Metaplasia in Male Rat Reproductive Accessory Glands Induced by Neonatal Estrogen Treatment

It is well established that neonatal sex steroid treatment causes irreversible changes in the hypothalamic regulatory mechanisms of pituitary gonadotrophic function, resulting in a permanent anovulatory condition in female rats[1,2]. In male rats injections of estrogen in the early postnatal days bring about an inhibition of spermatogenesis and a marked atrophy of reproductive accessory glands due to a permanent suppression of the pituitary gonadotrophic activity[3-5]. Especially in male rats given large doses of estrogen for the first 30 days of postnatal life, it was found that squamous stratification and cornification occur in the epithelium of the coagulating glands and seminal vesicles.

Estrone was injected in 20 male rats of the Wistar strain for 30 successive days from the day of birth. The daily dose was increased with age, 25 μg in 0.02 cm^3 sesame oil during the first 10 days, 50 μg in 0.04 cm^3 oil during the next 10 days and 100 μg in 0.08 cm^3 oil from day 21 to day 30. Six other male rats were given injections of estrone from days 21–50, successively. This daily dose was increased in a similar way, 50 μg in 0.04 cm^3 oil, 100 μg in 0.08 cm^3 oil and 200 μg in 0.16 cm^3 oil in 3 successive 10-day periods, respectively. All animals were sacrificed at 360 days of age.

As previously reported[5], the growth of testes was markedly inhibited (Table) and spermatogenesis was severely suppressed in neonatally estrogenized rats. Their reproductive accessory glands were quite atrophic (Table). Histological examinations showed that the seminal vesicles and the coagulating glands had only a narrowed unbranched cavity surrounded by hypertrophied fibromuscular tissues. The epithelial cells were devoid of secretion granules, showing deficiency of endogenous androgen. Interestingly enough, instead of the expected cuboidal condition, metaplasia of the epithelium with squamous stratification had taken place in the coagulating glands and seminal vesicles in 6 out of 12 neonatally estrogenized rats. The stratified squamous epithelium of the coagulating glands of these rats showed persistent cornification, in fact a section of the gland is almost identical with that of the vagina of a female in persistent estrus (Figure 1). There was also squamous stratification in the epithelium of the seminal vesicles (Figure 3), but cornification was recognized only in 2 of them. Metaplastic changes were not marked in the prostate glands, but there was a tendency for the formation of stratified epithelium where infiltration of leucocytes was striking.

In addition, to test the effects of orchidectomy and adrenalectomy on these metaplastic epithelia, 4 rats whose coagulating glands had shown metaplasia upon biopsy, were orchidectomized 30 days prior to autopsy and adrenalectomized 10 days before autopsy, then given a 1% NaCl solution as drinking water. The squamous stratification was found to be well maintained in the coagulating glands and the seminal vesicles of these animals, but cornification seemed to be influenced by the removal of the testes and adrenals, since it was visible only in the coagulating glands of 1 animal.

In the animals in which the estrone treatment was started day 21, there was no sign of metaplastic changes in the reproductive accessory glands. Moreover their histological appearance was normal (Figures 2 and 4).

It is of interest to note the earlier findings of PFEIFER[6] that a similar metaplasia in the epithelia of reproductive accessory glands occurred only in the male rats which were castrated and received ovarian grafts at birth. It has been reported that administration of large amounts of estrogen to adult male rats or mice causes squamous metaplasia

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Mean weights (± S.E.) of body, pituitary, testes and reproductive accessory glands/100 g body weight of control and estrone-treated rats

Treatment	Body (g)	Pituitary (mg)	Testes (mg)	Seminal vesicles and coagulating glands (mg)	Ventral prostaes (mg)
Control (7)[a]	428 ± 9	3.8 ± 0.70	1031 ± 40	531 ± 18	167 ± 6.1
Day 1–30 estrone (12)	418 ± 7	2.4 ± 0.02	186 ± 12	26.5 ± 6.8	11.3 ± 3.1
Day 21–50 estrone (6)	409 ± 11	3.4 ± 0.09	984 ± 12	443 ± 18	148 ± 9.1

[a] No. of animals. For doses of estrone, see text.

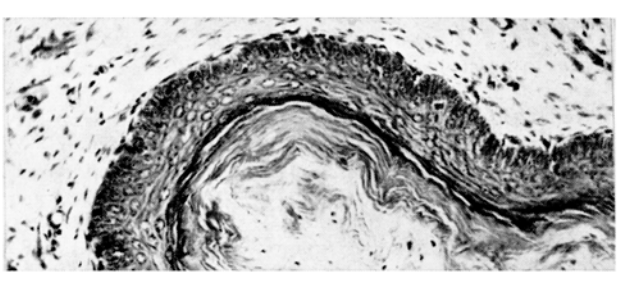

Fig. 1. Coagulating gland of neonatally estrone-treated rat sacrificed at 360 days of age. Epithelium stratified and cornified. × 150.

