

RADIATION TOXICOLOGY

by

R.J.M. Fry, J.B. Storer and R.L. Ullrich

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R. J. M. Fry, J. B. Storer and R. L. Ullrich

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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Radiation, unlike some of the chemicals about which there is concern today, has been present through man's evolution. The heat from the radioactive elements in the earth's crust has helped shape its surface. We know less about the way in which radiation has influenced the design of man although it seems reasonable to believe exposure to both ionizing and non-ionizing radiation has influenced the development of systems capable of repairing damage to DNA and recovery from both the consequences of DNA damage and also from lesions in molecules and structures not associated with DNA. Although a small fraction of the incidence of cancer is attributed, by some, to environmental radiation it is not known with certainty that background radiation does cause cancer.

The natural radiation background varies, depending on geographical location, by as much as a factor of 20.² Unfortunately, there are no adequate studies of the human populations such as those on the Kerala coast in India that are exposed to about 2 rem/yr background radiation or those in Brazil exposed to high background radiation from the monozite sand. It would be very valuable to compare the effect of background irradiation in these populations and those exposed to less than 100 mrem/yr. Despite the profound difficulties in such an epidemiological approach Frigerio and Stone³ considered it so attractive that they examined cancer rates in relation to the varying levels of background radiation within the U.S. Although the results suggested a negative correlation between radiation levels and cancer mortality the problems of lack of uniformity of medical treatment, the recording of cancer mortality, and the fact that the range in the background levels is small compromise any conclusions.

In the case of ultraviolet radiation (UVR) a very clear cut association has been established between the environmental level of the radiation at different latitudes and the incidence of skin cancer.^{4,5}

Time and Radiation

Time after irradiation is important for the expression of the biological effects and the interval, or latent period, between the exposure and the development of tumors is long. Time-dose relationships, such as dose-rate and fractionation have a profound influence on the effect of irradiation.

Latent Period

The amount of time necessary for the expression of different types of radiation-induced lesions varies with the nature of the lesion and is also influenced by the total dose of radiation. Some effects, such as cancer, are expressed months or years after exposure and this is one of the difficulties in epidemiological studies of radiation carcinogenesis. Obviously, exposure to many other factors in the interval between radiation exposure and tumor appearance complicates the studies. Similarly in animal experiments competing risks from diseases other than that under study confound the analyses. It is established that radiation can act as a complete carcinogen, an initiator, and also interacts with other carcinogens. But the role of irradiation, particularly low dose protracted irradiation, in enhancing or promoting the expression of tumors induced by various other agents is not understood. The importance of such interaction is shown by the finding that uranium miners who smoke had 10 times the excess of lung cancers than miners who did not smoke.⁶

In the case of UVR, Blum suggested that the effect of many of the later fractions in the multifraction regimes necessary for carcinogenesis was on the expression of the initiation events.⁷ This idea has been confirmed. When the

promoter phorbol ester is used after a regime of UVR a comparable incidence of tumors is produced with many less fractions of UVR.⁸ In the case of protracted or fractionated exposures of ionizing radiation there has been very little work that allows a separate examination of the effect on initiation and an expression of the initial lesions.

Experimentally, the term "latent period" has various meanings depending on the organ, the endpoint, and the methods of detection of the selected endpoint. For example, the latent period for skin tumor development may be from the time of exposure to the appearance of the first tumor of a size that can be recognized. As very small tumors can be recognized in the skin the estimate of time of appearance may not be much greater than the time for the necessary cell divisions for the growth to a size that can be seen by the eye, and any time that may exist between the exposure and the onset of tumor growth. The latent period, for lung tumors, if based on the time from the exposure to time of death due to the tumor, will depend very much on the degree of malignancy of the tumor. Admittedly, most of the natural history of a tumor is over by the time it is detected. Despite the lack of understanding of the biology of the latent period, Blum,⁷ Druckery,⁹ and more recently Albert and Altshuler¹⁰ have found that the time of tumor appearance has a log normal distribution that is dependent on dose rate and that the dose rate multiplied by a power of the median time to appearance is a constant. It has been accepted by some authors that higher doses result in earlier appearance of tumors than with lower doses.^{11,12} Unfortunately, there is no body of data that show unequivocally that the time of appearance is dependent on dose and independent of the change in incidence that accompanies higher doses of the carcinogenic agents.

