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PROGESTATIONAL SUPPORT OF THE DECIDUAL REACTION BY TOPICALLY ADMINISTERED STEROIDS

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SUMMARY

Steroids, impregnated into hemicylindrical rods formed of Silastic, were applied topically as intraluminal implants into pseudopregnant rats. Progesterone and Provera (2 mg/implant and 0·5 mg/implant respectively) administered in this manner on the 4th day of pseudopregnancy effectively supported the decidual reaction in ovariectomized animals. 5α-Pregnane-3,20-dione, in the range of 0·5 to 25 mg, similarly administered, was ineffective. Allopregnanedione does not appear to be the primary progestational hormone in the decidual reaction.

INTRODUCTION

The decidual reaction is a definite and specific indicator of corpus luteum activity in the rat and has been used effectively as an assay for progestational potency [1]. Topical application of test steroids dissolved in sesame oil and administered locally into the unilateral uterine segment has been used successfully in a modification of the decidual reaction assay [2]. Certain progesterone metabolites including 5α-pregnane-3β,20α-diol, 5α-pregnane-3β,20β-diol, and 3β-hydroxy-5α-pregnan-20-one, demonstrated anti-deciduomatogenic responses following local administration, but were not active when given subcutaneously [2]. The possibility that topical application might prove more definitive than systemic administration [3] in assessing progestational potency warrants the development of a new assay procedure. The sustained release of hormonal steroids from intraluminal silastic implants [4–6] would provide an effective and long-acting source of the test substance. Such a procedure is described in the present report, and the failure of the progesterone metabolite, 5α-pregnane-3,20-dione, to be progestationally effective is reported.

MATERIALS AND METHODS

Preparation of implants. Crystalline steroids were finely pulverized and mixed thoroughly into an appropriate volume of medical-grade elastomer, Silastic 383, (Dow Corning). After mixing, the elastomer was polymerized by the addition of catalyst M, stannous octoate, (Dow Corning) and while still fluid the preparation was "buttered" into Lucite molds, hemicylindrical in shape, having radii of 0·75 mm and lengths of 4 cm. Steroid impregnated implants were allowed to cure at room temperature overnight and were then removed from the molds. Implants were prepared free of steroid and with the following test substances: progesterone, 2 mg/implant; 5α-pregnane-3,20-dione, 0·5 to 23 mg/implant; and Provera, 0·5 mg/implant. Examination of implant at sacrifice showed a weight loss equal to approximately 90% of embedded steroid in all cases.

Animals. Young adult female rats were obtained from the Holtzman Company, Madison, Wisconsin, weighing 200 to 225 g. They were kept in an air-conditioned room with a controlled lighting system, 14 h of light (5.30 a.m. to 7.00 p.m.) and 10 h of darkness and were given Purina Rat Chow and water without restriction. Pseudopregnancy was induced by electrical stimulation of the uterine cervix on the morning of vaginal cornification. Animals were bilaterally ovariectomized at midday on the 4th day of pseudopregnancy. The right uterine horn was traumatized by passing a burred needle through the lumen from a point just above the cervix to the tubal junction and withdrawing it slantwise scratching the antimesometrial aspect of the endometrium throughout its length. Silastic implants were inserted into the lumen of the right uterine horn through a small incision placed near the cervix. Expulsion of the implants was prevented by a single suture placed near the cervix, through the uterine wall near the site of insertion. Initially all animals were traumatized by needle scratching whether or not they were given silastic implants. Later it was noted that the insertion of the implant provided equivalent trauma and scratching was omitted. On the 9th day of pseudopregnancy the rats were killed, the uteri were removed, and the two horns were separated at the cervix. Silastic implants were removed, and each horn was weighed on a semi-micro balance to the nearest 0·1 mg. Representative uterine horns were selected from each group for histological examination. They were fixed in formalin, sectioned, and stained with hematoxylin-eosin.

Table 1. Decidual response induced by endogenous and topically administered steroids*

Group	Content of implant	n	Ovariectomy	Cont. horn (C)	Uterine wet weight Traum. horn (T) (mean ± S.D.)	% Increase†
A	No implant	6	–	140 ± 4	1702 ± 52	1116 ± 37
B	Steroid-free‡	9	–	171 ± 10	869 ± 64	408 ± 29
C	Steroid-free	5	+	111 ± 4	200 ± 34	80 ± 28
D	Progesterone, 2 mg	6	+	156 ± 26	478 ± 85	206 ± 28
E	Provera, 0·5 mg	4	+	161 ± 11	617 ± 115	284 ± 39
F	Pregnanedione, 0·5 mg	2	+	91,125	127,167	36
G	Pregnanedione, 23 mg	9	+	135 ± 16	278 ± 18	106 ± 27

* Medical silastic implants impregnated with indicated steroid (for details of preparation see text).

† $\frac{(\text{wt. traum. horn}) - (\text{wt. cont. horn})}{(\text{wt. cont. horn})} \times 100$

‡ Group composed of intact animals given steroid-free implants with and without prior needle scratching.

