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On the cover is an interference photomicrograph of a monolayer of B16-F10 melanoma cells illustrating morphologic changes induced by treatment for 30 minutes with cytochalasin B and colchicine in the culture medium. The tumor cells are rounded up, show no evidence of strong attachment to the substrate, and have blebs attached to their surfaces (see paper by Hart et al. on pages 891–900).

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Announcements

Induction of Mammary Neoplasms in the Sprague-Dawley Rat by 430-keV Neutrons and X-Rays [1,2,3]

Claire J. Shellabarger, [4] Danielle Chmelevsky, [5] and Albrecht M. Kellerer [5,6,7]

ABSTRACT—Female noninbred Sprague-Dawley rats were exposed to single doses of 0.28, 0.56, and 0.85 gray (Gy =1 J/kg or 100 rads) of X-rays or 0.001, 0.004, 0.016, and 0.064 Gy of 430-keV neutrons at 62±1 days of age and were then observed over the rest of their lives for the appearance of mammary neoplasia. As mammary neoplasms were detected, they were removed and given a classification of adenocarcinoma(s) (AC) or fibroadenoma(s) (FA) after microscopic study. All irradiated groups exhibited an increased incidence of mammary neoplasia. The tumor rate increased steeply with age of the animals, and the effect of the irradiation could be adequately described as a forward shift in time of the spontaneous incidence. The cumulative prevalence was derived from first neoplasms only, and a formalism was presented that makes it possible to derive the integral tumor rate from all neoplasms in all animals. Mortality-corrected cumulative prevalences and integral tumor rates as a function of age were given for the different doses and separately for FA and AC. The mammary FA response and the total mammary neoplastic response (including both FA and AC) were approximately proportional to the absorbed dose of X-rays or the square root of the neutron dose. The relative biological effectiveness (RBE) of the neutrons increased with decreasing dose and reached values exceeding 100 at a neutron dose of 1 mGy; the single dose of 1 mGy of neutrons produced a significant increase of the tumor rate that corresponded to a foward shift or roughly 35 days of the spontaneous incidence. The AC, taken separately, were subject to considerable statistical uncertainties due to their small numbers. However, their RBE-dose dependence was consistent with that for the FA and, even at the highest dose studied, the RBE value exceeded 10. The nonrandom development of multiple FA within individual animals appeared to be the result of differences in susceptibility to radiation. However, mammary FA and AC within individual animals were not statistically correlated.—JNCI 64: 821-833, 1980.

This article presents the results of an extensive experimental investigation on the induction of mammary tumors in the Sprague-Dawley rat by 430-keV neutrons and by X-rays. Earlier studies with various biologic systems revealed a maximum of the effectiveness of neutrons at an energy around 400 keV; they also showed that RBE increases with decreasing neutron dose and that it can exceed values of 100. These results, which are in agreement with microdosimetric considerations (1-3), have suggested the present experiment designed with special emphasis on the effects of low neutron doses. The Sprague-Dawley rat is well suited for studies in the low-dose range because it is particularly prone to develop early mammary neoplasms (4) in response to radiation and to certain chemical carcinogens.

Earlier studies dealt with the effects of low LET radiation (5-8) and of high-energy neutrons (9-11) and even more recently with the effects of high LET particles (12). From data for fission neutrons (9, 11) and X-rays (10), Vogel and Zaldivar inferred RBE

values in the range of 20-60. The present experiment comprises concurrent neutron and X-irradiations and is aimed at the determination of dose-effect relations as well as the dependence of RBE on neutron dose.

An interim report on the result of the experiment up to 400 days after irradiation has been given earlier (13). The conclusions, drawn in this earlier report on the RBE of neutrons versus X-rays and gamma rays, have now been confirmed by the results of the completed experiment. However, current results permit, in addition, a more detailed analysis of the tumor rate as a function of time and absorbed dose of X-rays or neutrons. Due to the sharp increase in spontaneous tumor rate with increasing age of the animals, the analysis poses a number of conceptual and formal problems that will be dealt with in detail.

MATERIALS AND METHODS

Weanling female Sprague-Dawley rats were purchased from Sprague-Dawley Inc., Madison, Wisconsin, and maintained on commercial rat chow and water ad libitum in temperature-controlled (22±2° C) and humidity-controlled (55±10%) animal rooms under conditions of 12 hours of fluorescent light per day. A single lot of 742 rats, all born on the same day, was numbered consecutively by the withdrawal of 1 rat

from each of the 30 shipping cartons. From the consecutively numbered animals, the required numbers of rats were then assigned to each experimental group and to the control group. When they were 61–63 days old, the animals were exposed either to neutrons, X-rays, or sham radiation. Five rats were then assigned to a single cage on a random basis independently of treatment. Each rat was examined frequently, and when breast tumors were noted by palpation, the tumors were removed surgically from the rats under ether anesthesia, and the rat was then returned to the experiment. No animal was killed, unless death appeared imminent, until 1,033–1,053 days of study when 19 rats, from a starting number of 742, were killed to terminate the experiment. Individual records were kept for each rat, and the time of appearance and anatomic location of the breast tumors were recorded. Mammary neoplasms that occurred in different quadrants in the same animal were considered to be separate neoplasms. If successive mammary neoplasms were noted in the same quadrant, they were considered to be different neoplasms only if they were of a different pathologic type. If they were of the same pathologic type, they were considered different neoplasms provided that at least 6 months had elapsed between removal of the first neoplasm and detection of the second neoplasm. All mammary neoplasms, after microscopic study, were given a pathologic classification of either AC or FA according to criteria published previously (7). All data concerned with death or time of appearance of mammary neoplasia are reported in days after the 62d day of age.

Neutron irradiations were accomplished as follows: Twenty-three rats were rotated in a vertical carousel or Ferris wheel 30 cm from a water-cooled tritium target. Each rat was placed in a cylinder 5 cm in diameter and 17.5 cm long made of Lucite 0.3 cm thick and weighted so that the animal remained in its normal horizontal standing position while the wheel was rotating.

A Van de Graaff generator was operated to bombard the target with 2.43-MeV protons to produce neutrons with an energy of 430 keV at the midpoint of each cylinder. The geometry was such that within the rat maximal deviations of the neutron energy from +28 to −14% of the midpoint energy occurred. All rats were reversed in their cylinders after one-half of the dose had been given to reduce nonuniformity in the energy and absorbed dose distributions. The mean tissue kerma in free air was measured at the locations of the rats by integration of the response of a rat-sized, homogeneous tissue-equivalent ionization chamber of minimal mass placed in the 24th cylinder. A compensated Geiger-Müller dosimeter indicated a gamma ray component to the kerma of 3.5%. Depth-dose measurements were made to determine the ratio of absorbed dose to neutron kerma. For this purpose, a 0.6-cm spherical tissue-equivalent multiplication chamber was inserted into a nylon rat phantom at four radial depths. The ratio of absorbed dose to neutron kerma varied between 0.75 and 0.88 over the entire presumed volume of the

rat mammary tissue. In the following, a factor of 0.8 was used to convert neutron kerma to neutron-absorbed dose. The neutron kerma rate was approximately 40 mGy/hour. The rats were exposed to a total kerma of 1.25, 5.0, 20, or 80 mGy, which is considered to be the equivalent of 1.0, 4.0, 16, or 64 mGy of absorbed dose, respectively.

