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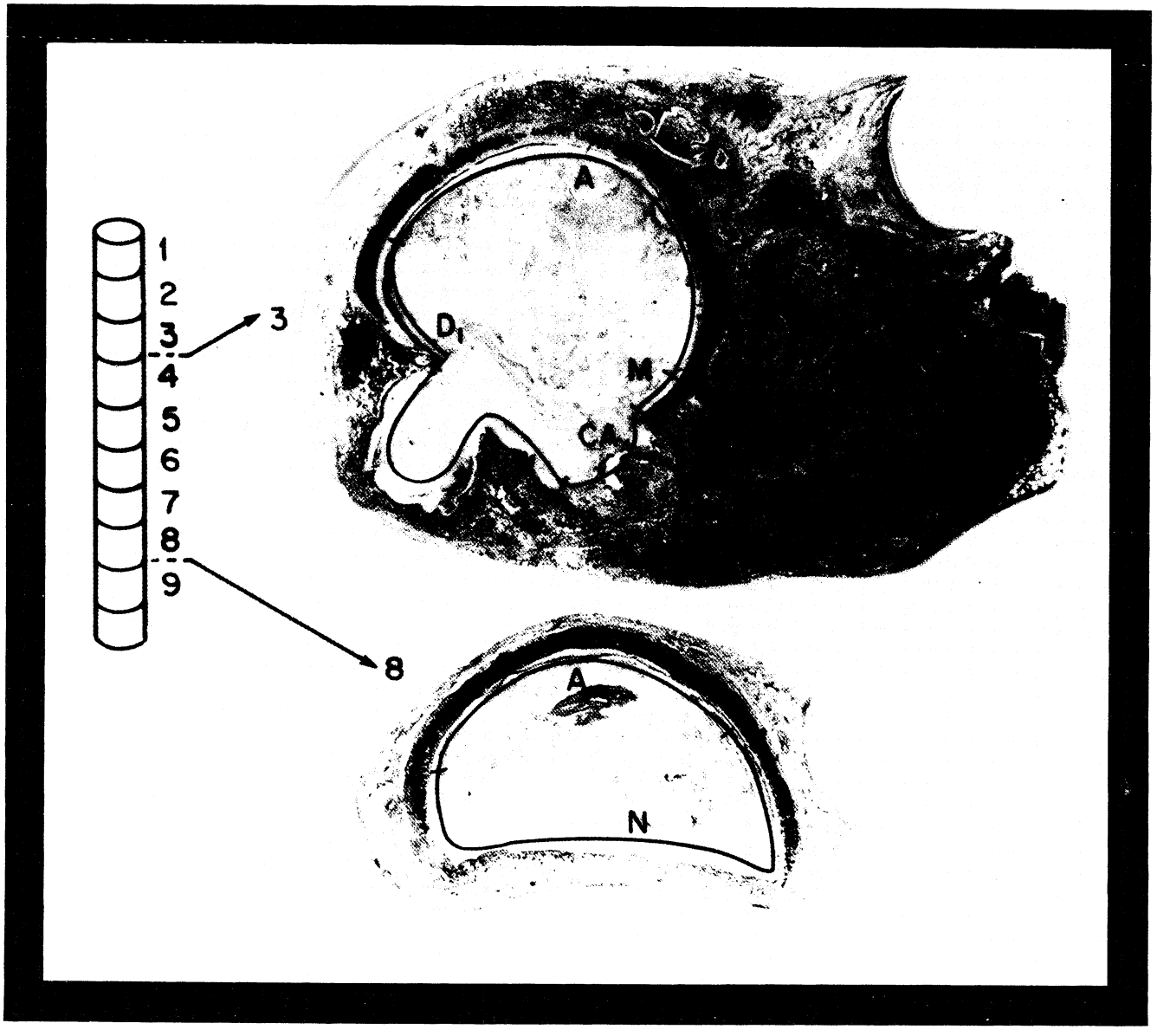

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

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Investigations on Man

Announcements

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

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ABSTRACT—Mammary tumorigenesis was studied in female ACI rats after treatment with X-irradiation or neutron-irradiation, with or without diethylstilbestrol (DES) treatment. The mortality-corrected cumulative tumor rate based on all mammary neoplasms and the mortality-corrected incidence based on the first neoplasms only have been derived. In non-DES-treated animals, at the relatively high radiation doses studied, all dose-effect relationships were consistent with relative biological effectiveness (RBE) values slightly in excess of 10. In DES-treated rats definite findings were observed at neutron doses as low as 0.01 Gy (1 rad). The dose-effect relationship in DES-treated rats showed a strong sublinearity (dose exponent <1) at low neutron doses. RBE values in DES-treated rats increased in inverse proportion to the square root of the neutron dose, and exceeded 100 at a neutron dose of 0.01 Gy (1 rad).—JNCI 1982; 69:1135-1146.

In female rats of the ACI strain, both low LET and high LET radiations induce mammary AC and mammary FA (*1, 2*). In this strain of rat, a form of synthetic estrogen, DES, induces mammary AC, but no mammary FA, when given in the form of a 20-mg pellet of 25% DES and 75% cholesterol (*3*). Furthermore, in ACI rats, the effect of DES and ionizing radiations appears to be synergistic for mammary AC formation, as shown in studies with X-rays by Segaloff (*4*) and later by Stone et al. (*2*), and in studies with neutrons by Shellabarger et al. (*5*).

The present work is primarily aimed not at the further elucidation of the synergistic effect but at the comparison of the effects of X-rays and neutrons at low doses. Therefore, graded X-ray and neutron doses were applied and only one dose of DES was utilized. The interest in this investigation is due to the theoretical prediction (*6*) and the experimental observation in various systems (*7-9*) of high-neutron RBE, which may be of considerable impact to radiation protection because they support the suggestion that the quality factor of 10, now used for neutrons, should be reconsidered (*10*). The only source of human data for neutrons, the Japanese atomic bomb survivors, may be lost due to the revision of neutron dosimetry (*11*); thus data from animal studies with low doses of neutrons have gained importance.

In an earlier experiment with Sprague-Dawley rats, very low doses of neutrons were used, and for a neutron dose of 1 mGy an RBE in excess of 100 was found (*12, 13*). However, it remained unclear whether this finding was specific to the Sprague-Dawley rats only, with the high spontaneous incidence and with a considerably larger number of FA than AC characteristic to this strain. Therefore, it appeared desirable to conduct analogous studies with ACI rats that have a very low spontaneous incidence of mammary neoplasms and show predominantly AC when treated with DES.

A second aspect of the earlier findings on Sprague-Dawley rats was of particular importance and has been an additional motivation of the present study. This was the finding of a substantial *sublinearity*, i.e., a negative curvature of the dose-effect relationship, at small neutron doses. Such a sublinearity implies that the effect per unit dose is largest at smallest doses and is therefore of evident concern in radiation protection, for which risk assessments are commonly based on the linear hypothesis. Sublinearity is also of interest because it occurs at doses that are sufficiently small that only few cells are traversed by any neutron recoil, and the probability of several recoils can be disregarded (*14, 15*). In this dose range the tendency is to postulate linear dose dependences for neutrons, because the number of cells that receive any energy deposition is merely proportional to dose. The failure to find a linear relation has therefore been interpreted as evidence of a multicellular reaction or a radiation-induced tissue factor (*15*). Examination of the linearity or nonlinearity of the dose-effect relationship for neutrons was accordingly another main goal in the present investigation.

MATERIALS AND METHODS

Rats and treatment schedules.—Approximately 800 weanling, virgin, female ACI rats were purchased from M.A. Bioproducts, Walkersville, Md. Rats were delivered to this laboratory in eight shipments of at least 50 rats at weekly intervals. From each shipment 3 rats were assigned to each of 14 groups that were to be irradiated, and 4 rats were assigned to each of 2 groups that were reserved as nonirradiated controls. This procedure was repeated with another 400 rats. Thus all 16 groups were matched closely for age both in regard to the average age and the range of ages. Neutron irradiation was given to rats with an average age of 88 days, and X-irradiation was given to rats with an average age of

Abbreviations used: AC=adenocarcinoma(s); DES=diethylstilbestrol; FA=fibroadenoma(s); Gy (gray)=unit of absorbed dose in the International System of Units (Gy=J/kg or 100 rad); LET=linear energy transfer; RBE=relative biological effectiveness.

[1] Received February 17, 1982; accepted July 6, 1982.

