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**RADIATION CARCINOGENESIS -
LABORATORY STUDIES**

by

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MASTER

In the words of Peter Alexander, (1), it is a paradox that radiations both cure and cause cancer. In today's world of an increased concern for the environment we are aware that we live in a sea of hazardous agents. This has resulted in a sea of benefit-risk equations. I have a suspicion, or better a conviction, that no radiation therapist, today, assumes that the risk value is absolutely zero for all forms of radiation therapy.

To evaluate the carcinogenic "risk", there are at hand data from both animals and from man. Drs. Wasserman and Berlin and Dr. Hutchison will deal with the human data in the 2 following presentations. In regard to animal data, I fully subscribe to the conclusion of the 1973 United Nations Report on Ionizing Radiation, page 402, "The carcinogenic effects of radiation are best evaluated by human population studies. Because of the great differences in susceptibility to cancer induction between human beings and other species, studies with experimental animals provide information of more qualitative than quantitative significance." (2) In a similar vein, this U.N. report goes on to state that animal studies are most useful in the establishment of generalizations and help clarify the mechanisms of radiation carcinogenesis.

I now propose to follow the format of this U.N. Report, both in regard to providing information of qualitative rather than quantitative usefulness, and in regard to subject headings, such as RBE, dose-rate, etc. adding to, and differing from, their conclusions where I think such differences are appropriate in regard to the subject of this symposium. I thus hope to avoid a lack of completeness and personal bias while at the same time allowing for the inclusion of some newer data that seem of interest. I will limit myself largely to animal studies of carcinogenesis following external radiation since internal emitters are to be covered in another presentation.

First, what may seem to be a digression, a brief discussion of radiation-induced life shortening. Most laboratory experiments have shown that life span is shortened by radiation exposure. The question of mechanism remains. The U.N. report tends to down-grade the possibility that radiation-induced life shortening is due to the advancement in time of all causes to death, in favor of the possibility that radiation-induced neoplasia itself is the more important cause of life shortening. Cited in this regard are the experiments of Mole (3) with neutrons, Grahn and Sacher (4) with low LET radiation, and Bustad (5) with internal emitters. However, the committee that produced this U.N. Report asks for additional experiments to verify the concept that radiation-induced neoplasia is largely responsible for radiation-induced life shortening.

Now, I want to attempt to deal with dose-effect or dose-response relationships. You will all remember the possible usefulness of dose-response relationships to predict effects of exposure to radiation at levels too low to examine experimentally along with the attendant theoretical curves that may or may not have a threshold. You will recall also that dose-response curves can be altered by radiation quality. And you will remember that dose-response curves can be altered by the end-point selected for study, that is, the particular type of tumor to be studied and the choice of age specific incidence or final cumulative incidence.

The data (Figure 1) are the data taken from Figure II, page 385, of the now familiar U.N. Report. The data are the same, but I have chosen to plot them on a log-log scale. Each axis extends over some 3 orders of magnitude. Curve A is the data of Upton (6) on myeloid leukemia in mice as induced by total body x-irradiation. Curve B, my own data (7) on mammary tumors in rats as produced by total body gamma-irradiation. Curve C, the data of Kaplan on thymic lymphoma in mice produced by total body x-irradiation. Curve D, the data of Maldaque (9) on kidney tumors in rats produced by localized x-irradiation. Curves E and F come from Burns (10) on skin tumors in rats produced by localized alpha particles or electrons. Single, acute, dose is plotted against percent incidence with the control incidence subtracted. This plot illustrates very well the complexities of dose-response relationships.

Clearly, the doses at which the rise in incidence becomes detectable, the dose at which the maximum incidence is reached, and the doses at which the decline in incidence begins, differ for different neoplasms. Just as it seems likely to many that there will be no single "cancer cure" it seems equally unlikely that a single dose-response relationship will describe radiation carcinogenesis.