The distinction between advancement of the time of appearance of naturally occurring tumors and the induction of tumors is not a trivial matter and is fundamental to the understanding of mechanisms and susceptibility to cancer induction. There are a number of tumors that occur with a high natural incidence in rodents; in some strains the incidence may reach 100%. Presumably the observed radiation effect in such cases can only be related to the time of appearance. In Figure 1 the dose-dependent change in time at death from lung tumors after exposure to fission neutrons is shown. In this hybrid mouse no dose-dependency for the number of mice dying from lung tumors was found but only a change in time of death after exposure.¹³

The marked difference in time to appearance of tumors in various species is one of the facets that must be understood if extrapolations across species are to be made. The equally marked difference in life span between species has encouraged interest in the idea that the temporal pattern of tumor response is dependent on life span.^{14,15} The idea of finding a correction factor for life span differences that allows meaningful comparisons or even extrapolations for tumor rates across species is attractive but perhaps too optimistic.

Dose-Response Relationships

The data for radiation-induced cancer in humans is still insufficient to determine the shape of the dose response curves. Furthermore, human studies have shed little or no light on the mechanisms of tumorigenesis. Studies in radiation carcinogenesis in experimental animals are useful for the investigation of mechanisms, for the determination of time-dose relationships, such as the effect of dose rate and fractionation, and to obtain dose response curves at least of sufficient quality to test models.

The simplest models suggested for the dose response curves for high linear energy transfer (LET) radiation, such as neutrons, and low-LET radiation such as gamma radiation are linear and curvilinear respectively (Fig. 2). Nowadays the equations that describe the curves usually have some correction for cell killing. It is not surprising that these models, if it is appropriate to call them that, are too simple. First of all it is clear that the mechanism of tumorigenesis, though not necessarily induction, involves different factors. For example, the mechanisms involved in the production of hormonal dependent tumors must surely be different from the production of a sarcoma. So there is no a priori reason that one model will be suitable for more than one or a small number of tumor types. In tumor dose-response curves tumor incidence is often plotted as a function of dose but the occurrence of a tumor involves not only the malignant transformation of a cell but also all the factors that influence the subsequent expression (and repression). The models for dose response curves are really based on the dose response of initial events or transformation and not sufficient for what many workers believe to be a multistage process.

An example of the influence of hormones on radiation-induced cancer is shown in Fig. 3. Pituitaries were grafted into the spleens of mice to increase the level of prolactin. This treatment advanced the time of appearance of the small number of naturally occurring Harderian-gland tumors (data not shown in Fig. 3) without any significant increase in the incidence. When pituitaries were grafted into mice after they had been irradiated there was marked increase in the tumor prevalence compared to mice exposed only to radiation. For Harderian gland tumors the increased prolactin level appears to act as a promoter.

The effect of the pituitary isografts on mammary tumors was quite different. The cumulative natural incidence of mammary tumors in B6CF₁ mice is about 1%. The increased prolactin levels from the pituitary isograft resulted in an incidence of 43% of mammary tumors. If we assume that the raised prolactin levels maximize the expression of the radiation-induced transformation in both the mammary and Harderian glands then the excess tumors in mice exposed to radiation compared to the tumor incidence in mice with pituitary isografts and irradiation should be a measure of the radiation induced initial events. It was found that the effect of both gamma and neutron irradiation on the incidence of mammary tumors was very small compared to the effect of altering the prolactin level. Furthermore the effect on tumorigenesis of the combined hormone and radiation treatment that could be attributed to the radiation was much less in the mammary gland than with the Harderian gland.

