The Effects of 7,12-Dimethylbenz(a)anthracene on the Ovarian Response of Mice and Rats to Gonadotrophins

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SUMMARY

Administration by stomach tube of 7,12-dimethylbenz(a)-anthracene (DMBA) (30 mg/100 gm body weight) in oil caused a decrease in the ovarian weights of C57BL/6J, C3D2F1, CAF1, B6AF1, B6D2F1, and C57BL/6J X C3HeB/FeJ mice, but it did not affect the ovarian weights of C3HeB/FeJ X NZY mice or of Sprague-Dawley or Fischer rats.

DMBA treatment also eliminated the ovarian weight gain, which normally is seen 48 hours after chorionic gonadotrophin (CG) injection, in C57 mice 34 days of age or less, but there was no significant effect on the action of CG in stimulating ovarian weight gain in C57 mice 56 days of age or more. In C3D2F1 mice, the ovarian weight response was eliminated up to 23 days of age, but in older mice of this strain the inhibition was not complete and was more variable in degree.

The ovarian weight response to CG was reduced within 3 days of DMBA treatment in mature B6D2F1 or immature B6AF1 mice, and within 7 days in mature CAF1 mice. However, the carcinogen had no significant effect on ovarian weight stimulation by CG in mature B6AF1, C57BL/6J X C3HeB/FeJ, or C3HeB/FeJ X NZY mice.

In a less extensive series of experiments, substitution of pregnant mare serum (PMS) as the gonadotrophin after DMBA treatment gave results parallel to those obtained with CG. Ovarian weight gain following PMS was inhibited in immature Sprague-Dawley rats, but not in Fischer rats at any of the 3 weights studied.

Uterine weight was lower in most DMBA-treated immature mice and Sprague-Dawley rats than in the controls and increased less following gonadotrophin injection. In the majority of mature mice and rats, however, uterine weights did not differ significantly between DMBA-treated and control animals either before or after gonadotrophin injection.

Amounts of DMBA in arachis oil equivalent to or less than 20 mg/100 gm administered through a stomach tube or 2 mg/100 gm injected intraperitoneally were ineffective in inhibiting the action of gonadotrophins on the ovaries of immature C57BL/6J mice.

The number of viable ovarian follicles was greatly reduced in mice within 3 days after DMBA treatment, and there were none present 100 days afterwards. There was no obvious toxic effect of DMBA on the rat ovaries.

Carcinogen treatment was followed by a loss of total body weight in some, but not all, rats and mice. There was no absolute correlation between the inhibition of ovarian response to gonadotrophin and the decrease in total body weight following treatment with the carcinogen.

An interpretation of the results is given in terms of the action of DMBA on pituitary secretion in both mice and rats, and in terms of direct action on ovarian follicles in mice only.

INTRODUCTION

Although there had been previous accounts of occasional ovarian neoplasms in mice following treatment with DMBA, the induction of a high incidence of granulosa cell tumors with this carcinogen was first reported in 1954 by Howell et al. (21), using the IF strain. Since then there have been reports of ovarian tumor induction in other strains of mice (4, 5, 28, 36, 37), but the incidence and time at which the lesions were noted has varied considerably from one strain to another. It has been observed, however, that the course of events following exposure of the mouse ovary to DMBA is very similar to that produced in both the mouse and the rat by radiation (36, 39, 43).

Ovarian changes in mice after irradiation were detailed in the early reports of Parkes and his collaborators (7-9, 44-47). Since then there have been coherent and sequential investigations by Mandi and her colleagues on both the mouse and the rat (25, 26, 31-35). Many other workers have added to the large body of information which has been extensively reviewed by Lacassagne et al. (29).

The course of events is similar in rats and mice. Primordial oocytes disappear within 18 hours after a sterilizing dose of radiation, and, although some follicles (up to 150 μ in size) persist for a few weeks, there is a complete absence of follicles by 14 weeks (34). Progesterone secretion is inhibited at an early stage, but estrogen secretion continues, with some suggestions of cyclic variations in both mice (45-47) and rats.

