MASTER

RADIATION TOXICOLOGY*

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INTRODUCTION

The study of radiation toxicology is about as old as the proverbial life span of man. Despite intensive research and the development of a remarkable body of information about the effects of radiation of different qualities, there is still no absolute agreement on how radiation kills cells or induces tumors. Not only is there a practical need for an understanding of various radiation effects but there is still the excitement of investigating the mechanisms by which deposition of energy results in major biological effects.

Radiations of different wavelengths vary in their biological effects (Table 1). Radiations with wavelengths greater than 320 nm are considered noncarcinogenic but very few systematic late effects studies have been carried out with the longer wavelengths. With radiations such as infrared and radio-waves, thermal damage is the common feature. There are no data on the carcinogenic effects of hyperthermia in animals, and heat does not transform cells[1]. The relative amounts of the different types of radiation-induced macromolecular damage vary considerably for the spectrum of radiations that are carcinogenic. It is clear that the more densely ionizing radiations (probably up to about 100 kev/μ) are more effective than the sparsely ionizing for several biological endpoints including tumorigenesis. But there is yet no evidence that a DNA lesion common to the various radiation qualities is involved in carcinogenesis. In the case of ultraviolet radiation there seems to be a correlation between the wavelengths that interact with DNA and those that result in skin cancer. The spatial and temporal characteristics of the deposition of energy of radiation of different qualities provide a potentially powerful probe for investigating the mechanisms of malignant transformation.

TABLE 1

Type of Radiation	Source Natural	Other	Wavelength NM	Molecular Lesions	Adverse Biological Effects
Gamma	Radioactive minerals	Medical	1×10^{-4} 1.4×10^{-1}	Single strand breaks Double strand breaks Base Damage	Cell killing Mutagenesis Teratogenesis
X-rays	Sun	Medical	5×10^{-4} - 20		Carcinogenesis
Ultraviolet	Sun		40 - 390	Pyrimidine dimers Base damage Thymine photoproducts DNA-protein crosslinks	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 TV Radio	$10^{5} - 3 \times 10^{13}$	Thermal damage	Cell killing Deafness ?
Power A.C.		Electric Power lines	10^{5}	?	?

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 nonionizing 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 whether or not this level of 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 effects 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 Stone[3] 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 distinct 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 diffi- culties in epidemiological studies of cancer. 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, as an initiator, and also interacts as a co-carcinogen. 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 fewer 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 and site 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 Altshuler[10] 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 shows unequivocally the time of appearance to be 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

LUNG TUMORS B6CF₁ MICE
TIME TO A MORTALITY RATE OF
10 / 10⁵ MICE / DAY
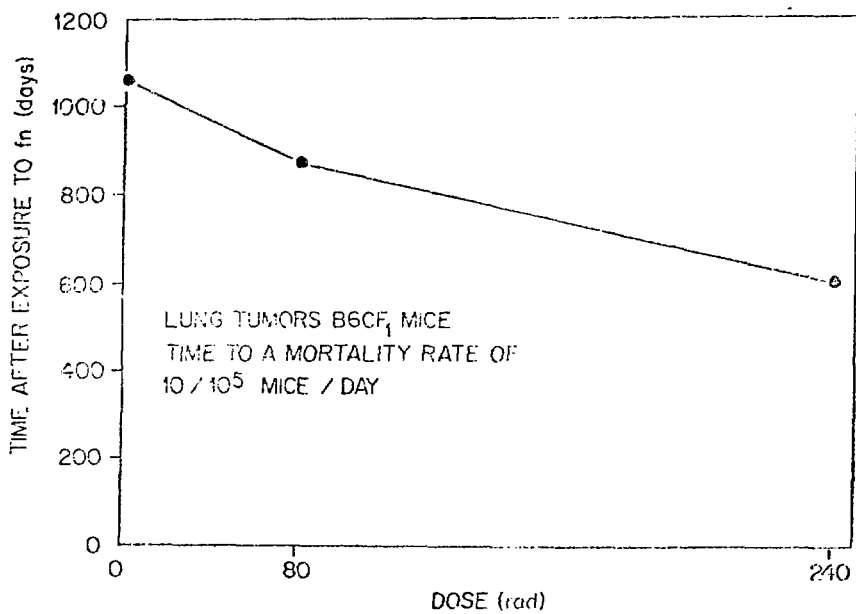
occurring tumors and the induction of tumors is not a trivial matter and is
fundamental to the understanding of mechanisms and susceptibility to cancer
induction. There are a number of tumors which occur with a high natural
incidence in rodents; in some strains the incidence may reach nearly 100%, for
example, liver tumors in C3H mice, and mammary tumors in Sprague Dawley rats.
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].

