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Radiation-Induced Experimental Cancer of the Esophagus

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THE ESOPHAGUS—where squamous epithelium is rapidly replaced, where the mucosal surface is large in area and mobile, where there are no glands, where the mucous membrane is but little exposed to trauma and infection, and where spontaneous carcinoma is almost unknown [1] in laboratory animals—provides a simple experimental model for study of evolution of cancer of keratinized mucous membranes.

Only a few experiments, and these chiefly with chemical carcinogens, have been directed to the induction of cancer of the esophagus. Schoental [2] reported cancer of the esophagus in rats given N-methyl-N-nitrosourethane (NMU) by stomach tube. Long and Jenner [3] also induced esophageal cancer in rats by feeding dihydrosafrole.

By implanting ^{60}Co wires in the lungs of mice, the mucosa of the esophagus can be continuously irradiated at various dose rates and for varying lengths of time. We reported in 1960 the first experimental radiation-induced esophageal carcinomas as an incidental finding in our studies on induction of bronchial carcinoma using this technique.[4] The lesions thus induced in the esophagus range from radiation reaction through precancer to cancer.

It is a paradox that while carcinoma of the esophagus rarely appears in untreated animals it may be induced by radiation, whereas in man, in

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whom the lesion is rather frequent spontaneously,[5-7] it is very rarely seen as a sequel to exposure in the course of therapeutic irradiation of adjacent lesions.

The radiosensitivity of esophageal mucosa of animals and of man has had very little study: It is considered slight [8] although greater than that of the trachea.[9] The clinical signs of radiation-induced esophagitis, dysphagia, and substernal burning—long recognized in patients receiving intensive radiation to the thorax or neck—have not been completely correlated with factors of radiation. Seaman and Ackerman [10] found the frequent occurrence of esophagitis a major limiting factor in treatment of intrathoracic cancer with the betatron. The maximum tolerance dose (recognizing individual differences in response) was around 6000 R at a rate of 1000 R per week.

As with clinical signs, so it is with the pathology of radiation-induced esophagitis with respect to dose and time relations of injury and recovery. In man, pathologic material is seldom available. However, it has been shown that even after intense irradiation, epithelial regeneration may be complete, and the residual fibrosis of the submucosa and muscle compatible with a functioning organ.[10] In the rat, early and late phases of response following 3000 R (250 kvp X-ray) have been described by Jennings and Arden.[11] Necrosis of the mucosa developed about the sixth day, regeneration by the twenty-fifth day, and epithelial atrophy, subepithelial scarring, and diverticula at 3 months. The relation of scarring and epithelial atrophy to cancer was early recognized in radiologists who had radiodermatitis and occupational skin cancer.[12]

Chemical radiation-induced cancer has consistently shown a long latent period, especially in the lower dose ranges, thus limiting the number of cases that have been observed or recognized as such. Perhaps Goolden and Morgan [13] are right in estimating that only a small proportion of the total number of the cancers following therapeutic irradiation have been reported. There are in the literature two cases of post-irradiation cancer of the upper esophagus observed 42 and 31 years after treatment for thyrotoxicosis and tuberculous lymphadenitis, respectively.[13,14] We have recently seen a patient with carcinoma of the upper esophagus found 18 years after treatment for hyperthyroidism.* With the steady advance in sophistication of therapeutic irradiation techniques and the possibilities of environmental radiation exposure in the atomic age, cases of radiation-induced cancer in man involving unusual sites may well become more frequent, and experiments are needed to throw further light on radiation-induced carcinogenesis and the factors which relate to it.

* We are indebted to the Department of Pathology, Indiana University, for this case.

Methods

A ^{60}Co gamma radiation source about 2 × 0.5 mm. was placed in the lung near the esophagus of mice 4–10 weeks old, as in our previous experiment on cancer of the lung.[4] This was done by means of a trocar inserted after incision of the skin at the sixth costal interspace, to the right of the vertebra. Nembutal anesthesia was used. That the source remained fixed at the site of implantation was substantiated, with few exceptions, by serial roentgenograms. The animals were tested daily with a Geiger counter to check against loss of the source. Rarely, as a result of radiation-induced necrosis, sources sloughed free into the pleural cavity or, very rarely, into the esophagus and were discharged in the feces.

The mice (202 males, 160 females) represented eight strains selected on the basis of varied tendencies to develop spontaneous tumors. The RAP strain, randomly bred (Swiss), made up the largest single unit of 140 mice, as compared with units of 26–48 animals in each of seven strains: Ajax, $C_{57}B1$, CF_1, C_3H, dba, LAF and BALB. These represent randomly bred, inbred, and hybrid lines.

The mice received Purina Laboratory Chow and water ad libitum. The farm was kept at 70° F. with the humidity at 50%. The mice were healthy and free of ecto-parasites; relatively little murine pneumonia developed. Mice were killed only when moribund. Complete necropsies were performed. The esophagus was removed in entirety and fixed in formalin, and longitudinal serial sections of the entire organ were stained with hematoxylin and eosin.

