CONF-8205170--2

CONF-8205170--2

DE83 014053

THE RELEVANCE OF EXPERIMENTAL ANIMAL STUDIES TO THE HUMAN EXPERIENCE*

R. J. M. Fry

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

Running Title: Experimental Radiation Carcinogenesis

---

*Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

EMB

INTRODUCTION

The estimates of risk for radiation-induced cancer have been based on human experience. This has been the case because it has been considered that the data for effects of exposures of humans were adequate and because it is maintained it is not possible to extrapolate quantitative risks from animal experiments. Both these reasons might be questioned. Atomic bomb survivors have provided much of the data on which risk estimates are based and yet more than sixty percent of the survivors are still living. An experimenter that came to conclusions on the basis of data from less than forty percent of his experimental sample would, at best, be thought to be speculating. To be fair the available information for leukemia in the atomic bomb survivors may be at hand. However, different forms of leukemia are usually pooled when dose responses are considered, a luxury denied the experimenter. Necessity has made the use of experimental data for the estimation of radiation-induced genetic effects acceptable and so far the evidence is that protection standards for genetic effects based on experimental data have served us well (16).

The Role of Animal Experiments

In Table I the factors that influence radiation responses are listed. (T-I) All of these can be investigated experimentally but of course the question is whether the experimental results can be applied quantitatively to the estimate of risk in humans. Despite the breadth of research in radiation carcinogenesis the understanding of the factors listed in Table I is far from complete and confidence in the interpretation of empirical data is

tempered by the complexity of mechanisms. Animal and allied experiments must provide much of the information that is required to understand mechanisms since cancer is not only a disease of cells but clearly involves tissue factors and interactions.

Dose-Response Relationships

Animal experiments have provided an increasing insight into dose-response relationships (14, 20, 21). First, the shapes of the dose-response curves for radiation induction of cancer in different tissues vary in form suggesting that the mechanisms involved also vary. From both written and spoken discussions it often appears there is an implicit assumption that the reason for the differences in the responses of different tissues lies in the events involved in initiation. However, the differences in the responses may also result from the factors that influence expression. If the current opinion that carcinogenesis, at least in some tissues, is a multistage process is correct then the opportunities for systemic and tissue-dependent factors to influence expression are considerable. An example taken from ultraviolet carcinogenesis (UVR) may illustrate the point.

It can be seen from Fig. 1 that the plot of skin cancer as a function of UVR dose is curvilinear with perhaps a threshold. If the animals are treated with the promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) after the regime of UVR treatments is completed, the shape of the dose-response curve is changed dramatically. Since the treatment with TPA is after the completion of the regime of UVR exposures, it is assumed the action of the TPA is on the expression of the events initiated by UVR.

F-1

Similar findings have been obtained with beta-irradiation (17) and psoralen-UVR exposures (5). These findings suggest that the curve for cancer incidence as a function of dose is a complex resultant of the radiation induction of potential cancer cells and the factors that influence the expression of those cells. One explanation of the differences in the response curve in Fig. 1 is that there are tissue and perhaps systemic factors that are capable of suppressing the growth of the cells transformed by exposure to UVR and the effects of TPA overcome the suppression. Since the dose-response curves for various tissues are different the question arises whether the difference in response is due to differences in the induction of the initial lesions or in their expression. It is possible that the major difference in the responses between different tissues in the same species or the same tissue in different species is due to the factors that influence expression.

The fact that the shape of the dose-response curve can be changed by altering the expression of the initial events underlines the problem of the use of models for dose-responses that are based entirely on the biophysical aspects of the induction of initial events.

In order to study the dose response of initiation or malignant transformation methods are required for maximizing the expression of initiation in a number of different tissues. Experimental systems have been introduced that permit modulation of the expression of cells exposed to carcinogenic agents (2, 3, 18). The success of these systems depends on the ability to expose tissues to radiation, or other carcinogens, in vivo that are then removed and the cells dissociated; the separated cells are either cultured in vitro or transplanted into appropriate sites of syngeneic animals.