Fig. 1. Time to a selected mortality rate due to lung tumors in female B6CF$_1$
(Anl) mice as a function of dose of Janus fission neutrons.

TUMOR INCIDENCE

HIGH LET

LINEAR
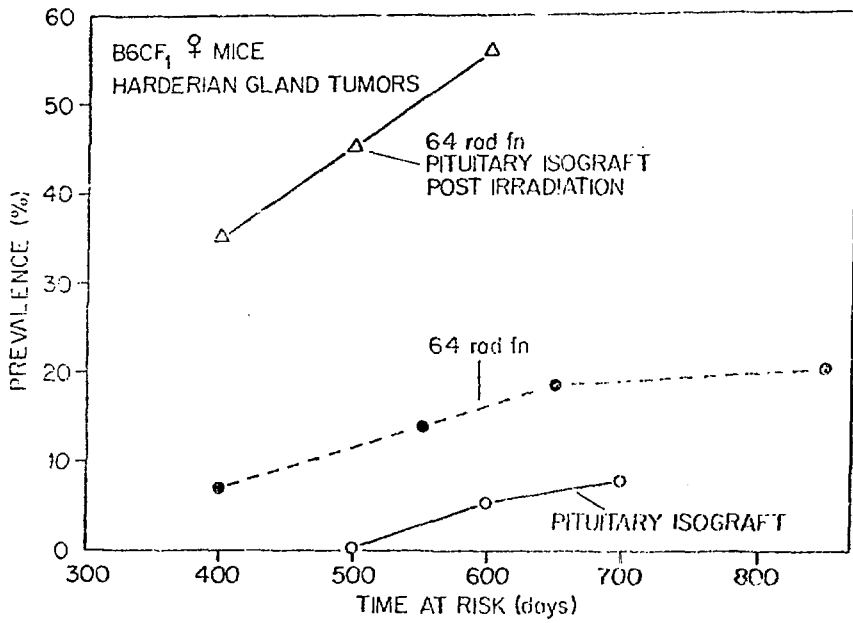INTERPOLATION

LOW LET

LOW DOSE RATE

DOSE
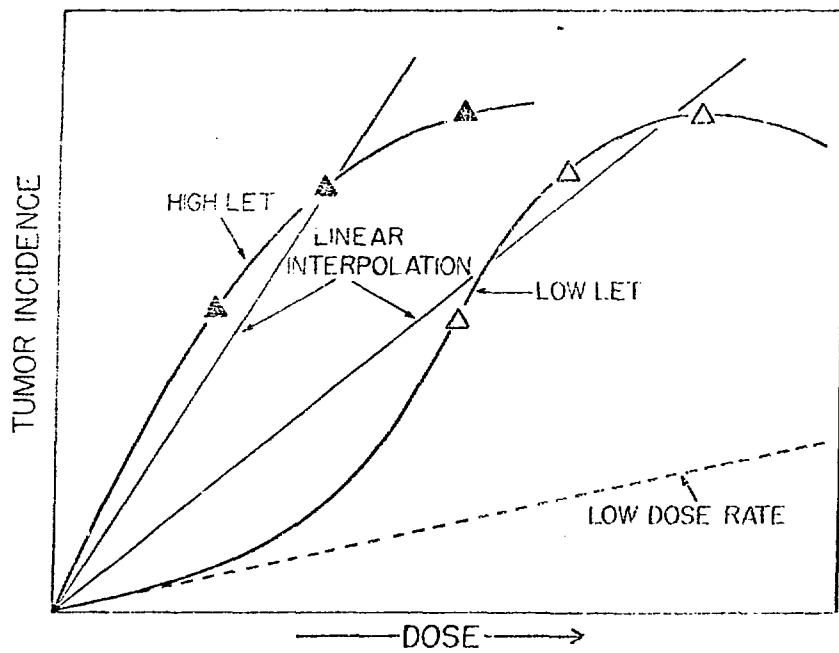
The marked difference in time to appearance of tumors in various species is one of the facets that must be under stood if extrapolations across species are to be made. The equally marked difference in life span between species has encouraged interest in the idea that the temporal pattern of tumor response 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
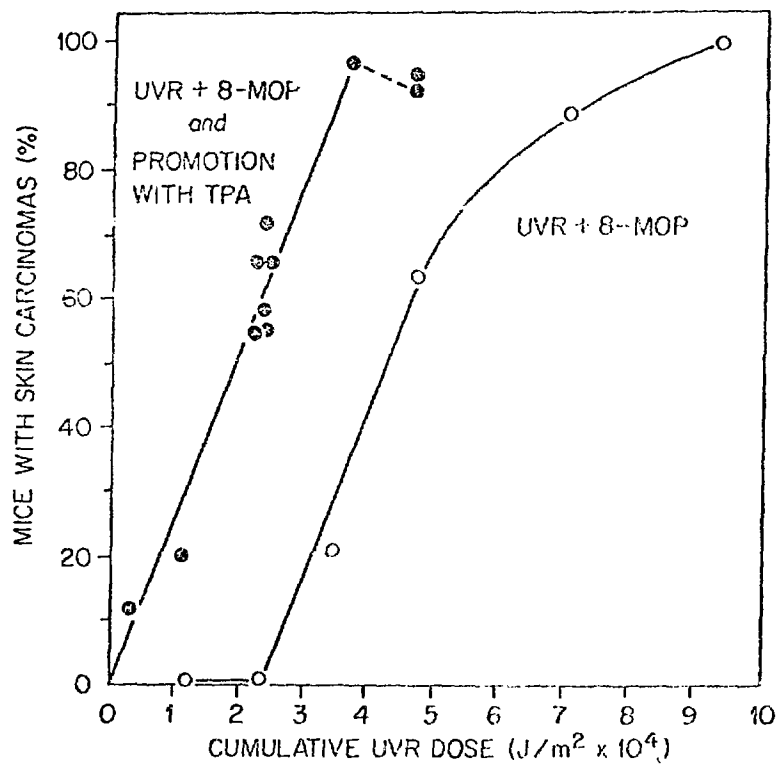
Fig. 2. Schematic dose response curves for tumor incidence after exposure to high and low LET radiation with linear interpolations through selected points of the curves.

BECF₁ ♀ MICE
HARDERIAN GLAND TUMORS

64 rad fn
PITUITARY ISOGRAFT
POST IRRADIATION

64 rad fn

PITUITARY ISOGRAFT

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 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

Fig. 3. The prevalence of Harderian gland tumors in female $B6CF_1$(Anl) after pituitary isograft only o——o after a single exposure to 64 rad fn only o---o and to 64 rad fn followed by pituitary isograft on the same day Δ——Δ.

UVR + 8-MOP
and
PROMOTION
WITH TPA

UVR + 8-MOP

MICE WITH SKIN CARCINOMAS (%)

CUMULATIVE UVR DOSE (J/m² x 10⁴)

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 B6CF$_1$ 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

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-methoxy-psoralen o——o and similar exposures but followed at the end of the fractionation regime by treatment with 5 µg of TPA 3/week.

