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CONTENTS

NEUTRON SOURCES, SPECTROMETRY AND DOSIMETRY
(Sessions I and II)

NEUTRON EFFECTS AT SUBCELLULAR AND CELLULAR LEVELS (Sessions III and IV)

NEUTRON EFFECTS ON MULTICELLULAR SYSTEMS
(Sessions V and VI)

USE OF NEUTRONS IN PLANT BREEDING (Session VII)

OTHER PRACTICAL IMPLICATIONS OF NEUTRON RADIATION BIOLOGY (Session VIII)

RAT MAMMARY CARCINOGENESIS FOLLOWING NEUTRON OR X-RADIATION*

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Abstract

RAT MAMMARY CARCINOGENESIS FOLLOWING NEUTRON OR X-RADIATION.

Female 61 to 63-day-old Sprague-Dawley rats were exposed once to a single dose of either 0.43-MeV neutrons or 250-kV X-rays. For neutrons 23 rats were exposed in plastic tubes rotated around and 31 cm from a water-cooled tritium impregnated target bombarded with 2.45-MeV protons from a Van de Graaff generator. The mean kerma was measured at the rat location by integrating the response of a rat-sized homogeneous tissue equivalent ionization chamber of minimum mass. The ratio between absorbed dose and kerma is under investigation and is anticipated to be approximately 0.7. A compensated GM gamma-ray dosimeter indicated that the gamma-ray doses were 3.5% of the total dose. All rats were examined weekly for the presence of breast tumours and these were removed, fixed, stained and verified histologically as mammary neoplasms. At 10 months after exposure 98% of the rats were alive. The neutron kerma, the per cent of rats with mammary neoplasia, and the number of rats were, respectively: 0.125 rads, 8.2%, 182; 0.5 rads, 9.0%, 89; 2 rads, 20.6, 68; and 8 rads, 31.1%, 45. The X-ray results were: 30 R, 7.4%, 95; 60 R, 27.1%, 48; and 90 R, 35.4%, 48. A 3.0% incidence was found in 167 control rats. At 10 months after exposure the mammary neoplastic response after 8 rads of neutrons corresponds approximately to that after 60 - 90 R of X-rays. Similarly, the response after 2 rads of neutrons was intermediate between 30 and 60 R of X-rays and the response after 0.125 and 0.5 rads of neutrons was similar to that after 30 R of X-rays. This demonstrates that the RBE for 0.43-MeV neutrons is much lower at high doses than at low doses. Determination of the confidence limits for the dose-RBE dependence and dose-incidence relationship will be determined as additional data are collected.

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,

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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° ± 3° F) and humidity controlled (55% ± 10%) animal rooms under conditions of 12 hours of flourescent 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 elasped 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 carrousel 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 ½ 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 of absorbed dose respectively.

Table I. MORTALITY AND MAMMARY NEOPLASTIC 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
0.1 rad	All tumors					3	3	7	7	9	16	18	26	32	36	
	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
0.4 rad	All tumors			1	2	2	2	3	7	7	9	11	11	11	17	
	Adenocarcinomas			1	2	2	2	2	2	2	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
1.6 rad	All tumors				1	4	5	7	7	11	14	14	19	24	25	
	Adenocarcinomas				1	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	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
6.4 rad	All tumors		1	1	1	3	4	5	12	15	18	26	28	31	38	
	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
28 rad	All tumors			1	1	2	3	4	5	7	8	10	15	20	23	
	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
56 rad	All tumors			1	3	5	6	7	10	12	14	16	24	27	30	
	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
85 rad	All tumors					3	6	8	9	14	19	25	27	31	33	
	Adenocarcinomas					1	1	2	2	3	3	3	3	4	4	

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 of absorbed dose respectively.

