The Effect of Methylcholanthrene on Rat Prostate Glands Grown in Natural and Semi-defined Medium

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SUMMARY

The effects of 20-methylcholanthrene (MC) on organ cultures of ventral prostate glands from rats of different ages grown in natural and semi-defined medium were studied.

In the natural medium MC induced extensive epithelial hyperplasia and partially inhibited the growth of the connective tissue within a short period of treatment. The hyperplastic epithelium consisted of undifferentiated and columnar cells without orientation, some of which were transformed into prickle cells. These were similar in explants derived from young and older animals. In explants treated for 10 days and transferred to control medium the hyperplasia was reduced, and the growth of the stroma resumed. In the semi-defined medium the effect of the carcinogen was much less marked and limited to an increase in cell size and formation of papillary projections.

In earlier work it has been shown (4, 5, 8) that 20-methylcholanthrene directly provoked changes of a preneoplastic nature in organ cultures of mouse ventral and anterior prostate glands and that this effect could be modified by addition of estrone.

The present paper is part of a larger investigation of the direct action and interaction of 20-methylcholanthrene and various hormones on the ventral prostate gland of the rat grown in organ culture. The first part of the work is concerned with the effect of 20-methylcholanthrene alone.

To ascertain whether the age and functional state of the gland influence its response to the carcinogen, explants from two age groups, 5- to 7-week and 10-week rats were grown in natural medium in the presence of methylcholanthrene. Natural medium, however, contains small, unknown amounts of hormones; these as well as other nutritional factors may modify the effect of the carcinogen. To explore this question cultures from the younger age group were also kept in semi-defined medium, and the action of the carcinogen under the two conditions was examined and compared.

MATERIALS AND METHODS

The material was obtained from a laboratory inbred strain of hooded rats, 5-7 and 10 weeks old. The animals were killed by dislocation of the cervical vertebrae, and the ventral prostate gland was removed under sterile conditions. The organ was freed from its surrounding sheath of fat and connective tissue and pulled apart...
gently into its natural subdivisions. The use of the knife was avoided as much as possible to prevent a regenerative hyperplasia at the cut edge. For cultivation in natural medium Shaffer's (11) modification of the watch-glass technic of Fell and Robison (2) was used. The pieces were approximately 2 × 4 × 2 mm. in size and were arranged on strips of rayon acetate; two of these strips holding three cultures each were then placed on a clot contained in a watchglass. The clot consisted of 1 mm. of medium made up of cock plasma, horse serum, and dilute chick embryo extract in a proportion of 3:1:1.

The carcinogen was first dissolved in acetone and the solution suspended in horse serum. A given quantity of this suspension was added to the medium before it clotted, and the final concentration was 4 μg/ml of medium. The control cultures received medium to which a corresponding amount of acetone had been added.

For cultivation in semi-defined medium the rayon strips holding the explants were supported on metal grids placed in small, flat-bottomed dishes of borosilicate glass; the dishes were filled with medium, usually 2.5 cm., up to the level of the explants. The medium used was Parker 199 with 5 per cent horse serum.

The watchglass containing the clots and the dishes holding the grids and the fluid medium were enclosed in Petri dishes carpeted with damp cottonwool; the Petri dishes were stacked in a tightly closed desiccating jar and placed in an incubator. The desiccating jar was perfused with a mixture of 95 per cent oxygen and 5 per cent CO₂ for 45 minutes at a flow rate of 100 ml/min after explantation and after each transfer; between transfers it was perfused for 20 minutes only.

The cultures were incubated at 37.5°C. and transferred to fresh medium every 3–4 days; fresh carcinogen was added at each transfer. The explants grown in natural medium were cultured with the carcinogen for periods of up to 18 days and fixed after 10, 14, and 18 days' growth. One set was treated with the carcinogen for 10 days and transferred to normal medium for 4 days. Cultures kept in the semi-defined medium were exposed to methylicholanthrene for 10 days.

The explants were fixed in 3 per cent acetic Zenker's solution, embedded in paraffin, and sectioned at 6μ. The sections were stained by the periodic acid-Schiff technic.