X-ray exposures were accomplished with a conventional therapy X-ray machine. The exposure conditions were: 100 cm target-to-specimen distance; 0.5 mm Cu plus 1.0 mm aluminum filter; maximum backscatter, 250 kVp; and an exposure rate of 30 R/minute measured with a 100-R Victoreen chamber at the dorsal-ventral midpoint of the rats. Rats received an exposure of 30, 60, or 90 R. With a conversion factor of 0.0094, this exposure corresponded to 0.28, 0.56, or 0.85 Gy of absorbed dose, respectively.

RESULTS AND DISCUSSION

Summary of observations.—In this section a synopsis of the observations will be given in elementary form. The inadequacies of this conventional description will make it apparent, however, that a more rigorous analysis is required. This kind of analysis will be the subject of the subsequent sections.

Mean number and mean time of neoplasm appearance.—Radiation doses and numbers of animals in the irradiated groups are listed in table 1. Because the experiment was directed toward the elucidation of the effects of extremely low doses, the largest numbers of animals were assigned to the control group and to the group that was exposed to 1 mGy of neutrons. Table 1 also gives the numbers of animals with specified numbers of FA and AC. It lists the mean numbers of tumors per animal throughout their lives and the standard errors of these numbers. The mean values and their standard errors are obtained by the formula

$$\bar{\nu}=\frac{1}{N}\sum_{\nu=1}^{\nu_{max}}\nu\mu_\nu\pm\sqrt{\sum_{\nu=0}^{\nu_{max}}(\nu-\bar{\nu})^2\mu_\nu/N(N-1)}, \quad [1]$$

where N is the total number of animals in the group and μ_ν is the number of animals that develop ν neoplasms throughout their lifetimes. All the distributions of the number of tumors per animal in table 1 are overdispersed; i.e., the variances of the distributions are broader than those of Poisson distributions. However, this in itself cannot be taken as proof that there are inherent variations in the tumor rates within each group of experimental animals. In fact, substantial deviations from the Poisson distributions arise from the variable life-spans of the animals together with the sharp increase in tumor rate with age. The detailed analysis in the subsequent sections of this article will nevertheless provide evidence for inherent variations in tumor rate among animals.

Table 2 gives the fraction of animals with at least 1 neoplasm each. In addition, the mean times of appearance of the first neoplasms and of all neoplasms are

TABLE 1.—*Number of rats with specified numbers of mammary neoplasms throughout their life-spans*

Dose type	Initial No. of animals	No. of animals with specified No. of FA throughout lifetime											Mean No. of FA/ animal through-out life-time[a]	No. of animals with specified No. of AC throughout lifetime					Mean No. of AC/ animal through-out life-time[a]
		0	1	2	3	4	5	6	7	8	9	≥10		0	1	2	3	4	
Controls	167	57	42	30	15	8	4	4	2	2	3	0	1.68±0.15	141	22	3	1	0	0.19±0.04
Neutron dose, Gy																			
0.001	182	61	42	28	23	7	10	6	2	2	1	0	1.76±0.14	154	24	3	1	0	0.18±0.03
0.004	89	27	24	12	6	7	6	2	3	1	0	1	1.98±0.23	67	19	2	1	0	0.29±0.06
0.016	68	16	14	11	9	3	5	2	1	1	3	3	2.74±0.34	53	11	3	1	0	0.29±0.08
0.064	45	11	6	8	7	4	1	1	1	0	2	4	3.18±0.46	28	10	6	1	0	0.56±0.12
X-ray dose, Gy																			
0.28	95	31	30	9	7	9	1	4	1	2	1	0	1.75±0.21	73	17	5	0	0	0.28±0.06
0.56	48	13	7	8	6	6	2	3	1	0	0	2	2.56±0.36	35	10	3	0	0	0.33±0.09
0.85	48	10	10	7	4	7	3	2	0	2	1	2	2.88±0.39	31	11	2	4	0	0.56±0.12

[a] Values are means ±SE.

TABLE 2.—*Mean life-span, fraction of rats with neoplasms, mean time of appearance of first FA and AC, total number of neoplasms, and mean time of appearance for all FA and AC*

Dose type	Fraction of animals with neoplasms	Mean time of appearance of first FA, days[a]	Mean time of appearance of first AC, days[a]	Mean time of appearance of first neoplasms, days[a]	Total No. of neoplasms	Mean time of appearance of all FA, days[a]	Mean time of appearance of all AC, days[a]	Mean time of appearance of all neoplasms, days[a]
Controls	112/167=0.67	587±15	771±25	590±15	311	687±10	788±83	697±9
Neutron dose, Gy								
0.001	127/182=0.70	543±16	712±39	536±15	353	663±10	732±34	670±10
0.004	67/89 =0.75	544±18	613±49	527±20	202	655±12	637±43	653±12
0.016	54/68 =0.79	481±25	668±71	460±25	206	666±15	745±62	674±15
0.064	38/45 =0.84	411±26	415±53	368±26	164	572±15	463±43	556±14
X-ray dose, Gy								
0.28	68/95 =0.72	551±23	655±46	533±23	193	644±14	677±41	648±13
0.56	37/48 =0.77	421±28	521±86	380±30	138	580±16	531±72	575±16
0.85	38/48 =0.79	418±31	637±52	397±30	165	589±17	698±37	607±15

[a] Values are means ±SE.

listed; the information is also given separately for the two types of neoplasms.

In table 3 the mean tumor-free life-span is compared to the mean total life-span of the animals; this is done separately for all neoplasms and for the two types of neoplasia. A reduction of the mean tumor-free life-span reflects both radiation-induced mortality and tumor incidence. Even the comparison of the mean tumor-free life-span with the mean total life-span may not afford a reliable measure of radiation-induced tumors; this is evident if one considers substantial doses whereby animals may die too fast to develop tumors. However, in the present experiment radiation-induced mortality plays a minor role, at least at the lowest doses.

Text-figure 1 represents, as a function of absorbed dose, the mean numbers of all neoplasms per animal throughout its life. Because the neutrons are far more effective than X-rays, dose scales differing by a factor of

TABLE 3.—*Mean life-spans and mean life-spans free of mammary neoplasms for different neutron and X-ray doses[a]*

Dose type	Mean life-span, days	Mean tumor-free life-span, days	Mean FA-free life-span, days	Mean AC-free life-span, days
Controls	748±13	597±13	599±13	734±13
Neutron dose, Gy				
0.001	717±14	543±13	544±13	696±14
0.004	724±17	541±17	564±16	673±19
0.016	774±20	500±24	518±23	734±23
0.064	643±29	389±28	424±26	552±35
X-ray dose, Gy				
0.28	708±18	539±18	557±18	675±20
0.56	729±21	443±31	476±27	667±32
0.85	667±26	415±26	432±27	628±27

[a] Values are means ±SE.
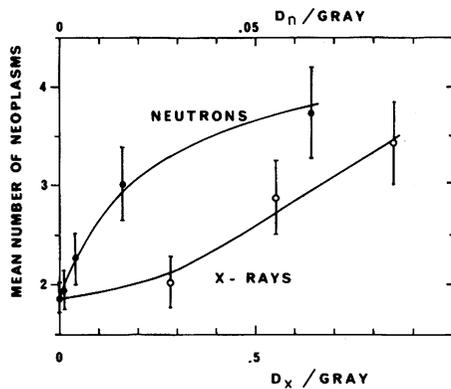
TEXT-FIGURE 1.—Mean No. of mammary neoplasms of any type, FA and AC, throughout life per animal as a function of neutron dose (D_n) and X-ray dose (D_x). Scales for the neutron dose and the X-ray dose differ by a factor of 10.