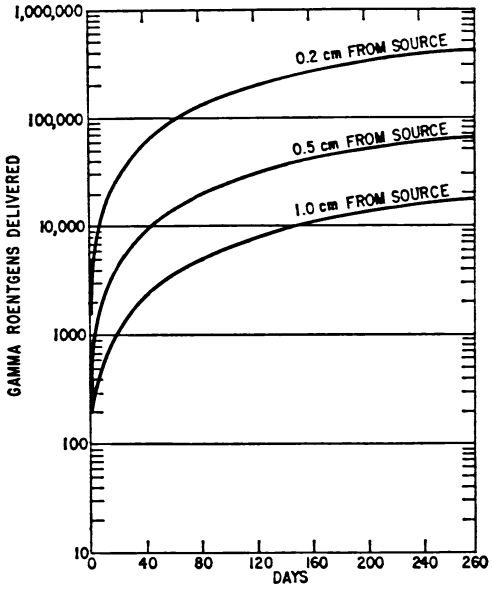
Radiation sources varied from 70 to 488 μc. in activity with the majority between 150 and 250 μc. Their activities were determined prior to each implantation using a Landsverk analysis unit (ionization chamber) with radium as a standard. The measurements, expressed in microcuries, are accurate to within ±6%. The gamma-ray component (1.4 Mev) alone is significant. The beta output of ^{60}Co is negligible owing to self-absorption in the cobalt and also absorption by adjacent necrotic tissue. Conversion of roentgens to rads was unsatisfactory because of the varying density of tissues and varying distances through those tissues. However, a factor of 0.9 would be reasonably accurate. Doses in roentgens were plotted against time for each ^{60}Co source at distances of 0.2, 0.5, and 1.0 cm., corrections being made for the radioactive decay (half-life 5.3 years). Such a graph, for a 250-μc. source, is shown in Text-fig. 1. Interpolations were made for calculations at other distances.

The distance from the source to the nearest point of the esophagus varied from contact to 8 mm., but no measurements less than 2 mm. from the mucosal surface were used in calculation of doses, since cells closer than this were nonviable. Figure 1 demonstrates roentgenographically a ^{60}Co wire in place and shows the decrement in dose at measured intervals from the source.

Since the cancers were small and not always clearly distinguishable, on gross examination, from surrounding radiation reaction, the measurements of their distance from the source could only be approximate, probably accurate within 1 mm. With the numbers of animals involved and the long periods of observation, minor inconsistencies tend to disappear.

Findings

This report is based on the 286 mice (150 male, 136 female) living more than 40 days exposed to continuous focal radiation. The 76 additional mice dying within the first weeks after insertion of the source were not suitable objects for the purposes of this study, as nearly all

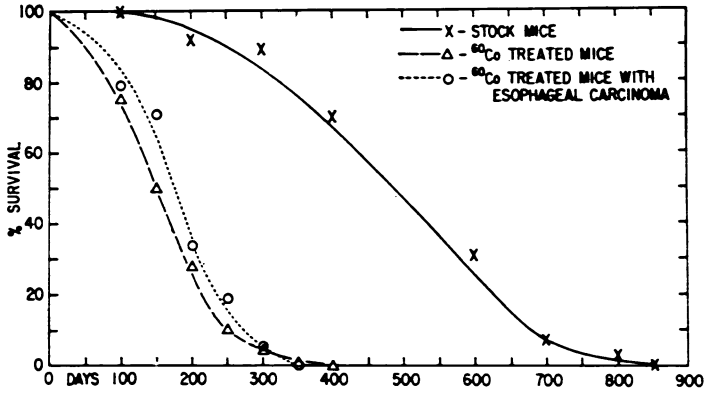


TEXT-FIG. 1. Gamma roentgens from a ^{60}Co, 250-μc. source at different distances and times of exposure. Corrections made for decay.

died of acute radiation-induced necrosis of the esophagus and of its sequelae: rupture, hemorrhage, and infection. The resulting distortion of tissues added to the difficulty of evaluating the radiation-induced epithelial abnormalities. These epithelial changes often occurred within a week, and cell for cell, the abnormalities of radiation reaction could not be distinguished from carcinoma in situ or early invasive carcinoma. Although most of the clear-cut cancers are found between 120 and 200 days after implantation of the source, cellular changes indistinguishable from those seen in cancer often appear 1–4 weeks after exposure. One of the most striking examples of this change, which we have excluded from the series because of the time limit chosen, is shown in Fig. 2.

The incidence of epidermoid carcinoma of the esophagus was 15%. These tumors showed the typical features of epidermoid carcinoma in man: undifferentiated and invasive growth; conjunction of mature and less differentiated keratinized cells; sometimes tumor giant cells and disproportionately large and irregular nuclei, and often numerous mitotic figures (Fig. 3 and 4). Multiple foci of advanced cancer in the esophagus were rarely seen, although multiple precancerous or early lesions were not unusual (Fig. 5). No metastases were found.

The survival curves of control mice and of treated mice with and without cancer are shown in Text-fig. 2. The marked reduction in life span of treated mice was due mainly to radiation-induced lesions other than cancer. A comparison of mortality rates and cancer rates at 50-day pe-



TEXT-FIG. 2. Survival curves of untreated and treated mice and those developing cancer of esophagus. Curve for stock mice represents life spans of our control mice supplemented by pertinent published data from Russell.[15]

riods shows this contraction of life span. It also shows the higher incidence of cancer in longer-lived mice (Table 1). Both of these effects are related to dose rate.

Dose rates delivered to the portion of the esophageal mucosa nearest the source ranged from 100 to 4000 R per day, and those rates which were carcinogenic ranged from 220 to 4000 R per day. The cancer incidence rose to a peak of 46% with 751–1000 R per day and then decreased, even though the majority (60%) of the 286 mice were exposed to lower dose rates (Table 2). Contrarily, life expectancy decreased with successively higher rates. The median survival times for all mice fell from 194 days at 100–250 R per day to 67 days at 3001–4000 R per day, and the median survival time of mice dying with cancer was 286 and 43 days, respectively, at these rates.

