The production of ovarian neoplasms in female mice by x-rays was described in 1936 (7, 10). The tumors arose about 7 to 10 months following irradiation with single or multiple doses of 200 r to 400 r. These tumors were of 3 histological types: (a) granulosa cell tumors, (b) luteomas, and (c) tubular adenomas. Whether the gland or duct-like structures of some of these adenomas are related to arrhenoblastomas has not been determined. Frequently, different parts of the same induced tumor had a different appearance.

Beginning about 5 months after irradiation hyperplastic changes were present in the ovaries of most x-rayed mice not bearing tumors. The borderline between neoplasia and hyperplasia was indistinct (3). Brambell and Parkes (17) have carefully described the hyperplastic ovarian changes that follow irradiation but they terminated their experiments about 6 months after irradiation, and were apparently unaware of the preneoplastic character of these alterations.

During the past decade little attention was given to the induction of ovarian tumors by x-rays and it was questioned whether the bulky masses of ovarian cells, which are formed after irradiation, were truly neoplastic. Traut and Butterworth (19) noted a great similarity between ovarian neoplasms of women and the experimentally induced tumors of mice, and traced the development of granulosa cell tumors of mice to granulosa cells that escaped destruction by x-rays. Geist, Gaines and Pollack (13), the only ones to repeat our experimental work, produced ovarian tumors in 22 of 38 mice by a single exposure to 200 r. They believe that the neoplastic granulosa cells arise from undifferentiated cells of the ovarian parenchyma.

Among the problems remaining to be solved were: (a) Is irradiation of the entire body necessary for the induction of ovarian tumors or would irradiation of ovaries alone be sufficient? Is the irradiation of the pituitary or some other organ with or without irradiation of the ovaries necessary? (b) What is the smallest dose of x-ray necessary for the induction of ovarian tumors and what is the relationship between the x-ray dose and the rate of induction of these growths? (c) Are these growths autonomous? (d) Do they secrete hormones?

Affirmative answers to the third and fourth questions have already appeared in preliminary publications (5, 9) in which it has been shown that granulosa cell tumors are actually neoplastic, occasionally metastasizing to distant organs, and proving readily transplantable to other hosts of the same inbred line of mice; and that animals carrying the growths often exhibit secondary changes indicative of hormone production (5, 11). The luteomas also proved transplantable and the secondary changes in luteoma-bearing hosts suggest progesterin secretion (12).

The following previously unpublished experiments, undertaken in 1936 to 1938 with Dr. H. Traut to seek an answer to the first question, remained inconclusive. However, since they disclosed some technical difficulties and have guided subsequent work they are worthy of brief record.

Irradiation of the pituitary region with shielding of the ovarian region.—Five mice, approximately 3 months of age, received 800 r over the upper third of the body and head including the pituitary region. They died at 9 to 11 months following irradiation. The ovaries of these mice contained ova and exhibited none of the changes that precede the development of ovarian tumors in mice that have been exposed to x-rays. Another 4 mice that had been similarly irradiated at 2 months of age died at 14 to 17 months after irradiation. Their ovaries exhibited only the usual changes of senility.

These experiments strongly suggest that irradiation of the pituitary region with shielding of the ovarian region is sufficient for the induction of ovarian tumors in mice.
Irradiation of the pituitary gland with shielding of the ovaries does not produce ovarian neoplasms.

Irradiation of the ovarian region with shielding of the pituitary region.—Twelve mice approximately 4 to 7 months of age were irradiated with 450 r over the lower half of the body, including the ovaries, while the head and upper half of the body were well shielded. These mice died from 5 to 13½ months after irradiation. Postmortem examinations showed the characteristic degenerative changes and atrophy that follow irradiation but no regenerative changes. Numerous mice of these two experimental series, which died earlier of intercurrent disease, have been omitted from this survey.

It remains uncertain whether regenerative changes leading to ovarian tumors were merely delayed, and if so whether this delay was due to the more advanced age of the mice at the time of irradiation or to the shielding of the pituitary.