DeOme (3) demonstrated that the expression of hyperplastic alveolar nodules in mammary glands of mice could be enhanced by cell dissociation and transplantation and modified by the presence of normal cells. The results of experiments carried out on a number of tissues using similar techniques and also from studies in which the expression of cells initiated by radiation has been enhanced by hormones indicate that initiation is a more common event than the appearance of cancer. For example, exposure of BALB/c mice to 100 rads gamma radiation resulted in an incidence of about 14% mammary cancer. When mammary tissue was removed from a number of mice similarly exposed and the cells were dissociated and implanted in cleared fat pads foci of neoplastic change, indicative of initiated cells, were found in all of the hosts. This indicates that there were initiated cells in all of the irradiated donor mice although when left in their normal environment these cells gave rise to tumors in only 14% of the mice (4).

In a tissue with its normal integrity the expression of transformed cells may be suppressed by cell-to-cell relationships. Disruption of the normal tissue integrity may contribute to the more marked carcinogenic effect of high doses of irradiation. Although tumor cell dormancy is well documented and many explanations have been offered its importance (23), in quantitative terms is not clear, but it is clear that a greater understanding of host factors that influence both initiation of cancer cells and their development to overt cancer is essential.

Estimation of Low Dose Effects

The direct determination of the initial slope of the low-LET radiation

dose-response curve for humans is impossible and therefore we must rely on models and indirect models of estimating the slope. The biophysical characteristics of the interaction of radiation and cells suggest that the initial slope of the dose-response curve is linear and dose-rate independent (8, 12) although this is still a matter some dispute (14). The question is how to estimate the initial slope with reasonable economy and accuracy. Clearly extrapolation from data obtained at high doses is dependent on the choice of the model for the dose-response curve. Since we know the models in common use, such as the so-called linear quadratic, are more appropriate as models for the induction of the initial events (6) than for the incidence of cancer it seems sensible to examine the possibility of empirical experimental approaches. One such approach is to determine the slope of the relationship of cancer incidence as a function of dose when the dose is given at a rate sufficiently low to ensure that the response is total dose dependent and dose-rate independent. A slope for the dose response obtained in this manner should be equal to that of the initial slope of a response obtained at very low doses given at high dose rates. Multiple fractions of very small doses should provide similar results. The effects of lowering dose rate and the implications for the estimation of the effects of low doses is discussed at length in NCRP Report No. 64 (15).

Although the use of low dose-rate exposures to estimate the initial slope of the response to very small single doses of high dose-rate low-LET radiation has not been examined systematically there is sufficient information to suggest some problems. In order to get an incidence of tumors significantly higher than the natural incidence with a reasonable

number of experimental animals it requires relatively high total doses and therefore relatively long periods of exposure. When exposures are protracted the effects of protraction, that are independent from those due to lowering instantaneous dose rate, must be taken into account. In some experimental systems susceptibility can change over a relatively short period of certain parts of the life span (11). A decrease in the induction of some tumors from exposures delivered at low dose rates may be due, in part, to a reduction in susceptibility due to the increase in age over the period of protraction. Contrariwise, in other systems it is possible that protraction enhances the radiation-induced effect. For example, it is not clear that the reason that protracted low dose-rate irradiation is required to induce myelogenous leukemia in dogs is purely due to the fact that protraction allows high total doses to be incurred (19). The effectiveness of the protracted irradiation may be due to repeated damage to marrow cells which in turn plays a part in the production of the disease. In the case of skin tumors induced by UVR or PUVA multiple exposures are required and it appears that the effect of many of the later fractions is to increase the expression of initiated cells (5). Such results raise the question whether the reduction in tumor induction with low dose rates is due entirely to the reduction of initiation, as is assumed, or that lowering the dose rate reduces the probability of expression.

The results shown in Fig. 2 suggest, that at least in the case of exposures to multiple small fractions to the Harderian gland, that reduction in the tumorigenic effect of ^{60}Co gamma radiation is, in part, due to a reduction in expression of initiated cells.