1 This study was supported by the National Cancer Institute of Canada, Toronto, Canada.

2 Abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; SD, Sprague-Dawley (rats); F, Fischer (rats); CG, human chorionic gonadotrophin; PMS, pregnant mare serum; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LTH, luteotrophic hormone.

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(31), for several weeks. Period of constant estrus usually follow, with the development of prolonged anestrus culminating in the appearance of granulosa cell tumors in the majority of mice and to a lesser extent in rats.

There is evidence that irradiated ovaries show a marked decline in their response to endogenous and exogenous gonadotrophins (13, 15, 24, 33). It should be noted, however, that some of the latter observations, based solely on variations in the total weight response to gonadotrophins of the ovary plus uterus, are not reliable measures of either the anatomic or secretory changes which occur in the ovaries. Changes in the weight of the ovary do not make a significant alteration in the total weight of the ovary/uterine complex; differences in uterine weight can be effected by variations in the absolute amounts, or by the proportions of any of the ovarian secretions, among which the estrogens, progesterins, and androgens are well established. Despite the marked decline in its response to gonadotrophin, the irradiated ovary apparently secretes some estrogen independently of pituitary stimulation (25, 26).

Gonadotrophin injection into immature rats prior to irradiation confers some protection, as there is a decreased rate of degeneration of follicles (35). This supports the concept that more mature follicles are relatively resistant to radiation damage.

There is no doubt (43) that the rat ovary is susceptible to tumorigenesis following splenic implantation or irradiation, although the dosages of radiation required are higher than for the mouse. The rat ovary, however, is completely insensitive to the carcinogenic action of DMBA, whereas the mouse responds at least as readily to DMBA treatment as it does to other tumorigenic procedures.

Although there are a few descriptions of the toxic effects of DMBA alone on the mouse (36, 39, 43) and the rat (52) ovary, the action of gonadotrophins following the administration of this carcinogen have not been extensively investigated in either the mouse or the rat. Flaks (16) was responsible for the original observation that the related compound 3-methylcholanthrene (20-methylcholanthrene in his terminology) inhibited the subsequent induction by chorionic gonadotrophin of follicle hemorrhage and luteinization in the ovaries of immature CBA and Strong A mice. He concluded that there was no evidence as to whether this action was direct or whether it was mediated via the pituitary gland. In a brief report of experiments confined to rats, Hipkin (20) concluded that DMBA has no direct action on the endocrine function of the gonads.

The following experiments were undertaken to determine the response of the mouse and the rat ovary to gonadotrophins following treatment with DMBA, and to see whether this response could be used to assess the susceptibility of various strains to ovarian tumor induction by this carcinogen.

MATERIALS AND METHODS

Mice

C57BL/6J strain and hybrid CDF (C3D2F1, C3HeJ 9 X DBA/2J d), CAF (CAF1, BALB/cJ 9 X A/J d), B6A (B6AF
C57BL/6J 9 X A/J d), and BDF (B6D2F1, C57BL/6J 9 X DBA/2J d) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine (50). B6C (C57BL/6J 9 X C3HeB/FeJ d) and CNZ (C3HeB/FeJ 9 X NZB d) hybrid mice were bred in this laboratory. The ages given in the experimental section were accurate, to the day, for all of the mice used.

Rats

Those designated SD were obtained from the Sprague-Dawley Co. Ltd., Madison, Wisconsin. Inbred Fischer strain rats (F) were raised in this laboratory. Rats were grouped according to body weight rather than by age.

Gonadotrophins

CG and PMS were purchased from the Sigma Chemical Co., St. Louis, Mo. The dry powder was dissolved in normal saline to give solutions containing 125 IU/ml. The standard dose used throughout was 25 IU/0.2 ml for mice and 50 IU/0.4 ml for rats, injected subcutaneously.

DMBA

DMBA was obtained from the Sigma Chemical Co. Ltd. The crystals were dissolved in arachis oil, at a concentration of 10 mg/ml, by heating in an oven at 60°C.