Before going on to discuss how dose rate and type of radiation may change dose-response relationships, I would like to make an additional observation about these data. Curve B is different from all other curves because in this experiment all of the rats were killed one year after exposure while all of the other experiments were continued over the life-span of the animals. Curve B represents then, an acceleration of the neoplastic response, or what Casarett (11) calls a "temporal advancement", or in a statistical sense, a radiation-induced increase in age-specific incidence. It should not be forgotten, that in a health physics or radiation protection sense, the early appearance of a tumor may be of even more biological consequence to the animal that carries the tumor than simple tumor incidence statistics might indicate. The problem is not yet solved where one wishes to quantitate, in biological terms or even human terms, the consequences of the risk of developing an early appearing and early killing tumor vis a vis the risk of increasing the incidence of a late appearing tumor.

The data on this plot can also serve to point out something about thresholds, although I realize that a linear plot would be better for this purpose. If a threshold exists for Curve B, it must be below about 15 rads. On the other hand, an apparent threshold of some 800 rads obtains for Curve D. Even here, it has not been disproved that there is an extremely low but slowly increasing risk with increasing dose at doses below 800 rads. To learn if there is an increasing risk with increasing doses below 800 rads, in the case of Curve D, requires massive and expensive experiments. The UN committee would give such experiments a low priority. So would I.

Before leaving these data, again look at how complex they are. Although the carcinogenic mechanisms are not well understood, it seems reasonable in all curves, except possibly at the lower end of Curve B, that enough radiation has been applied to cause considerable cell killing and some tissue destruction. A viral etiology obtains for Curve C, possibly for Curve A, but probably not for Curves E and F. Only the tumors in Curve B can be produced easily also by chemical carcinogens. It is known that hormones can modify the tumor response to radiation in Curves A, B, and probably C. Curve B represents a scopal carcinogenic mechanism, where induced tumors occur largely only in the irradiated volume, while Curve C represents an abscopal carcinogenic mechanism because the thymus that develops a thymoma does not itself need be irradiated.

I would now like to turn to the even more complicated situation, that of dose-response relationships confounded by dose-rate effects. First, the general theory. If the induction of a neoplasm requires that two or more events occur within a small volume, within a sufficiently small amount of time to preclude repair of the initial event, then low LET radiations delivered at low dose-rates would be expected to result in a low probability of the required events occurring simultaneously. ~~Thus~~ sufficient time might elapse between the events for repair to take place. This is the postulated mechanism of the "sparing" effect of low dose-rate. The sparing effect of low dose rate, would be expected to be confined to low LET radiations. Almost all fractionated and protracted, low dose-rate, low LET animal experiments show, or can not exclude, a lower tumor induction efficiency per rad when the radiation exposure is spread out in time. There may be either exceptions, or experiments not precise enough to allow detection of a sparing effect. One experiment that perhaps did not show a sparing effect, is shown in the next (2nd) figure. Again, this is an experiment on the induction of mammary tumors in rats, with up to 32 fractions of gamma radiation spread over 16 weeks (12). I suggested, when this was first published, that if a sparing effect was present, it was small. Others (13) thought a sparing effect was present. This unresolved argument is, I think, really over when the tumors are scored, either from the time of the first exposure, or from the time of the last exposure. In any event, in the case of low LET radiation, it is likely that low dose-rates will be less efficient per rad than high dose-rates even though the loss of carcinogenic efficiency has not been quantitated on a general basis, although some numbers have been given for a single tumor system.

