EXPERIMENTAL INDUCTION OF OVARIAN TUMORS IN MICE TREATED WITH SINGLE ADMINISTRATION OF 7,12-DIMETHYLBENZ[a]ANTHRACENE, AND ITS HISTOPATHOLOGICAL OBSERVATION
(Plates XXXVII～XLI)

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Synopsis

1) Induction of ovarian tumor with a single intragastric instillation of a large but tolerable doses of 7,12-dimethylbenz[a]anthracene (DMBA) was investigated on young female mice of C3H and C57BL strains, rats of Sprague-Dawley strain, guinea pigs, and rabbits. The ovarian tumor occurred in a high incidence only in C3H mice.

2) The induction of ovarian tumor in C3H mice with DMBA by various routes of administration such as intragastric instillation, intraperitoneal or intravenous injection was studied. The following tumor incidences were obtained in the treated mice: Single feeding, 59% until 7th month; intraperitoneal injection, 36% until 4th month; single intravenous injection, variable percentages at each month and average 57% after 6 months. Intraperitoneal injection of DMBA induced ovarian tumor earlier and in higher incidence than by other two routes but mortality of mice was unfortunately very high.

3) Histological observations revealed no normal ovaries in C3H mice given DMBA intravenously. Follicular structures were destroyed and oocytes vanished within one month. Proliferation of granulosa cell following its earlier non-specific pathological change progressed from microscopical nodule to a sizable gross tumor. These tumors were all granulosa-celled type.

4) Granulosa cell tumor arose probably from surviving granulosa cells in the partially degenerated follicles and its growth was stimulated by pituitary gland but there was still a probability that granulosa cell tumor might have originated from theca cells.

5) Frequent study of vaginal smears demonstrated possible association between the presence of tumor and estrogen activity.

INTRODUCTION

It has been shown that several methods are available for the induction of ovarian tumor in animals. Brambell et al.5~11,69) were the first to observe neoplastic changes in the ovaries of mice after irradiation. The second experimental method of inducing ovarian tumors was discovered by Biskind et al.,5) who obtained such neoplasms in rats by intrasplenic graft of an ovarian fragment. The third method was the repeated cutaneous application of a carcinogenic compound. Tumorigenic action of 7,12-dimethylbenz[a]anthracene (DMBA) on murine ovarian tissue was discovered by Engelbreth et al.,22) but unfortunately its tumor incidence was low. Radiomimetic, non-carcinogenic compounds such as triethyleneemelamine or 1,4-dimethanesulfonylbutane could alone

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induce ovarian tumor in mice. Recently, the authors reported that a single administration of a large amount of DMBA induced several kinds of ovarian tumors in a certain strain of mice in a high percentage.

In the present work, neoplastic process in the ovaries was initiated by a single intravenous injection or an intragastric instillation of DMBA. The present paper describes the incidence of induced tumors, their histogenetic origin, and growth behavior of experimented ovarian tumors induced by DMBA. It also describes the tumor incidence in several laboratory animals utilizing this technique.

**Materials and Methods**

**Animals** Normal virgin female mice of C3H and C57BL strains were originally supplied from the Central Animal Supply, Kyoto University School of Medicine, and bred by brother-sister mating in our Laboratory for the past 3 years. They were certified to be free from murine milk agent. They were provided commercial mouse chow, Oriental CMF, and water *ad libitum*, and kept in metal cages, up to 7 to a cage, in an air-conditioned room.

**Chemicals** 7,12-Dimethylbenz[a]anthracene (Eastman Organic Distillation Products, U.S.A.) (DMBA) was recrystallized and dissolved in sesame oil for instillation into a mouse stomach. Fat emulsion of DMBA (15% emulsion, The Upjohn Co., U.S.A.) was used for injection into a caudal vein or the peritoneal cavity.

