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RAT MAMMARY CARCINOGENESIS FOLLOWING NEUTRON- OR X-RADIATION *

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INTRODUCTION

Both the United Nations (1) and the United States National Academy of Sciences - National Research Council (2) have reviewed recently the relative biological effectiveness (RBE) of neutrons for the induction of mammary neoplasia in the female rat. The UN report, page 387, states "Mammary tumors in Sprague-Dawley rats have also been studied extensively. These data are more difficult to interpret since most of the x- and gamma-ray data come from one laboratory and the data for fission neutrons from another. In either case the RBE approaches a value of one between 350 and 400 rads of x rays and increases with decreasing dose." The NAS-NRC report, page 141, states, "Experimental studies with rats have shown an RBE of approximately 2 for fast neutrons, for the induction of mammary gland tumors following exposure at relatively high doses. The RBE value for exposure at lower doses is higher, approximately 10 to 20."

Because the relationship between dose and RBE is not well known for radiation-induced mammary neoplasia of the rat, a study was begun to compare the incidence of mammary neoplasia, in a single experiment, over the entire life-span of rats irradiated with either x-rays or neutrons. The purpose of this communication is to present interim results up to 14 months after exposure.

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 ($72^{\circ} \pm 3^{\circ}$ F) and humidity controlled ($55\% \pm 10\%$) animal rooms under conditions of 12 hours of floures-

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cent light per day. When they were 61-63 days old they were exposed to either neutron-radiation, x-radiation or sham-irradiation. Five rats were then assigned to a single cage on a random basis independently of treatment. Each rat has been examined frequently, and when breast tumors were noted by palpation, the tumors have been removed surgically under ether anesthesia and the rat then returned to the experiment. No animal has been killed unless death appeared imminent. Individual records are kept for each rat, and the time of appearance and the anatomical location of the breast tumors are recorded. Those mammary neoplasms which occurred at different locations in the same animal were considered to be separate neoplasms. If mammary neoplasms were noted at closely related anatomical sites, they were considered to be different neoplasms only if they were of a different pathologic type or, if the same pathologic type, if at least six months had elapsed between removal of the first neoplasm and detection of the second neoplasm. The mammary neoplasms were given a pathologic classification of either adenocarcinoma, or a combined classification of adenofibroma-fibroadenoma, according to the criteria published previously (3).

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 5 cm diameter cylinder 17.5 cm long, made of 0.3 cm thick lucite and weighted so that the rat remained in its normal, horizontal, standing position. A Van de Graaff generator was operated to bombard the target with 2.43 MeV protons to produce neutrons with an energy of 0.43 MeV at the mid-point of each cylinder. Calculations of kinematic conditions indicated an energy spread over the extent of the rat of +28% to -14% of the mid-point energy. All rats were reversed in their cylinders at mid-dose to reduce nonuniformities in the energy and absorbed dose distributions. The mean tissue kerma in free air was measured at the rat locations by integrating the response of a rat-sized, homogeneous tissue equivalent ionization chamber of minimal mass placed in the 24th cylinder. A compensated Geiger-Muller dosimeter indicated gamma-ray kermas 3.5% of the total kermas. Depth-dose measurements have been made to determine the ratio of absorbed dose to neutron-kerma. For this purpose a $\frac{1}{2}$ inch spherical tissue equivalent multiplication chamber was inserted into a nylon rat phantom at 4 radial depths. Subject to additional calculations, the absorbed dose to neutron-kerma ratio varies between 0.75 and 0.88 over the entire presumed volume of the mammary tissue of the rat. We have chosen, for the purpose of this report, to use a factor of 0.8 to convert neutron-kerma to rad. The neutron kerma-rate was approximately 4 rads per hour. The rats were exposed to total kermas of 0.125, 0.50, 2.0, or 8.0 which is considered to be the equivalent of 0.10, 0.40, 1.6, or 6.4 rads.

X-ray exposures were accomplished by using a conventional, therapy x-ray machine. Exposure conditions were: target to specimen distance=100 cm; 0.5 mm Cu + 1.0 mm Al; maximum tissue-like backscatter; 250 kVp; dose rate=30 R per minute measured with a 100 R Victoreen chamber at the dorsal-ventral midpoint of the rats. The rats were exposed to either 30, 60, or 90 R. These values were converted, by multiplying by a factor of 0.94 to 28, 56 or 85 rads.