F-2

In this study increased prolactin levels, maintained by using pituitary isografts, promoted the expression of radiation-induced initiation events in Harderian glands. It can be seen that fractionated exposures followed by pituitary isografts resulted in higher incidences than after fractionated exposures alone. It can also be seen that the incidence of tumors induced by fractionated exposures followed by promotion by prolactin from pituitary isografts is less than after exposure to single doses and promotion. One explanation of these results is that fractionation reduces the effectiveness of the radiation not only by reducing the induction of initial events but also their expression. In Figure 3 it can be seen from the data of Ullrich and Storer (22) that the incidence of thymic lymphoma is reduced markedly by reducing the dose rate but with a total dose of 200 rad the reduction in incidence is less than at lower doses. Since this tumors shows a marked reduction in susceptibility with increasing age the increase in incidence is surprising. These results suggest that estimation of the initial slope of the dose-response curve from data obtained at higher total doses but at low dose rates may not be an easy matter. It is of some importance to determine unequivocally whether lowering dose rate affects the expression as well as initiation of radiation-induced initial events. (F-3)

The potential magnitude of the influence of host factors in radiation carcinogenesis is illustrated in Fig. 4. The hybrid $B6CF_1$ Anl (C57BL/6 x BALB/c) mouse has a very low natural incidence of mammary carcinoma. In the untreated control group of the experiment illustrated no carcinomas were noted. However, transplantation of pituitaries into the spleens of of unirradiated control mice resulted in a marked increase in mammary (F-4)

carcinomas. It is thought that the increased cancer incidence was due to the elevated levels of prolactin maintained by secretion from the pituitary isografts. It appears that the only effect of exposure to the two dose levels of JANUS reactor fission neutrons prior to the pituitary transplantation was to advance the time of appearance of the cancers. It should be noted that irradiation alone resulted in little increase in the incidence of mammary carcinomas in this hybrid mouse. One interpretation of these results is that the raised prolactin levels revealed the inherent cancer potential of the mammary cells rather than induced malignant transformation. Whatever the correct interpretation is it is clear that inherited genomic and host factors are of fundamental importance in the genesis and growth.

Extrapolation across Species

I suggested above that, at least in some cases, the explanation of the differences in susceptibility between tissues might lie in differences in the expression of the induced events and not in the initial events. Similarly, differences in susceptibility between species might be due to differences in host factors. It seems a reasonable assumption that there are more cells at risk in a human then in a mouse and yet the natural incidence of cancer is less despite the fact that humans probably have greater exposure to carcinogens than the laboratory mouse. The explanation of the marked difference in cancers per cell at risk is not known. It is likely that the difference in susceptibility to cancer between species is due to a number of factors, one of which may be a major difference in the expression of initiated cells. When comparing the effects of radiation across the species it is important to know the relationship of susceptibility

of induced cancer to the natural incidence. This relationship is of interest in the investigation of mechanisms and is relevant to the choice between relative and absolute risk models for risk estimates. This is the type of question for which animal experiments can be used to answer.

Despite the considerable body of data for the experimental induction of tumors by radiation in mice there is a dearth of data suitable for examining the relationship of natural incidence of specific tumors and the induction of those tumors by radiation. In order to examine this question, both life time tumor rates and adequate dose-response data are required for a specific tumor type in two strains, or in both sexes for tumors that show a significant sex-dependency. The available data that were considered suitable are shown in Table II. It can be seen that the radiation-induced increase for each of the tumors is greater in the strain or sex with the higher natural incidence. For those tumor types that show this pattern relative risk would appear to be the method of analysis of choice. However, the number of tumor types and mouse strains examined is small and it is not clear that the relationship between natural incidence and susceptibility will hold as a general rule. For example, the natural incidence of myeloid leukemia in CBA/H mice is extremely low but the response to radiation is comparable to that in RFM mice (13).