Total doses were between 11,000 and 430,000 R; carcinogenic doses between 35,000 and 297,000 R. As with dose rates, the middle ranges of the dose were those most productive of cancer: a 43% cancer rate

Table 1. Death Rates Resulting from Continuous Radiation with ^{60}Co

Days with ^{60}Co (No.)	All deaths (%)	Deaths due to esophageal cancer (%)
41–50	5	21
51–100	20	10
101–150	24	4
151–200	32	18
201–250	10	21
251–300	6	38
301–350	2	33
351–400	<1	—

Table 2. Incidence of Esophageal Cancer in Relation to ^{60}Co Dose Rate and Median Time Exposed

Dose rate/day (R)	% of all exposed mice	% of exposed mice with cancer	Median time irradiated (days)	
			All mice	Mice with cancer
100–750	60	6	170	252
751–1000	14	46	143	176
1001–1500	16	23	114	177
>1500	9	7	94	65

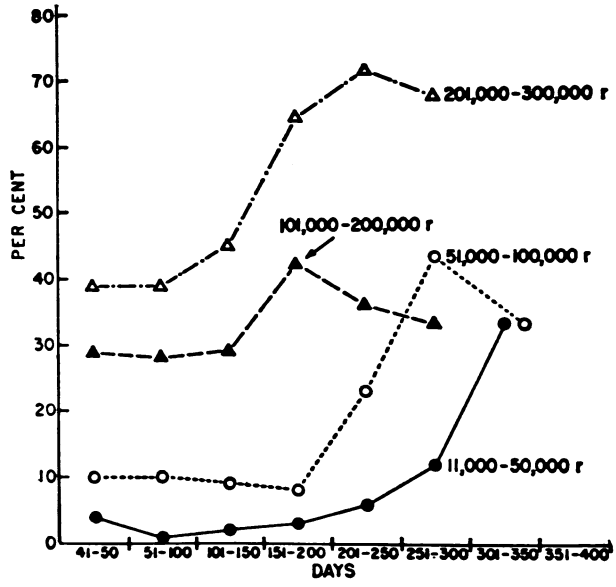
among those receiving 151,000–200,000 R (Table 3). The significance for cancer induction of the added increments in dose as related to time cannot be surely determined for want of knowledge of the latent periods of the cancers. We only know that the latent period may be short, 6 weeks or less, and that cancers are not always sufficiently far advanced to be the cause of death when they occur. However, it is of interest that the probability of ever dying of carcinoma of the esophagus within any one time period increased with the total dose, and the cancer rate was higher with higher doses irrespective of time (Text-fig. 3). The importance of survival time is illustrated especially at total doses under 100,000 R, when appreciable numbers of mice develop cancer only after 200 days.

We have thus far been concerned with mice of both sexes and the eight strains as a unit. We shall now consider whether there is any evidence that sex or genetic type affected the response to irradiation. Because of possible effects of these factors on survival time, we have presented in Text-fig. 4 the survival times of the treated male animals of the several strains, and in Text fig. 5 the treated females. In general, there is more difference between strains than between sexes within the strains. However, it must be remembered that doses and dose rates varied between the different sexes and strains. The relation of time, survival period, and dose and dose rate to cancer is presented (Text-fig. 3 and Table 2).

The sexes were fairly evenly represented in the group as a whole (150 males, 136 females) and in the separate strains, except for CF_1 mice,

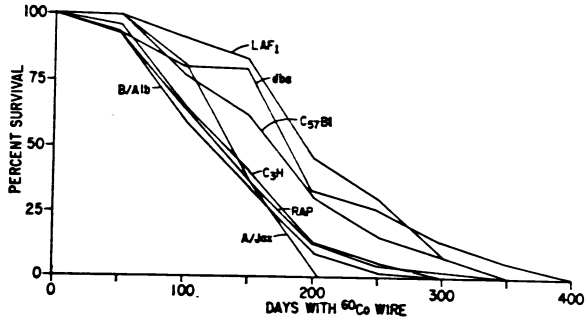
Table 3. Relation of Mice Exposed to Those Developing Cancer at Various Ranges of Total Doses of ^{60}Co Radiation

Total dose (R)	% exposed	% with cancer
11,000–150,000	80	9
151,000–200,000	10	43
201,000–300,000	8	39
301,000–450,000	2	—

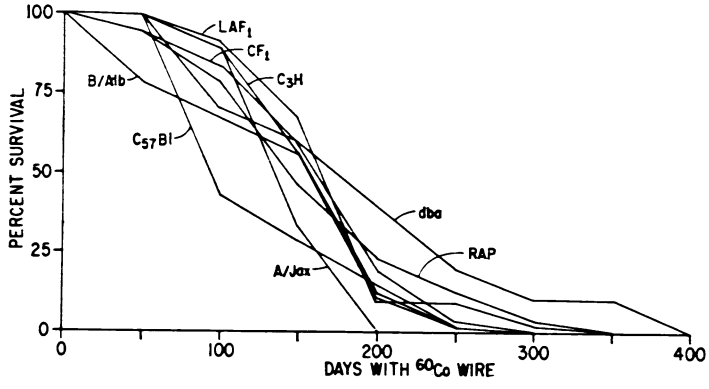


TEXT-FIG. 3. Probability of a treated mouse ever dying of carcinoma of the esophagus at a given time; grouped by total dose.

which were all females. The overall incidence of cancer was 14% in males and 16% in females. Females, whether with or without cancer, lived slightly longer than males—up to 150 days. Differences in cancer incidence in males and females occurred at the various dose rate levels—notably at 100–250 R per day (3% in males and 15% in females) and at 1251–1500 R per day (29% in males and 17% in females)—but the numbers of mice with cancer were small. At the optimal dose rate of 751–1000 R per day, the cancer incidence was the same in both sexes—45% for males, 48% for females. Variations in the cancer incidence in



TEXT-FIG. 4. Survival times of male mice of the several strains used. Note fairly close correspondence of Ajax, BALB, C3H, and RAP strains.

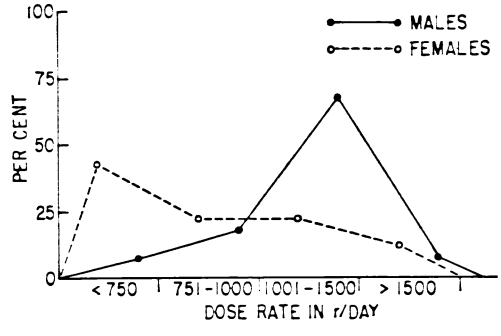


TEXT-FIG. 5. Survival times of female mice of the several strains used. Note the fairly close correspondence among all strains except Ajax, BALB, and $C_{57}B1$.

