OBSERVATIONS ON RATS TREATED WITH THE SEX HORMONES ESTRIN AND TESTOSTERONE

C. S. McEUEN, M.D.¹

(From the Department of Biochemistry, McGill University, Montreal)

TUMOURS IN RATS FED WITH A CRUDE ESTRIN PRODUCT

Previous publications from this laboratory (1, 2, 3) have described morphological abnormalities observed in rats after prolonged administration of estrone by intraperitoneal, intra-uterine, or subcutaneous injection or by vaginal application. The occurrence in such animals of tumours histologically diagnosed as cancer was reported in a preliminary note in 1937, and more fully in the following year (4). It was suggested that the appearance of these cancers was related to estrone administration, and a note recently published by Geschickter (5) lends additional support to this view as far as mammary cancer is concerned.

The present paper deals with the findings in three experimental series of rats of our hooded strain, which were fed with a crude but potent estrin prepared from pregnancy urine. The estrin product was mixed in the food (ground Purina fox chow) in the doses indicated. This strain was originally crossed with Wistar whites, and its genetic constitution is unknown to us.

In these experiments it was decided to permit the animals to live as long as possible; post-mortem findings were thus unavailable in many rats which were found dead. The tumours observed are deemed to be of sufficient interest to warrant description in detail.

The first series (436.S.1) consisted of 28 males, half of which were castrated at the age of 24 or 25 days. Each animal was fed 0.05 c.c. of the estrin product (approximately 250 to 375 international units) per day, beginning on the 26th or 27th day of life; four weeks later the dose was raised to 0.2 c.c. (approximately 1000 to 1500 international units) and maintained at that level until death. In each subgroup, 3 rats were painted with tar (horizontal retort gas-house tar received from the Gas, Light and Coke Company, London, England) on the interscapular area thrice weekly from the age of 109 days, and 2 animals received 30 mg. of dry kieselguhr in the upper left mammae at 82 days of age. A series of 10 controls, 5 intact and 5 castrates, were fed on plain Purina, one in each sub-group being tared and one receiving kieselguhr.

The following lesions occurred among the experimental animals. Intact rat 1190 at the age of 611 days (502 days of tar treatment) showed ulceration of the tarred area of the skin. Tarring was stopped at 646 days (537 days of tar treatment). On the 720th day of life a tongue-like process of induration was palpated, extending out into the skin surrounding the ulcer, and a biopsy revealed a squamous carcinoma. At the age of 771 days the carcinomatous ulcer measured about 50 × 30 mm. and the rat, being emaciated, was killed. Metastases were found in the left axillary nodes.

¹ Finney-Howell Research Foundation Fellow.
Another intact rat, II.50 PB, sacrificed on the 921st day of life, showed a small papillomatous growth approximately 3 mm. in diameter, situated on the inner surface of the squamous portion of the stomach, near the junction of the proventriculus with the ventriculus. Histologic examination proved this tumour to be a carcinoma (Fig. 1).

In castrate rat 1105, killed at the age of 895 days (786 days of tarring), there were a tar papilloma of the skin and a mammary adenofibroma.

Pituitary adenoma of the anterior lobe was seen in only one member of this series, a castrate. In two instances (one intact and one castrate) the mucosa of the glandular portion of the stomach was found to be fibrous (Figs. 2 and 3) and in one case there was a chronic gastritis (intact rat). These stomach lesions all occurred in animals which were never in contact with tar.

Among the controls, castrate rat 1150 at the age of 857 days (763 days of tar treatment) bore a tar papilloma, while intact rat 1151 at the age of 900 days (806 days of tar treatment) first showed a tar wart, which at death, on the 1024th day of life (930th day of tar treatment), was found histologically to be a basal-cell carcinoma with pearls (Fig. 4).

