The Role of Ovarian Hormones in Initiating the Induction of Mammary Cancer in Rats by Polynuclear Hydrocarbons*

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SUMMARY

The current studies demonstrate the critical importance of ovarian hormones in the initiation and promotion of carcinogenesis of mammary glands in rats by polynuclear hydrocarbons. Removal of ovarian hormones from the rats immediately after the administration of the carcinogenic hydrocarbons either inhibited or profoundly reduced the incidence of mammary cancer. Excision of ovaries beyond 7 days after feeding of carcinogenic hydrocarbon, however, was unable to prevent or to reduce significantly the incidence of mammary cancer in these rats. Likewise, feeding of carcinogenic hydrocarbons to already castrated rats failed to induce any mammary cancer if the time between castration and feeding of carcinogen was prolonged. These experiments suggest that neoplastic transformation in the cells of the mammary glands in rats cannot take place in the absence of the participation of ovarian hormones, since subsequent supply of ovarian hormones from the functioning grafts failed to produce any mammary cancers.

The experiments also indicate that there may be a quantitative balance between the carcinogenic polynuclear hydrocarbon and the hormones which govern the induction of mammary cancer in rats. In a small number of rats, a potent carcinogen was capable of inducing cancer in the mammary glands in which the metabolic activity was decreasing after the removal of ovarian hormones, whereas a “less potent” carcinogen failed to do so under the same circumstances. In these animals receiving a less potent carcinogen, mammary cancer could be induced if optimal ovarian hormones are present.

Some of the rats receiving a toxic dose of DMBA (30 mg.) died of adrenal apoplexy. DMBA caused necrosis and hemorrhage mostly in the inner zones, especially the zona reticularis of the cortex, whereas zona glomerulosa and medulla were frequently spared.

An incidental finding in the present experiment was the observation of chloroleukemia in two out of 100 rats receiving a single dose of 30 mg. DMBA.

The concept that carcinogenesis involves two separate components with independent mechanisms arose out of observations made by Rous and Kidd (15) in their study of the regression of skin tumors in the rabbit, and by Berenblum (1) in his study of the action of crotone oil in the induction of skin tumors. Dao and Sunderland (5) reported a similar observation that in mammary carcinogenesis by 3-methylcholanthrene (3-MCA), two independent processes seem to be operating: initiation of carcinogenesis by the carcinogen and promotion of tumor growth by hormones. The mammary cancers in these experiments appeared to be carcinogen-induced, and the hormones seemed to serve as promoters or cocarcinogens; nonetheless, 3-MCA, when administered alone, in the absence of ovarian hormones, was unable to elicit mammary cancer, and this fact raised the question whether ovarian hormones may also be of critical importance in the initiating process of carcinogenesis. Although it was conclusively demonstrated that grafting a pair of ovaries would significantly increase the incidence of tumors in male rats receiving 3-MCA (4), this observation did not distinguish whether ovarian hormones were directly involved or not.
involved in the initiating process or were merely functioning as cocarcinogens.

This paper is concerned with the possible role of ovarian hormones in initiating mammary cancer in rats receiving polynuclear hydrocarbons. During an experimental study of this problem the significant observation was made that successful tumor induction does indeed require the presence of ovarian hormones, not merely to promote carcinogenesis, but also to play an essential role in the initiating process.

MATERIALS AND METHODS

Sprague-Dawley rats, both male and female, 50–55 days old, were used in all experiments, and there were ten rats in each group, unless stated otherwise. In experiments involving ovarian transplantation, 30- to 60-day-old male and female littermates were used.

The experiments were designed on the basis of our earlier observation that in mammary carcinogenesis by 3-MCA, the carcinogen and the hormones were operating independently. The carcinogens used in the present experiments were 3-methylcholanthrene (3-MCA) and 7,12-dimethylbenz(a)anthracene (DMBA), and the ovarian hormones were derived from ovarian grafts. The carcinogens were dissolved in sesame oil in a concentration of 10 or 30 mg/ml for 3-MCA, and 20 or 30 mg/ml for DMBA. A single feeding of either of these compounds through a stomach tube was done in all experiments.