The results shown in Fig. 4 (unpublished data, Fry, R. J. M., Grube, D. and Ley, R. D.) provide a good example how the expression of the initial tumor induction events influence the shape of the dose response curve. The curve on the right representing the incidence of skin cancer as a function of total dose of UVR in mice photosensitized with 8-methoxypsoralen (8-MOP) appears sigmoid in shape and shows a threshold. When the total doses were reduced by decreasing the number of fractions but the exposure to UVR + 8-MOP regimes were followed by promotion by 12-O-tetradecanoyl phorbol-13-acetate (TPA) the shape of the dose response curve appears to be more linear and without a threshold. These results suggest: (1) that with the UV radiation some of the transformed cells did not express their tumor potential, and (2) accurate dose response relationships for the induction or initial events of transformation

are not necessarily represented by the incidence of cancer as a function of dose.

For other tissues and organs, we need similar techniques that will allow us to dissect out the radiation-induced initial events from the influence of endogenous and exogenous factors that influence the final tumor incidence.

The Influence of Exponential Results on Human Risk Estimates

The use of linear interpolation from higher dose levels for estimating the human risk of cancer has been accepted as a conservative approach for handling the data for low-LET radiation and a reasonable method for high-LET radiation. Recently, it has been suggested that even in the case of low-LET radiation that interpolation could underestimate the effects.¹⁶ The available experimental data do not support such a suggestion.

In an attempt to illustrate some aspects of this question we have made a comparison of linear fits of the data for dose responses from a number of different tumor types obtained after exposure to radiation at (a) high dose rate (above 7 rad/min) and (b) low dose rate (below 0.06 rad/min).¹⁷⁻²⁰ The results are shown in Fig. 5. The solid lines indicate the range of slopes obtained from linear fits to the data for the responses of the selected tumors after exposure to radiation at a high dose rate. The slopes of the linear fits of the data for the responses of the same tumor types after exposure to low dose-rate irradiation are shown individually. It can be seen that all of the responses to irradiation at low dose rates are less than the range of responses to irradiation at high dose rates. The relative range of dose levels is indicated for the different tumor types. For example the zero slope for thymic lymphoma is for the data up to 100 rad. It is clear that the effect of

lowering the dose rate is tissue dependent and varies considerably. The results for myeloid leukemia after low dose irradiation are of particular interest. The experiments of Upton et al., and Ullrich and Storer were carried out on the same strain of mouse (RFM) but in the case of Ullrich and Storer's experiment the mice were maintained in a specific-pathogen-free facility. The explanation for the difference in the results from these studies is not known but perhaps the different microbial environment results in a difference in the number of myeloid stem cells at risk. What is clear is that simple interpretations of dose response curves based purely on biophysical aspects are unwise. It can be seen from Fig. 2 that in the case of low-LET radiation that linear interpolation will overestimate risks for tumors with a curvilinear dose-response. But without a precise knowledge of the dose response relationships for different types of radiation-induced cancer there was no choice for the advisory bodies concerned with radiation protection; they had to recommend the use of a linear no-threshold model.

In the case of high-LET radiation if a linear interpolation of the data obtained from relatively low doses is made and the dose response curve bends as shown, the risk for low doses of high-LET radiation will be underestimated. In Fig. 6 it can be seen that the curve for the incidence of Harderian gland tumors as a function of dose of fission neutrons bends over. As the mice in this experiment had pituitary isografts before irradiation, which we assume maximizes the expression, the bending is probably due to factors influencing the initial events of induction. The reason for the bending over is not known but may reflect cell killing.²¹ Another possibility is that with high-LET radiation there is a linear increase as the number of effective targets traversed by a particle increase but as soon as the dose reaches a level at

which each effective target has been traversed by a particle the curve saturates. The distinction between cell killing and the saturation effect is difficult and, of course, both may be involved.

