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Radiation-Induced Hair Follicle Damage and Tumor Formation in Mouse and Rat Skin[1]

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DISCLAIMER

Running Head: Radiation Tumors in Mouse and Rat Skin

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SUMMARY

The dorsal skin of 172 Swiss Millerton mice was irradiated with electrons in single exposures at 6 dose levels ranging from 500 to 4000 rads. The mice were observed every 4 weeks for skin tumor formation over a period of 88 weeks. At death each tumor was examined histologically and whole skin mounts were used to determine the survival of intact hair follicles and the incidence of atrophic follicles. These data were compared to the dose-response characteristics for tumor induction and hair follicle survival in the rat from previous experiments. The incidence of epithelial skin tumors was markedly lower in mice compared to rats even at optimal tumorigenic doses. This difference was shown to be due to the failure of mice to develop adnexal tumors, which are the predominant type of skin tumor in the rat. The radiation sensitivity of the mouse skin for hair follicle destruction was at least double that of the rat. The virtual absence of atrophic follicle formation in the mouse skin as a consequence of radiation damage may be related to the failure to develop adnexal skin tumors, since there is a close association in the rat skin between the incidence of atrophic hair follicles and skin tumors.

INTRODUCTION

There is a strong association in the rat skin between the production of irreparable hair follicle damage by ionizing radiation and the magnitude of the skin tumor response. This correlation has been observed in a series of experiments involving single exposures of the skin with resting follicles to electrons (1), alpha particles (2), beta rays (3) and x-rays (4) by using various irradiation configurations, including sieve patterns (5) and several different depth-dose patterns (6, 7). It has been found consistently that as the radiation dose increases the tumor response, in terms of the incidence at 80 weeks after exposure, rises to a maximum and then declines in association with increasingly severe acute ulceration.

There is also a dose-dependent progression in the severity of permanent hair follicle damage. With increasing radiation dose, there is a decline in the proportion of hair follicles which remain structurally normal and an increase in the proportion of follicles which are completely destroyed. There is also an intermediate form of hair follicle destruction in which the follicles are atrophic. The incidence of atrophic follicles reaches a peak at about the same dose which produces the maximum tumor yield, and there is an excellent correspondence between the shapes of the dose-incidence curves for tumors and atrophic follicles. The number of atrophic follicles per epithelial tumor is in the domain of 4000. There is a correspondence between the histological structure of the atrophic follicles and the skin tumors most of which are adnexal. Growths on the atrophic follicles have occasionally been observed on whole skin mounts which resemble early tumors (8).

For these reasons, it has been suggested that the atrophic follicles might be the precursor lesion for the majority of the radiation induced epithelial skin tumors in the rat (1). However, the basis for an association between atrophic follicles and skin tumors is unknown, and it is also not clear whether the association is merely a peculiarity of the rat.

It appeared to us that further insight into the matter might be obtained by comparing the radiation tumor and hair follicle injury response of the rat and mouse. Reports from other laboratories indicate that the mouse has a very different skin tumor response pattern than the rat; relatively few tumors can be produced by ionizing radiation, and the majority of the epithelial tumors are epidermoid carcinomas rather than adnexal tumors as in the rat (9, 10). We therefore undertook the experiment reported here to assess the tumorigenic response of mice to a series of graded single electron exposures and to characterize the hair follicle damage on whole skin mounts obtained at the end of the experiment. These data are compared with the experimental findings from similar experiments on rats that have been reported previously.

MATERIALS AND METHODS

Swiss Millerton female mice were irradiated at 30 days of age with monoenergetic electrons from a Van de Graaff accelerator. The energy of the beam was 0.4 MeV and the maximum penetration in the skin was adjusted to 0.4 mm by use of an absorber in the beam. The electron beam was diffused by about 3 feet of air and only the portion of the beam within 2 inches of the center line was used for the exposures. The depth-dose curve was measured by placing plastic absorbers on a 1.0 mm gap ionization chamber. The depth-dose curve was approximately linear and the dose rate at the skin surface was 550 rads/min. (5).