RESULTS and DISCUSSION

There was little mortality during the 14 month follow-up period except in the groups that received either 6.4 rads of neutrons or 85 rads of x-rays where the mortality reached 20.0% and 13.3% respectively (Table I). The number of rats with mammary neoplasia, the number of mammary neoplasms, the number of mammary neoplasms, and the number of mammary adenocarcinomas is given for each group, month by month, as well as the percent incidence of rats with mammary neoplasia at 14 months in Table I. The percent of rats with mammary neoplasia at the end of 1 thru 14 months has been plotted in Fig. 1. Similarly, tumors per rat, that is, the number of mammary neoplasms divided by the initial or starting number of rats, has been plotted in Fig. 2. Inspection of these data indicate that a dose of 5.4 neutron rads was followed by a mammary neoplastic response that was similar to the response following 85 rads of x-rays. However, 0.1 or 0.4 rads of neutrons was followed by a mammary neoplastic response that was similar to the response following 28 rads of x-rays. Thus, it is suggested that 0.43 MeV neutrons show a much higher RBE at lower doses than at higher doses.

The curves in Figs. 1 and 2 are not corrected for mortality. This leads to no serious errors because mortality during the first 14 months has been insignificant in all but two groups. As the experiment continues, mortality will, however, be more extensive, and accurate analysis as well as rigorous definition of the quantities of interest are therefore essential.

The mean number of tumors per animal at time t after irradiation will in the following be designated by $R(t)$. The probability of an animal to have at least one tumor, i.e., the cumulative incidence, is related to $R(t)$ by the equation

$$I(t) = 1 - e^{-R(t)} \quad (1)$$

From this equation it follows that the cumulative incidence, $I(t)$, and the mean number, $R(t)$, of tumors per animal have nearly the same value whenever the two quantities are small as compared to 1; this can also be seen from a comparison of Figs. 1 and 2. A third quantity of interest is the tumor rate, $r(t)$, i.e., the probability for the development of a tumor per unit time and per animal. The tumor rate is the time derivative of the mean number of tumors per animal:

$$r(t) = \frac{d R(t)}{dt} \quad (2)$$

and accordingly $R(t)$ is the time integral or the tumor rate:

$$R(t) = \int_0^t r(\tau) d\tau \quad (3)$$

$R(t)$ can therefore be estimated from the observed incidence in a finite sample of irradiated animals as:

$$R(t) = \sum_{i=1}^n \frac{1}{N(t_i)} \quad (4)$$

where t_i are the times of incidence of the n observed tumors, and $N(t_i)$ are the numbers of animals still under observation at these times. A more detailed discussion of the estimation of $I(t)$ and $R(t)$ can be found in references 4-6.

The cumulative incidence, $I(t)$, is determined by the resulting value of $R(t)$ according to 1. A condition for the validity of this equation is that all scored tumors are primary tumors, that is, that the occurrence of a tumor neither increases nor decreases the probability of additional tumors being found in the same rat. In order to avoid this assumption concerning the statistical independence of multiple tumors, one can derive the quantities $I(t)$ and $R(t)$ by only scoring first tumors and by excluding all animals with a second tumor from further consideration. Both types of analyses have been performed and no systematic differences in the resulting values have been found. The assumption that one deals with independent primary tumors is therefore valid, and the complete analysis based on all observed tumors is preferable because it leads to slightly smaller statistical inaccuracies.

The quantity $R(t)$ is plotted in Figs. 3 and 4 for the different experimental groups. A comparison with Fig. 2 shows that the results are nearly identical except at the highest doses. The logarithmic scale has been chosen in order to obtain a clearer representation of the tumor frequency at small doses. One should note that the abscissa scale in Figs. 3 and 4 is twice as wide as the ordinate scale. A straight line of slope 1 corresponds therefore to the equation:

$$R(t) = k t^2 \quad (5)$$

and accordingly to a linear increase of the tumor rate with time after irradiation:

$$r(t) = 2k t^2 \quad (6)$$

It appears that the results are not inconsistent with such a dependence, but an exact analysis will have to be based on more complete results as the observations continue.

Standard deviations are not given in Figs. 3 and 4, but approximate values of the standard deviations of $R(t)$ are obtained by the equation:

$$\sigma = R(t) / \sqrt{n(t)} \quad (7)$$

where $n(t)$ is the cumulative number of tumors at time t as given in Table I.

