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MODIFYING FACTORS IN RAT MAMMARY GLAND CARCINOGENESIS

Claire J Shellabarger

Medical Department

Brookhaven National Laboratory

Upton, New York 11973

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Running head title: Rat mammary carcinogenesis

INTRODUCTION

Mammary tumors often appear in large numbers in rats following exposure to ionizing radiation, or administration of chemical carcinogens, or modification of hormonal status, or combinations of these manipulations, or often, simply as the result of old age. Further, mammary tumor incidence, either experimental or spontaneous, depends strongly on the strain and sex of the rat chosen for study. Although an enourmous literature exists on all of these topics, simple generalizations are not easily made, largely because few systematic comparison studies have been done, and partially because many papers fail to mention the strain of rat used, animal care conditions, statistics, or pathological criteria. In attempting to deal with the topic of modifications of rat mammary gland carcinogenesis, special emphasis will be given to reports that are both comparative in nature and contain sufficient detail of materials and methods so that the results can be evaluated. In some areas, to illustrate a particular point, only a few references will be cited and an exhaustive completeness of a literature search will not be attempted.

Before incidence statistics of rat mammary tumors can be understood, some consideration must be given to tumor pathology. Although mammary tumors may be first detected as subcutaneous nodules in mammary gland areas, anatomical location is not enough to be sure that the tumor is of mammary gland origin. Any lump or swelling in the region of the mammary gland should be examined to exclude salivary gland tumors, preputial gland tumors, epidermoid cysts, basal cell carcinomas, squamous cell carcinomas, lymph nodes, hibernomas, and lobular hyperplasia of the mammary gland. These exclusions then leave three types of mammary neoplasms, fibroepithelial, epithelial, and mesenchymal. The fibroepithelial neoplasms

are clearly benign, and are given names ranging from adenoma thru fibroma. In this report, the general term fibroadenoma will be used. Epithelial neoplasms of mammary gland origin are given names ranging from adenocarcinoma through anaplastic carcinoma, this report will use adenocarcinoma. Adenocarcinomas show some aspects of macroscopic, histological, cytological, and biological malignancy. They do invade and metastasize, but only very rarely. The important point is that almost all mammary neoplasms are either fibroadenomas or adenocarcinomas and they can easily be diagnosed and separated from H and E sections. Sarcomas can be easily classified as sarcomas, however, verifying their origin is extremely difficult, at least for me, but fortunately so few sarcomas occur that they can usually be ignored. The classification of mammary neoplasms here used follows generally the very useful paper of Young and Hallowes (1973).

RAT STRAIN DIFFERENCES

The strain of rat chosen for study has important consequences in regard to both spontaneous and induced mammary neoplasia incidence. Some selected illustrations of strain specificity follow. Females of the A X C strain have been reported to have "an essentially zero incidence of spontaneous incidence of breast cancer" (Segaloff and Maxfield, 1971) or "In 5329 normal adult and aged A X C rats necropsied at the Institute, the frequency of... mammary tumors was 1.8%" (Iglesias, 1974). In my own laboratory, we are following a small sample of A X C females, and they do have a reasonably low incidence of mammary neoplasia especially when compared to females of the Sprague-Dawley strain studied previously (Shellabarger, Bond, Aponte, and Cronkite, 1966). The difference in

spontaneous incidence between the two strains (Figure I) holds for both mammary adenocarcinomas and mammary fibroadenomas. No explanation has been put forward to account for this, or any other, particular strain difference. However, this one example of strain difference should suffice to illustrate that there are strain differences in regard to the spontaneous incidence of mammary neoplasia and to introduce the subject of rat strain differences in regard to the induction of mammary neoplasms.