Induction of mammary cancer in female rats castrated at different intervals after a single feeding of the carcinogen.—This experiment was designed to study the critical importance of the time required for induction of mammary cancer after carcinogen administration. It also aimed to demonstrate how ovarian hormones function in the process of carcinogenesis. The experiment, involving seven groups of rats, was carried out twice, each time with a different carcinogen at a different dose level. After a single feeding of a 10 mg. (suboptimal) dose of 3-MCA to all animals, Group 1 was kept as a control throughout the experiment, and Groups 2 through 7, each containing ten female rats, were castrated at intervals of 1, 3, 7, 15, 20, and 30 days, respectively. Fifty days after 3-MCA feeding, half of the animals (i.e., ten rats from each group) received a pair of ovarian grafts, and the other half of the animals served as controls. The experiment was then repeated, with seven groups identical to those just described, except that single doses of 20 mg. of DMBA were used.

Induction of mammary cancer in castrated female rats.—The experiment was designed to determine whether the time interval between administration of a carcinogenic hydrocarbon and subsequent ovarian grafting influences tumor induction. The experiment consisted of three groups of rats, divided according to the time interval between castration and subsequent administration of DMBA. There were 48 rats in Group 1, and they were all castrated at the age of 55 days. Seven days after castration, all castrated rats were fed a solitary dose of 20 mg. DMBA. Twenty rats were kept as controls for this group. The remaining 28 castrated rats were divided into four subgroups, and each rat received a pair of ovarian grafts from their littermates on the following days: (a) on the same day when DMBA was given, (b) 7 days after feeding DMBA, (c) 15 days after DMBA feeding, and (d) 30 days after feeding DMBA. Group 2 consisted of 48 rats which were all castrated and were fed a single dose of 20 mg. DMBA 15 days after castration. Twenty of these 48 rats were kept as controls throughout the experiment. The other 28 rats were again divided into four subgroups, and each rat received a pair of ovarian grafts on an identical schedule as was described earlier. In Group 3, also consisting of 48 rats, a single dose of 20 mg. DMBA was given to all these rats 1 month after castration. Subsequently the experiment was carried out exactly the same as in Groups 1 and 2.

Time of ovarian transplantation and subsequent development of mammary cancer in male rats treated with a chemical carcinogen.—The experiment involved four groups of male rats. Rats in Group 1 were castrated at the age of 50 days, and a single dose of 30 mg. of 3-MCA was fed to all the rats the day after castration. These rats were then kept as controls. In Group 2, castration and ovarian transplantation were carried out simultaneously. A single dose of 30 mg. of 3-MCA was given, 25 days later, to these castrated male rats bearing pairs of ovarian grafts. In Group 3, all the male rats were castrated, and a single dose of 30 mg. of 3-MCA was given the next day. A pair of ovaries was transplanted into each of these animals 25 days after castration (24 days after 3-MCA administration). In Group 4, a single dose of 30 mg. of 3-MCA was fed to all mature male rats. Each of these carcinogen-treated rats was castrated and grafted with a pair of ovaries simultaneously, 25 days later.

The rats in all these experiments were examined at frequent intervals for palpable tumors. We define the "latent period of tumor appearance" as the interval from the first feeding of the carcinogen to the detection of the first palpable tumor.

All rats were killed at the end of 6–8 months. At necropsy, the pituitaries, adrenals, ovarian
grants, seminal vesicles, prostates, and tumors were removed and weighed. Serial histological sections were prepared for all pituitaries, ovarian grafts, and mammary glands. All pituitaries were fixed in Zenker-Formol and were stained according to a modification of Masson's trichrome technic. The histology of the mammary glands was also studied by the whole-mount technic. Throughout this paper the term "optimal carcinogenic dose" refers to the effective amount of carcinogen given to the rats to produce 100 per cent tumor incidence.