TEXT-FIGURE 3.—Fraction of animals dead or alive with and without a mammary neoplasm of any type, either FA and/or AC, as a function of time after irradiation. ■, Fraction of animals dead without tumor; ▨, fraction of animals dead with at least 1 tumor; ▧, fraction of animals alive with at least 1 tumor; □, fraction of animals alive without tumor.

10 are used for the two radiations. In text-figure 2 the difference between the mean tumor-free life-span in the irradiated groups and the control group is plotted as a function of absorbed dose. Again, the dose scales differ by a factor of 10.

Both text-figures 1 and 2 indicate RBE values of neutrons versus X-rays of 100 or more at small doses. In addition, the two plots show a "sublinear" dose-effect relation (dose exponent <1) for neutrons. The extraordinary character of such a dose dependence at low neutron doses makes it desirable to subject the data to a more rigorous analysis. Such an analysis must take into account the time dependence of the tumor appearance.

Time dependence of observations.—Text-figure 3 gives a rough representation of the observed time dependences. The data for each group are given in a separate panel. Four different categories of animals are shown, and the relative numbers at any time after irradiation are represented by differently marked areas.

The solid line between the two shaded areas in text-figure 3 indicates cumulative mortality. In these plots the mean life-spans listed in tables 2 and 3 are equal to the areas above the lines representing cumulative mortality. The mean tumor-free life-spans listed in

table 3 are equal to the upper unshaded areas. Strictly speaking, the curves in text-figure 3 should consist of discrete steps. However, here and in subsequent text-figures the midpoints of the vertical steps are simply connected by sloping line segments. This makes the text-figures easier to read.

The increase in the plain shaded areas with increasing dose reflects the increased tumor incidence. However, these areas are not a proper quantitative measure for the incidence of neoplasms. The enhanced mortality at higher doses, i.e., the decreased mean life-spans

TEXT-FIGURE 2.—Loss of tumor-free life in days for any type of mammary neoplasm, FA, and/or AC as a function of neutron dose (D_n) and X-ray dose (D_x). Scales for the neutron dose and the X-ray dose differ by a factor of 10.

of the animals, reduces the number of observed tumors. In text-figure 3 this leads to a decrease in the plain shaded areas and a corresponding increase in the cross-hatched area.

Text-figure 3 does not convey all the information obtained from the experiment. A rigorous analysis will have to be based on the time dependence of the tumor rate, i.e., a quantity that requires somewhat more complicated calculations.

DETAILED ANALYSIS

Definition of Tumor Rate r(t) and Integral Tumor Rate R(t)

For numerical analysis, it is necessary to give first a rigorous definition of essential quantities. Particularly important among these quantities is the tumor rate. The tumor rate $r(t)$ is the probability per unit time interval for an animal to develop a neoplasm at time t. Because the actual time of origin of a neoplasm is unknown, it is identified with the time of first observation. The analysis is not restricted to first neoplasms in an animal but includes all separate neoplasms.

In a group of animals subjected to the same dose, the tumor rate may vary from animal to animal; i.e., some animals may have a greater inherent probability than others to develop neoplasms. This problem will be considered in a subsequent section; at present it is sufficient to note that the quantity $r(t)$ must be interpreted as the average tumor rate in a group of animals at time t. In principle, the tumor rate at a specified time t in a group of N animals at risk can be estimated from an observation of the number n of tumors appearing during a time interval Δt. This estimate of the tumor rate is

$$r(t) = \frac{n}{N \cdot \Delta t}. \qquad [2]$$

In reality, such estimates may be difficult to obtain. For a moderate number of animals at risk and for a reasonably short time interval Δt of not more than a few months, the number n of tumors appearing during the time interval may be small or even zero. This is particularly true in the initial phase of the experiment when the tumor incidence is much lower than at older ages. For the purpose of the numerical analysis, it will therefore be more practical to deal with a quantity that will here be called the integral tumor rate $R(t)$:

$$R(t) = \int_0^t r(t') dt'. \qquad [3]$$

This quantity is less affected by statistical fluctuations than is $r(t)$ and is therefore more readily derived from experimental observations. $R(t)$ is the expected number of neoplasms per animal at risk up to time t. The slope of $R(t)$ versus t is equal to the tumor rate $r(t)$.

Estimation of Integral Tumor Rate and Its Standard Error

There are different ways to estimate $R(t)$ from the observed data. The most straightforward method is to equate $R(t)$ with the mean number of neoplasms that have occurred up to time t in the surviving animals; this method is the so-called reduced sample estimate (14). It has, however, the disadvantage that all observations are disregarded that are made on animals not surviving up to time t. This results in large statistical uncertainties at later times when few of the animals in each group survive. Moreover, the estimate could be biased by a correlation between mortality and incidence of neoplasms.

Equations 2 and 3 permit an estimate of $R(t)$ that is based on all tumors observed up to time t and that is closely related to the so-called actuarial estimate (14-16). An estimate $R(t)$ of the integral tumor rate can be obtained from the relation

$$\hat{R}(t) = \sum_i \frac{n_i}{N_i \Delta t} \Delta t = \sum_i \frac{n_i}{N_i}, \text{ for all } i \text{ with } i\Delta t \leq t, \qquad [4]$$

where n_i is the number of neoplasms appearing in the time interval $(i-1)\Delta t$ to $i \cdot \Delta t$, whereas N_i is the number of survivors at this time. Choosing sufficiently short time intervals Δt, one reaches the point at which no neoplasm will appear in most of the intervals and the rest of the intervals will contain only 1 neoplasm. Equation 4 then reduces to

$$\hat{R}(t) = \sum_k \frac{1}{N(t_k)}, \qquad [5]$$

where t_k are the times of appearance of the individual neoplasms up to time t, and $N(t_k)$ are the numbers of animals at risk at these times. This formula is utilized for the numerical evaluations. For simplicity of notation, the estimated values of the integral tumor rate will be designated by $R(t)$ rather than by $\hat{R}(t)$. The less rigorous but also less technical term, mean number of tumors per animal, is used interchangeably with integral tumor rate.

The numerical values resulting from equation 5 are represented by the upper solid curves in text-figure 4 for FA and in text-figure 5 for AC. The curve for the controls is given together with each of the curves to facilitate comparison.