Experimental Design

The objective of each experiment was to determine the effect of DMBA administration on the increase in ovarian and uterine weights induced by the injection of either CG or PMS. Groups of 5 animals each were used for the following treatment regimes: (a) Group 1 received 30 mg/100 gm body weight of DMBA in solution on Day 0 through a stomach tube while under ether anesthesia. (b) Group 2 was the same as Group 1 with additional subcutaneous injections of gonadotrophin at various times after DMBA administration. (c) Group 3 received the same volume of arachis oil as was given to Group 1 on Day 0. (d) Group 4 received the same as Group 3 plus the subcutaneous injection of gonadotrophin at the same time as in Group 2.

Groups 1 and 3 were killed with ether on the day of gonadotrophin injection, and Groups 2 and 4 were killed with ether 48 hours after gonadotrophin injection.

Total body weight of each animal was determined to within 0.1 gm after death.

The uterus was dissected free from mesentery and fat, and removed by cutting through the vagina just below the cervix and through the Fallopian tubes just below their junction with the ovarian capsule. The intruterine fluid did not escape during this and subsequent procedures.

The ovaries were dissected free from their capsules using iridectomy knives and small scissors under a dissecting microscope. The greatest diameter of the ovary was measured in mm with an ocular micrometer and used as a comparative check in subsequent weighings. The ovary was invariably obtained intact with only an occasional shred of connective tissue attached.
Immediately after dissection the tissues were immersed in 10% formal-saline solution. They were removed from the fixative, briefly blotted on filter paper to remove excess fluid, and weighed to the nearest 0.01 mg. All weighings were completed within 1 hour after the animals were killed. Repeated weighings showed no changes in the weights of ovaries or uteri after immersion in the fixative for this length of time. The ovaries from each group were processed; they were embedded in a single paraffin block, which was cut at 5 μ to give representative sections, and were stained with hematoxylin and eosin.

Statistical Analysis

The results were analyzed by computer, using a program which calculated an analysis of variance for a two-way classification with replication in each cell. The calculation contained an interaction component which evaluated the response to DMBA and gonadotrophin in combination, that is, whether the two combined to produce an added effect not due to one of them alone. In the tables, the heading “Interaction of CG (or PMS) and DMBA” refers to a statistical comparison between ovarian, uterine, or total body weights of “(Normal + CG/PMS) and Normal”; and “(DMBA + CG/PMS) and DMBA alone.” When no significant difference occurred, it was concluded that the response to gonadotrophin was similar in carcinogen-treated animals and in normal animals. Values of \( P = 0.025 \) or less were considered significant.

RESULTS

The Effect of Age at the Time of DMBA Administration on the Subsequent Response of Mice to CG

The alterations in the mean weights of the ovaries, uteri, and the whole body are detailed separately in Tables 1–3 for mice of the CDF strain which received DMBA and CG treatment at various ages. Similar data for C57 mouse ovaries are given in Table 4, but only significant alterations in the uterine and body weights due to treatment are indicated by superscript notations in this table.

In these and all of the following experiments, gonadotrophin injection significantly increased the ovarian weights of normal mice at all of the ages studied. With the exception of one experiment (Table 1, Expt. 14), DMBA caused a reduction in ovarian weights 3 days after its administration to CDF and C57 mice at all of the ages studied. DMBA eliminated or markedly reduced the response of the ovary to gonadotrophin in C57 mice up to 34 days of age; but at 56 days and older, the interaction of these two factors was no longer significant (Table 4). A more persistent, though less absolute, interaction between DMBA and CG was observed in ovaries of CDF mice of age in the other mice.

The uterine weight increased after gonadotrophin treatment of immature mice, but this response was variable after 34 days of age in C57 mice and after 26 days of age in CDF mice. At these times there was a greater initial weight of this organ, reflecting the spontaneous ovarian secretion occurring with maturity. DMBA given prior to the gonadotrophin reduced the initial uterine weights and the response in immature mice but had no effect in most experiments after maturity was reached.

Total body weight was decreased after administration of DMBA in C57 mice up to 56 days of age and in CDF mice up to 36 days. There was no interaction between DMBA and gonadotrophin in this weight loss and, in some of the older groups, while there was a significant inhibition of the action of gonadotrophin on the ovary, there was no depression of body weight generally.