Mitosis was assessed in control and experimental cultures by counting all epithelial cells in alternate sections of at least six cultures of each group, and the result was expressed as a percentage of resting cells.

In all, 111 explants were studied.

RESULTS

The ventral prostate gland of the rat consists of tubuli and alveoli lined with slightly convoluted columnar secretory epithelium. The sections show very little connective tissue between and around tubuli and alveoli (Fig. 1).

GROWTH PATTERN IN CONTROL MEDIUM

Natural Medium

Explants from 5- to 7-week rats.—After 10 days' growth the cultures had undergone a striking transformation, which resembled the regression seen in the glands after castration of the animal (Figs. 2, 3). There were fewer tubuli and alveoli per unit area; their lumina had narrowed greatly, the lining epithelium had become lower owing to a loss of cytoplasm, and the nuclei were more intensely basophilic. In some alveoli a slight heaping of cells could be recognized. The cells and fibers of the connective tissue had considerably increased and filled the inter-alveolar spaces.

Semi-defined Medium

Explants from 10-week rats.—In these the normal structure and function of the epithelium was much better maintained for at least 2 weeks in culture (Fig. 4), and there was no increase in the connective tissue. Although the cells were slightly lower than in the older gland in vivo, they secreted more actively; secretory matter could be seen exuding from their apical surface and filling the alveolar lumen (Fig. 9). In explants grown for 18 days, however, the cells had become lower and showed less secretion, the lumina were diluted, and the connective tissue was slightly increased.

EFFECT OF 20-METHYLCHOLANTHRENE (MC)

Natural Medium

The carcinogen stimulated mitosis and cell proliferation and induced a striking hyperplasia of the alveolar epithelium (Table 1). This effect was identical in explants from both age groups. After 10 days' growth in the presence of MC, eighteen out of 22 treated cultures showed hyperplastic changes, and in most of them at least half the number of alveoli were affected (Fig. 5). In any given explant different stages of the process could be observed. Usually the first recognizable response to the carcinogen was an enlargement of cells; this was followed by increased proliferation of the reserve cells situated between the secretory cells and the basement membrane, resulting in a protrusion of several cell layers into the alveolar lumen (Fig. 10). At a later stage the newly formed cells partially or completely occluded the cavity. Alveoli with such extensive hyperplasia were usually found at the periphery of the explant.

The hyperplastic epithelium (Fig. 12) consisted of small cells with round nuclei and columnar elements with oval nuclei crowded closely together, sometimes in a disorderly manner. Both cell types varied greatly in nuclear and cell size and in a few cultures were seen to be connected by fine tonofibrils. The secretory cells lining the lumina were preserved in many hyperplastic alveoli and continued to function until the cavities were almost occluded.

The increase of the connective tissue which was so
prominent in the control explants was much less in the presence of MC, but the stroma contained more cells and fibres than the organ in vivo.

In all explants exposed to the carcinogen for 14 and 18 days many alveoli showed pronounced hyperplasia, and the connective tissue was diminished (Figs. 6, 8). Some of the alveoli were filled with a dense mass of cells; in others the lumen persisted, and the newly formed cells spread in an outward direction. In such alveoli the secretory elements had usually been shed and not replaced. The hyperplastic epithelium was largely composed of columnar, small round, or irregularly enlarged cells, but in some areas prickle cells with pale nuclei and connecting tonofibrils were found; such cells were more frequent after the longer periods of treatment (Fig. 11) than in cultures fixed after 10 days.

In explants exposed to MC for 10 days and then transferred to normal medium for 4 days, the number of hyperplastic alveoli was considerably smaller than in cultures exposed continuously to the carcinogen. The effect was mainly confined to the peripheral alveoli, which showed extensive hyperplasia, but in the remainder of the explant many of the alveoli were normal (Fig. 7). There was also more connective tissue than in explants treated continuously with MC.

Mitosis.—Mitotic counts of control and experimental cultures (Table 1) showed a significant increase of cell division in the explants treated continuously with the carcinogen. After 10 and 14 days’ exposure mitosis was approximately 5 times, and at 17 days 9 times, that of the control value. In explants transferred to normal medium after 10 days’ growth in the presence of MC, mitosis had fallen and was no longer significantly raised above that of the controls.