**Experimental Groups** The mice were grouped according to the route of the administration of DMBA.

- **Group 1**: Consisting of 25 C3H mice and 26 C57BL mice. Single intragastric instillation of 0.5 ml of 2% DMBA in sesame oil (10 mg), given at the age of 50 days.
- **Group 2**: Consisting of 41 C3H mice, aged 45 and 65 days. Single or repeated intraperitoneal injection of 0.5 ml of 0.5% fat emulsion of DMBA (2.5 mg) at weekly intervals.
- **Group 3**: Consisting of 69 C3H mice, aged 60 and 90 days. Single intravenous injection of 0.5 ml of 0.5% fat emulsion of DMBA (2.5 mg).
- **Group 4**: Control group of 50 C3H and 21 C57GL mice, observed for a one-year period without any treatment.
- **Group 5**: Miscellaneous animal group consisting of 10 Sprague-Dawley rats, 10 hamsters, 14 guinea pigs, and 6 rabbits. Each animal was given DMBA by intragastric instillation in an amount calculated according to their body weight; 30 mg to a rat, 30 mg to a hamster, 50 mg to a guinea pig, and 500~1,000 mg to a rabbit.

The mice were weighed every day for 2 weeks after DMBA administration and once a week thereafter. Following a given period of observation, the animals were sacrificed. In addition to routine postmortem examination, the ovaries and the uterus were carefully examined, weighed on a torsion balance, and processed for histological examinations. The animals found dead were necropsied and processed promptly in a similar manner. The specimens were fixed either in 10% neutral formaldehyde solution or 80% ethanol and embedded in paraffin in a usual manner. The serial sections were taken and stained routinely with Hematoxylin-Eosin, van Gieson’s method, and Bielschowsky’s method for reticulum fiber. Sudan stain for lipid was applied to frozen sections of

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*2 Both chemicals were the kind donation of Professor Charles B. Huggins of the University of Chicago.*
formaldehyde-fixed tissues. Vaginal smears were examined frequently by means of Giemsa stain.

All the C3H mice, receiving a single intravenous injection of DMBA, were sacrificed at monthly intervals, up to 12 months. Group 5 animals were observed for a maximum period of 7 months and the survivors were sacrificed at the end of this period. Their tissues were examined as above.

**RESULTS**

**Incidence of Ovarian Tumor in Mice**

All the mice given DMBA lost their weight in a few days following the administration of the carcinogen, but their weight regained before long. No immediate fatality due to the toxic effect of DMBA was noted after intragastric instillation or intravenous injection. Many of the C3H mice injected intraperitoneally with DMBA, however, died of severe peritonitis within 2 months and its mortality was 27/41 (61%) at the end of 4 months. There were a few animals in each group which died of benign disease such as pneumonia during the course of observation. None of these animals harbored neoplastic processes.

<table>
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<tr>
<th>Strain</th>
<th>Mode of administration</th>
<th>Autopsy (month)</th>
<th>Total No. of mice</th>
<th>Mice bearing tumor No. (%)</th>
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Only macroscopic ovarian tumors were counted as tumor in this Table. Total No. of mice indicates the number of survivals at the time of necropsy.

<table>
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<tr>
<th>Months after injection</th>
<th>Total No. of mice</th>
<th>No. of ovary with tumor histologically confirmed</th>
<th>No. of ovary with tumor macroscopically determined</th>
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* Bilateral
An ovarian tumor, measuring more than 2 mm in diameter and/or weighing more than 20 mg, has been arbitrarily classified as macroscopic tumor. The monthly incidence of macroscopic ovarian tumor, induced with DMBA by different administration route, is shown in Table I, and the monthly incidence of ovarian tumor including microscopic ovarian tumor in C3H mice, given DMBA intravenously, is listed in Table II.

Group 1 (Table I): Nine out of 26 C57BL mice, instilled DMBA into the stomach, died of thymoma and other benign disease within 7 months. Single intragastric instillation of DMBA did not induce ovarian tumor in the 17 survivors of C57BL mice but 10 splenic tumors, 2 liver tumors, and 1 skin papilloma were detected in the strain during the 7 months.