INDUCED MAMMARY NEOPLASIA

The Lewis strain differs from the Sprague-Dawley strain in regard to incidence of mammary neoplasia in females following either total body x-irradiation or dimethylbenzanthracene (DMBA) administration (Table 1). Examination of these data, taken from a published paper (Shellabarger, 1972), shows clearly, in this direct comparison over a 10 month period, that both the Lewis strain and the Sprague-Dawley strain show a large response to either DMBA or x-ray in regard to the incidence of mammary adenocarcinomas. However, only the Sprague-Dawley strain shows a large fibroadenoma response to either carcinogenic agent. Again, no explanation has been put forward to account for this strain difference. Less well studied, is the strain difference between Long-Evans and Sprague-Dawley rats. However, it is clear, from papers of Syndnor, Butenandt, Brillantes, and Huggins (1962), and our own studies (Brown and Shellabarger 1974) that in regard to mammary adenocarcinoma induction, the Long-Evans strain shows a smaller response than does the Sprague-Dawley strain to either x-irradiation of chemical carcinogens. Again, although there is no clear known rationale for this strain difference one experiment based on the in vitro, direct, application of DMBA to mammary gland tissue was interpreted to mean that

the strain difference was not at the level of carcinogen-tissue interaction (Brown and Shellabarger, 1974). The results of experiments cited above are all consistent with the thesis that strain sensitivity holds for both chemical carcinogens and for the physical carcinogen-ionizing radiation. That is to say, those strains that are relatively sensitive to DMBA are also apt to be sensitive to x-irradiation.

In addition to the differential susceptibility to chemical carcinogens shown by different strains there are additional variables in regard to the mammary carcinogenic responses to chemical carcinogens and to radiation. There is probably a relationship between age at the time of carcinogen administration and the size of the carcinogenic response. The mammary adenocarcinoma response to methylcholanthrene (MCA) in the female Sprague-Dawley rat appears to be at a maximum when the rats are about 50-65 days of age (Huggins, Grand, and Brillantes, 1961). If the rats are younger or older at the time of MCA administration, a smaller mammary adenocarcinoma response occurs. Similarly, when total body x-irradiation was used as the carcinogenic agent, the juvenile Sprague-Dawley rat shows a smaller response than a younger, sexually mature rat (Shellabarger, 1974a). However, where the MCA treated rat shows a diminished response at ages older than 65 days of age, no loss of response to x-rays has been found in rats of 225 days of age at time of exposure to x-irradiation (Table 2). What has not yet been studied is the morphological stage of development of the mammary gland at the time of the administration of the carcinogenic agents, or the effect of age on the distribution of the chemical carcinogen, or the effects of the carcinogenic agents on ovarian function and on pituitary function. Perhaps one situation that is understandible is the following. MCA given to lactating

rats produced no mammary adenocarcinomas (Dao, Bock and Greiner, 1960) while, in contrast, total-body x-irradiation of the lactating rat (Shellabarger, 1974b) does produce mammary adenocarcinomas (Table 3). This lack of response to MCA in the lactating rat may well be due to a reduced carcinogenic stimulus to the mammary gland itself because the lactating gland rapidly eliminates the carcinogen via the milk. In the case of radiation, the dose to the mammary gland is little changed by the process of lactation. Even so, the factors responsible for the changing carcinogenic sensitivity of the mammary gland with age and with endocrine system maturation are not understood nor well studied. This would appear, to me at least, a fruitful area of investigation.

RADIATION

Any of several types of low or high LET radiation produce mammary neoplasia in any of several strains of rats. There is general agreement that ionizing radiation acts by a scopal mechanism, that is, most of the mammary neoplasms following exposure are found in the irradiated volume of mammary gland tissue. There are two general types of evidence for the scopal mechanism. First, when Sprague-Dawley rats were shielded and then exposed to x-rays (Bond, Shellabarger, Cronkite, and Fliedner, 1960), most of the mammary neoplasms were found in the irradiated mammary gland tissue (Figure II). A second approach, was to remove mammary gland tissue, expose the tissue to x-irradiation in vitro, and then return the tissue to the rat from whence the tissue came (Shellabarger, 1971). When this was done, more mammary neoplasms were found in the irradiated tissue than in the non-irradiated tissue (Table 4).