In these experiments, it appeared that a sub-optimal dose of 3-MCA fed to female rats subsequently castrated within 7 days was unable to initiate carcinogenesis, since later treatment with ovarian grafts failed to produce any significant number of mammary tumors. In groups in which castration was done on the 15th-30th day after administration of 3-MCA, there were no palpable

### TABLE 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. rats</th>
<th>No. rats with tumors</th>
<th>Total no. tumors</th>
<th>Appearance of palpable tumors (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>10 (50%)</td>
<td>18</td>
<td>Range: 72–176 Mean: 114</td>
</tr>
<tr>
<td>Castration day 1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Castration day 1 + Ov. grafts*</td>
<td>10</td>
<td>1 (10%)</td>
<td>2</td>
<td>Mean: 112</td>
</tr>
<tr>
<td>Castration day 3</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Castration day 3 + Ov. grafts*</td>
<td>10</td>
<td>1 (10%)</td>
<td>1</td>
<td>Mean: 70</td>
</tr>
<tr>
<td>Castration day 7</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Castration day 7 + Ov. grafts*</td>
<td>10</td>
<td>2 (20%)</td>
<td>2</td>
<td>Range: 89, 88 Mean: 88</td>
</tr>
<tr>
<td>Castration day 15</td>
<td>10</td>
<td>1 (10%)†</td>
<td>1</td>
<td>Mean: 181</td>
</tr>
<tr>
<td>Castration day 15 + Ov. grafts†</td>
<td>10</td>
<td>2 (20%)</td>
<td>2</td>
<td>Range: 72, 78 Mean: 75</td>
</tr>
<tr>
<td>Castration day 30</td>
<td>10</td>
<td>3 (30%)†</td>
<td>4</td>
<td>Range: 181, 181 Mean: 181</td>
</tr>
<tr>
<td>Castration day 20 + Ov. grafts†</td>
<td>10</td>
<td>6 (60%)</td>
<td>13</td>
<td>Range: 75–115 Mean: 99</td>
</tr>
</tbody>
</table>

* Ovarian transplantations were carried out on day 40 after administration of 3-MCA.
† Ovarian transplantations were carried out on day 50 after administration of 3-MCA.
‡ Tumors found at autopsy.

### RESULTS

**Induction of mammary cancer in female rats castrated at different intervals after a single feeding of the carcinogen.**—After a single feeding of a 10 mg. (suboptimal) dose of 3-MCA to adult female rats, only 50 per cent of the animals had developed mammary cancer by the end of 6 months. Castration at intervals of 1, 3, and 7 days following the single feeding of 3-MCA inhibited tumor induction. Histological examination of the mammary glands removed at necropsy did not reveal any microscopic tumors. When ovaries were grafted into these castrated rats on the 40th day after 3-MCA administration, mammary cancer developed in one rat in each of the 1- and 3-day castration groups. In the 7-day castration group, two rats had mammary cancer (Table 1). Histological examination of the mammary glands of the remaining rats bearing ovarian grafts failed to demonstrate any microscopic tumors.

In these experiments, it appeared that a sub-optimal dose of 3-MCA fed to female rats subsequently castrated within 7 days was unable to initiate carcinogenesis, since later treatment with ovarian grafts failed to produce any significant number of mammary tumors. In groups in which castration was done on the 15th-30th day after administration of 3-MCA, there were no palpable tumors in animals which did not receive ovarian grafts. At the time of autopsy, however, one tumor was found in the 15-day castration group, three in the 20-day castration group, and four in the 30-day castration group. Histological examination, however, failed to reveal any microscopic tumors in the 20th- and 30th-day castration groups. On the other hand, in rats receiving ovarian grafts, the tumor incidence was similar to that in the control group. These results demonstrate that castration on the 15th-30th day after MCA administration evidently only retarded tumor appearance but did not reduce tumor incidence. It is apparent that initiation of carcinogenesis had occurred before the deprivation of ovarian hormones in these animals, since subsequent grafting of ovaries brought about the appearance of palpable mammary tumors.
which had been unable to grow in the absence of ovarian hormones.

DMBA, fed to adult female rats at a single-dosage level of 20 mg., produced 100 per cent tumor incidence. The tumors not only often appeared early, but also were present in almost all the breasts of the rats. A single dose of 30 mg. of DMBA was lethal to 70 per cent of the animals so treated. These rats apparently died of "adrenal apoplexy" due to massive hemorrhage and necrosis in the adrenal cortex. Death usually occurred on the 3d or 4th day after administration of the carcinogenic hydrocarbon. The animals bled both from the nostrils and from the rectum, and often died suddenly while running around the cage, apparently not in acute illness.

