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MAMMARY CARCINOGENESIS IN RATS:

BASIC FACTS AND RECENT RESULTS IN BROOKHAVEN[1] [2]

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Our interest in neutron-induced mammary carcinogenesis in rats stemmed from 2 publications dealing with the interaction of chemical carcinogens and x-radiation (1), and the interaction of x-radiation and estrogen (2).

First, we wished to know if the additive interaction of x-irradiation and 3-methylcholanthrene (MC) on mammary adenocarcinoma formation in female Sprague-Dawley rats (1) could be extended to neutron irradiation. Quite simply, the answer was yes. The interaction of neutron-irradiation and MC and the interaction of x-irradiation and MC were very similar as seen in Table 1 made-up of data taken from references 1 and 3. In the x-ray and MC study, 40 mg of MC was given in 4 ml of sesame oil by stomach tube on the 40th day of age, or 376 cGy on the 50th day of age, or both with the sequence reversed for half of the rats. The percent of rats with one or more mammary adenocarcinomas and the total number of mammary adenocarcinomas were scored at 140 days of age. In the fission neutron and MC study, the same experimental plan was followed except that neutron dose was 100 cGy and the observation period ran from 42 to 142 days of age. From these results it is possible to conclude that neutron-irradiation and MC and x-irradiation and MC produced qualitatively similar mammary carcinogenic results of additivity. We would of course, feel more secure of the interpretation of additivity if we had dose-response curves for both agents.

Secondly, we wished to know if the synergistic interaction between x-irradiation and diethylstilbestrol (DES) shown by Segaloff and Maxfield (2) on mammary adenocarcinoma formation in female ACI (AxC) rats could be extended to neutron-irradiation. Again, the answer was yes, as shown in Table 2 made-up of data taken from reference 4. At 60 days of age, female ACI rats were

implanted subcutaneously with a single 20 mg pellet composed of 5 mg DES and 15 mg of cholesterol, or given 9.6 cGy of 0.43 MeV neutrons on the 62nd day of age, or given both treatments. Mammary adenocarcinomas were tabulated for 50 weeks. The synergistic interaction between DES and neutron radiation, in this experiment, pertains to the total number of mammary adenocarcinomas per rat.

At this point, we remembered a previous publication (5) that reported, in contrast, in female Sprague-Dawley rats, that DES inhibited the mammary neoplastic response to x-irradiation. We then compared directly female Sprague-Dawley rats to female ACI rats. As seen in Table 3, data taken from reference 6, there is indeed a strain difference between Sprague-Dawley rats and ACI rats in regard to the interaction of radiation and DES. A single pellet containing 5 mg of DES and 15 mg of cholesterol was implanted subcutaneously in 80 day old female ACI rats. Two days later, half of the DES rats were given 4 cGy of 0.43 MeV neutrons. Other rats were given neutrons only. The same experimental plan was followed using 57 day old female Sprague-Dawley rats. Both strains were irradiated on the same day, kept in adjoining animal rooms, and followed for 48 weeks. Slightly older ACI rats were used to make sure that both strains were sexually mature at the time of treatment. The ACI strain exhibited a radiation dose-dependent (only the largest neutron dose data is here shown) synergistic interaction between DES and neutrons, both in terms of the percent of rats with mammary adenocarcinomas and the average number of mammary adenocarcinomas per rat. The Sprague-Dawley strain did not. This strain difference is not due to a difference in DES release rate as will be discussed below. An additional strain difference was found by Holtzman, et al (7). When female Fischer rats were irradiated with

x-rays and given DES, synergism was found in terms of the percent of rats with mammary adenocarcinomas but not in terms of the number of mammary adenocarcinomas per rat as shown in Table 4 taken from data from reference 7. Here, one compressed 20 mg pellet containing cholesterol only or cholesterol mixed with 0.98 to 3.9 mg of DES was implanted subcutaneously into F344 rats. Two days later, half of the animals in each group were exposed to 140 cGy of x-rays and all rats studied for 350 days. Thus the synergism between radiation and DES in ACI rats holds for the total number of mammary adenocarcinomas , and only partially for the percent of rats with adenocarcinomas , while in female Fischer rats the synergism holds only for the percent of rats with adenocarcinomas. This strain difference could be possibly due to the chosen doses of DES. Stone, et al (8), has shown previously in female ACI rats that the synergism between radiation and DES is dose-dependent for DES.