The shaded areas give the standard errors of $R(t)$ calculated according to the formula (see "Appendix")

$$\sigma_{R(t)} = \pm \sqrt{\sum_k \frac{1}{N(t_k)^2}}, \text{ summation over all } k \text{ with } t_k \leq t. [6]$$

Dose-effect relations and the RBE–dose relation will be considered in detail in a later section. However, the very high effectiveness of low doses of neutrons is apparent even without further analysis. It is particularly striking that a single dose of 1 mGy of neutrons leads to a visible increase in the incidence of FA. Application of the test that has earlier been described (17) and that is in essence a generalization of the so-
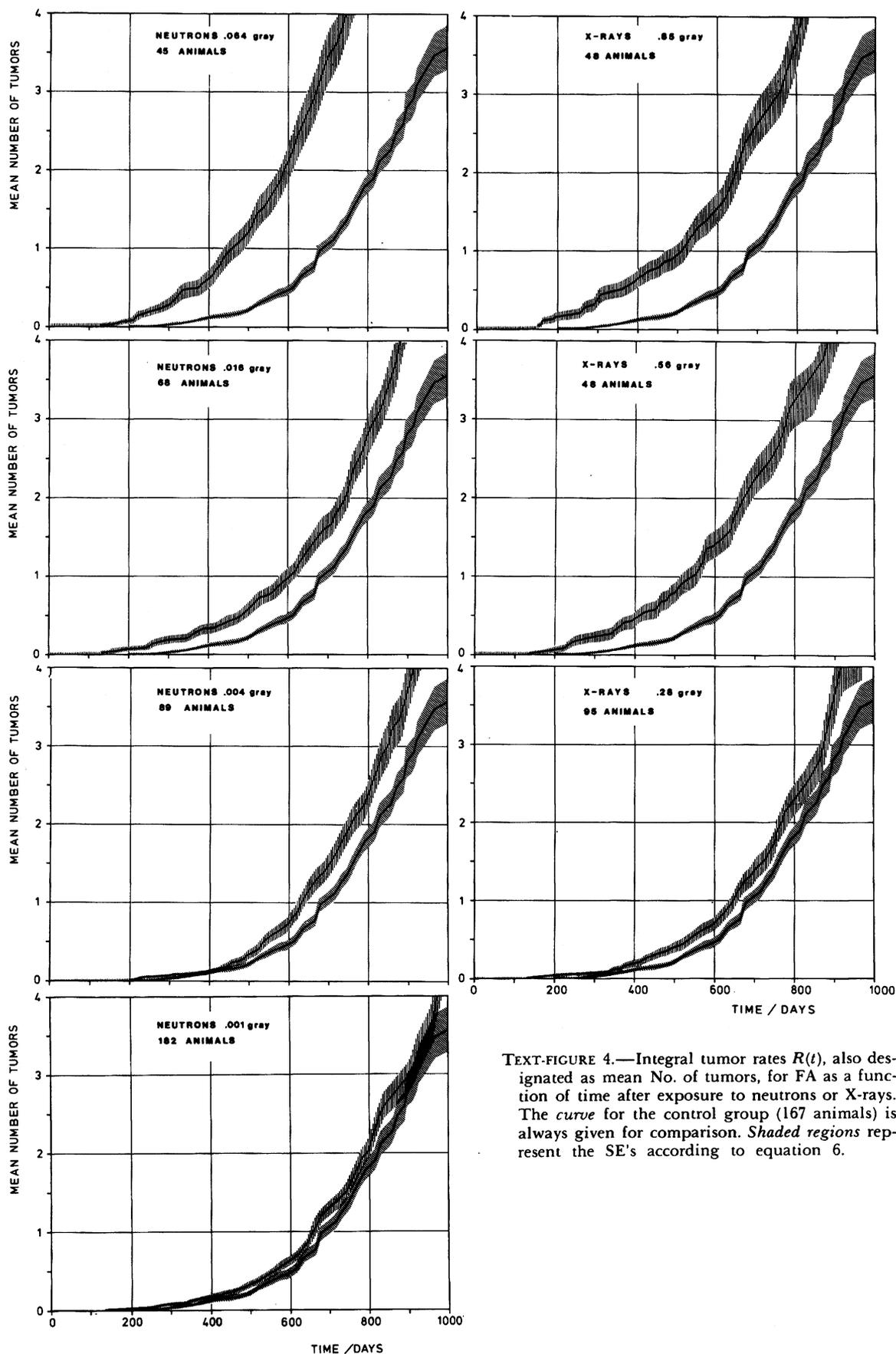
TEXT-FIGURE 4.—Integral tumor rates $R(t)$, also designated as mean No. of tumors, for FA as a function of time after exposure to neutrons or X-rays. The *curve* for the control group (167 animals) is always given for comparison. *Shaded regions* represent the SE's according to equation 6.
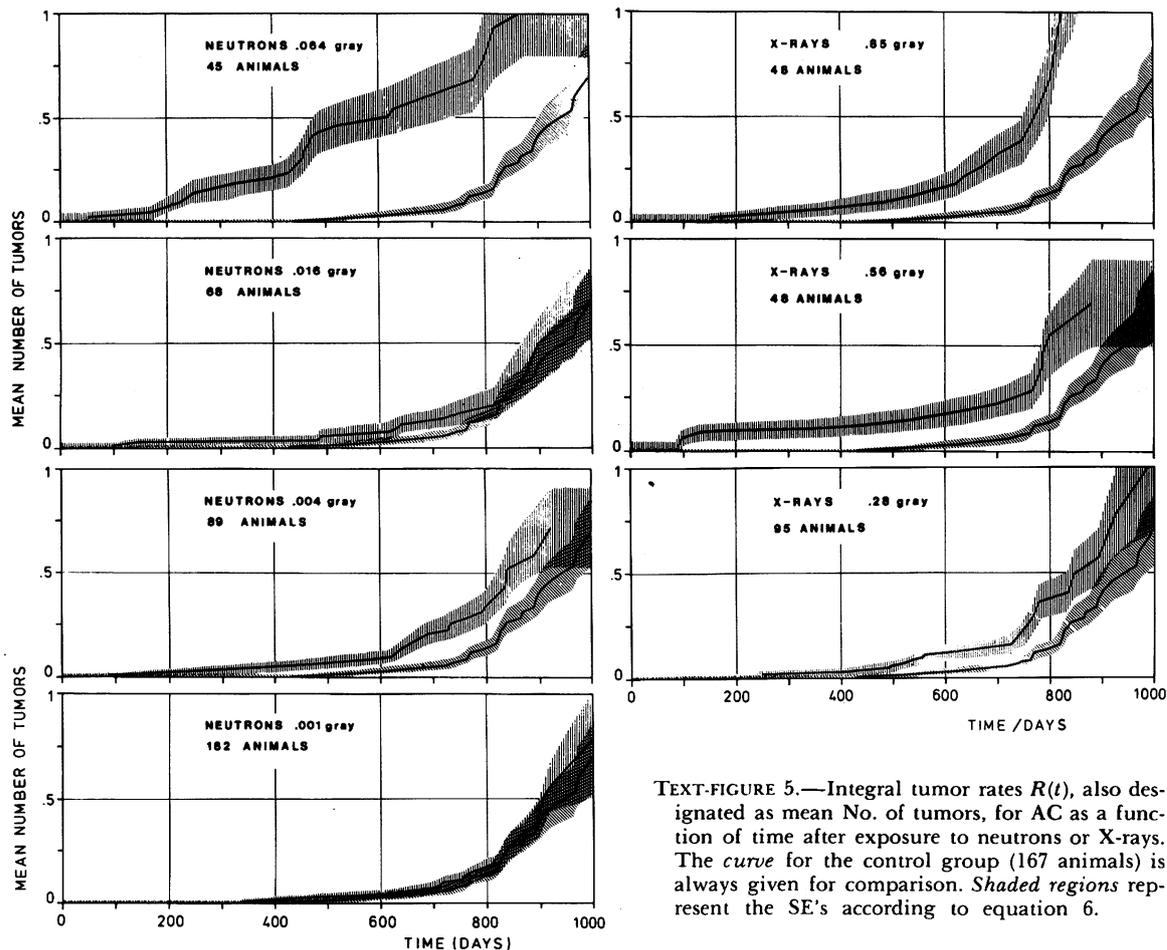
TEXT-FIGURE 5.—Integral tumor rates $R(t)$, also designated as mean No. of tumors, for AC as a function of time after exposure to neutrons or X-rays. The *curve* for the control group (167 animals) is always given for comparison. *Shaded regions* represent the SE's according to equation 6.

called log-rank test (15, 16) leads to the conclusion that the increase is significant with an error level of less than 5%.