At the time of necropsy, both adrenals were markedly enlarged and distended with blood. Splenomegaly was a frequent finding in these animals. Histological examination of the adrenal glands showed almost complete destruction of the zona reticularis and lesser damage to the zona fasciculata. These layers often were replaced with blood and necrotic materials, little recognizable cortical tissues remaining. Interestingly, the zona glomerulosa and medulla often were not involved (Fig. 1). Huggins and Morii (9) described in a recent publication the selective necrosis of adrenal gland in rats treated with DMBA. Our findings are in agreement with Huggins' observation. In addition, we found, however, that hemorrhage also occurred in the gastrointestinal tract, liver, and spleen. In the surviving animals, previous adrenal hemorrhage was evident at the time of autopsy several months later. Sections of the glands showed areas of hemorrhage in the cortex. Histological examination revealed areas of patchy hemorrhage in the zona fasciculata and zona reticularis, but regenerative cortical tissues were also visible (Fig. 2).

**TABLE 2**

**INDUCTION OF MAMMARY CANCER IN RATS CASTRATED FOLLOWING A SINGLE DOSE OF 20 MG. DMBA**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO. RATS</th>
<th>NO. RATS WITH TUMORS</th>
<th>TOTAL NO. TUMORS</th>
<th>APPEARANCE OF PALPABLE TUMORS (DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Intact 9)</td>
<td>10</td>
<td>10 (100%)</td>
<td>40</td>
<td>41–98</td>
</tr>
<tr>
<td>Castration day 1</td>
<td>10</td>
<td>1 (10%)</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>Castration day 1 + Ov. graft*</td>
<td>10</td>
<td>5 (50%)</td>
<td>8</td>
<td>60–116</td>
</tr>
<tr>
<td>Castration day 3</td>
<td>10</td>
<td>1 (10%)</td>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td>Castration day 3 + Ov. graft*</td>
<td>10</td>
<td>6 (60%)</td>
<td>10</td>
<td>62–108</td>
</tr>
<tr>
<td>Castration day 7</td>
<td>10</td>
<td>2 (20%)</td>
<td>5</td>
<td>79, 87</td>
</tr>
<tr>
<td>Castration day 7 + Ov. graft*</td>
<td>10</td>
<td>6 (60%)</td>
<td>10</td>
<td>62–90</td>
</tr>
<tr>
<td>Castration day 15</td>
<td>10</td>
<td>2 (20%)</td>
<td>5</td>
<td>74, 87</td>
</tr>
<tr>
<td>Castration day 15 + Ov. graft†</td>
<td>10</td>
<td>8 (80%)</td>
<td>24</td>
<td>62–87</td>
</tr>
<tr>
<td>Castration day 20</td>
<td>10</td>
<td>2 (20%)</td>
<td>6</td>
<td>79, 87</td>
</tr>
<tr>
<td>Castration day 20 + Ov. graft†</td>
<td>10</td>
<td>10 (100%)</td>
<td>32</td>
<td>62–81</td>
</tr>
</tbody>
</table>

*Ovarian transplantations were carried out on day 40 after administration of DMBA.
†Ovarian transplantations were carried out on day 50 after administration of DMBA.
‡Number of rats bearing mammary tumors which were found at autopsy (182 days after DMBA feeding).

It appears that a single dose of 20 mg. of DMBA is a maximal carcinogenic dose. The data in Table 2 show that a maximal dose of a potent carcinogen, such as DMBA, is able to elicit some mammary cancers in female rats even when the ovaries have been removed. These tumors evidently were unable to grow in the absence of ovarian hormones. When ovarian hormones were provided by grafts, however, the tumors appeared. As the interval between carcinogen feeding and castration was prolonged the tumor incidence in rats bearing ovarian grafts rose until it reached the level of the controls. The tumor incidence in the nongrafted animals, however, was similar among the different groups in this experiment. At autopsy, numerous small,
In 15-day-old castrates, the tumor incidence was about 42 per cent if ovaries were grafted on the same day of DMBA feeding. As the time interval lengthened, the tumor incidence remained about the same even if the ovaries were grafted 30 days after DMBA feeding (Table 3). In the 30-day-old castrates, mammary tumors have not been observed either in the castrated controls or in those with functioning ovarian grafts (Table 3).

