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Incidence and Nature of Tumors Induced in Sprague-Dawley Rats by γ-Irradiation[1]

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ABSTRACT

In our previous studies carried out on inbred rats of the Sprague-Dawley strain (L. Gross and Y. Dreyfuss, Proc. Natl. Acad. Sci. USA, *76:* 5910–5913, 1979), the tumor incidence was increased following irradiation (150 rads, 5 times, at weekly intervals), from 22 to 93% in females and from 5 to 59% in males. Experiments here reported suggest that 2 consecutive total-body γ-irradiations of 150 rads each are sufficient to induce in rats the development of tumors, some malignant; 18 of 19 females (94.7%) developed tumors at an average age of 11.4 mo, and seven of the 14 males in this group (50%) developed tumors at an average age of 10.4 mo. In the second group, which received 3 consecutive γ-irradiations, 20 of 23 females (86.9%) and 5 of 13 males (38.4%) developed tumors at average ages of 9.1 and 7.5 mo, respectively. In the third group, among rats which received 4 consecutive γ-irradiations, 17 of 19 females (89.4%) and 4 of 12 males (33.3%) developed tumors at average ages of 9.4 and 10.5 mo, respectively.

The etiology of tumors either developing spontaneously or induced by irradiation in rats remains to be clarified. Our attempts to detect virus particles by electron microscopy in such tumors or lymphomas have not been successful. As a working hypothesis, we are tempted to theorize that tumors or lymphomas developing spontaneously or induced by gamma irradiation in rats are caused by latent viral agents which are integrated into the cell genome and are cell associated, *i.e.*, not separable from the rat tumor cells by conventional methods thus far used.

INTRODUCTION

Benign as well as malignant tumors develop spontaneously in rats of different strains. The incidence observed in different laboratories varies, depending on the strain of rats used and the length of observation (1–9). Tumors occur more frequently in females than in males; many of the tumors observed are those of the mammary glands. Frequently, two or three tumors develop in short sequences in the same animal; some of the tumors are malignant. Tumors developing in males are usually s.c. or often soft tissue fibromas or fibrosarcomas (1). Most of the spontaneous tumors in rats are hard, fibrous, and difficult to dissect. With only very rare exceptions, the spontaneous rat tumors and primary spontaneous rat lymphomas do not reveal the presence of virus particles on electron microscopic examination and cannot be transmitted by filtered extracts to other rats.

The incidence of tumors in rats can be considerably increased by total-body γ-irradiation (1, 10–15). In our recent study carried out on inbred rats of the Sprague-Dawley strain (1), the tumor incidence was increased following irradiation from 22 to 93% in females and from 5 to 59% in males; in rats of the Long-Evans strain, the incidence of radiation-induced tumors was increased from 28 to 63% in females and from 10 to 42% in males. The relative incidence of malignant tumors was higher in the irradiated rats as compared with nontreated controls that developed tumors spontaneously.

It is of considerable interest that the incidence of radiation-induced tumors in Sprague-Dawley rats could be significantly reduced by restriction of food intake (16). Similar studies have not yet been carried out on rats of the Long-Evans strain, but it would be surprising if rats of that particular or any other strain would react in a different manner.

Our routine irradiation procedure consisted of 5 consecutive total-body γ-irradiations of 150 rads each. In the study here reported, we have tried to determine whether a reduction of the number of irradiations would still lead to an increase in the incidence of tumors. Furthermore, as part of this study, we have also reviewed the morphology of radiation-induced tumors, as compared with those that develop in rats spontaneously.

MATERIALS AND METHODS

Animals. From a nucleus of random-bred Sprague-Dawley rats received in June 1960 from the Animal Production Unit, NIH, a colony of rats has been raised in our laboratory by brother-to-sister mating. No animals from outside sources have been added; only those bred in our laboratory have been used in this study.

Technique of Total-Body γ-Irradiation. Young adult Sprague-Dawley rats received at the Radiotherapy Department of this Medical Center five consecutive total-body γ-irradiations of 150 rads (1 rad, 0.01 gray) each at weekly intervals. The animals were 3- to 4-wk old when they received the first irradiation; they were placed in small plastic compartments, 10 cm wide and 15 cm long, each holding one rat; up to six rats in six compartments were irradiated at one time. The technical factors were as follows: Picker Cobalt-60 teletherapy unit, 80-cm source-to-surface distance, 81.5-cm source-to-midplane of rat distance, portal size 33 × 30 cm for six compartments, dose rate of 100 rads per min.

RESULTS

The rats, all litter mates, were divided when they were weaned, prior to irradiation, into 3 different experimental groups. In the first group, the rats received 2 consecutive total-body γ-irradiations of 150 rads each at weekly intervals; in the second group the rats received 3, and in the third group 4 consecutive irradiations (Table 1).