[2] Supported by Public Health Service contract X01CP-60219 from the Division of Cancer Cause and Prevention, National Cancer Institute; by contract DE-AC02-76CH00016 from the U.S. Department of Energy; and by Euratom Research contract BI0-286-81 D (B).

[3] Animals were maintained under the guidelines set forth by the Animal Welfare Act in facilities accredited by the American Association for Accreditation of Laboratory Animal Care.

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89 days. All irradiations were done as described previously (*5*). DES (5 mg) was given, in pellet form, 2 days before irradiation, with methods described earlier (*4*). All data were recorded in days after the date of DES administration and, for simplicity, all ages, life-spans, and times quoted in this article refer to the date of DES administration as time 0.

All rats were kept in temperature-controlled (21–23°C) and humidity-controlled (45–65%) animal rooms with 12 hours (8 a.m.–8 p.m.) of fluorescent light. The animals were maintained on commercial rat chow and water ad libitum. Each rat was examined at least weekly for the presence of mammary tumors, and all mammary tumors were studied microscopically and given a pathologic classification of either AC or FA according to criteria consistent with those of Young and Hallowes (*16*).

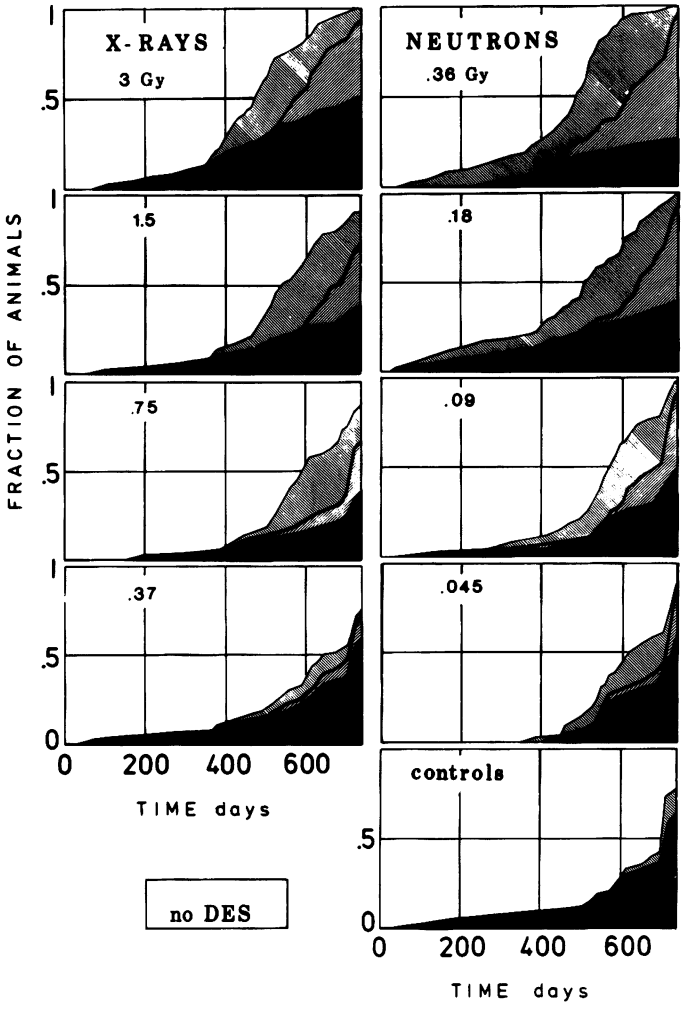
Tumor methodology.—Each rat was identified by a numbered ear tag. The anatomic location of each mammary tumor was recorded with the nipples as reference points. Because two distinct types of mammary tumor responses were found, individual or multiple, each was recorded separately. Individual mammary tumors were removed under ether anesthesia at a size of about 2 cm. If a second tumor was found at the site of a previously removed tumor, it was not recorded as a second individual tumor unless a 10-week period had elapsed between removal of the first tumor and the detection of the subsequent tumor. In the other type of mammary tumor response, which was confined to mammary AC in DES-treated rats, multiple AC were often detected within a single quadrant of mammary tissue over about 1 week. Because too many tumors existed to be counted by palpation alone, the entire quadrant was removed, fixed, defatted, stained with hematoxylin, cleared, and stored in methyl salicylate. Each quadrant was examined under a dissecting microscope at magnification 10X, and all presumed pathologic areas were removed individually for sectioning. The minimum number of these multiple AC was never less than 4 per quadrant. Even though there were often too many multiple AC per quadrant to be counted accurately, an individual quadrant was assigned a maximum number of 4 AC. Thus an individual rat could then have a maximum number of 16 AC. The multiple AC response within a single quadrant was usually found within a 1–3-week period. However, quadrants were removed not more often than twice per week. Rats were killed when they showed a multiple tumor response in all four quadrants, when they became moribund, or when tumor removal was no longer feasible. All remaining DES-treated rats were killed at days 523–525. Of the non-DES-treated rats still alive, approximately half of each treatment group was killed either on day 712 or on days 726–730. At autopsy, confirmation of DES treatment was made by locating the DES pellet. An abnormal pituitary gland was recorded as a pituitary tumor if the pituitary was hemorrhagic and fragile and exceeded a weight of 50 mg. Pituitary tumors were found in 30% of the untreated control rats, in 39% of the irradiated only rats, in 90% of the DES only rats, and in 79% of the irradiated plus DES-treated rats. The combined incidence of pituitary tumors in all nonirradiated rats was 58% compared to 52% in all irradiated rats, and the combined incidence in all non-DES-treated rats was 38% compared to 81% in all DES-treated rats. These results are in agreement with those of Stone et al. (*3*).

RESULTS

Of a total of 789 rats studied, 348 rats received DES and 441 did not receive DES. Of the rats that received DES, only 6.3% developed FA. Because of the small yield of FA in DES-treated rats (a total of 32 FA vs. 2,474 AC), the FA response in DES-treated animals was not analyzed. Among the non-DES-treated animals, 47% showed mammary neoplasms, 35% developed FA, and 26% developed AC; in view of their limited number, both types of neoplasms were pooled in the analysis.

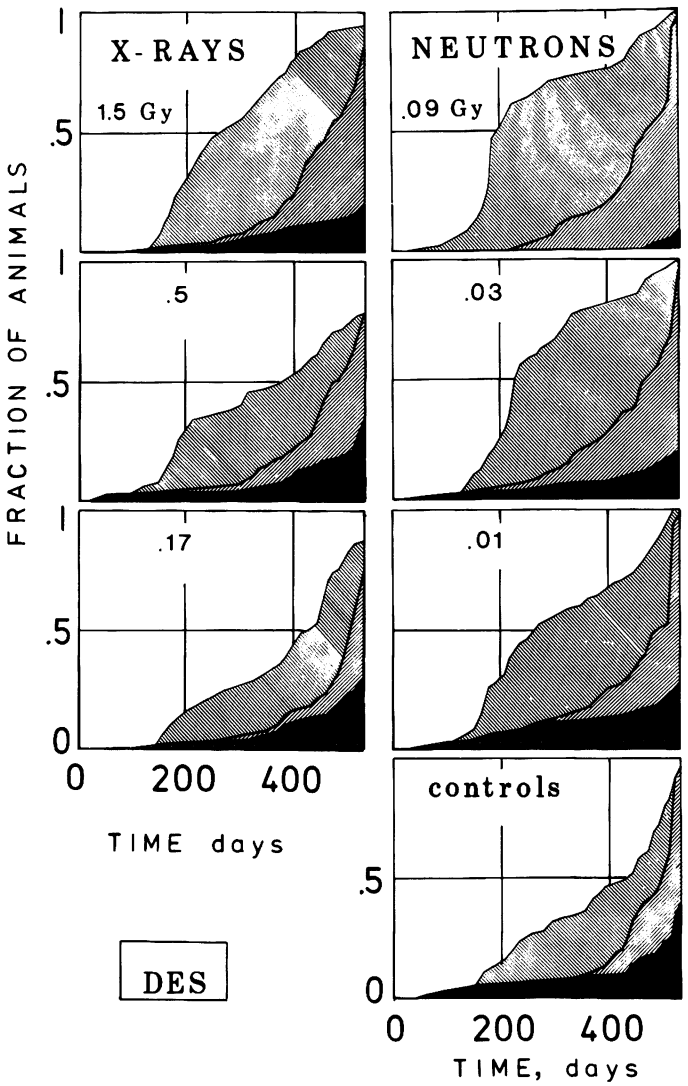
Summary of Observations

The results of this study will first be presented in elementary form; the more rigorous actuarial analysis will be given



TEXT-FIGURE 1.—Fraction of the non-DES-treated animals dead or alive with and without FA and/or AC as a function of time after irradiation. Group sizes are given in table 3. ■, Fraction of animals dead without neoplasm; ▨, fraction of animals dead with at least 1 neoplasm; ▧, fraction of animals alive with at least 1 neoplasm; □, fraction of animals alive without a neoplasm.

in a second part. Text-figure 1 represents the time course of tumor incidence and mortality of the animals not treated with DES. The heavy solid lines indicate the fraction of animals dead at the specified times. The lower black areas indicate the fraction of animals dead without neoplasms; the lower shaded areas give the fraction of animals that have died with at least 1 neoplasm. The upper shaded areas give the fraction of animals alive with at least 1 mammary neoplasm. For all doses of X-rays and neutrons, the increased incidence of neoplasms is readily evident. A substantial increase of mortality is apparent only for the highest X-ray and neutron doses. The analysis of radiation-induced life shortening cannot, however, be an objective of the present study, because the surgical procedures and the killing after multiple tumor response enhance mortality.