Two significant conclusions can be drawn from this experiment: (a) in rats deprived of ovarian hormones, the incidence of mammary tumors in DMBA-treated rats decreased as the interval between castration and carcinogen administration lengthened, and (b) the interval between DMBA feeding and subsequent ovarian grafting, however, has little or no effect on the mammary tumor incidence in both 7-day and 15-day castrates.

**Effect of the time interval between castration, administration of carcinogen, and subsequent ovarian graft on mammary tumor induction.—**DMBA in a single dose of 20 mg. fed to twenty adult intact, female rats (as control) produced 100 per cent mammary tumor incidence. There was a total of 93 tumors in these twenty rats. The latent period of tumor appearance ranges from 31 to 100 days, with a mean of 53 days. When a maximal dose of DMBA was given to the rats 7 days after castration, 43 per cent of the rats so treated had mammary cancer if ovaries were transplanted on the same day of DMBA feeding. Prolongation of the interval between DMBA feeding and ovarian grafting did not influence significantly the tumor incidence (Table 3).

**TABLE 3**

**Effects of Ovarian Grafts on Incidence of Breast Cancer in Castrated Female Rats Receiving a Single Dose of 20 Mg. DMBA**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rats fed DMBA 7 days after castration</th>
<th>Rats fed DMBA 15 days after castration</th>
<th>Rats fed DMBA 30 days after castration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. rats with tumors</td>
<td>Total no. of tumors</td>
<td>Appearance of palpable tumors (days)</td>
</tr>
<tr>
<td>Control (castrated)</td>
<td>1/20 (5%)</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>Ov. graft 0 days after DMBA</td>
<td>3/7 (43%)</td>
<td>9</td>
<td>43, 47, 50</td>
</tr>
<tr>
<td>Ov. graft 7 days after DMBA</td>
<td>2/6 (33%)†</td>
<td>4</td>
<td>43, 43</td>
</tr>
<tr>
<td>Ov. graft 15 days after DMBA</td>
<td>3/7 (43%)</td>
<td>7</td>
<td>43, 43, 49</td>
</tr>
<tr>
<td>Ov. graft 30 days after DMBA</td>
<td>2/7 (29%)</td>
<td>2</td>
<td>92, 101</td>
</tr>
</tbody>
</table>

* No. rats with tumors/no of rats.
† One rat died 1 week after feeding of DMBA.

*No. of differences in the potency and the dose of the carcinogen.*

**Effect of the time interval between castration, administration of carcinogen, and subsequent ovarian graft on mammary tumor induction.—**DMBA in a single dose of 20 mg. fed to twenty adult intact, female rats (as control) produced 100 per cent mammary tumor incidence. There was a total of 93 tumors in these twenty rats. The latent period of tumor appearance ranges from 31 to 100 days, with a mean of 53 days. When a maximal dose of DMBA was given to the rats 7 days after castration, 43 per cent of the rats so treated had mammary cancer if ovaries were transplanted on the same day of DMBA feeding. Prolongation of the interval between DMBA feeding and ovarian grafting did not influence significantly the tumor incidence (Table 3).

**Time of ovarian transplantation and subsequent development of mammary cancer in male rats treated with a chemical carcinogen.—**In male rats a dose of 30 mg. of 3-MCA given in a single feeding failed to induce any mammary cancer. It was demonstrated earlier that, when castrated male rats bearing functional ovarian grafts were treated with a maximal carcinogenic dose of 3-MCA (10 mg/day X 15 days), tumors were induced in 65 per cent of the rats so treated (4). The present experiment shows that castrated males bearing functional grafts and receiving a single feeding of 30 mg. of 3-MCA have a tumor incidence of 53 per cent (Table 4). If, however, the carcinogen was given to the castrated males first, and ovaries were trans-
planted to these castrated rats 30 days later, mammary tumor incidence was only 13 per cent. In noncastrated rats, 3-MCA given prior to ovarian grafting was similarly unable to induce any mammary cancer. These experimental results evidently indicate that 3-MCA alone is incapable of initiating carcinogenesis in the absence of ovarian hormones.