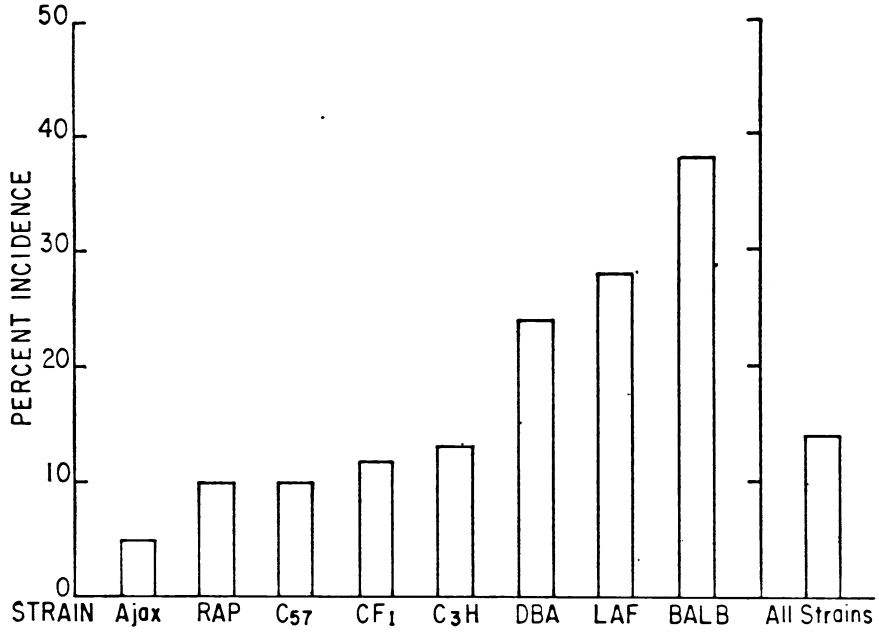
the sexes of different strains were inconsequential except in BALB strain, with females having a higher incidence of cancer at dose rates under 750 R per day and males more cancer at 1001–1500 R per day (Text-fig. 6).

Between strains a statistically significant difference in cancer incidence was found only between Ajax, 5%, and BALB, 38% (Text-fig. 7). This is comparable to the range of difference in response that prevails between species (Text-fig. 8). Of the different species tested, mice showed the earliest, but least frequent, occurrence of cancer; rats almost as early and three times as frequent; and the rates in other kinds of rodents fell between these figures.[16]

Differences in the general pattern of causes of death among the several strains might influence the likelihood of development of esophageal cancer; pertinent data are presented in Table 4. Radiation-induced can-



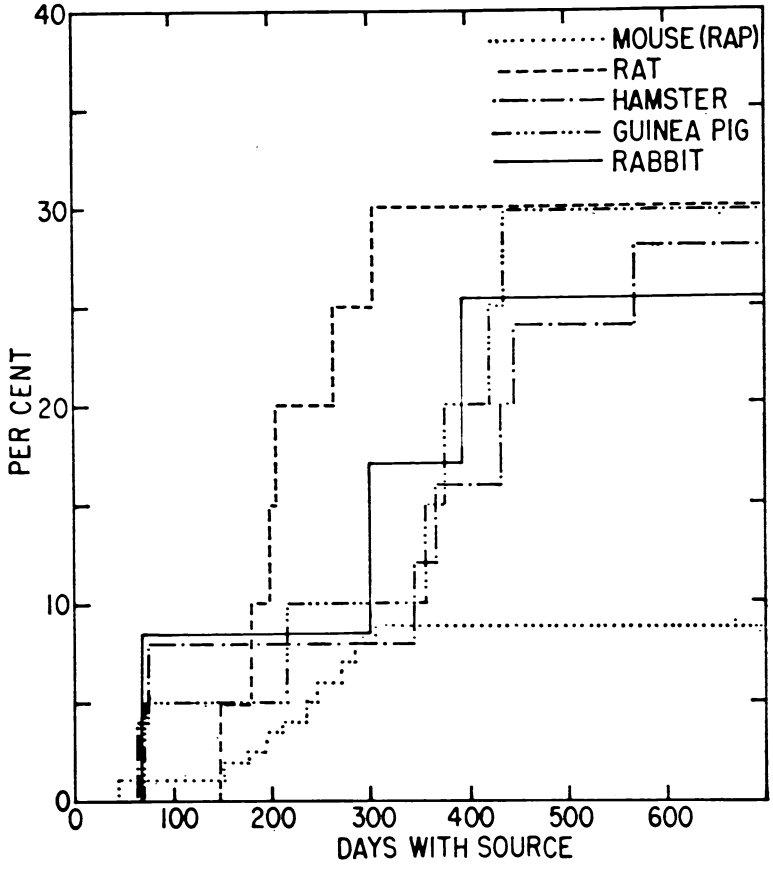
TEXT-FIG. 6. Radiation-induced carcinoma of the esophagus in male and female BALB strain mice exposed to varying dose rates of ^{60}Co radiation.



TEXT-FIG. 7. Range of difference in incidence of radiation-induced carcinoma of the esophagus among eight strains of mice.

cers were the commonest cause of death in all but Ajax mice, followed by reaction to focal irradiation. The usual type of infection was pneumonitis or mediastinitis secondary to implantation of the source, or to local radiation-induced necrosis; reaction to focal irradiation usually involved the lung or the esophagus. Epizootic disease or processes common to senescence were not contributors to mortality.

