The Effect of Estrone Alone and Combined with 20-Methylcholanthrene on Mouse Prostate Glands Grown in Vitro

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In previous experiments, which form part of a larger investigation on the direct action of hormones and carcinogens on mammalian tissues in vitro, the effect of 20-methylcholanthrene on mouse prostate glands was analyzed. This carcinogen was found to produce by direct action increase in cell division, foci of anaplastic cells with polyploid mitotic figures, hyperplasia, and squamous metaplasia of the alveolar epithelium (19).

The second part of the work is concerned with the influence of the sex hormones on this process. Estrogens used in the therapy of prostatic cancer in man usually cause a temporary regression of primary and secondary tumors (12, 15, 16, 23). In experimentally induced glandular carcinomas of the mouse prostate, the administration of estrogen is likewise followed by a retardation of growth (10). Two different modes of action may be responsible for this effect: (a) estrogen restricts the production of androgenic hormones by inhibiting the output of gonadotropic hormone in the pituitary, and the cancer cells still dependent on hormonal supplies break down; (b) estrogen damages the cancer cells directly (4).

The organ culture method is admirably adapted to the study under controlled experimental conditions of the direct effect of the hormone alone, and of the hormone and carcinogen combined. Any changes in the gland can be followed in detail from an early stage.

Before examining the combined action of estrogen and methylcholanthrene on the mouse prostate in vitro, it seemed necessary to study the effects of estrogen alone. In vitro, the hormone induces atrophy, followed by hyper- and metaplasia of the epithelium and an increase of the fibromuscular stroma in the prostate glands of most animal species (2, 3). Recently, it was claimed that estrogen does not affect the organs as such, but is activated by the liver (24); the addition of the hormone to organs growing in vitro should settle this point.

The paper is divided into two parts: in the first, mouse prostate glands were grown in vitro with the addition of two doses of estrone; in the second part, estrone and 20-methylcholanthrene were added either simultaneously or successively to the culture medium. In two series prostates obtained from young as well as older mice were used, to see whether age would influence the results.

MATERIALS AND METHODS

Prostate glands were obtained from CSH mice approximately 6 weeks of age and from the same strain, aged 6 months. The ventral prostates were used. They were grown by the watchglass technic in a medium consisting of 4 drops of fowl plasma, 2 drops of rat plasma, and 4 drops of chick embryo extract. To prevent infection, 250-300 units of Penicillin G (Glaxo) were added to this mixture. The explants were placed, well flattened out, on the surface of the clot to which they became attached, and were transferred to a fresh medium every 3 days.

The addition of estrone.—The hormone used was estrone (Menformon, Organon) in aqueous solution. One lobe of the paired gland was treated with the hormone, while the other was kept as control. The experiments were designed as follows:

1. Administration of 2 µg or 4 µg of estrone/ml of culture medium for periods of 10 days to 3 weeks, respectively, to glands from 6-week-old mice.
2. Administration of 2 µg of estrone to glands from 6-week-old mice for 10 days, followed by cultivation in normal medium for 15 days.
3. Administration of 2 µg or 4 µg of estrone to glands obtained from 6-month-old mice for periods of 10 days and 3 weeks, respectively.

The control explants received a corresponding amount of sterile distilled water.

The addition of combined estrogen and 20-methylcholanthrene.—In three experiments (see "Results," III, 1a, 2, and 3) the glands were divided into four corresponding parts: one received normal medium, the second estrone alone, the third methylcholanthrene (MC control), and the fourth methylcholanthrene and estrone combined. In the fourth (see "Results," III, 1b) the glands were halved, one half receiving methylcholanthrene (MC control), the other methylcholanthrene and estrone. The experiments were designed as follows:

1. Administration of 2 µg of estrone and 4 µg of methylcholanthrene/ml of medium for a period of 10 days to glands from (a) mice aged 6 weeks and (b) mice aged 6 months.

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2. Administration of 2 µg. of methylcholanthrene to glands from young animals for a period of 10 days followed by cultivation in a medium containing 1 µg. of estrone (M+E).