In 22 C3H mice given a single intragastric instillation of DMBA, the incidence of tumors after 7 months was 13/22 (59%) of ovarian tumor and 6/22 (27%) of mammary tumor. These ovarian tumors were mainly composed of granulosa-cell tumor and the others were thecoma or papillary adenocarcinoma.

Group 2 (Table I): In 14 C3H mice given a single intraperitoneal injection of DMBA, ovarian tumor developed in 5 mice (36%) and a skin papilloma in 2, after 4 months of observation.

Group 3: In 44 surviving C3H mice out of 69, given a single intravenous injection of DMBA at the age of 60 and 90 days, macroscopic ovarian tumors were noted in 25 mice (57%) after 6 months. The other types of tumor found were: Mammary cancer, 4; lymphoma, 2; stomach cancer, 1; adrenal tumor, 1.

Group 4: Neither ovarian tumor nor lymphoid tumor was observed in 21 untreated C57BL mice. No mice with ovarian tumor but only 2 with mammary cancer were found in 50 non-treated C3H mice.

Group 5: The animals were given a single intragastric instillation of a large dose of DMBA and observed for 6 months. No neoplastic process was found in any of the animals during this period except in the rats, who showed notable tendency for mammary cancer. No ovarian tumors were found even in the rats.

**Biological and Histopathological Observation on Preneoplastic and Neoplastic Changes in the Ovary of C3H Mice**

The histological changes in murine ovaries were investigated monthly in Group 3 animals and results are summarized in Table III.

At the 1st month, the ovaries were almost normal in gross appearance and weight (about 2 mm in size), roughly spherical in shape, with fine nodular surface and pale white-yellowish in color. Histologically, there were all stages of follicles which numbered 16 on an average and four corpora lutea in the largest section. The oocytes were markedly damaged. The nuclei of oocyte were fused and slightly basophilic in Hematoxylin-Eosin stain. The karyorrhexis of these nuclei was prominent. The protoplasm of these oocytes was fused, stained slightly eosinophilic, and was sometimes vacuolated (Photo 1). There were degeneration of granulosa cells and thin eosinophilic vacuolated substance in Graafian follicle. In some cases, there were calcified areas surrounded by theca cells which appeared after degeneration of oocytes (Photo 2). In one ovary there was an aggregation of pale or thin eosinophilic polygonal epithelial cells surrounded irregularly by a single layer of spindle-shaped cells. The latter seemed to invade into the former, and these spindle-shaped cells were connected to stroma cells (Photo 3). Oc-
Table III. Histological Structures in Ovaries of C3H Mice at Monthly Intervals after Intravenous Injection of DMBA

<table>
<thead>
<tr>
<th>Months after DMBA injection</th>
<th>Follicle</th>
<th>Granulosa cells</th>
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<td>Graafian follicle</td>
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<th>Months after DMBA injection</th>
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<th>Months after DMBA injection</th>
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- absent, ± sometimes present, sometimes absent, + present, ++ abundant
casionally granulosa cells with varying degree of luteinization, which were not separated by theca cells spread out from the follicle (Photo 4).

At the 2nd month, all ovaries were almost normal in gross appearance except some ovaries which greatly varied in size and weight. They had a few hemorrhagic spots on its surface. In general, weight of murine ovary treated with DMBA was less than those of untreated animals. In all the ovaries, normal follicles were replaced by abnormal ones. Number of follicles in the largest section fell considerably and each follicle became smaller; sometimes became anovular and was formed with a single layer of flattened cells. There were also a few follicles formed with a single layer of cuboidal cells with dark stained nuclei and with a small amount of cytoplasm. These cells have a strong resemblance to the cells of the normal granulosa membrane (Photo 5). There were also follicles which were composed of the degenerated granulosa cells, surrounded by spindle-shaped cells, and contained mucinous fluid in its center (Photo 6). Some follicles were cystically dilated in which granulosa cells degenerated to the extreme. The mouse with such a cystic follicle showed estrus vaginal smears at the time of sacrifice. Nodular hyperplasia of granulosa cells was seen in some part of ovary and this was clearly demarcated with a layer of theca cells. Corpora lutea was smaller, decreased in number, and of different degree of luteinization. Interstitial tissues were loose and edematous. Several calcified foci were also seen in the follicle.