T-II

Grahn et al. (10) concluded that induced tumor rate was correlated positively with the natural incidence and that the relative risk or mortality ratio is an appropriate method of estimating the excess risk of radiation-induced cancer. Our initial attempts to compare risks of specific tumor types and death from all tumors relative to natural incidence between humans

and mice showed reasonable agreement (7) and this approach warrants further examination. In the case of humans the similarlity of the excess risk of breast cancer per rad in patients in North America exposed to x-rays, and in atomic bomb survivors, although the natural incidences in Japan and the U.S. and Canada are very different, has been used to support the use of the absolute risk model. It will require longer periods of study to conclude which model is appropriate for different tumor types.

There have been attempts to use scaling factors to adjust for the difference in life spans between species in order to compare tumor rates (1, 9). In an attempt to examine whether there was a general relationship between life span and susceptibility for tumor induction we have investigated the induction of fibrosarcomas in three species: Peromyscus leucopus (deer mouse), Rattus norvegicus (Sprague-Dawley rat), and Canis formiliaris (beagle) using mylar discs with ^{90}Sr-^{90}Y incorporated as a radiation source. These species were chosen because of their different mortality rates which are shown in upper right panel of Fig. 5. It can be seen that over the period of observation no tumors occurred in the beagles and high incidences occurred in the rat and deer mouse. Although the life span of the deer mouse and the rat are significantly different the time pattern of appearance and cumulative incidence were similar. These results suggest that neither susceptibility to radiation-induced sarcomas nor their appearance in time show a general relationship to life span.

(F-5)

Currently, we must rely on empirical methods of extrapolation but with increasing understanding about mechanisms such exercises will be on a sounder basis. Until mechanisms are understood we will not know the

significance or the sense of using the same name for cancers of specific cell types in different species.

SUMMARY

Animal experiments are being used to examine a number of physical and biological factors that influence risk estimations though not usually in coordination with epidemiologists. It is clear that the different mechanisms involved in different types of tumors are reflected in the diversity of dose-response relationships. The forms of the dose-response relationships are influenced by both the initial events and their expression. Evidence is accumulating that many initiated cells do not get expressed as overt cancers and host factors may play a major role in the expression of potential tumor cells. There is a need for information about the relationship of the natural incidence and susceptibility to radiation induction for more tumor types. Such experiments will help answer the question of which risk estimate models are appropriate for different tumor types and can be carried out on animals. Perhaps because of the importance of host factors risk estimates as a percentage of the natural incidence appear to be similar for human beings and mice for a small number of tumor types. The elucidation of the mechanisms involved in different tissues while a slow business remains an important role of animal experiments.

REFERENCES

1. Albert, R. E., Burns, F. J., and Shore, R. (1978): Comparison of the incidence and time patterns of radiation-induced skin cancer in humans and rats. In: Late Biological Effects of Ionizing Radiation, Vol. I, pp. 499-505, IAEA, Vienna.

2. Clifton, K. H., Mulcahy, R. T. and Demott, R. K. (1979): The radiobiology of thyroid epithelium: Cell survival and neoplasia. In: Radiation Research Proceedings of the 6th International Congress of Radiation Research, edited by S. Okada, M. Imamura, T. Terasima and H. Yamaguchi, pp. 753-760, Tokyo.

3. DeOme, K. B., Faulkin, L. J., Bern, H. A., and Blair, P. B. (1959): Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free fat pads of C3H mice. Cancer Res. 19: 515-520.

4. Ethier, S. P., and Ullrich, R. L. (1982): Depression of ductal dysplasia in mammary outgrowths derived from carcinogen-treated virgin female BALB/c mice. Cancer Res. 42: 1753-1760.

5. Fry, R. J. M., Ley, R. D., and Grube, D. D. (1978): Photosensitized reactions and carcinogenesis. In: Ultraviolet Radiation Carcinogenesis, edited by M. L. Kripke. Natl. Cancer Inst. Monogr. 50: 39-43.

6. Fry, R. J. M. (1981): Experimental radiation carcinogenesis: What have we learned? Radiat. Res. 87: 224-239.

7. Fry, R. J. M., Ullrich, R. L., and Storer, J. B. (1982) On the concordance of some estimates of the relative risk of radiation-induced cancer in mouse and man. Radiat. Res. (accepted for publication).