Four days before irradiation the hair was removed with an electric clipper. Mice with evidence of hair growth on the day of irradiation were discarded. The mice were anesthetized with a dose of 25 mg/kg of pentobarbital approximately 15 min. before irradiation. The anesthetized mice were taped in a head-up position to one-eighth inch thick plastic plates, which were mounted vertically on the rim of a circular table and rotated through the electron beam at a rate of 18 revolutions per minute. The rig permitted exposure of up to 36 mice at the same time. Apertures in the plastic plates served to mask the radiation exposure to a 1.5 cm x 3.0 cm area on the dorsal skin with the long axis of the irradiated area centered along the spine.

After the irradiations the mice were observed every 4 weeks until the end of the experiment at 88 weeks. The cumulative tumor incidence was obtained by adding, in successive 4 week periods, the ratio of new tumors appearing to the average number of mice alive during the period.

At death the full thickness of the entire dorsal skin was removed; all tumors were taken for histological examination. Whole mounts were made by soaking the remainder of the skin overnight in 0.5% crude trypsin at 4°C; the epidermis and attached hair follicles were peeled away from the dermis and mounted on a slide in glycerol jelly after being stained with Sudan III and hematoxylin. Tumor sections were stained with hematoxylin and eosin.

Normal and atrophic follicle counts were made on whole skin mounts by using a stereomicroscope with a field size of 0.44 mm^2 which, in the non-irradiated areas, encompassed about 15 follicles. The number of follicles (both atrophic and structurally normal) was measured in successive 0.44 mm^2 fields with a minimum of three traverses across the irradiated area of each mouse skin.

The total number of normal and damaged (atrophic) follicles in the irradiated area was obtained by multiplying the follicle density by the total area of the irradiated skin. The irradiated area was measured from photographs of each animal made at the time of sacrifice. Where the site was not discernible in terms of hair loss or the presence of a scar, the area was assumed to be 4.5 cm^2 which was the size when originally irradiated.

RESULTS

As shown in Table 1, a total of 172 mice were irradiated in groups of 20 to 24 at doses ranging from 500 to 4000 rads. All of the mice in each dose group were given their radiation exposure at the same time. There was comparable survival of mice in the irradiated and control groups ranging from 52% to 67% at 88 weeks except for the 800 rad group where survival was 37%.

The tumor incidence with respect to time after irradiation is shown in text-figure 1. Separate curves are shown for sarcomas (A) and epidermoid carcinomas (B) which occurred in approximately equal numbers and which accounted for all of the tumors. The first sarcoma occurred at 40 weeks and the first epidermoid carcinoma occurred at 48 weeks. For sarcomas there was a trend toward earlier tumor formation at higher doses; this was not clearly the case with the epithelial tumors. No further tumor formation occurred between 68 and 88 weeks.

Text-figure legend 1. The incidence (in tumors per mouse) of epidermoid carcinomas and sarcomas with respect to elapsed time after irradiation.

The tumor incidence at 88 weeks as a function of surface dose is shown in text-figure 2. For epidermoid carcinomas the maximum incidence of 0.14 tumors per mouse occurred at 2540 rads while at 1690 rads and 4000 rads the incidence was 0.08 and 0.05 respectively. For sarcomas the incidence was 0.04 at 1690 rads and 0.14 at 4000 rads. No sarcomas were observed at doses less than 1690 rads.

Text-figure legend 2. The incidence versus surface dose of sarcomas and epidermoid carcinomas for mouse skin at 88 weeks after irradiation.