At the 3rd month, all ovaries were small and uniform in size, and contained anovular follicle and/or follicles with degenerated oocytes. Normal Graafian follicles were entirely absent. Diffuse proliferation of granulosa cells was seen in one ovary (Photo 7). The contralateral ovary was composed of a nodule of granulosa cell tumor. (Photo 8). In other ovaries, there were many small aggregations of granulosa cells without mitotic figures. In one ovary, there was one nodule of granulosa cell tumor. It was rather interesting that the tumor granulosa cells grew centropetally from one point of theca cell line (Photo 9). It seemed that granulosa cells could have originated from theca cells. The mice with these nodules showed estrus vaginal smears at necropsy. These findings at the 3rd month were the first obvious microscopic ovarian tumor in this experiment, even though the mice bore cystic follicle and showed continuous estrus vaginal smears two months after DMBA treatment.

At the 4th month, all ovaries were variable in size and had less follicles with thin eosinophilic mucous substance in their center. In some cases, there were small nodules composed of degenerated, fused granulosa cells surrounded by spindle-shaped cells. Some mice with continuous estrus vaginal smears had larger ovary on one side than the other (Photo 10). The larger ovary was spherical with fine nodular surface, measuring 3 mm in diameter, and yellowish brown in color. There were calcified foci and large nodule of tumor granulosa cells with scattered thecoma-like cells (Photo 11). In another case, a diffuse proliferation of granulosa cells mixed with pigmented cells was observed. The contralateral small ovaries were white-yellowish, atrophic, and composed chiefly of many small anovular degenerated follicles.

At the 5th month, one ovary was large, spherical, and measured 15 mm in diameter. It was mottled purple red with whitish spots and streaks. The ovary also contained hemorrhagic cyst and adhered to the posterior viscera (Photo 12). Histologically, it was a typical granulosa cell tumor with focal hemorrhage, folliculoid (follicular) patterns,
the so-called Call-Exner body (Photo 14), and mucoid degeneration. In some area, calcification, pigmentation, hyalinization of stroma, and necrosis were seen. Another ovary was small and composed of proliferated granulosa cells in which many follicular structures were arranged beneath the tunica albuginea. The mice with this type of ovary showed continuous estrus vaginal smears. Other ovaries were small. They contained degenerated granulosa cell nodules or showed diffuse luteinization and hyalinization.

At the 6th month, 3 out of 6 mice had unilateral ovarian tumor which was spherical or ellipsoid, measuring 13 mm in diameter and weighed 700 mg in maximum, grey-whitish in color, and mottled with purple red spots. They showed a differentiated type of granulosa cell tumor with bud-like alveolar structure (Photo 15). One mouse had a granulosa-cell tumor nodule in the other ovary at the same time (Photo 13). In the other mice, there were large hemorrhagic cysts, diffuse hyperplasia of luteinizing interfollicular tissues (Photo 16), and a nodule of granulosa cells or complete atrophy.

At the 7th month, there were two ovaries with a typical granulosa cell tumor. One was a large ellipsoid, measuring 15 × 12 × 10 mm, weighing 950 mg, and white-yellowish in color. The other was smaller but showed similar features. In the other ovaries, there were granulosa cell tumor nodules mingled with small adenomatous follicular structures beneath the tunica albuginea, hemorrhage, and necrosis. The contralateral ovaries were atrophic. One mouse had atrophic ovaries on both sides.

At the 8th month, all mice had ovaries with localized or diffuse granulosa cell tumor unilaterally except in three mice. One of these neoplastic ovaries weighed 291 mg and was whitish in color. It also contained serous cystic cavity (Photo 17).

