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^{20}NEON ION- AND X-RAY-INDUCED MAMMARY CARCINOGENESIS IN FEMALE RATS

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INTRODUCTION

The acceleration of heavy ions ($^{12}_{6}C$, $^{20}_{10}Ne$, $^{40}_{18}Ar$) to energies of 400-500 MeV/amu has been achieved at the BEVALAC of the University of California in Berkeley[1]. In addition to radiation therapy, one of the proposed uses of heavy ion irradiation is to image lesions of the human female breast[2]. It is now generally accepted that radiation exposure of the human female breast will increase the risk of breast cancer development in the irradiated breast[3-5]. It is known that radiation-induced mammary carcinogenesis in the female rat occurs by a scopal mechanism[6] that is similar to the scopal mechanism of radiation-induced breast cancer in the human female[7]. Thus, the rat model system was chosen to assess the carcinogenic potential of heavy ion irradiation in the belief that data obtained from rat studies would have a qualitatively predictive value for the human female. Accordingly, female rats were exposed to ^{20}Ne ions at the BEVALAC and studied for the development of mammary neoplasia for 312 $\pm$ 2 days at Brookhaven along with rats exposed concurrently to x-irradiation or to no irradiation.

MATERIALS AND METHODS

Female Sprague-Dawley rats were purchased from Taconic Farms, Germantown, N.Y. and shipped by air to California. Two days after their arrival, they were irradiated at 43 $\pm$ 1 days of age, and two days later all rats were returned to Brookhaven via air shipment, thence studied at BNL for some 312 days.

A 7.36 GeV ^{20}Ne ion beam from the BEVALAC facility was passed through a 2 cm inch lead scatterer to provide a uniform field approximately 5 cm in diameter at the rat position. The resulting beam energy at the sample

position was estimated at 6.6 GeV. ^{20}Ne ions of this energy have a linear energy transfer (LET) and range in water of 33 KeV/μm and 20 cm respectively. ^{20} Ne ion irradiation and dosimetry were done as follows. Nominal doses of 2,6,18, or 54 rad (100 rad = 1 Gy) were given in 1-3 minutes. Each rat was exposed individually, facing the beam, in a plexiglass cylinder 5 cm in diameter and 14 cm long. The cylinder was placed concentric with the beam. Dose was measured upstream, downstream, and radially, using ionization chambers normally employed at the BEVALAC facility, and supplemented by TLD and film dosimeters placed anteriorly and posteriorly to the rat position. Each rat was placed in the plateau region of the depth dose pattern, immediately before the Bragg-peak, as evidenced by the slightly higher dose measured at the posterior dosimeters than at the anterior dosimeters. Based on these measurements, and available information on the ^{20}Ne ion depth dose pattern[8], it was concluded that dose increased by 50% from the anterior end of the rat to the posterior end, and dose decreased from the central longitudinal axis of the beam out to the wall of the rat holder by approximately 15%. The increased dose and LET near the posterior end contributed an estimated 14% greater mean dose and mean LET to the posterior half of the rat than to the anterior half of the rat. As a positive control, and as reference radiation, either 60 R or 180 R (1 R = 0.95 rad) of total body 230 kVp x-irradiation was given at an exposure rate of approximately 14 R per minute by operating a Phillips x-ray machine at 15 mA with 0.5 mm Cu and 1.0 mm Al filtration. Fifteen rats at a time were exposed, from the top, at a target to sample distance of 95 cm. The exposure was measured in air, under maximum backscatter conditions, with a Victoreen ionization chamber. The calculated nominal given were 57 and 171 rad.

