INFLUENCE OF OVARIAN FUNCTION ON INCIDENCE OF RADIATION-INDUCED OVARIAN TUMORS IN MICE.[1] [2]

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INTRODUCTION

Tumors have long been known to develop in the ovaries of mice exposed to roentgen irradiation (*1–5*). More recently similar neoplasms have been observed in nonirradiated ovaries grafted into the splenic or pancreatic tissue of gonadectomized rats and mice (*6–8*). The histologic appearance and biologic behavior of these neoplasms have been described by Furth and his co-workers (*2, 9–12*) and others (*13*).

The mechanism by which irradiation leads to the development of ovarian tumors has attracted considerable interest. Theoretically, it is possible that such tumors may result from the *systemic* effect of whole-body irradiation, or from *local* action upon the ovary, or from both. The local ovarian response to suitable doses of radiation is a dual one. It involves such *direct* effects as the destruction of ova and follicles, as well as a diminished production or secretion of estrogenic hormone with secondary, *indirect* disturbances in endocrine interrelationships. Since both are sequelae of the same stimulus, experimental attempts to isolate and evaluate each of these effects independently are necessarily attended by serious difficulties.

A number of investigators have presented evidence indicating that the development of tumors in intrasplenic ovarian grafts is at least partially the result of a hormonal imbalance (*6, 14–16*). It has been postulated that estrogens secreted into the portal circulation by the ovarian grafts are inactivated by the liver, with presumptive depletion of systemic circulating estrogens and secondary hypersecretion of pituitary gonadatropic hormone. Prolonged stimulation by this hormone is thought to result in hyperplasia and ultimate neoplasia of the ovarian grafts.

In earlier studies, Li, Gardner, and Kaplan (*17*) failed to obtain definite evidence that a similar mechanism is involved in the induction of such tumors by irradiation. More recently, evidence for such an indirect mechanism was presented by Lick, Kirschbaum, and Mixer (*18*). They found that local irradiation of a single ovary yielded a neoplasm in that ovary only when the opposite ovary was excised or also irradiated. This paper presents the confirmatory results of a quite different experiment designed to study the same problem.

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EXPERIMENTAL PROCEDURE

Four experimental groups of female F_1 hybrid mice of strain L × A parentage were established. Litter-mate pairs were assigned respectively to groups I and II, or to groups III and IV. Three variables: (*a*) irradiation of the host, (*b*) *in vivo* irradiation of ovarian grafts, and (*c*) ovariectomy, were manipulated in an attempt to evaluate possible direct and indirect, and local *versus* systemic, effects of irradiation on ovarian tumor induction. The experimental procedures that characterize each group are summarized in table 1.

TABLE 1.—*Incidence of ovarian and lymphoid tumors in spayed and intact LAF_1 female mice bearing irradiated and nonirradiated ovarian grafts*

Group	Whole-body irradiation	Bilateral ovariectomy	Intramuscular ovarian grafts	Net number of mice	Mice with tumors in ovarian grafts		Mice with lymphoid tumors	
					Number	Percent	Number	Percent
I	Yes	Yes	Nonirradiated	31	[a] 1	3	20	64
II	No	Yes	Irradiated	26	19	73	9	35
III	Yes	Yes	Irradiated	28	17	61	13	46
IV	No	No	Irradiated	30	0	0	14	47

[a] This tumor was a sarcoma that could not definitely be said to have originated in ovarian tissue.

Mice of group I received whole-body irradiation in a single dose of 300r at about 1 month of age.[3] Bilateral ovariectomy was performed 7–21 days later, at which time the freshly excised ovaries of a group II mouse, usually the original littermate, were implanted into the adductor muscles of each hind leg.

Group II mice similarly were ovariectomized and received intramuscular grafts of the ovaries of group I mice, but were not irradiated.

Animals of group III were exposed to a single whole-body dose of 300r at about 1 month of age.[3] Bilateral ovariectomy was performed 7–21 days later. One of the excised, preirradiated ovaries was autologously grafted into the adductor muscle of the right hind leg of each mouse. The other excised ovary was simultaneously implanted intramuscularly into the corresponding litter-mate animal of the intact, nonirradiated fourth group.

Thus, the experimental design presents several possibilities. The occurrence of tumors in the irradiated grafts of otherwise untreated hosts (group IV) would indicate clearly that a direct local effect of irradiation exists and is an adequate stimulus. The failure of tumors to appear in this group would not exclude a direct local effect, but would imply that such an effect, if it exists, is unable to elicit tumors in the absence of an indirect hormonal imbalance. A direct local effect could be dismissed only if tumors were to occur with significant frequency in the nonirradiated grafts

[3] Physical factors of irradiation were: 186 KVP, 20 ma., 0.25 mm. Cu and 0.55 mm. Al filter, focus-skin distance 47 cm., output 66 r/min.

of irradiated, spayed group I mice. Finally, an estimate of the influence of whole-body irradiation might be gained from the comparative results in groups II and III (both of which were composed of spayed hosts bearing irradiated grafts), since only the latter group was irradiated.