The Effect of Genetic Differences and the Interval between Administration of DMBA and CG on the Ovarian Response of Mature and Immature Mice

Mature Mice. The variations in ovarian weight of mature mice of various strains with DMBA and CG dosage are shown in Table 5. Details of uterine and body weights are not shown, but significant alteration of these by DMBA or CG treatment is indicated by superscript notations. CG injection caused an increased ovarian weight in all strains. After DMBA dosage there was a significant drop in ovarian weight in all of the mouse strains examined except the CNZ. There was no interaction between the effects of DMBA and CG on the ovaries of mature mice of the B6A, BCH, and CNZ strains at any of the time intervals examined, but, with BCH and CNZ mice, the interaction of these two factors was studied only with an interval of 3 days between the two factors. The experiments with CAF mice (Expts. 22–24) demonstrate that a significant relationship in the ovary between DMBA dosage and subsequent response to CG may not be demonstrable in 3 or 5 days but can become apparent after 7 days. There was significant interaction between DMBA and CG given to BDF mice after an interval of 3 days and to C57 mice given DMBA when mature with a 100-day interval before the administration of CG (cf. Expt. 7, Table 4).

DMBA treatment depressed the uterine weight in a minority of the mice treated when mature (Expts. 19, 27, 28, and 31), and CG injection alone was also followed by decreases in uterine weight in 4 experiments (Nos. 22, 26, 29, and 33). However, there was significant interaction in the effects of the trophic hormone and DMBA only in Experiment 22.

Immature Mice. In 4 of the 5 experiments in which DMBA was given to mice before maturity (Table 6), the ovarian weight fell below the pretreatment level and did not rise in any of the four strains after 30 to 38 days, and up to 100 days in one case (Expt. 18). Although the data in Tables 1 and 4 show a significant interaction between the action of DMBA and CG on the ovary in immature mice, when the latter was administered after an interval of 3 days, it is evident (Table 6) that when the interval between CG injection is increased to 30–38 days this interaction no longer exists. However, the ovaries of C57 mice returned to an unresponsive state and did not react to CG when the interval after DMBA was increased to 100 days.
DMBA dosage depressed uterine weight (Table 7) in strain C57 (Expt. 17) and CAF mice (Expt. 25) when these were examined 30 to 33 days afterwards, but 100 days after exposure to the chemical, strain C57 uteri were similar in weight to those of untreated controls (Expt. 18).

**Effects on Body Weight**

Body weight was depressed by DMBA in 7 of the 16 experiments in Tables 5 and 6, but only in one such instance was there interaction between the DMBA and CG in their effects on the ovarian weights.

**The Reaction of Mice and Rats to PMS Injection after DMBA**

Data for the ovarian response of 3 mouse and 2 rat strains to DMBA and subsequent PMS administration are given in Table 8. These are comparable to similar experiments for mice...
### Table 4

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Mean weights of ovaries of C57 mice with and without treatment with CG and DMBA at different ages. CG, chorionic gonadotrophin; DMBA, 7,12-dimethylbenz(a)anthracene.

*Associated with depression of total body weight.

*Associated with depression of uterus weight.

*Associated with increase of uterus weight.

### Table 5

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<th>Expt. No.</th>
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Mean weights of ovaries of various strains of mice with and without CG and DMBA at varying ages and time intervals. CG, chorionic gonadotrophin; DMBA, 7,12-dimethylbenz(a)anthracene.

*Associated with depression of total body weight.

*Associated with depression of uterus weight.

*Associated with increase of uterus weight.

### Table 6

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<th>Expt. No.</th>
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Mean weights of ovaries of mice with and without DMBA when immature followed by CG after an extended time interval. CG, chorionic gonadotrophin; DMBA, 7,12-dimethylbenz(a)anthracene.

*Associated with depression of total body weight.
Mean uterus weights of mice with and without DMBA when immature followed by CG after an extended time interval. CG, chorionic gonadotrophin; DMBA, 7,12-dimethylbenz(a)anthracene.

