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An abstract of a dissertation in the Department of Biology submitted to the faculty of the Graduate School of Arts and Sciences in partial fulfillment of the requirements of the Doctor of Philosophy at New York University.

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DISCLAIMER

A Comparison between the Early Cellular Response to Electron Radiation and the Production of Tumors

The aim of this study was to compare radiation induced cell population changes in various epithelial components of the skin with the subsequent production of tumors of different types. The dorsal skin of rats in the growing phase of the hair cycle was irradiated with an electron beam. Two penetration depths of the beam were used, one shallow (1.0 mm) which gave a lower dose to the follicle matrix than to the upper part of the follicle, and the other deep (2.0 mm) which gave an essentially uniform dose to the whole follicle. Doses of 1000 rads, 2000 rads, 4000 rads and 8000 rads were given.

Size changes in the epidermis, sebaceous glands and follicles were measured from histological sections during an 85 day period post irradiation. The sizes were estimated by determining the number of eyepiece grid intersections contained within each structure in a series of microscopic fields.

Cell loss and repopulation were measured biochemically during the first 10 days post irradiation. Rats which had been injected with ^{3}H-thymidine 6 days prior to irradiation were biopsied at

2 day intervals. DNA was extracted from the biopsies and the total DNA and ^{3}H content of the DNA was determined. In separate groups of animals the tumor incidence was determined monthly and all tumors were classified histologically at the death of the animal. Abnormal follicle incidence was also determined from whole mounts of the rats' skin.

The rate of loss of ^{3}H-thymidine labelled DNA was found to be the same in all irradiated groups as in controls, indicating that the irradiated cells were eliminated at the normal turnover rate. At the deep penetration the rate of loss of total DNA was the same as the rate of loss of ^{3}H-thymidine during the first 4 days post irradiation, indicating that there was essentially no replacement of irradiated cells during this period. The grid counts showed that after the initial cell loss there was in general a hyperplastic reaction in the epidermis and sebaceous gland. The degree and time of occurrence of the hyperplasia was dependent on dose and penetration; for example, in the epidermis there was a transient hyperplasia at 1000 rads deep and 2000 rads shallow and a later occurring but persistent hyperplasia at 2000 rads and 4000 rads deep. The reaction of the sebaceous gland was similar

to the epidermis but varied from gland to gland, some glands did not regenerate at all while those that did were frequently hyperplastic. The grid counts showed an initial marked decrease in the size of the hair follicles at high doses, i.e., 2000 rads and over, however, by 15 days post irradiation asynchrony in the hair-cycle made comparisons with controls meaningless due to the vast difference in size between resting and growing follicles.

No correlation between acute or chronic damage and the tumor incidence was found. The peak tumor incidence for the shallow penetration occurred at 1000 rads, a dose which produced only minimal morphological changes and very few abnormal follicles; while very few tumors were induced at 4000 rads shallow, a dose which produced epidermal and sebaceous gland hyperplasia, and a significant number of abnormal follicles. No association between tumor type and persistent hyperplasia was found either. The incidence of keratinizing tumors was higher at 1000 rads deep, where there was only transient epidermal hyperplasia, than it was at 4000 rads deep where the hyperplasia was persistent. Hyperplastic sebaceous glands were not necessarily associated with a large

number of sebaceous tumors.

The high incidence of tumors at 1000 rads shallow implied that there might exist a population of stem cells susceptible to oncogenic transformation which were located at a relatively shallow depth in the skin. It was suggested that a possible source of this population might be the germ cells of the resting follicle, and that the location of these cells did not change between the resting and growing phases of the hair cycle.

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1.0 Introduction

The aim of this study is to quantitate radiation-induced cell population changes in various epithelial components of rat skin and to correlate them, if possible, with the subsequent production of tumors.

The experiment reported here is part of a continuing series of experiments carried out in this laboratory, in which a number of relationships between radiation-induced damage and oncogenesis have been established. The model used was the dorsal skin of albino rats (strain CD) which is susceptible to the production of radiation-induced tumors and which has a low spontaneous tumor incidence.

It was shown that the peak tumor incidence occurred at intermediate doses of radiation, that is, there was an increase in tumor yield up to a maximum which was followed by a decline associated with doses which produced some degree of ulceration (Albert, Newman and Altshuler, 1961). A possible interpretation was that at higher doses insufficient cells survived to give rise to an appreciable number of tumors. The peak tumor yield occurred at a dose just below the ulcerative dose and thus must have resulted

in a considerable amount of cell death.

The association between damage and tumor formation was studied in greater detail in resting skin by counting the number of damaged (atrophic) hair follicles in whole mount preparations of irradiated skin (Albert, Burns and Heimbach, 1967 b). It was found that the tumor incidence was directly proportional to the number of atrophic follicles. Thus, a relationship between chronic damage and oncogenesis was established for the resting phase follicles.

The correlation between damaged follicles and tumors was pursued in two experiments in which the degree of damage to different parts of the follicle was varied. This was done by two methods. The first method utilized electron beams of varying penetration (Albert, Burns and Heimbach, 1967 a). It was found that the surface dose had to be considerably increased at low penetrations to give the same tumor yield as at high penetrations. The dose response curves for the different penetrations could be made to coincide if the dose at a depth of 0.27 mm was plotted against the tumor yield. This depth corresponded roughly with the tip of the resting follicle. The second method used the Bragg peak of a 40 MeV α-particle beam to irradiate the follicle at selected depths (Heimbach, Burns and Albert, 1969). The Bragg peak which

is about 0.1 mm wide was placed at three different depths below the skin surface. In no case was the tumor incidence elevated above that expected from the Bragg curve plateau dose. It was suggested that tissue repair could in some circumstances reduce the oncogenic damage, but that lack of complete repair, as evidenced by permanently damaged follicles, increased the susceptibility to tumors.

In all prior experiments cited thus far, the irradiations were carried out in the resting phase of the hair cycle. In the experiments reported here the irradiations were carried out in the growing phase. The resting follicle is a mitotically inactive structure. During the period of growth, there is intense mitotic activity in the follicle accompanied by an enormous increase in size. In the mature growing follicle mitotic activity is concentrated in the matrix of the hair bulb.

It has been established (Geary, 1962) that the growing phase of the follicle is much more susceptible to radiation-induced morphological damage than the resting phase. Comparison of the tumor data in the present experiment with that already collected

in the experiments on the resting follicle will determine whether or not the growing phase is also more sensitive to tumor induction either in view of the increased mitotic activity or the increased number of cells present.

In addition, since radioactive label could be built into the growing follicle, it was possible to obtain information on cell loss from cell kinetic studies. Two penetration depths were used in order to modify the injury response. The shallower of the two penetrations essentially spared the matrix of the hair bulb while the deeper penetration delivered a more uniform dose to the whole follicle. Thus, information on the relationship between injury and oncogenesis was obtained for both penetrations.

1.1 Radiation Oncogenesis

Much of the literature on radiation carcinogenesis is concerned with the indirect or systemic effects of radiation. Since electron irradiation of the skin is a localized effect, systemic effects will not be considered. Reviews of systemic effects are included in articles by Casarett (1965) and Glucksmann, Lamerton and Mayneord (1957). However, the exclusion of a discussion of systemic effects does not preclude a consideration of the indirect effect of radiation on the cellular environment as opposed to the direct effect on the cells from which the abnormal growth arises.

The problem of understanding the mechanism of radiation carcinogenesis cannot be adequately dealt with until certain basic relationships have been elucidated. These relationships include the dependence of tumor production on radiation dose, on fractionation of the dose, on the volume of tissue irradiated, and on the inherent sensitivity of the tissue. In addition, determining the antecedents of tumor production in terms of morphological and other changes in the tissue will contribute to the solution.

Despite the large amount of data reported on the carcinogenic effects of radiation, the mechanism is still obscure. The following sections will attempt to summarize the present information on the subject.

1.1.1 Dose Response Relationships

Considerable interest in the relationship between radiation dose and carcinogenesis has stemmed from the controversial concept of a "threshold dose" for the induction of cancer. Lewis (1957) calculated that the probability of leukemia per individual per rad per year was constant over a wide range of doses in the case of Japanese atomic bomb survivors and in the case of patients treated with radiation for ankylosing spondylitis. He took this as evidence that the relationship between leukemia incidence and radiation dose was linear. He also found no evidence in the Japanese data to support a threshold dose. This finding was supported by the fact that relatively low doses of about 0.1 rad per day appeared to increase the incidence of leukemia in radiologists. Lewis concluded that the linear dose response data could be explained by a somatic mutation, presumably occurring in one of the precursor cells destined to give rise to mature leukocytes.

However, he did point out that the somatic mutation hypothesis and other hypotheses for the origin of radiation-induced malignancies were not mutually exclusive.

On the other hand, Brues (1958) emphasizing the uncertainties in the dosimetry of the Japanese data suggested that the data were at least as compatible with a physiological mechanism linking leukemogenesis with initial severe hematopoietic damage as they were with a mechanism which was linear with dose. Further, he claimed that more critical evaluation of the data on ankylosing spondylitis patients and on radiologists failed to support the linear, no threshold dose hypothesis.

Gray (1965) analyzed the data of Upton (1961) on murine myeloid leukemias and found two components to the dose response curve. The first was an increasing incidence of leukemia at relatively low doses which was dependent on approximately the second power of dose, and the second was a decreasing incidence at high doses due to cell killing. The dose squared relationship suggests that more than one process is involved in leukemogenesis.

Perhaps the most widely studied radiation induced carcinogenesis, after leukemias, is that in skin. This area of study was

made feasible by the use of electron beams and β-particles from radioactive sources which enabled the skins of laboratory animals to be irradiated without causing injuries to deep seated organs. Raper, Henshaw and Snider (1951) reporting on the delayed effects of exposures to external β-rays on the skin in a variety of laboratory animals presented data on tumor induction in the skins of mice and rats. Though the data were sketchy, they suggested a low incidence at low doses with a sharp increase at higher doses (3000 rem in mice and 4000 rem in rats). The lack of survivors 6 months after irradiation at even higher doses precluded any further analysis. However, earlier data by Henshaw, Snider and Riley (1949) indicated a decreasing tumor incidence with increasing dose in the range 4500 rep to 8500 rep.

Both of these experiments were essentially confirmed by the work of Albert et al.(1961) who completed dose-tumor incidence curves for single doses of β-radiation ranging from 230-10,000 rads applied to the backs of rats. The resultant curves showed that the incidence of all tumor types increased abruptly at around 2000 rads, reached a peak at about 4000 rads and declined at higher doses. The sharp increase in tumor production occurred at

doses which produced mild to moderate skin damage, while markedly ulcerative doses had a suppressing effect on tumor production.

Hulse (1967) found the same relationship for skin tumors in mice produced by β-irradiation in the dose range from 750 to 12,000 rads. In a further analysis of this data, Hulse, Mole and Papworth (1968) found that it could be accounted for by assuming that tumor induction was proportional to the square of the dose, i.e., the same relationship found by Gray (1965). It is worthy of note that the tumor incidence recorded by Albert et al. (1961) is also roughly proportional to the square of the dose.

The value of dose-response relationships lies in the potential to decide whether two types of carcinogenesis may have a similar mode of action or even in deciding between two different hypothesis of cancer induction. In radiation carcinogenesis two separate but not mutually exclusive hypotheses may be presented -- that radiation converts normal cells into potentially neoplastic cells, and that radiation causes tissue damage the imperfect repair of which is responsible for tumor production (Mole, 1958).

It is possible then to surmise that the dose squared dependence found in the data presented above may imply the

involvement of both the aforesaid processes.

1.1.2 Dose Fractionation and Repair

Though dose fractionation has been used routinely in the radiation therapy of malignant neoplasms, e.g., see Ellis (1969), it has not been widely used in oncogenesis. The rationale for its use in therapy is that homeostatic repair mechanisms are operative in normal tissue but not in the tumor. It would be of interest to know whether or not these same mechanisms could cause a reduction in the oncogenicity of a given dose of radiation.

Henshaw et al. (1949) exposed a group of rats to surface β-radiation for months and another group to single doses. The tumor incidence from daily doses of 50 rep cumulating to 24,600 rep was equivalent to a single dose of 4600 rep, suggesting that the single dose was much more efficient.

The implication that there is recovery with respect to tumor induction was illustrated in a recent experiment by Burns, Albert and Sinclair (1971 Abstract) in which dose response curves were compiled on two groups of rats. The first group was given a subtumorigenic dose of electron radiation (or at least a dose at which there was a very low incidence of tumors) of 750 rads. One

month later both groups were given graded doses from 750-3000 rads. The dose response curves for tumor incidence for both groups coincided if one subtracted 650 rads from the first group. Thus, the effectiveness of the initial dose in the production of tumors was largely reduced by the time of the second irradiation.

The evidence from other fractionation studies in skin is equivocal on repair. For example, Boag and Glucksmann (1956) reported the same tumor incidence after 12,000 rads in a single exposure as after 4600 rads in two equal exposures 2 months apart. Hulse and Mole (1969), on the other hand, found that with doses of 12,000 rads and 6000 rads of β-radiation given in different fractionation regimes, there was a decrease in the incidence of dermal tumors and increase in epidermal tumors with fractionation of the dose. However, the radiation doses employed in these two investigations were high, the single doses being in the ulcerative range. It is possible that there was a decrease in the tumor incidence for the single doses due to excessive cell killing.

Experiments involving fractionation or protraction of dose in the induction of leukemia seem to indicate that fractionation

increases the incidence of leukemias. Cole and Nowell (1963) compared the induction of leukemia in LAF mice by a dose of 260 rads of X-rays either as weekly fractions or as a single dose at the beginning or end of the year. The results show a significantly higher incidence of leukemia from fractionation. This supports the conclusions drawn by Berenblum and Trainin (1963) that divided small doses of radiation are more effective than a single large dose for leukemia induction.

However, the murine leukemias are a varied group including thymic leukemia and generalized leukemias and there is considerable evidence from the work of Berenblum and Trainin (1963) and others of indirect effects and the role of a virus, so that perhaps they constitute a special case. Certainly the role of repair in the oncogenic processes has not been specifically determined by fractionation studies.

1.1.3 Volume of Tissue Irradiated

The question of whether or not non-uniform irradiation patterns produce tumors in proportion to the number of cells irradiated is pertinent to the verification of any hypothesis on radiation carcinogenesis. Proportionality between the number of

cells irradiated and the number of tumors produced would suggest that the development of a tumor depends on radiation-induced changes within the cells and not on the spatial arrangement of the tissue or interactions between the cells and their environment.