A comparison with the curve for 0.28 Gy of X-rays leads us to the conclusion that the RBE for a neutron dose of 4 mGy exceeds 70 for FA. For AC one also obtains an RBE of 70 from the observed approximate equality of the effect of 4 mGy of neutrons and 0.28 Gy of X-rays. The small incidence of AC in the group exposed to 16 mGy of neutrons is not in line with the other observations, but this may be a fluctuation due to the small absolute numbers of AC. At the highest neutron dose studied, the statistical accuracy is better and, as discussed in the subsequent section, it can be shown that the rate of AC is significantly higher for 0.064 Gy of neutrons than for 0.85 Gy of X-rays. This implies that even at the highest dose the neutron RBE for AC exceeds 10.

Comparison to Integral Tumor Rate Obtained From First Neoplasms per Animal and Connection Between $R(t)$ and Cumulative Prevalence $I(t)$

Estimates of the integral tumor rates could also be obtained by restriction of the analysis to the first neoplasm in each animal. The same equation 5 applies

in this case. The only differences are that the summation extends only over first neoplasms and that the number $N(t_k)$ of animals at risk at time t_k is not that of the survivors but that of the animals without neoplasms. In other words, the same formalism is applied, but the animals are removed from the analysis as soon as the first neoplasm is found. Regardless of whether all animals or only animals without previous neoplasms are retained in the analysis and provided all animals have the same inherent tumor rate that is unaffected by the occurrence of previous neoplasms, one should, on the average, obtain the same estimate $R(t)$. Under this condition, the two estimates are equivalent; however, the estimates from all neoplasms are subject to less statistical error because they are based on more data.

A comparison of the values $R(t)$ obtained from first tumors indicates, however, that animals with a previous neoplasm have a somewhat higher probability of developing further neoplasms. This phenomenon can be seen in text-figure 6 in which comparison of the two estimates is given for FA. In the early phase of the experiment, the two estimates coincide; at later times, the value $R(t)$ obtained from all neoplasms is substantially larger than the value obtained from first neoplasms only. The slopes of the solid lines in text-figure

6 are estimates of the tumor rate among all surviving animals, whereas the slopes of the broken lines are estimates of the tumor rate among animals with no previous neoplasms. One concludes, therefore, that there is indeed a somewhat higher tumor rate among animals with previous neoplasms. Inhomogeneity of the population is also evident from a consistent over-dispersion of the distribution of the number of FA among animals surviving at a specified time.

In other rat strains and mainly for carcinomas, Clifton and Crowley (18) have also demonstrated that animals with previous mammary neoplasms have a higher probability of developing further neoplasms. They concluded from these observations that the occurrence of a neoplasm increased the probability for further neoplasms, and they consequently restricted the analysis to first neoplasms.
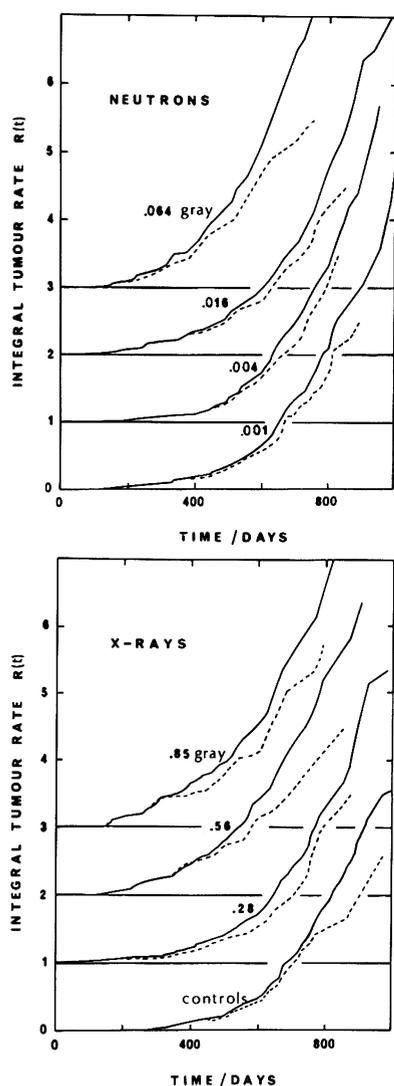
TEXT-FIGURE 6.—Comparison of integral tumor rates $R(t)$ for FA from computations based on all FA (*solid lines*) and on first FA only (*broken lines*). To avoid overlap, different shifts of the ordinate values are applied to the curves. The numerical values on the ordinate apply, therefore, only to the unshifted pairs of curves. For the other curves, the ordinate values must be adjusted.

There is no direct evidence that the presence of an FA in a Sprague-Dawley rat predisposes the animal to the development of additional neoplasms. In fact, it has been found (19) that there is no abscopal effect, i.e., no increased tumor rate in the unirradiated tissue if the mammary tissue is partially irradiated. In fact, overdispersion does not require that the incidence of a neoplasm increases the probability for further neoplasms in an animal. Instead, there may be inherent differences in the tumor rate between animals. Restriction of the analysis to first tumors only would bias the results toward animals with the smallest tumor rate, and all neoplasms are therefore considered in the present analysis.

There is no similar evidence for differences in the rate of AC (text-fig. 7), but the number of AC is so small that only a very substantial correlation would be observable. It would be easier to detect a correlation of the occurrence of AC with the number of FA in the same rat. However, no such correlation has been found.

The probability of an animal to incur no neoplasm up to time t is equal to

$$\rho_0(t) = e^{-\int_0^t r(t')\,dt'} = e^{-R(t)},\qquad [7]$$

where $r(t)$ and $R(t)$ are the tumor rate and the cumulative tumor rate, respectively, for animals with no previous neoplasms.

$R(t)$ obtained from first neoplasms only can therefore be used for the calculation of the probability $I(t)$ of an animal to incur at least 1 neoplasm up to time t:

$$I(t) = 1 - e^{-R(t)}.\qquad [8]$$

$I(t)$ is usually called cumulative prevalence (20) and is obtained by the product limit estimate of Kaplan and Meier (14). The computation from $R(t)$ for first neoplasms, i.e., the computation based on equation 5, is used in the present analysis because it is somewhat simpler. The results are given in text-figure 8 for the two types of neoplasms separately.