In these experiments, it is further demonstrated that the time of the introduction of ovarian hormones is critical. The fact that grafting ovaries into castrated rats receiving 3-MCA prior to the transplantation failed to induce mammary cancer is conclusive evidence that initiation of carcinogenesis cannot take place in the absence of ovarian hormones.

Physiological effects of functioning ovarian grafts. — In castrated females a pair of functioning ovaries will produce a four- to eightfold increase in uterine weight as compared with that of the castrated females without ovarian graft. The uterine weights in 150 castrated rats without ovarian grafts (controls) ranged from 52.6 to 88.8 mg., with a median of 71.2 mg. and a mean of 70.6 mg. In the castrated rats with functioning grafts the uterine weights ranged from 215.6 to 611.0 mg., with a median of 371.2 mg. and mean of 339.9 mg. In the normal intact female rats of the same age the uterine weights in twenty rats ranged from 247.8 to 748.2 mg., with a mean of 427.5 mg. When only one ovarian graft is functioning or recovered, the uterine weights were smaller, ranging from 123 to 170 mg. Uterine weights below 100 mg. indicate that the ovarian grafts are nonfunctioning. In a total of 175 castrated female rats to which ovaries were transplanted, 148 (82 per cent) rats had both grafts functioning, 27 (15 per cent) rats had only one graft recovered which was functioning, and there were only five (3 per cent) rats from which grafts could not be recovered.

The presence of a pair of functioning ovarian grafts abolishes the typical castration changes in the pituitary glands of the castrated rats. This observation has been described in an earlier paper (4). The histology of a functioning graft resembles that of a normal ovary, containing both primordial and mature follicles and in many instances corpora lutea. In the castrated male rats, the presence of a pair of functioning ovarian grafts induces a significant increase in weight of the seminal vesicles. It seems that estrogen stimulates connective tissue metaplasia (4, 14).

**Development of chloroleukemia in rats receiving toxic dose of DMBA.**—During necropsy of the surviving 30 rats, in a total of 100 previously treated

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. RATS</th>
<th>NO. RATS WITH PALPABLE TUMORS</th>
<th>TOTAL NO. TUMORS</th>
<th>APPEARANCE OF PALPABLE TUMORS (DAYS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrated ♂</td>
<td>10</td>
<td>1 (10%)</td>
<td>1</td>
<td>126</td>
</tr>
<tr>
<td>Castrated ♂+Ov. graft→3-MCA*</td>
<td>19</td>
<td>10 (53%)</td>
<td>11</td>
<td>40-106</td>
</tr>
<tr>
<td>Castrated ♂+3-MCA→Ov. grafts†</td>
<td>23</td>
<td>3 (13%)</td>
<td>3</td>
<td>135, 145, 148</td>
</tr>
<tr>
<td>Intact ♂+3-MCA→Castration+Ov. grafts‡</td>
<td>27</td>
<td>1 (3%)</td>
<td>1</td>
<td>158</td>
</tr>
</tbody>
</table>

* Castration and ovarian graft done simultaneously in 35-day-old males, 3-MCA 25 days later.
† 3-MCA fed to 35-day-old male rats just being castrated; ovaries were transplanted to these rats 25 days later.
‡ 3-MCA fed to intact 35-day-old male rats; 25 days later castration and ovarian graft done simultaneously.

With a single dose of 30 mg. of DMBA, we discovered that in two rats there was a striking green pigmentation in all the lymph glands. Histological examination of the lymph nodes and spleen revealed leukemia cells of myeloid type (Figs. 3 and 4). Chloroleukemia in rats was first reported in 1936 by Wilens and Sproul (16). The details of our present findings will be reported in a separate paper.