TEXT-FIGURE 2.—Fraction of the DES-treated animals dead or alive with and without AC, as a function of time after irradiation. Group sizes are given in table 1. ■, Fraction of animals dead without AC; ▨, fraction of animals dead with at least 1 AC; ▧, fraction of animals alive with at least 1 AC; □, fraction of animals alive without AC.

Text-figure 2 is the analogous representation for the animals treated with DES. The graphs extend over a shorter period after irradiation, since the DES-treated animals have a significantly reduced life-span. In comparison to the controls in text-figure 1, the high incidence of mammary neoplasms due to DES alone is recognizable. A considerable further increase due to the irradiations is evident for the highest X-ray dose and for all neutron doses. Mortality is not substantially increased due to the irradiations in comparison to that of the control group that is treated only with DES.

Text-figures 1 and 2 are only restricted representations of the neoplastic response. In particular, multiple mammary neoplasms in an animal are not taken into account. Furthermore, no distinction is made between the two types of neoplasms, i.e., FA and AC, that occur in the non-DES-treated animals. The DES-treated animals develop almost exclusively AC.

Table 1 lists the number of animals with specified numbers of mammary AC throughout life for the DES-treated animals. In addition, it gives the mean number of AC per animal, the fraction of animals with AC, and the total number of AC. Mean lifetimes of the animals, mean AC-free lifetimes, and mean times of the first AC and of all AC are given in table 2. The analogous data for the non-DES-treated animals are given separately for FA and AC in tables 3–5.

Different types of dose-effect relationships can be derived from the raw data in the tables. Text-figure 3 gives the conventional raw incidence versus absorbed dose or the fraction of animals that develop at least 1 mammary neoplasm throughout life. The standard errors are derived from the binomial distribution. The dose scales for the X-rays

TABLE 1.—*Mammary AC response in DES-treated ACI rats*

Absorbed radiation dose, Gy	Initial No. of animals	No. of animals with specified No. of AC throughout lifetime												Mean No. of AC/animal throughout lifetime[a]	Fraction of animals with AC	Total No. of AC
		0	1	2	3	4	5	6	7	8	9	10	>10			
Controls	63	15	6	5	5	2	3	3	4	5	4	3	8	4.8±0.5	48/63=0.76	304
Neutrons																
0.01	48	8	2	0	1	1	2	5	3	3	2	2	19	8.1±0.8	40/48=0.83	388
0.03	48	8	2	2	1	1	5	3	3	1	1	4	17	7.9±0.8	40/48=0.83	380
0.09	47	2	1	2	1	1	4	2	1	3	3	3	24	10.0±0.7	45/47=0.95	468
X-rays																
0.17	47	9	5	1	1	3	1	4	4	3	3	1	12	6.1±0.5	38/47=0.81	287
0.5	47	10	4	2	1	3	2	5	4	4	0	2	10	5.9±0.5	37/47=0.79	278
1.5	48	8	4	2	3	2	0	1	1	5	1	2	19	7.7±0.8	40/48=0.83	369

[a] Values are means ± SE.

and the neutrons in this and in the subsequent text-figures differ by a factor of 10. Data for the non-DES-treated animals indicate an RBE of neutrons somewhat larger than 10. For the DES-treated animals, RBE is substantially larger and may apparently exceed 100 at the lowest dose; however, it is difficult to infer accurate values of RBE from this crude analysis.

An RBE-versus-dose relationship for neutrons will be given at the end of this paper. The curves drawn through the data in text-figure 3 correspond to this RBE–dose relationship. The same applies to the dose–effect relationships given in subsequent text-figures. These curves will not always be the best fits to the individual data, but they are always consistent with them, and they are inserted to permit a judgment of the RBE–dose relationship of the last text-figure as an overall fit to the experimental data.

Text-figure 4 gives the mean number of mammary neoplasms per animal throughout life. The approximate coincidence of the two curves for X-rays and neutrons for the non-DES-treated animals indicates again RBE values close to 10. For the DES-treated animals the RBE appears to be well in excess of 100 at small doses.

Similar conclusions can be drawn from the values of the mean tumor-free life-span in tables 2 and 5. This will be substantiated by a competing-risk-corrected analysis in a later section.

Text-figures 3 and 4 show, for the DES experiments, not only RBE values in excess of 100 but also *sublinear* dose–effect relationships (dose exponent <1) for neutron irradiation. Similarly, high RBE values and sublinear dose–effect relations were also obtained in earlier experiments with Sprague-Dawley rats (*12, 13*). In view of the evident importance of such observations for general considerations on the effects of small doses of ionizing radiations, it is essential to submit the data to a rigorous analysis. Such an analysis corrects for differences in life-span. It also permits an evaluation of the time course of the appearance of mammary neoplasms; this is particularly important in the experiments

TABLE 2.—*Temporal mammary AC response in DES-treated ACI rats*[a]

Absorbed radiation dose, Gy	Lifetime	AC-free lifetime	Time of appearance of first AC	Time of appearance of all AC
Controls	456±13	374±18	372±19	434±6
Neutrons				
0.01	446±17	305±20	314±22	396±6
0.03	435±17	268±17	253±16	374±6
0.09	455±12	260±19	251±19	354±6
X-rays				
0.17	471±13	378±19	381±21	448±6
0.5	445±16	341±23	338±26	405±7
1.5	432±16	301±18	292±20	373±6

[a] Values are mean No. of days ± SE.

TABLE 3.—*Mammary FA response in non-DES-treated ACI rats*

Absorbed radiation dose, Gy	Initial No. of animals	No. of animals with specified No. of FA throughout lifetime								No. of FA/animal throughout lifetime[a]	Fraction of animals with FA	Total No. of FA
		0	1	2	3	4	5	6	7			
Controls	61	59	0	2	0					0.07±0.05	2/61=0.03	4
Neutrons												
0.045	46	32	11	1	1	0	1			0.46±0.14	14/46=0.30	21
0.09	48	24	11	7	5	0	1			0.94±0.17	24/48=0.50	45
0.18	48	23	12	7	3	0	1	1	1	1.10±0.23	25/48=0.52	53
0.36	48	19	11	5	6	0	4	2	1	1.63±0.29	29/48=0.60	78
X-rays												
0.37	48	43	4	0	1					0.15±0.07	5/48=0.10	7
0.75	48	29	13	5	1					0.54±0.11	19/48=0.40	26
1.5	46	27	8	4	5	1	0	1		0.89±0.20	19/46=0.41	41
3	48	29	8	9	1	0	1			0.71±0.15	19/48=0.40	34

[a] Values are means ± SE.