3. Administration of 1 µg. of estrone to glands from young animals, for a period of 10 days followed by cultivation in a medium containing 2 µg. of methylcholanthrene (E+M). Approximately 100 mice were used for the experiments. Six to ten explants of each type, control and experimental, were fixed for each point of observation. They were fixed in 3 per cent acetic Zenker after 10 days' and 3 weeks' growth and serially sectioned; slides were stained with hematoxylin-eosin or with Schiff's reagent following periodic acid treatment.

The incidence of mitosis was assessed by counting all the cell divisions present in every second section, i.e., in about twenty sections of experimental and control explants. In the first part of the paper, dealing with the effects of estrone alone, this was expressed as a percentage of the mitoses found in untreated control explants. In the second part, the mitotic rate in cultures treated with estrone and methylcholanthrene was expressed as a percentage of that observed for cultures treated with methylcholanthrene alone.

RESULTS

The ventral prostate gland consists of alveoli lined with cylindrical, slightly folded epithelium of ducts and alveoli, especially in those situated in the center of the explant, combined with dilatation of their lumen; the folding seen in vivo was usually reduced in vitro. The newly formed peripheral alveoli were mostly straight, lined with cuboidal epithelium, and often possessed one layer of reserve cells; mitoses were present in the luminal epithelium but, as in vivo, were not frequent. The stroma was always well developed, and there was usually a diffuse proliferation of fibroblasts and fibers throughout the gland which invaded the spaces not occupied by the parenchyma.

Of 109 control cultures analyzed, eighteen showed slight epithelial hyperplasia, i.e., an increase in the layers of reserve cells confined to one side of the alveolar wall (Fig. 8). In such cultures the rest of the alveoli showed little or no de-differentiation and were lined with high secretory epithelium arranged in folds. The distribution of this mild hyperplasia among untreated cultures fixed after different intervals of growth suggested that the incidence is related to the length of the culture period; thus, in groups fixed up to the 20th day it was of the order of 10-14 per cent, while after 30 days it rose to 50 per cent, a significant difference (Table 1).

I. OBSERVATIONS ON UNTREATED GLANDS IN VITRO

The living explants appeared clear, with translucent edges and, as a sign of active growth, liquefied the medium in their immediate neighborhood. Two types of proliferation could be distinguished: the migration of unorganized fibroblasts and epithelial cells to form a diffuse zone of growth which was removed at each transfer and the formation of new differentiated alveoli at the edge of the explant which were preserved at subcultivation.

Stained serial sections of untreated cultures (Fig. 1), fixed after 10 days' and 3 weeks' growth in vitro, showed a flattening of the lining epithelium of ducts and alveoli, especially in those situated in the center of the explant, combined with dilatation of their lumen; the folding seen in vivo was usually reduced in vitro. The newly formed peripheral alveoli were mostly straight, lined with cuboidal epithelium, and often possessed one layer of reserve cells; mitoses were present in the luminal epithelium but, as in vivo, were not frequent. The stroma was always well developed, and there was usually a diffuse proliferation of fibroblasts and fibers throughout the gland which invaded the spaces not occupied by the parenchyma.

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II. ADMINISTRATION OF ESTRONE ALONE

1. The effect of 2 µg. and 4 µg. of estrone on explants from young mice (Table 2).—Explants treated with estrone were often larger and more translucent than the control cultures at the be-

Table 1

<table>
<thead>
<tr>
<th>Duration of cultivation (days)</th>
<th>Percentage of hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>35</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Effect of Estrone on Prostate Glands from 6-Week-Old Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µg.</td>
</tr>
<tr>
<td>10 days in vitro</td>
</tr>
<tr>
<td>No. explants</td>
</tr>
<tr>
<td>Hyperplasia</td>
</tr>
<tr>
<td>Metaplasia</td>
</tr>
<tr>
<td>Struma increase</td>
</tr>
<tr>
<td>Mitoses, per cent controls</td>
</tr>
</tbody>
</table>

* = Slight.  ++ = Pronounced.
ginning of the culture period but became denser toward the end.