Although there may be a sparing effect when low LET radiation is spread out in time, over days or weeks by lowering the dose-rate (Shellabarger and Brown, 1972) or fractionation and protraction, this has not been demonstrated unequivocally for mammary neoplasia in irradiated rats. Total body x-irradiation split into 2 equal doses separated by intervals up to 24 hours did not seem to diminish the mammary neoplastic response to 400 R of total body irradiation (Shellabarger, 1970) in Sprague-Dawley rats (Table 5) over a 315 day follow-up period. Also, split doses of 550 R plus 550 R of partial-body irradiation, again with intervals up to 24 hours, did not diminish the mammary neoplastic response to 1100 R (Table 6) over a 300 day follow-up period. If repair or recovery does occur, it was not of sufficient magnitude to modify tumor incidence in these 2 experiments.

High LET radiation seems to be more efficient, rad for rad, than low LET radiation in inducing mammary neoplasia, provided that the comparisons are made at low doses, and that the tumor observations are made relatively soon after exposure (Vogel 1973, Shellabarger, Brown, Rao, et al 1973). Inspection of the plot of percent of rats with mammary neoplasia against dose, for neutrons or for x- or gamma-rays suggests (Figure III) that neutrons have a reasonably high RBE, that the RBE is larger at low doses than at high doses, and that the shapes of the dose-response curves may be different for the two types of radiations. Rossi and Kellerer (1972) Kellerer and Rossi (1972) and Kellerer (1975) have discussed the implications of these results in terms of mechanisms of action at the biophysical and cellular levels. However, from a biological standpoint, it should be remembered that these results obtain only for one

strain of rat, for overall mammary neoplasia, and for the temporal advancement of mammary neoplasia. Several laboratories have in progress experiments designed to study different strains of rats, with sample sizes large enough to look at both mammary adenocarcinomas and fibroadenomas, and over the life span of the rats, and at a wider range of doses in order to understand the RBE of neutrons.

COMBINATIONS OF CARCINOGENIC AGENTS

Because some chemical carcinogens seem to produce the same mammary neoplasms with about the same latent period as does ionizing radiation, it seemed logical to study the interaction of chemical carcinogens and radiation on the induction of mammary neoplasia.

When methylcholantrene (MCA) was given to Sprague-Dawley female rats with and without x-rays, or the sequence reversed, and the incidence of mammary adenocarcinomas determined over a short period of time, the 2 agents appeared to act in an additive fashion (Shellabarger, 1967). Again, when MCA and fission neutrons were studied (Shellabarger and Straub, 1972), the interpretation was that the 2 agents were more nearly additive than either inhibitory or synergistic. An additional observation is that what is close to the optimum dose of MCA seems to produce a larger mammary adenocarcinoma response that does a similar dose of neutron radiation. In other words, the maximum response, in terms of percent of rats with one or more mammary adenocarcinomas that can be produced with radiation seems to not exceed 20-30% while it is not unusual to produce almost a 100% incidence with chemical carcinogens. In any event, if the additive result is accepted it is then a usual step to continue to speculate and conclude that the 2 agents, one physical and one chemical, act at a common level of biological organization. Proof of a common pathway for chemical and physical carcinogens has not yet been provided. Addition experiments are underway to learn, at lower doses of the two agents and over longer follow-up periods, if an additive result obtains also for fibroadenoma induction.

A long-standing concept in chemical carcinogenesis has been the idea of initiation and promotion. Recently, Armuth and Berenblum (1974) have, for the first time, extended this concept to rat mammary carcinogenesis. They administered a relatively small amount of DMBA in a single dose and then gave phorbol 2 times per week for 10 weeks. Their results can be easily interpreted as showing that DMBA acted as initiator and phorbol as a promotor. Despite the trouble that I have in reconciling the fact that DMBA itself can act as a "complete" carcinogen as well as appearing to serve as an initiator in the experiments of Armuth and Berenblum, we plan to test the effect of phorbol given after x-irradiation of rat mammary carcinogenesis.