8. Goodhead, D. T. (1982): An assessment of the role of microdosimetry in radiobiology. Radiat. Res. 91: 45-76.

9. Grahn, D. (1970): Biological effects of protracted low dose radiation exposure of man and animals. In: Late Effects of Radiation, edited by R. J. M. Fry, D. Grahn, M. L. Griem and J. H. Rust, pp. 101-136, Taylor & Francis, London.

10. Grahn, D., Sacher, G. A., Lea, R. A., Fry, R. J. M., and Rust, J. H. (1978) Analytical approaches to and interpretations of data on time, rate and cause of death of mice exposed to external gamma irradiation. In: Late Biological Effects of Ionizing Radiation, Vol. II, pp. 43-58, Intern. Atomic Energy Agency, Vienna (STI/PUB/489).

11. Kaplan, H. S. (1948): Influence of age on susceptibility to the development of lymphoid tumors after irradiation. J. Natl. Cancer Inst. 9: 55-56.

12. Kellerer, A. M., and Rossi, H. H. (1982): Biophysical aspects of radiation carcinogenesis. In: Cancer: A Comprehensive Treatise, edited by F. F. Becker, 2nd Edition, pp. 569-616, Plenum Press, New York.

13. Major, I. R., and Mole, R. H. (1978): Myeloid leukemia in X-ray irradiated CBA mice. Nature 272: 455-456.

14. Mole, R. H. (1982) Dose-response relationships. In: Radiation Carcinogenesis Epidemiology and Biologic Significance, edited by J. D. Boice and J. F. Fraumeni, Raven Press, New York.

15. National Council on Radiation-Protection and Measurements, (1980): Influence of dose and its distribution in time and dose-response relationships for low-LET radiations. NCRP Report No. 64, Washington, DC.

16. Schull, N. J., Otake, M., and Neel, J. V. (1981): Genetic effects of the atomic bomb: A reappraisal. Science 213: 1220-1227.

17. Shubik, P., Goldfarb, A. R., and Ritchie, A. C. (1953): Latent carcinogenic action of Beta-irradiation on mouse epidermis. Nature 171: 934-935.

18. Terzaghi, M., and Nettesheim, P. (1979): Dynamics of neoplastic development in carcinogen-exposed tracheal mucosa. Cancer Res. 39: 4003-401

19. Tolle, D. V., Fritz, T. E., Seed, T. M., Cullen, S. M., Lombard, L. S., and Poole, C. M. (1982): Leukemia induction in beagles exposed continuously to ^{60}Co gamma irradiation: Hematology. In: Experimental Hematology Today (in press).

20. Ullrich, R. L., and Storer, J. B. (1979a): Influence of γ irradiation on the development of neoplastic disease in mice. I. Reticular tissue tumors. Radiat. Res. 80: 303-316.

21. Ullrich, R. L., and Storer, J. B. (1979b): Influence of gamma ray irradiatio on the development of neoplastic disease in mice. II. Solid tumors. Radiat. Res. 80: 317-324.

22. Ullrich, R. L., and Storer, J. B. (1979c): Influence of gamma ray irradiati on the development of neoplastic disase in mice. III. Dose rate effects. Radiat. Res. 80: 325-342.

23. Wheelock, E. F., Weinhold, K. J., and Levich, J. (1981): The tumor dormant-state. In: Advances in Cancer Research, edited by G. Klein and S. Weinhouse, Vol. 34, pp. 107-139, Academic Press.

TABLE I

Factors that influence the estimates of risk of radiation-induced cancer

Physical	Biological	Analytical
Radiation Quality	Genetic Factors	Choice of:
Dose	Age	(a) Models for dose-response
Dose Rate	Sex	(b) Projection models:
Protraction		Absolute risk
Fractionation		Relative risk
		Independence

TABLE II

Relationship of natural incidence and susceptibility

Tumor Type	Mouse Strain	Natural Incidence (per cent)	Response to Radiation Increase in tumor Incidence per rad (per cent)
Ovarian	RFM	2.4	0.39
Ovarian	BALB/c	6.4	1.2
Mammary Gland	BALB/c	7.5	0.07
Mammary Gland	$B6CF_1$	1.2	0.01
Myeloid Leukemia	RFM ♂	1.3	0.14
Myeloid Leukemia	RFM ♀	0.8	0.09