Exposed to DMBA and CG shown in Tables 5 and 6, and it is seen that there are no qualitative differences between the responses of mice to PMS or CG after previous treatment with DMBA.

SD rats of about the same starting weight were used in Experiments 35A and B. When the delay before PMS injection was only 3 days, there was a significant inhibition of gonadotrophic activity on the ovary, but when PMS was injected after 5 days its action was actually enhanced. It should be noted in the second of the two experiments, however, that at this period of rapid growth there were marked changes in both the initial whole body and ovarian weights. F rats showed little effect of DMBA on the ovaries or their response to PMS at any of the three weights studied.

The gonadotrophin alone enhanced uterine weight in all rat experiments. Depression of uterus weight by DMBA occurred in Experiments 35A and 38, and depression of total body weight occurred in both Experiments 35A and B. There was no interaction between DMBA and PMS in their effects on these parameters.

Effect of Dose of DMBA

The response of immature C57 mice to CG 3 days after the administration of different doses of DMBA by stomach tube or by intraperitoneal injection in oil is given in Table 9. Administration of 4 mg DMBA by stomach tube or injection of 0.5 mg DMBA i.p. completely inhibited response to gonadotrophin either by ovarian hypertrophy or by increase in uterine weight. The necessity for doses of this order is demonstrated by the findings that 2 mg or 1 mg by stomach tube or 0.25 mg or 0.1 mg i.p. had much less inhibitory activity.

Histologic Observations

Follicles in various stages of maturity were seen in all normal mice, their size distribution varying with age. Some follicles appeared to be undergoing atresia in all of the ovaries examined, and in the older ovaries, the remnants of degenerated follicles could be distinguished.

Three days after administration of DMBA the ovaries of mice contained a greater proportion of follicles which were obviously degenerating. Evidence of this was the disintegration of the granulosa cells and the shrinking and fragmentation of the ovum. All follicles smaller than 80 to 100 μ seemed to be involved in this process, but the larger ones were relatively unaffected and were often seen to contain healthy-looking ova.

At 30 to 38 days after exposure to DMBA, the ovaries

| Expt. No. | Species and age at start | Weight from DMBA to PMS (days) | Ovarian weights (mg) | Values of P for effect on ovarian weight of:
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<td>F 80 gm</td>
<td>3</td>
<td>9.5</td>
<td>46.7</td>
<td>8.8</td>
<td>51.3</td>
<td>0.001c</td>
<td>0.1</td>
</tr>
<tr>
<td>38</td>
<td>F 115 gm</td>
<td>3</td>
<td>22.8</td>
<td>42.7</td>
<td>19.3</td>
<td>46.3</td>
<td>0.001c</td>
<td>0.1a</td>
</tr>
</tbody>
</table>

Mean weights of ovaries of rats or mice with and without PMS and DMBA at various ages and time intervals. PMS, pregnant mare serum; DMBA, 7,12-dimethylbenz(a)anthracene; SD, Sprague-Dawley rats; F, Fischer rats.

*Associated with depression of total body weight.
*Associated with depression of uterus weight.
*Associated with increase of uterus weight.
General Reactions to DMBA

At the doses used, there were no general toxic effects other than weight loss in mice. Some rats in Experiments 37 and 38, however, had diarrhea and adrenal hemorrhage after DMBA.

DISCUSSION

An analysis of the effects of DMBA on ovarian response to either endogenous or exogenous gonadotrophins must be based on fundamental concepts of the hormonal factors which regulate ovarian growth and secretion.

The development of the ovary in the mouse and the rat has been reviewed in detail by Franchi et al. (17) and by Harrison (19). Follicular enlargement, up to diameters of about 250 μ, occurred independently of the pituitary, with proliferation of granulosa cells and the establishment of the theca interna. Subsequent development is dependent upon stimulation by gonadotrophins, their precise nature and interrelationships remaining unestablished (10). The concept of three gonadotrophins (2), i.e., FSH, LH, and LTH, explains the observed sequence of events, and the three factors can be identified in different fractions of pituitary extracts. Rupture of the mature follicle and ovulation precede conversion to a corpus luteum by luteinization of the existing granulosa cells, together with proliferation of the theca interna. The extent to which the theca interna contributes to luteinization is not yet clear (12).