The second series (436.S.2,3,4,5) consisted of 30 females, half of which were ovariectomized at the age of 24 days. Feeding of 0.2 c.c. of the estrin product (approximately 1000 to 1500 international units) per day per rat was begun on the 26th or 28th day of life and maintained at that level till death. Three intact and three castrate rats were repeatedly tarred from the age of 90 or 92 days, while 4 other animals received 30 mg. of kieselguhr in the upper left mammae at 78 or 80 days. Ten controls (5 intact and 5 castrate) were fed plain Purina.
Among the experimental animals the following lesions occurred: In castrate tarred rat 1100 PB3, at the age of 717 days (625 days of tar treatment) a cutaneous horn was observed among the hairs of the skin about 1/4 inch distant from the tarred area (Fig. 5). The multicentric origin of cancer of various types in this animal is of interest. Fig. 6, a sketch made when the rat was killed on the 900th day of life (808 days of tar treatment), shows the several
discrete tumours. Tumour No. 1 was a cutaneous horn, the base of which revealed a squamous-cell carcinoma. No. 2 was a hard nodule showing a parenchymatous carcinoma with a few pearls (Figs. 7 and 8). Nodule No. 3 was histologically diagnosed as an immature parenchymatous adenocarcinoma. In addition to the regions of acinus formation, however, there were areas presenting the appearance of basal cells and squamous pearls. In the area indicated by No. 4, the skin eventually broke down over a dry abscess, and sections of the skin removed from the margin of this ulcer at two different sites (numbered 5) revealed a carcinoma cutis showing basal-cell proliferation with nucleated squamous cells and pearls (Fig. 9). Furthermore, in this same rat a mass (numbered 6) was found overlying part of the left tympanic bulla, which histologically proved to be a squamous-cell carcinoma. In addition a large fibromyoma was discovered growing from the posterior aspect of the cervix uteri. The pituitary was of normal size and the uterine horns showed no metaplasia.

Castrate tarred rat 1100 Pl.5 (1154) at the age of 594 days (502 days of tar treatment) presented a small ulcer covered by a heaped-up scab approximately 1/2 inch distant from the tarred area: 91 days later (593 days of tar treatment) this lesion had developed into a cornified conical mass 30 × 26 mm. in diameter at its base, with the apex projecting 25 mm. from the skin level. Histologic examination of the base showed a squamous-cell carcinoma.

In castrate rat 1101 Pl.10, killed on the 819th day of life, an abscess was found at the ovarian end of the right uterine horn, accompanied by a possibly precancerous metaplasia of that horn below the abscess. Moreover, a large tumour composed of edematous fibrous tissue arising from the cervix was dis-
OBSERVATIONS ON RATS TREATED WITH SEX HORMONES

FIG. 5. CUTANEOUS HORN ABOUT 1/4 INCH FROM TARR ED AREA IN RAT 1100 PB3

FIG. 6. SKETCH OF TAR TUMOURS AT AUTOPSY IN RAT 1100 PB3 AT AGE OF 900 DAYS


covered prolapsed through the vagina to the exterior. The pituitary weighed 33 mg. Castrate 3 YB5, at 988 days, showed a similar tumour prolapsed through the vagina. Castrate 3 Pl.4, killed at the age of 494 days, had a large abscess enclosing the uterine horns. Sections through the abscess wall showed a squamous-cell carcinoma (Fig. 10). The pituitary was normal and weighed 8 mg.

Papillomatosis of the stomach (Figs. 11 and 12) was met with in intact un tarred animal 4 Vb.8, while lymphosarcomata of the ileocecal lymph nodes
occurred in 4 members of the series. Among the controls one mammary fibroma and one case of papillomatosis of the urinary bladder with calculi were seen, but no malignant neoplasms.

The third series comprised 12 intact males, and was designed for observation of the effects of diminishing dosage. Half of the members of this series
received 0.02 c.c. (approximately 100 to 150 international units) and the other half 0.004 c.c. of the estrin product (approximately 20 to 30 international units) per day per rat, feeding being begun at the age of 24 days and continued until death. Six controls received plain Purina. Tarring was not resorted to.

No malignant growth occurred in these experimental animals except for one case of lymphosarcoma of ileocecal nodes. Rat C.37.1145PB, however, presented a pigmented adenoma of the middle lobe of the pituitary and two chromophobe adenomata of the anterior lobe, reported elsewhere (6). Nothing of interest was found in the controls with the exception of one rat with a benign glandular structure simulating prostatic tissue situated at the external side of the right superior vena cava.

Discussion: It is necessary to emphasize that in this work the diagnosis of carcinoma has been made on the basis of histologic examination alone. It is felt that the estrin content of the animals' food does not entitle us to draw any conclusions, especially as to extragenital carcinogenesis. In view of the fact, however, that our hooded strain of rats is regarded as of relatively low susceptibility to spontaneous cancer (4), it is desired merely to record these tumours and the conditions under which they occurred.

It may be pointed out that, whereas in previous experiments (4) in which estrone was administered subcutaneously or per vaginam, the carcinomata were found in the genital tract and breast, in the series now reported the occurrence was mostly in other sites. Moreover, utero-rectal fistulae were observed in the earlier experiments but not in the present series.
No particular importance is attached to the appearance of lymphosarcoma, as this tumour was frequent among animals in the colony breeding cages and various experimental series at the time of these observations.