There are various ways to deduce dose-effect relations from the observed time dependence of tumor incidence. An obvious way is to plot the cumulative incidence or the mean number of tumors per rat at a given time as a function of dose. Such a procedure has, however, the disadvantage that it utilizes only part of the experimental information, and it is therefore sensitive to statistical fluctuations. One can avoid this by plotting the time integral of $R(t)$ as a function of dose. In doing this one effectively averages over the whole observation period. The procedure is also justified because tumors are then weighed according to their time of incidence. The integral:

$$P(t) = \int_0^t R(\tau) d\tau \quad (8)$$

can be considered as an "effect period", namely the mean number of tumors per rat multiplied by the number of days the rat is bearing these tumors. The "effect period" minus its control value, in units of "tumor-days" is plotted in Fig. 5 as a function of neutron and x-ray dose. One finds that, in agreement with an earlier analysis (7) the dose-effect relation for neutrons has a slope considerably less than 1, that is, the effect of neutron irradiation is proportional to a power of absorbed dose less than 1. The dose-effect curve for x-rays is considerably steeper, and it appears that the RBE of neutrons approaches values near 100 at low doses while at 6.4 rad of neutrons it is closer to 10.

A separate statistical assessment of the dose dependence of the RBE of neutrons has been performed by comparing the effect of each x-ray dose with that of each neutron dose. These comparisons have been based on a statistical evaluation of the full time dependence of tumor incidence in the groups of animals which are compared. Details of this approach have been described earlier (8, 9). One finding of particular interest is that the effect of 0.1 rad of neutrons is larger on a 95% level of statistical significance than the control levels and that it also exceeds on the same level of statistical significance the earlier observed effect (10) of 15 rad of gamma rays. Because only a limited number of x-ray doses is applied in the present experiments, the earlier data obtained with gamma rays are also utilized in the statistical analysis. The results are represented in Fig. 6. In this representation the vertical bars cover those ranges of RBE of neutrons which are excluded on a level of statistical significance exceeding 95%. The solid curve of slope -0.5 is the best estimate of the dependence of neutron RBE on dose. This relation is in agreement with the dose-RBE dependence observed in a large number of higher organisms (11). As in the case of lens opacification by 0.43 MeV neutrons (8) and in the induction of YG_2 - mutations by fission neutrons (12) the RBE at low doses approaches values exceeding 100.

The observation that the effect of neutron irradiation increases with a power of absorbed dose which is less than 1 has been discussed earlier (7), and it has been concluded from this observation that the tumor induction can not be solely determined by lesions in individual cells. It is necessary to postulate interaction between a number of affected cells. It is significant that in this system the same dose-RBE relation is found which has been postulated on general biophysical grounds for all radiation effects on higher organisms. The finding is also of interest because the induction of mammary tumors in the Sprague-Dawley rat presents a special case inasmuch as the spontaneous incidence becomes very large as the age of

the rats increases. The mammary neoplastic response to neutrons and to x-rays in Sprague-Dawley rats can therefore not be used to predict the radiation induction of other tumors in other systems. However, the current observations confirm that even in a complicated system the dose-RBE dependence agrees with the dependence predicted by the theory of dual radiation action (11).

The data here reported are in general agreement with the reports of Vogel (13-15) although there are some important differences in experimental techniques. Some of these differences, and their importance, include the following. The current data are based on 0.43 MeV neutrons, which would be expected to be more efficient biologically than the fission neutrons used by Vogel. The current data comes from animals that had their tumors removed while Vogel allows the tumor to remain in place. Tumor removal should allow for more tumors to be reported because a tumor left in place may "overgrow" subsequently arising tumors and may tend to kill the tumor bearing rat sooner than if the tumor were removed. In the present experiment rats that were born on the same day, were from the same shipment, and were housed together, were used. Vogel, on the other hand, began his studies with rather large neutron doses, and over a period of years, added additional neutron doses and x-ray controls. Since Jull (16) has shown that mammary tumor incidence may be changed significantly by housing conditions, we tend to place more confidence in data collected from a single experiment than from experiments that were done at different times and under different housing conditions.

SUMMARY

Sprague-Dawley female rats, 61-63 days of age, were given a single dose of either 0.10, 0.40, 1.6, or 6.4 rads of 0.43 MeV neutrons, or 28, 56, or 85 rads of 250 kVp x-rays, or no radiation in a single experiment. The interim results, either in terms of percent of rats with mammary neoplasia or mean number of mammary neoplasms per rat, at the end of 14 months after irradiation, indicate that 0.10-0.40 neutron rads was followed by a mammary neoplastic response much like that following 28 x-ray rads. The mammary neoplastic response to either 6.4 neutron rads or 56-85 x-ray rads was similar. Thus the RBE for neutrons for the induction of mammary neoplasia in the Sprague-Dawley rat is much larger at low neutron doses than at high doses.