Two µg. of estrone: Serial sections of most cultures fixed after 10 days' growth showed hyperplasia of some alveoli, while in others the epithelium was flattened and the lumen dilated (Fig. 2).

In general, the degree of hyperplasia varied in different alveoli of the same explant. In the initial stage, the cuboidal cells lining the alveolus were enlarged, and the layers of reserve cells had increased from one to three or four; cell division was frequent among both luminal and reserve cells (Fig. 3), and the mitotic count was 151 per cent of that seen in untreated controls. At a later stage, the layers of reserve or basal cells had multiplied to ten or twelve, spreading in a centrifugal direction and pressing the basement membrane outwards. The cells were unchanged at this stage and show large oval or round nuclei with relatively little cytoplasm.

Each tubule was surrounded by a concentric ring of muscle and connective tissue fibers. Among the latter were fibroblasts which were orientated with their long axes parallel to the alveolar lumen and may be identical with those described by Wells (27) in the prostate gland of estrogen-treated ground squirrels. This concentric layer was more conspicuous around alveoli which were not yet hyperplastic or in the early stages of hyperplasia; it became thinner with increasing proliferation of the basal cells and often disappeared if two or more hyperplastic alveoli fused.

After 3 weeks' growth the hyperplasia was more extensive. The proliferation of the basal cells which was originally orientated outward, i.e., away from the lumen, was now directed inward, either compressing or obliterating the cavity in the process (Fig. 4).

Cell division was abundant, and the mitotic count was 524 per cent of that in the controls. Often abnormal mitotic figures could be distinguished in meta- and anaphase showing clumping, breakage, and dislocation of chromosomes. Polyploidy was not observed in contrast to the methylcholanthrene-treated glands.

Squamous metaplasia (Fig. 5) began in cells occupying the central parts of a hyperplastic alveolus which became irregular in shape and enlarged while their nuclear membranes became indented. The enlargement was due to an increase of the cytoplasm which now stained a deeper pink with eosin. In more advanced stages eight to ten layers of such squamous cells were surrounded by one or two layers of basal cells which still showed mitotic activity; cornification was present but not marked.

2. The effect of 4 µg. of estrone on explants from young mice.—Explants treated with 4 µg. of estrone showed similar changes, but the dilatation of the alveolar lumina and the flattening of the epithelium was greater and led to degeneration and desquamation, while the incidence and extent of hyperplasia were somewhat reduced as compared with glands treated with the lower dose. The squamous changes, on the other hand, were slightly more pronounced. There was a small rise in the number of mitotic cells at 10 days' growth to 116 per cent of controls, but at 3 weeks it had fallen below the control value to 76 per cent as against the steady rise observed after the lower dose of hormone.

3. The effect on explants from young mice of 2 µg. of estrone for 10 days, followed by cultivation in normal medium for 15 days.—In glands treated in this manner epithelial hyperplasia was observed in nine of eleven explants after 25 days' growth (Fig. 6). Mitotic counts, however, were below the control level, while squamous metaplasia was absent (Table 2).

4. The effect of 2 µg. and 4 µg. of estrone on glands taken from 6-month-old mice.—In such explants epithelial hyperplasia was less frequent and less extensive than in glands from young animals and varied inversely with the dose. After exposure to the lower concentration, half the cultures showed slight hyperplasia; after exposure to the higher, only one out of ten (Table 3). In the latter, cystic dilatation of alveoli was widespread and necrosis of the alveolar epithelium common.

The fibromuscular stroma, on the other hand, was greatly increased, particularly after the higher dose. Most of the alveoli were surrounded by a wide dense zone of collagenous and muscle fibers; stromal elements filled the interalveolar spaces and absorbed and replaced necrotic alveoli in the process (Fig. 7). After the lower dose, mitosis

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**TABLE 3**

<table>
<thead>
<tr>
<th>No. explants</th>
<th>2 µg. in vitro in 10 days</th>
<th>4 µg. in vitro in 10 days</th>
<th>2 µg. in vitro in 21 days</th>
<th>4 µg. in vitro in 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td>1+/*</td>
<td>3++</td>
<td>2++</td>
<td>2++</td>
</tr>
<tr>
<td>Metaplasia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stroma</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td>10++</td>
</tr>
<tr>
<td>Mitoses, per cent controls</td>
<td>130 ± 10.2</td>
<td>48 ± 5.9</td>
<td>294 ± 30.0</td>
<td>160 ± 14.8</td>
</tr>
</tbody>
</table>

* + = Slight.
++ = Pronounced.
was slightly and temporarily increased; after the higher dose the mitotic increase was greater. In spite of the numerous cell divisions, hyperplasia occurred in only one out of ten explants, and it must be assumed that it was counter-balanced by degeneration of the epithelial elements.