FIGURE LEGENDS

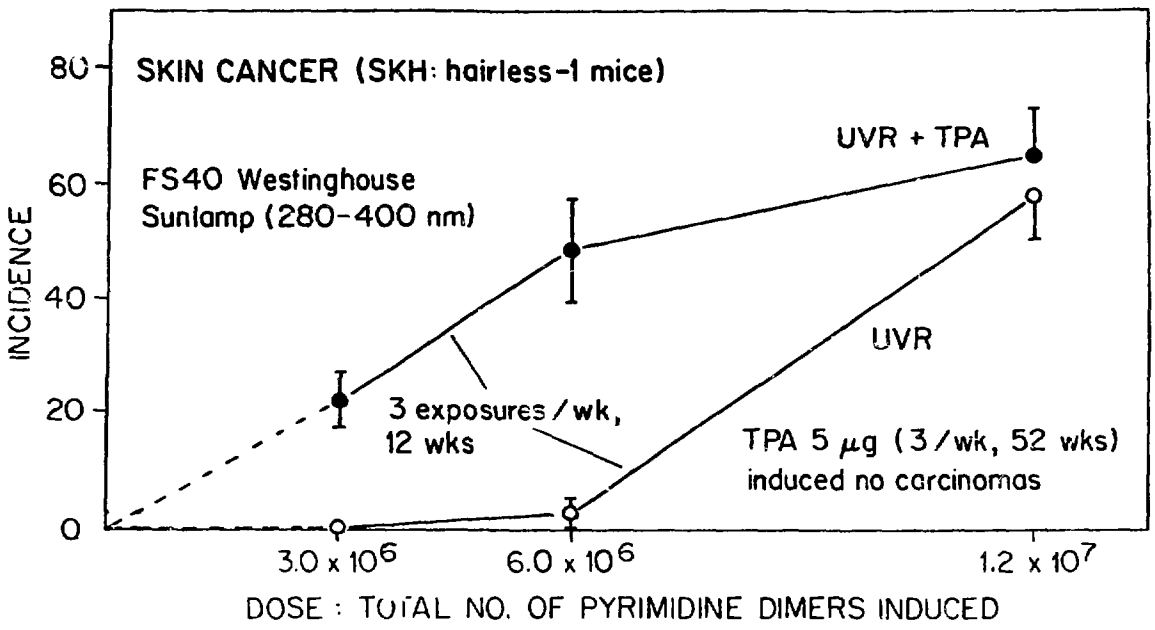
Fig. 1. Incidence of skin carcinomas in SKH:hairless-1 mice as a function of the number of pyrimidine dimers estimated from assays of the UVR-induced endonuclease sensitive sites (o—o):UVR exposures 3/week for 12 weeks of 250, 500 and 1000 J/m^2 (●—●):the same UVR exposure regimens followed by treatment with 5 µg TPA 3/week for 52 weeks.

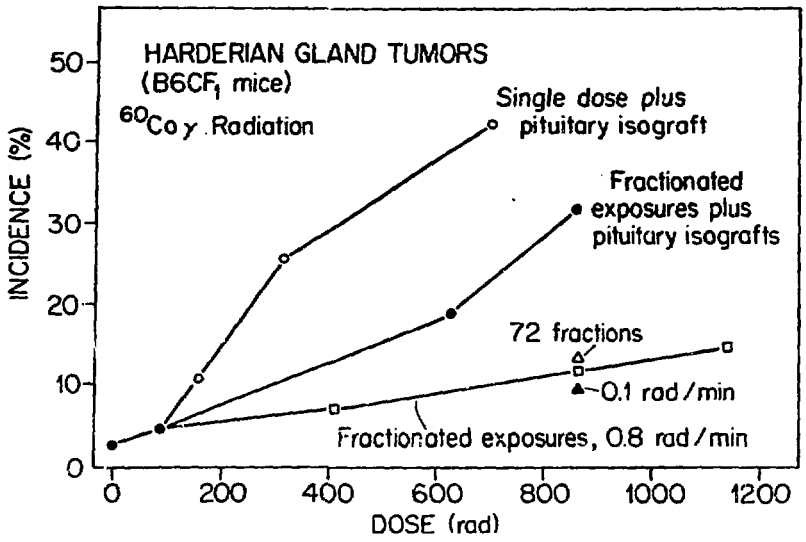
Fig. 2. Incidence of Harderian gland tumors as a function of dose of ^{60}Co gamma radiation (□—□): fractionated exposures, (dose rate 0.8 rad/min, ▲: 0.1 rad/min), (●—●): fractionated exposures; pituitary isografts were made after the completion of the radiation regimes, (o—o): single doses plus pituitary isografts.