Text-figure 3 shows the survival of hair follicles in the mouse as a function of surface dose. Follicle survival and the incidence of atrophic follicles are expressed as percent of the number of normal follicles in an equivalent control area. There was virtually no follicle loss at 800 rads but as the dose increased there was a very rapid reduction in follicle survival. The dose for 50% follicle survival was 1580 rads. The yield of atrophic follicles was very low and the occurrence of these lesions was essentially limited to doses of 1690 rads and greater with a peak incidence of 7% at 2540 rads. It can be seen in text-figure 3 that there is a similarity between the curves for the incidence of epidermoid carcinoma and atrophic follicles in the sense that the general shapes are similar with maxima occurring at about the same dose, i.e., 2540 rads. At this dose the overall follicle survival was reduced to about 15% of the number of follicles in control skin. Text-figure 3 also includes a curve representing the size of the residual irradiated area as a percentage of the equivalent control area. The discrepancy between the curve of the residual irradiated area and the curve of follicle survival indicates that not all of the follicle lethality can be ascribed to ulceration and gross tissue loss. For example, at 1690 rads

the follicle population was reduced to 25% of control while the skin area was reduced to only 75% of control. This was evident on the whole mounts as large areas of epidermis completely devoid of hair follicles.

Text-figure legend 3. The dose-response curves in the mouse at 88 weeks after irradiation are shown (as percent of controls) for hair follicle survival (total and atrophic), the area of irradiated skin, and the incidence of epidermoid carcinomas. The data are related to the dose in kilorads at the skin surface.

DISCUSSION

The results of this experiment confirm the reports of other investigators that ionizing radiation has a very low efficiency for the induction of skin tumors in the mouse, and that the few tumors that do occur are epidermoid carcinomas whereas the majority of radiation-induced skin tumors in the rat are adnexal (9, 10).

The reason for the difference in the radiation skin tumor response of the two species is clearly related to the marked difference in the pattern of hair follicle injury. This is illustrated by a comparison of the data for follicle survival in mice shown in text-figure 3 with the combined data from a series of rat experiments presented in text-figures 4 and 5.

Text-figure 4 shows the total survival of all follicles (normal and atrophic) and the incidence of atrophic follicles for rat skin as a function of the minimum follicular dose which is defined as the smallest dose occurring within 0.3 mm of the surface, i.e., within the depth of the hair follicle. The minimum follicular dose was used because it best reconciled the results of experiments with radiations having diverse penetrations (6, 7).

Text-figure legend 4. The dose-response curves in the rat at 80 weeks after irradiation are shown as a function of the minimum hair follicle dose (in kilorads) for the survival (in percent of controls) of total follicles (upper curve) and atrophic follicles (lower curve). The total follicle survival curve includes normal as well as atrophic follicles. The various experiments are indicated in the key.

The follicle survival curve has a shoulder (extrapolation number approximately equal to 5), and 50% survival occurs at a dose of just under 3000 rads. There is considerable scattering of the points for the incidence of atrophic follicles in the vicinity of the peak incidence. The curve shown in text-figure 4 was drawn through the average of the points and the best estimate of the position of the maximum is 2700 $\pm$ 700 rads.

The tumor incidence as a function of the minimum dose to the hair follicle for rat skin and the curve for the incidence of damaged follicles from text-figure 4 are shown in text-figure 5. The approximate congruence of the two curves demonstrates the correlation between tumor incidence and atrophic follicle incidence for rat skin for several different types of radiations and skin penetration depths.

Text-figure legend 5. The dose-response curves in the rat at 80 weeks after irradiation are shown as a function of the minimum follicle dose (in kilorads) for the incidence of epithelial skin tumors (tumors per rat) and the incidence of atrophic follicles (percent of control). The various experiments are indicated in the key.

There are 2 major differences between the follicle injury and tumor response in the mouse compared to the rat: (1) as shown in text-figure 3, there is virtually no radiation-induced follicle atrophy in the mouse skin; by and large, either the follicles remain intact or they are completely destroyed; (2) the mouse hair follicles are much more radiosensitive than those of the rat. Text-figure 3 shows that the surface dose for 50% survival of the mouse hair follicles is 1500 rads which is equivalent to a minimum follicle dose of 950 rads. This is to be compared with a minimum follicle dose of 3000 rads in the rat for the survival of both structurally intact as well as atrophic follicles and a minimum follicle dose of about 2200 rads for the survival of only those follicles which remain structurally intact. It is perhaps more appropriate to make the survival comparison on the basis of structurally intact follicles since the incidence of atrophic follicles in the mouse is negligible. Regardless of how the comparison is made, the sensitivity of the mouse hair follicles is at least double that of the rat.