5. Comparison of the effects of estrone on mice aged 6 weeks and 6 months.—The experiments described above show that estrone added to the medium of prostate glands derived from 6-week-old mice produced hyper- and metaplasia as well as some atrophy of the alveolar epithelium, and that with the smaller dose hyperplasia was more pronounced and atrophy less than with the higher, and vice versa. Cell division was increased to 5 times the control value after continuous application of the small dose but was reduced to half the control value after the higher. In contrast to in vivo results hyperplasia persisted after withdrawal of the hormone.

TABLE 4

<table>
<thead>
<tr>
<th>No. explants</th>
<th>Hyperplasia</th>
<th>Metaplasia</th>
<th>Stroma present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal medium</td>
<td>12</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Estrone 4 µg.</td>
<td>2+</td>
<td>1</td>
<td>4+</td>
</tr>
<tr>
<td>Methylcholanthrene 4 µg.</td>
<td>10</td>
<td>4+</td>
<td>3+</td>
</tr>
<tr>
<td>Estrone and Methylcholanthrene 4 µg.</td>
<td>11</td>
<td>3+</td>
<td>3+</td>
</tr>
</tbody>
</table>

Mitoses, (M+E)/MX100: 81 ± 3.4
Degeneration of hyperplastic epithelium: + = Slight, ++ = Pronounced.

Estrone treatment of explants from 6-month-old mice inhibited hyperplasia; after the smaller dose it was infrequent, and after the higher dose it was not only extremely rare, but atrophy of the secretory epithelium was often observed. There was, however, a considerable increase in the fibromuscular stroma of the older glands after treatment.

III. SIMULTANEOUS AND SUCCESSIVE ADMINISTRATION OF ESTRONE AND Methylcholanthrene

1. Simultaneous addition of 2 µg. of estrone and 4 µg. of methylcholanthrene for a period of 10 days.—
   a) Glands from young mice (Table 4): The incidence of hyperplasia in these explants was about the same as in glands treated with either the hormone or the carcinogen alone, if we take into consideration the fact that the concentration of estrone used was half that of the carcinogen. Squamous metaplasia was greater in explants grown with both substances (Fig. 9) than in MC controls in which the epithelium retained its glandular character during the first stage of hyperplasia (Fig. 10). Glands grown with either estrone or with estrone and carcinogen combined usually contained more stroma than those grown with methylcholanthrene alone or than untreated controls, results which indicate that the hormone stimulates both metaplasia and stromal growth. Mitosis in cultures treated with both substances was slightly lower than in those grown with the carcinogen only.

   b) Glands from 6-month-old mice (Table 5): Explants treated with both substances (Fig. 11) showed much less hyper- and metaplasia than those grown with the carcinogen alone. Many of the alveoli were well differentiated, had a winding course, and high cylindrical epithelium. Such hyperplasia as existed consisted of one or two rows of reserve cells. Mitoses were scarce and reduced to 40 per cent of those counted in MC controls, but the connective tissue was greatly increased. Trabeculae of muscle and collagenous fibers surrounded alveoli in dense concentric layers and spread diffusely into the interalveolar spaces.