TABLE 4.—*Mammary neoplastic response in non-DES-treated rats*

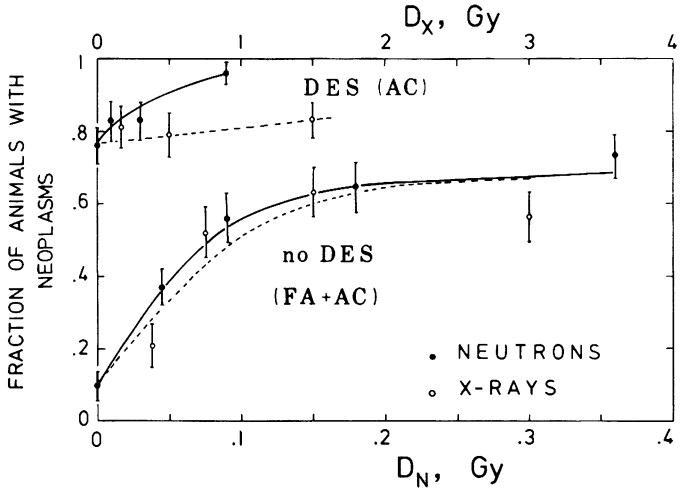
Absorbed radiation dose, Gy	Initial No. of animals	No. of animals with specified No. of AC throughout lifetime										No. of AC/animal throughout lifetime[a]	No. of AF and AC[a]	Fraction of animals with AC	Fraction of animals with AC and/or FA	Total No. of AC
		0	1	2	3	4	5	6	7	8	>8					
Controls	61	56	5	0								0.08±0.04	0.15±0.06	5/61=0.08	6/61=0.10	5
Neutrons																
0.045	46	40	4	0	1	0	0	0	0	1	0	0.33±0.18	0.78±0.26	6/46=0.13	16/46=0.35	15
0.09	48	39	4	2	1	2						0.40±0.14	1.33±0.23	9/48=0.19	27/48=0.56	19
0.18	48	32	8	2	3	3						0.69±0.17	1.79±0.30	16/48=0.33	31/48=0.65	33
0.36	48	26	8	7	1	1	2	1	0	1	1	1.31±0.24	2.94±0.42	22/48=0.46	36/48=0.75	63
X-rays																
0.37	48	42	4	1	0	0	1					0.23±0.11	0.37±0.15	6/48=0.12	10/48=0.21	11
0.75	48	38	5	2	1	1	1					0.44±0.15	0.98±0.20	10/48=0.21	25/48=0.52	21
1.5	46	25	12	6	0	1	1	0	1			0.87±0.21	1.76±0.32	21/46=0.46	29/46=0.63	40
3	48	29	10	6	1	0	0	0	1	0	1	0.90±0.25	1.60±0.32	19/48=0.40	27/48=0.56	43

[a] Values are means ± SE.

TABLE 5.—*Temporal mammary neoplastic response in non-DES-treated ACI rats*[a]

Absorbed radiation dose, Gy	Lifetime	AC-free lifetime	FA-free lifetime	Tumor-free lifetime	Time of appearance of:			Time of appearance of:		
					First AC	First FA	First tumor	All AC	All FA	All tumors
Controls	630±21	627±21	626±21	624±21	690±11	590±11	658±23	690±11	631±24	663±15
Neutrons										
0.045	655±13	644±14	619±16	614±16	603±35	572±28	566±26	615±19	587±22	599±15
0.09	629±20	617±19	560±21	586±20	574±21	534±22	533±20	602±13	567±15	577±11
0.18	580±26	501±26	533±24	472±28	429±53	572±19	494±32	523±31	616±13	580±15
0.36	590±18	496±26	500±15	444±21	426±43	507±19	437±26	509±25	576±12	546±13
X-rays										
0.37	614±25	603±25	602±25	591±25	542±36	580±60	549±34	585±27	573±43	580±23
0.75	646±20	621±20	603±19	585±19	560±29	595±15	574±15	585±22	616±14	602±12
1.5	617±24	577±23	558±23	535±22	603±18	545±24	555±18	613±12	588±15	600±10
3.	537±24	498±22	487±22	460±20	523±20	505±22	498±18	573±13	529±14	554±10

[a] Values are mean No. of days ± SE.



TEXT-FIGURE 3.—Fraction of animals with mammary neoplasms as a function of the neutron or the X-ray dose. Scales for the neutron (D_N) and the X-ray (D_X) doses differ by a factor of 10. Data are given with standard errors. *Solid lines* refer to the neutron irradiations; *dashed lines*, to the X-irradiations.

with DES where the raw incidence is always close to 100% regardless of radiation dose, and where, accordingly, dose-effect curves can be constructed only on the basis of the temporal distribution of neoplasms.

Actuarial Analysis

Estimates of Cumulative Tumor Rate and Cumulative Tumor Incidence

The same basic quantities will be utilized that have been employed in the preceding article on the induction of mammary neoplasms in the Sprague-Dawley rat (*13, 17*). 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 cumulative tumor rate $R(t)$ is defined as time integral of the tumor rate:

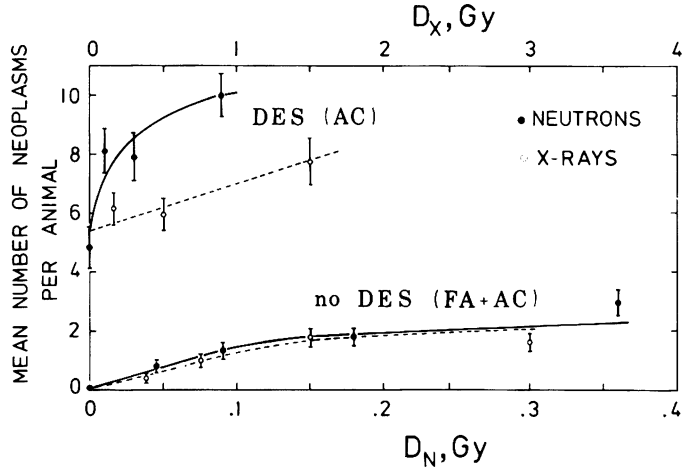
$$R(t) = \int_0^t r(t')\,dt'. \qquad [1]$$

It is difficult to estimate the tumor rate with sufficient precision, and its integral $R(t)$ is therefore a quantity more suitable to characterize the time course of the incidence of neoplasms. $R(t)$ can be considered as the expected number of neoplasms up to time t for animals that are at risk throughout the whole time period from 0 to t. However, $R(t)$ may differ from the actual mean number of neoplasms in those animals that survive up to time t; the reason is that $R(t)$ is estimated on the basis of the complete information, including tumor incidence in those animals that do not survive up to time t.

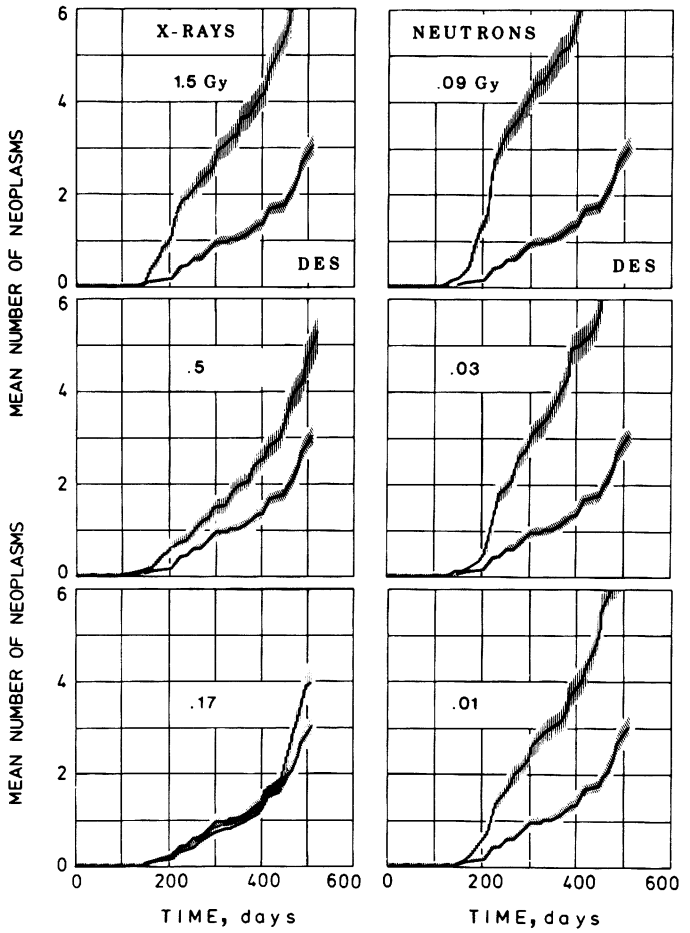
As shown in our preceding article (*13*), the cumulative tumor rate can be estimated from the formula:

$$\hat{R}(t) = \sum_i \frac{1}{N(t_i)} \pm \sqrt{\sum_i \frac{1}{N(t_i)^2}}, \qquad [2]$$

where t_i are the times of appearance of the individual neoplasms up to time t, and $N(t_i)$ are the numbers of animals still at risk at these times. The numerical values and the standard errors resulting from equation 2 are given in text-figure 5 for the DES-treated animals.