VIRUSES

Any consideration of rat mammary gland carcinogenesis must include a consideration of viruses. Two recent reports are most pertinent. First, (Ankerst, Jonsson, Kjellen, Norrby and Sjogren, 1974) have reported that inoculation of adenovirus type 9 into 7 new born Wistar/Furth rats was followed by fibroadenoma development in all 7 rats within 25 weeks, while none of their sisters not inoculated, or inoculated with adenovirus type 5, developed fibroadenomas. This report is of great interest, and I look forward to the results of attempts to confirm it. Secondly, Bogden, Cobb, Ahmed, Alex, and Mason (1974) reported that when R-35 mammary tumor virus, a C-type virus isolated from a transplantable rat mammary tumor, was inoculated into female, neonatal Sprague-Dawley rats, 10.6% of them developed mammary adenocarcinomas over a 20 month period and only 1.6% of the controls developed adenocarcinomas during the same period. However, until survival rates are given for the two groups the difference in tumor yield must be accepted with some reservations.

Implanation of a 17 beta-estradiol pellet in 20 virus treated rats was followed by 7 rats developing mammary adenocarcinomas within 13 months, while no tumors were noted in 21 estrogen only treated rats over the same period. Since the survival rates appeared to be similar in this part of the experiment, a synergistic interaction between estrogen and virus may be postulated. Also, C-type virus was isolated from some of the tumors. At the very least, these two reports strongly suggest that viruses must be considered when discussing mammary carcinogenesis in the rat.

HORMONES

One of the most striking, recent reports on the modification of rat mammary carcinogenesis was that of Segaloff and Maxfield (1971). Working with the A X C strain, they reported a synergistic interaction between x-irradiation and diethylstilbesterol (DES) on mammary adenocarcinoma formation. Since we have been able to reproduce their findings in A X C rats, using 0.43 MeV neutrons rather than x-rays, the findings with neutrons and DES will be described in these rats and contrasted with the findings in Sprague-Dawley strain. In both strains, a 20 mg cholesterol pellet containing 25% DES was implanted 2 days before 3.92 rads of neutrons. Even though the experiment is not yet finished, the interim results are of interest (Table 7). Three hundred and thirty-six days after DES administration it is clear that DES has the capacity to induce mammary adenocarcinomas in A X C rats but not in Sprague-Dawley rats. Radiation induced mammary fibroadenomas in Sprague-Dawley rats but not in A X C rats. Only in A X C rats did a synergistic interaction between estrogen and radiation occur and then only in regard to mammary adenocarcinomas. The A X C rats that received both DES and neutron treatment

developed mammary adenocarcinomas somewhat sooner that with either treatment alone but what is more spectacular is that only the A X C rats that received both treatments developed what Segaloff and Maxfield (1971) called "essentially" total carcinogenesis (Figure IV). That is, so many adenocarcinomas were present that they were difficult to count. A possible clue as to the difference between strains may be the fact that only A X C rats developed pituitary tumors. These pituitary tumors were judged to be prolactin secreting tumors. This has been proven to be true, since Dr. J.P. Stone has found, in a separate experiment, that prolactin blood levels are much higher in A X C rats with a DES pellet than in Sprague-Dawley rats with the same pellet, and because Dr. S. Holtzman has found, by immunofluorescent techniques, prolactin secreting cells in these pituitary tumors. These results well illustrate the complexities of rat mammary gland carcinogenesis. There are strain differences in response to exogenous estrogen, in response to radiation, and to the response to interactions of radiation and estrogen. These results suggest that prolactin may play a key role in the strain differences and differences in response to radiation. It must also be mentioned that the results of this experiment are in full accord with the report of Yokoro Furth (1961) who reported a synergism between prolactin and radiation on mammary carcinogenesis some 15 years ago.

SUMMARY

The spontaneous incidence of mammary adenocarcinomas and mammary fibroadenomas is related to the strain of rat studied.

Strains of rats that are sensitive to chemical carcinogens in regard to induced mammary neoplasia tend to be the same strains of rats that are sensitive to radiation.

MCA and x-rays appear to act in an additive fashion on the induction of mammary adenocarcinomas when they were given together.