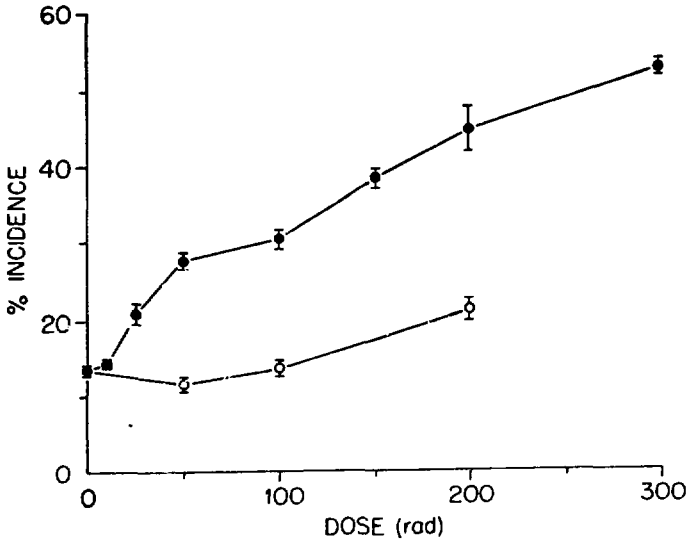
Fig. 3. Incidence of thymic lymphoma in RFM mice as a function of dose of gamma radiation (●—●): 45 rad/min, (o—o): 8.3 rad/min. Data from Ullrich and Storer ref. (22).

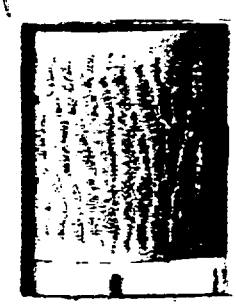
Fig. 4. Mammary carcinomas in $B6CF_1/An1$ mice as a function of time: unirradiated with pituitary isografts, : (●—●)
Single doses of JANUS reactor fission neutrons, 32 rad: (Δ—Δ) and 64 rad: (o—o).

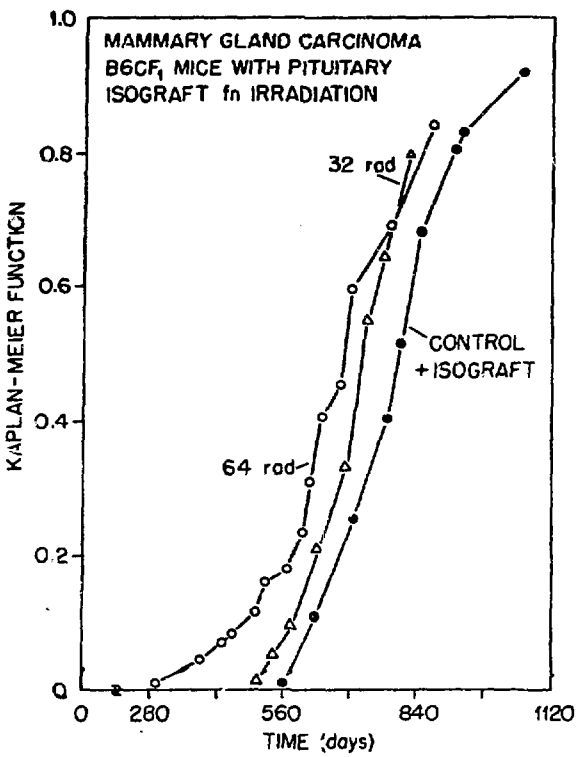
Fig. 5. Top left panel: Percentage of adult weight as a function of time. Top right panel: Percentage of cumulative mortality as a function of time. Bottom panel: Cumulative incidence of fibrosarcomas as a function of time: beagle: (o), rat: (o—●), deer mouse: (x).