After their return to Brookhaven National Laboratory, all rats were kept 5 per cage on corncob bedding in rooms maintained at 21-23°C under conditions of 7AM-7PM fluorescent light, and given commercial rat chow and water ad libitum. Each rat was identified by a numbered ear tag. Each mammary tumor, as it was located by once per week palpation, was recorded as to anatomical location using the nipples as reference points. All mammary tumors were removed under ether anesthesia at a size of about 2 cm. All suspected mammary tumors were studied microscopically, and were classified as either mammary adenocarcinomas or mammary fibroadenomas using criteria consistent with those published by Young and Hallowes[9]. In the case of a mammary neoplasm appearing at the site of a previous neoplasm, if the second neoplasm was of a different pathological type, or if more than 90 days had elapsed and the neoplasm was of the same pathological type, it was considered a separate neoplasm. The time of appearance of mammary tumors was taken as the date of the initial palpation of a tumor that proved to be a mammary tumor upon subsequent histological study. The time of tumor appearance, and death, was reckoned as days after the date of the exposure to radiation. The experiment was ended 312 + 2 days after the day of irradiation when all rats were killed and examined for pathology.

RESULTS AND DISCUSSION

In the ^{20}Ne ion irradiated rats, 41 mammary neoplasms were found in the posterior half of the rats, and 17, were found in the anterior half. In contrast, the anterior-posterior distribution was approximately equal, 31 and 33 respectively, in the x-ray irradiated rats. The right-left distribution was approximately the same in both the ^{20}Ne ion irradiated rats, 28 and 30, and in the x-ray irradiated rats, 29 and 35. The finding, in the ^{20}Ne ion

irradiated rats, of more neoplasms in the posterior half than in the anterior half is in agreement with the calculation of a larger ^{20}Ne ion dose in the posterior half than in the anterior half. This result, more mammary neoplasia in the volume with the larger ^{20} Ne ion dose is in agreement also with a dose related mammary neoplastic response as well as the concept that the mode of action of radiation-induced mammary neoplasia is scopal in nature.

There are some uncertainties about whether or not the development of a mammary adenocarcinoma and a mammary fibroadenoma within the same rat is an interdependent process[10]. In the current experiment, using 301 rats, 13 developed only an adenocarcinoma, 46 developed only a fibroadenoma, and 12 rats developed both types. A X^2 analysis of these data indicate ($P<0.001$) dependence between the occurrences of the two types of tumor. Even so, we have chosen to analyze the percent of rats with at least one adenocarcinoma (with or without a fibroadenoma), or at least one fibroadenoma (with or without an adenocarcinoma). We accept as proven that the irradiated female rat and the irradiated human female are at risk for the development of both benign and malignant mammary neoplasia[4].

We have chosen not to "correct" or modify the final incidence of rats with either an adenocarcinoma or a fibroadenoma for intercurrent mortality. Some 98% of the rats survived the 312 day study period (Table 1). Only 7 of the 301 rats died and these deaths did not appear to be related to the type of radiation.

Similarly, we have chosen not to study the time-to-tumor data. This option was selected, in part, because the experiment was severely truncated and no account was taken of possible late appearing mammary neoplasia. Also, some statisticians[11] argue that no meaningful distinction can be made between earlier onset (acceleration) and extra onset (higher incidence).

Data on the mean number of mammary neoplasms per rat were not analyzed. It has been reported[12,13] that the distribution of the number of rats with a specified number of mammary neoplasms (rats with no mammary neoplasms, rats with 1 mammary neoplasm, rats with 2 mammary neoplasms ..., etc) departs from a Poisson distribution. Thus, even though the mean number of mammary adenocarcinomas and mammary fibroadenomas tended to increase with dose, data not shown, there were too few rats with multiple tumors to allow a meaningful analysis of these data.

The percent of rats with one or more mammary adenocarcinomas, and the percent of rats with one or more mammary fibroadenomas tended to increase as the dose of ^{20}Ne ion-irradiation, or x-irradiation was increased (Table 1). The two highest doses of ^{20}Ne ion-irradiation, 18 rad and 54 rad, and the higher dose of x-irradiation 180 R, increased the incidence of rats with mammary adenocarcinomas, as compared to the non-irradiated controls, using the X^2 test. The highest ^{20}Ne ion dose, 54 rad, and both doses of x-rays, 60 R and 180 R, increased the incidence of rats with mammary fibroadenomas above the control value.