The fact that 0.3 MeV electrons produced no skin tumors in mice in contrast to 0.7 MeV electrons (Glucksmann, 1963) is evidence that spatial distribution of the irradiated cells is a contributory factor in carcinogenesis. This was reinforced by the studies already alluded to in the introduction (Albert et al. 1967 and Heimbach et al. 1969) in which rats were irradiated with electrons of varying penetrations and with α-particles with the Bragg peak placed at varying skin depths. The conclusion was that the entire follicle should be irradiated in order to produce a significant tumor yield. Additional evidence for the existence of an effect extrinsic to the irradiated cells was the decreased tumor yield obtained from grid and sieve irradiation (Albert et al. 1967). The implication from the above papers was that the oncogenic action of radiation could be reduced by the presence of cells which had received minimal amounts of radiation.

1.1.4 Inherent Susceptibility of Tissue

Practically no experimental work has been carried out to ascertain the possible variation in the susceptibility of a particular organ to radiation carcinogenesis as a function of the different physiological states of the organ. The only data from which a possible inference might be drawn was that on the increased incidence of leukemia and other neoplasms in children irradiated in utero (Stewart, Webb, Giles and Hewith, 1956), which suggested that developing organs were very susceptible. This effect might be due to the increased radiosensitivity of dividing cells.

It is certainly well documented (Chase and Montagna, 1951, Geary, 1952 and Argyris, 1954) that hair follicles are much more sensitive to the lethal effects of radiation in the growing stage than in the resting stage. For example, Geary (1952) showed, in a histological study of the effect of low voltage X-rays on the hair follicles of rats, that with doses up to 2000 R the matrix cells of the growing follicle were among the most radiosensitive elements in the skin. Argyris (1954) using higher doses, 3000 rads and 5000 rads in mouse skin, found earlier and more abrupt

epilation in the growing phase than in the resting phase. However, no investigations into differences in oncogenicity of the two phases have previously been carried out.

Data on the effect of the hair cycle on chemical carcinogenesis, however, is not lacking. Andreasen and Engelbreth-Holm (1953) demonstrated that skin in the resting stage of hair growth was much more susceptible to tumor formation after a single painting of 7,12-dimethylbenz(a)anthracene than skin in the active phase. This was confirmed by Borum (1954). However, Berenblum, Haran-Ghera and Trainin (1958) showed that this effect was due to the greater retention time of the hydrocarbon in the resting skin rather than any increased inherent susceptibility of the cells in the resting stage.

The importance of the hair follicle in the production of skin tumors have been investigated in chemical carcinogenesis by comparing the incidence in a strain of hairless mice with that in the same strain of mice with hair. Giovanella, Liegel and Heidelberger (1970) recently reviewed the conflicting reports on this subject and concluded that there are markedly fewer cells in the skin of the adult hairless mouse capable of undergoing

neoplastic transformation. They stressed the role of the hair follicle in the neoplastic process.

It would be of interest, therefore, to compare the oncogenic response of the skin in the active and resting stages of hair growth, to a carcinogen, the dose level of which could be calculated at any level in the skin -- namely, radiation.

1.1.5 Morphological Antecents of Tumor Production

Radiation-induced injury has been described in virtually every organ of the body. A recent monograph on the pathology of irradiation edited by Berdjis (1971) would serve as a general guide.

Although the degree of susceptibility of different tissues vary the general patterns are similar. The injury is basically characterized by cellular degeneration followed by structural disorganization. Glucksmann et al. (1957) concluded in their discussion that radiation-induced cancer arose from a tissue environment that had suffered severe disorganization either as a result of local radiation damage or hormonal disturbance.

Radiation damage to skin has been described by numerous investigators, for example, Henshaw et al. (1949) described abnormal

tissue development in rats exposed to beta rays. Tessmer and Brown (1962) describe lesions in bovine skin exposed to radioactive fallout, and Conrad, MacDonald, Meyer, Cohn, Sutow, Karnofsky, Jaffe and Rikton (1962) describe skin changes in Marshallese islanders exposed to radioactive fallout. Several histological studies have been reported; for example, Geary (1952) described damage to the skins of rats irradiated with low energy X-rays, and Tessmer, Andrews and Jennings (1960) reported observations on changes in porcine skin following electron beam irradiation.

Doses of above 1000 rads were sufficient to cause observable damage. The lesions of acute radiation dermatitis could be observed in the epidermis after 48 hrs. and were well developed within a few weeks. Histologically, these lesions could be seen to consist of exfoliation of the stratum corneum, parakeratosis, swelling and vacuolization of prickle cells, liquefactive degeneration of the basal layer, loss of nuclear polarity and pleomorphism. Sufficiently large doses resulted in necrosis and ulceration. In severe damage the epithelial cells of the hair follicles underwent swelling and vacuolization or frank necrosis resulting in epilation. Such epilation characteristically occurred

at about 3 weeks and could be either temporary or permanent. Sebaceous glands showed reduced secretion at about one week and then became atrophic with thickening of the basement membrane. Eventually many of the glands disappeared completely. In the dermis the collagen bundles became swollen, edematous and homogenized. Elastic fibres were swollen, fragmented and frayed. There was an associated inflammatory reaction with edema and cellular infiltrate. The blood vessels were edematous, often dilated and showed endothelial swelling and proliferation. In severe damage the blood vessels underwent necrosis.

With irreversible damage, there was a slow progression of changes over a period of months. The epidermis became atrophic in areas. Elsewhere there was hyperkeratosis and acanthosis, disorderly arrangement of cells, dyskeratosis and increased mitotic activity. Irregular downgrowths of epidermal cells around telangiectatic vessels were observed. Such changes frequently lead to invasive carcinoma. In the dermis the collagen bundles become atrophic, sclerotic and hyalinized and contained pleomorphic giant fibroblasts. The blood vessels become thickened and hyalinized and their lumens were reduced.

What role the above changes play in the subsequent development of tumors is unknown. However, since in the rat the highest tumor incidence was associated with a dose, which was just below the ulcerative dose (Albert et al. 1961), and since the tumor incidence was proportional to the number of damaged follicles (Albert et al. 1967), then it is possible that a considerable degree of injury is necessary for tumor formation.

2.0 Materials and Methods

2.1 Experimental Animals

Male albino Sprague Dawley rats, CD strain from the Charles River Company, Brookline, Massachusetts were used in the experiment. A 12 cm^2 area on the backs of these animals was irradiated. This region was irradiated during the growing phase of the hair cycle, i.e., when the rats were 37 days of age. In order to insure greater uniformity in the phase of the hair growth than is obtainable by following the normal growth patterns, the dorsal hair of these animals was plucked at the 28th day of age which was close to the end of the first resting period (telogen) of hair growth. Plucking of the hair, which was accomplished by using the depilatory wax compound ZIP (Jean Jordeau Inc., N.Y.), synchronized the onset of the elongation of the follicles in the region to be irradiated (Johnson and Ebling, 1964).

The animals were anesthetized with 35-40 mg per kg Nembutal by intraperitoneal injection prior to irradiation. Following the irradiation procedure, the animals were allowed to recover from the anesthesia and housed two to a cage while on test. They were given food and water ad libitum.

2.2 Irradiation Procedure

The irradiations were carried out on the 1.0 MeV Van de Graaff accelerator located in the Department of Nuclear Engineering on the University Heights campus of N.Y.U. The energy of this accelerator is variable over wide limits permitting the adjustment of the depth of penetration of the electron beam into the skin. The beam was collimated with lead bricks.

The dosimetry of the electron beam was carried out with a 1.0 mm gap parallel plate ionization chamber. The saturation current was converted to dose rate using the Bragg-Gray principle (see Appendix I). The rats were irradiated in boxes attached to the rim of a rotating table. The average dose rate was calculated from 10 passes of the chamber through the beam.

Depth dose curves were constructed by placing tissue equivalent absorbers in front of the ionization chamber and measuring the dose rate for each absorber thickness.

The rats were irradiated with either of two penetration depths, one shallow and one deep. The depth dose curves for the two penetrations are shown in Figure 1. The shallow penetration delivered a substantially lower dose to the follicle matrices

than to the upper part of the follicle. The deep penetration delivered an essentially uniform dose to the whole follicle. The doses at different levels of the growing follicle are shown in Fig. 2 for both penetrations.

Four surface dose levels 1000 rads, 2000 rads, 4000 rads and 8000 rads were used at the shallow penetration and three, 1000 rads, 2000 rads and 4000 rads at the deep; in order to establish dose response curves for tumor production and related effects.

Approximately 32 rats were irradiated in each group making a total of 256 including controls.

The beam current of the accelerator was adjusted to make the surface dose rate about the same for both penetrations. This dose rate was about 1050 rads/min.

2.3 Observations and Analysis

Sixteen animals in each group were observed for tumor production over an eighty week period. The remaining animals in each group were used to study various aspects of the acute response, and chronic damage. The analyses carried out are summarized in

Table 1.

2.3.1 Appearance of the Skin

After the irradiation, the rats were observed every other day for the first 4 weeks to assess the acute response, and at 4 week intervals thereafter to assess chronic damage and tumor formation.

The acute responses recorded were:

1. Retardation of hair growth resulting in a reduction of hair length in the irradiated area called hair suppression.
2. Decrease in the amount of brown scale normally found on those animals called blanching.
3. Areas of complete tissue breakdown or necrosis called ulceration.

The observations carried out at 4 week intervals were used to assess the chronic effects of irradiation which included scar formation, permanent suppression of hair growth, ulceration and tumor production.

2.3.2 Tumor Incidence Calculation

The tumor incidence rate, expressed as number of tumors

per rat, was calculated by dividing the number of new tumors with a diameter greater than 2 mm which appeared during each 4 week interval, by the average number of rats alive during that interval (Altshuler, 1970). Summation of the tumor incidence rates through any post-irradiation time then gave the tumor incidence at that time. All tumors included in the analyses were verified histologically at death.

2.3.3 Histological Analysis

Direct evidence of tissue damage and of possible repair was obtained by examination of histological sections prepared from skin biopsies. Eight rats per group were used to provide the sections, each animal provided 6 to 9 biopsies each taken at a different time after irradiation. Biopsies were taken from six rats per group at 5 to 10 day intervals for up to 60 days post-irradiation. In addition, two rats per group provided sections at intermediate time intervals to see whether or not multiple biopses affected the response to irradiation. The biopsy schedule is shown in Table 2.

The tissue biopsies were preserved in formalin until cut and stained with hematoxylin and eosin. Acute damage was

determined from estimations of the size of the follicle and sebaceous gland compartments made by using an eyepiece grid and counting the number of grid intersections which superimposed the compartment in question (Chalkley, 1943). A 10 line x 10 line eyepiece grid was superimposed on a 460 μ diameter field. For the follicle compartment only intersections falling on cellular parts of the follicle were counted. For the sebaceous gland all intersections falling on the boundaries or lumen of the gland were counted. For the epidermis a line inscribed on a grid eyepiece was superimposed on the epidermis at right angles to it. The number of intersections this line made with epidermal cells was taken as a measure of the cellularity and thickness of the epidermis. Since all comparisons were made with sections from unirradiated controls, no attempt was made to convert the "counts" to a measure of the volume of the compartment.

2.3.4 Biochemical Analyses

Four animals in each group were used to make an estimate of acute damage by measuring the loss of DNA from prescribed areas within the irradiated region. Decreases in DNA levels

were equated with a net reduction in cell number, i.e., the number of cells destroyed minus those replaced by repopulation repair. In order to estimate the overall rate of cell loss, the cell population was pretagged with ^{3}H-thymidine. An increased loss of ^{3}H-thymidine in the irradiated areas over the controls was taken as a measure of the number of cells destroyed by the radiation. A relatively slower rate of loss of total DNA as compared to that of ^{3}H-thymidine would indicate that the DNA was being replaced by repopulation repair.

The DNA analysis was carried out on biopsies taken from the irradiated area on the day of irradiation and at 2 day intervals up to 10 days post-irradiation. Epilation forceps were used to take the biopsies in order to get tissue samples of constant size. In order to incorporate radioactive label into the growing follicle, ^{3}H-thymidine was injected intraperitoneally 2-3 days after plucking. Four injections at approximately 12 hour intervals were given to a total of 1 μCi ^{3}H-thymidine per gm of rat weight. Autoradiographs were made of the pretagged skin. The biopsies used for autoradiography were fixed in Carnoy's solution and

stained by the Feulgen procedure.

A description of the method of extraction of the DNA is given in Appendix II. The analyses were performed using Burton's colorimetric technique for DNA (Burton, 1956) and liquid scintillation counting of the tritium.

2.3.5 Whole Mount Preparation

The number of surviving follicles and the number of abnormal follicles produced by the irradiation were determined from whole mount preparations of the skin. These whole mounts were processed by soaking biopsy sections overnight in a Ringers solution containing 0.5% crude trypsin at about 3°C. The dermis could then be peeled off from the epidermis and attached hair follicles. The epithelium was then stained with hematoxylin and sudan III, embedded in glycerin and sealed with a cover glass.

Follicle counts were made under low magnification using a stereoscopic dissecting microscope using a field delineated by a square of area 1.21 mm^2. Twenty fields were counted on each animal. The size of the irradiated area at the time of sacrifice in animals suffering severe radiation damage was smaller than the size of the irradiated area in animals suffering less damage, due

to scar formation and contracture. Therefore, the follicle densities were corrected by multiplying by the ratio of the size of the irradiated area to the known size of a similar area in unirradiated controls.

Follicles were considered abnormal if they displayed a marked loss of normal structure. The two most common abnormalities were (1) small, hairless, follicle remnants and (2) aggregations of sebaceous cells and/or sebum.

Four animals in each group were used for the preparation of whole mounts as soon as ulcerative damage was healed, i.e., around 16 weeks post-irradiation. In addition, whole mount preparations were made from biopsies taken from tumor bearing animals at the time of sacrifice, i.e., at about 80 weeks. This provided a comparison between early and later incidences of follicle abnormalities.

2.4 Tumor Pathology

At 80 weeks the rats were killed and sections stained in hematoxylin and eosin were made of all tumors. Any rats dying before 80 weeks were processed provided autolysis was not too advanced. Ninety-nine per cent of the tumors were verified

histologically and classified according to their differentiation pattern following a system used in this laboratory (Albert, Phillips, Bennett, Burns and Heimbach, 1969).