**DISCUSSION**

By the use of a single-dose technic in our present experiments, it has been possible to provide a unique situation such that the actions of the carcinogen and the hormones can be studied separately. Whether carcinogenesis might be a discontinuous process consisting of multiple stages with different characteristics in each stage, or whether it indeed arises in a single step, cannot yet be decided. In mammary carcinogenesis by chemical
Huggins et al. (7) and Dao et al. (5) demonstrated previously that castration in female rats prior to administration of a carcinogen reduced only the mammary cancer incidence, but it did not prevent tumor induction. It must be noted that, in these experiments, 3-MCA was given either immediately or shortly after castration. It is now evident that if carcinoogenic hydrocarbons are given to female rats 30 days after castration, mammary cancers are not induced. In these rats deprived of ovarian hormones carcinoengenesis had never occurred, despite the feeding of optimal doses of the carcinoogenic hydrocarbons which otherwise will effectively induce mammary tumors.

Although it could be argued that initiation could have taken place but that the removal of ovarian steroid hormones killed the "initiated cells," hence subsequent grafting of ovaries failed to promote the appearance of tumors. Nonetheless, in experiments in which ovaries were grafted into the castrated female rats subsequently fed DMBA, no significant difference in mammary tumor incidence was observed in animals receiving ovarian grafts immediately following or 30 days after DMBA feeding (Tables 3, 4). This observation suggests that, once the neoplastic transformation of cells is completed, these malignant cells can survive under prolonged period of ovarian hormone deficiency. Later contribution of ovarian hormones by the grafts only serves to promote the growth of the mammary cancer.

Thus ovarian hormones seem to possess a dual function in mammary carcinogenesis by polycyclic hydrocarbons. That the presence of carcinogen alone in the mammary gland is insufficient to initiate carcinoengenesis has been conclusively demonstrated by the fact that 3-MCA fails to induce mammary cancer in male rats, irrespective of the doses given and despite the fact that the concentration of 3-MCA is the same in the mammary glands of both male and female rats (3). The experimental results presented in this paper have provided additional evidence that in male rats the tumor incidence rises only when the carcinoogenic hydrocarbon is given at a time when ovarian hormones are present in the recipient. 3-MCA given to the castrated males and subsequently followed by ovarian grafts, as well as 3-MCA given to intact males and followed later by castration and ovarian grafts, fails to induce mammary tumors. This fact clearly demonstrates that initiation has never occurred in these rats. If early neoplastic transformation could take place without ovarian hormones, the mammary cancers should certainly develop after castration and ovarian transplantation.

Previous reports by Huggins et al. (8) and the experimental results presented in this paper show that DMBA is highly effective in the induction of mammary cancer in female rats. A solitary feeding of optimal dose of DMBA can elicit 100 per cent tumor incidence with a shorter latent period. In addition, the number of mammary cancers per rat receiving DMBA is twice as many as that in the rat receiving similar optimal carcinogetic doses of 3-MCA. This interesting phenomenon denotes the difference in carcinogenicity of these two polynuclear hydrocarbons. It is apparent that not all the millions of epithelial cells of the mammary glands are affected and become malignant after feeding an optimal dose of the carcinogen. The present data show that an effective amount of DMBA can inflict injury to twice as many cells of the mammary gland as an optimal carcinogetic dose of 3-MCA can. The fact that the incidence of mammary cancer was significantly higher in DMBA-treated rats than in the 3-MCA-treated rats, when both groups were castrated 1, 3, and 7 days following the feeding of carcinoegen, does not mean that DMBA may induce mammary cancer without the

significant difference in mammary tumor incidence is demonstrated by the fact that ovaries were grafted into the carcinoegenic hydrocarbon, the incidence of mammary cancer rose as the interval between the time of feeding and subsequent pregnancy lengthened. These experiments clearly demonstrated the critical significance of time between initiation and promotion in tumor induction. The results of the present experiments uniformly demonstrate that there is definitely a time factor in the initiating process and that, beyond the critical time, the affected cells are irreversibly transformed. The experiments in which female rats are castrated at different intervals following a single feeding of the carcinoegen show that between 10 and 15 days after the administration of carcinoegen, deprivation of ovarian hormones cannot inhibit tumor induction; but it does retard tumor appearance, since subsequent contribution of ovarian hormones by the ovarian grafts brings about the appearance of tumors which otherwise may remain "dormant." These data also indicate that the ovarian hormones play a critical role in the initiating process of carcinoengenesis, since the removal of ovarian hormones immediately after the administration of carcinoegen markedly reduced tumor induction. It appears that initiation of carcinoengenesis does not occur in the absence of ovarian hormones.