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, 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. Similarily, 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 6.4 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 o.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 that 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

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

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:

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

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

$$R(t) = \int_0^t r(\tau)\, d\tau \tag{3}$$

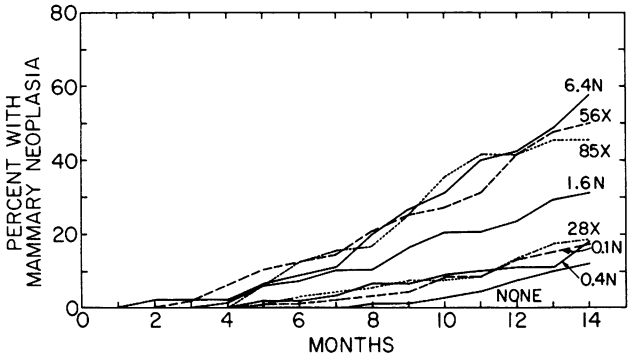

FIG. 1. The percentage of initial number of rats with mammary neoplasia plotted at the end of 1-14 months after radiation on the 61-63rd day of age; rectangular co-ordinates.

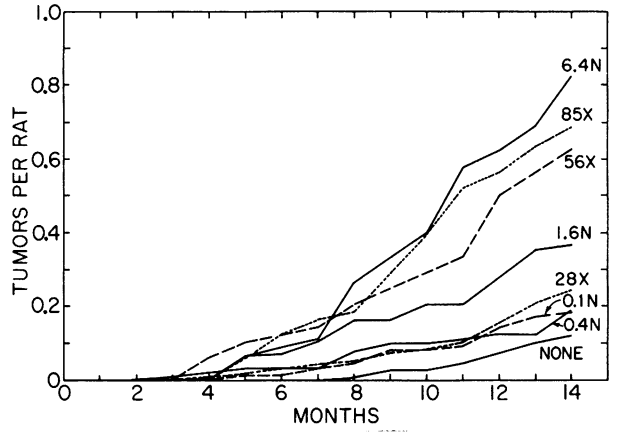

FIG. 2. The mean number of tumours per rat, obtained by dividing the total number of mammary neoplasms in any one group by the initial number of rats in that group, plotted at the end of 1-14 months after radiation on the 61-63rd day od age; rectangular co-ordinates.

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

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

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

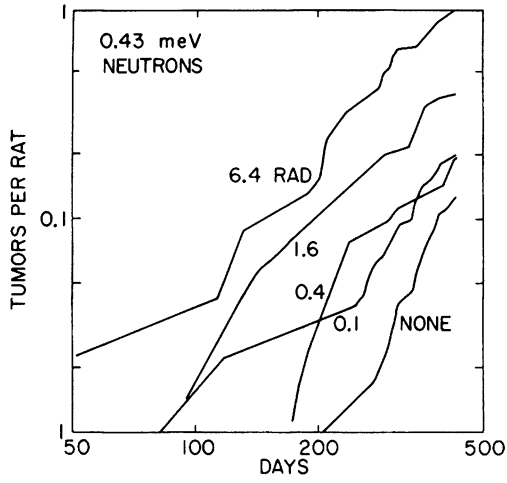

FIG. 3. Mean number of mammary neoplasms per rat as a function of time, R(t), for the different doses of neutrons; log-log plot with the abscissa scale twice the magnitude of the ordinate scale.

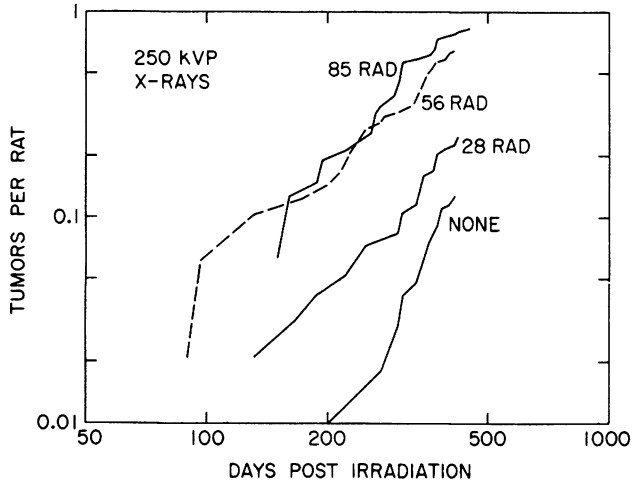

FIG. 4. Mean number of mammary neoplasms per rat as a function of time, R(t), for the different doses of X-rays; log-log plot with the abscissa scale twice the magnitude of the ordinate scale.