TEXT-FIGURE 4.—Mean number of mammary neoplasms as a function of the neutron or the X-ray dose. Scales for the neutron (D_N) and the X-ray (D_X) doses differ by a factor of 10. Data are given with standard errors. *Solid lines* refer to neutron irradiations: *dashed lines*, to X-irradiations.



TEXT-FIGURE 5.—Cumulative tumor rates $R(t)$ also designated as mean number of neoplasms, as a function of time after exposure to X-rays *left panels* or to neutrons *right panels* for DES-treated animals. Curves are given with their standard errors. Absorbed doses are noted in the individual *panels*. The *curve* for the unirradiated but DES-treated animals is repeated in *each panel*.

The results in text-figure 5 are obtained on the basis of all neoplasms, i.e., animals are not removed from the analysis after the occurrence of a first neoplasm but remain at risk and are not distinguished from animals that had no previous neoplasms. A comparative analysis in the subsequent section shows that the tumor rate is above average in those animals that had previous neoplasms. Therefore, estimates based on first neoplasms only will be lower than those represented in text-figure 5. Furthermore, the formula for the standard error applies strictly only to independently occurring neoplasms; the actual standard errors will therefore be somewhat larger than those indicated in text-figure 5.

A synopsis of the cumulative tumor rates at all doses both for the DES-treated and the non-DES-treated animals is given in text-figure 6. The spontaneous incidence of mammary neoplasms is very low in non-DES-treated animals, and the incidence is greatly enhanced in DES-treated animals. All radiation doses except the lowest X-ray dose enhance markedly the incidence of mammary neoplasms in DES-treated animals.

The most striking observation is the very substantial enhancement of tumor incidence due to 0.01 Gy (1 rad) of neutrons in DES-treated animals. Even without further detailed analysis, it is evident that the dose dependence for neutron irradiation of DES-treated animals is sublinear; i.e., it corresponds to a dose exponent <1. This will be substantiated in the section dealing with dose-effect relationships.

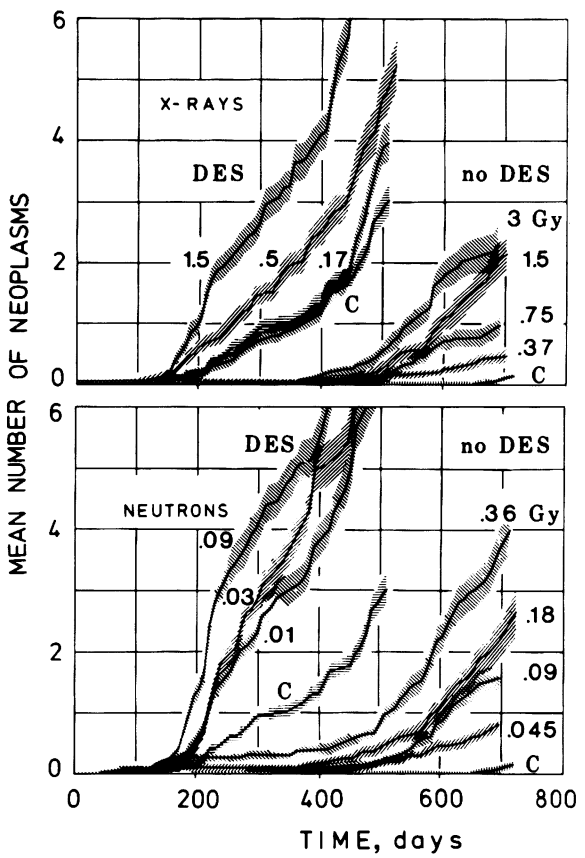
The cumulative tumor rates are not given separately for the two types of neoplasms in DES-treated animals. The absolute numbers of these neoplasms are too small to permit the identification of possible differences in the time dependence for the two types of neoplasms. Instead of the cumulative tumor rate $R(t)$, one can also derive the cumulative incidence $I(t)$, i.e., the mortality-corrected probability per animal to have at least 1 tumor. This quantity is derived by the Kaplan-Meier product limit estimate (*18*):

$$\hat{I}(t) = 1 - \prod_i \left[1 - \frac{1}{N(t_i)}\right] \pm [1 - \hat{I}(t)] \cdot \sqrt{\sum_i \frac{1}{N(t_i)^2}}. \qquad [3]$$

One may note that very nearly the same numerical values are obtained from the sum limit estimate (*see* equation 2):

$$\hat{I}(t) = 1 - \exp\left[-\sum_i \frac{1}{N(t_i)}\right]. \qquad [4]$$

In equations 3 and 4 the numbers $N(t_i)$ of animals at risk include only those animals that have not incurred an earlier



TEXT-FIGURE 6.—Cumulative tumor rates $R(t)$ also designated as mean number of neoplasms, as a function of time after exposure to X-rays *upper panel* or to neutrons *lower panel*. Curves and their standard errors are given both for DES-treated and non-DES-treated animals. Absorbed doses are noted on the *curves*. *C* designates controls, i.e., unirradiated animals. For non-DES-treated animals, both FA and AC are included; for DES-treated animals, only AC are included.

neoplasm. If all neoplasms were statistically independent, and if one were to deal with an entirely homogeneous population, one could include all animals and would then obtain somewhat better statistical accuracy, and, in particular, one would then have the relation:

$$I(t) = 1 - \exp[-R(t)]. \quad [5]$$

However, it will be seen in the subsequent section that these conditions are not fulfilled in the present experiment. $I(t)$ is therefore determined from equation 3 by considering only those animals that had no earlier neoplasms.

Text-figures 7 and 8 represent the time course of the cumulative incidence $I(t)$ for non-DES-treated and for DES-treated animals. In both text-figures the control curve is repeated in each panel.

The difference between the estimates of $R(t)$ from all neoplasms and first neoplasms only will be considered next.

Difference of Cumulative Tumor Rates Obtained From All Neoplasms, From First Neoplasms per Quadrant, and From First Neoplasm per Animal

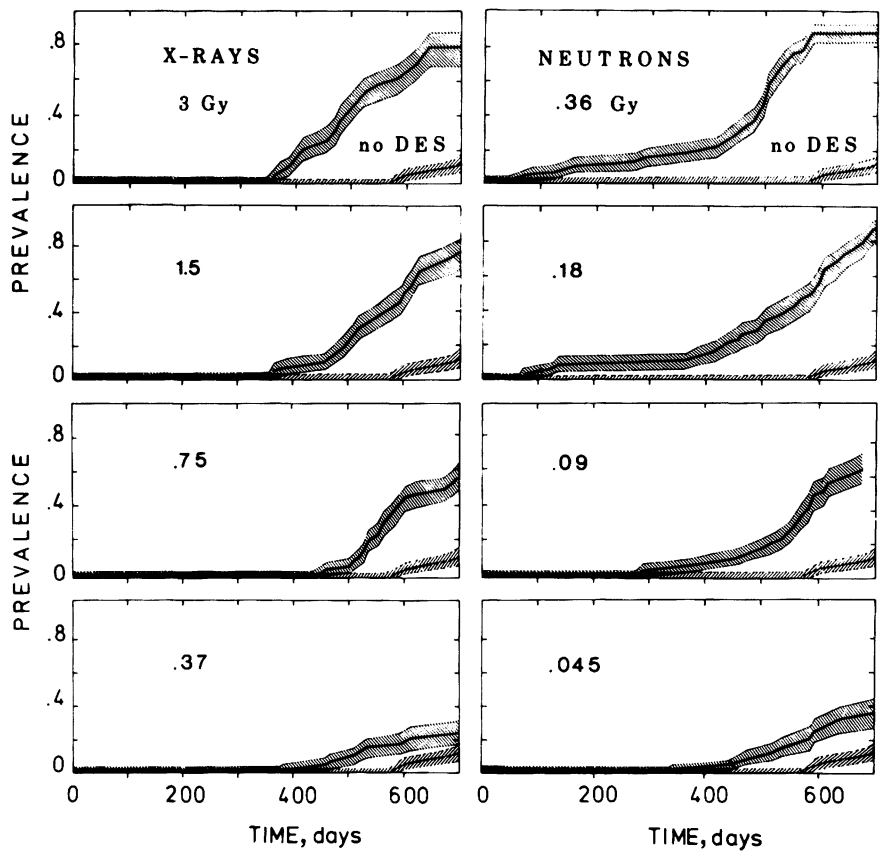
Text-figure 9 gives the estimates of the cumulative tumor rates for DES-treated animals that are obtained if all mammary neoplasms, if only the first neoplasms per quadrant of the mammary tissue, or if only the first mammary neoplasms per animal are utilized in the analysis. As stated, one should obtain essentially the same estimates if the tumor rate in the animals, or in the quadrants with previous neoplasms, were equal to the tumor rate without previous neoplasms. The difference would merely be that taking all neoplasms into account would result in a somewhat better statistical accuracy. In reality, the estimates are considerably higher if all neoplasms are included, i.e., if the animals with previous neoplasms are retained in the analysis. The estimates are much lower if only the first neoplasm per animal is taken into account, i.e., if an animal is removed from the analysis as soon as a first neoplasm has occurred. If only first neoplasms per quadrant are considered, one obtains intermediate values of the cumulative tumor rate. In this latter type of analysis, an animal is taken to be *partially at risk* according to the fraction of quadrants still unaffected.