The tumors were classified into 4 principle categories: keratinized, keratosebaceous, sebaceous, and nondifferentiated. The tumors were also subclassified on the basis of the predominant mode of growth as exophytic, endophytic, and subepidermal. Exophytic tumors projected more than 3/4 above the skin surface and subepidermal lesions were completely below the skin surface.

Subtypes were established with similar structural characteristics for each of the 4 principle categories. Keratinized tumors were subdivided into four types. Type-1 was composed of tightly-packed lobules with cells resembling those of the basal layer of the surface epidermis. Granules of keratin were scattered sparsely throughout each lobule. Type-2 resembled type-1 except the keratization was more extensive and tended to form discrete nodules. Type-3 consisted of lobules with cells on the periphery surrounding a large amount of keratin in the core. Intermediate stages of keratinization with keratohyalin granules could be recognized.

There were no subtypes of the keratosebaceous tumors. These

tumors were lobulated with sebaceous cells at the core and keratinizing cells at the periphery of the lobule.

Two types of sebaceous tumors occurred. Type-1 were solid tumors with predominantly sebaceous cells. Type-2 were cystic lesions.

The nondifferentiated tumors were subdivided into 2 subtypes. Type-1 resembled keratinized type-1, except no keratin was evident. Type-2 resembled type-1, except for the presence of cells with a clear non-reticulated cytoplasm. Some connective tissue tumors occurred, but they were relatively infrequent and were not included in the analysis. Illustrations of the tumors in the various categories and sub-categories may be found in the article by Albert et al. 1969.

3.0 Results

3.1 Appearance of the Skin

Hair emerged on all rats 7 days after plucking, which confirmed the synchronization of the growth phase. However, by 6 days post-irradiation the length of hair inside the irradiated areas was noticeably shorter than outside in all groups except 1000 rads shallow. This suppression of hair growth depended on both dose and depth of penetration. The average response for animals in each group was estimated 6 days after irradiation and the degree of suppression was graded 0 to 4, these values are presented in Table 3. At 8 days post-irradiation, there was a loss of hair from the irradiated areas in most groups and this obscured the suppression of hair growth. The degree of epilation also depended on dose and penetration depth and ranged from complete epilation at 2000 and 4000 rads deep to only the slightest suggestion of a thinning of the hair at 1000 rads shallow.

It can be seen from Table 3 that in terms of hair suppression 4000 rads shallow is equivalent to 1000 rads deep and 8000 rads shallow is equivalent to 2000 rads deep. A ratio of deep to shallow surface doses of 4:1 delivered the same dose at about

0.8 mm depth in the skin (Fig. 2). This was about the level of the matrix of the growing follicle. Thus, the degree of hair suppression was related to the dose received by the matrix (Table 3).

In control rats hair growth continued until 18 days post-irradiation, however, it continued up to 32 days post-irradiation in the irradiated area on rats in which only partial epilation occurred.

Ulceration, that is the complete breakdown of the skin, occurred extensively in the highest dose groups. The onset of ulceration was between 10 and 12 days post-irradiation and was maximal by 14 days. The average response was estimated at 14 days post-irradiation and is shown in terms of percentage of irradiated area ulcerated in Table 4. From this it can be seen that the deep penetration is more effective in causing ulceration than the shallow penetration. It is evident then that ulceration is dependent on the dose relatively deep in the dermis. The variability of the response precludes a good estimation of a critical depth but it is likely that a critical level lies between 0.6 and 0.8 mm. In this region the deep penetration delivers a dose 2 to 4 times higher than the shallow penetration, yet the shallow

penetration can still deliver a substantial dose (Fig. 2). The critical structures are probably the larger blood vessels.

Small areas of ulceration healed and were replaced by scar tissue by about 36 days post-irradiation. Large ulcerated areas were replaced by a laterally contracted scar between 50 and 100 days post-irradiation.

Ulceration was usually preceded by a serous oozing and sloughing of the epidermis, which is known as moist desquamation. However, in groups in which ulceration was limited to less than 5% of the irradiated area, i.e., 2000 rads shallow and 1000 rads deep, the epidermis sloughed off in dry flakes, this is known as dry desquamation. At 1000 rads shallow, there was no noticeable desquamation. The similarity of the epidermal response at 2000 rads shallow and 1000 rads deep indicated that the epidermis required about twice the dose at the shallow penetration as at the deep to produce the same degree of epidermal damage. Thus, it is evident that damage to the epidermis is dependent on more than epidermal irradiation but is also affected by irradiation of deeper structures, possibly blood vessels.

Another radiation effect which was observed was blanching, that is, the loss of the brown substance which is present on male

rats of this strain. Blanching was noted by 10 days post-irradiation, and was the only really observable effect in the 1000 rads shallow group. If the brown substance is associated with secretions of the sebaceous gland as has been suggested by Nikkari (1965), then its disappearance implies damage to this gland. The average response for each group was graded 0 to 4, at its peak at 21 days post-irradiation, and is shown in Table 5. The dose to the sebaceous gland was 10 to 30% higher for the deep penetration than for the shallow. This may explain at least in part why the response for 1000 rads deep was more marked than for 1000 rads shallow. However, the similarity of the response at 2000 rads shallow and 1000 rads deep suggested that the deep penetration is more effective for a given dose to the gland.

The brown material returned after varying intervals which were dependent on dose and penetration. This is evidence of functional recovery of the gland. No evidence of the recovery of epilated hair follicles to produce hair could be deduced from the gross observations.

3.2 Histological Data

Examination of the histological sections revealed degeneration

in the bulbs of the follicles at 2 days post-irradiation in the deep penetration. This preceded the grossly observable response of suppression of hair growth. The average number of grid intersections, which occurred over cellular parts of the follicle per observed field, were counted as described in Section 2.3.2. These follicle "hits" are shown in Figs. 3 and 4. Representative standard errors are included.

The sudden decrease in "hits", which began around day 10 in the control group, reflected the decrease in the size of the follicles associated with the end of the growth phase of the hair cycle. This shrinkage of the follicle is known as catagen. The subsequent increase about 8 days later is due to the start of the third anagen or hair growth period of the rat's life. The number of hits in the third anagen is lower than the second due to the wider spacing of the follicles in the older animals. No attempt was made to correct for growth of the rats.

From Fig. 3 it can be seen that the 1000 rads shallow dose group did not differ significantly from controls, however, there was immediate evidence of a decrease in the size of the follicle compartment at 2000 rads shallow and a marked decrease at 4000 rads

and 8000 rads shallow. For the deep penetrations (Fig. 4), the initial rates of decrease in the size of the follicle compartment was the same for all 3 dose groups, however, the decrease halted at 5 days post-irradiation at 1000 rads but continued to complete destruction of the follicles at the higher doses.

No evidence of follicle regeneration is evident from this data. However, at long intervals after irradiation the presence of follicles in different phases of the hair cycle in the irradiated area makes it impossible to compare the count data with that of earlier synchronized phases.

Injury to the epidermis appeared first as a loss of definition in outline of the basal cells 4 days post-irradiation. By 6 days the squamous layers became amorphous and there was a reduction in the amount of keratin present. Table 6 lists the number of "layers" (i.e., epidermal cells intersected by a line superimposed on the epidermus and perpendicular to it as described in Section 2.3.2). The control number was relatively constant and consisted of a layer of basal cells, a layer intermediate between the basal and squamous layer, a squamous layer of cells containing keratohyalin granules and two layers of cells composed largely of

keratin in which it was not possible to delineate the individual cells.

One of the most noticeable observations from the data in Table 6 is the penetration effect, i.e., for the same surface dose the deep penetration was more effective in causing damage than the shallow. At 1000 rads shallow there was no significant difference from control values, whereas at 1000 rads deep there was a transient increase in the number of layers at 10 days post-irradiation followed by a return to essentially control values seven days later. At 2000 rads, the shallow penetration values were similar to 1000 rads deep, i.e., there was a transient hyperplasia, whereas at 2000 rads deep the epidermis was eradicated by 10 days post-irradiation. Re-epithelialization occurred at about 20 days post-irradiation at 2000 rads deep, and a markedly hyperplastic epidermis was maintained up to at least 86 days post-irradiation. At 4000 rads there was complete eradication of the epidermis at both penetrations but the shallow penetration was re-epithelialized earlier than the deep. Further, at 4000 rads shallow the hyperplastic epidermis appeared to return to normal by 49 days post-irradiation, whereas at 4000 rads deep the epidermis

was still markedly hyperplastic by 86 days post-irradiation.

The sebaceous glands were found to be extremely sensitive to irradiation and disappeared as recognizable structures by 10 days post-irradiation at all doses above 1000 rads. There was some regeneration at all doses but not all glands regenerated. Sebaceous gland data are presented in Table 7. This data was accumulated by the grid count method used for the follicles. However, since not all glands regenerated and since many of those that did were hyperplastic, "hits" per gland and glands per field were scored rather than the number of hits per field. Thus, the number and size of the regenerated glands is recorded in preference to the amount of sebaceous material per field. It can be seen from Table 7 that for 1000 rads shallow, there was a slight reduction in the size of the glands followed by a rapid return to control levels, whereas at 1000 rads deep there was a decrease in both the size and the number of glands followed by an overshoot in the size of the regenerating glands. This hyperplasia of the regenerating glands is accentuated at 2000 rads. It is also evident at 4000 rads. In general, the time of reappearance of the glands was dependent on dose and penetration, being later at the higher doses and deeper penetration.

There was a definite difference in response of the sebaceous gland to the same surface dose at the two penetrations. However, the dose to the gland was 10-30% higher for the deep penetration (Fig. 2) and this may account, at least in part, for the difference in response.

3.3 Biochemical Data

3.3.1 DNA Extractions

Total DNA measured in biopsy sections taken from the irradiated area at 2 day intervals are shown in Figs. 5 and 6. Standard errors of the determinations are included. Deficiency in DNA in the irradiated areas was taken as a measure of net cell loss. Overall cell loss measured by this method should reflect the weighted mean of the cell loss from all the components of the skin and should thus complement the histological data.

Figure 5 indicates that for the shallow penetrations, the rate of loss of DNA initially increased with dose. This may imply that as the surface dose increased, cytocidal doses were delivered to deeper structures. For the deep penetrations (Fig. 6), the initial rates of loss of DNA were about the same at all doses. From this it might be inferred that if a cytocidal dose

was delivered then the rate of cell loss was independent of dose and that only the maximal destruction was dose dependent.

After the initial decrease in DNA levels, the values leveled out and increased from 6-10 days post-irradiation. Macroscopic observations revealed progressive deterioration of the tissue during this period especially at the high doses. Thus, it was unlikely that such increases were due to repopulation repair. Examination of histological sections revealed that the increased DNA levels were due to the infiltration of the damaged area by cells associated with the inflammatory process. However, the sections indicated that this was not a serious problem until after the fourth day post-irradiation. Thus, the initial slopes of the DNA curves were not affected.

3.2.2 Measurement of Radioactive Label

The measurement of ^{3}H-thymidine in the biopsies enabled the rate of cell loss to be measured. Examination of the autoradiographs showed that at the time of irradiation, most of the heavily labelled cells appeared to be connective tissue cells in the dermis and connective tissue sheath of the follicle. Since the ^{3}H-thymidine had been injected 6-7 days earlier, the presence of these heavily labelled cells indicated a slow turnover time in those compartments.

The rest of the label was diluted in the compartments of the skin with the faster turnover rates.

The levels of tritiated thymidine incorporated into DNA, measured in disintegrations per minute per square centimeter (dpm/cm^2) of skin biopsied, are plotted against time in Figs. 7 and 8. Since the uptake of ^3H-thymidine varied considerably from animal to animal, the dpm/cm^2 were normalized at the day of irradiation. The actual dpm/cm^2 on the day of irradiation are shown in Table 8. Standard errors of the measurements are included.

For all doses at both penetrations, the slopes of the curves in Figs. 7 and 8 are approximately the same as controls. This is evidence that the rate of loss of cells after irradiation was similar to the turnover rate in the normal tissue.

3.4 Whole Mount Preparations

3.4.1 Hair Follicle Survival

Hair follicle survival was defined to include both normal and abnormal follicles and is expressed as per cent of the average number of follicles in unirradiated controls. The percentage of surviving follicles at about 16 weeks post-irradiation and at about

80 weeks post-irradiation are listed in Table 9 for both penetrations. It can be seen that there was no significant difference between the two periods.

It is evident that the number of surviving follicles decreased with increasing dose at both penetrations. The number of surviving follicles at 4000 rads and 8000 rads shallow penetration was relatively high, suggesting that the sparing of the deepest elements of the follicles helped to ensure their survival. Comparison of Tables 4 and 9 reveals a correlation between follicle survival and ulceration.

3.4.2 Abnormal Hair Follicles

Abnormal hair follicles expressed as per cent of normal follicles in the control rats is listed in Table 10 for both penetrations at both time intervals. Again, there is no significant difference between the incidence at 16 weeks and that at 80 weeks. This implies no repair of abnormal follicles in that interval. The peak for abnormal follicles occurred between 2000 rads and 4000 rads at the shallow penetration and between 1000 rads and 2000 rads at the deep penetration. Decreases in the number of atrophic follicles at the higher doses is associated with the

decrease in survivors at those doses.

3.5 Tumor Data

3.5.1 Tumor Incidence

The cumulative tumor incidence was calculated as described in Section 2.3.1, and is shown in Figs. 9 and 10. The first tumors were observed at twelve weeks after irradiation. Subsequently, there was an approximately linear increase in the incidence, at all doses and at both penetrations.

In Fig. 11 the cumulative tumor incidences at 72 weeks are plotted as a function of surface dose. The 72 week incidence was chosen rather than at 80 weeks, since it was considered desirable to have followed a tumor for at least two observation periods before it was included. In addition, abnormalities arising shortly before the rats were killed were often too small to obtain reliable pathology. The standard deviations in Fig. 11 were calculated by assuming that the total number of tumors was distributed according to a Poisson function and thus the standard deviation was equal to the square root of the number of tumors.

The peak tumor incidence appears to be between 1000 rads and 2000 rads surface dose at the shallow penetration and

around 1000 rads at the deep penetration. Since surface dose is a rather arbitrary parameter, dose-response curves for tumor incidence were calculated for the dose at 0.3 mm depth and at 0.8 mm depth in the skin, and are shown in Figs. 12 and 13. 0.3 mm was chosen since this is about the depth of the germ cells of the resting follicle, these cells are the stem cells of the cell population of the mature growing follicle. The 0.8 mm depth was chosen since it is about the depth of the matrix cells of the average growing follicle. The dose response curves for the two penetrations are irreconcilable when the dose at 0.8 mm is used, but they have a similar shape when either the surface dose or the dose at 0.3 mm is used. The shallow dose gave a consistently lower incidence.