DOSE-EFFECT RELATIONS AND RBE-DOSE RELATION

Dose-effect relations can be obtained readily on the basis of the excess number of neoplasms per animal at a specified time. The disadvantage of this procedure is that the choice of the time of reference is arbitrary, and one does not utilize all of the experimental data. Depending on the time of reference, one will obtain somewhat different curves. This is evident in text-figure 9 in which the mean number of FA $R(t)$ minus the control value is plotted for 400, 600, and 800 days as a function of neutron and X-ray doses. To accommodate all curves on a common graph, we chose the logarithmic representation. This representation has the additional advantage that linear dose-effect relations are easily recognized because they should appear as lines of unit slope. In spite of considerable statistical
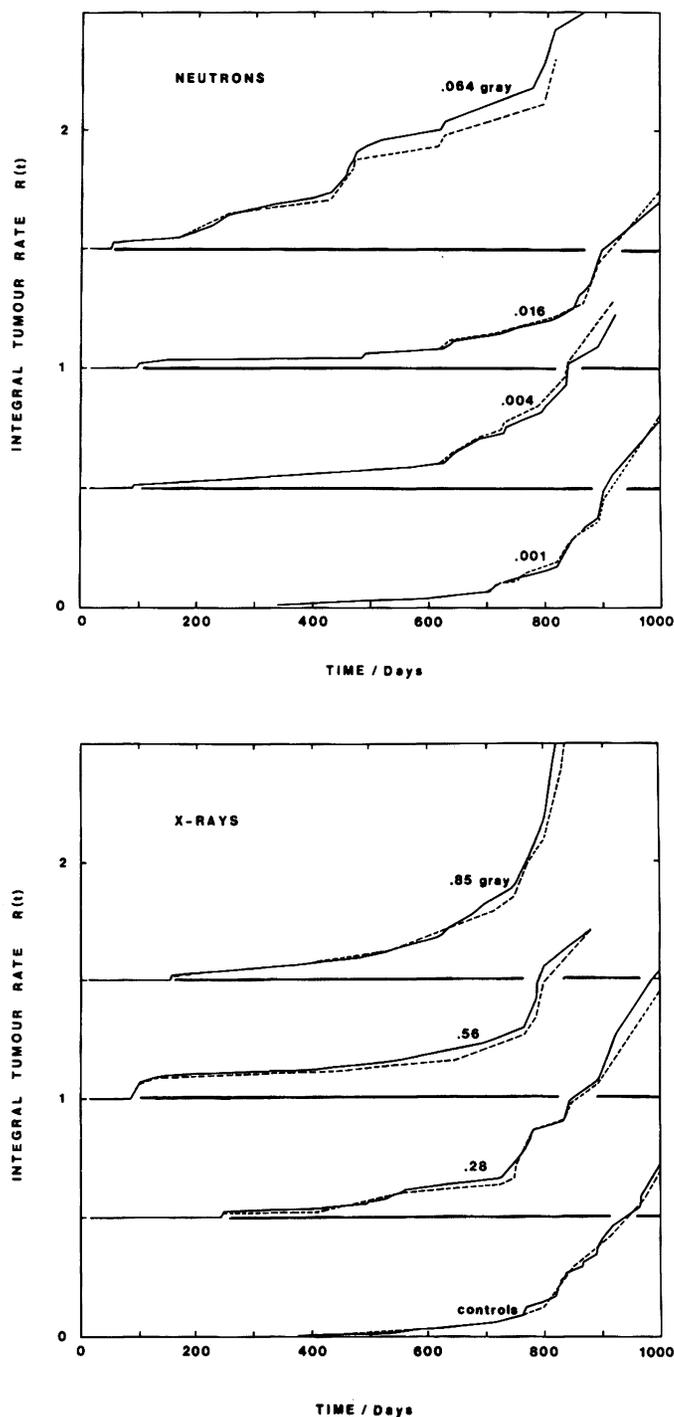
TEXT-FIGURE 7.—Comparison of integral tumor rates $R(t)$ for AC from computations based on all AC (*solid lines*) and on first AC only (*broken lines*). Vertical shifts of the curves are as in text-fig. 6.

fluctuations, the same observation that has been made from text-figures 1 and 2 is also apparent from text-figure 9; this is the sublinearity, i.e., the dose exponent less than 1, for neutrons. For X-rays, a linear dose dependence cannot be excluded. The observations are therefore in agreement with earlier analyses (*2, 3*), and a dose exponent less than 1 at very low neutron doses appears to be well confirmed. This need not be

inconsistent with the fact that no deviation from linearity was established in a similar experiment (*21*) involving smaller groups of animals, higher doses, and shorter times of observation.

It is unsatisfactory to represent the results of the full experiment by dose-effect relations that contain only a minor fraction of the total information. A more complete description is therefore desirable. One method to obtain this description is the representation of the observed integral tumor rates by certain analytic expressions and the examination of their dependence on absorbed dose.

A comparison of the curves for FA (text-fig. 4) indicates that their shape is similar. One may therefore surmise that the effect of the irradiation consists in a shift in the occurrence of neoplasms to earlier times. This would be in line with other analyses of late effects which have led to the notion that the effect of irradiation can be equivalent to an age increment (*22*). One can on the basis of text-figure 4 estimate the time shifts produced by the different neutron and X-ray doses in regard to mammary FA. For this purpose, the individual curves in text-figure 4 are approximated by the relation

$$R(t) = 7.5 \cdot 10^{-10}((t - \Delta t)/[d])^{3.3}, \qquad [9]$$

where $[d] = $ day and $R(t) = 0$ for $t < \Delta t$. This corresponds to the tumor rate

$$r(t) = 2.5 \cdot 10^{-9}((t - \Delta t)/[d])^{2.3} \cdot [d]^{-1} \qquad [10]$$

and $r(t) = 0$ for $t < \Delta t$.

For the control group, $\Delta t = 106$ days. For the irradiated groups the values Δt are smaller, and the difference to the control value is the radiation-induced time shift in the occurrence of the mammary neoplasms. The time shifts that yield a good fit to the data are plotted for FA in text-figure 10 in which the scales for the neutron and the X-ray dose differ again by a factor of 10. As with text-figure 9, results are given here only for FA. However, nearly the same time shifts result if the analysis is based on all neoplasms.

In contrast to the dose-effect relations in text-figures 1, 2, and 9, text-figure 10 has the double advantage of being based on mortality-adjusted data and of being representative for all observations. A disadvantage is that there is no simple method to derive standard deviations or confidence limits for the results. This will require further study. However, it is apparent that these dose-effect relations are also in agreement with the conclusion that the dose dependence of the induction of mammary neoplasms in Sprague-Dawley rats is sublinear at low doses of neutrons (dose exponent <1), whereas it is approximately linear for X-rays.