At the 9th and 10th month, all mice had large unilateral ovarian tumor which was composed predominantly of granulosa cell proliferation. The greater part of the tumor consisted mainly of nests of tumor granulosa cells and adenomatous proliferation invading the capsule. Hemorrhage, hemorrhagic cyst, pigmented cells, and necrosis were seen in some part. One of these mice harbored concomitantly breast cancer and thymic tumor.

At the 11th and 12th month, three mice had a large ovarian tumor of granulosa cell type on one side. The largest measured 15 × 15 × 10 mm, weighed 1200 mg, and was dark red in color with hemorrhage and hemorrhagic cyst (Photo 18). Ossification, many pigmented cells, and necrosis were seen in strongly hyalinized tumor stroma (Photos 19 and 20). Another mouse had a spherical granulosa-cell tumor with a small cyst (Photo 21). Ovaries of other three mice were all small but composed of granulosa-cell tumor nodules or diffuse granulosa cell proliferation on one side (Photo 22). The other ovaries were atrophic.

Uterus: When an ovarian tumor was present in a mouse, the uterus was always hypertrophic, but one mouse had a markedly atrophic uterus in spite of a large granulosa-cell tumor. Hypertrophy of these uteri was due to glandular proliferation manifested by excessively dilated glands. They were irregularly distributed and arranged in various directions with mucinous fluid or blood. Also hemangioma-like structure was seen in uterine muscle of some mice bearing granulosa-cell tumor.

To summarize the findings of ovarian change, all ovaries of C3H mice, given intravenous injection of DMBA, showed histopathological changes as early as in the 1st month. The contralateral ovary to the tumor and ovaries of tumor-free mice were all very small and atrophied at any given stage. Normal follicles or matured Graafian follicles were
seldom seen when ovarian tumor nodule had appeared, even on a microscopic level. The first granulosa cell tumor nodule was found microscopically at the 3rd month and macroscopically at the 4th month. Many large ovarian tumors appeared at the 5th month and its maximum size was 15 mm in diameter, 13000 mg in weight. Table II shows that, as time goes on, there is an increase of tumor and tumor nodule incidence, especially from the 4th month. The relative frequency of ovarian tumor between the left and right side was almost the same. The ovarian tumors were variable in shape and yellow whitish but sometimes greyish in color. In the case of cystic tumor, its color depended on the content of the cyst. No metastasis of these ovarian tumors was seen even after a thorough examination.

The first microscopic changes seen in these ovaries were the continuous disappearance of normal oocyte. They degenerated at the 1st and 2nd month, and then they vanished. Corpora lutea consisting of luteinizing cells in different degree were arranged, until the 2nd month, as in the ovary of untreated mice. Thereafter, these cells were fused, and eventually vacuolated and hyalinized or became cystic at the 4th month. These findings were invariably seen in all the ovaries of mice treated with DMBA. The luteal tissue was completely absent in the large ovarian tumor and there was no convincing evidence of neoplastic luteal growth in any of the treated mice. The germinal epithelium cells were cuboidal or short columnar in shape whether a mouse had ovarian tumor or not. These cells seemed to be dependent on the tumor growth.

**Vaginal Smears taken during Preneoplastic and Neoplastic Changes in Ovary induced by DMBA**

The vaginal smears were studied frequently throughout this experiment and also just prior to the sacrifice of animals. From the vaginal smears, it appeared that the estrus cycle was already disturbed in 10 out of 43 mice two weeks after DMBA treatment. Some of them, with adenomatous folliculoid ovary or atrophic ovary, showed continuous diestrus vaginal smears with frequent interruption of estrus picture for a few days. Continuous estrus smears were seen in three mice without ovarian tumor, in two of which diestrus periods appeared occasionally for a few days. In 29 mice, either continuous diestrus period or alternating diestrus and estrus cycles of short duration were observed. Twenty of these 29 mice had granulosa-cell tumors. Estrogenic influence in vaginal smears and uterus was found in 29 out of 40 mice with ovarian tumor (72%) prior to sacrifice. Eleven mice with granulosa-cell tumor did not show any estrogenic effect (27%). These statistics may indicate a high degree of correlation between estrogen activity and the presence of ovarian tumor. It was interesting to note that one mouse had an atrophic uterus and diestrus vaginal smears in spite of having a large ovarian tumor.

