INFLUENCE OF TESTOSTERONE IN INTACT AND MAMMECTOMISED FEMALE MICE*1

(Plate LI)

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Synopsis

Spontaneous mammary tumour incidence, at death, of intact C3H(Jax) virgin female mice was not altered by treatment with testosterone propionate, given up to the beginning of tumour age. In 6 out of 12 mice, testosterone delayed the appearance of tumours and thus added to their longevity significantly. The type of mammary tumours obtained in testosterone-treated female mice was largely similar to the spontaneous and unlike the chemically induced ones.

The yield of ovarian tumours induced by 7,12-dimethylbenz[a]anthracene (DMBA) was significantly greater in C3H(Jax) mammectomised mice when treated with testosterone. It is suggested that testosterone has a promoting action for chemically induced ovarian tumours in mice. Subcutaneous fibrosarcomas were induced in DMBA-treated mammectomised C3H(Jax) female mice given testosterone. They were not obtained in comparable groups treated with testosterone alone or with DMBA alone. This observation supports the suggestion that testosterone has a promoting rôle in the mechanism of chemical carcinogenesis.

Female mice of the milk factor-possessing C3H(Jax) strain have a very high incidence of spontaneous breast tumours. It was planned to find the possible effects of a male hormone in this essentially female carcinogenesis. Secondly, since enhancement of ovarian tumorigenesis by pseudopregnancies12) was suspected to be associated with andromimetic activity,13) it was decided to investigate how treatment with testosterone might influence chemical induction of ovarian tumours in mammectomised C3H(Jax) mice.

MATERIALS AND METHODS

The three experimental groups consisted of 34 C3H(Jax) virgin female mice. Twenty-two of these were prepuberally mammectomised by a technique described previously14) whereby all the five pairs of mammary glands with their nipples were surgically removed. In all 34 mice, 0.1–0.2 ml of 1% testosterone (Organon) in olive oil was injected subcutaneously, beginning at 10–12 weeks of age. Weekly injections for a period of 6–8 weeks did not affect the estrous cycle nor produced clitoral hypertrophy. It was, therefore, replaced by 1% testosterone propionate (BDH) in olive oil, 0.1 ml of which was injected weekly for 16–18 weeks. In addition to testosterone treatment, 12 of the mammectomised mice received 1% 7,12-dimethylbenz[a]anthracene (DMBA) in

*1 Paper read at the Ninth International Cancer Congress, Tokyo, October 1966.
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olive oil; also beginning at 10–12 weeks of age. Cutaneous applications of this carcinogen were given once a week for 8 weeks.

Animals were killed as and when they appeared emaciated. The maximum survival in Group 1 of 12 intact testosterone-treated females, was up to 24 months of age; in Group 2 of 10 mammectomised testosterone-treated females, up to 21 months; and in Group 3 of 12 mammectomised testosterone-and-carcinogen-treated females, up to 11 months. Observations on groups of untreated intact and mammectomised animals and only carcinogen-treated mammectomised mice have been described previously.14,15

RESULTS

Mammary Glands

In Group 1, 11 of 12 females developed breast tumours (Table I). On the other hand, the mammectomised testosterone-treated groups of females did not develop breast tumours; even when DMBA was given.

The tumours in Group 1 animals first became palpable at an age between 7 and 23 months. After tumour development the animals survived for 1 to 4 months and occasionally longer. The tumours were usually single, large, and located in the second and third pairs of glands of either side. Histologically, the tumours were adenocarcinomas of the milk-agent types as described elsewhere.1 There were small and irregular tubular types with areas of polygonal cells but intratubular growth pattern was frequently seen in this group. Hemorrhage and necrosis were found in parts. Heiman4 found that histologically the tumours remained unaffected by testosterone treatment.

Ovaries

In Group 1, there were large clear cystic ovaries in 11 of 12 females (Table I). Patchy luteinisation was noted wherever a little ovarian tissue was left. In Group 2 also, there were 5 of 10 females with cystic ovaries. In the absence of cyst formation, luteinisation and atrophic changes as of senility were observed. Ovarian tumours developed in Group 3 where DMBA was given. There were 11 of 12 females with tumours (Table I). The contralateral ovaries of the tumour-bearing mice, which were reduced in size, were precociously atrophic and occasionally partly cystic. There were 9 granulosa cell tumours of which 6 were visible to the naked eye. Two tumours were of mixed tubular adenoma type (Photo 1) with a structure described in an earlier paper.15 The larger tumours were about 1 cm in diameter. They were soft, fleshy growths with hemorrhagic and necrotic areas. The predominant histological patterns were pseudofollicular (Photo 2), papillary, or undifferentiated (Photo 3). There was myxomatous degeneration (Photo 4) in one of the large granulosa cell tumours.