The substantial differences in the estimates of $R(t)$ in the DES experiments indicate large differences of the tumor rates between animals with and without previous neoplasms. A similar but less substantial effect has been found in the earlier experiment with the Sprague-Dawley rats (*13*). It has also been pointed out earlier that the effect could either be due to an increase of the tumor rate after the incidence of neoplasms or, and this may be more likely, to inherent differences in sensitivity within the strain.

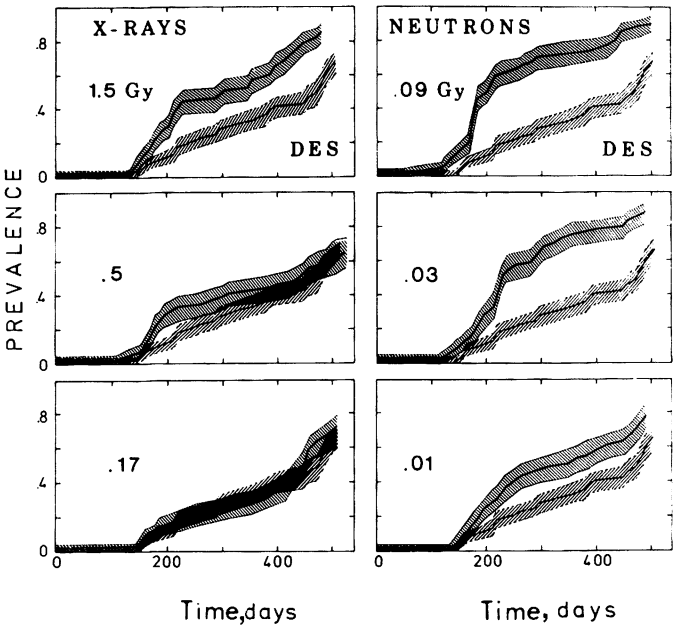
Text-figure 10 shows that there is also a certain decrease of the cumulative tumor rate in non-DES-treated animals if first neoplasms only are considered. However, the difference is smaller, and, in view of the modest size of the effect, only the curves for all neoplasms and for the first neoplasm per animal are plotted.

Dose-Effect and RBE-Dose Relationships

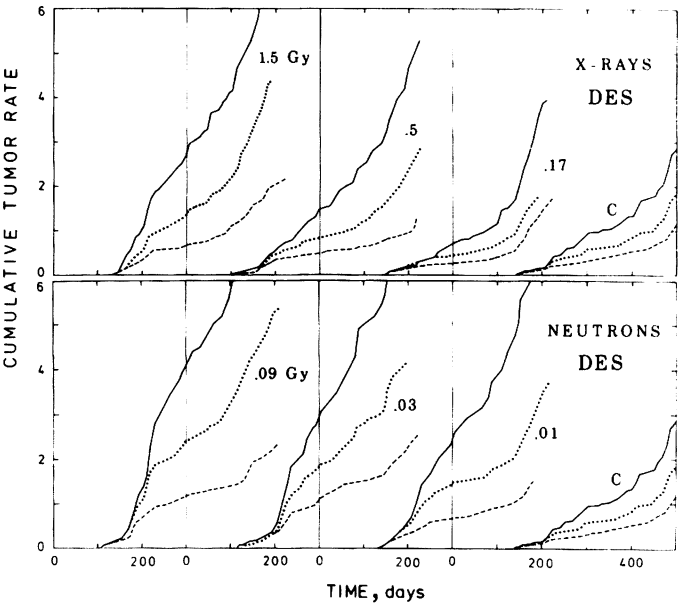
The results from the actuarial analysis can be used in several ways for the construction of dose-effect relations; it



TEXT-FIGURE 7.—Cumulative incidences $I(t)$ and their standard errors as a function of time after exposure to X-rays *left panels* or to neutrons *right panels* for non-DES-treated animals. Results are based both on AC and FA. Absorbed doses are given in the individual *panels*. The *curve* for the controls is repeated in *each panel*.



TEXT-FIGURE 8.—Cumulative incidences $I(t)$ and their standard errors as a function of time after exposure to X-rays *left panels* or to neutrons *right panels* for DES-treated animals. Results are based on AC only. Absorbed doses are given in the individual *panels*. The *curve* for the unirradiated, but DES-treated controls is repeated in *each panel*.



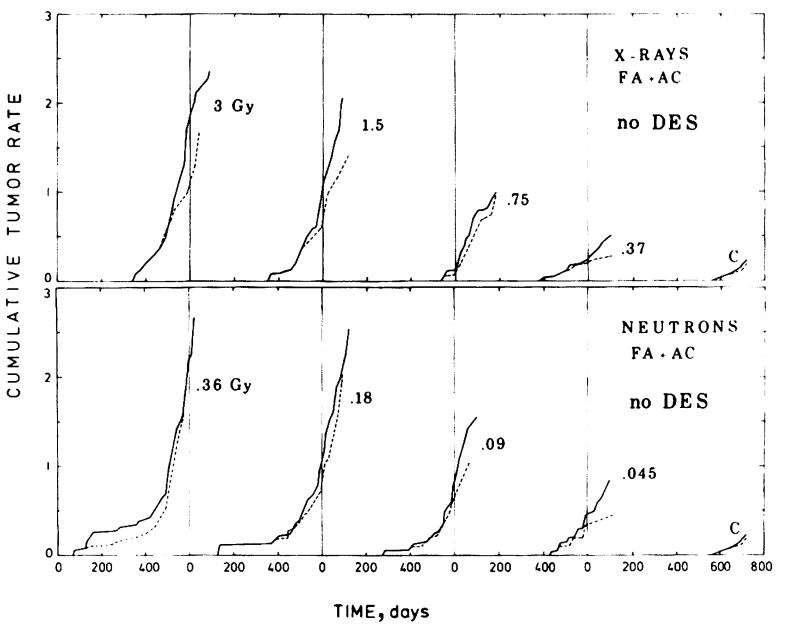
TEXT-FIGURE 9.—Different estimates of the cumulative tumor rate $R(t)$ for DES-treated animals as a function of time after exposure to X-rays *upper panel* and to neutrons *lower panel*. *Solid lines* are obtained on the basis of all AC, *dotted lines* on the basis of first AC in a quadrant, and *dashed lines* on the basis of the first AC in an animal. Absorbed doses are noted on the *curves*; *C* designates unirradiated but DES-treated animals.

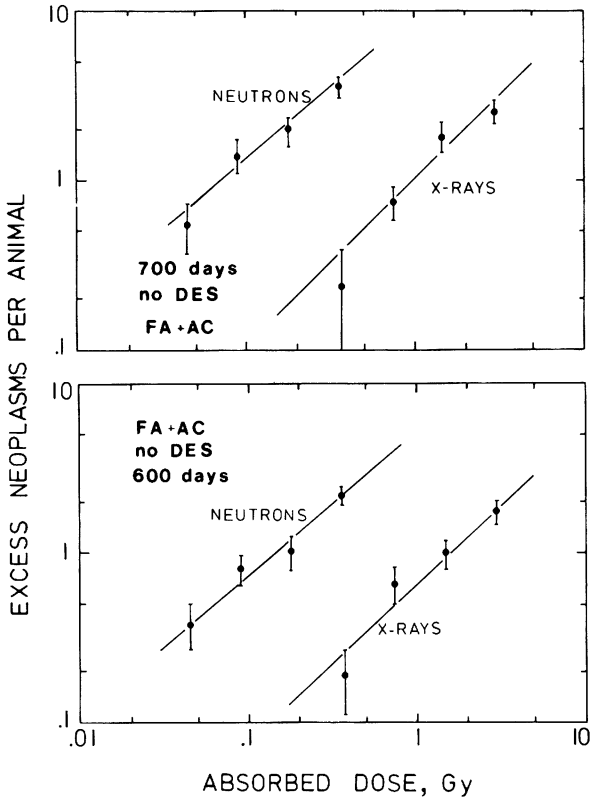
is of particular interest to examine these possibilities and to assess the consistency of their conclusions as well as those from text-figures 3 and 4 that had been based on the conventional analysis.