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:

$$R(t) = k\, t^2 \tag{5}$$

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

$$r(t) = 2k\, t \tag{6}$$

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:

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

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:

$$P(t) = \int_0^t R(\tau)\, d\tau \tag{8}$$

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 assesment 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

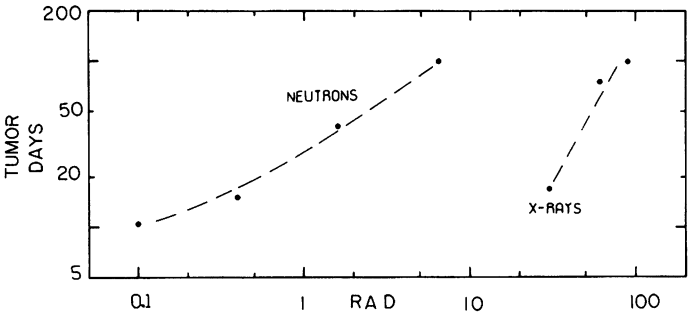

FIG. 5. Effect-period, in units of tumour-days, calculated as the time integral of R(t), minus its control value (see text) as a function of neutron or X-ray dose; log-log plot. The observations extend to 400 days after irradiation. The broken lines have no mathematical significance and only serve to indicate the trend of the data.

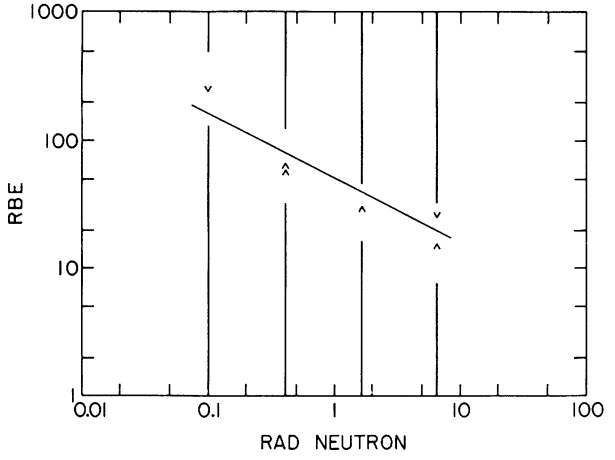

FIG. 6. The dependence of RBE on neutron dose. Vertical bars indicate the ranges of RBE that are excluded on a level of statistical significance exeeding 95%, wedges indicate non-significant differences. The statistical analysis [8, 9] is based on the current data for neutrons and X-rays and on earlier results for X-rays [10].

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 insomuch 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. 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 come 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. The present experiment used rats that were born on the same day, were from the same shipment, and were housed together. 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 irradiation 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 a dose of 0.10-0.40 rads of neutrons was followed by a mammary neoplastic response much like that following a dose of 28 rads of x-rays. The mammary neoplastic response to either a dose of 6.4 rads of neutrons or 56-85 rads of x-rays was similar. Thus the RBE for neutrons is much larger at low doses of neutrons than at high doses.

REFERENCES

(1) IONIZING RADIATION: LEVELS and EFFECTS. A report of the United Nations Scientific Committee on the Effects of Atomic Radiation to the General Assembly, with annexes. Volume II. Effects. (1972).

(2) THE EFFECTS on POPULATIONS of EXPOSURE to LOW LEVELS of IONIZING RADIATION. Division of Medical Sciences, National Academy of Sciences, National Research Council, Washington, D.C.,(1972).

(3) SHELLABARGER, C.J., CRONKITE, E.P., BOND, V.P., and LIPPINCOTT, S.W. The occurrence of mammary tumors in the rat after sublethal whole-body irradiation. Rad. Res. 6 (1957) 501.

(4) KAPLAN, E.L., and MEIR, P. Nonparametric estimation from incomplete observations. Amer. Statistical Assoc. J. 53 (1958) 485.

(5) ROSENBLATT, L.S., HETHERINGTON, N.H., GOLDMAN, M., and BUSTAD, L.K. Evaluation of tumor incidence following exposure to internal emitters by application of the logistic dose-response surface. Health Physics 21 (1971) 869.

(6) KELLERER, A.M. Quantification of radiation induced carcinogenesis. Annual Report on Research Project, USAEC-COO-3243-2(1973).

(7) ROSSI, H.H., and KELLERER, A.M. Radiation carcinogenesis at low doses. Science 175 (1972) 200.

