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Cornification of Vaginal Epithelium of Ovariectomized Rat Produced by Smearing.

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The histological examination of the vaginae of a number of spayed rats showing an unexpected response to the examination of the vagina by the smear method revealed that smearing alone, without the injection of an estrogenic hormone, may produce, under certain conditions, full cornification of the vaginal epithelium. The results of some of our experiments are summarized in Table I.

TABLE I.

No. animals	Smears per day	Rat units of theelin daily	Smear when killed	Cell layers in vaginal epithelium
13	none	none	none	2
14	1	none	—	3-6
6	2	none	±, ±±	4-8
35	3	none	±, ±±, ±±±, +	6-12
3	1	1	+	8-12
3	3	1	+	8-12

The spayed rats which were neither smeared nor injected presented a thin, smooth vaginal epithelium usually 2 cell layers in thickness with leucocytes migrating into the lumen. When the animals were smeared once, twice, and 3 times daily the typical section from the vagina showed a progressive thickening of the epithelium up to 12 or more layers with the usual desquamation of the surface cells, thus presenting a picture very similar to that obtained after the administration of estrogenic material.^{1, 2}

¹ Allen, E., *Sex and Internal Secretions*, Chap. IX, 1932, Williams and Wilkins, Baltimore.

² Allen, E., Doisy, E. A., Francis, B. F., Gibson, H. V., Robertson, L. L., Colgate, C. E., Kountz, W. B., and Johnson, C. G., *Am. J. Anat.*, 1924, **34**, 133.

The vaginal smear picture³ likewise underwent a progressive change when the animals were examined once, twice, and 3 times daily. The usual — smear observed when the animals were examined once daily changed to a $\pm$ or occasionally a $\pm\pm$ on and after the third day if the animals were examined twice daily. When the vaginal smears were made 3 times daily about 25% of the animals showed full + smears on the third or fourth day of treatment.*

In order to observe whether smearing 3 times daily would change the typical picture observed after the administration of theelin, 3 animals injected daily with one rat unit of theelin and smeared 3 times each day were compared with 3 other animals injected with the same amount of theelin but smeared once daily. No difference in response could be detected in sections of the vagina or in the vaginal smears.

Since a deficiency of vitamin A may cause cornification of the vaginal epithelium^{4, 5} 8 rats which had been maintained on a high A diet from the time of weaning and 7 regular stock animals from the same source† were spayed and examined. These animals likewise showed cornified smears and the usual hyperplasia of the vaginal epithelium after being smeared 3 times daily, thus eliminating a deficiency of vitamin A as a factor.

The ultimate cause of the hyperplasia of the vaginal epithelium has not been determined but it is apparently produced by mechanical stimulation of the vagina either as a localized cellular reaction or as a response to nervous stimulation. Since irritation of the vaginal mucosa appears to be the chief cause of the hyperplasia, the method of making the vaginal smears in this laboratory may be mentioned briefly. A small amount of cotton wrapped about the end of a toothpick and moistened with water is inserted into the vaginal canal and gently rotated a few times, after which the accumulated material is spread on the slide, stained and examined. Although this procedure produces no gross injury to the vaginal mucosa it appears to be sufficient to stimulate the proliferation of cells in the vaginal epithelium. It does not, however, produce a generalized condition of estrus since no macroscopic changes were observed in the uterus.

³ Kahnt, L. C., and Doisy, E. A., *Endocrinology*, 1928, **12**, 760.

* Dr. S. A. Thayer will discuss the relation of these facts to the vaginal smear method of assay in a subsequent paper devoted to certain phases of bio-assay.

⁴ Evans, H. M., *J. Biol. Chem.*, 1928, **77**, 651.

⁵ Evans, H. M., and Bishop, K. S., *Anat. Rec.*, 1922, **23**, 17.

† These animals were secured from Purina Mills through the courtesy of Mr. H. C. Schaefer.

The age of the animal, within a range of 4 to 8 months, and the fact that the animal has or has not been used for assay of the estrogenic hormones apparently plays no rôle in this response to smearing. Likewise the reaction is not confined to our own strain of rats since the group of animals secured from an unrelated colony responded in the same manner. These same animals, as noted earlier, also eliminated a deficiency of vitamin A as a factor. The participation of the hypophysis may be eliminated also since a group of hypophysectomized rats showed a similar hyperplasia of the vaginal epithelium in response to smearing. This behavior is not peculiar to rats alone since a similar, though less extensive growth of the vaginal epithelium was observed in a group of mice smeared 3 times daily.

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Nuclear Inclusions Suggestive of Virus Action in Salivary Glands
of the Monkey, *Cebus fatuellus* L.*

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Evidence is fast accumulating that we must recognize a special group of salivary gland viruses. All of them have been discovered by chance. They are so benign that attention was not directed to them by distinctive clinical symptoms. What attracted notice was the extraordinary hypertrophy of certain acinous, or duct, cells accompanied by the formation in their nuclei of inclusions resembling those caused by viruses.

The first inclusion-laden cells were reported under the heading of "protozoan-like bodies" in the parotids of 2 infants by Ribbert¹ and in the submaxillary glands of guinea pigs by Jackson.² Credit is due to Goodpasture and Talbot³ for recognizing the close resemblance between the bodies in humans and guinea pigs and for pointing out the similarity of both to the intranuclear inclusions described by Tyzzer⁴ in varicella. Lipschütz⁵ then rediscovered the intranu-

*Aided by a grant from the Rockefeller Foundation for Research in virus disease.

¹ Ribbert, H., *Centr. allg. Path. u. path. Anat.*, 1904, **15**, 945.

² Jackson, L., *J. Infect. Dis.*, 1920, **26**, 347.

³ Goodpasture, E. W., and Talbot, F. B., *Am. J. Dis. Child.*, 1921, **21**, 415.

⁴ Tyzzer, E. E., *J. Med. Res.*, 1906, **14**, 361.

⁵ Lipschütz, B., *Arch. f. Dermat. u. Syph.*, 1921, **136**, 428.