We have noticed when "American" female Sprague-Dawley rats are irradiated with neutrons by us (3), or "French" female Sprague-Dawley rats by Jacrot (9), mammary adenocarcinomas begin to appear after only a few weeks. In contrast, when "Dutch" female Sprague-Dawley rats are irradiated with neutrons, no mammary neoplasms (and thus no adenocarcinomas) begin to appear until some months after exposure (10,11). Thus, even within a strain, different "stocks" of rats show different mammary carcinogenic responses to neutron irradiation. To confirm clearly this stock (substrain) difference, TNO (Dutch) Sprague-Dawley rats were shipped to Brookhaven where they were given DMBA and compared to Brookhaven (American) Sprague-Dawley rats given the same DMBA dose. The "American" Sprague-Dawley rats showed a more rapid and larger mammary adenocarcinoma response during a 10 month period than did the TNO

rats. The Dutch Sprague-Dawley rats are inbred while the American Sprague-Dawley rats are not inbred. It may be that results from different laboratories can be compared only with difficulty even though the same strain of rat is used in both laboratories.

Turning to the "synergism" between the interaction of DES and neutron irradiation, the initial finding of Segaloff and Maxfield (2), and our own studies (4,5,6), were all done with DES. We wanted to know if this synergism held only for the synthetic estrogen DES or if the synergism held for "estrogen". Accordingly, Holtzman et al (12) irradiated female ACI rats with x-rays and then gave them either 17-ethinylestradiol (EE-2) or DES pellets. In this experiment, 140 cGy of 250 kVp x-rays was given 2 days before implanting a single pellet of either 2.3 mg of DES and 17.7 mg of cholesterol or 1 mg of EE-2 and 19 mg of cholesterol. All rats were studied for 190 days.It can be seen in Table 5, made-up from data in reference 12, that ethinylestradiol, as well as DES, interacted in a synergistic fashion with with irradiation on the number of mammary adenocarcinomas per rat. We concluded that the synergistic interaction between DES and radiation is not confined to just DES. Instead, these results implied that the synergistic interaction is a synergistic interaction between the estrogenic activity of DES and radiation and is not due to some unique factor of DES. Additionally, Broerse, et al (10) and van Bekhum et al (11) have shown that estradiol and radiation interact synergistically on mammary tumor formation.

Early in our studies we noted that female ACI rats treated with DES almost always developed pituitary tumors. Since it is widely known that estrogens are stimulators of prolactin secretion (13) we postulated that prolactin levels

might prove to be high in female ACI rats. The data in Table 6, taken from reference (14) show clearly that female ACI rats give a much larger pituitary response, serum prolactin response, and mammary adenocarcinoma response than did female Sprague-Dawley rats. The release of DES from the pellets was not different in the two strains. In this experiment 84 day old female ACI and Sprague-Dawley rats received a compressed pellet containing 5 mg of DES and 15 mg of cholesterol. [G-3H] DES (32.9 nCi/mg of DES) was incorporated into each pellet. Rats were killed at 2,10,28,56,130, and 214 days later along with control rats of the same age. Mammary adenocarcinomas appeared only in DES treated ACI rats. Pituitary weight was larger in ACI rats than in Sprague-Dawley rats. Holtzman, et al (15) found, data not shown, from day 56 onward that gross pituitary tumorigenesis was evident in the ACI but not in the Sprague-Dawley rats. At the microscopic level, adenomatous lesions involving prolactin cell hyperplasia were observed in both strains of DES treated rats. Multiple centers of marked vascularization predominated by granular acidophilic cells were more extensive and larger in pituitaries from DES treated ACI rats than in DES treated Sprague-Dawley rats. Of the two types of immunoreactive prolactin cells the polyhedral, strongly reactive and hypertrophic cells were more numerous in the ACI rats while the weakly reactive cells were found in both strains. Plasma prolactin levels were higher in the ACI rats than in the Sprague-Dawley rats at all time per/rats after DES. Mammary development, data not shown, was greater in the DES treated ACI rats than in the DES treated Sprague-Dawley rats. Increased uterine weights and pyometritis, data not shown, were found only in DES treated Sprague-Dawley rats. There is then, a positive correlation, in female ACI rats, between serum

