43. Critical Exposure Time to Estrogen for Cornification of Vaginal Epithelium in Spayed Rats

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(Comm. by Teizo OGAWA, M. J. A., March 12, 1968)

It is well known that cornification of the vaginal epithelium is highly dependent upon the presence of estrogen in normal and ovariectomized rats (see, Allen *et al.*, 1939; Burrow, 1949). The influence of estrogen on the vaginal epithelium is thought to be the results of the direct action of estrogen on the epithelial cells (Jensen and Jacobson, 1962). Recently we found that a non-steroidal anti-estrogen, CN-55,947, 27¹⁾ inhibited effectively estrogen-induced vaginal cornification in ovariectomized rats when it was given at the same time as estrogen, but cornification of the vaginal epithelium could not be inhibited by CN when this antiestrogen given 24 hours after estrogen (Arai and Gorski, 1968). On the basis of these findings we attempted to study the minimal exposure time to estrogen needed for inducing vaginal cornification.

Two weeks after ovariectomy female rats of the Wistar strain weighing 180 to 240 g were used for the experiments. In general, estradiol benzoate (EB, 0.75 μ g/0.1 ml sesame oil) was given subcutaneously once in the morning (10–11 a.m.) and killed 48 hours later. CN (500 μ g/2.0 ml aqueous vehicle) or aqueous vehicle²⁾ was given twice 24 hours apart by gavage according to the experimental designs (see Table I). In each animal vaginal smear was taken on the day of autopsy. The vaginae were fixed in Bouin's fluid and processed routinely for histologic study.

As shown in Table I, EB treatment alone (Group 1) resulted in massive cornification and marked proliferation of the vaginal epithelium (Fig. 1). In contrast, when two administrations (24 hours apart starting with the injection of EB) of CN were superimposed on EB treatment (Group 2), proliferation of basal cells of the vaginal epithelium was markedly inhibited and cornification failed to occur (Fig. 2). The epithelium consisted of only 3–4 cell layers, the

1) CN-55, 945, 27: 1-[2-(*p*-[α -(*p*-methoxyphenyl)- β nitrostyryl] phenoxy) ethyl] pyrrolidine, monochlorate.

2) The aqueous vehicle was composed of 0.9% NaCl, 0.4% polysorbate 80, 0.5% carboxymethylcellulose and 0.9% benzylalcohol.

Table I. Critical exposure time to EB for vaginal cornification determined by CN administration

Group	EB at time zero	Time of CN administration (hours after EB)	Cornification of vaginal epithelium		
			+	± ^{*)}	—
1	EB	None	6**)	0	0
2	EB	Zero	0	1	5
3	EB	3	0	7	0
4	EB	6	4	1	2***)
5	EB	12	7	0	0
6	None	None	0	0	4

*) The appearance of eosinophilic squamous cell layer, but no cornified cells.

***) Number of animals.

***) Mucification.

appearance of squamous cell layer being rare (1/6). The effect of CN was not as marked when this antiestrogen was given at 3 hours after EB, but the epithelium still contained no cornified cells. Moderate proliferation of the basal cells and hypertrophy of old superficial cells were observed and the development of eosinophilic squamous cell layers³⁾ was striking. It appeared as a clear stripe interposed between old superficial cell and polyhedral cell layers.

In the rats of Group 4 which received CN at 6 hours after EB, CN was no longer effective in preventing the occurrence of vaginal cornification. Complete vaginal cornification and marked proliferation of the epithelium were encountered in 3 out of 7 rats (Fig. 3). Their vaginal histology was virtually the same as that of the rats which received EB alone (Group 1). Localized cornification was observed in the vaginal epithelium of 1 animal. In the remaining 3 rats, the vaginal epithelium was thick and covered with denucleated squamous cells (1 rat) or mucified cells (2 rats), although cornification was not observed. When the administration of CN was delayed until 12 hours (Group 5), the effect of CN completely disappeared, well differentiated, thick, cornified epithelium being observed in all animals of the group.

The mechanism of the inhibitory action of CN is still unknown in the present study. Since CN inhibits the action of estrogen on the uterus apparently by blocking its uptake (Callantine *et al.*, 1966), it might exert a similar effect in the vagina, perhaps at the level of the epithelial cells.

In the present study, the induction of cornification could not be inhibited by CN when given 6 hours after EB. It is suggested that the length of exposure time to EB needed for vaginal cornification

3) This layer consisted of denucleated cells or cells with flattened nucleus.

Fig. 1. Vagina of rat treated with EB alone. Note well differentiated, cornified epithelium.

Fig. 2. Vagina of rat treated with EB and CN at the same time. Note atrophic epithelium.

Fig. 3. Vagina of rat treated with CN 6 hours after EB. Note thick, cornified epithelium.

Fig. 4. Vagina of ovariectomized rat without the treatment. Note atrophic epithelium.

is less than 6 hours. This conception is in harmony with the findings of Hori and Miyake (1966) that estrogen stimulated vaginal TTC⁴⁾ reduction (supposed to be an earlier transient phenomenon in the process of vaginal cornification) was markedly inhibited by an antiestrogen, MER-25, when administered simultaneously with estrogen, but the inhibition was abolished when MER-25 given 6 hours after estrogen. Cornification of the vaginal epithelium did not appear yet 24 hours after EB (preliminary observation). Beyond a critical exposure, more time is necessary to develop the process

4) TTC: 2, 3, 5-triphenyltetrazolium chloride.

of vaginal cornification. However, this process requires time during which the continued presence of estrogen, at least in the blood, might not be essential. It would appear that CN antagonizes only the early stage of this complex process.

The authors express their gratitude to Dr. H. A. DeWald of Parke, Davis Research Laboratories, U.S.A. for supplying the CN and to Dr. M. Sawai of Chemical Laboratories, Teikoku Hormone Mfg, Co., Ltd. for the aqueous vehicle and EB. We are also grateful to Dr. T. Kimura, University of Tokyo, for valuable suggestions.

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