Lactating and older rats lose responsiveness to chemical carcinogens but do not lose responsiveness to radiation.

Radiation appears to act in a scopal fashion in the induction of mammary neoplasia.

Mammary neoplasia induction was not changed when low LET radiation was split into 2 equal fractions.

High LET radiation is more effective than low LET radiation in inducing mammary neoplasia.

One report suggests that DMBA can act as an initiator for the induction of mammary adenocarcinomas and that phorbol can act as a promotor.

Two reports were cited where it is thought that viruses may induce mammary neoplasia.

DES and radiation appear to act synergistically in the induction of mammary adenocarcinomas in one strain of rat but not in another strain.

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Table 1. Mammary neoplasia in female rats of the Lewis or Sprague-Dawley strains.

Treatment	Strain	
	Lewis	Sprague-Dawley
start	22	44
end	21	40
rats with AC	0	1
total AC	0	1
rats with FA	0	2
total FA	0	2
DMBA		
start	29	29
end	22	21
rats with AC	20	18
total AC	43	39
rats with FA	1	16
total FA	1	53
X-Ray		
start	40	40
end	38	36
rats with AC	3	10
total AC	4	11
rats with FA	3	23
total FA	4	39

AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. DMBA, 13.3 mg per 100g body weight, or 350 R of 250 kVp total-body x-irradiation on the 50th day of age and followed until autopsy 10 months later.

Table 2. Effect of age at time of exposure on mammary neoplasia in female Sprague-Dawley rats.

	Days of age at time of exposure to 350 R			
	24	42	84	225
start, number	29	30	30	30
end, number	28	27	24	25
rats with AC	2	10	12	12
total AC	2	13	12	12
rats with FA	15	19	19	19
total FA	19	33	35	35

AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. X-rays were 250 kVp given as total body radiation on the days of age as indicated and all rats studied for 300 days. Three of 30 control rats, matched in age with those irradiated at 225 days exhibited a total of 4 FA.

Table 3. Effect of lactation on mammary neoplasia response to 350 R of total body x-irradiation in Sprague-Dawley rats, strain 784.

	Treatment			
	350 R Lactating	350 R Virgin	Control Lactating	Control Virgin
start, number	30	30	19	29
end, number	27	27	19	28
rats with AC	11	11	0	0
total AC	15	13	0	0
rats with FA	6	5	0	1
total FA	6	6	0	1

AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. X-ray, 250 kVp, given on the 4th day of lactation, or to virgin rats of the same age, or non-irradiated rats of the same age, and all rats studied for 300 days.

Table 4. Mammary neoplasia in transplants of tissue exposed *in vitro* to 800 R of 250 kVp x-rays, or no irradiation.

	Irradiated	Sham-irradiated
Transplants with:		
No neoplasia	94	109
1 FA	11	1
2 FA	2	0
1 FA and 1 AC	1	0
1 AC	2	0
Total	110	110

AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. Transplants from 49-53 day old female Sprague-Dawley rats, both the irradiated and non-irradiated mammary gland tissue retruned to the rat from whence they came. Transplants recovered up to 9 months after transplantation.

Table 5. Effect of split doses of 250 kVp x-ray, total body irradiation on mammary gland neoplasia in female Sprague-Dawley rats.

	Hours Between 200 R + 200 R				No Radiation
	0	2	6	24	
start, number	24	24	24	24	15
end, number	22	23	21	23	15
rats with AC	9	9	6	7	0
total AC	10	12	7	9	0
rats with FA	17	18	18	19	3
total FA	37	27	43	32	4

AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma.

Rats irradiated on the 55th day of age and followed for 315 days.

Table 6. Mammary neoplasia in irradiated volume after split doses of 250 kVp x-rays administered to the anterior half of female Sprague-Dawley rats.