A much more elegant experiment has been published recently by Yuhas (14) on the induction of ovarian tumors in BALB/c mice, looking at both dose-rate effects and dose-response effects. Dr. Yuhas used total doses of 49, 98, 196, or 392 rads, at dose-rates of 1.75, 3.5, 7, 14, 28, 56, or 112 rads per day to form a 4 x 7 matrix of 28 points with 200 mice per point. When the excess cases of ovarian tumors are plotted against dose (Fig. 3) it can be seen that there was a reasonably linear dose-response relationship that showed a decreasing slope as dose-rate decreased. Since the longest exposure time was 224 days (1.75 rads per day x 224 days = 392 rads), Yuhas has done an additional experiment to determine the effect of age at the time of exposure. If the observed decline of sensitivity with age is then "subtracted" from the overall result, then he concludes that the response, that is excess cases, increases with the square of dose but decreases with the square root of exposure time. I look forward to, what I am sure will be, alternative interpretations of these data. I have cited this experiment of Yuhas partly because it demonstrates both the magnitude and complexity of experiments that must be done, before there can be a real understanding of the interaction of dose and dose-rate effects. And, it should be reaffirmed again that the dose rate sparing effect found by Yuhas for ovarian tumors in mice obtains only for this tumor system.

As a way of summarizing dose-response and dose-rate effects of low LET radiation on carcinogenesis and as a way of introducing the next subject-high LET radiation, I would like to examine the results of Upton (15). While at Oak Ridge, Dr. Upton completed an enormously large experiment that involved some 11,669 mice, neutron and x- or gamma-ray dose rates ranging from 10^{-6} to 10^2 rads per minute, and total doses of neutrons ranging from 1.6 to 930 rads, and total doses ranging from 25 to 7875-rads for x- and gamma-rays. I have selected the following two statements from the conclusions of Upton because I believe these statements fairly represent his findings, "Incidence of myeloid leukemia and the thymic leukemia increased with dose, also varying with dose rate of gamma-rays but not of neutrons." And, "Neutrons were equally or slightly less leukemogenic than x- or gamma-rays at high dose rates but several times as leukemogenic as gamma-rays at low dose rates." In other words, neutrons may appear to have an increasing RBE, for carcinogenesis, as neutron radiation is compared to x-rays at lower and lower dose rates and at lower and lower doses, not because neutrons become increasing efficient but because x-rays become decreasingly less efficient.

From the above, it seems logical to anticipate that fractions of neutron radiation will be additive, in contrast to low LET radiation, where fractions will be less than additive. There are in fact, two instances, that I know of, where high LET radiation has been claimed to be more than additive. An experiment in progress, (16) at the Argonne National Laboratory prosecuted by Ainsworth, has already produced the following result, "... administration of the total neutron dose of 240 rads in 6, 24, or 72 fractions produces higher death and tumor rates than occur when the same total dose is administered as a single fraction." This finding of Ainsworth is even of more interest when placed in context with the report of Spiess and Mays (17) where they find that ^{224}Ra given to children produced more bone sarcomas as the dose was protracted. I have been sufficiently titillated by these two provocative reports to set-up an experiment designed to investigate the possibility that split doses of neutrons might produce more breast tumors in rats than the same dose given only once.

A somewhat unique approach toward the understanding of the interaction of RBE, and of dose and dose rate effects has been taken by Drs. A.M. Kellerer and H.H. Rossi. They have put forward a theory, called the dual radiation injury theory (18) which states, in part, that the dependence of RBE on dose for certain biological effects is related to primary lesions that may be produced by one or two high LET particles but that require the action of at least two electrons when electromagnetic radiation is applied. When this theory of dual radiation radiation injury was applied to the induction of mammary neoplasms in the female Sprague-Dawley rat by either neutrons or x-rays, they concluded (19) tumor induction was dependent upon action of more than one cell and that a linear relation between incidence and x-ray dose was unlikely.

After the publication of these conclusions, I have provided Kellerer and Rossi with additional data, and I will now show the data and the conclusions, skipping over the exact data analysis (20). First, (Figure 4) small doses of 0.43 MeV neutrons induce mammary neoplasia in the Sprague-Dawley rat. At 14 months after exposure, 1.6 rads produced an incidence larger than the control incidence. At even earlier time periods, 10 months, even 0.1 and 0.5 rads produced an incidence larger than the control incidence. When these data are plotted, (Figure 5), at 10 and at 14 months after exposure, it can be seen that there is a high RBE. Finally, when these data are plotted in terms of RBE versus dose (Figure 6) the RBE is much larger, about 100, at the low dose region than at the high dose region, about 8. These findings are in accord with the Kellerer-Rossi theory of dual radiation injury and these data are in accord with their (21) analysis of the Japanese leukemia data in regard to neutron RBE. Again, these findings must be related to only the tumor system studied.