For reasons unknown the few atrophic follicles that do occur in the mouse are found at doses where the total follicle survival is very low. In the rat the peak atrophic follicle incidence occurs at a level of follicle survival of about 60%. In the mouse the corresponding peak occurs at about 15% follicle survival. Nevertheless, the correspondence between atrophic follicles and tumors is not greatly different than in the rat, i.e., in the mouse the number of atrophic follicles per epidermoid carcinoma when averaged for the 4 highest dose groups was 7200 (σ = 2400). This ratio is only about double that which occurs in the rat for epithelial skin tumors.

The maximum tumor incidence for mouse skin is very low, i.e., 0.14 tumors per mouse (text-figure 2) compared to 4.0 or more for rat skin (text-figure 5) which is about a 30 fold excess of rat tumors compared to mouse tumors. One contributing factor to the difference might be the comparatively small size of the mouse. The relative proportion of the total skin surface that was irradiated was roughly comparable for the two species although the actual irradiated area in rats was much greater at 24 cm^2 than that in the mouse at 4.5 cm^2. Approximately 15,000 follicles were irradiated in the mouse compared to 60,000 follicles in the radiation experiments with rats. Thus, the number of irradiated follicles and the irradiated area were about 4 and 5 times greater respectively in the rat. Even after correcting for the difference in areas, the epithelial tumor incidence for rat skin exceeded that of mouse skin by about 6 fold.

On a per unit area basis, the susceptibility to sarcoma induction was not significantly different for mouse and rat skin. The best estimates are as follows: for mouse skin the average number of sarcomas per cm^2 in the dose range from 1690 rads to 4000 rads was 0.026 ± 0.02 while the comparable figure for rat skin in the dose range from 1500 rads to 6000 rads was 0.10 ± 0.005.

The relatively low tumor yield for mouse skin compared to rat skin can be attributed solely to the lack of formation of adnexal (hair follicle) tumors. Text-figure 6 shows the epidermoid carcinoma incidence per animal as a function of surface dose for rat skin from the experiments referred to in text-figures 4 and 5 and for mouse skin after multiplying by 24/4.5 from the present experiment. It can be seen from the data in text-figure 6 that there is no significant difference in epidermoid carcinoma incidence per unit area for mouse and rat skin which means that the elevated incidence of epithelial tumors for rat skin was due solely to the occurrence of adnexal tumors.

Text-figure legend 6. The incidence of epidermoid tumors for rats and mice, at 80 and 88 weeks respectively after irradiation, are shown as a function of the surface dose. The mouse tumor incidence data has been scaled up by a factor of 24/4.5 to correct for the difference in the irradiated areas. The rat data was from the same experiments used in text-figures 4 and 5.

The lack of formation of adnexal tumors in mouse skin may be related to the inability to form atrophic (damaged) follicles. In rat skin atrophic follicles occurred at intermediate doses and seemed to represent an intermediate stage of damage, whereas in comparable dose ranges for mouse skin, relatively few atrophic follicles formed even though many follicles were completely destroyed. At higher doses a low incidence of atrophic follicles was found but only at the edges of the irradiated area.

The explanation for the relative lack of formation of atrophic follicles in mouse skin is unknown but it cannot be related simply to the increased sensitivity of the mouse follicles to the lethal action of the radiation. Our current hypothesis is that the radiosensitivity of the cells at the various levels of the hair follicle is relatively uniform in the mouse so that the follicle tends to be destroyed completely or not at all. In the rat, however, there is a tendency for the destruction to progress from the lower end of the follicle toward the surface. The hair germ of the follicle tends to be destroyed selectively at lower doses leaving a sebaceous gland and follicle orifice attached to the skin surface; with more severe damage the follicles tend to be reduced to stumps of epithelium attached to the surface (i.e., without a sebaceous gland). It is this latter form of follicle atrophy which is also found in the mouse at high doses in association with epidermoid tumors; similarly, in the rat, the epithelial-stump variety of atrophic follicle is associated with a predominance of epidermoid tumors.