	Hours Between 550 R + 550 R			
	0	2	6	24
start	35	35	35	35
end	33	34	33	33
rats with AC	7	8	6	14
total AC	8	8	7	16
rats with FA	10	16	13	17
total FA	13	24	15	22

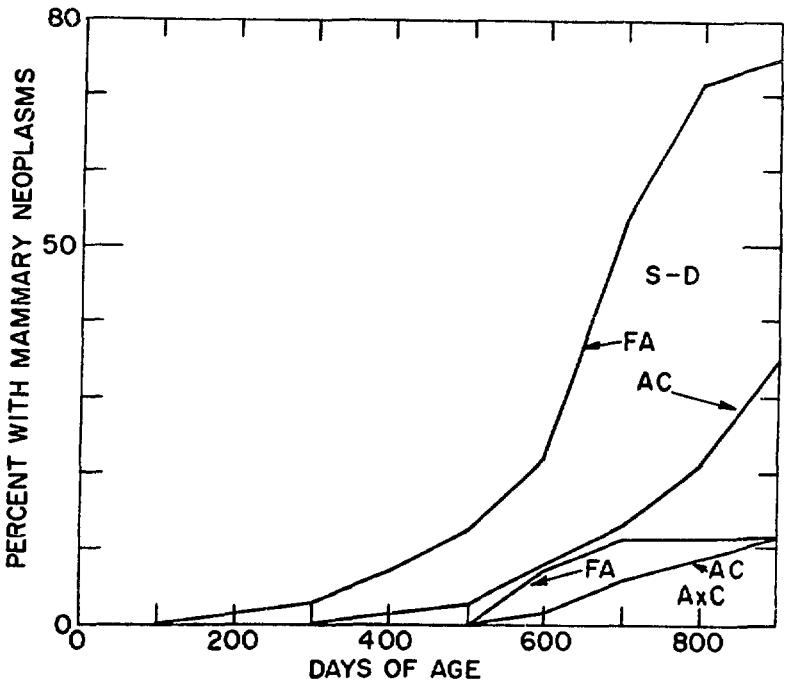
AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. Rats exposed, with posterior half shielded on 60th day of age and studied for 300 days. No neoplasia found in 30 control rats.

Table 7. Mammary neoplasia in AxC or Sprague-Dawley female rats given a DES pellet (5 mg diethylstilbestrol + 15 mg cholesterol), or DES and 3.92 rads of 0.43 MeV neutrons 2 days after DES, or neutrons only.

	TREATMENT					
	3.9 Rads		DES		Both	
	AxC	S-D	AxC	S-D	AxC	S-D
start	23	34	23	34	23	34
end	22	32	0	16	0	16
rats with AC	2	4	11	0	20	1
total AC	2	6	48	0	300*	2
rats with FA	1	14	0	0	0	0
total FA	1	17	0	0	0	0

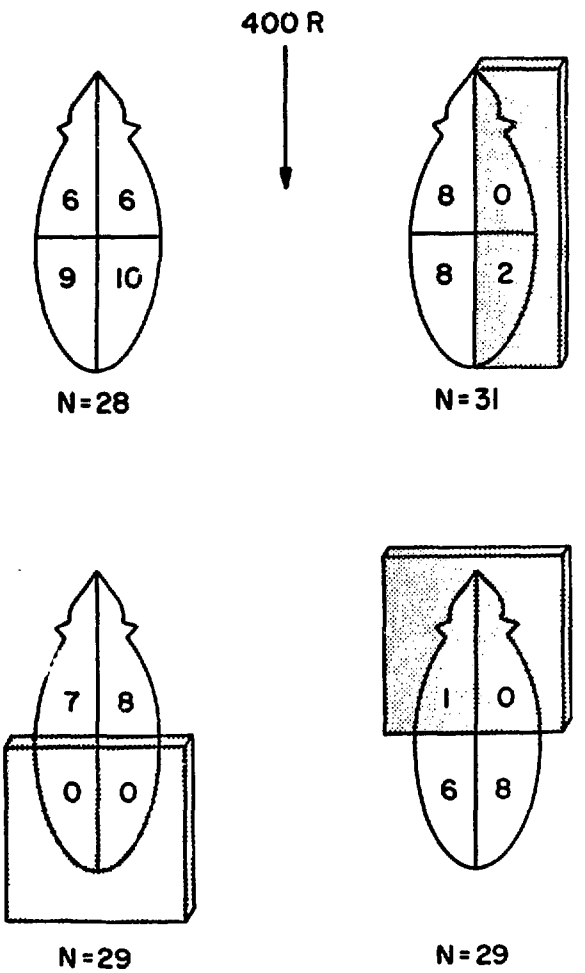
* Estimated. AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma. Rats studied for 336 days after DES.