For my summation, I shall return to my beginning. Studies of experimental radiation carcinogenesis can provide information of more qualitative than quantitative significance in regard to human radiation carcinogenesis. From the animal experiments that I have reviewed above, the following points emerge.

1. It is likely that the major component of radiation-induced life shortening comes from radiation-induced carcinogenesis.
2. For low LET radiation, a wide range of dose-response relationships obtain, over a wide range of doses.
3. For low LET radiation, lengthening the time over which radiation is given, generally but not always, lowers the risk of tumor induction.
4. For high LET radiation, the dose-response relationship usually tends to appear to be linear.
5. For high LET radiation, lengthening the time over which the radiation is spread does not appear to lower the risk of tumor formation, and in two instances, fractionation of neutron radiation appears to have increased the tumor risk per rad.
6. No single RBE for high LET radiation for carcinogenesis can be given. The trend of current studies is clearly pointed in the direction of higher RBEs at lower doses.
7. Recent studies, taken together and at face value, appear to support the concept that at low doses and at low dose rates, low LET radiation may have a lower carcinogenic potential than thought earlier but that high LET radiation may be even more effective than previously thought.

Finally, to provide quantitative estimates of risk for the human situation from animal data, I think that one must proceed on a tumor by tumor basis. For each specific human tumor, a model system must be developed where the burden of proof is on the one who proposes the model. In regard to breast tumors, Young and Hallows (22) are of the opinion that both structurally and functionally induced rat mammary tumors bear a marked resemblance to their human counterparts. From my ~~own~~ work (23) it seems clear that radiation induces rat mammary tumors by a scopol mechanism. The report of Myrden and Hiltz (24) states, "breast carcinoma frequently occurred in the breast on the same side as the fluoroscopic rays were concentrated." These three ideas, when taken together along with the previously discussed suggestion that neutron induced mammary tumors of the rat are a function of dose, lead to the following statement. There is no risk-free dose of neutrons for human breast tissue when the breast tissue itself is irradiated. This statement is better taken as a qualitative estimate. It can, be taken as a quantitative estimate of risk only in the sense that the value of the risk is some number other (larger) than zero.

Figure 1. Data from Figure II of the United Nations Report, Ionizing Radiation: Levels and Effects, Volume II, page 385, 1972, reference 2. Log-log scale. Percent with neoplasms after control incidence was subtracted. Curve A, myeloid leukemia in mice after x-rays, reference 6. Curve B, mammary tumors in rats after gamma-rays, reference 7. Curve C, thymic lymphoma in mice after x-rays, reference 8. Curve D, kidney tumors in rats after x-rays, reference 9. Curves E and F, skin tumors in rats after electrons of alpha particles, reference 10. (BNL 12-262-74)

Figure 3. Ovarian tumors in mice plotted against dose at different gamma-ray dose rates. Reference 14. (BNL 12-263-74)

Figure 1. Incidence of mammary tumors at 14 months after either neutrons or x-rays.

Radiation	Measure	Months														Σ
		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	9	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	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	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
8.5 rad	All tumors					3	6	8	9	14	19	25	27	31	33	
	Adenocarcinomas					1	1	2	2	3	3	3	3	4	4	

Figure 5. Incidence of mammary tumors at either 10 or 14 months after either neutrons or x-rays. Reference 20. (BNL 8-1140-73)

Figure 6. Log-log plot of RBE versus dose for 14 month data of rat mammary tumors. Reference 20. (BNL 8-1139-73)

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