**DISCUSSION**

**Spontaneous Ovarian Tumor in Animals**

The spontaneous ovarian tumor in mice is believed to be extremely rare, but there are some reports of spontaneous ovarian tumor in mice and rats. In mice, the following spontaneous ovarian neoplasms have been reported: One bilateral papillary cysto-adenoma, a non-functional ovarian tumor in 44/2200 unknown strain, two transplantable functional ovarian tumors in 32/99 EBA strain, and bilateral granulosa
cell tumor of a 23-month-old mouse. In rats, non-functional ovarian tumor in 6/14038 unknown origin strain and a transplantable estrogen-producing tumor, which can metastasize to kidney, in AxC line have been reported.

Experimental Ovarian Tumor in Animals

There have been many different experimental methods and techniques of ovarian carcinogenesis with varying latency, such as irradiation in mice or rats, grafting an ovarian tissue of mouse, rat, guinea pig, or rabbit into its own spleen, or administration of a carcinogenic or radiomimetic substance to the mice of various strains.

X-Ray Induction of Ovarian Tumors

Some workers reported that the sterilizing dose of an X-ray caused first the disappearance of oocyte, followed by the destruction of Graafian follicle and the growth of the cells of the membrana granulosa and of theca interna. Later changes leading to the development of ovarian tumors in animals after irradiation were noted by many workers. Recently, radiomimetic substance, such as triethyl-melamine or 1,4-dimethanesulfonoxybutane, was tried to induce ovarian tumors in mice. The mechanism of induction of ovarian tumors after irradiation has been discussed by many workers. Most of them held that one of the mechanisms involved is the radiation injury of the ovaries as a direct action of the X-ray, but others claimed the disturbance of pituitary ovarian function due to ovarian changes induced by the X-ray.

Ovarian Tumors in Intrasplenic Graft

The growth of granulosa cell by implantation of ovarian fragment into the spleen of gonadectomized rat was first reported by Biskind et al. This method is often accompanied by parabiosis of the grafted animal to the castrated one. Such an intrasplenic grafted ovarian tumor was observed in some mice, rabbits, and guinea pigs. Intrapancreatic ovarian graft also induced granulosa-cell tumor in the castrated mice but subcutaneous ovarian graft failed to induce the tumor. An ovarian fragment irradiated prior to splenic grafting also induced an ovarian tumor in mice. There are many other reports concerning the influence of gonadal and gonadotrophic hormones and antigonadotrophic serum in the development of ovarian tumors in intrasplenic ovarian grafts to supply an evidence on the possible rôle of these hormones and serum in the production of ovarian tumors. These experiments have led to a hypothesis that an increased or inhibited pituitary stimulation to the intrasplenic ovary could be an important factor in the development of ovarian tumors. It could be considered also that the pituitary stimulation, such as an excessive and continuous stimulation, chiefly produced by over-action of gonadotrophic hormones from pituitary gland following the hormonal imbalances, is the only major factor involved in the formation of these ovarian tumors.

Chemical Induction of Ovarian Tumors

There are many carcinogens but none produces any ovarian tumors by the chemical compound alone but must be combined with the use of irradiation or parabiosis method. Howell et al., and Marchant et al., however, reported that ovarian tumor could be induced in significant numbers of a certain species of mice by treatment with a chemical compound alone. They observed tumors of granulosa-cell origin in mice
after fortnightly painting the skin with an 0.5% olive oil solution of DMBA. Later repeated painting of the skin of mice with an oil solution of some carcinogens, such as 3-methylcholanthrene, benzopyrene, dibenz[a,h]anthracene, and DMBA, was used to test ovarian tumor production.1, 2, 37, 57, 59, 64, 67, 68) Ovarian neoplasms are induced in mice by DMBA or benzopyrene but not with 3-methylcholanthrene or dibenzanthracene. DMBA showed the shortest carcinogenic latency and the highest incidence. Bonser IF, F1 hybrid derived from crossing IF female with Strong A or C57BL males, A, C3H, C57BL, and stock albino mice have been tried for ovarian tumor induction with skin application of DMBA.1, 2, 37, 57, 59, 64, 67, 68)