Figure I. Percent of rats "at-risk" (by life table technique) with mammary neoplasia in Sprague-Dawley (S-D) female rats and A X C female rats plotted against days of age. AC stands for mammary adenocarcinoma and FA for mammary fibroadenoma.



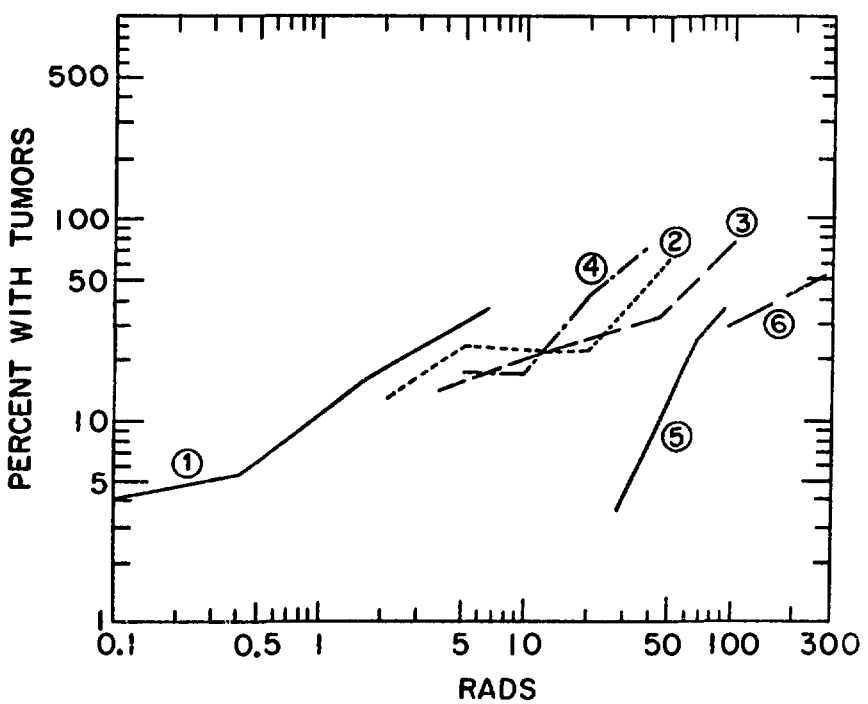
BNL Neg. No. 3-1051-75

Figure II. Number of mammary neoplasms found in each quadrant of mammary tissue after 400 R of partial-body 250 kVp x-rays 11 months after exposure.



BNL Neg. No. 6-417-73

Figure III. Percent of rats with mammary neoplasia minus control values 11 months after exposure plotted against dose of radiation on log-log scales. Curve 1, 0.43 MeV neutrons, from Shellabarger, Brown, Rao, et al (1973). Curve 2, fission neutrons, from Vogel (1973). Curve 3, fission neutrons, from Shellabarger, unpublished. Curve 4, cyclotron (about 35 MeV) neutrons, from Montour (1975). Curve 5, 250 kVp x-rays, from Shellabarger, Brown, Rao et al (1973). Curve 6, 250 kVp x-rays, from Shellabarger, unpublished. Curves 1 and 5 were done in a single laboratory at the same time. Curves 3 and 6 were done in a single laboratory at the same time.



BNL Neg. No. 3-1050-75

Figure IV. A single quadrant of mammary gland tissue removed from an A X C female rat that received DES and neutron radiation that illustrates a multiple tumor response.

BNL Neg. No. 5-842-74