2. Addition to glands from young mice of 2 µg. of methylcholanthrene for 10 days followed by 1 µg. of estrone for 11 days (Table 6).—The administration of estrone to explants previously treated with the carcinogen was followed by a reduction of hyperplasia but an increase of metaplasia, as compared with cultures transferred to normal medium after withdrawal of the carcinogen (Figs. 12, 13). Analysis of individual metaplastic foci
usually showed a larger proportion of differentiated, squamous cells than in those produced by the carcinogen alone. Frequently, widespread destruction occurred in the hyperplastic epithelium (Fig. 14) before and after squamous transformation. In the former, the cells enlarged, the cytoplasm became vacuolated, and nuclei underwent pyknosis. Degeneration of squamous epithelium usually appeared in occluded alveoli; here most of the "filling," consisting of squamous cells and cells in various stages of keratinization, was shed in one mass, leaving an almost empty alveolus behind (Fig. 15).

Cell division was decreased to half of that seen in explants treated with the carcinogen alone, a result which fits in well with the inhibition of hyperplasia and extensive degeneration observed.

On the other hand, the addition of estrone alone to cultures previously grown in normal medium did not produce any marked degeneration or increase of squamous metaplasia (Table 6).

3. Addition to glands from young mice of 1 μg. of estrone for 10 days followed by administration of methylcholanthrene for 11 days (Table 7).—These explants showed an incidence of hyperplasia similar to that in those which received the carcinogen only (Table 7), but degeneration of the hyperplastic epithelium was widespread and similar in type to that observed in series 2. Cell division was also considerably less than in the MC controls (61 per cent). In explants treated with estrone and transferred to normal medium, degeneration and metaplasia were absent.

4. Comparison of the effect of estrone and 20-methylcholanthrene with that of the carcinogen alone.—Simultaneous administration of estrone and carcinogen to prostate glands of young mice slightly increased squamous metaplasia and the connective tissue, while the incidence of hyperplasia was similar to that in explants grown with the carcinogen alone; in explants from older mice it inhibited the hyperplasia normally seen after methylcholanthrene treatment and produced considerable stromal growth.

Addition of estrone to cultures previously treated with the carcinogen and of methylcholanthrene to explants previously grown with estrone inhibited cell division, increased the incidence of metaplasia, and caused widespread degeneration of the hyperplastic and metaplastic epithelium. These effects were more pronounced if estrone was added after the carcinogen than vice versa.

**DISCUSSION**

The experiments show that estrogens are capable of affecting the prostatic epithelium by direct action and cause similar changes to those described in vivo. This result casts some doubt on the contention of Szego and Roberts (24) that estrogens do not become effective unless activated by the liver and on that of Chamorro (5), who claims that the action of estrogens on the prostate gland is an indirect one, by restriction of androgenic hormones.

In glands derived from young animals, the type and degree of response vary with the dose employed; thus, stimulation is prevalent with the smaller dose, while atrophic changes and squamous metaplasia are increased with the higher concentration.

The squamous transformation observed clearly arises among undifferentiated basal cells, which fact agrees with the findings of Bern (1) on estrogen-treated rat prostates in vivo as well as with those of Meyer (20) and Motylloff (21), who studied spontaneous squamous changes in the human uterus. Meyer strongly denies the existence of any transformation other than that origi-
nating in basal cells, in contrast to Kaufmann (17), who claims that a direct metaplastic change of the fully differentiated glandular epithelium is possible.

The alteration in response to age may be due to the higher level of androgen present in older animals. Laqueur (18) and de Jongh (13) demonstrated that the interaction of androgen and estrogen may be antagonistic or synergistic, depending on the relative amounts of hormones present. It is possible that the longer exposure to androgens of the prostate from older mice may cause an antagonistic response to estrogen, i.e., atrophy of the epithelium and stimulation of the stroma, while a similar dose administered to prostate glands from young mice may produce a synergistic effect, i.e., stimulation of the epithelium.

A similar change in effect was reported by Wells (27) in the prostate gland of ground squirrels treated with estrogen in and out of the breeding season.

The stromal stimulation seen in this work also recalls the hypertrophy of the fibromuscular stroma in elderly men which, according to Burroughs (4) and de Jongh (14), is due to a relative increase of the estrogen level following a lowered androgen output.

The prolonged persistence in vitro of epithelial hyperplasia after withdrawal of estrone is probably due to the absence of a circulatory system which favors the retention of the hormone within the cells, while in vivo it is quickly removed and inactivated by the liver (22, 25, 26).