It is not clear whether the mouse fails to produce adnexal tumors because the relatively high sensitivity of the follicle for lethal damage precludes the survival of cells irradiated at effective transforming doses or because of the inability to form atrophic follicles. Evidence obtained from experiments where the irradiations were done on the rat skin with the hair follicles in the resting stage suggests that atrophic follicles might be a necessary factor in the tumorigenic process because of the relatively constant proportionality between the incidence of atrophic follicle and tumors under conditions where the dose distribution along the follicles varied widely (1, 8).

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FIGURE LEGENDS

Figure 1 - The incidence (in tumors per mouse) of epidermoid carcinomas and sarcomas with respect to elapsed time after irradiation.

Figure 2 - The incidence versus surface dose of sarcomas and epidermoid carcinomas for mouse skin at 88 weeks after irradiation.

Figure 3 - The dose-response curves in the mouse at 88 weeks after irradiation shown (as percent of controls) for hair follicle survival (total and atrophic), the area of irradiated skin, and the incidence of epidermoid carcinomas. The data are related to the dose in kilorads at the skin surface.

Figure 4 - The dose-response curves in the rat at 80 weeks after irradiation are shown as a function of the minimum hair follicle dose (in kilorads) for the survival (in percent of controls) of total follicles (upper curve) and atrophic follicles (lower curve). The total follicle survival curve includes normal as well as atrophic follicles. The various experiments are indicated in the key.

Figure 5 - The dose-response curves in the rat at 80 weeks after irradiation are shown as a function of the minimum follicle dose (in kilorads) for the incidence of epithelial skin tumors (tumors per rat) and the incidence of atrophic follicles (percent of control). The various experiments are indicated in the key.

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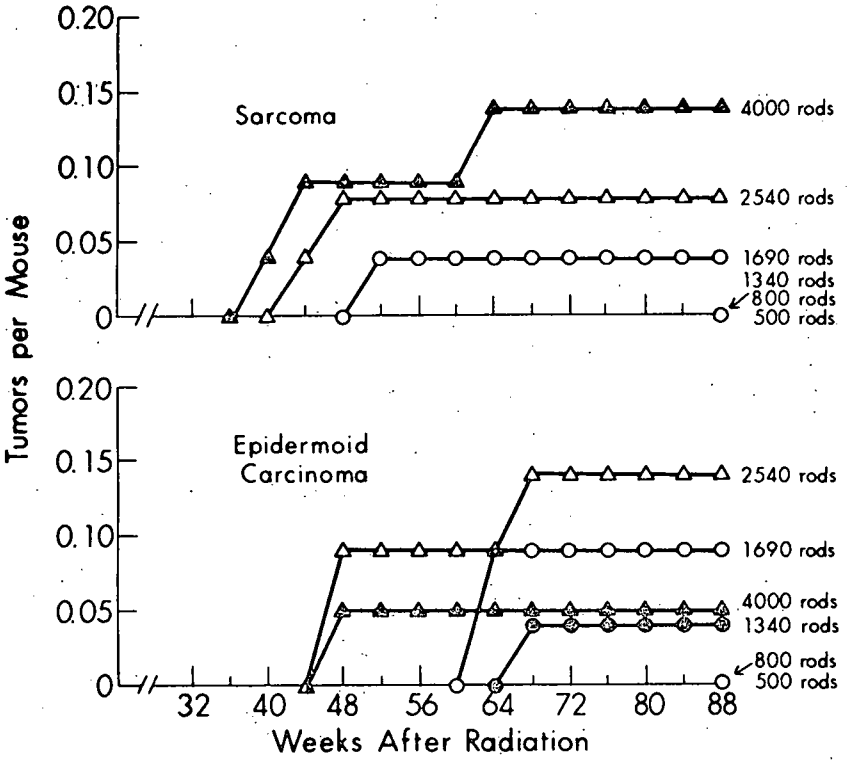