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Table I. Mortality and Tumor Results

Radiation	Measure	Months														%
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
0.43 MeV	Number of rats	182	182	182	180	180	180	180	179	178	177	175	173	172	169	92.9
Neutrons	Rats with tumors					2	2	6	6	8	15	16	24	28	28	17.6
0.125 Kerma	All tumors					3	3	7	7	9	16	18	26	32	36	
0.1 Rad	Adenocarcinomas							1	1	1	1	1	2	2	3	
0.43 MeV	Number of rats	89	89	89	89	89	89	89	88	88	88	86	85	85	85	95.5
Neutrons	Rats with tumors			1	2	2	2	3	6	6	8	9	10	10	16	18.0
0.50 Kerma	All tumors			1	2	2	2	3	7	7	9	11	11	11	17	
0.4 Rad	Adenocarcinomas			1	2	2	2	2	2	3	3	3	3	3	4	
0.43 MeV	Number of rats	68	68	68	68	68	68	68	68	68	68	68	68	68	67	98.5
Neutrons	Rats with tumors			1	4	5	7	7	11	14	14	16	20	21	31.0	
2 Kerma	All tumors			1	4	5	7	7	11	14	14	19	24	25		
1.6 Rad	Adenocarcinomas			1	2	2	2	2	2	2	2	2	2	2	2	
0.43 MeV	Number of rats	45	45	45	45	45	44	42	42	42	42	39	38	36	80.0	
Neutrons	Rats with tumors		1	1	1	3	4	5	9	12	14	18	19	22	26	57.8
8 Kerma	All tumors		1	1	1	3	4	5	12	15	18	26	28	31	38	
6.4 Rad	Adenocarcinomas		1	1	1	1	2	3	5	6	6	8	8	8	8	
None	Number of rats	167	167	167	167	167	167	167	167	167	165	163	163	162	159	95.2
	Rats with tumors								2	2	5	8	13	17	20	12.0
	All tumors								2	2	5	8	14	18	21	
	Adenocarcinomas														0	
250 kVp	Number of rats	95	95	95	95	95	95	95	95	94	93	92	92	91	89	93.7
x-rays	Rats with tumors			1	1	2	3	4	5	7	7	8	13	17	18	18.9
30 R	All tumors			1	1	2	3	4	5	7	8	10	15	20	23	
28 Rad	Adenocarcinomas			1	1	1	1	1	1	2	2	2	2	2	2	
250 kVp	Number of rats	48	48	48	48	48	48	48	48	48	48	47	47	47	46	95.8
x-rays	Rats with tumors			1	3	5	6	7	10	12	13	15	20	23	24	50.0
60 R	All tumors			1	3	5	6	7	10	12	14	16	24	27	30	
56 Rad	Adenocarcinomas			1	3	4	4	4	4	4	4	4	5	5	5	
250 kVp	Number of rats	48	48	48	47	46	46	46	45	45	45	45	45	45	42	86.7
x-rays	Rats with tumors					3	6	7	8	12	17	20	20	22	22	45.8
90 R	All tumors					3	6	8	9	14	19	25	27	31	33	
85 Rad	Adenocarcinomas					1	1	2	2	3	3	3	3	4	4	

FIGURE CAPTIONS

- Fig. 1. The percent of initial number of rats with mammary neoplasia plotted at the end of 1-14 months after radiation on the 61-63rd day of age, rectangular coordinates.
- Fig. 2. The mean number of tumors per rat, obtained by dividing the total number of mammary neoplasms in any one group by the initial number of rats in that group, plotted at the end of 1-14 months after radiation on the 61-63rd day of age, rectangular coordinates.
- Fig. 3. Mean number of mammary neoplasms per rat as a function of time, $R(t)$ -see text for method of calculation, for the different doses of neutrons, log-log plot with the abscissa scale twice as much as the ordinate scale.
- Fig. 4. Mean number of mammary neoplasms per rat as a function of time, $R(t)$, for the different doses of x-rays, log-log plot with the abscissa scale twice as much as the ordinate scale.
- Fig. 5. Effect-period, in units of tumor-days, calculated as the time integral of $R(t)$, minus its control value-see text, as a function of neutron or x-ray dose, log-log plot. The observation extends to 400 days after irradiation. The broken lines have no mathematical significance and only serve to indicate the trend of the data.
- Fig. 6. The dependence of RBE on neutron dose. Vertical bars indicate the ranges of RBE which are excluded on a level of statistical significance exceeding 95%, wedges indicate nonsignificant differences. The statistical analysis (8,9) is based on the current data for neutrons and x-rays and on earlier results for x-rays (10).

Fig. 1 BNL Neg. 9-29-73
Fig. 2 BNL Neg. 9-30-73
Fig. 3 BNL Neg. 8-1133-73
Fig. 4 BNL Neg. 9-28-73
Fig. 5 BNL Neg. 8-1139-73
Fig. 6 BNL Neg. 9-118-73