A comparison of the direct effects of either estrogen or 20-methylcholanganthrene shows that both substances cause stimulation of cell division leading to hyperplasia of basal cells and squamous metaplasia, but, in the case of estrogen, this effect is confined to a narrow dose range and depends on the age of the animal.

Withdrawal of the carcinogen is followed by further proliferation with high mitotic activity, but after withdrawal of the hormone the changes are gradually reversed. The hormone and the carcinogen also produce different types of abnormal mitotic figures: breakage and dislocation of chromosomes are seen after estrone, often leading to cellular disintegration, whereas polyploidy follows treatment with methylcholanganthrene and results in the formation of viable multinucleate or large mononucleate daughter cells.

In these experiments the combined effect of the two substances, like that of estrone alone, varies with the age of the animal.

Except for a slight increase in stroma and acceleration of metaplasia, the addition of estrone to methylcholanganthrene has little influence on explants from young animals. Horning (11), using a combination of stilbestrol and methylcholanganthrene on prostate grafts in young mice, reported an acceleration of carcinogenesis as compared with grafts treated with the carcinogen only. However, the experiments are not strictly comparable, since in his work the presence of androgenic hormones during carcinogenesis may have influenced the final results.

Simultaneous addition of estrone and methylcholanganthrene to the medium of older glands inhibits the incidence of hyperplasia and strongly enhances stromal growth as compared with MC-treated explants. This result suggests that the hormone counteracts the effect of the carcinogen by stimulating stromal proliferation and reducing epithelial hyperplasia.

The cellular breakdown and increase in squamous metaplasia which occur if estrone is added to explants previously grown with methylcholanganthrene and vice versa cannot be due to a summation effect, since trebling the dose (see Table 2) does not produce anything similar if the two agents are given simultaneously. It must be remembered, however, that when the two compounds are administered together they act on unchanged differentiated cells, while successive administration entails exposure of an already altered epithelium. The degeneration found is histologically similar to that described recently by Fergusson and Franks (7) in cases of human prostatic cancer following estrogen therapy, a result which indicates that, apart from possible indirect effects via the restriction of androgenic hormones, estrogens are able to affect prostatic cancer cells directly.

The occurrence of spontaneous hyperplasia in normal untreated cultures is of interest in connection with Earle’s (6) and Gey’s (9) findings on the malignant transformation of mouse and rat fibroblasts after prolonged cultivation in vitro. More recently, Goldblatt and Cameron (8) induced a malignant change in mouse fibroblasts kept alternately under aerobic and anaerobic conditions in vitro and concluded that enforced anaerobiosis may cause a somatic mutation of adaptable cells.

In the present experiments the incidence of hyperplasia is related to the length of the culture period. It is unlikely, however, that the increased proliferation of the reserve cells is due to somatic mutation caused by adverse conditions in vitro, since it usually occurs in healthy and well growing explants. An alternative explanation may be the absence of a control mechanism in vitro which in vivo would regulate the balance of cell division.
and differentiation. Thus, reserve cells which in
the organism would form secretory epithelium
may not differentiate in vitro but continue to
divide instead.

SUMMARY

Ventral prostate glands from 6-week- and 6-
month-old mice were grown in vitro by the watch-
glass technic for 10–21 days with addition of two
doses of estrone, with 20-methylcholanthrene, or a
combination of estrone and 20-methylcholanthrene,
given either simultaneously or successively.

Addition of 2 µg. of estrone to the culture
medium of glands derived from young mice directly
induced hyper- and squamous metaplasia of the
prostatic epithelium. A dose of 4 µg. of estrone
was followed by a lower degree of hyperplasia and
some atrophy.

After withdrawal of the hormone the hyper-
plastic changes persisted longer than in vivo.

The addition of estrone to glands derived from
older animals caused considerable stimulation of
the fibromuscular stroma, with atrophy of the
alveolar epithelium.

20-Methylcholanthrene induced hyperplasia
and squamous metaplasia of the prostatic epithe-
ilum in glands derived from both young and
older animals.