Intrafollicular hemorrhage preceding or accompanying luteinization is a common sequel to the experimental administration of gonadotrophins, although its frequency varies considerably from one species to another, and also between different strains of the same species. It has not been recorded as being normally associated with ovulation in the mouse and the rat, but it has been noted as a spontaneous event in other species (1). The secretion products of the follicle and corpus luteum are related to stimulation by FSH, LH, and LTH (14, 49).

Both CG and PMS have FSH and LH activity. The follicle-stimulating activity of CG is apparently dependent upon the possession of an intact pituitary, although luteinization of existing follicles occurs in the hypophysectomized rat (42). PMS, however, is active in the absence of the pituitary, and it tends to have a more marked effect on the stimulation of follicles.

There are two variables in the experimental treatments we have used: the presence or absence of exogenous gonadotrophin and the presence or absence of DMBA. Gonadotrophin treatment alone (either CG or PMS) consistently caused marked increases in ovarian weight, due to follicular maturation and luteinization, in all strains of mice and rats studied. Whether the administered hormone was CG or PMS made no qualitative difference. In immature mice, the ovarian hypertrophy was accompanied by a large increase in uterine weight from the low level for the atrophie uterus found in controls. With maturity, however, changes in weight of the ovary were not necessarily paralleled by changes in uterine weight.

The administration of DMBA had two distinct effects on the ovaries of mice. With few exceptions, there was a decrease in ovarian weight 3 or more days after giving DMBA, no matter at what age this was done. The response to subsequent gonadotrophin injection was reduced in all immature mice, and, in some mature mice, the effect was more pronounced with PMS than with CG but qualitatively the same. The most pronounced effect of DMBA was on the follicles, and the development of granulosa cell tumors in mice in long-term experiments may be an ultimate reflection of this. Follicular damage affecting their response to exogenous gonadotrophin varied in degree and was related to the age of the animal and follicle size at the time of exposure to the chemical. In immature mice (Tables 1 – 4), inhibition of response to gonadotrophin was complete or almost so. If, however, DMBA was given to immature mice but gonadotrophin administration was delayed for 28–33 days (Table 6), ovarian hypertrophy...
was proportionately almost normal, although the starting weight of the ovary was in fact less than at the time of DMBA administration. Delay of gonadotrophin until 100 days after DMBA dosage eliminated any response to the hormone by hypertrophy of the ovary, which is understandable in light of the histologic finding of complete absence of follicular tissue at this time.

Histologic observations showed that, in the mouse, DMBA eliminated all primordial follicles and damaged some larger follicles. In the case of immature mice, those follicles which remained were inhibited in their response to gonadotrophin for periods of up to 7 days, but they recovered this response by 28 days after DMBA administration. The immediate effect on the response of surviving follicles to gonadotrophins was therefore transitory and was followed by recovery, termination of which was probably due to exhaustion of the remaining follicles as they degenerated or luteinized.

In mature mice, the action of DMBA in eliminating primordial follicles seems to have paralleled that in immature mice, but the inhibition of response to gonadotrophin among the surviving follicles was probably absent or only partial. This would account for the undiminished, or only partially decreased, response of the mature ovaries to exogenous stimulation. Susceptibility of the mouse ovarian follicle to DMBA is probably linked to the degree of maturation, varying from destruction at the earliest stages to inhibition at intermediate stages and to no effect in the more mature follicles.

There is a parallel between the effects of DMBA and the initial uterine weights 30 to 33 days after DMBA administration. Spontaneous secretion returned thereafter in some cases, and, in the remainder, the capacity to secrete returned and became evident with an adequate stimulus.