Figure 1

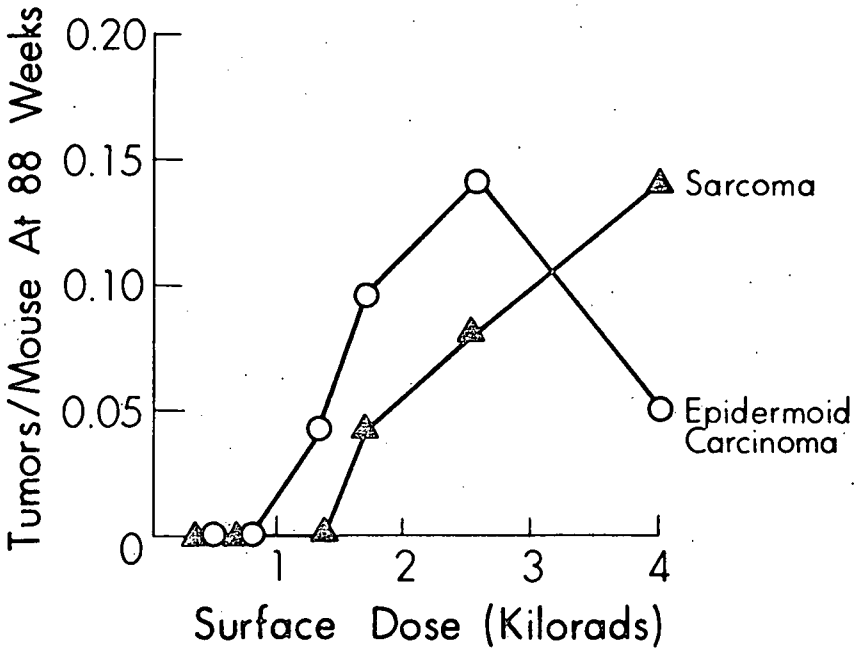


Figure 2

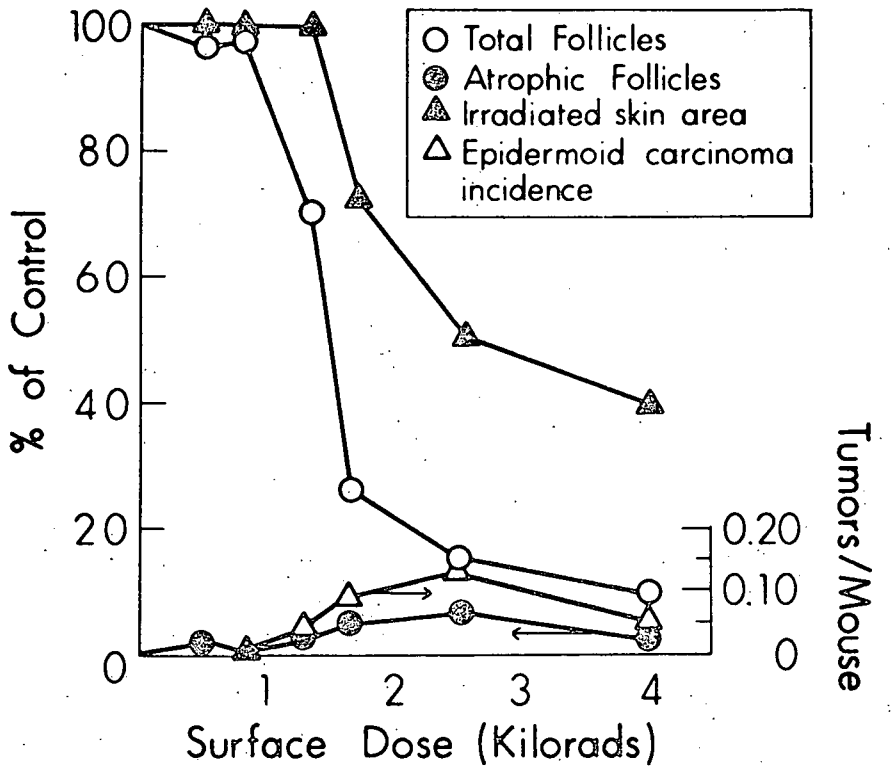