In the first group which received 2 consecutive γ-irradiations, 18 of 19 females (94.7%) developed tumors at an average age of 11.4 mo. Seventeen tumors developed in the right or left axillary or inguinal regions, corresponding to the location of the mammary glands; two of these tumors were adenocarcinomas; the remaining 15 were benign (14 fibroadenomas and 1 leiomyofibroma). In addition, 1 female developed a s.c. sarcoma in the dorsal area. Among the 14 males in this group, seven (50%) developed tumors at an average age of 10.4 mo. Six of these tumors were s.c., developing on left or right flanks; 3 of these tumors were sarcomas, and 3 were carcinomas. One

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Table 1 *Effects of γ-irradiation on the incidence of tumors in Sprague-Dawley rats*

No. of irradiations[a]	Sex	No. of rats observed	No. that developed tumors[b]	% that developed tumors	Age tumors developed (mo)			No. that developed leukemia	% that developed leukemia	Average age of rats developing leukemia	No. of rats died without tumors	Age negative rats died (mo)		
					Minimum	Maximum	Average					Minimum	Maximum	Average
2	F	19	18	94.7	5	18	11.4	0			1			21
	M	14	7	50	5.5	13	10.4	0			7	7	20	15.3
3	F	23	20	86.9	3	13	9.1	0			3	9	23	15.3
	M	13	5	38.4	6	8.5	7.5	0			8	5.5	18.5	12.1
4	F	19	17	89.4	3	18	9.4	0			2	6.5	17	11.7
	M	12	4	33.3	9.5	12	10.5	0			8	8	21	15.6
Controls[c]	F	293	77	26	9	28	18.7	5	2	15.8	211	6	28	16.9
	M	301	10	3	9	26	16.9	4	1	20.6	287	6	31	16.8

[a] Total-body, 150 rads each, at weekly intervals.

[b] In the first group (2 irradiations), of the tumors that developed in 18 females, 3 were malignant (17%). Among the tumors that developed in 7 males, 6 were malignant (86%). In the second group (3 irradiations) of the 20 tumors that developed in females, 7 were malignant (35%). Among the tumors that developed in 5 males, 4 were malignant (80%). In the third group (4 irradiations), of the 17 tumors that developed in females, 4 were malignant (24%). Among the tumors that developed in 4 males, 2 were malignant (50%).

[c] Controls from 1979 to 1985. Most of the females in the control group had litters, whereas those in the experimental groups were virgin females.

additional rat in this group developed 2 benign fibroadenomas in the left and right axillary areas.

In the second group which received 3 consecutive γ-irradiations, 20 of 23 females (86.9%) developed tumors at an average age of 9.1 mo. Again, as in the preceding group, 17 of these tumors developed in the right or left axillary or inguinal areas corresponding to the location of mammary glands. Among these tumors were 1 carcinoma and 3 sarcomas; the remaining 13 were benign fibroadenomas. In addition, 2 females developed tumors on the neck (an undifferentiated carcinoma in one rat and an angiosarcoma in another animal) and 1 female developed a fibrosarcoma on the skin. Among 13 males, 5 developed tumors (38.4%) at an average age of 7.5 mo. Two of these tumors developed in the area of the neck; both were adenocarcinomas; one rat developed a liposarcoma in the right inguinal region and another a liposarcoma of the skin; the fifth rat developed a fibroadenoma in the axillary region.

In the third group which received 4 consecutive γ-irradiations, 17 of 19 females (89.4%) developed mammary tumors at an average age of 9.4 mo. Among these tumors 13 were benign fibroadenomas and 4 were malignant (3 adenocarcinomas and 1 sarcoma). Of the 12 males, 4 (33.3%) developed tumors at an average age of 10.5 mo. Two tumors were in the axillary area (one sarcoma and one fibroadenoma). The tumors in the remaining 2 rats were abdominal, one, a sarcoma in the mesentery, and another, an angiolipoma in the area of the left kidney.

The animals with tumors were observed for 1 to 2 mo; the rats were sacrificed when the tumors became large or partially necrotic. The remaining rats, free from tumors, were observed until they died from causes apparently unrelated to cancer or leukemia. A few in this group were sacrificed when they appeared sick and almost terminal, in almost all instances with a lung infection, but exhibited no presence of tumors.