The fact that, in Experiments 17, 18, 25, 30 (Table 6), and 42 (Table 8), the ovaries 30 to 100 days after DMBA administration were smaller than those of immature animals must mean either that they were insensitive to endogenous gonadotrophin stimulation, or that they were not exposed to a sufficient level of gonadotrophin after DMBA administration. In all but Experiment 18, where follicular tissue was absent, these ovaries responded to injected gonadotrophin by an increase in weight. It must therefore be inferred that the DMBA had some action which inhibited or considerably reduced the secretion, release, or relative amounts of the trophic hormones from the animal’s own pituitary. This could be achieved by an inhibition of LH or FSH, either, probably, being ineffective in the absence of the other. In Experiments 18 and 30 in which, despite atrophic ovaries, the uterine weights were not abnormally low, it must also be inferred that despite the absence of ovarian growth a secretory stimulus was present.

Effects of polycyclic hydrocarbons on pituitary synthesis and release of hormones in rats have been demonstrated or postulated by a number of workers. Huggins and Pollice (23) found a decrease in the ovarian weight of rats treated with 3-methylcholanthrene and attributed this to an effect on gonadotrophin production because the ovaries still responded to the injection of PMS. Moon (40) showed a decrease in pituitary prolactin content of lactating rats which had been given 3-methylcholanthrene and found this to be associated with increased progestin activity in the estrone-primed breast which he attributed to increased release of prolactin by the carcinogen. Using androgen-sterilized rats, Stern et al. (51) found that DMBA administration interrupted the characteristic vaginal keratinization in these animals and induced mammary lobular development with sustained secretion. They also attributed this effect to LTH (prolactin) release.

Recently Dao et al. (11) have shown that the incidence of mammary tumors in transplants of breast from SD rats treated with DMBA is maximal if the transplantation is carried out within 24 hours, that is to say, at a time when the amount of DMBA present in the transferred tissue is still high. Grants made later than this survive better but have a much lower incidence of tumors. A possible explanation for this apparent paradox could be that there is sufficient DMBA carried over at the earlier time to influence significantly the function of the host pituitary, and it is this activity which stimulates the tumor yield in the grafts, although the level of the carcinogen is too low to induce tumors in the host’s own mammary tissue. The high mammary tumor incidence regularly achieved in intact SD rats with this carcinogen could, therefore, be due in part to pituitary modification, causing enhanced LTH release and, consequently higher levels of progesterone secretion. High progesterone levels are known to favor mammary tumor development (22). Such a dual action of some mammary carcinogens, causing a change in hormonal status favorable to tumor development as well as a change in the target tissue, has previously been suggested by Moon (40).

At first sight these results, which are compatible with the promotion of LTH release by DMBA administration (11, 23,
Ovarian Response to Gonadotrophins

40, 51), are contradicted by the clearly presented evidence of Wong et al. (52) that DMBA causes necrosis of the most recent corpora lutea in the rat. Their description and illustrations of this change, however, agree closely with the descriptions of spontaneous involution of corpora lutea (3, 6). The apparent paradox is that corpora lutea are undergoing necrosis at a time when LTH secretion is apparently augmented. It should be noted (52), however, that a substantial rim of luteal cells is maintained and that the necrosis is centralized. The observations of Wong et al. are in agreement with the postulate that DMBA causes an abrupt termination of LH release and so initiates luteal degeneration, presumably by interfering with the passage of the LH-releasing factor from the hypothalamus to the anterior pituitary. The sequel to this inhibition of LH is the stimulation or release of inhibition of LTH (38). The circulation of LTH might be expected to maintain such cells in the corpora lutea in which involution has not yet occurred, and, as involution commences centrally, these will be at the periphery. It has been shown (27) that DMBA has some of the biologic properties of progesterone. The inhibition of LH discussed here conforms to this observation, as it is well known that progesterone inhibits LH secretion (3, 38, 48).

DMBA administration alone had no significant effect on the weights of the ovaries in either immature SD or in F rats, and histologic observations showed no morphologic changes in them. The action of PMS was inhibited 3 days after DMBA in SD rats, but at 5 days the response to PMS was greater than normal. Similar ovarian weight increases greater than those of the controls were shown by F rats 3 days after DMBA, although these differences were not significant.

