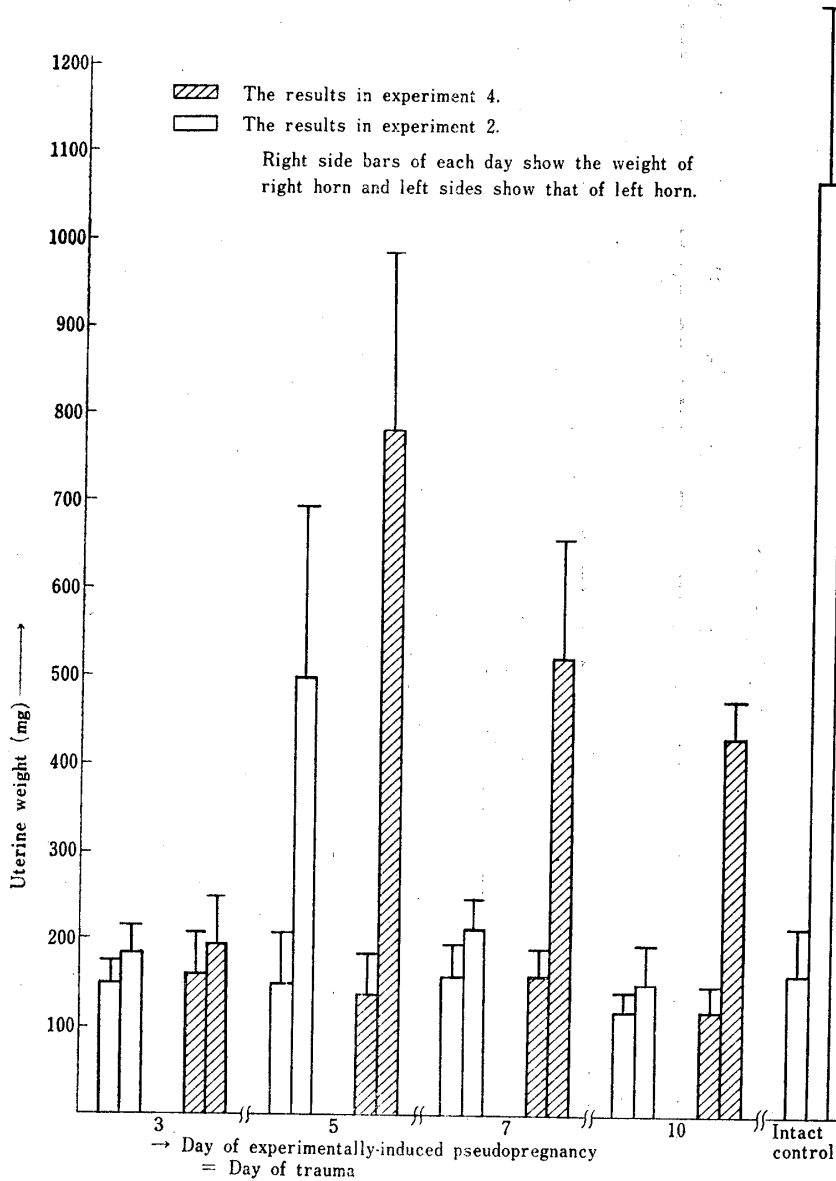


FIG. 4. The effect of a single dose of estrone administration at 12 hr before traumatization on various days of experimentally-induced pseudopregnancy on deciduoma formation in comparison with the results in experiment 2.

Therefore, it may be concluded that period of 12 to 24 hours after the estrogen surge is the essential factor for the occurrence of decidual reaction during the progestational stage in the rat uterus and that the effect of estrone at least exists up to 48 hours after estrone administration. The relationship between the blastocyst of delayed implantation and the single dose of estrogen have been investigated by Yasukawa and Meyer (2) and Takeuchi et al (7). These authors have demonstrated that the delayed blastocyst begins to grow and increases in each area 12 to 24 hours after a single administration of estrone. The authors have reported that when a single dose of estrone was injected into ovariectomized rats on the 8th day