It became evident that meticulous care of the animal is of prime importance in the study of the production of ovarian tumors by x-rays because of the long induction period of these tumors and the possible interference by intercurrent diseases. It also seemed advisable to use young mice for such studies and to obtain more information on the relation of the age of mice and the dose of x-rays to the rate of induction of ovarian tumors. The experiments here described were undertaken to answer the latter questions and to find out if, and to what extent, the application of methylcholanthrene might modify the carcinogenic action of x-rays.

MATERIALS AND METHODS

Groups of mice 4 to 6 weeks of age were irradiated with 87 r, 175 r, and 350 r respectively. Additional groups of similarly x-rayed mice were also “painted” with methylcholanthrene and a third group of control mice was similarly painted with methylcholanthrene but received no irradiation.

All mice were F1 hybrids of RF females and Ak males and were thus genetically alike. This stock was used because it has a low incidence of neoplastic diseases, because it appears relatively resistant to the usual intercurrent infections and because of its longevity.

The factors of irradiation were as follows: 140 kv., 5 m. amp., 30 cm. target skin distance, with an inherent filtration of 1 mm. of aluminum.

Methylcholanthrene was applied as follows: An area of skin of about 1 × 1.5 cm. was gently “painted” with a 0.5 per cent solution of methylcholanthrene in benzol, making 50 strokes with a fine brush. The paintings were done twice weekly at 8 different sites, in rotation.

Most mice were kept until natural death; a few were killed in extremis. Six mice in this series are still living at over 31 months of age and all have large ovarian tumors proved by laparotomy. The incidence of neoplasms other than those of the ovary in these mice is the subject of a future report. Stimulation of the leukemogenic action of methylcholanthrene by pre-irradiation has already been described (8).

RESULTS

Table I shows the frequency and time of occurrence of ovarian tumors in the different groups of

<table>
<thead>
<tr>
<th>Treatment X-ray dose, r</th>
<th>Methylcholanthrene, weeks</th>
<th>Age at death in months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>87</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>87</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>175</td>
<td>1</td>
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<td>175</td>
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<tr>
<td>175</td>
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<td>175</td>
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</table>

Table I: The Incidence of Ovarian Tumors in Mice Exposed to X-Rays and “Painted” with Methylcholanthrene

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experimental animals. The results are summarized in Table II.

**Table II: Combined Data on the Incidence of Ovarian Tumors in X-Rayed and Methylcholanthrene-Treated Mice**

<table>
<thead>
<tr>
<th>Age at death in months</th>
<th>X-rayed</th>
<th>Mice x-rayed and painted*</th>
<th>Painted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-8</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>9-12</td>
<td>6</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>13-16</td>
<td>3</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>17-20</td>
<td>30</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>&gt;21</td>
<td>72</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Total living longer than 5 months: 123, 21, 69, 150, 2, 138.

Total living longer than 18 months: 92, 0, 2, 1, 0, 11.

*With methylcholanthrene.

The first ovarian tumor in mice x-rayed only was found 9 months after irradiation and at 17 months of age every mouse had an ovarian tumor irrespective of the dose of irradiation (87 r, 175 r, or 350 r). The percutaneous application of methylcholanthrene in both irradiated and normal mice brought about an earlier death of the mice mainly because it had induced leukemia, and cutaneous, breast and lung tumors.

It likewise remains doubtful whether or not methylcholanthrene alone is capable of producing ovarian tumors. Two ovarian tumors occurred among the 138 mice treated with methylcholanthrene only. The microscopic appearance of ovarian tumors and ovaries of these mice were indistinguishable from those of x-rayed mice. Ovarian tumors are practically nonexistent in normal mice of this stock. Most methylcholanthrene-treated mice died at a relatively early age and their early death might have masked a slight ability of methylcholanthrene to induce ovarian tumors.