Such effects are compatible with the concept that the chemical inhibits pituitary LH secretion for a few days. This action would explain the variations in ovarian weights observed by us and other workers and would be in accord with the histologic evidence of a lack of direct ovarian damage. The effects of DMBA on rat ovaries are in marked contrast to those observed in mice. These differences may possibly be related to the fact that DMBA has no tumorigenic action at all on the rat ovary.

The fact that carcinogenic polycyclic hydrocarbons may cause growth inhibition was noted by Haddow et al. (18), and there are examples of this activity in these experiments. Flaks (16) also found a general loss of weight in his mice treated with 3-methylcholanthrene, in which there was inhibition of response to gonadotrophins. He concluded that the loss of weight was not responsible for the effect, as inhibition of gonadotrophic stimulation was observed before the general loss in weight occurred.

Hipkin (20) in his experiments on rats considered that the decrease in the response of the ovaries to minimal doses of gonadotrophin was due to the concurrent loss in body weight. He achieved a comparable weight loss by fasting rats before injecting the gonadotrophin and found a decreased response similar to that induced by DMBA. Some support for this point of view is derived from the work of Lang and Lamond (30); these investigators have shown that fasting significantly decreases the ovarian secretory response to gonadotrophins in mice as judged by uterine weights. They did not, however, show that there was an effect on the weight increase of the ovaries.

Our experiments are not strictly comparable with those of Hipkin or Lang and Lamond in view of the fact that we used a large dose of gonadotrophin, but DMBA did significantly inhibit weight gain in a number of groups. In C57 mice (Table 4), a significant interaction between the effects of DMBA and gonadotrophin on the ovary between 21 and 34 days was paralleled by a depression of body weight due to the chemical treatment. Although there was inhibition of body weight in CDF mice by DMBA treatment in 5 experiments between 21 and 36 days of age (Table 3), there was an effect on the ovarian weight response to gonadotrophin in only three of these experiments (Table 1). Mature CDF mice, aged 104 days, did not lose weight after receiving DMBA but showed inhibition of ovarian response to exogenous CG. In other experiments (Table 5), ovarian inhibition occurred in only 1 of 5 experiments in which there was a significant effect of DMBA on body weight.

Thus it does not seem possible to explain the effects of DMBA on the ovarian weight response to gonadotrophins by using the simple premise of general body weight loss. This conclusion is supported by the observation that mice, in which DMBA inhibited the ovarian response to gonadotrophins, appeared to eat normally and had no diarrhea. F rats on the other hand (Table 8), which showed no inhibition of ovarian response to PMS after DMBA, had marked inhibition of growth and some diarrhea and adrenal hemorrhage. Our conclusion is that the decrease in body weight, sometimes observed after DMBA administration, reflects a direct action on pituitary function.

The possibility that there is a DMBA-induced modification of the pituitary secretion of gonadotrophins and growth hormone seems to be common to both mice and rats, although there are considerable differences in the relative sensitivities of different strains. There is a clear distinction, however, between mice and rats in the susceptibility of their pituitary-dependent target tissues to modification by the carcinogen. In mice the ovary, but not the adrenal, is very susceptible; in the rat, the susceptibility to damage is reversed. The ovaries and the adrenals in both species possess steroidogenic capacities which probably follow common paths some part of the way. It is possible, therefore, that in the process of tissue differentiation the ovary of the mouse and the rat adrenal inherit similar steps in their steroidogenic mechanisms which are susceptible to DMBA. In this regard, it is pertinent that the dose requirements for ovarian tumorigenesis and gonadotrophin inhibition in the mouse are comparable with that for adrenal necrosis by DMBA in the rat.

We hope to publish in the future considerable information regarding the responses of various strains of mice to the tumorigenic action of DMBA. The data will show that there is no simple relationship between the interactions of DMBA and gonadotrophins discussed here and the susceptibility of mice of different genotypes to granulosa cell tumor induction by the chemical.

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REFERENCES


12. Deanesly, R. Development and Vascularization of the Corpus


15. Essenberg, J. M. Response of Germ Cells to Gonadotrophic


The Effects of 7,12-Dimethylbenz(a)anthracene on the Ovarian Response of Mice and Rats to Gonadotrophins

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