3.5.2 Tumor Incidence and Abnormal Follicles

A direct proportionality between the incidence of abnormal follicles and the tumor incidence had been found for the irradiation of the resting follicles (Albert et al. 1967). Thus, the ratio of abnormal follicles to tumors was calculated for each irradiated dose group and the data are presented in Table 11. It can be seen that the ratios for the deep penetration

are relatively constant. This is consistent with the hypothesis that a relationship exists between abnormal follicles and tumors. However, the ratios are widely disparate for the shallow penetration, notably the high number of abnormal follicles per tumor at 4000 rads shallow and the relatively low ratio at 1000 rads shallow. It is evident then, that the correlation between abnormal follicles and tumors is not valid for the shallow penetration.

3.5.3 Tumor Pathology

All tumors occurring in the irradiated area were examined histologically at the death of the animal and classified as described in Section 2.4. After combining subtypes, the numbers of tumors in the four main categories are given in Table 12 for all dose groups. The tumor types are similar to those obtained in previous experiments on irradiation of skin in the resting phase of hair growth, no new tumor types were observed. The data shows no clear dependence of tumor type on penetration. However, it can be seen from Table 11 that there is an excess of keratinized tumors over non-differentiated tumors at doses which produced a marked degree of residual injury, i.e., 2000 rads and 4000 rads deep and 8000 rads shallow. Whereas the non-differentiated tumors

were in the majority at 1000 rads shallow, where the chronic injury was minimal. This finding is consistent with a previous report which summarized the tumor morphology of a number of experiments carried out in this laboratory (Albert, Phillips, Bennett, Burns and Heimbach, 1969).

4.0 Discussion

The purpose of the experiment was to compare early changes in the cell populations of various components of irradiated skin, with the subsequent production of tumors.

Both biochemical and histological techniques were utilized to measure the cell population changes. The measurements of the persistence of ^{3}H-thymidine in the DNA of the skin showed that the rate of loss of ^{3}H was the same in all the irradiated groups as in the controls (Figs. 7 and 8). This indicates that the rate of loss of cells in irradiated areas was similar to the normal turnover rate. The slopes in Figs. 7 and 8 indicate a 50% reduction in ^{3}H labelled DNA in 5 days which implies a 50% turnover of labelled cells in this interval. At the deep penetration the total DNA also declined at a rate of 50% in 5 days (Fig. 4), which implies that complete reproductive death occurred without any regeneration during the initial 4 days post-irradiation. The growth of the skin was estimated during the interval after irradiation by measuring the increase in size of an area tattooed on the back of several rats. The growth rate was found to be approximately 4% per day. When a correction for

growth is applied to the slopes of the curves for total DNA and ^{3}H-label, the turnover time is increased from 5 to 6 days.

The equality between the rates of loss of ^{3}H and total DNA also implies that at the time of irradiation, the ^{3}H was distributed fairly uniformly between the different components of the skin. That is, 6 days after injection the label in the compartments with a high turnover rate (and consequently high incorporation) was diluted, so that the levels of ^{3}H in those compartments was approximately the same as that in the compartments with a low turnover rate (and consequently low incorporation).

The histological data on the sebaceous gland (Table 7) shows that the glands are almost completely eradicated by 5 days post-irradiation at the high doses. This implies a greater than 50% loss of cells in that interval. Similarly, the decrease in size of the follicle compartment at the high doses in the deep penetration (Figs. 4) also implies a greater than 50% loss of cells in 5 days. The difference between the rate of loss of cells in the follicles and sebaceous glands (greater than 50% in 5 days) and the rate of loss of total DNA (about 50% in 5 days) indicates that the turnover rates of these epithelial components is faster

than the average for the whole skin. Thus, the turnover rate of the connective tissue must be slower than that for the whole skin. This explains why the heavily labelled cells in the autoradiographs were mainly connective tissue cells.

One interesting consequence of the irradiation was over-compensatory hyperplasia. In the epidermis and sebaceous glands, the acute cell loss was followed, in general, by regeneration which resulted in an increase in thickness of the epidermis and an increase in size of the sebaceous glands compared to controls. Although cell counts per se were not performed, the size increases were equated with hyperplasia since the individual cells were not larger than those in controls. At most dose levels the hyperplasia was temporary and there was a return to normal size. The degree of hyperplasia and the time at which normal size returned were dependent on dose and penetration. In the epidermis, for example, the response ranged from a slight hyperplasia with a rapid (within 21 days) return to normal at 1000 rads deep, to a late occurring (38 days post-irradiation) and persistent (up to at least 86 days post-irradiation) hyperplasia at 4000 rads deep (Table 6). The response of the sebaceous gland was similar to

the epidermis. However, it was a more individual response, since some glands did not recover at all, but those that did were frequently hyperplastic (Table 7).

By 15 days post-irradiation, differences in the hair growth phase between the irradiated group and the controls made the comparison of follicle size difficult, since a high "count" could represent either a few growing follicles or a larger number of resting follicles. Follicles in intermediate stages, that is, in the process of elongating or in transition from growing to resting (catagen) also added uncertainty to the interpretation of the follicle count data. Asynchrony in the hair growth cycle after 15 days post-irradiation made it impossible to assess regeneration in the follicles.

The histological and biochemical data can be summarized as follows. The irradiation resulted in complete reproductive death in the first 4 days post-irradiation. The sequel to cell death was either a permanent reduction cell number or a return to control levels or to a hyperplastic state. The hyperplasia was either temporary or persistent depending on the dose and penetration of the beam. For example, temporary hyperplasia occurred in the

epidermis at 1000 rads deep and 2000 rads shallow, and in the sebaceous gland at 1000 rads shallow and 1000 rads deep (Tables 6 and 7), while persistent hyperplasia occurred in the epidermis at 2000 and 4000 rads deep and in the sebaceous glands at 2000 deep and 4000 rads shallow (Tables 6 and 7). The persistent hyperplasia was present until at least 86 days post-irradiation and this is about the time of appearance of the first tumors in all the irradiated groups. In addition, there were abnormal follicles in all dose groups (Table 10).

The aim of the study was to determine whether or not these early changes could be correlated with oncogenesis. The correlation between the incidence of abnormal follicles and the tumor incidence, which was established in earlier experiments on irradiation of skin in the resting phase of hair growth (Albert et al. 1967 b), was not supported by the data in the present experiment. Although the ratio of abnormal follicles to tumors was relatively constant for the deep penetration and similar to that which had been obtained for the resting phase, it was widely disparate for the shallow penetration (see Table 11). The most significant departures from the previous ratios

were the high incidence of tumors at 1000 rads shallow which was associated with the production of relatively few abnormal follicles, and the very low incidence of tumors at 4000 rads shallow where the number of abnormal follicles was high. Thus, it appears unlikely that abnormal follicle incidence alone determines the tumor incidence.

Do other forms of morphological abnormalities correlate with tumor induction? Again, the above mentioned groups are difficult to reconcile with such an hypothesis. At 1000 rads shallow, the effects on the epidermis and sebaceous glands were relatively mild and transient, yet there was an appreciable tumor yield; whereas at 4000 rads shallow a persistent epidermal hyperplasia was found together with frequent hyperplastic sebaceous glands, yet the tumor yield was low. In other dose groups no correlation could be found between the type of damage produced and the tumor type. For example, one might expect a high incidence of keratinizing tumors to be associated with a persistently hyperplastic epidermis. However, the incidence of this type of tumor was lower at 4000 rads deep at which there was a persistent epidermal hyperplasia, than at 1000 rads deep where the hyperplasia

was transient (Table 12). Nor can a correlation with transient hyperplasia be substantiated since the degree of hyperplasia at 2000 rads shallow is the same as that at 1000 rads deep, but the incidence of keratinizing tumors at 2000 rads shallow is only half that at 1000 rads deep (Table 12). Thus, it is evident that, in the case of partial irradiation of the growing hair follicle, no relationship between persistent morphological damage and oncogenesis can be established.

An alternative hypothesis, consistent with the present data and which explains the previous experiments on irradiation of the resting follicle, is that there exists an oncogenically susceptible population of cells at a relatively shallow depth in the skin, irrespective of the phase of the hair cycle. In the resting phase the "critical" depth was found to be 0.3 mm, this is the level of the "germ" cells, i.e., the stem cells of the growing follicle. The fate of the "germ" cells in the growing phase is unknown, however, it is possible that they remain at or about the same depth, this would place them in the body of the follicle about the level of the sebaceous gland. The results of the present experiment are consistent with the susceptible cells being located at a depth of

around 0.3 mm, since the dose dependence of the tumor incidence for the two penetrations are similar when the incidence is plotted against dose at 0.3 mm (Fig. 12). However, this similarity also holds for doses plotted at other superficial depths, for example, it holds for the surface dose (Fig. 11). In order to determine the exact depth more accurately more data must be accumulated; this is being done in an experiment which is currently in progress, in which additional doses and penetration depths were used.

The hypothesis that the oncogenically susceptible cells remain permanently at a relatively superficial level while the follicle length varies, can explain the correlation between abnormal follicles and tumor incidence for the resting follicle and for the deep penetration irradiation of the growing follicle, and the lack of correlation for the shallow penetration irradiation of the growing follicle. According to the hypothesis, in the resting phase the oncogenically susceptible cells are located at the distal end of the follicle and thus to irradiate them it was necessary to irradiate the entire follicle thereby producing abnormal follicles. In the deep penetration irradiation of the growing phase, the whole follicle received a uniform dose, thus the susceptible cells received approximately the same dose as the entire follicle, and a similar

situation to irradiation of the resting follicles was produced. On the other hand, for the shallow penetration irradiation of the growing follicle, the susceptible cells received a higher dose than the lower part of the follicle and tumors developed independent of follicle damage.

The correlations of Albert et al. (1967 b) between abnormal follicles and tumor production and the hypothesis of Glucksmann (1963) that severe tissue disorganization is required for radiation carcinogenesis, are not then necessary conditions. Glucksmann's position which grew out of the observation that severe radiation dermatitis preceded skin tumors in radiation workers has received much attention. Dunham (1972) has reviewed the incidence of tumors arising in benign lesions in man and the suggestion that the morphological abnormalities associated with these lesions are necessary for oncogenesis is strong. However, the present work suggests that in rat skin at least the association may be only coincidental.

The vast majority of radiation induced skin tumors in man are associated with a degree of skin damage which suggests a radiation dose of several thousand rads (Medical Research Council, 1956).

There are, however, a few reports of tumors appearing in skin which had been irradiated but which clinically appeared normal (Ridley, 1962 and Lazar and Cullen, 1963). Hulse (1967) found a few skin tumors in mice whose pelvic and thoracic area were irradiated with ^{204}Tl, a beta emitter with a maximum range in soft tissue of 3.0 mm, at an incidence dose of 750 rads. The only acute effect of this dose was depigmentation of some hair in half the irradiated mice. However, the vast majority of the tumors arose at radiation doses which caused more severe damage. The significance of Hulse's experiment is obscured by the fact that the mice ranged in age from 2-4 months and consequently the hair phase was variable at the time of irradiation. Zackheim, Krobock and Langs (1964) were also able to produce tumors in rats with Grenz ray irradiation with certain dose levels and treatment schedules which produced no dermatitis, although an increased tumor incidence was generally associated with severe radiation dermatitis. The present investigation is the only instance to the author's knowledge where the peak tumor incidence (in the case of the shallow penetration) occurred at a dose which produced only minimal grossly observable damage (i.e., slight blanching).

Heimbach et al. (1969) using an α-particle beam placed the Bragg peak at different levels in the resting phase skin and showed that tumors were not produced when the Bragg peak was placed at 0.3 mm and only cells around this depth were heavily irradiated. They suggested that the minimally damaged part of the follicle could effect recovery with respect to tumor induction in the rest of the follicle. This is not true in the case of the growing follicle. The lower part of the follicle escaped serious damage at the low doses in the shallow penetration, but the tumor incidences, although lower than those for the deep penetrations, are appreciable. The cells below 0.3 mm in the growing phase are well differentiated, and this may restrict their capacity for repair.

The peak of the tumor incidence occurred at a dose of about 2000 rads at 0.3 mm in the resting phase (Albert et al. 1967 a). In the growing follicle the peak was definitely below 2000 rads and could be as low as 1000 rads at 0.3 mm (Fig. 10). This difference in sensitivity may be expected since in the resting phase the follicle cells are mitotically inactive, whereas in the growing phase the follicle cells are dividing rapidly. Thus,

although the susceptible cells may remain at a fixed location as the follicle length varies, they differ in oncogenic radiosensitivity between telogen and anagen.

5.0 Summary

The aim of this study was to quantitate radiation induced cell population changes in various components of anagen phase rat skin and to correlate them, if possible, with the subsequent production of tumors. The dorsal skin of rats in the growing phase of the hair cycle was irradiated with an electron beam. Two penetration depths of the beam were used: one shallow (1.0 mm) and the other deep (2.0 mm). Doses of 1000 rads, 2000 rads, 4000 rads and 8000 rads were given.

Size changes in the epidermis, sebaceous glands and follicles were measured from histological sections by counting the intersections of an eyepiece grid which fell within each structure. Measurements of total DNA and ^{3}H-thymidine labelled DNA in prelabelled rats, were made from biopsy samples taken at 2 day intervals post-irradiation. Tumor incidence was recorded monthly and the tumors were verified histologically at the animal's death. Abnormal follicle incidence was determined from whole mounts of the rats' skin.

The rate of loss of ^{3}H-thymidine labelled DNA was found to be the same in all dose groups as in controls, indicating that the

irradiated cells were eliminated at the normal turnover rate. At the deep penetration the initial rate of loss of DNA was the same as that of ^{3}H-thymidine indicating that there was no initial cell replacement. The grid counts showed that after the initial cell loss there was, in general, a hyperplastic reaction in the epidermis and sebaceous gland. The degree and time of occurrence of the hyperplasia was dependent on dose and penetration. The follicle grid counts provided no evidence of regeneration in the follicles.

No correlation between acute or chronic damage was found. The peak tumor incidence for the shallow penetration occurred at 1000 rads, a dose which produced only minimal morphological changes and very few abnormal follicles; while very few tumors were induced at 4000 rads shallow a dose which produced epidermal and sebaceous gland hyperplasia and a significant number of abnormal follicles. No association between tumor type and hyperplasia was found either. The incidence of keratinizing tumors was higher at 1000 rads deep, where there was only transient hyperplasia, than it was at 4000 rads deep where the hyperplasia was persistent. Hyperplastic sebaceous glands were not necessarily associated with a large number of sebaceous tumors.