prolactin levels and mammary development, mammary adenocarcinoma formation, pituitary weight, and pituitary tumor formation. In fact, we believe that the synergistic interaction between radiation and DES may be better described as a synergistic interaction between radiation and prolactin. In accord with this speculation are the numerous studies (16,17,18,19) showing a synergistic interaction between radiation and elevated serum prolactin produced by grafts of prolactin-secreting pituitary tumors.

Both radiation and diethylstilbestrol (DES) are carcinogens for the mammary gland of female ACI rats. When DES is given at about the same time as radiation, DES and radiation interact in a synergistic fashion particularly in regard to the number of mammary adenocarcinomas per rat. We have studied the effect of increasing the time interval between radiation and DES on the capacity of DES to enhance (promote?) radiation-induced mammary carcinogenesis in female ACI rats. DES, in the form of a compressed pellet containing a mixture of cholesterol and DES, formulated to average 1.25mg of DES per 100 grams of body weight, was given to groups of approximately 28 rats at either 2 days before, or 50, or 100, or 200 days after 6.4 cGy of 0.43 MeV neutron radiation. At each time that DES was given to irradiated rats, DES was also given to non-irradiated rats. All rats were studied for 375 days after the date of the DES administration. When the total number of mammary adenocarcinomas was evaluated (20) as a percentage of 24 sites per rat at-risk, the combination of DES and radiation always produced a response that was larger than the sum of the responses of DES alone plus radiation alone, Table 7 -- data taken from reference (21). This result suggests that these two agents can interact in a synergistic fashion. The interaction between radiation and DES

did not decline as the time interval between radiation and DES was lengthened. That is, the ratio of observed to expected, where observed is the combination of neutron and DES treatments and expected is the sum of neutron treatment alone plus DES treatment alone, ranged from 1.71 at -2 days (62.7/0.2 +36.5), to 3.11, to 2.54, to 1.65 at +50, +100, and +200 days respectively. Similarly, if the observed value at -2 days is taken to be 100%, then the 83% at +50 days, the 88% at +100 days, and the 124% at +200 days shows no decline as the interval is between neutron treatment and DES treatment is increased to 200 days. These results suggest that radiation induced (initiated?) mammary carcinogenesis is not subject to repair since DES enhancement (promotion?) continues to be effective over long intervals after radiation. These results are in full accord with those of Yokoro et al (18,19), where pituitary tumor grafts were used as a source of prolactin. These results are also in full accord with the initiation-promotion hypothesis.

We (22) have studied also the effect of increasing time intervals between 2 equal fractions of 3.2 cGy of 0.43 MeV neutrons on the total number of mammary adenocarcinomas in DES treated female ACI rats. In this experiment, female ACI rats were given two doses of 3.2 cGy with intervals of 0,1,2,4, or 8 weeks between doses. At 163 days of age, 2 days after the second neutron dose, they were given a 20 mg pellet containing 2.5 mg of DES and 17.5 mg of cholesterol. Other rats of the same age were given DES only or radiation only, or no treatment. All rats were studied for 290 days after DES administration. When the total number of mammary adenocarcinomas was evaluated (20) as an at-risk percent of a maximum of 24 per rat, Table 8 taken from reference (22),

no effect of increasing the interval between the two fractions was observed. We interpret these results as again indicating no repair of neutron-induced initiating events. These results also are in accord with the results of Yokoro et al, (18), who found no repair of x-ray-, or neutron- or N-butyl-N-nitrosourea-induced "initiation" when "promoted" by prolactin from grafts of prolactin secreting pituitary tumors. These results of Yokoro et al (18), along with our own finding (23) of synergism between DES and DMBA on mammary adenocarcinoma formation in female ACI rats, suggest that chemicals, as well as radiation, can act as initiating agents. In fact, we have suggested (23) that chemical carcinogens and radiation can be thought of as "interchangeable" carcinogenic agents.