In the earlier experiment with Sprague-Dawley rats, it has been found that the results can adequately be described in terms of a radiation-induced forward shift in time in the occurrence of tumors. In the present experiment no such conclusion can be drawn for the non-DES-treated animals because the spontaneous incidence is too low to permit a comparison to the time course of the radiation-induced incidence. Alternatively, the neoplasms appear so early in the DES-treated animals that there can be only limited further shortening of the latent period and, accordingly, any simple linear relationship appears unlikely. The construction of dose–effect curves must therefore be based on other considerations.

One relatively simple way to construct dose–effect curves is the determination of the increment of the cumulative tumor rate at specified times after irradiation. The disadvantage of the method is that there are no obvious criteria for the proper choice of the reference time. In text-figure 11 the resulting dose–effect relationships are given for non-

TEXT-FIGURE 10.—Different estimates of the cumulative tumor rate $R(t)$ for non-DES-treated animals as a function of time after exposure to X-rays *upper panel* or to neutrons *lower panel*. Both FA and AC are taken into account. *Solid lines* are obtained on the basis of all mammary neoplasms; *dashed lines*, on the basis of first neoplasms in an animal. Absorbed doses are noted on the curves; *C* designates unirradiated animals.





TEXT-FIGURE 11.—Excess number of mammary neoplasms in non-DES-treated animals, i.e., increase of the cumulative tumor rate $R(t)$ over the control level, at 600 *lower panel* and at 700 days *upper panel* after irradiation. Data are given with their standard errors.

DES-treated animals at two different times after irradiation (600 and 700 days). An analogous plot is given in text-figure 12 for DES-treated animals; in keeping with the shorter lifespans, shorter reference times, 300 and 500 days, are chosen. Consistent with the results of the conventional analysis, the RBE appears to exceed 100 at the lowest neutron dose in the DES experiment.

It would be tempting to investigate separately the dose-effect relationships and the RBE for FA and AC in the experiments without DES. Text-figure 13 gives such a separate analysis that appears to indicate somewhat larger RBE values for the FA; however, the absolute numbers are too small to establish a confirmed difference.

Dose-effect curves also can be constructed in a somewhat more general way, and two possibilities will be considered. The first possibility utilizes $R(t)$ and a quantity that has been termed *effect period* Θ (*12, 17*):

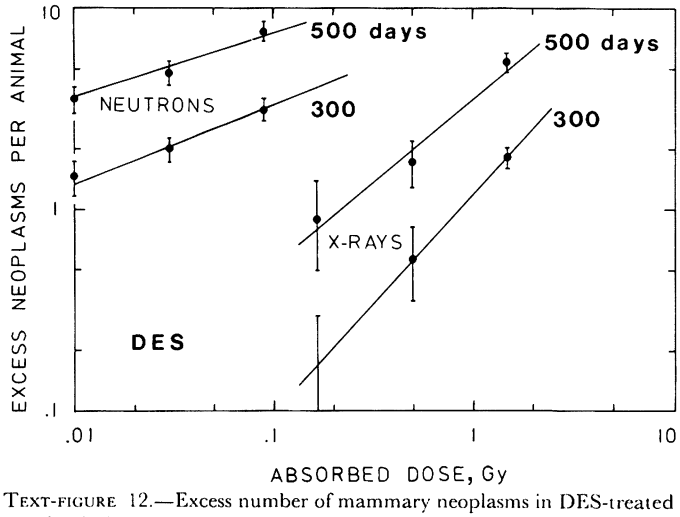
$$\Theta = \int_0^{t_{max}} R(t)\,dt. \qquad [6]$$

In this definition t_{max} is the maximum time for which $R(t)$ is obtained in the experiment. The quantity Θ can then be understood as the expected time with neoplasms for an animal that is at risk up to t_{max}; multiple neoplasms are counted with a corresponding factor so that Θ can ultimately, at high doses, exceed the period of observation.

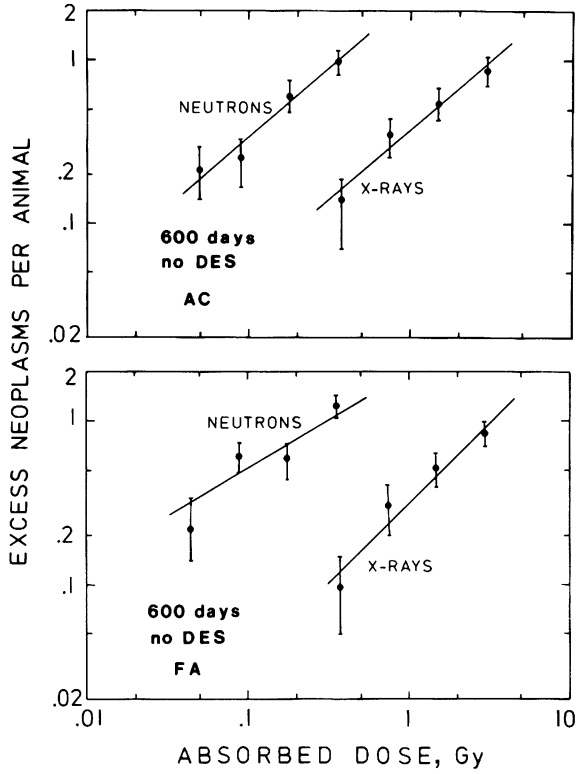
The quantity Θ is estimated by

$$\hat{\Theta} = \sum_i \frac{1}{N(t_i)}(t_{max}-t_i) \pm \sqrt{\sum_i \frac{1}{N(t_i)^2}(t_{max}-t_i)^2}. \qquad [7]$$

The summation extends over all $t_i < t_{max}$, and t_i and $N(t_i)$ have the same meaning as in equation 2. Text-figure 14 gives a plot of $\hat{\Theta}$ as a function of dose. t_{max} has been taken as 500 days in the DES experiment and as 700 days in the experiment without DES.



TEXT-FIGURE 12.—Excess number of mammary neoplasms in DES-treated animals, i.e., increase of the cumulative tumor rate $R(t)$ over the control level at 300 and 500 days after irradiation. Data are given with their standard errors.



TEXT-FIGURE 13.—Excess number of mammary neoplasms in non-DES-treated animals, i.e., increase of the cumulative tumor rate $R(t)$ over the control level, separately for FA *lower panel* and AC *upper panel* at 600 days after irradiation. Data are given with their standard errors.

An alternative construction of a dose-effect plot is based on a quantity that is similar to Θ but differs from it in depending on first neoplasms only. Hoel and Walburg (*19*) have defined a quantity

$$\mu = \int_0^\infty [1 - I(t)/I(\infty)]dt \qquad [8]$$

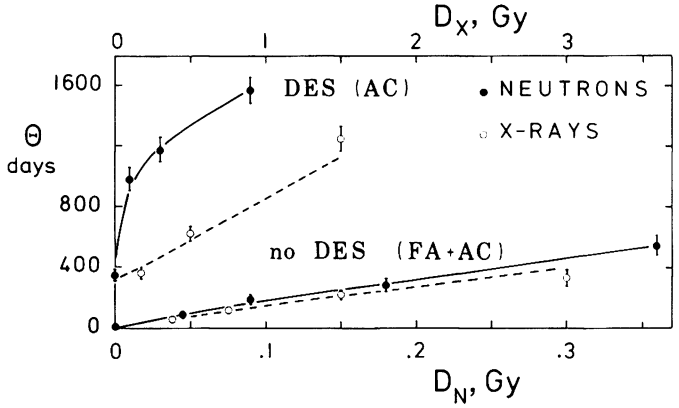
that is frequently applied in studies of life shortening, i.e., in the analysis of lethal diseases (*20, 21*). In these studies it is called "mean age at death," and $I(t)$ is either the overall cumulative mortality or the cumulative mortality due to a particular risk. However, it is evident that the quantity proposed by Hoel and Walburg can also be applied in the analysis of nonlethal diseases. In the present case $I(t)$ is the cumulative incidence of mammary neoplasms (*see* equation 5), $I(\infty)$ is set equal to 1 and, as in the determination of Θ, the integration is terminated at t_{max}=500 or 700 days. The formula for the estimation of μ is then:

$$\hat{\mu} = \Sigma[\hat{I}(t_i) - \hat{I}(t_{i-1})] \cdot t_i \pm \sqrt{\{\Sigma t_i^2[-\hat{I}(t_i)]/N(t_i) - \hat{\mu}^2\}/(n-1)} \qquad [9]$$