The high incidence of tumors at 1000 rads shallow implied that there might exist a population of stem cells susceptible to oncogenic transformation which were located at a relatively shallow depth in the skin. It was suggested that a possible source of this population might be the germ cells of the resting follicle, and that the location of these cells did not change between the resting and growing phases of the hair cycle.

6.0 References

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7.0 Tables

Table 1 Performed Analyses

No. of Rats/Group	Analyses	Post Irradiation Interval Covered
16	Tumor Formation	560 days
8	Histology	90 days
4	Acute DNA Loss and Retention of Label	10 days
4	Whole Mounts	~90 days
32 Total/Group		560 days

Total # of Rats = 256

Table 2 Schedule of Histological Sections

No. of Rats/Group	Days Post Irradiation	No. of Sections/Group
6	0 5 10 15 21 29 38 49 60	54
1	21 86	2
1	29 86	2
	Total No. of Sections/Group	58

8 groups, i.e., 8,000, 4,000, 2,000 and 1,000 rads at the shallow penetration and 4,000, 2,000 and 1,000 rads at the deep penetration plus the control group, were used to provide histological data. Thus, there was a total of 448 sections.

Table 3 Hair Growth Suppression--Maximum Response-Seen at 1 Week

	Shallow Penetration		Deep Penetration	
Surface Dose (rads)	Dose at 0.8mm (rads)	Response	Dose at 0.8mm (rads)	Response
1000	280	0	1090	2-3
2000	560	2	2180	3
4000	1120	2-3	4360	3-4
8000	2240	3		

0 - No effect
1 - Just noticeable
2 - Noticeable
3 - Marked
4 - Complete

Table 4 Ulceration - maximum response at 2 weeks. Percentage of irradiated area.

	Shallow Penetration 1.0 mm	Deep Penetration 2.0 mm
1000 rads	0	5%
2000 rads	5%	75-100%
4000 rads	25-100%	100%
8000 rads	50-100%	

Table 5 Blanching--Maximum Response Seen at 3 Weeks

Surface Dose (rads)	Shallow Penetration		Deep Penetration	
	Dose at 0.3mm (rads)	Response	Dose at 0.3mm (rads)	Response
1000	1020	1-2	1200	3
2000	2040	3	2400	xx
4000	4080	4	4800	xx
8000	8160	4	9600	

0 - None
1 - Just Noticeable
2 - Noticeable
3 - Marked
4 - Complete
xx - Epidermis too damaged for meaningful observation

Table 6 Epidermal "Layers"

Days Post Irradiation	Number of Epidermal "Layers"									
	0	5	10	15	21	29	38	49	60	86
Control	$5.2^{\pm}0.2$	$4.8^{\pm}0.3$	$4.8^{\pm}0.3$	$5.2^{\pm}0.3$	$5.2^{\pm}0.4$	$5.0^{\pm}0.2$	$5.3^{\pm}0.3$	$4.5^{\pm}0.3$	$5.3^{\pm}0.2$	$5.8^{\pm}0.7$
1000 rads Shallow	$5.3^{\pm}0.2$	$5.0^{\pm}0.5$	$5.3^{\pm}0.2$	$5.0^{\pm}0$	$5.0^{\pm}0.4$	$4.5^{\pm}0.2$	$4.0^{\pm}0.2$	$4.7^{\pm}0.2$	$4.9^{\pm}0.6$	$4.0^{\pm}0.4$
2000 rads Shallow	$5.2^{\pm}0.2$	$5.3^{\pm}0.4$	$7.3^{\pm}0.8$	$5.7^{\pm}0.5$	$6.0^{\pm}0.5$	$5.3^{\pm}0.2$	$5.2^{\pm}0.4$	$5.5^{\pm}0.3$	$6.0^{\pm}0.8$	$5.0^{\pm}0.4$
4000 rads Shallow	$5.0^{\pm}0.2$	$5.2^{\pm}0.3$	$0^{\pm}$ --	$9.5^{\pm}2.0$	$8.7^{\pm}1.8$	$8.2^{\pm}0.5$	$8.5^{\pm}1.2$	$6.2^{\pm}0.6$	$5.8^{\pm}0.2$	$4.5^{\pm}0.6$
1000 rads Deep	$4.8^{\pm}0.2$	$4.8^{\pm}0.2$	$7.3^{\pm}0.6$	$5.8^{\pm}0.2$	$5.3^{\pm}0.6$	$5.3^{\pm}0.5$	$4.3^{\pm}0.5$	$5.0^{\pm}0.7$	$4.8^{\pm}0.6$	$4.5^{\pm}0.5$
2000 rads Deep	$4.8^{\pm}0.2$	$5.0^{\pm}0.3$	$0^{\pm}$--	$0^{\pm}$--	$12.0^{\pm}1.6$	$9.3^{\pm}1.5$	$7.5^{\pm}2.0$	$9.0^{\pm}1.4$	$9.5^{\pm}0.9$	$12.3^{\pm}3.2$
4000 rads Deep	$5.3^{\pm}0.2$	$5.8^{\pm}0.2$	$0^{\pm}$--	$0^{\pm}$--	$0^{\pm}$--	$0^{\pm}$--	$12.4^{\pm}0.8$	$10.0^{\pm}2.1$	$11.0^{\pm}0.9$	$11.2^{\pm}1.4$

Table 7 Sebaceous "Hits"

Days post Irradiation	Sebaceous "Hits" per gland (glands per field)									
	0	5	10	15	21	29	38	49	60	86
Control	15.8±2.0 (1.7±0.2)	16.0±1.0 (1.6±0.2)	14.2±1.7 (1.2±0.1)	14.8±1.8 (1.2±0.2)	21.8±3.0 (0.9±0.1)	18.5±3.1 (1.1±0.2)	19.1±1.7 (1.1±0.1)	23.2±1.8 (0.8±0.1)	22.8±4.5 (0.8±0.1)	20.7±2.9 (0.9±0.1)
1000 rads Shallow	16.0±0.7 (2.2±0.2)	13.3±1.1 (1.6±0.2)	18.3±1.0 (1.7±0.1)	24.0±4.8 (1.3±0.1)	30.3±5.8 (1.0±0.1)	32.6±4.9 (1.0±0.2)	22.7±3.9 (0.9±0.1)	24.3±4.8 (1.0±0.2)	31.1±5.9 (1.0±0.1)	22.2±2.1 (0.8±0.1)
2000 rads Shallow	14.0±2.3 (2.0±0.2)	4.3±0.2 (0.4±0.1)	0± -- (0± --)	9.0±2.0 (0.4±0.1)	19.0±4.6 (0.2±0.1)	24.3±8.2 (0.5±0.1)	23.7±5.1 (0.6±0.2)	33.2±8.0 (0.9±0.1)	46.8±6.5 (1.1±0.1)	34.5±3.7 (0.9±0.1)
4000 rads Shallow	14.4±1.7 (2.2±0.1)	4± -- (0.1±--)	0± -- (0± --)	13.0±1.1 (0.1±0.1)	5.5±0.3 (0.1±0.1)	14.2±3.8 (0.5±0.1)	26.0±7.6 (0.7±0.1)	34.0±3.1 (1.4±0.3)	45.8±11.7 (1.0±0.1)	37.5±2.3 (1.2±0.3)
1000 rads Deep	17.5±1.0 (2.1±0.1)	8.8±2.1 (0.9±0.2)	39.3±5.5 (0.8±0.2)	21.8±4.0 (0.9±0.1)	33.3±8.1 (1.0±0.1)	26.0±4.5 (1.2±0.3)	29.3±2.6 (1.0±0.1)	39.3±7.8 (1.0±0.1)	35.8±7.2 (1.1±0.1)	22.0±5.2 (0.7±0.1)
2000 rads Deep	17.3±1.7 (2.2±0.5)	4± -- (0.1±0.1)	0± -- 0± --	0± -- 0± --	53.0±6.4 (0.1±0.1)	22± -- (0.3±--)	27.3±3.3 (0.6±0.2)	27.3±6.9 (0.6±0.2)	27.5±2.9 (0.5±0.4)	86.7±41.2 (0.8±0.2)
4000 rads Deep	22.5±3.5 (1.9±0.1)	0± -- 0± --	0± -- 0± --	0± -- 0± --	0± -- 0± --	0± -- 0± --	0± -- 0± --	0± -- 0± --	0± -- 0± --	30.5±20.7 (0.2±0.2)

Table 8 DPM/cm^2 on Day of Irradiation

Dose	Pen[a]	DPM/cm^2 day 0 (not normalized) individual rats in each group			
		1	2	3	4
Control		19,659	14,056	18,937	15,825
Control		29,925	32,893	28,441	--
1000 rads	Shallow	18,450	20,750	22,450	1,790
2000 rads	Shallow	27,125	28,528	11,322	9,966
4000 rads	Shallow	16,610	10,040	21,965	21,205
8000 rads	Shallow	5,140	19,869	7,031	4,540
1000 rads	Deep	19,770	27,595	11,725	24,095
2000 rads	Deep	20,529	15,044	15,200	18,050
4000 rads	Deep	13,631	26,684	15,978	--

Table 9 Surviving Follicles

Surface Dose	Pen[2]	Survivors % of controls	
		16 weeks	80 weeks
Control		100.0	100.0
1000 rads	Shallow	100.0 ± 4.5	107.1 ± 2.7
2000 rads	Shallow	88.5 ± 3.7	84.5 ± 2.4
4000 rads	Shallow	68.2 ± 5.5	82.6 ± 3.1
8000 rads	Shallow	54.4 ± 6.8	57.4 ± 5.1
1000 rads	Deep	87.0 ± 6.3	97.3 ± 5.3
2000 rads	Deep	23.4 ± 3.0	30.3 ± 2.8
4000 rads	Deep	4.7 ± 1.5	9.0 ± 1.8

Table 10 Abnormal Follicles

Surface Dose	Pen	Abnormal % of Normals in Controls 16 weeks	80 weeks
Control		0	$1.3^{\pm}0.5$
1000 rads	Shallow	$3.6^{\pm}1.1$	$5.8^{\pm}1.4$
2000 rads	Shallow	$8.3^{\pm}2.6$	$13.5^{\pm}1.8$
4000 rads	Shallow	$12.2^{\pm}2.5$	$14.2^{\pm}1.8$
8000 rads	Shallow	$5.2^{\pm}1.8$	$8.3^{\pm}0.9$
1000 rads	Deep	$10.9^{\pm}1.9$	$23.2^{\pm}3.2$
2000 rads	Deep	$16.1^{\pm}3.0$	$16.1^{\pm}1.4$
4000 rads	Deep	$3.6^{\pm}1.1$	$5.2^{\pm}0.9$

Table 11 Ratio of Abnormal Follicles to Tumors

Surface Dose	Pen[a]	Abnorm. foll. / tumor
1000 rads	Shallow	1,370± 370
2000 rads	Shallow	4,110± 1,180
4000 rads	Shallow	40,615±21,120
8000 rads	Shallow	4,061± 1,425
1000 rads	Deep	2,410± 434
2000 rads	Deep	4,150± 772
4000 rads	Deep	2,032± 581

Table 12 Cumulative Tumor Incidence in 4 Main Categories

		Cumulative Tumor Incidence				
		Cat 1	Cat 2	Cat 3	Cat 4	Total
Surface Dose	Pen	K	KS	S	U	ΣI
1000 rads	Shallow	.28	.07	.08	.92	1.35 ± 0.34
2000 rads	Shallow	.32	.06	.06	.65	1.09 ± 0.26
4000 rads	Shallow	0	0	0	.13	0.13 ± 0.09
8000 rads	Shallow	.41	0	.12	.12	0.65 ± 0.24
1000 rads	Deep	.78	.63	.16	1.28	2.85 ± 0.48
2000 rads	Deep	.90	.20	.11	.38	1.59 ± 0.33
4000 rads	Deep	.57	.20	0	.14	0.91 ± 0.26

K - Keratinized
KS - Keratosebaceous
S - Sebaceous
U - Undifferentiated

8.0 Figures

Figure 1 - Depth dose curves in skin for the two penetrations of the electron beam.

Figure 2 - Depth doses at various depths in the skin relative to the growing hair follicle.

Figure 3 - Follicle "hits" per microscope field as a function of days post-irradiation for the shallow penetration beam.

Figure 4 - Follicle hits per microscope field as a function of days post-irradiation for the deep penetration beam.

Figure 5 - Total DNA in mgms per biopsy sample as a function of days post-irradiation for the shallow penetration beam.

Figure 6 - Total DNA in mgms per biopsy sample as a function of days post-irradiation for the deep penetration beam.

Figure 7 - Amount of ^{3}H-thymidine per biopsy sample in disintegrations per minute per cm^2 of tissue as a function of days post-irradiation for the shallow penetration beam. All counts standardized on the day of irradiation.

Figure 8 - Amount of ^{3}H-Thymidine per biopsy sample in disintegrations per minute per cm^2 of tissue as a function of days post-irradiation for the deep penetration beam. All counts standardized on the day of irradiation.

Figure 9 - Tumor incidence in tumors per rat as a function of post-irradiation elapsed time for the shallow penetration.

Figure 10 - Tumor incidence in tumors per rat as a function of post-irradiation elapsed time for the deep penetration.

Figure 11 - Tumor incidence in tumors per rat versus the incident dose in rads.

Figure 12 - Tumor incidence in tumors per rat versus the dose in rads at 0.3 mm depth in the skin.

Figure 13 - Tumor incidence in tumors per rat versus the dose in rads at 0.8 mm depth in the skin.