In nearly one-quarter of the mitotic cells abnormalities of various types could be distinguished. These included dislocation and clumping of chromosomes, diminution or complete absence of one-half of the chromosome complement in anaphase, and occasional binucleate cells in division (Fig. 13).

### TABLE 1

**Effect of 20-Methylcholanthrene on Rat Ventral Prostates Grown in Natural Medium**

<table>
<thead>
<tr>
<th>Duration of treatment (days)</th>
<th>Hyperplasia</th>
<th>Percentage of mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MC-treated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4/22†</td>
<td>12/22‡</td>
</tr>
<tr>
<td></td>
<td>0.13 ± 0.021</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>14</td>
<td>4/8‡</td>
<td>4/8†</td>
</tr>
<tr>
<td></td>
<td>0.14 ± 0.03</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td>10 on MC, 4 on normal medium</td>
<td>3/7‡</td>
<td>4/7‡</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.03</td>
<td>0.22 ± 0.024</td>
</tr>
<tr>
<td>18</td>
<td>6/6†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.03</td>
<td>0.9 ± 0.13</td>
</tr>
</tbody>
</table>

* Number of treated cultures.
† Most of the alveoli show marked hyperplasia.
‡ Half to two-thirds of the number of alveoli show medium to marked hyperplasia.
§ Less than half the number of alveoli show mild hyperplasia.

In nearly one-quarter of the mitotic cells abnormalities of various types could be distinguished. These included dislocation and clumping of chromosomes, diminution or complete absence of one-half of the chromosome complement in anaphase, and occasional binucleate cells in division (Fig. 13).

### TABLE 2

**Effect of 20-Methylcholanthrene on Rat Ventral Prostate Glands Grown in Semi-defined Medium**

<table>
<thead>
<tr>
<th>Duration of treatment (days)</th>
<th>No change</th>
<th>Cellular enlargement only</th>
<th>Cellular enlargement, papillary projections, spreading of cells into lumen</th>
<th>Mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/14*</td>
<td>5/14</td>
<td>4/14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.04 ± 0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of treated cultures.

### DISCUSSION

In the natural medium the ventral prostate glands from the younger rats rapidly undergo regressive changes in culture, but in explants derived from older animals the normal structure and secretory activity of the gland is preserved for longer periods. A similar variation in growth pattern with age has been observed in the ventral prostates from young and older mice (6).
In the natural medium a short treatment with methylcholanthrene induces hyperplasia of the epithelium in explants from both age groups. As the period of treatment is extended, the mitotic rate of the epithelium rises from 5 to 9 times the control value and with it the number of hyperplastic alveoli and the degree of hyperplasia.

It is interesting, however, that, even in cultures showing extensive hyperplasia, all the alveoli are never involved, and apparently normal alveoli lined with one row of secretory cells often lie adjacent to hyperplastic structures. This might be owing to either of two factors: availability of the carcinogen or difference in sensitivity. Since both normal and hyperplastic alveoli can be seen in the same region, it seems more likely that some cells remain refractory to the carcinogen.

The increase of the connective tissue in the controls is partly inhibited by methylcholanthrene, but it is still increased relative to that in the organ in situ.

If the carcinogen is withdrawn and the cultures continued in control medium, mitosis fails to the control level, and the increased proliferation is halted. Hyperplasia persists only at the periphery of the explant, and elsewhere the alveoli appear normal and the growth of the connective tissue is resumed. This suggests that at this stage the maintenance of the hyperplasia depends on the constant stimulus of the carcinogen.

The effects of the carcinogen are similar to those described for the ventral prostate glands of mice of various ages (4, 7, 8), but there are certain differences. In the rat tissue the hyperplasia appears earlier, and the connective tissue growth is less severely inhibited. Unlike the mouse prostate, which shows pronounced squamous metaplasia of the hyperplastic epithelium, the squamous transformation in the rat gland is limited to the formation of prickle cells with tonofibrils, and the secretory epithelium is preserved for longer periods. In the mouse gland the hyperplastic changes persist and progress even after withdrawal of the carcinogen, whereas in the rat the hyperplasia is reduced under the same conditions.