Other Organs

Squamous papillomas of the skin were found in several animals of Group 3 while 7 of 12 developed subcutaneous fibrosarcomas (Photos 5 and 6). A moderate degree of glandular hyperplasia of the uterine endometrium was found in some animals of each group (Table I) but was not correlated to ovarian histology. Areas of hydrosalpinx were noted especially in Group 1 animals. The adrenal glands did not show significant histological differences.

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<th>Group</th>
<th>Mammary tumours in</th>
<th>Ovarian histology</th>
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<td>(3)</td>
<td>Mammect. testost. and DMBA</td>
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* Normal size, luteinised ovaries
DISCUSSION

In the clinic, testosterone propionate is reported to give objective improvements in varying percentages of advanced breast cancer cases. However, many conflicting views are presented in the literature on the uses of testosterone propionate, either as a therapeutic agent or as a carcinogen. It is reported as having anti-cancer properties and also as having carcinogenic properties.

In mice, Jones found a lowered spontaneous tumour incidence when testosterone propionate treatment was begun prepuberally in C3H(Jax) animals. In the present experimental study, it was noted that 6 of 12 females of Group 1 did not develop palpable tumours before 18 months of age; while in a previous group all untreated C3H(Jax) virgin females had died by that age bearing large tumours. The testosterone-treated, non-tumour-bearing animals survived for 22–24 months of age. The increase in life span appears to be due to the delay in development of breast tumours which became palpable after 18 months of age. At death, however, there was no difference in the breast tumour incidence between the testosterone-treated and the untreated mice. Also the differences found, in the span of survival after developing a palpable tumour and in the location, in the size or the number of tumours in a female, did not prove to be truly significant.

It is concluded that testosterone given in physiologically active doses from maturity up to the beginning of tumour age (8–10 months) significantly delayed the appearance of spontaneous tumours in 50% of C3H(Jax) virgin mice and thereby lengthened their life span considerably. Although testosterone has been suggested as a prophylactic measure in women with family history of breast cancer, extensive prior evaluation by animal experiments is yet called for. On the other hand, if mammectomy is done, the animals do not develop breast tumours even after repeated exposure to carcinogenic stimuli.

In the ovaries, it was found that in Groups 1 and 2, the effect of testosterone was not truly specific, as clear cystic ovaries have been observed in untreated old females of this strain. The number of animals with cystic ovaries was, however, much larger with treatment. Jones had also obtained clear cystic ovaries after treatment.

The ovarian tumour incidence in Group 3 was significantly higher as compared to a group studied earlier (5/12 tumours) where mammmectomised females had received DMBA alone. The larger yield of tumours might be due to testosterone treatment. In some ways the action of testosterone was not unlike pseudopregnancy which aggravated and enhanced ovarian tumorigenesis. In pseudopregnancy, ovulation was prevented because the follicles remained quiescent and retarded. In the testosterone-treated animals, the ovulation was inhibited by lipoid degeneration of follicles. While pseudopregnancy suppressed vigorously growing follicles, testosterone caused their degeneration. Estrus was inhibited in both as a consequence.

DMBA, which is a known initiator, induces ovum and follicle degeneration and inhibits new growth of follicles. Absence of follicles was seen to be an essential prerequisite for abnormal thecal luteinisation which lead to development of granulosa cell tumours. On the other hand, testosterone caused follicle degeneration but it did not affect their formation potential, that is, new follicles continued to develop.
In Group 2, where testosterone alone was given, ovarian tumours were not induced, so that testosterone can be considered only a promoter and not an initiator.

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REFERENCES
3) Hartwell, J.L., "Survey of Compounds which have been Tested for Carcinogenic Activity," (1951).
   Charles C. Thomas, Springfield, Ill.

EXPLANATIONS OF PLATE LI

Photo 1. Part of a mixed tubular adenoma type of ovarian tumour. C3H(Jax) mouse aged 11 months. H.-E. x 190

Photo 2. Part of a granulosa cell tumour showing pseudofollicles. C3H(Jax) mouse aged 11 months. H.-E. x 380

Photo 3. Undifferentiated part of the tumour shown in Photo 2. H.-E. x 380

Photo 4. Part of a large granulosa cell tumour undergoing myxomatous type of change. C3H (Jax) mouse aged 9 months. H.-E. x 190

Photo 5. Early beginnings of a subcutaneous fibrosarcoma. C3H(Jax) mouse aged 7 months. H.-E. x 380

Photo 6. Part of a large subcutaneous fibrosarcoma. Note the degeneration and liquefaction of the muscle layer towards the right. C3H(Jax) mouse aged 8 months. H.-E. x 114

H.-E. = Hematoxylin and Eosin stain