Figure 3

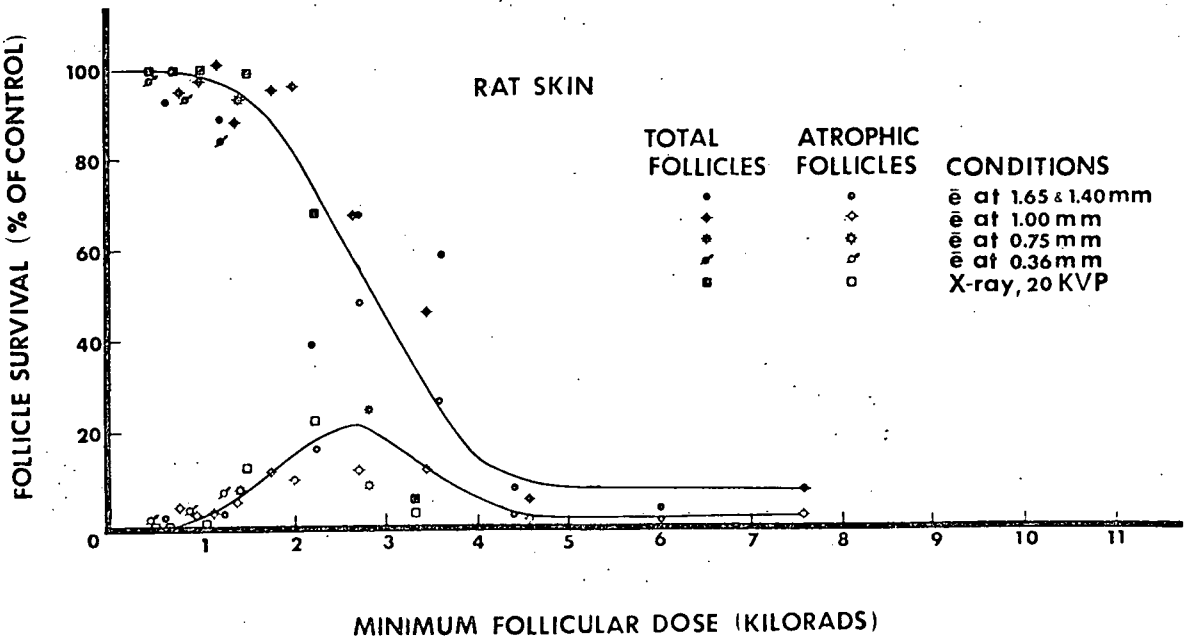
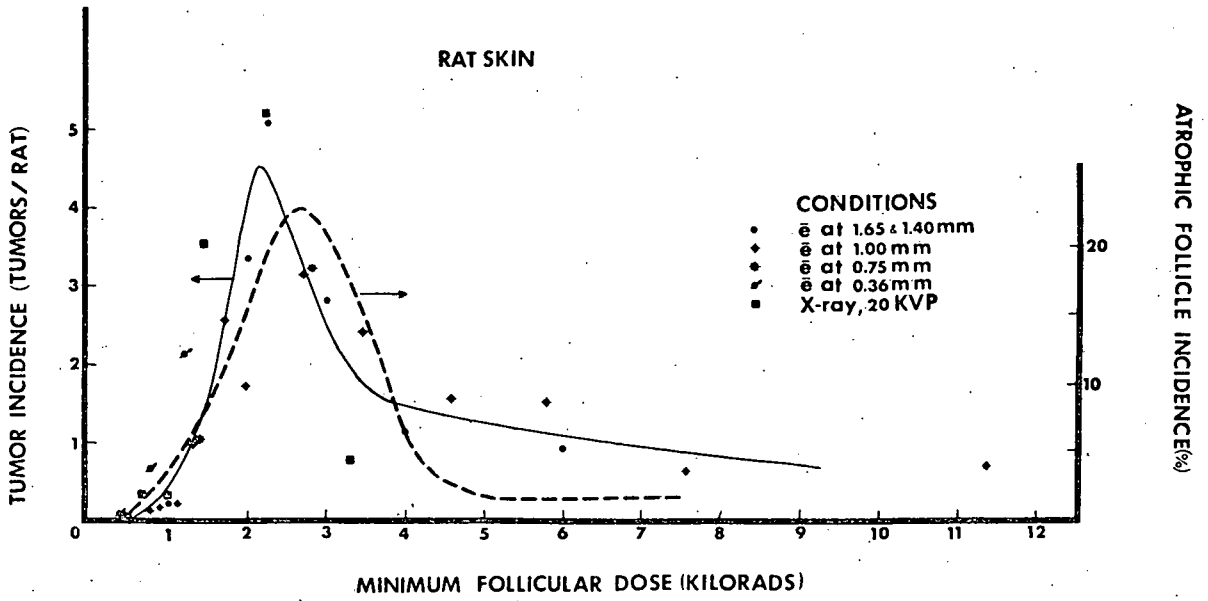