In the semi-defined medium the action of methylcholanthrene is much less marked. It is unlikely that this difference in response is related to the variation in growth pattern or functional state of the tissue in the two media, since in the natural medium the carcinogen induces hyperplasia in explants from young animals which undergo regression and in those from old animals in which the epithelium is better preserved and resembles that of the young gland in the semi-defined medium.

It is possible that the solubility of methylcholanthrene in the protein-low, semi-defined medium is reduced and hence its concentration in the tissue. However, this is probably not the only factor. The absence of mitotic activity in the control explants suggests that the semi-defined medium cannot provide the building stones necessary for rapid cell multiplication, and, in addition, the hormones present in the natural medium may also promote the action of the carcinogen.

ACKNOWLEDGMENTS

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REFERENCES

All figures are stained by the periodic acid-Schiff technic after diastase digestion.

FIG. 1.—Section through the ventral prostate gland of a 5-week intact rat before explantation. Mag. X 165.

FIG. 2.—Section through a similar gland in situ 9 days after castration of the animal. Note low epithelium, narrow lumina, and increase of connective tissue as compared with those in Fig. 1. Mag. X 165.

FIG. 3.—Section through an explant from the same gland after 10 days' growth in natural medium. Note flattening of epithelial cells, narrow lumina and increase in connective tissue. Mag. X 165.

FIG. 4.—Section through an explant from a ventral prostate from a 10-week rat after 10 days' growth in natural medium, showing preservation of epithelium, secretion, and absence of connective tissue. Mag. X 165.

FIG. 5.—Section through an explant from a 5-week rat grown for 10 days in natural medium in the presence of 4 μg/ml methylcholanthrene, showing hyperplasia of peripheral alveoli. Mag. X 165.

FIG. 6.—Section through an explant from a 10-week rat grown for 2 weeks in natural medium with 4 μg/ml methylcholanthrene; note extensive hyperplasia of most alveoli. Mag. X 165.

FIG. 7.—Section through an explant from the same gland as in Fig. 6 grown for 10 days in natural medium with methylcholanthrene and transferred to control medium for 4 days. There is less hyperplasia than in Fig. 6. X 165.

FIG. 8.—Section through an explant from a ventral prostate from a 10-week rat grown for 18 days in natural medium with 4 μg/ml methylcholanthrene, showing pronounced hyperplasia. X 165.

FIG. 9.—Alveolus in an explant from a 10-week gland grown for 10 days in natural medium showing columnar secretory epithelium. X 400.

FIG. 10.—Two alveoli in an explant from a younger rat grown for 10 days in natural medium with 4 μg/ml methylcholanthrene, showing early hyperplasia. X 400.

FIG. 11.—Hyperplastic alveolus in an explant from younger gland treated with methylcholanthrene in natural medium for 2 weeks showing tonofibrils between the cells. X 1000.

FIG. 12.—Alveolus in a similarly treated gland showing crowded undifferentiated cells with mitotic figures in periphery and transformation to prickle cells in central region. X 400.
FIG. 13.—Abnormal mitosis in explant treated with methylcholanthrene in natural medium; note dislocation and clumping of chromosomes. × 2400.

FIG. 14.—Section through an explant from a 6-week rat after 10 days' growth in semi-defined medium. Note maintenance of epithelium, but not of secretion and absence of connective tissue. × 165.

FIG. 15.—Explant from a young prostate grown for 10 days in semi-defined medium with 4 μg/ml methylcholanthrene showing two alveoli partially filled with weakly staining cells. × 400.

FIG. 16.—Section through a similarly treated explant. The epithelium is slightly higher and shows some folding, as compared with the control (Fig. 14), but there is no hyperplasia. × 165.

FIG. 17.—Alveolus from an explant grown for 10 days in semi-defined control medium. × 400.

FIG. 18.—Alveolus from an explant grown for the same period in semi-defined medium with 4 μg/ml methylcholanthrene. Note enlargement of cells as compared with that in the control (Fig. 17). × 400.
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