In all of the eight strains the interaction of dose rate and life span in the induction of cancer follows, in general, the same pattern. For instance, RAP and BALB mice exemplify the responses to low and high dose rates. The proportion of mice in these two strains living more than 200 days with cancer was 63% and 12%, respectively. That is what would be expected in view of the fact that the majority of all the cancers in RAP followed dose rates of less than 750 R per day, while all the cancers in BALB mice developed at dose rates above 750 R per day. The behavior of Ajax mice is worth noting (Table 5): All mice were dead at 200 days, and cancer developed in only one, although the radiation exposures—i.e., the proportion of mice exposed to the various dose rates —were comparable to those delivered to six of the eight strains. The low cancer incidence would not seem to be due to short survival time, since the median life times of Ajax mice exposed at various dose rates were



TEXT-FIG. 8. Comparison of radiation-induced carcinoma of the esophagus in five species of rodents: cumulative percentage of deaths by time.

Table 4. Causes of Death in Various Strains of Treated Mice* Expressed in % of Each Group of Animals

Strains	Carcinoma of esophagus	All other cancer	Infections	Focal radiation effect	Other or undetermined cause
Ajax	5	15	20	45	15
RAP	10	22	30	29	9
$C_{57}B1$	10	30	15	25	20
CF_1	11	31	22	22	14
C_3H	13	21	21	25	21
dba	24	16	16	20	24
LAF	28	12	12	12	36
BALB	38	10	5	19	29
Total	15	21	22	26	17

* Cancers were included only when the cause of death; thus all skin cancers and a number elsewhere were excluded.

Table 5. Variations in Carcinogenic Response of Strains of Mice to Continuous ^{60}Co Irradiation

	% distribution of cancer by time		% distribution of cancer by dose rate		
	<200 days	>200 days	<750 R/day	751–1000 R/day	1001–4000 R/day
Ajax	100	0	0	100	0
RAP	36	63	63	0	36
$C_{57}B1$	100	0	0	50	50
CF_1	77	22	50	25	25
C_3H	66	33	0	66	33
dba	83	17	0	100	0
LAF	57	43	28	57	14
BALB	87	12	0	37	62

comparable to those of other strains. Furthermore, BALB mice were also short-lived. The median life span for Ajax mice was 122 days and for BALB mice 126 days, but 80% of Ajax mice were exposed to dose rates under 1000 R, and 57% of BALB mice were exposed to dose rates over 1000 R. Ajax mice, however, showed the highest proportion of deaths due to focal radiation-induced reactions (Table 4). The difference in cancer rates of the two strains might be thus accounted for, but not the differences in life span.

While all strains developed cancer at dose rates between 751 and 1250 R per day, only three (RAP, CF_1, and LAF) strains did so at dose rates below 750 R per day, even though 50–70% of mice of each strain, excepting BALB, were treated at these latter levels, and even though these mice so treated had a long life span and thus accumulated high total doses. Furthermore, these three strains did not respond consistently to dose rates of 100–250, 251–500, or 501–750 R per day.

The trends of response to irradiation are sufficiently consistent to indicate that in this experiment intensity of radiation is a key element in the mechanism of radiation-induced carcinogenesis, but the possibility that genetic structure or sex may modify the general response to irradiation cannot be dismissed. Possible examples of this have been noted in the variations in cancer rates of males and females of BALB strain, which showed but little difference in life span (Text-fig. 4 and 5); in the shortened life span of Ajax; and in the distribution of cancer at dose rates under 750 R per day.

In this paper we have limited ourselves to recording measurable conditions associated with carcinogenesis. The numbers of mice under particular experimental conditions were small, especially when dealing with subdivisions of strain and sex. This very limitation makes correspondences between exposures, cancer rates, and life spans the more

compelling. Yet these data should not be thought to bear more meaning than they logically permit. Among influences which may impinge upon the carcinogenic process, the local tissue reaction, as evidenced by its stromal and epithelial response, must be considered. It is clear that stromal changes such as fibrosis and alterations of vascularity, which affect growth and differentiations of epithelium, would be quite dissimilar at low and high radiation exposures at comparable time intervals. Many of the factors in radiation-induced carcinogenesis and in changes in life span, as well as in the organism's attempt at repair, are as yet unknown.[17]

Summary

1. Carcinoma of the esophagus, virtually unknown as a spontaneous murine tumor, has been induced in eight strains of mice by continuous gamma irradiation.

2. The development of carcinoma was dependent primarily on the intensity of the radiation exposure.

3. Irradiation was a sufficiently powerful carcinogenic stimulus to eliminate possible differences in sex or strain sensitivities, except perhaps for the Ajax and BALB strains.

4. There appeared to be optimal dose rates for the development of cancer of the esophagus, varying slightly with strain.

5. The rarity of cancer induced by very high doses is consistent with an optimal dose rate. However, the possibility of transiently existing lesions cured by further irradiation must be considered.

6. Local interactions of damaged tissues may account for the variation in rates of cancer induction, especially at low exposure levels.

References

1. Crain, R. C. Spontaneous tumors in the Rochester strain of the Wistar rat. *Amer J Path 34*:311–335, 1958.
2. Schoental, R. Experimental induction of squamous carcinoma of the lung, oesophagus and stomach. The mode of their induction. *Acta Un Int Cancr 19*:680–683, 1963.
3. Long, E. L., and Jenner, P. M. Esophageal tumors produced in rats by the feeding of dihydrosafrole. *Fed Proc 22*:275, 1963.
4. Gates, O., and Warren, S. The production of bronchial carcinomas in mice. *Amer J Path 36*:653–671, 1960.
5. Wynder, E. L., and Bross, I. J. A study of etiological factors in cancer of the esophagus. *Cancer 14*:389–413, 1961.
6. Marcial, V. A., Tomé, J. M., Ubiñas, J., Bosch, A., and Correa, J. N. The role of radiation therapy in esophageal cancer. *Radiology 87*:231–239, 1966.