Figure 1

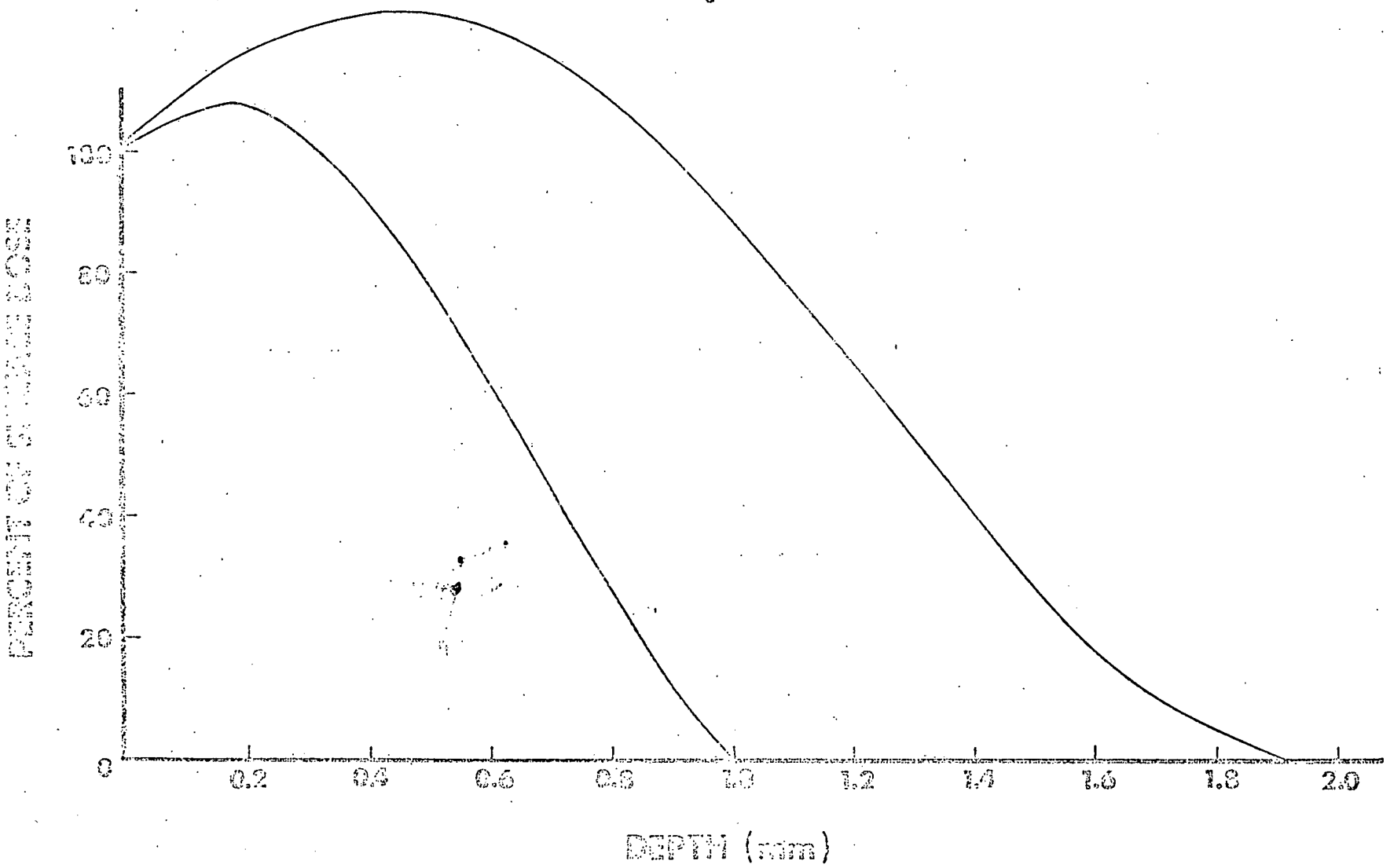

PERCENTAGE OF MAXIMUM ENERGY
100
80
60
40
20
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
DEPTH (mm)

Figure 2

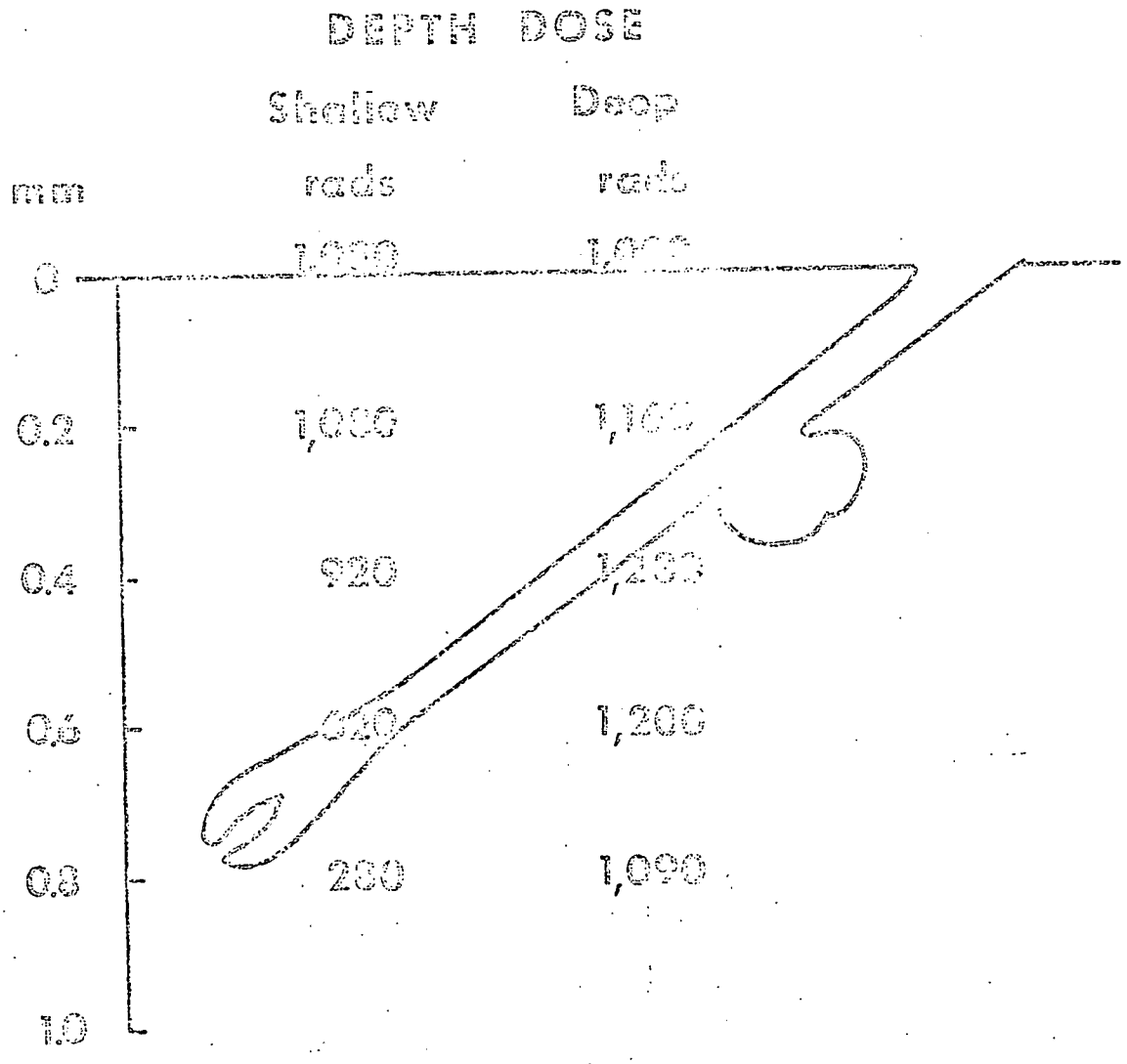

DEPTH DOSE
Shallow
Deep
mm
rads
rads
0
0.2
0.4
0.6
0.8
1.0
1,200
1,200
1,098

Figure 3

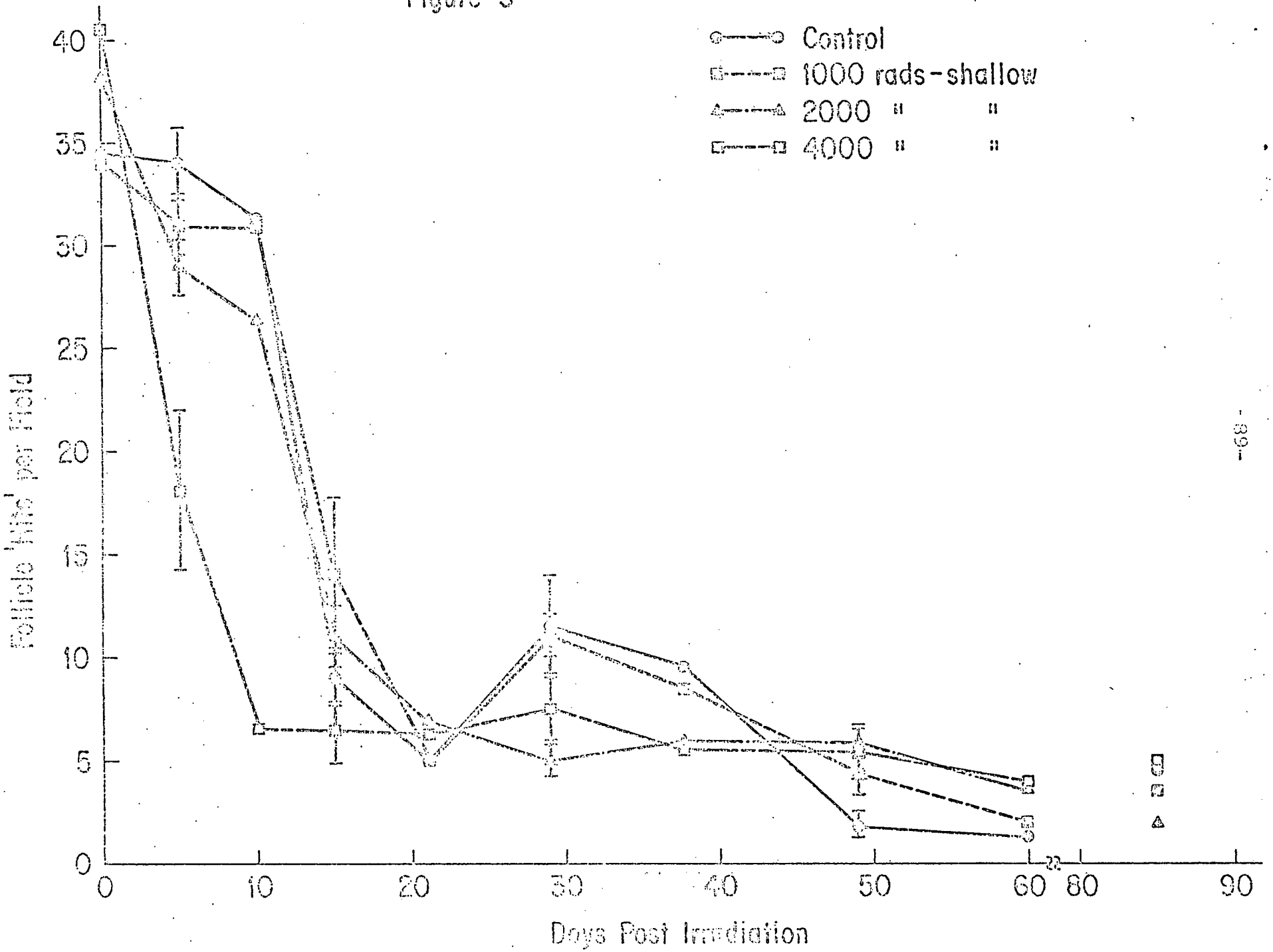

Control
1000 rads - shallow
2000 " "
4000 " "
Follicle hits per Field
Days Post Irradiation
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
80
90

Figure 4

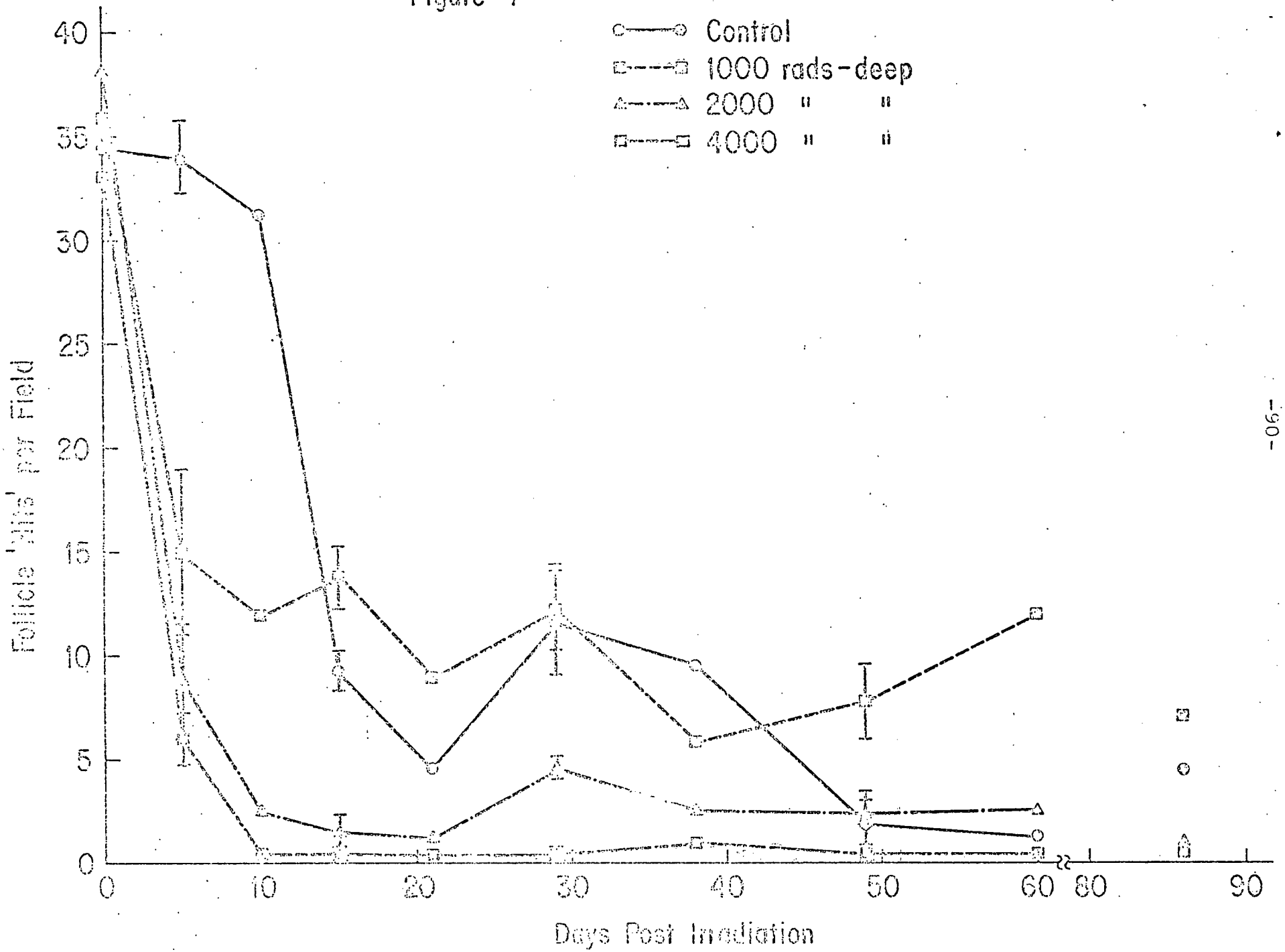

Control
1000 rads-deep
2000 " "
4000 " "
Follicle 'Hits' per Field
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
80
90
Days Post Irradiation