All animals received Purina laboratory chow and water *ad libitum*, and were maintained under identical laboratory conditions. Daily vaginal smears were made for several weeks in selected mice from all four groups. After an interval of about 4 months, vaginal smears of the same mice were again studied daily for approximately 2 weeks.

Animals were observed for the development of cysts and tumor masses in the ovarian transplants and were killed when they developed very large tumors or became moribund for other reasons. Autopsy was performed on all animals, with routine examination of the ovarian grafts, the uterus, adrenals, kidneys, liver, spleen, lungs, thymus, lymph nodes, and the intact ovaries in mice of group IV. The mammary glands and the vagina were also studied in a few mice. Histologic sections of all of these tissues were routinely prepared and examined, except where excessive autolysis had occurred.

OBSERVATIONS AND RESULTS

The incidence of ovarian tumors by groups is summarized in table 1. Irradiated grafts implanted into mice with intact ovaries (group IV) yielded no tumors. Irradiated ovaries implanted into ovariectomized mice, either irradiated (group III) or nonirradiated (group II), gave rise to a high incidence of tumors (61 and 73 percent, respectively). Only 1 (3 percent) of 31 irradiated ovariectomized mice bearing nonirradiated ovarian grafts developed a tumor at the site of the implant (group I). This was a sarcoma that could not definitely be said to have originated in ovarian tissue.

Table 2 indicates that most of the observed ovarian neoplasms were granulosa-cell tumors, similar to those described elsewhere (*8, 9, 13, 17*). Metastasis to the liver was noted in one instance. There were also 4 luteomas, 6 tubular adenomas, and 1 thecoma; several other tumors exhibited mixtures of more than one tumor-cell type. Granulosa-cell tumors were more frequent among tumor-bearing mice of group II than among those of group III. Cysts of various sizes, filled with either clear or bloody fluid, were often found in the nontumorous grafts as well as in association with tumors.

Cyclic vaginal smears were observed in all animals so studied among the nontumor-bearing groups, I and IV; all but one of these again exhibited cyclic smears on the repeat study about 4 months later (table 2). In contrast, 15 of 18 mice in groups II and III showed persistent anestrous smears on the initial study. However, on subsequent re-examination, 7 of 9 mice in group II, and 4 of 9 in group III had developed either cyclic or persistently cornified vaginal smears. The return of cornified cells to the vaginal smears of animals bearing irradiated ovaries has been previously described by Geist, Gaines, and Escher (*19*).

TABLE 2.—*Ovarian tumors and vaginal smears in LAF_1 female mice bearing intramuscular ovarian grafts*

Group	Status of host	Status of graft	Number of grafts	Number of mice	Ovarian tumors [a]							Vaginal smears [b]					
												Initial			Repeat		
					G	L	M	Th	Ta	S	None	+	±	−	+	±	−
I	Spayed	Nonirradiated	2	31	0	0	0	0	0	1	30	10	0	0	10	0	0
II	Spayed	Irradiated	2	26	17	2	3	0	4	0	7	0	2	7	7	0	2
III	Spayed	Irradiated	1	28	6	2	4	1	2	0	[c] 11	1	0	8	4	2	3
IV	Intact	Irradiated	1	30	0	0	0	0	0	0	30	9	0	0	8	0	1

[a] G—Granulosa-cell tumor; L—luteoma; M—mixed-cell tumor; Th—thecoma; Ta—tubular adenoma; S—sarcoma.

[b] +=cyclic or persistently cornified; ±=occasional cornification; −=persistent anestrus.

[c] Two other tumors were autolyzed and not histologically classifiable.

The longevity of LAF_1 hybrid mice is well known (*20*). In the present experiment, the average survival times of the four groups were, respectively, 613, 707, 654, and 809 days. The relatively greater number of early lymphoid tumors in mice of group I was probably responsible for their somewhat shorter life span. Since animals were not killed until they became moribund, and most of the ovarian tumors grew very slowly, survival time was not a good index of the latent period for ovarian-tumor development following irradiation.

Lymphoid tumors were observed in many of the animals in all groups (table 1). However, the differences in incidence among groups were not statistically significant at the 0.05 level. The observed incidence ranged from 35 to 64 percent, with an average of 49 percent, which tallies very closely with the incidence previously observed by Lorenz and associates (*20*) in LAF_1 hybrid mice. In general, the histologic character of these lymphoid tumors appeared to fall into two main groups that were at least roughly correlated with the survival time. Lymphomas appearing relatively early tended to be lymphocytic or lymphoblastic, and were essentially similar to those previously observed in other strains of mice following whole-body irradiation (*4*). Most of the lymphoid tumors that occurred in mice surviving 600 to 800 days had a more pleomorphic character, with a predominance of larger, less deeply staining cells and less evidence of invasion. These have been designated by various observers as stem-cell lymphomas, reticulum-cell sarcomas, or clasmatocytomas. Detailed studies aimed at clarifying the histologic nature of these tumors were not made in the present experiment.