In consideration of the 70 experimental rats with which this paper is concerned the following observations should be made. Of the 54 animals surviving 494 days (earliest known age at which we have so far detected rat carcinoma) 5 developed cancers, one uterine, one gastric, and 3 about the tarred skin. One control had a tar cancer.

On account of the smallness of the series it is impossible to say whether or not estrin had any bearing upon the dates of appearance of tar cancer. It is evident that these tumours may arise in the skin of the rat in situations apparently considerably removed from the tarred area. During a prolonged course of tar painting it is quite conceivable that the tarred area may gradually deviate to some extent from the original site. It is safe to say, nevertheless, that none of the carcinomatous areas shown in Fig. 6 were repeatedly tarred; nor were there any excoriations of the skin to suggest local irritation from scratching.

Tumours arising in mice at a distance from the tarred area were observed long ago. Woglom (7) refers to Maisin as being one of the first to record this, having "found carcinomata and papillomata on the lower jaw, the side of the thorax or on the back outside of the painted region." These findings are of considerable speculative interest at the present time. In the few cases observed in these laboratories in rats, the distribution of the tumours was in the region of the tarred areas, so that one is more inclined in these instances to visualize the transportation of an agent from the tarred area along the lymphatics than to think in terms of a general constitutional action of tar.
Figs. 11 and 12. Papillomatosis and Cyst Formation of the Stomach in Rat 4 VB8. (6741G)

The upper figure shows papillomata in the squamous portion; the lower shows cysts in the submucosa of the glandular part.
Rat skin has been regarded as resistant to tar cancer. Woglom (7) in 1926 observed that “although the skin of rats, guinea-pigs and fowls is almost totally resistant to the carcinogenic action of tar, other of their tissues are not.” He quotes Yamagiwa’s opinion that tar painting will not produce carcinoma in rats or fowls and relates the failures of numerous investigators (Paszkiewicz, Leitch, Polettini, Itchikawa, Buschke and Langer, Borrel, Boez and de Coulon, etc.) and the single success of Herly in one rat painted with tar and arsenious acid. In their review of 1933, Seelig and Cooper (8) refer to many failures and to the success of Watson after a preliminary application of petroleum ether to the skin. Commenting on the subject himself, Watson (9) remarks that the value of rats in carcinogenesis studies has been limited owing to the failure of even prolonged coal tar treatment to provoke skin tumours. Cameron and Meltzer (10) have referred to this difficulty, and reported two tumours among ten rats treated with ordinary gas-works tar, which proved histologically to be cancer after eighteen and a half months of treatment. It would appear from the work of the latter investigators and
from the series here presented, that previous attempts might have been more successful had the period of tarring been more prolonged.

It is interesting to compare the histopathology of these rat tar tumours with human skin cancer. In reference to basal-squamous-cell epithelioma, MacKee and Cipollaro in their monograph (11) have observed: "The tumor is composed mostly of basal cells with here and there a group of squamous cells and often a pearl or whorl. At times the cells composing the tumor correspond strictly neither to basal nor squamous cells, but rather are of an intermediary type."

In the series reported in this paper, vaginal estrus was constantly maintained in all of the 30 females both intact and ovariectomized, except during a few days on one occasion when feeding was stopped.

The growth of these animals, male and female, was obviously stunted (Figs. 13 and 14), the inhibition varying directly with the dosage of the estrin product. Nevertheless, in marked contradistinction to old rats, whether untreated or injected subcutaneously with estrone or testosterone for long periods,
these animals irrespective of sex or castration were in old age (700 days and upwards) alert and vigorous, with healthy coats. Indeed, it may be said that they looked like normal young rats 6 to 9 months old. The pituitary weights were found to be above normal limits in only 5 instances, and in 4 of these pituitary adenomata were found.

Observations in Rats after Prolonged Treatment with Testosterone

Two series of rats were treated with testosterone for long periods, for comparison with the observations made in rats following prolonged administra-

![Fig. 15. Metastatic Parenchymatous Carcinoma in Cervical Nodes of Rat 1175 PB. (6501F)](image)

The primary tumour was not found.
tion of the mammary glands as seen by biopsy after 14 and 22 days' treatment have been reported elsewhere (12). The following lesions were subsequently found.

Castrate rat 1175 PB was killed at the age of 699 days on account of dyspnea. Large anterior cervical nodes were found on each side, histologic examination of which demonstrated a parenchymatous carcinoma (Fig. 15). The primary tumour was not discovered.

Another castrate, 1175 PI, killed on the 570th day of life, carried a gelatinous bloody tumour on the left hind leg (Fig. 16), which is regarded as probably a cavernous haemangioma.