Simultaneous addition of estrone and 20-
methylcholanthrene to glands derived from young
animals increased squamous metaplasia, while the
incidence of hyperplasia was similar to that seen
in explants treated with 20-methylcholanthrene
alone.

The same combination administered to glands
from older mice inhibited the hyperplasia seen in
explants treated with the carcinogen alone, and
caused considerable stromal increase.

Successive administration of either estrone to
explants previously treated with the carcinogen or
of 20-methylcholanthrene to explants previously
exposed to estrone, resulted in inhibition of mitot-
ic stimulation, increase of squamous metaplasia,
and degeneration of the hyperplastic epithelium.

Normal untreated control explants showed a
mild degree of hyperplasia in 10–50 per cent, with
an average of 17 per cent of all cases; the incidence
depended on the length of cultivation.

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Compounds in the Coagulating Glands and Prostate of the

Fig. 1.—Ventral prostate gland from a young mouse after
10 days’ cultivation in vitro. Untreated control. Hematoxylin-
eosin, X 175.

Fig. 2.—Explant from a young mouse after 10 days’ growth
with 2 µg. of estrone, with hyperplasia of the alveolar epithe-
ilum mainly directed outwards. Hematoxylin-eosin, X 150.

Fig. 3.—Alveolus in similar explant showing development of
hyperplasia. Note multiplication of basal cells with normal and
abnormal mitosis. Hematoxylin-eosin, X 620.

Fig. 4.—Explant from young mouse grown for 8 weeks in
the presence of 2 µg. of estrone, showing pronounced hyper-
plasia and squamous metaplasia with occlusion of alveoli.
Hematoxylin-eosin, X 220.

Fig. 5.—Hyperplastic alveolus in similar explant showing
transition to squamous metaplasia and degeneration of
secretory epithelium. Hematoxylin-eosin, X 620.

Fig. 6.—Persistence of hyperplasia in explant treated with
estrone for 10 days and kept in normal medium for a further
15 days. Hematoxylin-eosin, X 90.

Fig. 7.—Inhibition of hyperplasia with atrophy and de-
genation of the alveolar epithelium and proliferation of the
fibromuscular stroma in explants from older mice grown for 8
weeks in the presence of 4 µg. of estrone. Schiff’s reagent,
X 100.
Fig. 8.—Control explant after 3 weeks' growth, showing mild hyperplasia of the alveolar epithelium. Hematoxylin-eosin, X 200.

Fig. 9.—Ventral prostate from young mouse treated with 4 μg. of 20-methylcholanthrene and 2 μg. of estrone for 10 days. Note increase in squamous metaplasia, as compared with Figure 10. X 165.

Fig. 10.—Similar explant treated with 4 μg. of 20-methylcholanthrene for 10 days. (MC control) showing hyperplasia and retention of glandular character of some alveoli. Hematoxylin-eosin, X 165.

Fig. 11.—Explant from older mouse treated for 10 days with 4 μg. of 20-methylcholanthrene and 2 μg. of estrone, showing very mild hyperplasia and increased proliferation of fibromuscular stroma. Hematoxylin-eosin, X 200.

Fig. 12.—Explant from young mouse treated for 10 days with 2 μg. of 20-methylcholanthrene and maintained in normal medium for a further 11 days, with marked hyperplasia and squamous metaplasia. Hematoxylin-eosin, X 150.

Fig. 13.—The same at higher magnification. Note predominance of basal cells and mitotic cells. Hematoxylin-eosin, X 370.

Fig. 14.—Similar explant grown with 2 μg. of 20-methylcholanthrene for 10 days then treated with 1 μg. of estrone for 11 days. Note degeneration of alveoli. Hematoxylin-eosin, X 150.

Fig. 15.—Degeneration in metaplastic alveolus in explants from young mice grown with 20-methylcholanthrene for 10 days followed by estrone treatment. Hematoxylin-eosin, X 285.
Lasnitzki—Effect of Estrone and MC on Prostate


The Effect of Estrone Alone and Combined with 20-Methylcholanthrene on Mouse Prostate Glands Grown *In vitro*

Ilse Lasnitzki


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