Human Risk Estimates

Estimates of risk are expressed in one of two ways, either in absolute or relative terms. For radiation protection, absolute risk estimates are usually used and the absolute risk is expressed as the number of excess cases of cancer assumed to be radiation-induced per unit of time in an exposed population of stated size per unit of dose; for example, 1 case/ 10^6 persons/year/rad. Such estimates normally assume a linear dose-response relationship for dose levels for which there are no data. The estimate of the total risk to a population exposed also requires knowledge about the period of years over which the excess risk exists. Except for radiation associated leukemia it is not known for how long an excess risk exists. In the case of solid cancers the excess risk may last to the end of life. In the case of leukemia the risk decreases after 20-25 years after exposure. The risk period is also dependent on the type of tumor and perhaps the age at time of exposure of the person. In persons exposed at older ages (over 50) the risk of developing a radiation-associated tumor is offset by competing risks. Relative risk is the ratio between the irradiated population and the risk in the nonirradiated population and is expressed as a multiple of the natural risk. The dose that doubles the natural incidence is referred to as the doubling dose. If the natural incidence determines the susceptibility and the increase in risk after radiation is proportional to the natural risk then the use of relative risk would be appropriate. The importance of understanding the relationship of the natural incidence of a tumor to the response to radiation is not just a

matter of interest in risk estimates but could provide a possible insight into mechanisms.

The determination of the relationship of the natural incidence with radiation response would seem amenable to experimental validation. Surprisingly the question has not been systematically investigated. The data in Table II show that in the case of the tumors selected it is not possible to eliminate the possibility that the risk of radiation carcinogenesis is influenced by the natural incidence. The paucity of cases of chronic lymphocytic leukemia in the atomic bomb survivors and in the rest of the Japanese population is consistent with the hypothesis that natural incidence does influence the response to radiation. It seems surprising that there has been so little attention to this problem.

Conclusions

The extensive studies on both human and experimental animal population have provided information that allow radiation protection standards to be set with greater confidence than for most if not all other carcinogenic agents. Furthermore, both international and national advisory bodies are continually updating the risk estimates and the standards as new information is available. However, it is clear that we need models that take into account the multistage nature of carcinogenesis. Studies in both ionizing and ultraviolet radiation carcinogenesis are more valuable to the general problem of elucidating the mechanisms involved in cancer than is indicated by the amount of work or support for this field of research.

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Table 1

TYPE OF RADIATION	SOURCE		WAVELENGTH NM	MOLECULAR LESIONS	ADVERSE BIOLOGICAL EFFECTS
	NATURAL	OTHER			
GAMMA	RADIOACTIVE MINERALS	MEDICAL	1×10^{-4}	SINGLE STRAND BREAKS DOUBLE STRAND BREAKS BASE DAMAGE	CELL KILLING MUTAGENESIS TERATOGENESIS CARCINOGENESIS
			1.4×10^{-1}		
X-RAYS	SUN	MEDICAL	5×10^{-4} - 20		
ULTRAVIOLET	SUN		40 - 390	PYRIMIDINE DIMERS BASE DAMAGE THYMINE PHOTOPRODUCTS DNA-PROTEIN CROSS- LINKS	CELL KILLING CARCINOGENESIS
					> 320 NM CONSIDERED NONCARCINOGENIC
VISIBLE	SUN		390 - 780		
INFRARED	SUN		$780 - 4 \times 10^5$	THERMAL DAMAGE	CELL KILLING
RADIO WAVES	SUN	RADAR	$10^5 - 3 \times 10^{13}$	THERMAL DAMAGE	CELL KILLING DEAFNESS! ?
		TV RADIO			
POWER A.C.		ELECTRIC POWER LINES	10^5	?	?

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Table 2

RELATIONSHIP OF NATURAL INCIDENCE AND SUSCEPTIBILITY		
TUMOR TYPE	NATURAL INCIDENCE %	RESPONSE TO γ RADIATION TUMORS/RAD
OVARIAN RFM	2.4	0.39
OVARIAN BALB/c	6.4	1.2
MAMMARY GLAND BALB/c	7.0	0.12
MAMMARY GLAND BCF ₁	1.2	0.01