One cannot assume that the different dose-effect relations that have been discussed here lead to precisely the same RBE of neutrons as a function of absorbed dose. The RBE values obtained from the various dose-effect relations could be different if the effect of neutrons were qualitatively different from that of X-rays, e.g., in the temporal distribution of tumors.
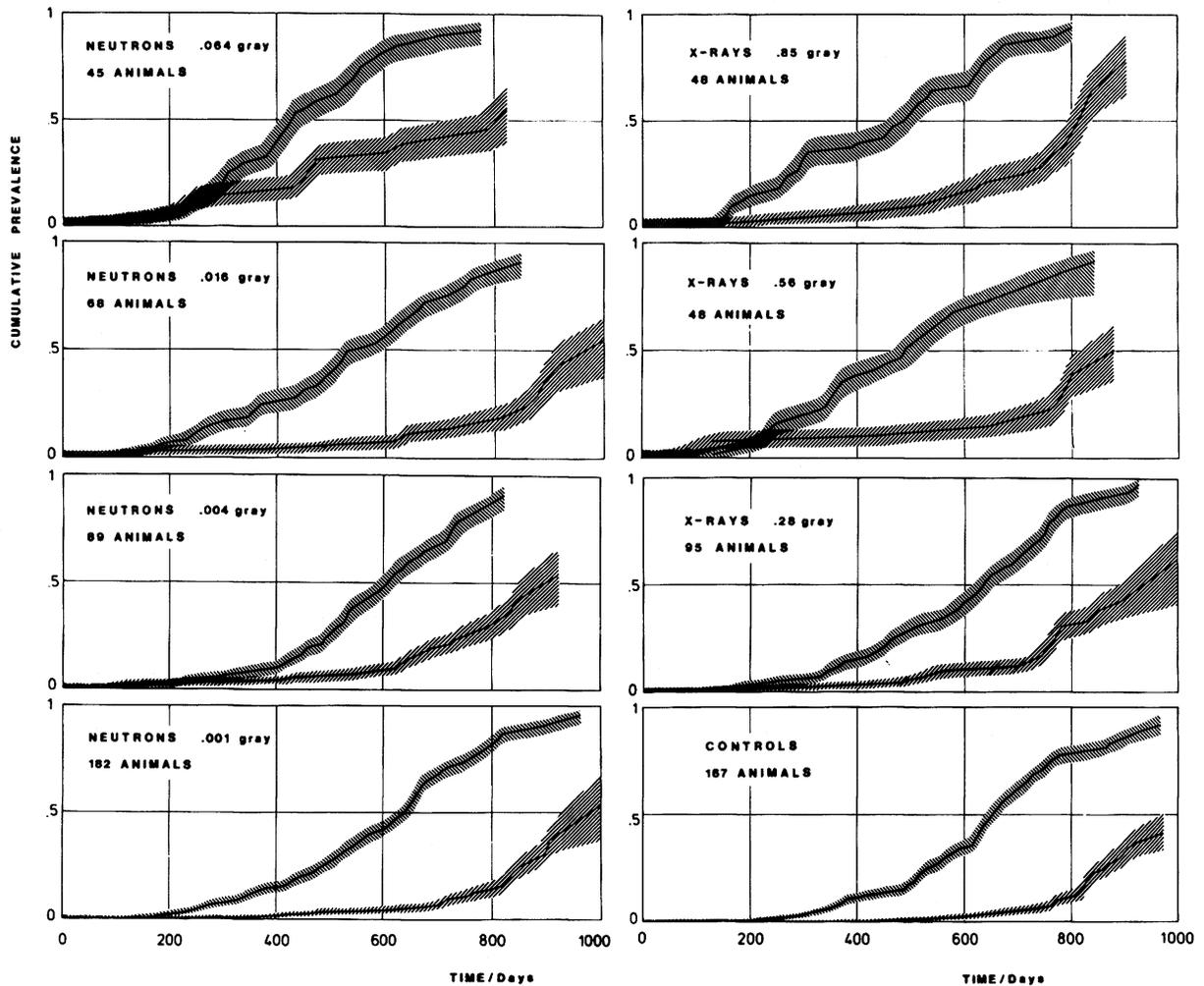
TEXT-FIGURE 8.—Cumulative prevalence $I(t)$ as a function of time after exposure to neutrons or X-rays. In *each panel*, the *curves* for FA and AC are given separately; *shaded areas* represent the SE's.
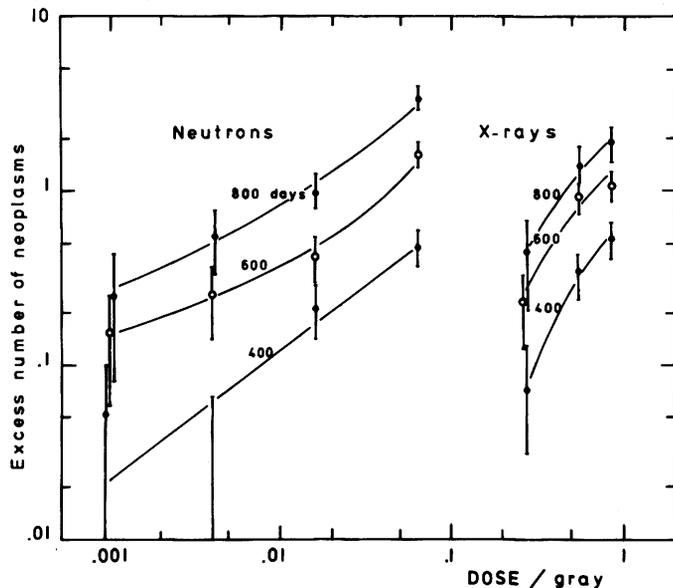
TEXT-FIGURE 9.—Excess No. of FA per animal at specified times after exposure to 430-keV neutrons or X-rays. Data correspond to those in text-fig. 4. *Bars* are SE's. *Solid lines* have no mathematical significance; they are inserted merely to clarify the representation.

However, no indication is found in the present experiment that such a qualitative difference exists. In fact, the dose-effect relations in text-figures 1, 2, 9, and 10 are all consistent with the RBE–dose relation that is plotted in text-figure 11.

The computation of confidence ranges for the RBE as a function of neutron dose would require the assumption of an analytic expression for the underlying dose-effect relations. This will not be considered in the present article. Certain boundaries for the RBE values can, however, be obtained from direct comparison of the effects of a neutron dose and an X-ray dose (23). Application of a test (17) closely related to the log-rank test (15, 16) to the full set of observations at, for example, a dose of 16 mGy of neutrons and 280 mGy of X-rays shows that the latter is significantly less effective. This implies that the neutron RBE at a neutron dose of 16 mGy must exceed 17.5. To indicate this, the excluded range RBE less than 17.5 is blocked out by a solid vertical line in text-figure 11. The other vertical lines are obtained in the same way from other comparisons. Because there are only 3 X-ray doses in the present experiment, earlier gamma ray data (7)
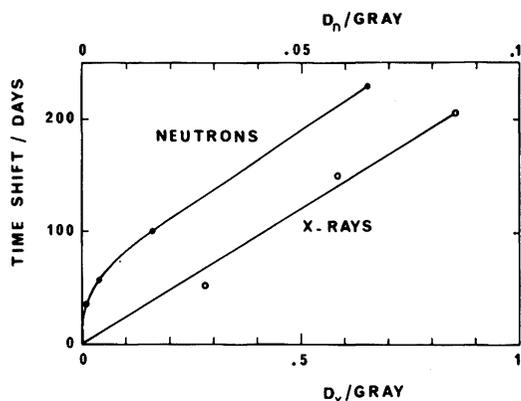
TEXT-FIGURE 10.—Forward shift in time of occurrence of FA due to neutron or X-ray exposure. Scales for the neutron dose (D_n) and the X-ray dose (D_x) differ by a factor of 10. Time shifts are obtained from the data in text-fig. 4.

have also been utilized for the comparisons. The vertical bars indicate that the effects are different on a significance level of more than 95%. The arrows indicate differences with less statistical significance. The band of RBE values between the set of vertical bars is consistent with the curve that corresponds to the dose-effect relations in text-figures 1, 2, 9, and 10.