7. DOLL, R., PAYNE, P., and WATERHOUSE, J., Eds. *Cancer Incidence in Five Continents*. Union International Contra le Cancer. Springer-Verlag, Berlin, 1966.
8. ENGELSTAD, R. B. Über die Wirkungen der Röntgenstrahlen auf Ösophagus und Trachea. *Acta Radiol 15*:608–614, 1934.
9. DESJARDINS, A. U. Action of roentgen rays and radium on the gastrointestinal tract. Experimental data and clinical radiotherapy. *Amer J Roentgen 26*:151–190, 1931.
10. SEAMAN, W. B., and ACKERMAN, L. V. The effect of radiation on the esophagus. *Radiology 68*:534–541, 1957.
11. JENNINGS, F. L., and ARDEN, A. Acute radiation effects in the esophagus. *Arch Path (Chicago) 69*:407–412, 1960.
12. WOLBACH, S. B. The pathological histology of chronic x-ray dermatitis and early x-ray carcinoma. *J Med Res 21*:415–449, 1909.
13. GOOLDEN, A. W. G., and MORGAN, R. L. Radiation cancer of the pharynx. Case report. *Acta Radiol [Ther] (Stockholm) 3/5*:353–360, 1965.
14. MCGRAW, R. W., and MCKENZIE, A. D. Carcinoma of the thyroid and laryngopharynx following irradiation. *Cancer 18*:692–696, 1965.
15. RUSSELL, E. S. "Lifespan and Aging." In *Biology of the Laboratory Mouse* (ed. 2), GREEN, E. L., Ed. McGraw-Hill, New York, 1966, p. 513.
16. WARREN, S., and GATES, O. Cancers induced in different species by continuous gamma radiation. *Arch Environ Health (Chicago)* In press.
17. UPTON, A. C., RANDOLPH, M. L., and CONKLIN, J. W. Late effects of fast neutrons and gamma rays in mice as influenced by the dose rate of irradiation: Life shortening. *Radiat Res 32*:493–509, 1967.

[*Illustrations follow*]

Legends for Figures

Fig. 1. Roentgenogram of mouse with source in place, showing decrements in dose with distance.

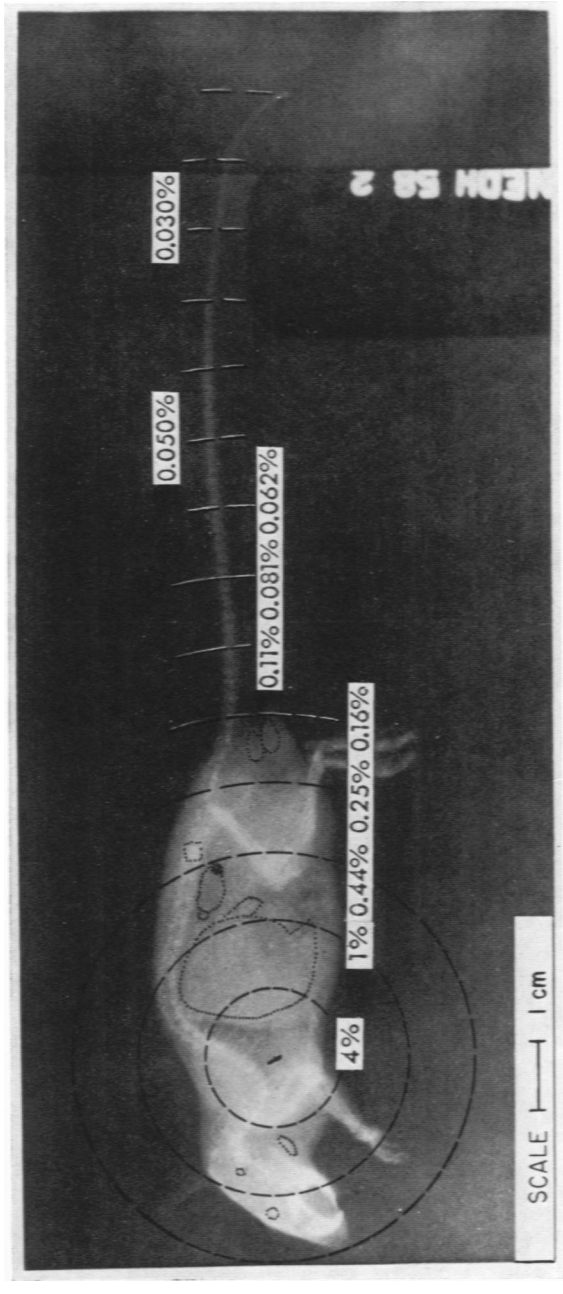
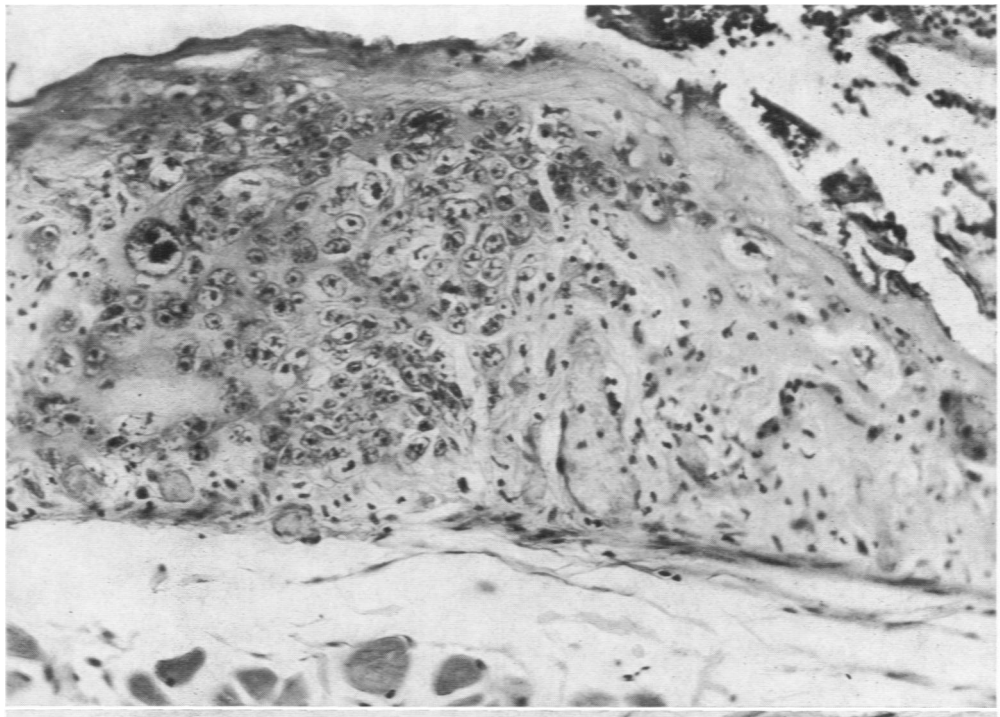