Up to date, DMBA is recognized to be the most effective and powerful ovarian carcinogen, and there has been no recognizable strain difference in mice in the induction of ovarian tumor by irradiation or by intrasplenic ovarian graft. Repeated painting of the skin of mice with DMBA revealed a definite strain preference to IF hybrid and no ovarian tumor induction in C3H mice. This fact suggests that the susceptibility of an ovary to DMBA may be due to the difference in the internal condition of various strains. Although a single skin painting would be sufficient to induce an ovarian tumor in mice, in general, a fewer application takes a longer time to produce ovarian tumor and decreases the tumor incidence.57)

In the present work, a single intravenous administration of DMBA (2.5 mg) in C3H mice consistently induced ovarian tumor rapidly and in a high percentage. This may indicate that the difference from other reports may be just due to the route of administration rather than to the susceptibility of ovaries of various strains to DMBA. Whereas a repeated skin painting of DMBA was required to induce an ovarian tumor, a single feeding or a single injection of DMBA was equally effective, less hazardous, and more convenient to induce an ovarian tumor rapidly and in high incidence.

Some investigators have reported that intact ovarian function inhibits the development of ovarian tumor in irradiated animals48) and in intrasplenic irradiated42) or non-irradiated6, 8, 52) ovary-grafted animals. Also certain steroid hormones inhibit the ovarian tumor induction in irradiated28) and intrasplenic ovary-grafted animals39, 44, 47, 52) The administration of 0.02% stilbesterol dipropionate, 2% progesterone, and 1% testosterone propionate in olive oil containing DMBA does not prevent the induction of ovarian tumor in IF and its hybrid mice.57) The ovary, grafted from mice not treated with DMBA to the one with intact ovarian-pituitary relationship, does not become neoplastic, but when DMBA-induced preneoplastic ovary is transplanted orthotopically into normal mice, it forms a tumor in high percentage after 15 months.58, 60, 61) Hypophysectomy of F1 hybrid mice does not prevent DMBA from rendering ovaries preneoplastic and such preneoplastic ovaries are capable of developing a tumor when they are grafted to the mice with intact pituitary. On the other hand, preneoplastic ovaries grafted into hypophysectomized mice fail to develop tumor.62, 63) Tumor development from these preneoplastic ovaries require pituitary stimulations of some degree and these pituitary stimulations eventually would occur as a result of the lack of ovarian hormones from the atrophied ovaries. Thus, the pituitary hormonal mechanism is involved in the ovarian carcinogenesis but there is still a possibility that some factors other than the mere elevation of pituitary hormone acting on ovary may be concerned in ovarian tumor induction.
Histological findings of these chemical-induced ovarian tumor are similar to those produced by X-rays or intrasplenic ovarian graft. Degenerative changes of the oocyte occurred at first, and then granulosa cells and finally lutein cells were more slowly damaged. These changes are brought about only by the action of DMBA.

Since DMBA exhibits a radiomimetic effect, it would be reasonable to assume that DMBA, whatever its dose, attacks earlier and stronger on the primary follicles than the mature follicles. The primary target appeared to be the oocyte. Subsequently, all follicles including oocytes were stimulated, degenerated, and vanished. The pathological changes in the granulosa cell were delayed and usually appeared after the degeneration of the oocyte. It is difficult to determine whether this delayed reaction of the granulosa cells is due to their intrinsic resistance to DMBA or to their dependence on the presence of oocytes. It is also interesting to note that after this intravenous administration of DMBA, neoplastic changes were mainly observed in the ovaries during the present experiment. Exhaustive attempts made to find any neoplastic changes in other organs proved negative. To explain this interesting observation, one may entertain the possibility of a strong affinity of DMBA to the hormone-producing cells from its stereochemical resemblance to steroids. Naturally, with the pituitary-ovarian interrelationship being disturbed by the destruction of ovaries, one could not ignore the fact that pituitary hormone may be involved as an indirect principle of ovarian carcinogenesis.