Figure 5

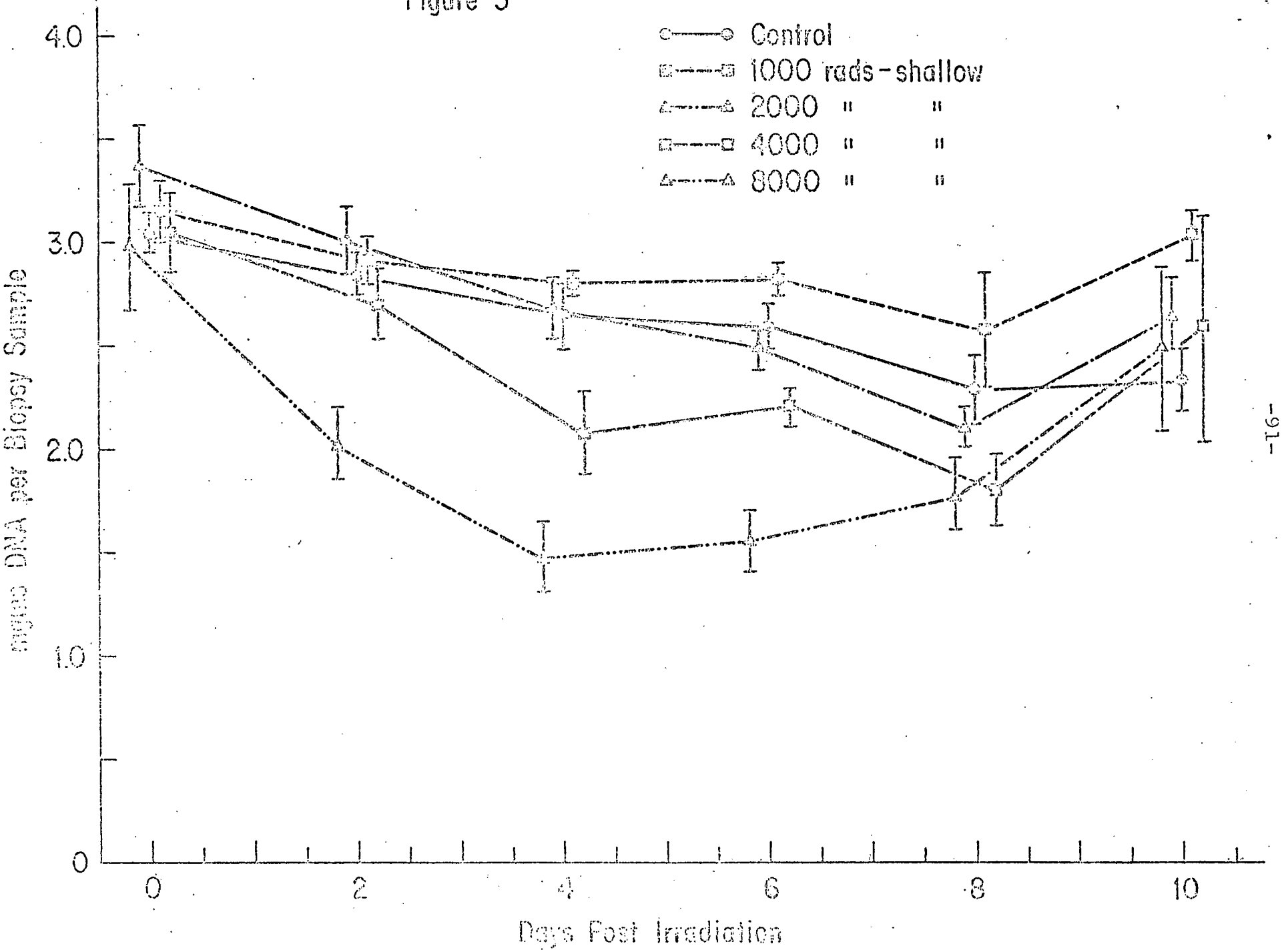

Control
1000 rads - shallow
2000 " "
4000 " "
8000 " "
Assay DNA per Biopsy Sample
4.0
3.0
2.0
1.0
0
0
2
4
6
8
10
Days Post Irradiation

Figure 6

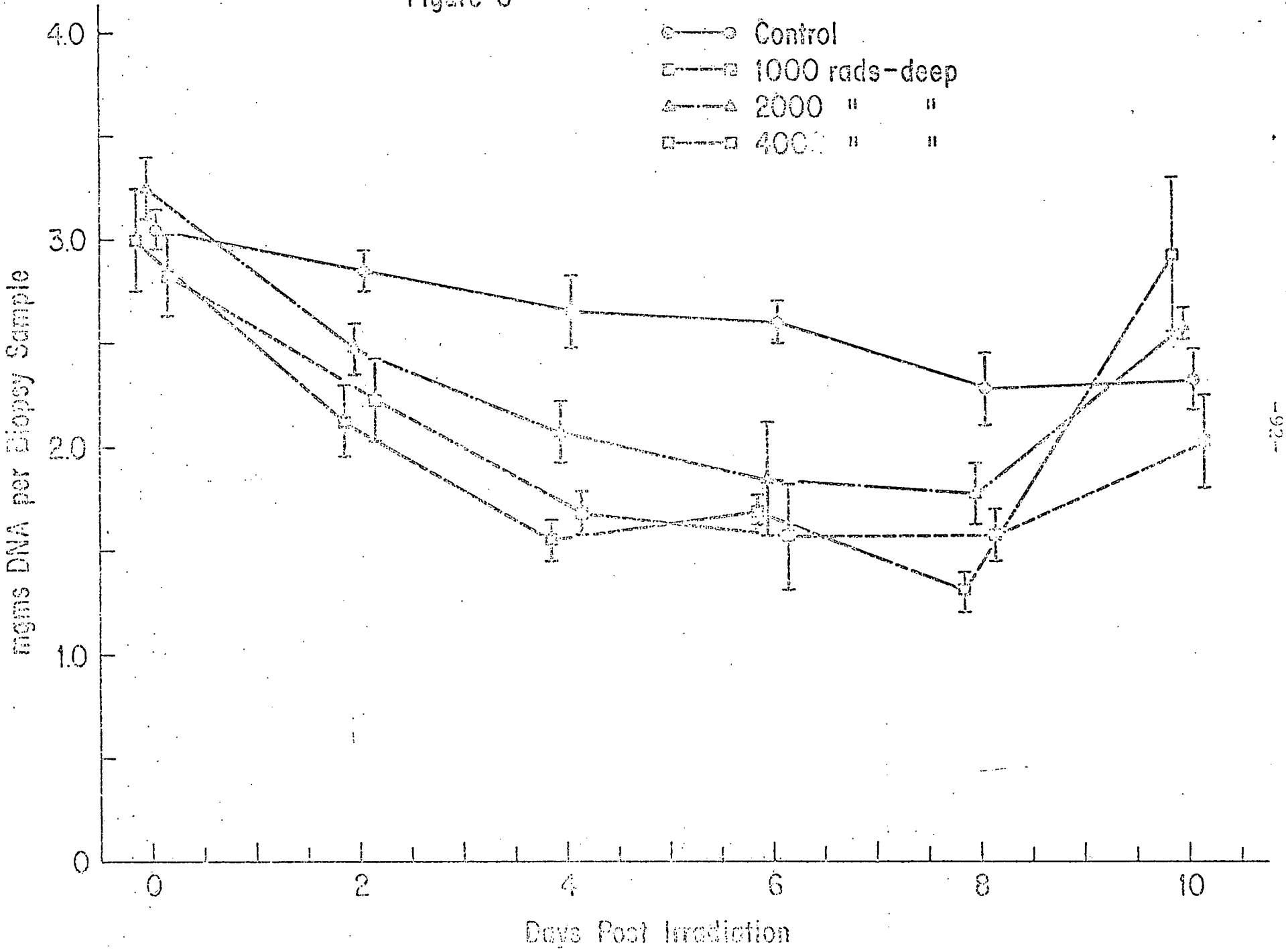

Control
1000 rads-deep
2000 " "
4000 " "
mgms DNA per Biopsy Sample
4.0
3.0
2.0
1.0
0
0
2
4
6
8
10
Days Post Irradiation

Figure 7

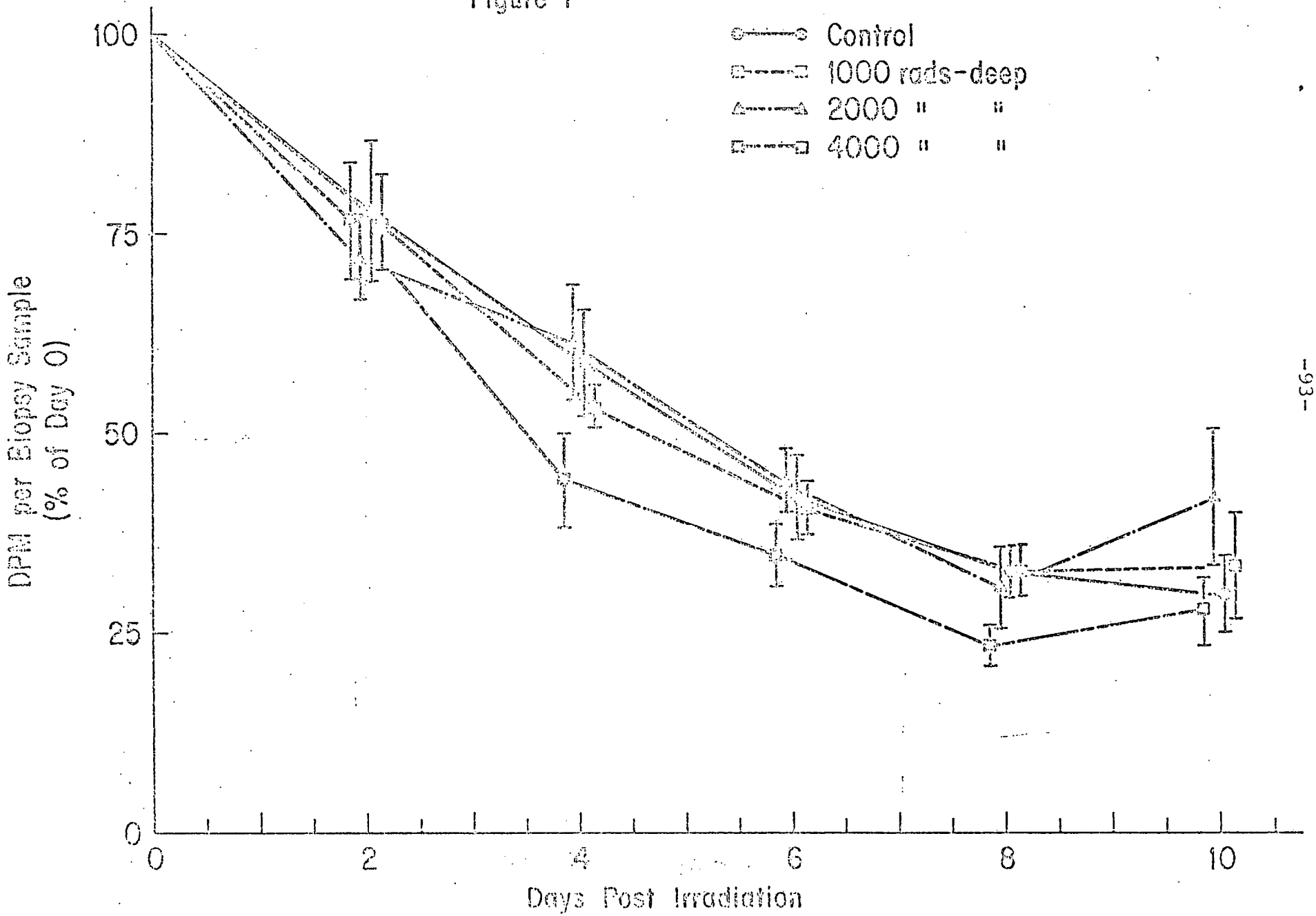

Control
1000 rads-deep
2000 " "
4000 " "
DPM per Biopsy Sample
(% of Day 0)
100
75
50
25
0
0
2
4
6
8
10
Days Post Irradiation