To compare the dose-response relationships for the two types of radiation, the percent of irradiated rats with one or more mammary adenocarcinomas, or with one or more mammary fibroadenomas, after subtracting the appropriate non-irradiated control value, were plotted in a log-log fashion, with the points joined by straight lines (Figure 1). At a prevalence of 20%, a response that is bracketed by all groups, the RBE (relative biological effectiveness) for rats with mammary fibroadenomas appears to be less than 2, and for adenocarcinomas, more than 5. No attempt to derive

confidence limits of the RBE values has been made because we believe the data are not adequate for this purpose. Also, it is not possible using these data to determine if the RBE values vary inversely with dose.

SUMMARY AND CONCLUSIONS

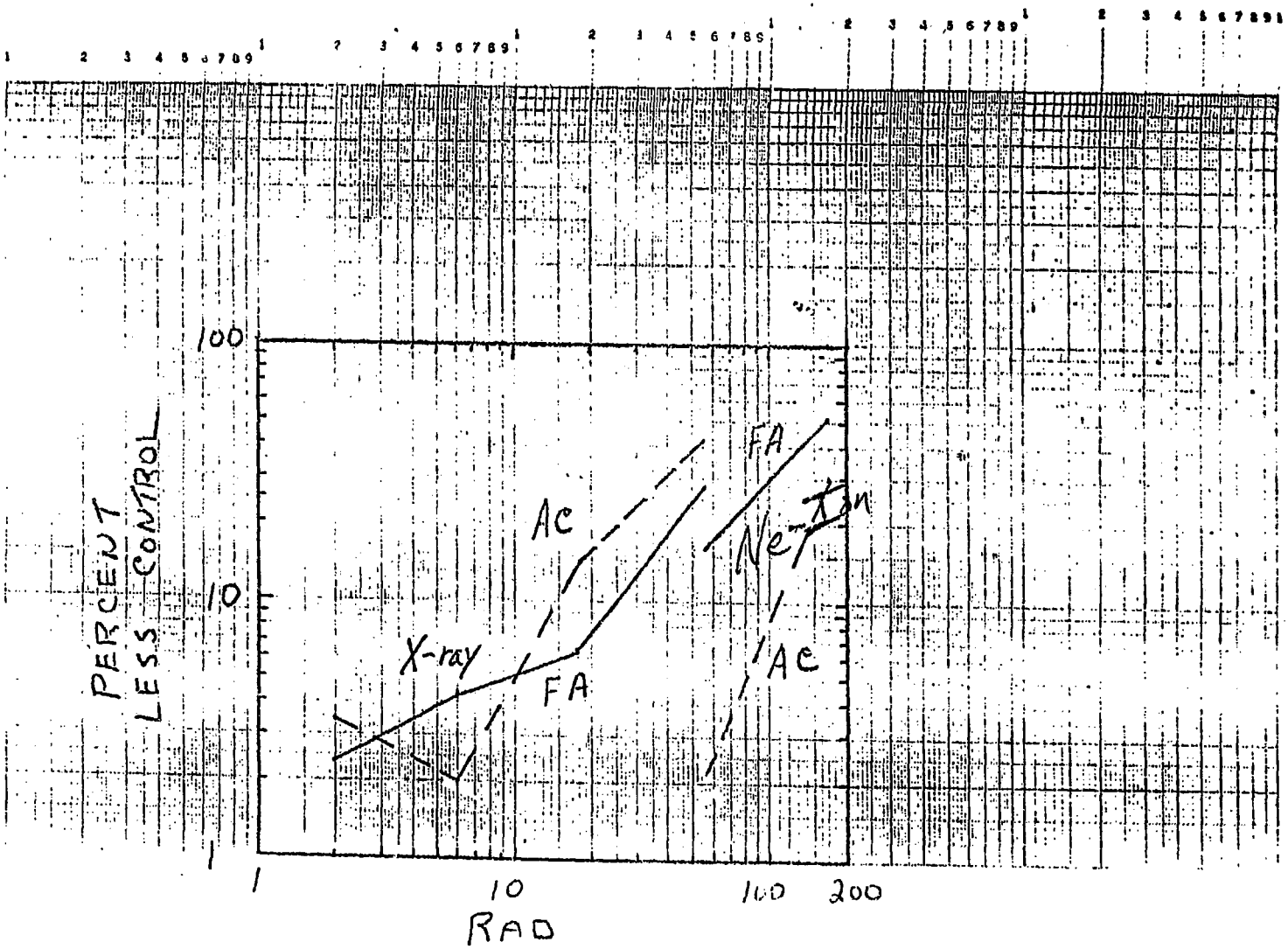
Female, Sprague-Dawley rats were given 6.6 GeV ^{20}Neon ions in the amount of 2,6,18 or 54 rad on the 43 $\pm$ 1 days of age. Concurrently, additional rats were either exposed to 60 R or 180 R of 230 kVp x-rays, or not irradiated. Mammary neoplasms were removed as they occurred and all rats were killed after 312 $\pm$ 2 days of study. As the dose of either type of radiation was increased the percent of rats with mammary adenocarcinomas, and the percent of rats with mammary fibroadenomas, tended to increase. At a prevalence of 20%, the RBE for ^{20}Neon ions for mammary adenocarcinomas was estimated to be larger than 5 and for mammary fibroadenomas the RBE was estimated to be less than 2. No conclusion was reached concerning whether or not the RBE might vary with dose. We suggest that ^{20}Ne ions do have a carcinogenic potential for rat mammary tissue and that this carcinogenic potential is likely to be greater than for x-irradiation. This finding is in accord with reports indicating that neutron radiation has a high RBE for mammary carcinogenesis in several strains of rats[13-18]. Thus, although we conclude that ^{20}Neon ions are likely to be carcinogenic for the human breast, the question of what the precise RBE might be remains unanswered.