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 exposures to UVR + 8-MOP regimes were followed by promotion by 12-0-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
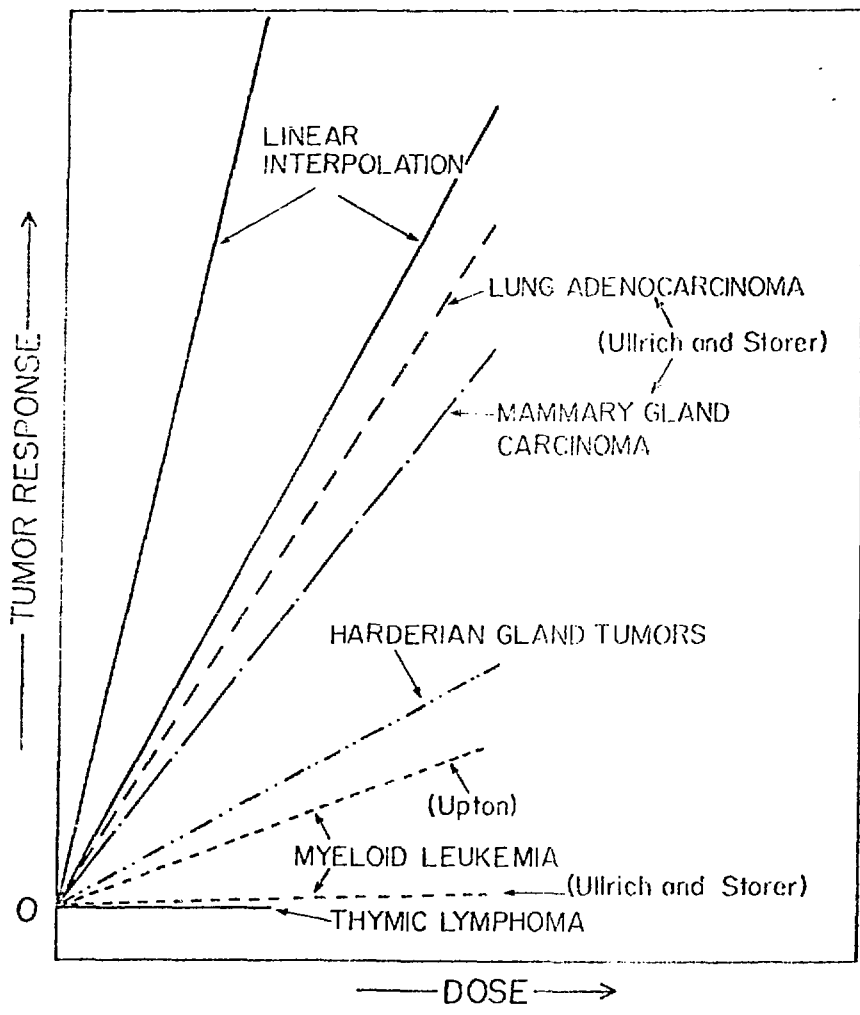
Fig. 5. Plot of the slopes of tumor incidence as a function of dose derived from linear interpolation of data assuming no threshold for exposures to high dose rate irradiation solid lines and for the same

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 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.

B6CF₁ MICE ♀
(Harderian gland tumors)
• pituitary isograft−fn
o fn−pituitary isograft
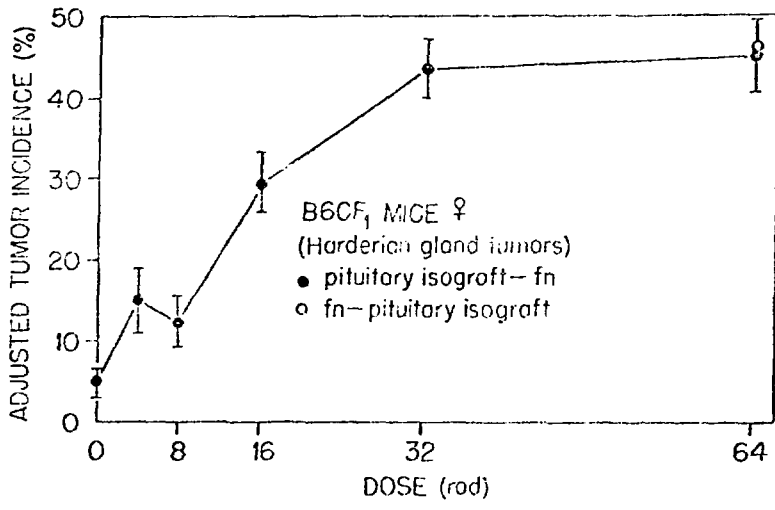
In Fig. 6 it can be seen that the curve for the incidence of Harderian gland tumors as a function of dose of fission neutrons bends over. As the mice in

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

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 non-
irradiated 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

TABLE 1

Tumor Site	Mouse Strain	Relationship of Natural Incidence and Susceptibility	
		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

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 the 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 allows 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.