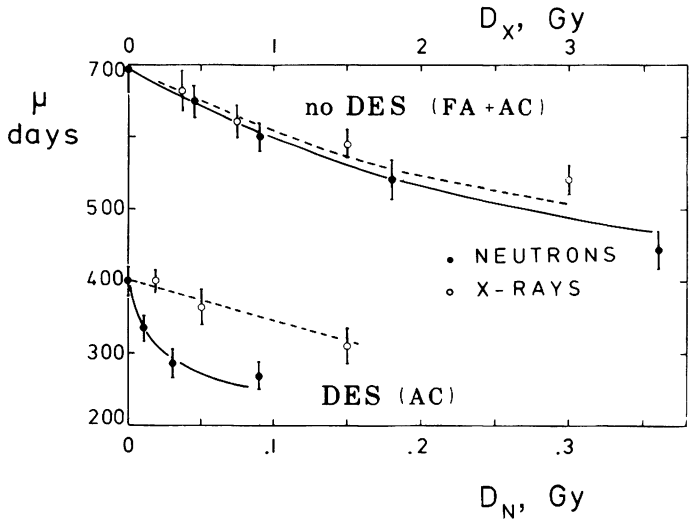
(summation over all t_i up to t_{max}; n is the number of animals with neoplasm). The standard error in equation 9 corresponds to the formula given by Hoel and Walburg (*19*).

The symbol μ stands for a competing-risk-corrected mean time without mammary neoplasm for the animals. The influence of the correction is substantial, as seen from a comparison of text-figure 15 with the mean tumor-free lifetimes in tables 2 and 5. The dose dependence of μ in text-figure 15 is yet another confirmation of the sublinearity in the DES experiment at small neutron doses; it also confirms the high RBE values in excess of 100 for the DES experiment and close to 10 for the experiment without DES.

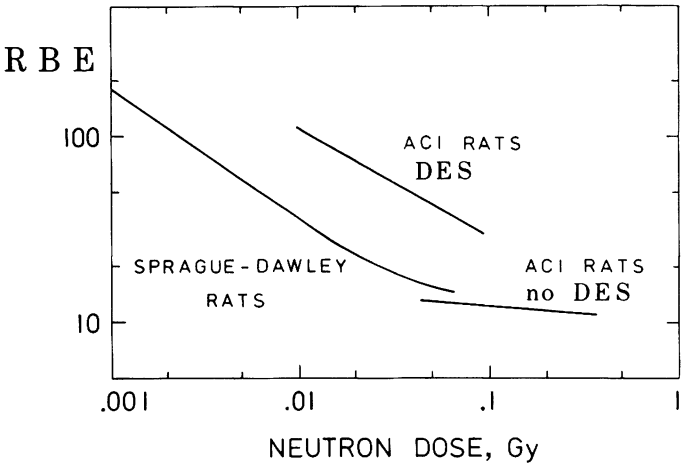
Text-figure 16 gives, both for the experiments with and without DES, the RBE of neutron irradiation as a function of dose. These curves have been derived from the mortality-corrected results, i.e., from text-figures 11, 12, 14, and 15. The curves inserted in text-figures 3 and 4 are in agreement with these RBE-dose relationships. The RBE-dose relationship from the earlier experiment with Sprague-Dawley rats (*13*) is inserted for comparison.



TEXT-FIGURE 14.—*Effect period* Θ and its standard error (*see* equation 7) for DES-treated and non-DES-treated animals. Scales for the neutron (D_N) and the X-ray (D_X) doses differ by a factor of 10. *Solid lines* refer to neutron irradiations; *dashed lines*, to X-irradiations.



TEXT-FIGURE 15.—Mean time without tumor and its standard error (*see* equation 9) for DES-treated and non-DES-treated animals. Scales for the neutron (D_N) and X-ray (D_X) doses differ by a factor of 10. *Solid lines* refer to neutron irradiations; *dashed lines*, to X-irradiations.



TEXT-FIGURE 16.—Dependence of the RBE of 430-keV neutrons on dose for the induction of mammary neoplasms. Results for the Sprague-Dawley rats are from an earlier study (*13*) (rats were not treated with DES). Results for ACI rats are from the present investigation. Line segments for ACI rats correspond to the curves in text-figs. 3, 4, 11, 12, 14, and 15.

DISCUSSION

Previous experiments (*12, 13*) with female Sprague-Dawley rats had led to the conclusion that the RBE of neutron irradiation for the induction of mammary neoplasms exceeds 100 at an absorbed dose of 1 mGy (0.1 rad). In agreement with earlier microdosimetric considerations (*6*), it had also been found that the RBE of neutron irradiation increases in inverse proportion to the square root of the neutron dose. A further finding was a strong sublinearity (dose exponent ≈0.5) of the dose-effect relationship at small neutron doses. In view of the potential implications of such findings for radiation protection, it seemed desirable to inquire whether similar results are obtained in other strains. The present experiment has been designed for this purpose.

The incidence of mammary neoplasms in control female ACI rats is low. However, the mammary carcinogenic response of non-DES-treated ACI rats to irradiation is relatively small, thus requiring relatively high doses of X-rays and neutrons to produce incidence values large enough to be analyzed statistically. In this part of the experiment, RBE values of neutron irradiation somewhat in excess of 10 are found for the FA and AC, which is in agreement with the values for Sprague-Dawley rats at comparable doses. Experiments with considerably larger numbers of animals would be required to determine the RBE of neutrons at smaller doses.

In ACI rats treated with DES, a large number of early mammary AC is found. With neutron doses as low as 0.01 Gy (1 rad), this incidence is further substantially enhanced. The RBE of neutrons at 0.01 Gy is in excess of 100, and this is even larger than the corresponding value for Sprague-Dawley rats at this neutron dose.

A similar experiment designed with three rat strains (Sprague-Dawley, Wistar WAG/Rij, and Brown Norway) is currently under way at the Toegepast Natuurwetenschappelijk Onderzock in The Netherlands. Interim results (*22, 23*) of this investigation indicate RBE values from 8 to 25 for neutrons of 0.5 MeV energy at somewhat higher doses than in our study; they also point at considerable differences in susceptibility between the three strains. Quantitative comparisons with the results of the present experiment will have to await completion of the experiment.

The high RBE for mammary tumor carcinogenesis in rats are in line with earlier findings of high neutron RBE in other biological systems. They are also consistent with predictions based on microdosimetric data and support the proposal that the quality factor of 10 presently used for neutrons in radiation protection may need to be reconsidered (*10*).

The observed nonlinear dose-effect relationship for neutrons has an even greater potential impact on radiation protection. At doses below 0.01 Gy of sparsely ionizing radiations, a mammalian cell is traversed by a substantial number of electrons. At comparable doses, the number of cells traversed by any neutron recoil is small. Accordingly, one should expect dose dependence diagrams with positive curvature for sparsely ionizing radiations and linear dose-effect relationships for neutrons; in the dose range where there is a quadratic dependence on X-ray dose and a linear dependence on neutron dose, the neutron RBE has to be inversely proportional to the square root of the neutron dose. In the present experiment, as with the earlier findings on Sprague-Dawley rats, the dose-effect relationships are not of the expected shape; they are not recognizably different from a linear relationship for X-rays and have negative curvature for neutrons. Nevertheless, one obtains the familiar dependence of the RBE of neutrons on the square root of the neutron dose. It appears, therefore, that the characteristic difference between the densely ionizing neutron radiation and the sparsely ionizing X-rays is preserved but that a multicellular reaction or a tissue factor causes a similar distortion for both dose-effect relationships. It must be concluded, that the dose-effect relationships for radiation tumorigenesis are not as yet fully understood, whereas the RBE-dose dependence reflects known biophysical principles.

It is evident that data obtained in animal experiments cannot provide numerical risk estimates for humans. However, the high values of RBE and the negative curvature of the dose-effect relationship at small neutron doses are of sufficient concern to necessitate further radiation carcinogenesis studies with low doses of neutrons.

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