0.030%
0.050%
0.11% 0.081% 0.062%
1% 0.44% 0.25% 0.16%
4%
SCALE 1 cm

Fig. 2. Lesion of esophagus resulting from 30 days of irradiation; ^{60}Co source; 287 μc. Dose rate per day 1050 R; total dose 31,500 R. Hematoxylin and eosin. × 250.

Fig. 3. Epidermoid carcinoma; after 42 days of irradiation; ^{60}Co source; 152 μc. Dose rate per day 1200 R; total dose 50,000 R. Hematoxylin and eosin. × 500.



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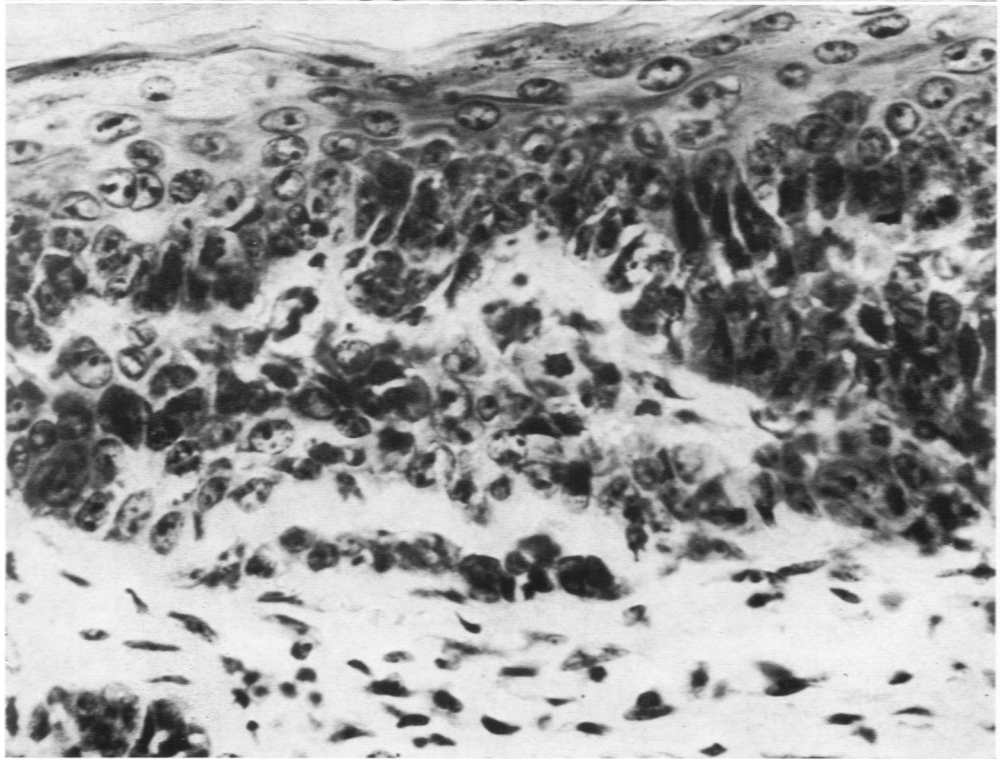