According to the tables, ovarian tumors were noted at an earlier age in mice that had received the combined treatment of x-rays and methylcholanthrene, than in mice that were x-rayed only. However, most mice that received 87 r or 175 r died after 19 months of age and no biopsies were taken on these mice at an earlier age. The induced ovarian tumors are known to grow very slowly. Therefore, the data summarized in the tables yield no information as to the actual time of onset of the ovarian tumors in the mice x-rayed.

On the genesis of ovarian tumors. Comparison of x-ray-induced granulosa cell tumors with the nodules resulting from grafts of normal ovarian tissue in spleens of gonadectomized mice.—Biskind and Biskind (1) discovered that implantation of ovaries into the spleens of adult gonadectomized female rats is followed several months later by the development of tumor-like masses of granulosa cells. The theory which led them to undertake this work is as follows: Estrogens are inactivated in the liver (15, 21). The estrogen level of the blood is the pace-maker for the discharge of gonadotropic hormones of the pituitary. Hence draining all estrogens into the liver where they are inactivated would result in an increased discharge of pituitary gonadotropic hormones with enhanced and seemingly uncontrolled growth of granulosa cells. Biskind and Biskind called attention to the similarity of the granulosa cell growths thus induced to those produced by x-rays but they did not prove the neoplastic character of their growths. Their work has been confirmed by Li and Gardner (16) and by ourselves (6).

In order to determine autonomy of the ovarian growths induced by the technic of Biskind and Biskind it seemed essential to graft these growths into the subcutaneous tissue or some other site not drained into the liver. Accordingly numerous first generation ovarian growths produced by this procedure in the spleens of mice were grafted into the spleens of gonadectomized and into the subcutaneous tissue of normal mice. No difficulty was encountered in making intrasplenic subpassages of these growths in gonadectomized mice but in only 2 instances did we succeed in establishing a progressively growing, subcutaneous neoplasm readily transplantable into normal mice (6).

This finding led us to accept with Li and Gardner (16) the theory of Biskind and Biskind and to assume that pituitary stimuli might play a role in the induction of ovarian neoplasms by x-rays. Several workers have shown (2, 14, 18) that following irradiation with about 140 r to 400 r there is a disturbance in the ovarian cycle suggestive of diminution of estrogenic output sometimes terminating in an anestrous state. Estrus reappears, however, either (a) because of the regenerative changes which follow atrophy of estrogen secreting cells of the ovary or (b) because of a compensatory mechanism.

In the experiments of Geist, Gaines and Escher (14) the destructive effects of 200 r were accompanied by temporary abolition of estrus during the period of 4 to 8 weeks after irradiation. Subsequently the cycles became irregular with marked variations in the duration of estrus and in the intervals between
cstrus. From 6 months to 1 year after irradiation there was a decrease in vaginal stimulation. After 1 year estrus was absent but it reappeared in animals that developed biologically active granulosa or theca cell tumors.

It seems probable that the diminished output of estrogens in x-rayed mice stimulates the discharge of gonadotropins by the pituitary and that these act as growth stimulants of granulosa and lutein cells. The discharge of hormones by these stimulated ovarian cells into the general circulation would then depress the pituitary and thus true neoplasms would not develop until a new type of granulosa or lutein cell arises which is emancipated at least in part from the growth control of the pituitary gland. Most, if not all, x-ray induced ovarian tumors are transplantable in the subcutaneous tissue of normal mice (6). They are, therefore, autonomous and not mere masses of normal granulosa or lutein cells. Most growths induced by the technic of Biskind and Biskind are not transplantable in the subcutaneous tissue and are to be regarded as extreme examples of hyperplasia, which however can lead to neoplasia, as indicated by the observations just described.

X-rays can elicit neoplasms in tissues that are not known to be directly under the influence of the pituitary gland (e.g., skin) and it is possible that ovarian neoplasms can also arise in the absence of pituitary stimuli. On the other hand the pituitary gland might play an auxiliary role in the induction of these neoplasms, in that it discharges cyclic growth stimuli to the ovary and could thereby magnify the tissue derangement brought about in this organ by x-rays.