Although it could be argued that initiation could have taken place but that the removal of ovarian steroid hormones killed the "initiated cells," hence subsequent grafting of ovaries failed to promote the appearance of tumors. Nonetheless, in experiments in which ovaries were grafted into the castrated female rats subsequently fed DMBA, no significant difference in mammary tumor incidence was observed in animals receiving ovarian grafts immediately following or 30 days after DMBA feeding (Tables 3, 4). This observation suggests that, once the neoplastic transformation of cells is completed, these malignant cells can survive under prolonged period of ovarian hormone deficiency. Later contribution of ovarian hormones by the grafts only serves to promote the growth of the mammary cancer.
presence of ovarian hormones. On the contrary, it suggests that a potent carcinogen may induce changes leading to neoplasm in mammary gland cells in which metabolic activities are declining owing to the deprivation of ovarian hormones. Whereas DMBA may still be capable of inducing mammary tumors in a few rats being castrated shortly prior to feeding of the carcinogen, it was nevertheless unable to induce any tumors in rats castrated for a long period of time. These observations led to an important consideration—that a quantitative balance may exist between the carcinogenic hydrocarbon and hormones which may govern the initiation of the carcinogenesis.

The exact mechanism by which ovarian hormones participate in carcinogen-induced carcinogenesis is not understood at present. Bock and Dao (2) recently reported that alteration of the endocrine state of the rats by hypophysectomy or castration had little effect on the concentration of 3-MCA in either the general body fat or the breast. Accordingly, the hormonal effects on tumor formation appear to be due primarily to alteration of the target cells rather than to changes in the amount of carcinogen to which they are exposed. A lack of understanding of the exact mechanisms of hormone action on the cell precludes any attempt to explain the present experimental phenomenon.

The observation by Krischbaum et al. (10, 11) that estrogenic hormones "augment" the capacity of x-rays and MCA to "incite" leukemia is in reality a similar example of the fact that hormones do indeed influence the actions of many carcinogenic substances on cells. Loeb (12) theorized (more than 4 decades ago) that hormones might cause the development of cancer and that substances normally produced in the body in the usual quantity may be responsible for the initiation of cancer. His conclusion was that hormones interact with genetic-hereditary factors, and this interaction leads to the formation of cancer. If neoplastic transformation represents some form of heritable cellular change, it can be induced by an agent either normally present in the organism or introduced from the outside.

Our present experiments supply some convincing evidence that the polycyclic hydrocarbons initiate mammary carcinogenesis, with one prerequisite—participation of ovarian hormones. Whether the polycyclic hydrocarbons do induce heritable cellular changes has not yet been conclusively demonstrated, although the work of Miller and Miller (13) and that of Heidelberger (6) has shown that chemical carcinogens interact with the cytoplasmic proteins. Whether this interaction may lead to a subsequent change in the behavior of the cells is still to be elucidated.

The unique similarity between the steric configurations of steroid hormones and polycyclic aromatic hydrocarbons has long suggested that steroid hormones possess carcinogenic properties similar to those of the polycyclic hydrocarbons. Recently, Yang et al. (17) again postulated that a carcinogenic hydrocarbon must bear steric resemblance to steroids, suggesting that the hydrocarbon may act on the same sites as steroid hormones. From our experiments it seems that ovarian hormones may indeed provide a favorable "substrate" for the interaction of carcinogenic hydrocarbon and cellular constituents. Further investigation along this line of thinking may prove to be fruitful for our understanding of the exact mechanism of carcinogenesis.

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FIG. 3.—The splenic pulp in a rat is replaced by myeloid leukemic cells. X100.

FIG. 4.—The lymph node in the same rat, 90 days after a single feeding of 30 mg. DMBA. Note the replacement by myeloid leukemia cells. X400.
The Role of Ovarian Hormones in Initiating the Induction of Mammary Cancer in Rats by Polynuclear Hydrocarbons

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