Table 1. The type and dose of radiation, the number of rats irradiated at 43 ± 1 days of age and alive 312 ± 2 days later, and the number and percent of rats with mammary adenocarcinomas (AC) or with mammary fibroadenomas (FA).

RADIATION		NUMBER		RATS WITH AC		RATS WITH FA	
		START	END	N	%	N	%
None	None	76	75	0	0	6	8
^{20}Neon	2 rad	58	57	2	3	6	10
^{20}Neon	6 rad	49	49	1	2	6	12
^{20}Neon	18 rad	21	20	3[a]	14	3	14
^{20}Neon	54 rad	22	19	9[b]	41	8[a]	36
X-ray	57 rad	45	45	1	2	11[b]	24
X-ray	171 rad	30	29	9[b]	30	18[b]	60

[a], different from control, non-irradiated value, X^2, $p<0.01$.

[b], different from control, non-irradiated value, X^2, $p<0.001$.


PERCENT
LESS CONTROL
100
10
1
1
10
100
200
RAD
X-ray
FA
Ac
FA
Net
Ac

EPILOGUE

When Dr. Cronkite moved to Brookhaven National Laboratory in 1954, he was interested in the study of the hematopoietic aspects of the acute radiation syndrome and in developing a model system to simulate the radiation conditions of the Marshallese in regard to the possible interaction of beta burns and total body gamma radiation. In regard to the study of hematopoietic cellular proliferation - the rest is history - witness the scientific content of this conference.

In regard to the possible interaction of beta burns and total body irradiation on the risk for the development of skin cancer in the Marshallese, Drs. Cronkite, Bond, and Shellabarger started an experiment where female Sprague-Dawley rats received either total body irradiation and/or beta irradiation of the skin. It turned out that the beta sources were calibrated incorrectly and only a trivial amount of beta dose was delivered to the skin. However, the rats that received total body irradiation, either 200 R or 400 R, began to develop subcutaneous tumors on the ventral surface some 60-90 days after exposure. With the help of Dr. Lippincott, it was soon shown that these tumors were of mammary gland origin. The incidence of rats with mammary neoplasms increased to approximately 60% at 11 months after starting the experiment while no mammary neoplasms developed in non-irradiated rats. It was soon recognized that this experimental system could be used to study radiation-induced mammary carcinogenesis (Shellabarger, Cronkite, Bond, and Lippincott, Radiation Research 6, 501-512, 1957) and indeed, many investigators did and still do use this rat model system.

The above account shows another example of serendipity at work.

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