One type of ovarian growth is probably not directly related to the pituitary. These are the tubular adenomas, most of which appear to be distinct from arrhenoblastomas, that produce male sex hormones and whose mother cells may respond to pituitary stimuli (4). Tubular adenomas are also transplantable but grow much more slowly than granulosa cell tumors and luteomas and are usually outgrown by the latter (6).

In order to test the validity of these views it will be necessary to ascertain (a) whether x-rays induce ovarian tumors in hypophysectomized mice; (b) whether added pituitary gonadotropic hormones hasten their induction; (c) and whether the common tubular adenomas of x-rayed mice do secrete hormones and are dependent on the pituitary gland.

Susceptibility of mice of various ages and of various stocks to the induction of ovarian tumors.—Because of the established success in inducing ovarian tumors by irradiation with x-rays in mice of weaning age (4 to 5 weeks), it is desirable that in future studies mice of this age should be used as reference standards. The relative susceptibility of younger and older mice and of mouse embryos remains to be determined. Experiments now in progress (6) indicate that following total irradiation with 150 r at 1 to 3 days of age palpable ovarian tumors appear in many mice. One group of 9 mice so irradiated was explored by laparotomy at 14 months of age and ovarian tumors measuring 4 to 7 mm. across were found in 6. The unpublished experiment mentioned earlier in this paper has shown that regenerative changes are delayed when the irradiated mice are mature young adults. Although the pituitaries of mice of the latter series were shielded we believe that the lack of regenerative change is due to age. These fragmentary observations suggest that the probability of development of ovarian tumors following general irradiation is great when mice are exposed to x-rays at birth up to about 4 to 6 weeks of age.

In our original study (10) mice of 3 different stocks were used; the present work was done with hybrids of 2 of these stocks. Geist, Gaines and Pollack (13) induced ovarian neoplasms in an unrelated stock. Since there are no negative experiments on record it seems probable that mice of most, if not all, stocks are susceptible to the induction of ovarian neoplasms by x-rays.

Ovarian neoplasms in x-rayed women.—The rare occurrence of granulosa cell tumors in women and the lack of data suitable for statistical analysis make it uncertain whether x-rays will produce ovarian tumors in women. X-rays have been extensively used to sterilize women. These irradiations are local and there are no experimental data to indicate that ovarian irradiation alone is sufficient to produce granulosa cell tumors, although we believe this to be true. Furthermore, most women exposed to x-rays are middle-aged and the available experimental data cast doubt on the possibility of producing ovarian tumors under such conditions.

However, a large scale human "experiment" is now in progress in which girls and young women have been exposed over the entire body to comparable doses of gamma rays. The observations of Shields Warren (20) indicate that the late effects of atomic bomb explosions are similar to those of x-rays and it remains to be seen whether this type of radiating energy is capable of producing in human beings the neoplasms, including ovarian tumors, which can be elicited in mice by x-rays.
SUMMARY AND CONCLUSIONS

Following irradiation of 4 to 6 week old mice with 87 r, 175 r, or 350 r, ovarian tumors began to appear when the mice were about 11 months of age. The frequency of these neoplasms increased with time and almost every mouse that lived 17 months developed a unilateral or bilateral ovarian growth, irrespective of the dose of irradiation.

These ovarian growths are compared as to pathogenesis and autonomous character with the hyperplastic nodules that result from implantation of normal ovaries into the spleens of castrated mice. It is concluded that the latter are not autonomous neoplastic growths (although they sometimes give rise to true neoplasms); while the x-ray-induced ovarian growths, on the contrary, are readily transplantable autonomous growths.

The factors necessary for the induction of ovarian growths in mice and the bearing of the observations made on the general problems of carcinogenesis are discussed.

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Induction of Ovarian Tumors in Mice by X-Rays

J. Furth and M. C. Boon


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