FIGURE LEGENDS

- Fig. 1. Time to a selected mortality rate due to lung tumors in female B6CF₁ (An1) mice as a function of dose of Janus fission neutrons.
- Fig. 2. Schematic dose response curves for tumor incidence after exposure to high and low LET radiation with linear interpolations through selected points of the curves.
- Fig. 3. The prevalence of Harderian gland tumors in female B6CF₁ (An1) after pituitary isograft only o—o after a single exposure to 64 rad fn only e—e and to 64 rad fn followed by pituitary isograft on the same day Δ—Δ.
- Fig. 4. The percent of mice with squamous cell carcinomas as a function of total dose of 320-400 nm UVR given in various numbers of fractions plus 8-methoxypsoralen o—o and similar exposures but followed at the end of the fractionation regime by treatment with 5 μg of TPA 3/week.
- Fig. 5. Plot of the slopes of tumor incidence as a function of dose derived from linear interpolation of data assuming no threshold for exposures to high dose rate irradiation solid lines and for the same
- Fig. 6. The adjusted incidence of Harderian gland tumor as a function of dose of Janus fission neutrons in mice with pituitary grafts before irradiation x—x. The incidence of tumors in mice exposed to 64 rad fn and that received pituitary isografts post irradiation is indicated: 0

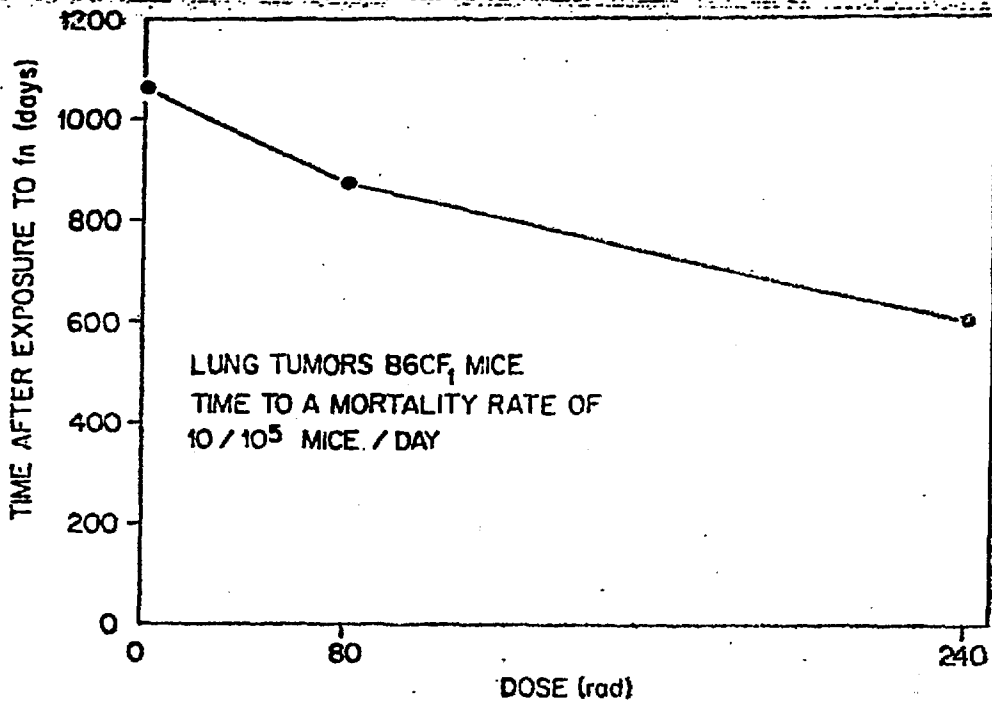


Fig 2

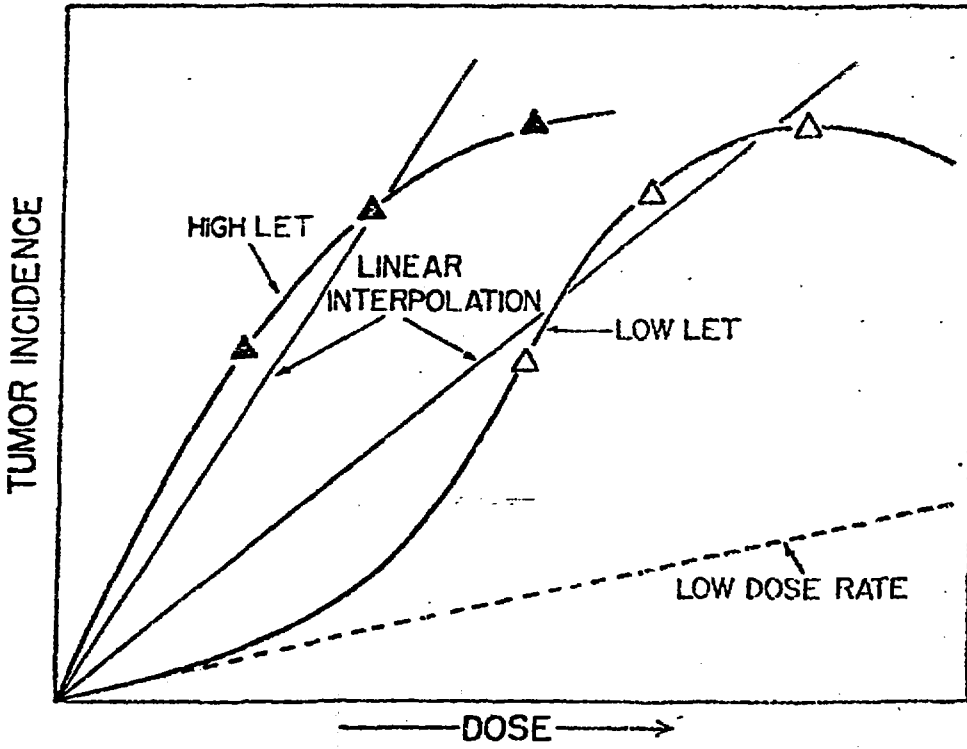
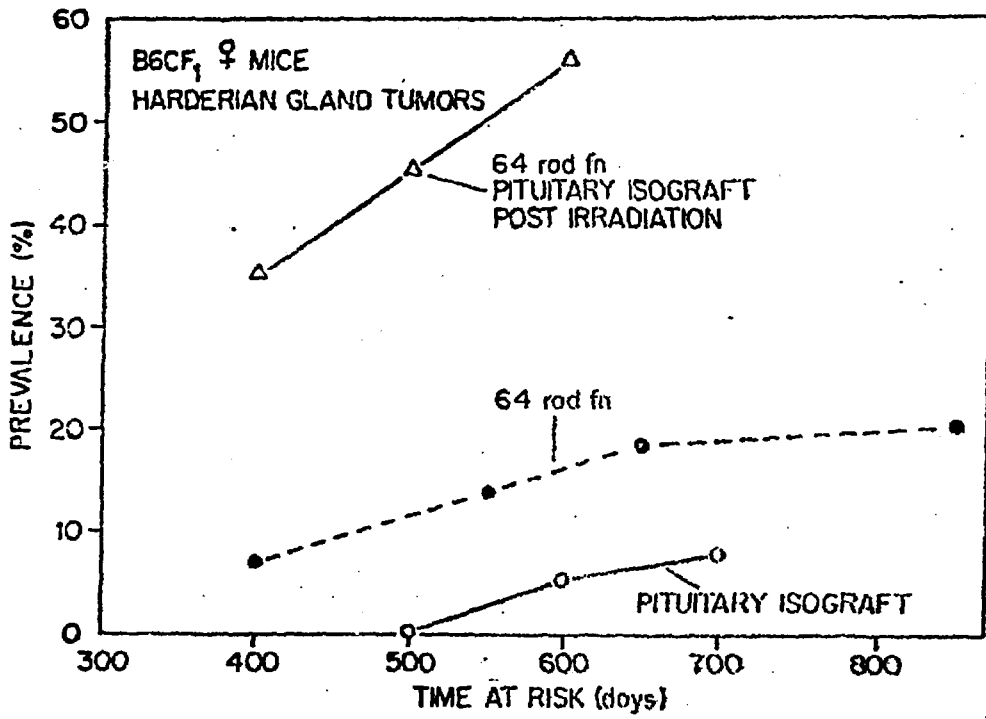
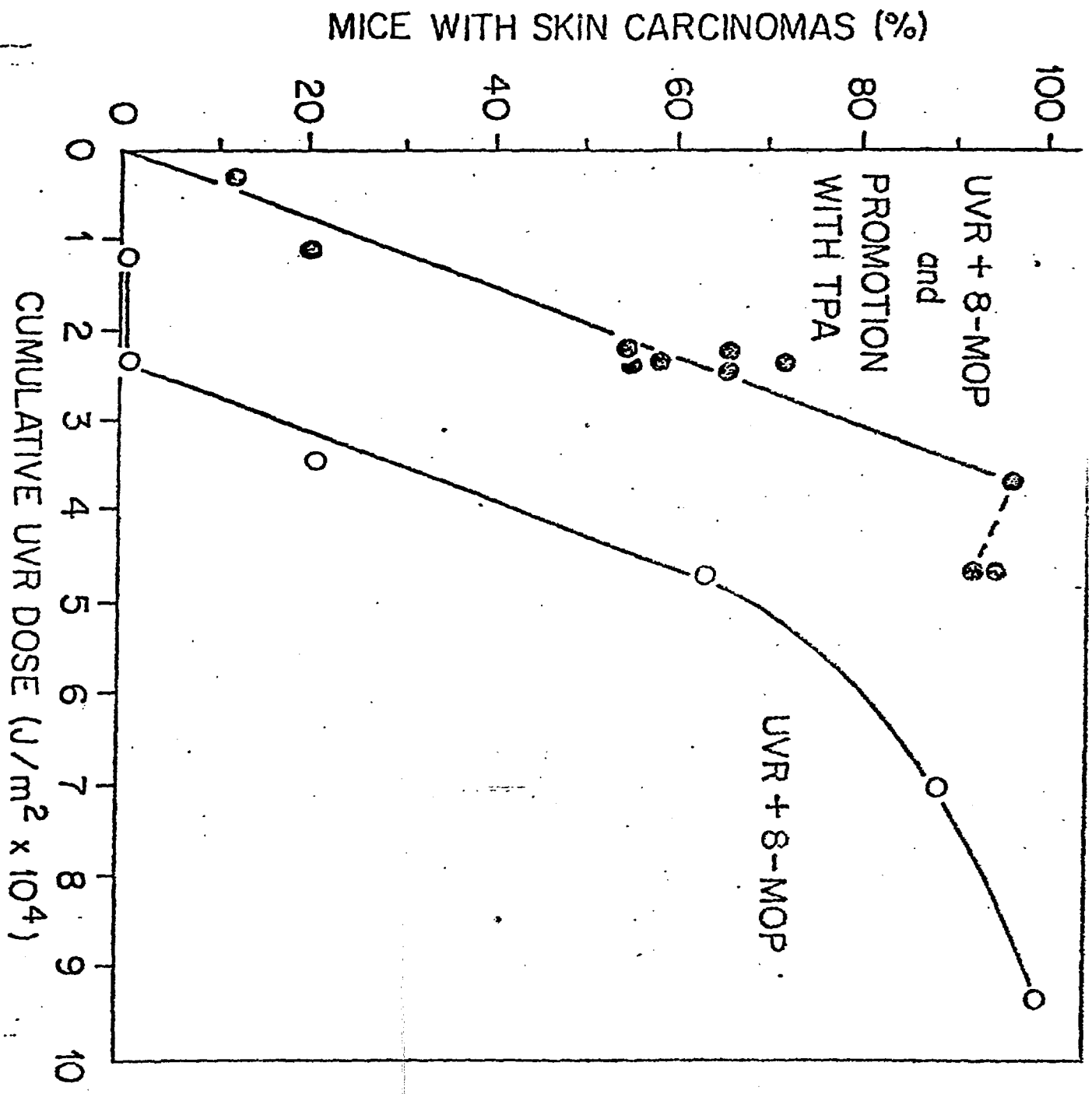