We have also looked at the RBE of 0.43 MeV neutrons in two strains of rats, Sprague-Dawley and ACI, and also ACI treated with DES. (Table 9: made up with data from references 24 and 25). Using the measure of all mammary neoplasms, as corrected for intercurrent mortality, in some cases an inverse with dose relationship has been found with RBE values ranging from 10 to more than 100. However, in other cases the data are not good enough to show whether or not an inverse RBE with dose obtains. However, these RBE values, as well as other literature values obtained with additional strains of rat (10,11,17,18,19) suggest that high RBE values are not confined to Sprague-Dawley rats (26,27,28,29,30). Rather, high RBE values, in excess of 10, may be the general case for several strains of rat. This, in our opinion, makes it difficult to accept as being correct the failure to find an RBE value in excess of one in the Japanese (31) for breast cancer.

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Table 1. Interaction of Radiation and 3-Methylcholanthrene (MC) on Mammary Adenocarcinoma Formation in Female Sprague-Dawley Rats -- 100 Day Study.

Treatment	N	MAMMARY ADENOCARCINOMAS	
		Rats with	Total Number
Fission Neutron (100 cGy)	76	14	17
MC (40 mg)	76	29	38
Both	76	38	49
(Expected)		(43)	(55)
X-ray (376 cGy)	72	14	18
MC (40 mg)	71	27	36
Both	68	35	47
(Expected)		(39)	(51)

Table 2. Interaction of Neutron Radiation and Diethylstilbestrol (DES) on Mammary Adenocarcinoma Formation in Female ACI Rats -- 50 Week Study.

Treatment	N	MAMMARY ADENOCARCINOMAS Rats (%) with	Total (Per Rat) Number
None	31	0	0
Neutron (9.6 cGy)	33	2 (6)	3 (0.1)
DES (5 mg)	25	22 (88)	182 (7.3)
Both	35	32 (91)	842 (24.1)

Table 3. Mammary Adenocarcinoma Formation in Female ACI and Female Sprague-Dawley Rats. Interaction of Neutron Radiation and Diethylstilbestrol (DES) — 48 Week Study.

Treatment	MAMMARY ADENOCARCINOMAS					
	SPRAGUE-DAWLEY			ACI		
	N	Rats(%) with	Total(Per Rat) Number	N	Rats(%) with	Total(per rat) Number
None	33	1 (3)	1 (0.03)	13	0	0
Neutron(9.6 cGy)	34	5 (15)	8 (0.2)	23	2 (9)	2 (0.1)
DES(5 mg)	33	0	0	23	12 (52)	63 (2.7)
Both	33	1 (3)	2 (0.1)	23	21 (91)	347 (15.1)

Table 4. Interaction of Radiation and Diethylstilbestrol (DES) in Female Fischer Rats. — 350 Day Study.

Treatment	N	MAMMARY ADENOCARCINOMAS Rats(%) with	Total(per rat) Number
None	20	0	
X-ray(140 cGy)	20	0	
DES(1.0 - 3.9 mg)	81	0	
Both	82	12 (15)	17 (0.2)

Table 5. Interaction of Diethylstilbestrol (DES) or 17 Ethinylestradiol (EE-2) and Radiation on Mammary Adenocarcinoma Formation in ACI Rats. -- 190 Day Study.