A miscellany of other tumors occurred sporadically in all groups. These included two adrenal medullary tumors (pheochromocytomas) similar to those recently reported by Smith, Gardner, Li, and Kaplan (*21*). Also noteworthy were an epidermoid carcinoma of the renal pelvis and an epidermoid carcinoma of the forestomach, the etiology of which was not apparent.

DISCUSSION

Irradiated ovaries grafted intramuscularly into untreated mice did not give rise to tumors, in contrast to the behavior of irradiated ovarian grafts in spayed hosts. This result is consistent with the observation of Lick, Kirschbaum, and Mixer (*18*) that the presence of an intact ovary inhibits the development of tumors in the locally irradiated opposite ovary. These findings appear to support the thesis that radiation-induced ovarian tumors of mice arise indirectly, presumably in response to hormonal imbalance resulting from radiation injury to the ovaries, with impairment of ovarian endocrine function. Such an interpretation is in keeping with available evidence concerning the mechanism of development of the histologically similar tumors that occur in intrasplenic ovarian grafts in gonadectomized mice (*15*).

However, the experimental observations reported here cannot properly be interpreted to exclude a direct local effect of irradiation in ovarian tumorigenesis. Such a conclusion would have been warranted only if a

number of tumors had been observed in mice of group I. All that can be said is that a direct local effect of irradiation, if it exists, is not an adequate stimulus for ovarian-tumor development in intact hosts (group IV) in which there is no reason to anticipate a pituitary-ovarian imbalance.

The concept of a direct local effect is not without some experimental support, albeit of inferential nature. Ovarian tumors have been observed in mice exposed to single x-ray doses of as little as 50r, as well as in animals chronically exposed to gamma radiation at a rate of only 0.1r per day (*20*). Animals exposed to such low doses exhibit cyclic vaginal smears and are capable of bearing normal litters with apparently normal frequency (*22*). There is no way, at present, of evaluating the sensitivity of the vaginal smear and of fertility as indices of ovarian endocrine function. Nevertheless, these are the best criteria currently available. Since ovarian tumors may be induced by doses of ionizing radiation so small as to effect no detectable disturbance of ovarian hormone secretion, a direct effect of radiation on ovarian tissue would appear to be implicated in the neoplastic process.

Evidence can therefore be adduced to support both an indirect hormonal mechanism and a direct local effect of irradiation in ovarian-tumor formation, under experimental conditions so designed as to minimize one factor and perhaps exaggerate the other. It seems likely, however, that under most conditions *both* factors would be effective, perhaps by synergism or summation, in leading to the development of ovarian tumors in irradiated mice.

Finally, brief comment should be made about the role of systemic irradiation. In unpublished incidental observations (*23*, Exp. 1), local irradiation yielded ovarian tumors only when the ovaries were included in the treatment field. This observation is supported by the work of Lick, Kirschbaum, and Mixer (*18*), and by certain of the results of the present experiment. The frequency with which mice of groups II (nonirradiated hosts) and III (irradiated hosts) developed ovarian tumors did not differ significantly. Thus, systemic irradiation does not appear to influence the incidence of these neoplasms. However, the relative frequency of granulosa-cell tumors was distinctly greater among mice of group II. It would be of interest to repeat this part of the experiment under more critical conditions, since this result carries the very interesting implication that systemic irradiation may influence the histology of induced ovarian neoplasms, possibly through some effect on the pituitary.

SUMMARY

Irradiated ovaries implanted intramuscularly into irradiated and nonirradiated groups of spayed LAF_1 hybrid mice, gave rise to many granulosa-cell tumors, luteomas, and related neoplasms. No such tumors occurred when irradiated ovaries were implanted into nonirradiated, nonovariectomized mice. Nonirradiated ovarian grafts in irradiated, spayed animals yielded only one sarcoma (3 percent) that could not definitely

be said to have originated in ovarian tissue. Cyclic vaginal smears were consistently observed in selected animals of the latter two groups, whereas anestrous smears occurred initially in 15 of 18 mice of the tumor-bearing groups, giving way within about 4 months to cyclic or persistently cornified smears in 11 of these. It is concluded that intact ovarian endocrine function inhibits the development of tumors in irradiated ovarian grafts, confirming the reported observations of Lick, Kirschbaum, and Mixer (*18*). The significance of these results is discussed in relation to other available evidence, and it is suggested that both a direct and an indirect mechanism are involved in the development of tumors in irradiated mouse ovaries.

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