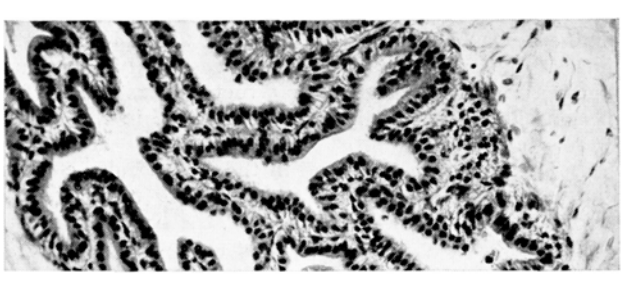

Fig. 2. Coagulating gland of a rat which received estrone treatment from day 21–50. Epithelium normal. × 150.

in the epithelia of reproductive accessory glands during the period of the treatment[7,8]. The metaplasia in the animals of the present experiment was still visible 11 months after the last injection of estrone. Further, the processes of the squamous stratification seem to be independent of the presence of testes and adrenals and their steroid hormones, although cornification might be affected by the removal of these endocrine glands. Thus this metaplasia appears to be caused by the neonatal treatment of a high dose of estrogen acting at least initially upon the epithelial cells of the reproductive accessory glands and might be permanent. It should be mentioned that permanent hyperplastic lesions in the vagina and uterus produced by neonatal estrogen treatment was recently reported in mice and rats[9-13].

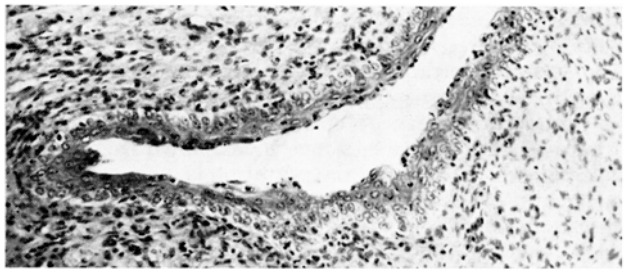

Fig. 3. Seminal vesicle of a neonatally estrone-treated rat sacrificed at 360 days of age. Epithelium stratified but no cornification. × 150.

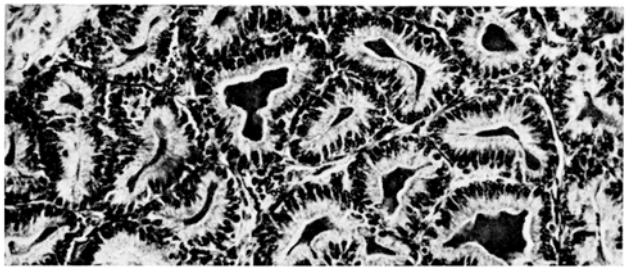

Fig. 4. Seminal vesicle of a rat which received estrone treatment from day 21–50. Epithelium normal. × 150.

Résumé. L'injection de larges doses d'estrone à des rats mâles au début de la période postnatale cause une stratification dans l'épithélium des glandes de coagulation et dans les vésicules séminales. Cette métaplasie a persisté 11 mois après la dernière injection d'estrone.

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Radiation-Induced Resistance to Dodine in *Hypomyces*

Resistance to agricultural fungicides in plant pathogenic fungi has not so far created problems comparable to bacterial resistance to antibiotics, or to resistance to certain organic insecticides in insects. Even under favourable laboratory conditions, well defined resistance to the majority of important fungicides has not been achieved[1]. The non-specific mode of action of most antifungal compounds in use, is considered as the most important factor for this rarity of resistant strains. There are, however, mechanisms by which resistance to non-specific toxicants can arise. Hence, suitable mutagenic treatments should be able to induce resistance to many important fungicides. This view is supported by our recent successful use of UV- and γ-radiation to obtain resistance to *n*-dodecylguanidine acetate, commonly known as dodine or Cyprex. This compound is one of our most successful agricultural fungicides and particularly effective against *Venturia inaequalis*. Like most of the present day fungicides, dodine is not known to act by specific enzyme inhibition. Its fungitoxic effect seems to be due to blocking vital anionic sites at the cell surface or inhibiting important enzymes located there[2,3]. This communication reports the development of resistance to dodine in *Hypomyces solani* f. *cucurbitae*, a plant pathogenic pyrenomycete suitable for genetic work, and gives some first data on its nature.

Conidia (*Fusarium* type) were obtained from potato dextrose agar slant cultures kept at room temperature and in diffuse daylight. For the UV-irradiation of the spore suspensions a 15 Watt Philips TUV germicidal lamp was used. This provided a flux of 20 ergs mm^{-2} sec^{-1} at the target distance employed. Eight ml of suspension containing approximately 32×10^5 of conidia were placed in an open Petri dish and agitated during irradiation. Exposure for 6 min was required to give around 95% lethality. In other attempts spore suspensions in small test tubes were exposed to 180,000 rads of γ-radiation from a 'Gammacell 200' Cobalt-60 source of approximately 3000 curies. This irradiation reduced colony formation by 90%. For the selection of mutants a potato dextrose agar medium of pH 5.1–5.4 containing about 5.0 μmoles dodine/100 ml was used. This concentration was 1.5 times the concentration required to prevent formation of colonies by wild type conidia plated at very high

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