A biopsy, done at the age of 790 days, of a tumour arising at the site of injections in the castrate 1176 PB showed a fibrosarcoma (Fig. 17) which 35 days later was found to have metastasized into the axillary nodes.

A large tumour arose in breast tissue in intact rat 1174 PB at the site of injections; at 918 days this proved histologically to be a spindle-cell sarcoma. Mammary fibromata were found in two other intact animals at the ages of 969 and 898 days, also in injected areas.

In intact rat 1174 PI a right supraclavicular mass was noticed at the age of 790 days. A biopsy was done and the histology showed a fibrous tumour, probably a fibrosarcoma. Five weeks later this animal was found dead. The regional nodes were enlarged.

One animal showed an anterior pituitary adenoma.

In the controls fibromata were found in two animals at the ages of 698 and 995 days, and a fibrous tumour, probably a fibrosarcoma, in another rat at 890 days, all in areas injected with corn oil. A lymphosarcoma was seen in one case.

The second series (435 Sv) consisted of 12 females, of which 6 were ovariectomized on the 23rd day of life. Daily subcutaneous injections of 200 \( \gamma \) of testosterone in corn oil per rat were begun at the age of 25 days and maintained until death. Twelve controls were similarly handled, except that they were injected with a like quantity of cholesterol in corn oil.

Among the experimental animals the castrate 40 PT3 was shown at the age of 752 days to have a round-cell sarcoma in an injected area of breast
Fig. 17. Fibrosarcoma at site of testosterone injections in rat 1176 PB. (6641C)

Fig. 18. Round-cell sarcoma at site of testosterone injections in rat 40 PT3. (6845A)
tissue (Fig. 18) and a papilloma of the vagina. Two intact animals at 879
days presented fibrous tissue tumours at the site of injections, and the castrate
40 PB2 a myxoma of the sacral region. Among the controls one castrate at
879 days showed a fibrous tissue mass at the site of injection, and another
animal a beginning lymphosarcoma of the ileocecal nodes.

Discussion: It is necessary again to emphasize that throughout this work
the diagnosis of malignancy has been made solely on the basis of histologic
examination. In comparing the findings of the present testosterone treated
series with observations on rats following prolonged administration of estrogenic hormones, the following points are of interest:

Whereas in the estrone series (4) carcinomata were encountered in the
genital tract and breast, in the 20 testosterone treated animals surviving 494
days (earliest known age at which we have as yet detected rat carcinoma)
one case of metastatic adenocarcinoma in the cervical nodes, already referred
to (4), was seen. Indeed, since 18 of the testosterone controls survived 494
days and lived mostly well on into the cancer age without the appearance of
carcinoma, the series reported in this paper may be regarded as further con-
trols to the estrone experiments.

Moreover, in the present series the predominant lesions seemed to be sub-
cutaneous fibrous tissue tumours arising about oil cysts in injected areas, some
of which went on into sarcoma. No importance is attached to the occurrence
of lymphosarcoma of the ileocecal nodes, which, as stated above, was frequent
in the colony at the time.

### Table I: Longevity of Testosterone Treated Rats and Controls

<table>
<thead>
<tr>
<th></th>
<th>6 mos. (180 days)</th>
<th>12 mos. (360 days)</th>
<th>18 mos. (540 days)</th>
<th>24 mos. (730 days)</th>
<th>30 mos. (900 days)</th>
<th>33 mos. (1000 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone Injected: 23 males and females</td>
<td>22</td>
<td>22</td>
<td>20</td>
<td>16</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Controls: 24 males and females</td>
<td>23</td>
<td>22</td>
<td>14</td>
<td>12</td>
<td>4</td>
<td>0</td>
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Furthermore, it should be stated that all the females injected with testo-
sterone went early into vaginal diestrus and remained thus throughout the
experiment, which is in agreement with previous experience (13). No inhibi-
tion of somatic growth was noticeable in either sex, nor were the pituitary
weights found to be above normal limits in any instance. One anterior pitui-
tary adenoma was seen.

The longevity of the animals is shown in Table I.

**Summary**

(1) The occurrence of 3 tar cancers of the skin, one uterine cancer, and
one gastric cancer are reported in rats fed with an estrin product obtained
from pregnancy urine. A tar cancer is recorded in one control rat.

(2) Whereas in rats injected with estrone for long periods carcinomata of
the genital tract and breast occurred accompanied by other usual estrin effects,
it appears that with prolonged injection of testosterone the females went into
permanent vaginal diestrus, the pituitary weights were not above normal limits,
somatic growth was not inhibited, and the predominant neoplastic lesions were fibrous tissue tumours in injected areas, some of which became sarcomatous.

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**References**