A vs B: $P < 0{\cdot}001$
A and B vs C: $P < 0{\cdot}001$
C vs D and E: $P < 0{\cdot}001$
C vs G: No significance

RESULTS

Table 1 summarizes the decidual cell response induced by endogenous and topically administered steroid hormones. Insertion of steroid-free silastic implants into uterine horns of intact animals resulted in equivalent deciduoma induction whether or not needle scratching preceded insertion of the implant. However, the mass of decidual tissue formed in intact animals bearing implants was not equivalent to that produced by scratching if no implant were inserted. Removal of endogenous steroids by ovariectomy prevented deciduoma induction by steroid-free implants. Implants containing 2 mg of progesterone elicited a two and one-half fold increase in decidual reaction over steroid-free implants. The response was not, however, equivalent to that produced by steroid-free implants in intact traumatized horns. A three and one-half fold increase was also obtained with Provera* at a level of 0·5 mg/implant. Topically applied 5α-pregnane-3,20-dione at the concentrations used (0·5 to 23 mg) gave no detectable decidual response. Microcrystalline preparations in the same amounts also gave no decidual response.

The data given in Table 1 also suggested the probability that silastic implants produced an inhibitory effect upon decidual cell development. This point was examined in intact rats whose uteri were traumatized along the entire length by needle scratching. Steroid-free silastic implants were placed in the lower segment of the horn leaving approximately one-half of the horn unoccupied. The data in Table 2 show that the intrauterine device did inhibit decidual cell development in directly adjacent tissue, but its effect was not manifest throughout the entire length of the horn. The percentage weight increase of the two segments of the traumatized horn compared with the contralateral control horn indicates that the implant inhibited full development of the decidual reaction approximately 50%.

Histologically, deciduomata induced by needle scratch could not be distinguished from those induced by insertion of the silastic implant alone. Sections from the latter group did not contain any more evidence of inflammatory reaction than did the former. The difference in total mass of tissue could be accounted for solely on the basis of the amount of decidual tissue present. The presence of deciduomata in animals receiving progesterone and Provera was confirmed by histological examination. Tissue from rats given 5α-pregnane-3,20-dione showed no decidual reaction. Those exposed to the highest dose of 5α-pregnane-3,20-dione were characterized by a fairly thick layer of edematous endometrial stroma with numerous irregularly narrow or slightly dilated endometrial glands.

Table 2. Inhibitory effect on silastic implants (IUD) on uterine sensitivity

	Wet weight* mg	% Increase†
Control horn (C)	147 ± 10	—
½ Traumatized horn (without IUD)	794 ± 46	980
½ Traumatized horn (with IUD)	450 ± 67	512

* Mean ± S.E. (n = 5).

† $\frac{(\text{wt. } \frac{1}{2} \text{ traum. horn}) - (\text{wt. } \frac{1}{2} \text{ cont. horn})}{(\text{wt. } \frac{1}{2} \text{ cont. horn})} \times 100$

* 6α-Methyl,17α-hydroxyprogesterone acetate (The Upjohn Company).

DISCUSSION

Unilateral sterilization is accomplished by insertion of a thread into one horn of the rat uterus [7]. The presence of such an intrauterine foreign body is thought to inhibit the decidual reaction (and therefore the implantation process) by interfering with certain normal endocrine processes [7]. Psychoyos and Bitton [8] and Psychoyos [9] obtained decidualization in uteri of progesterone-treated castrate animals containing a uterine foreign body. However, they attributed the apparent reduced sensitivity in the presence of the foreign body to an increased responsiveness to progesterone. Intrauterine devices have been shown to lower the uterine uptake of radioiodine [10] and to prevent the normal increase in cathepsin D activity associated with the period of maximal uterine sensitivity [11]. Wrenn *et al.* [12] showed that intrauterine devices had marked effects on glycogen, RNA, DNA, and histamine concentrations in the rat uterus near the time of expected blastocyst implantation.

The present study confirms the observation that deciduomata may be induced in the presence of an intrauterine foreign body but also reaffirms the inhibitory effect of such devices on decidual tissue growth. The possibility, however, that maximal growth may have been prevented mechanically by the presence of the device has not been eliminated. The present data suggest that increased tissue response may be achieved by confining the silastic implant to a smaller volume of the uterine lumen.

Topical application of progestational agents from silastic implants appears to provide advantages of convenience and duration of response over intraluminal injections of oily solutions [2]. This would be particularly true if the *trans*-cervical application of steroid-impregnated devices could be perfected [12]. A more adequate investigation into the question of minimal effective dose and maximal sensitivity of the bioassay technique has yet to be made. It appears that the higher potency index [13] of Provera has been retained.

It has been generally considered [2, 13–17] that 5α-pregnane-3,20-dione (allopregnanedione) is devoid of progestational activity. However, reports of biological activity being associated with this compound have appeared. Hisaw *et al.* [16] noted that allopregnanedione effectively prevented the antiprogestational action of 5β-pregnane-3,20-dione in the decidual reaction. Villee has reported [18] that allopregnanedione delayed blastocyst implantation in spayed rats more effectively than did progesterone, and Armstrong[19] has been able to support deciduomal proliferation in castrate, immature rats with subcutaneous injections of allopregnanedione. Since allopregnanedione is rapidly formed from progesterone in uterine tissue [20, 22] and in chick oviduct [23], the possibility that the metabolite may be a critical species at the site of hormonal action must be rigorously examined. The negative results obtained in this study, designed to contribute information on this point, argue against the possibility that allopregnanedione rather than progesterone is the ultimate progestational hormone in the decidual reaction.

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