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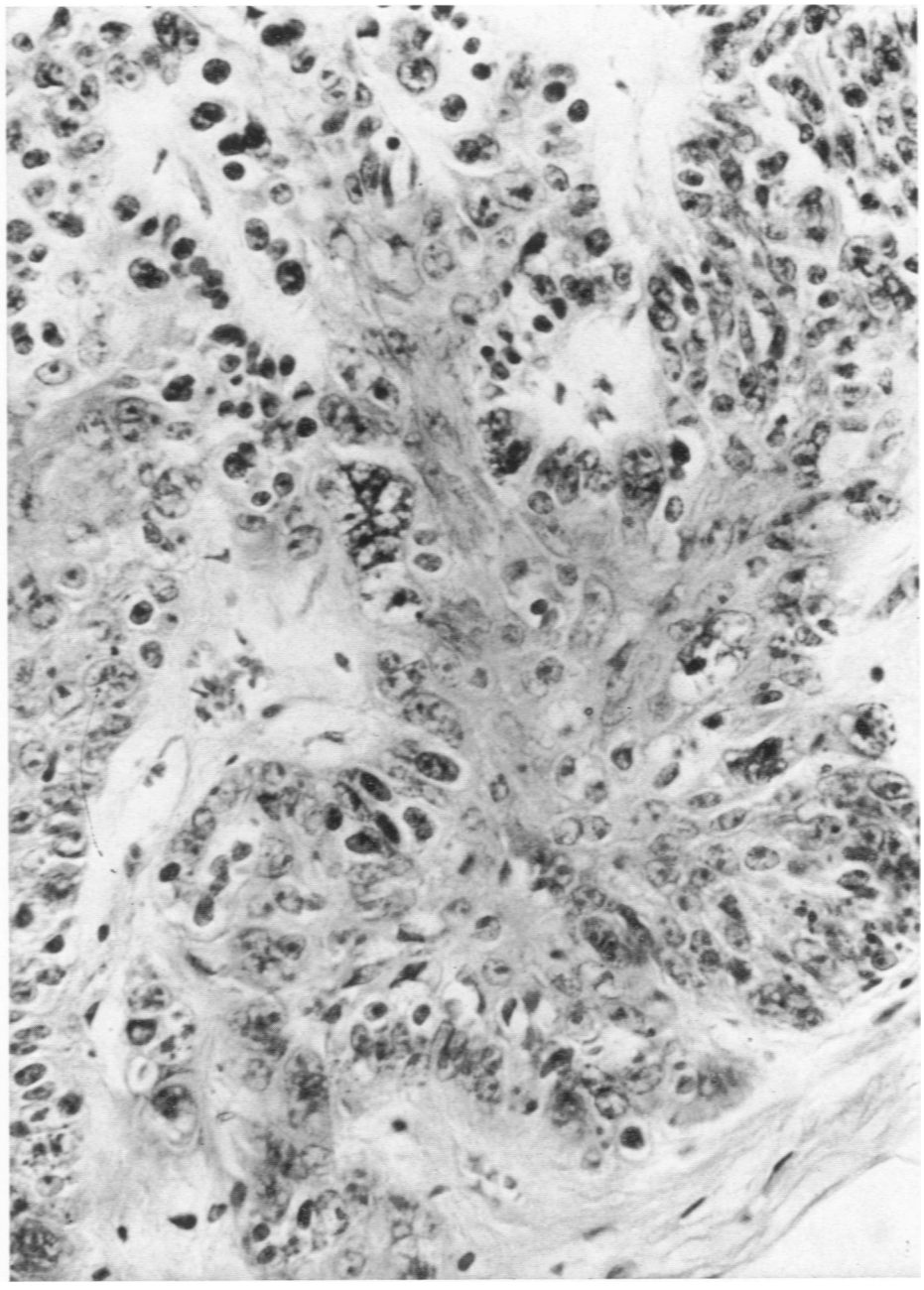
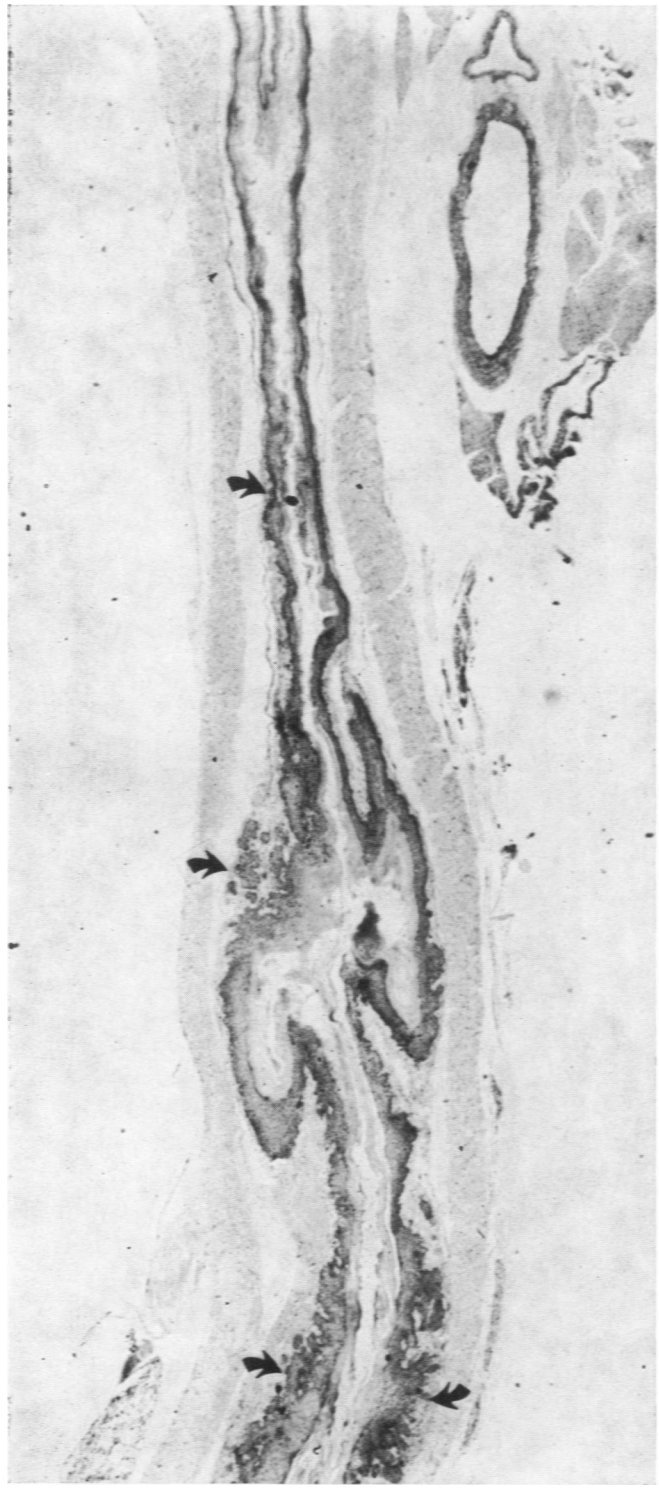


Fig. 4. Epidermoid carcinoma; after 276 days of irradiation; ^{60}Co source; 140 μc. Dose rate per day 550 R; total dose 152,000 R. Hematoxylin and eosin. × 500.

Fig. 5. Longitudinal and cross section of esophagus showing multiple foci *(arrows)* of early carcinoma; after 254 days of irradiation; ^{60}Co source; 113 μc. Dose rate per day 950 R; total dose 235,000 R. Hematoxylin and eosin. × 25.



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