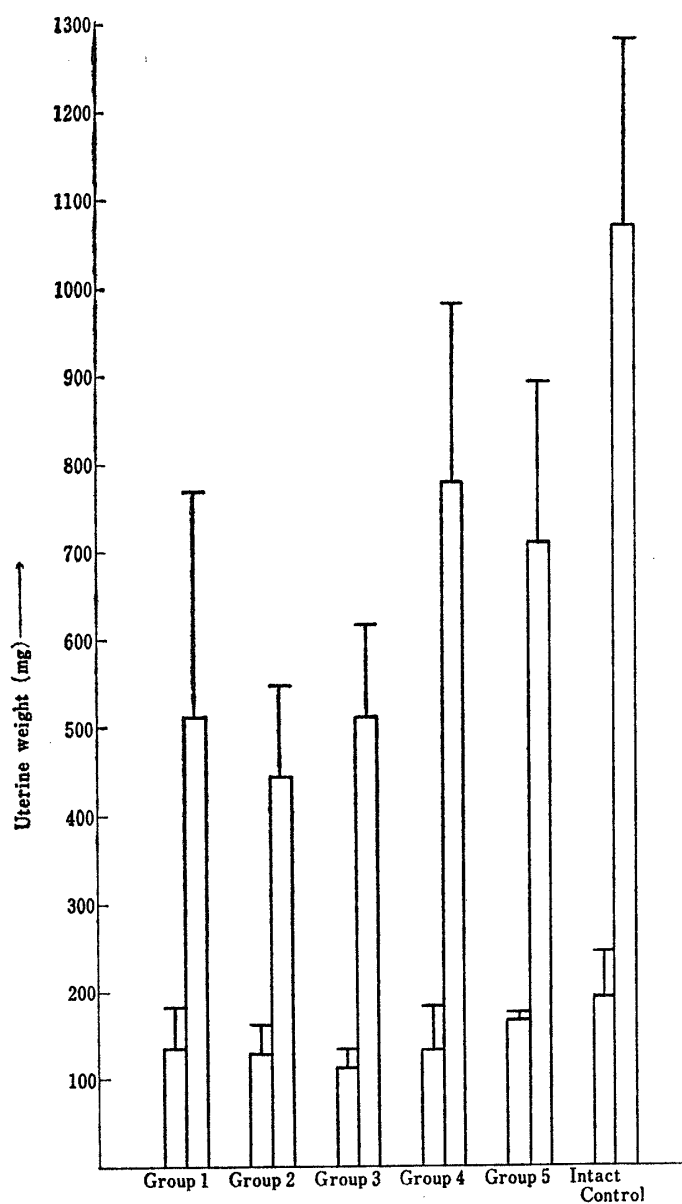


FIG. 5 The effect of a single dose of estrone administration 12 hours before traumatization at various stages on 3-5th day of experimentally-induced pseudopregnancy. Right side bars of each group show the weight of right horn and left sides show that of left horn.

of experimentally-induced pseudopregnancy the enzyme activities of dehydrogenases concerned with carbohydrate metabolism were comparatively high in epithelium, subepithelial layer and uterine gland at 12 hours after estrone administration (8). It is interesting that the response of deciduoma formation, enzyme activity of uterus and development of blastocyst to estrogen occurs simultaneously at 12 hours after hormone administration. Shelesnyak et al (4) have reported that the animals ovariectomized at noon on day 4 of pseudopregnancy failed to respond to the decidual inducing stimulus, and that if ovariectomy was performed 12 hours

later, the uterus responded normally, also they have concluded that the estrogen surge from ovary occurs at noon on day 4 of pregnancy and pseudopregnancy. If there is an estrogen surge, considering from both reports, it is estimated that the estrogen surge occurs between 10:00 A.M. to 10:00 P.M. on day 4 of normal pregnancy and pseudopregnancy. In experiment 4 and 5, whether or not a single dose of estrone injection in various days of pseudopregnancy allows the maximal formation of decuduoma was investigated. When the animals were traumatized at 12 hours after estrone injection on the 3rd day of pseudopregnancy, decuduoma formation was not seen at all. It might be that the animals had not received successive administration of progesterone more than three days. On the other hand, when uterine traumatization was applied on the 5, 7 and 10th day of experimentally-induced pseudopregnancy, the decuduoma formation was recognized in all these groups especially in the decuduoma formation induced on the 5th day was the same as those observed in normal pseudopregnancy (intact control). On the basis of the present experiment, it is speculated that the copulation stimulus may somehow influence the decidual reaction and that the maximal sensitivity for the decidual reaction on the 5th day may be induced as a result of successive secretion of progesterone more than three days in combination with estrogen secretion from ovary at 10:00 A.M. to 10:00 P.M. on the 4th day of normal pregnancy and pseudopregnancy.

Acknowledgement

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