Treatment	N	MAMMARY ADENOCARCINOMAS Rats(%) with	Total(per rat) Number
None	17	0	
X-ray(140 cGy)	18	0	
DES(2.3 mg)	19	9 (47)	
Both x-ray and DES	19	11 (58)	151 (8.0)
EE-2(1 mg)	23	20 (87)	123 (5.3)
Both x-ray and EE-2	24	21 (88)	300 (12.5)

Table 6. Responses of Female ACI Rats and Female Sprague-Dawley Rats to Diethylstilbestrol (DES) — 214 Day Study.

	Rats with Mammary Adenocarcinomas		Pituitary Weight(mg)		Plasma Prolactin ng/ml		DES Released (ug/day)	
Treatment	ACI	S-D	ACI	S-D	ACI	S-D	ACI	S-D
Control, Start	0/10	0/10	13	12	39	11	--	--
2 Day DES(5mg)	0/10	0/10	10	13	380	39	38	38
10 Day DES	0/10	0/10	13	12	412	68	16	15
28 Day DES	0/9	0/10	19	19	414	136	9	8
56 Day DES	0/10	0/5	28	16	1297	100	6	5
130 Day DES	9/10	0/10	42	12	1399	69	4	3
214 Day DES	9/10	0/6	61	13	3147	85	3	2
Control, End	0/10	0/10	12	16	88	23	--	--

Table 7. Effect of Increasing the Interval Between 6.4 cGy of 0.43 MeV Neutron Radiation and 1.25 mg of Diethylstilbestrol (DES) on Total Mammary Adenocarcinoma Response in Female ACI Rats as Percent At-Risk per 24 Tumor Sites per Rat per Group.— 375 Day Study. Approximately 24 Rats per Group.

ALL MAMMARY ADENOCARCINOMAS AS
PERCENT OF 24 SITES AT RISK PER GROUP

Days Between DES and Neutrons	Neutrons Only	DES Only	Both DES and Neutrons			
			OBS	EXP	OBS/EXP	% of -2
-2	0.2	36.5	62.7	36.7	1.71	100
+50	0.2	16.6	52.3	16.8	3.11	83
+100	0.9	20.8	55.1	21.7	2.54	88
+200	4.1	43.1	77.8	47.2	1.65	124

Table 8. Effect of Increasing Interval Between Two Doses of 3.2 cGy of 0.43 MeV Neutrons Plus 2.25mg of Diethylstilbestrol (DES) Two Days After Second Radiation on Total Mammary Adenocarcinoma Response as Percent At-Risk Per 16 Tumor Sites Per Rat Per Group. — 290 Day Study. Approximately 42 Rats Per Group.

Interval Days	Neutron Radiation cGy	DES mg	Percent At Risk
--	--	--	0
0	3.2+3.2	--	0.3
--	--	2.25	23.8
0	3.2+3.2	2.25	51.7
1 week	3.2+3.2	2.25	57.0
2 week	3.2+3.2	2.25	51.3
4 week	3.2+3.2	2.25	48.5
8 week	3.2+3.2	2.25	51.0

Table 9. Estimates of RBE.

STRAIN	TREATMENT	DAYS OF STUDY	MEASURE	DOSES STUDIED (cGy) NEUTRON	X-RAY	ESTIMATED RBE RANGE	RBE INVERSE WITH DOSE
S-D	None	1000+	Total AC+FA	0.1-6.4	28-85	10-100	Yes
S-D	None	1000+	Total FA	0.1-6.4	28-85	10-100	Yes
S-D	None	1000+	Total AC	0.1-6.4	28-85	>10	?
ACI	None	720	Total AC+FA	4.5-36	37-300	>10	No
ACI	None	720	Total FA	4.5-36	37-300	>10	No
ACI	None	720	Total AC	4.5-36	37-300	>10	No
ACI	DES	520	Total AC+FA	1.0-9.0	17-150	20-100	Yes
ACI	DES	520	Total FA	1.0-9.0	17-150	?	?
ACI	DES	520	Total AC	1.0-9.0	17-150	20-100	Yes