We have reviewed a representative number of microscopic sections of tumors induced in Sprague-Dawley rats by γ-irradiation and tried to compare such tumors with those developing spontaneously in untreated, control rats. Our general impression was that, essentially, tumors induced in rats by γ-irradiation were similar to those that developed spontaneously in untreated control animals, except that the incidence of malignant tumors was significantly higher in the irradiated animals, an observation consistent with that tentatively suggested in our previous study (1). In females mammary gland tumors were by far the most common. Histologically they were mostly benign adenomas or fibroadenomas; malignant breast tumors were mainly undifferentiated or poorly differentiated adenocarcinomas. Other types were also seen but they were rare. Occasionally, foci of malignant tumors were found within adenomas or fibroadenomas, possibly suggesting that benign tumors may become malignant in the course of time. In these instances, the benign tumors had a tendency to be rather cellular. Soft tissue tumors were also observed, although less frequently. Their morphology resembled those seen in humans and no particularly distinguishing histological features were noticed; malignant fibrous histiocytomas seemed to be slightly more frequent than the other varieties. There were also s.c. either benign or malignant tumors. Tumors developing in males were often s.c.; they were frequently sarcomas.

DISCUSSION

Experiments here reported suggest that 2 consecutive total-body γ-irradiations of 150 rads each administered to rats about 1-mo-old are apparently sufficient to induce in the irradiated animals the development of tumors, frequently multiple and often malignant, after latency periods varying from 3 to 18 mo (Table 1). Untreated rats of the same strain also develop tumors similar to those developing in irradiated rats, although more often of a nonmalignant nature.

It is difficult to assess with accuracy the incidence of tumors developing spontaneously in untreated rats. That incidence varies in different studies (1–9), depending on several factors, particularly on the strain of rats observed and the length of observation.

In our studies dealing with a strain of Sprague-Dawley rats inbred by brother-to-sister mating since 1960, we have observed a relatively high incidence of spontaneous tumors in females as compared with that in males, with only very occasional development of leukemia or lymphomas in either males or females. Of a total of 293 females observed, 77 (26%) developed tumors spontaneously at ages varying from 9 to 28 mo; in contrast, only 10 of 301 males (3%) developed tumors after similar latency periods. Only 5 females (2%) and 4 males (1%) developed leukemia or lymphomas.

In reviewing the factors which apparently determine the incidence of spontaneous tumors developing in rats, it is necessary to consider first the strain of animals observed. One could designate this factor as genetic, although the true nature of it still remains to be determined, since it is possible to theorize that what is now considered to be "genetic" may actually involve the presence or absence of vertically transmitted latent oncogenic viruses which may be responsible for the development of tumors in a given strain of laboratory animals, imitating an inherited tendency for "spontaneous" oncogenesis.

We have already mentioned that in the Sprague-Dawley strain of rats used in our study, we have observed that 26% of females and 3% of males develop spontaneously benign or malignant tumors, and not more than 2% develop leukemia or lymphoma. We also have in our laboratory rats of the Long-Evans strain that have been inbred by brother-to-sister mating since 1966 (1). About 28% of females and 10% of males of this strain develop tumors spontaneously and 3 and 5%, respectively, develop leukemia or lymphomas. Remarkably, for some as yet unknown reasons, when these two strains of rats were mated reciprocally, the incidence of tumors and to some extent also that of leukemia or lymphomas was significantly higher, increasing up to 69% in females and up to 41% in males (1).

Another very important factor influencing the development of tumors is quite obviously hormonal, since many more females than males develop tumors spontaneously.

Other factors should also be considered, which may determine the development of spontaneous tumors in laboratory animals; thus, exposure to total-body γ-irradiation may increase considerably the incidence of tumors in rats, up to 100% in some experiments (1, 16).

Among factors inhibiting the development of tumors, or leukemia, in rats and mice is the amount of food they consume during their lifetime. Rats on calorically restricted diets have a much lower incidence of tumors than those allowed to eat *ad libitum* (16). The same refers to leukemia and lymphomas occurring in mice spontaneously (17) or induced by γ-irradiation (18).

The etiology of tumors either developing spontaneously or induced by irradiation in rats is of considerable interest but remains to be clarified. Attempts to detect virus particles by electron microscopy in rat tumors and lymphomas developing either spontaneously or induced by irradiation have not been successful. Similarly, attempts to transmit to newborn rats, by filtered extracts, spontaneous or radiation-induced rat tumors, leukemias, or lymphomas, did not succeed.

As a working hypothesis, we are tempted to assume that tumors or lymphomas developing in rats spontaneously or induced by γ-irradiation are caused by latent viral agents which are integrated into the cell genome, and are cell associated, *i.e.*, not separable from the rat tumor cells by conventional methods thus far used.

It is very interesting that in contrast to tumors and lymphomas developing in rats spontaneously, or induced by γ-irradiation, leukemia or lymphomas transmitted to rats from mice by inoculation of the mouse leukemia (Gross) virus reveal consistently on electron microscopic examination the presence of C-type virus particles. Furthermore, the virus-induced lymphomas are transmissible from rats to rats or from rats to mice by filtered extracts (10, 19). It thus appears that, although morphologically indistinguishable, leukemia and lymphomas developing in rats spontaneously, compared with those induced in that same species by inoculation of the passaged (Gross) mouse leukemia virus, appear to be distinct disease entities.

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