REFERENCES

1. Harisiadis, L., Miller, R. G., Harisiadas, S. and Hall, E. J. Br. J. Radiol. (in press).

2. Hill, J. (1979) Br. J. Radiol., 52, 2-13.

3. Frigerio, N. A. and R. S. Stone (1976) Carcinogenic and Genetic Hazard from Background Radiation, in Biological and Environmental Effects of Low Level Radiation. Vol. II, pp 385-393, IAEA.

4. Urbach, F. (1974) Ultraviolet Carcinogenesis Experimental Global and Genetic Aspects in Sunlight and Man. (eds.) T. B. Fitzpatrick, M. A. Pathak, L. C. Harber, M. Seiji and A. Kukita, University of Tokyo Press, pp 259-283.

5. Magnus, K. (1977) Int. J. Cancer, 20, 477-485.

6. Blum, H. F. (1959) Carcinogenesis by Ultraviolet Light, Princeton University Press, Princeton, NJ.

7. Druckery, H. (1967) Quantitative Aspects of Chemical Carcinogenesis, in Potential Carcinogenic Hazards from Drugs, Evaluation of Risks, UICC Monograph Series (ed.) R. Truhaut. Vol. 7, Springer-Verlag, New York, pp 60-78.

8. Albert, R. E. and Altshuler, B. (1973) Consideration Relating to the Formulation of Limits for the Unavoidable Population Exposure to Environmental Carcinogens, in Radiationnuclid Carcinogenesis. AEC Symposium Series 29, (eds.) C. L. Sanders, R. M. Busch, J. E. Ballou and D. D. Mahlum, pp 233-253.

9. Evans, R. D. (1966) Br. J. Radiol., 39, 881-895.

10. Nelson, N. (1978) Environmental Health Perspectives, 22, 93-95.

11. Ainsworth, E. J. (1977) Dose-Effect Relationships for Life-Shortening, Tumorigenesis and Systematic Injuries in Mice Irradiated with Fission Neutron or ^{60}Co Gamma Radiation. Proceedings of International Radiation Protection Association IVth Intern. Congress, pp 143-151.

12. Grahn, D. (1970) Biological Effects of Protracted Low Dose Radiation Exposure of Man and Animals in Late Effects of Radiation. (eds.) R. J. M. Fry, D. Grahn, M. L. Griem and J. H. Rust. Taylor-Francis Ltd., London.

13. Albert, R. E., Burns, F. and Shore, R. (1978) Comparison of the Incidence and Time Patterns of Radiation-Induced Skin Cancer in Humans and Rats, in Late Effects of Ionizing Radiation, Vol. 2, IAEA, Vienna.

14. Lundin, F. E., Jr., Lloyd, J. W., Smith, E. M., Archer, V. E. and Haladay, D. A. (1969) Mortality of Uranium Miners in Relation to Radiation Exposure, Hard-Rock Mining and Cigarette Smoking - 1950 through September 1967. Health Physics, 16, 571-578.

15. Fry, R. J. M., Ley, R. Dand Grube, D. D. Photosensitized reaction and carcinogenesis Carcinogenesis. in, Ultraviolet Carcinogenesis. NCI Monograph (in press).

16. Brown, J. M. (1976) Health Physics, 31, 231-245.

17. Ullrich, R. L. and Storer, J. B. (1978) Influences of Dose, Dose Rate and Radiation Quality on Radiation Carcinogenesis and Life Shortening in RFM and BALB/c Mice. in, Late Effects of Ionizing Radiation, Vol. 2, pp IAEA, Vienna.

18. Ullrich, R. L. (1979) Carcinogenesis in Mice after Low Doses and Dose Rates, in Proceedings of 32nd Annual Symposium on Fundamental Cancer Research, Houston, Texas (in press).

19. Upton, A. C., Randolph, M. L. and Conklin, J. W. (1970) Late Effects of Fast Neutrons and Gamma Rays in Mice as Influenced by the Dose Rate of Irradiation. Induction of Neoplasia. Radiat. Res., 41, 467-491.

20. Ullrich, R. L. and Storer, J. B. (1979) Influence of Gamma Ray Irradiation on the Development of Neoplastic Disease. III. Dose Rate Effects. Radiat. Res. (in press).

21. Mole, R. H. (1975) Br. J. Radiol., 48, 157-169.