Fig. 3





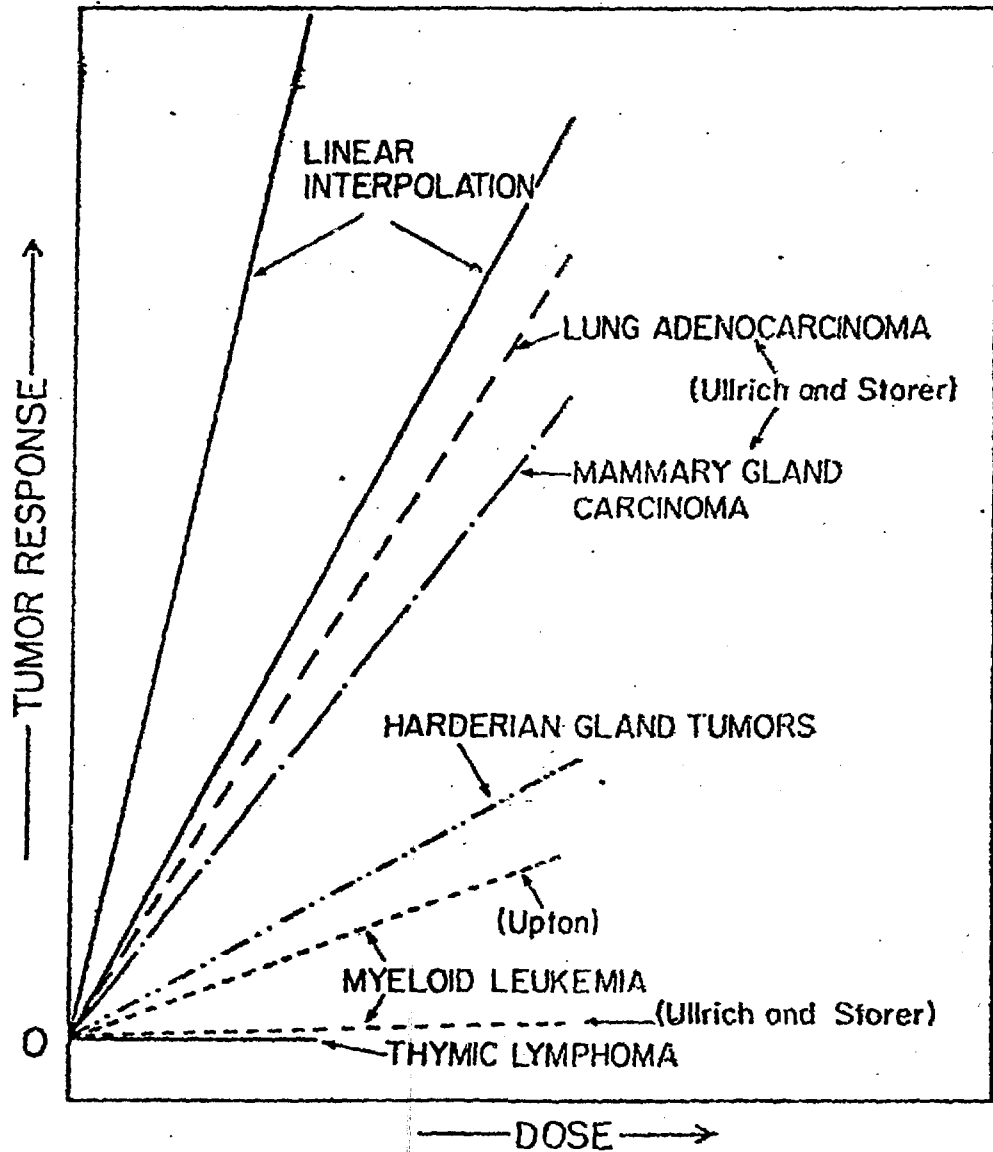


Fig. 6a