Figure 4



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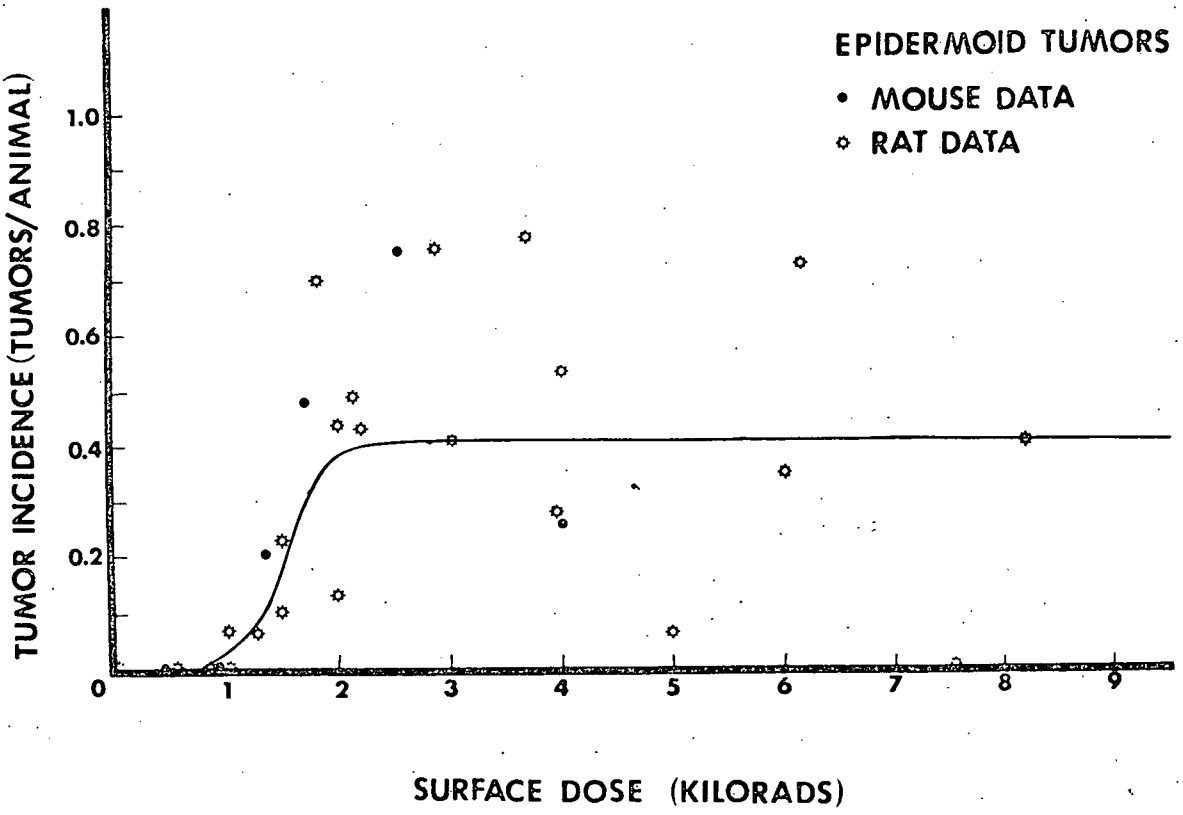

Figure 6