In some ovaries, within 3 months after intravenous injection of DMBA, granulosa cells with mitotic figures streamed out centrifugally from the follicle at the point where the line of theca cells was broken. The granulosa cells began to proliferate at the 3rd month. This suggests strongly as if granulosa cell tumor would originate from the remnants of granulosa cells. It is also possible from the histological studies that granulosa-cell tumor might have originated from theca cell. This could not be determined from the present experiment and a further study is required to elucidate this point.

The author wishes to express his appreciation to Professor Emeritus Fukuzo Oshima and Professor Sotokichi Morii for their kind advice and encouragement during this study, and also to Professor Ryuei Maeda and members of this Department. The author is also greatly indebted to Professor Charles B. Huggins, The University of Chicago, for his thoughtful gifts of purified compound and a fat emulsion of 7,12-dimethylbenz[a]anthracene.

(Received December 16, 1966)
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EXPLANATION OF PLATES XXXVII-XLI

Photos 1-22. Changes in the ovaries of C3H mice, given DMBA (2.5 mg) at the age of 60 and 90 days. (H.-E. = Hematoxylin-Eosin stain)

1. Degeneration of oocytes with fused cytoplasm and pyknotic nucleus or without nucleus and just mucinous fluid in follicles. 1st month. H.-E. ×370
2. Calcified areas at the upper right and the left lower corner are surrounded by a single layer of theca cells. 1st month. H.-E. ×470
3. Many small polygonal cells are surrounded irregularly by spindle-shaped cells and expanded at random around the degenerated follicles in the same ovary as in Photo 2. H.-E. ×270
4. The granulosa cells with mitotic figures are seen throughout from one point of the follicle to the other where cleft of the theca cell line can be seen. Same ovary as in Photo 2. H.-E. ×470
5. Small shrunken cells are gathered and also a few degenerated follicles can be seen. 2nd month. H.-E. ×270
6. Mucinous fluid in prominently altered Graafian follicle without even a trace of oocytes. 2nd month. H.-E. ×370
7. Diffuse granulosa cell proliferation with varying degree of luteinization without follicle. 3rd month. H.-E. ×75
8. Granulosa cell nodule in the other ovary from that in Photo 7. H.-E. ×56
10. Ovaries unequal in size; a small tumor in the left and atrophic ovary in the right. 4th month.
11. Tumor nodule, consisting chiefly of granulosa cells and of theca cells in some part, and calcified area in the larger ovary of Photo 10. H.-E. ×48
12. Cross-section of a large solid granulosa-cell tumor of ovary, weighing 1300 mg, with small blood cysts and multiple bleeding. 5th month.
13. Large, well-demarcated granulosa-cell tumor nodule. The contralateral ovarian tumor weighed 701 mg, shown in Photo 15. 6th month. H.-E. ×93
14. Follicular pattern, the so-called Call-Exner body, in well-differentiated granulosa-cell tumor. 5th month. Reticulum fiber stain. ×470
16. Diffuse hyperplasia of interfollicular tissue cells with luteinization and small glandular structures beneath the germinal epithelium. 6th month. H.-E. ×140
17. Large ovarian tumor, weighing 291 mg, with two large serous cysts. 8th month.
18. Cross-section of the large solid ovarian tumor, weighing 1200 mg, dark red, with blood cysts, bleeding, and necrosis. 11th month.
21. A large unilateral solid ovarian tumor, weighing 691 mg, composed of granulosa-cell tumor. 12th month.
22. Many small nests of granulosa tumor cells in a very small ovary. 11th month. H.-E. ×56