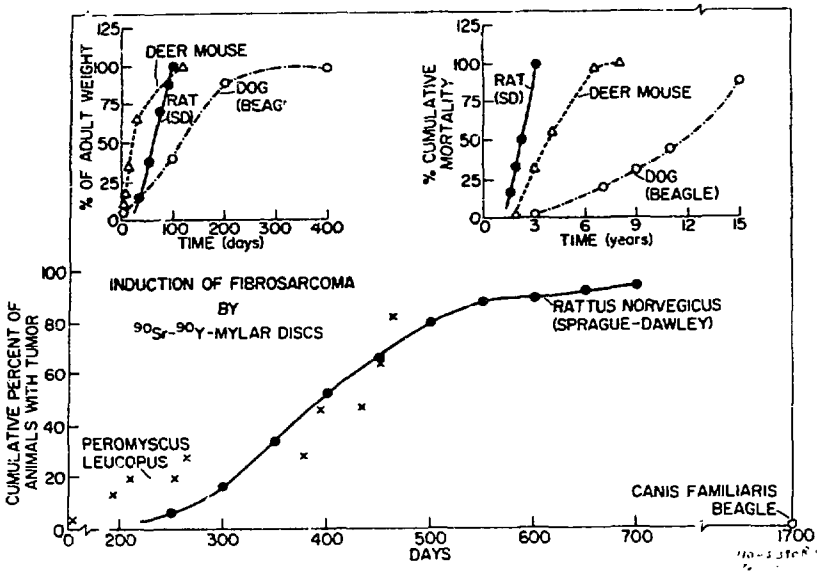

SKIN CANCER (SKH: hairless-1 mice)
FS40 Westinghouse
Sunlamp (280-400 nm)
UVR + TPA
UVR
3 exposures / wk,
12 wks
TPA 5 μg (3/wk, 52 wks)
induced no carcinomas
INCIDENCE
80
60
40
20
0
3.0 x 10^6
6.0 x 10^6
1.2 x 10^7
DOSE : TOTAL NO. OF PYRIMIDINE DIMERS INDUCED


HARDERIAN GLAND TUMORS
(B6CF1 mice)
60Co γ Radiation
Single dose plus pituitary isograft
Fractionated exposures plus pituitary isografts
72 fractions
0.1 rad/min
Fractionated exposures, 0.8 rad/min
INCIDENCE (%)
DOSE (rad)
0
10
20
30
40
50
0
200
400
600
800
1000
1200


60
40
20
0
% INCIDENCE
0
100
200
300
DOSE (rad)




MAMMARY GLAND CARCINOMA
B6CF1 MICE WITH PITUITARY
ISOGRAFT fn IRRADIATION
32 rad
64 rad
CONTROL
+ISOGRAFT
KAPLAN-MEIER FUNCTION
TIME (days)
1.0
0.8
0.6
0.4
0.2
0
0
280
560
840
1120


% OF ADULT WEIGHT
DEER MOUSE
RAT (SD)
DOG (BEAG
TIME (days)
% CUMULATIVE MORTALITY
RAT (SD)
DEER MOUSE
DOG (BEAGLE)
TIME (years)
CUMULATIVE PERCENT OF ANIMALS WITH TUMOR
INDUCTION OF FIBROSARCOMA BY ^{90}Sr-^{90}Y-MYLAR DISCS
RATTUS NORVEGICUS (SPRAGUE-DAWLEY)
PEROMYSCUS LEUCOPUS
CANIS FAMILIARIS BEAGLE
DAYS