Only a minor fraction (14%) of all mammary neoplasms found in this study were mammary AC. No separate dose-effect relations are given for AC, but there is no evidence that their dose dependence differs from that of the FA. In particular, the RBE-dose dependence plotted in text-figure 11 for FA and the combined group of FA and AC is consistent also for the more limited data pertaining to AC. Due to statistical uncertainties at low doses, differences in the occurrence of FA and AC cannot be excluded. However, statistical tests show a significantly faster rise in the AC rate for 0.064 Gy of neutrons than for 0.85 Gy of X-rays. The neutron RBE for AC at 0.064 Gy can therefore be taken to be approximately 13, and this value is in agreement with the value for FA.
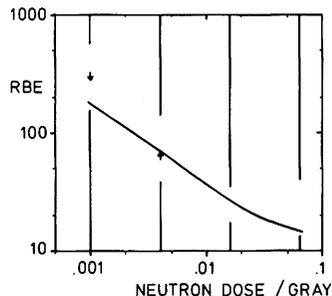
TEXT-FIGURE 11.—Dependence of the RBE of neutrons for the induction of mammary neoplasms on neutron dose. *Vertical bars* indicate the ranges of RBE that are excluded on a level of statistical significance exceeding 95%; *arrows* represent differences with lower level of significance. This analysis utilizes a nonparametric method (23) and is based on the current results and on earlier data (7) for gamma rays. The graph applies equally whether one considers FA only or both types of neoplasms.

CONCLUSIONS

The present experiment has been designed toward the assessment of the effect of very low doses of neutrons in comparison to that of sparsely ionizing radiations.

Even a single dose of 1 mGy of neutrons (0.1 rad) produces a significant increase in the tumor rate in regard to mammary FA and in regard to the total mammary neoplastic response which includes both FA and AC. A comparison of various exposed groups leads to RBE values of neutrons that increase beyond the value 100 at low doses. At high doses one obtains substantially smaller RBE values. This RBE-dose relationship is consistent with the theoretically expected (1) proportionality of RBE to the inverse square root of the neutron dose and with the high values of RBE at small neutron doses.

Dose-effect relationships can be obtained in various forms. Regardless of whether one considers the number of excess tumors at a specified time, the forward shift in tumor rates, or the loss of tumor-free life-span, the resulting dose-effect relationships for neutrons generally correspond to a dose exponent of approximately 0.5. The data for X-rays appear to be consistent with a dose exponent of 1; however, the apparent linearity of the dose-effect relationships for X-rays may be fortuitous.

The number of radiation-induced AC is too small to permit definite statements on the dose-effect relationships. However, the high RBE values for neutrons and the RBE-dose relationship that applies to FA appear to pertain also to AC.

APPENDIX

Estimates of Integral Tumor Rate $R(t)$ and Cumulative Prevalence $I(t)$

In agreement with the formula derived by Kaplan and Meier (14), one can use the so-called "product limit estimate" of the cumulative prevalence:

$$\hat{I}(t) = 1 - \prod_k \left(1 - \frac{1}{N(t_k)}\right), \quad t_k \leq t, \qquad [A.1]$$

where the product extends over all times t_k of occurrence of a first tumor. $N(t_k)$ is the number of animals without neoplasms before t_k.

In the present article, the relation between $I(t)$ and the integral tumor rate $R(t)$ (from first tumors)

$$I(t) = 1 - e^{-R(t)} \qquad [A.2]$$

is used, and $R(t)$ is estimated by the formula that, in analogy to equation A.1, can be termed the "sum limit estimate":

$$\hat{R}(t) = \sum_k \frac{1}{N(t_k)}, \quad t_k \leq t. \qquad [A.3]$$

Equation A.3 is more convenient numerically. It also has the advantage that it can be applied to all tumors instead of first tumors only.

The numerical values obtained with the two different estimates are very nearly equal provided the number of animals is not too small. If $N(t_k)$ is large compared to 1, one has the approximate equality

$$\ln\left(1-\frac{1}{N(t_k)}\right) \approx -\frac{1}{N(t_k)}. \quad \text{[A.4]}$$

Accordingly, one obtains

$$\hat{I}(t) = 1 - \prod_k\left(1-\frac{1}{N(t_k)}\right) = 1 - e^{\sum_k \ln\left(1-\frac{1}{N(t_k)}\right)} \approx 1 - e^{-\sum_k \frac{1}{N(t_k)}}$$

$$= 1 - e^{-\hat{R}(t)}. \quad \text{[A.5]}$$

Standard Errors of R(t) and I(t)

The standard deviation of the estimate of $R(t)$ can be deduced from equation 4. If the neoplasms are statistically independent, the variance of the observed quantity $R(t)$ is the sum of the variances of the individual terms n_i/N_i. Since n_i follows a Poisson distribution, the estimated variance of n_i is also equal to n_i. The estimated variance of n_i/N_i is therefore n_i/N_i^2. Accordingly, one obtains an estimate of the variance of $R(t)$ as the sum of variances of all terms in equation 4:

$$\sigma^2_{R(t)} = \sum_i \frac{n_i}{N_i^2}, \text{ summation over all } i \text{ with } i\Delta t \le t. \quad \text{[B.1]}$$

By considering sufficiently short time intervals, one obtains a relationship analogous to equation 5:

$$\sigma^2_{R(t)} = \sum_k \frac{1}{N(t_k)^2}, \text{ summation over all } k \text{ with } t_k \le t, \quad \text{[B.2]}$$

where t_k are the times of appearance of the individual tumors, and $N(t_k)$ are the numbers of survivors at these times. The corresponding standard error $\sigma_{R(t)}$ (see equation 6) has been represented in text-figures 4 and 5 by the shaded areas.

The assumption of the independence of mammary neoplasms is not strictly valid. An analysis of the frequencies of multiple tumors among the animals surviving at specified times indicates deviations from the Poisson distribution that are due to differences in tumor rate among the animals. The effect is significant toward the later part of the observation period; the indicated standard errors are then somewhat too small.

The standard error $\sigma_{I(t)}$ of the cumulative prevalence is usually obtained from the Greenwood formula (14):

$$\sigma_{I(t)} = (1-I(t)) \cdot \sum_k \frac{1}{N(t_k) \cdot N(t_{k-1})}, \ t_k \le t. \quad \text{[B.3]}$$

One obtains nearly the same values whether one plots $I(t) \pm \sigma_{I(t)}$ or, as in text-figure 8, $1 - \exp(-R(t) \pm \sigma_{R(t)})$. The consistency of the two standard errors follows from the fact that they are in the proper relationship:

$$\sigma_{I(t)} = \frac{d\,I(t)}{d\,R(t)}\,\sigma_{R(t)} \quad \text{[B.4]}$$

that results from

$$\frac{d\,I(t)}{d\,R(t)} = \frac{d(1-e^{-R(t)})}{d\,R(t)} = e^{-R(t)} = 1 - I(t) \quad \text{[B.5]}$$

and

$$\sigma_{I(t)} = (1-I(t))\sum_k \frac{1}{N(t_k)\,N(t_{k-1})} \approx \frac{d\,I(t)}{d\,R(t)}\sum_k \frac{1}{N(t_k)^2}$$

$$= \frac{d\,I(t)}{d\,R(t)} \cdot \sigma_{R(t)}. \quad \text{[B.6]}$$

The estimate for $\sigma_{R(t)}$ used in the present article is therefore consistent with the Greenwood formula.

In a detailed study of radiation carcinogenesis, the main objective is the analysis of the tumor rate and the integral tumor rate. Accordingly, the sum limit estimate and the corresponding formula for the standard error are used instead of the product limit estimate and the Greenwood formula.

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