(8) BATEMAN, J.L., ROSSI, H.H., KELLERER, A.M., ROBINSON, C.V., and BOND, V.P. Dose-dependence of fast neutron RBE for lens opacification in mice. Rad. Res. 51 (1972) 381.

(9) KELLERER, A.M., and BRENOT, J. Nonparametric determination of modifying factors in radiation action. Rad. Res. (1973) in press.

(10) SHELLABARGER, C.J., BOND, V.P., CRONKITE, E.P., and APONTE, G.E. Relationship of dose of total-body ^{60}Co radiation to incidence of mammary neoplasia in female rats. Radiation-Induced Cancer, IAEA-SM-118/9 (1969).

(11) KELLERER, A.M., and ROSSI, H.H. The theory of dual radiation action. Curr. Topics Radiat. Res. 8 (1972) 85.

(12) SMITH, H.H., ROSSI, H.H., and KELLERER, A.M. Relation between mutation yield and cell lethality over a wide range of x-ray and fission neutron doses in maize. IAEA/SM-179/28, this symposium.

(13) VOGEL, H.H.,Jr. Mammary gland neoplasms after fission neutron irradiation. Nature 222 (1969) 1279.

(14) VOGEL, H.H., Jr., and ZALDIVAR, R. Experimental mammary neoplasms: a comparison of effectiveness between neutrons, x-and gamma-radiation. AEC-tr-7387, ORNL (1969) 207.

(15) VOGEL, H.H., Jr., and ZALDIVAR, R. Neutron-induced mammary neoplasms in the rat. Can. Res. 32 (1972) 933.

(16) JULL, J.W. The effect of infection, hormonal environment and genetic constitution of mammary tumor induction in rats by 7,12-dimethylbenz-anthracene. Can. Res. 26 (1966) 2367.

DISCUSSION

V. KOFRÁNEK. Have you any radiobiologically convincing explanation for the widely discussed belief that the incidence of mammary neoplasms increases with increasing dose?

C.J. SHELLABARGER: I have neither the confidence nor the inclination to defend the Kellerer and Rossi theory of dual radiation action, RBE and the primary mechanism of radiation action. I will leave that to Kellerer and Rossi; but I think that their hypotheses do apply to rat mammary carcinogenesis due to low-and high-LET radiations.

Lola S. KELLY: Would you care to say something about the possibility of a virus being present in the Sprangue-Dawley and Long Evans rats and absent in the case of the rats that do not get the tumour? Multiple cell interactions might be due to virus infection or release.

C. J. SHELLABARGER: I know of no real evidence that a virus participates in the development of a rat breast tumour. However, a negative finding does not prove that a virus does not participate in mammary carcinogenesis.

G. W. BARENDSEN: If one wishes to interpret your data in terms of a radiobiological mechanism, it will be of interest to note that the dose distribution on a microdosimetric scale, with secondary short high-LET tracks of protons from neutrons and less densely ionizing electrons from X-rays, may account for the high RBE. The proton tracks are much more likely to damage two adjacent cells than the electrons, so if interaction between two damaged cells is required for tumour induction, protons may be expected to be much more effective because of their microdosimetric properties.

That interaction between two damaged cells is required was also indicated by experiments which I carried out with Dr. Klein in Rijswijk, when malignant transformation was studied in cultured cells derived from a hamster embryo: The addition of heavily irradiated 'feeder' cells to the cells to be transformed increased the frequency of transformation considerably. This indicates that some type of interaction enhances transformation.

A. G. SEARLE: In connection with the question whether more than one cell is involved in tumour induction, I should like to point out that there is now strong evidence that certain spontaneous tumours are derived from a single cell in females heterozygous for particular sex-linked markers, such as glucose 6-phosphate deficiency. I wonder if similar work has been done on induced tumours.

C. J. SHELLABARGER: As far as I know, work along these lines has not been carried out on rat breast tumours. It would be a worth-while approach.

H. H. VOGEL, Jr.: Would you care to comment on the relationship between low doses of neutrons and induced mammary tumours in the female Sprague-Dawley rat? Do you think there could be a linear dose-effect relation at very low doses?

C. J. SHELLABARGER: The dose-effect relationship could well be linear, of course, but our own data are not good enough to establish that with any degree of statistical confidence. Moreover, since the 'effect' changes with time after irradiation, one must be careful to specify the time in relation to the dose-effect curve.