Figure 8

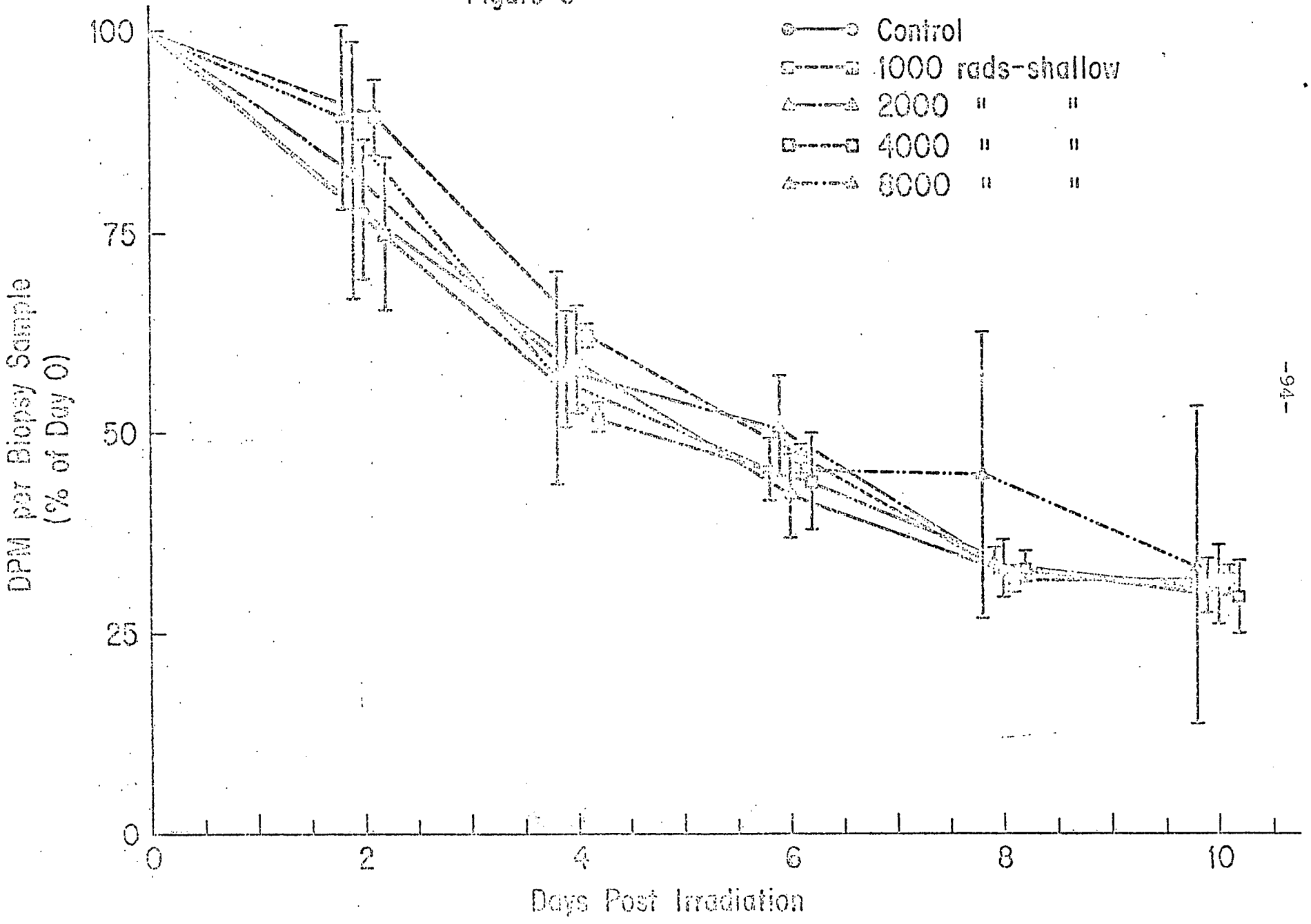

Control
1000 rads-shallow
2000 " "
4000 " "
8000 " "
DPM per Biopsy Sample
(% of Day 0)
100
75
50
25
0
0
2
4
6
8
10
Days Post Irradiation

Figure 9

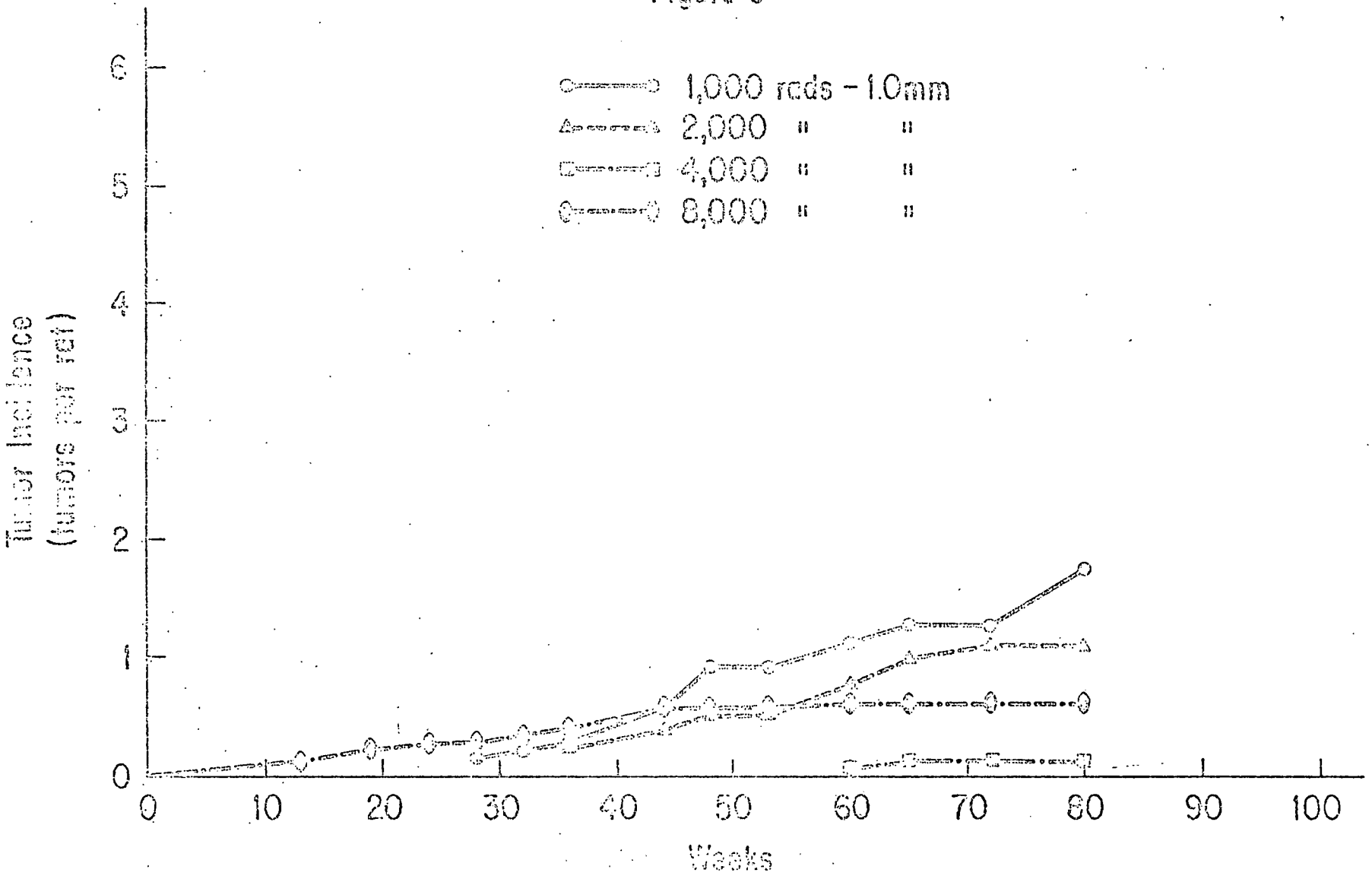

1,000 rads - 1.0mm
2,000 " "
4,000 " "
8,000 " "
Tumor Incidence
(tumors per rat)
0
1
2
4
5
6
0
10
20
30
40
50
60
70
80
90
100
Weeks

Figure 10

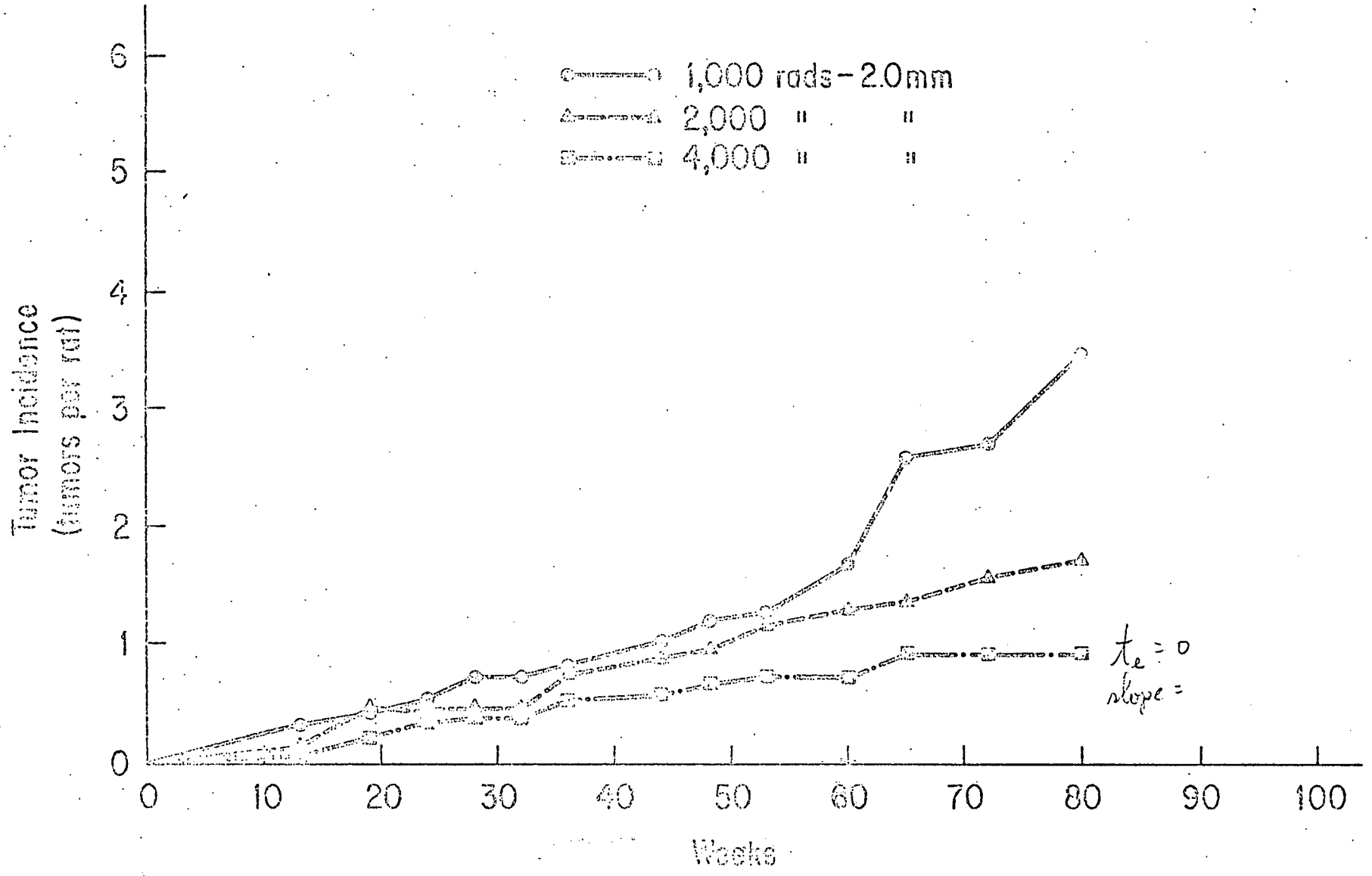

1,000 rads – 2.0mm
2,000 " "
4,000 " "
Tumor Incidence (tumors per rat)
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100
Weeks
$t_e = 0$
slope =

Figure 11

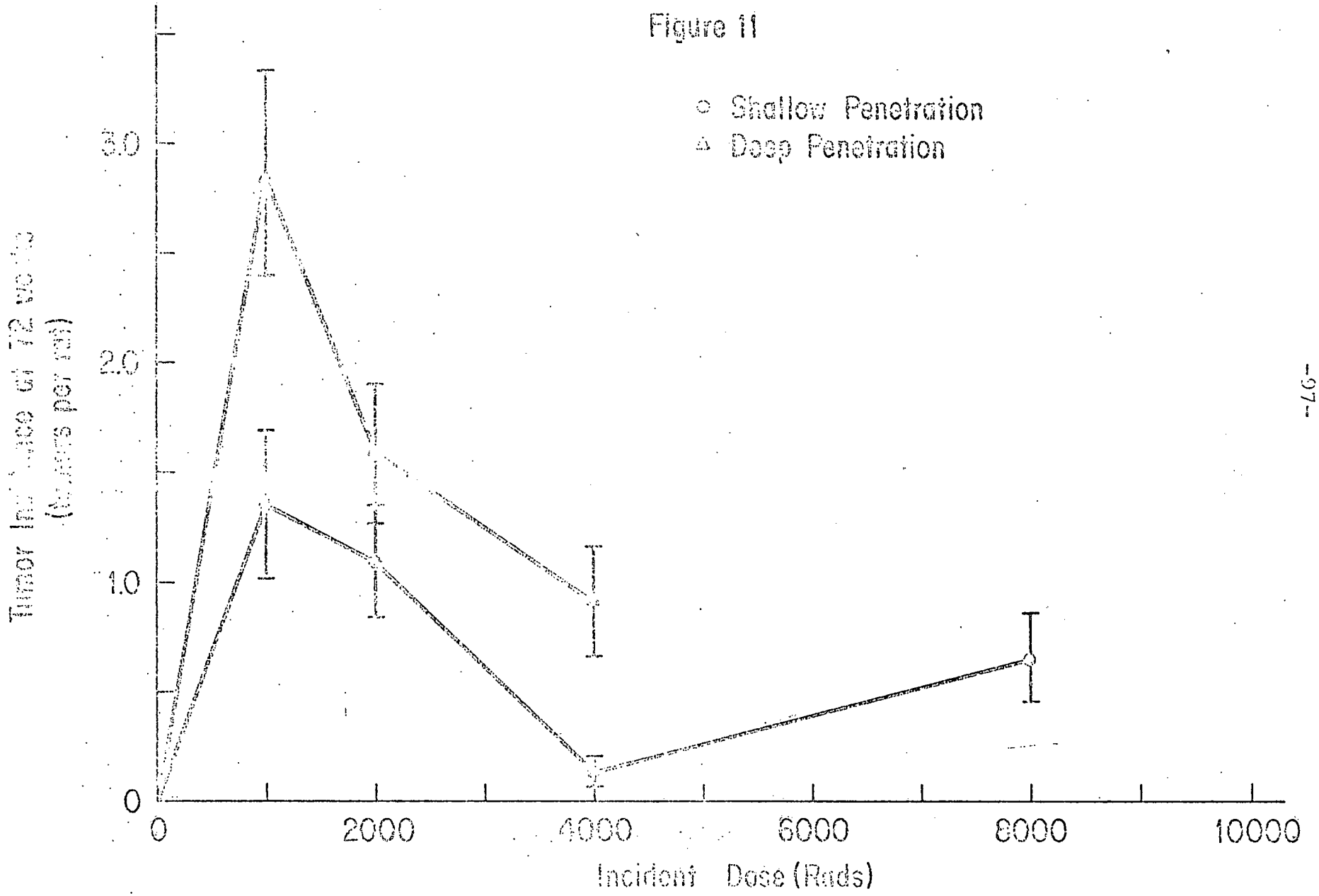

○ Shallow Penetration
△ Deep Penetration
3.0
2.0
1.0
0
0
2000
4000
6000
8000
10000
Incident Dose (Rads)

Figure 12

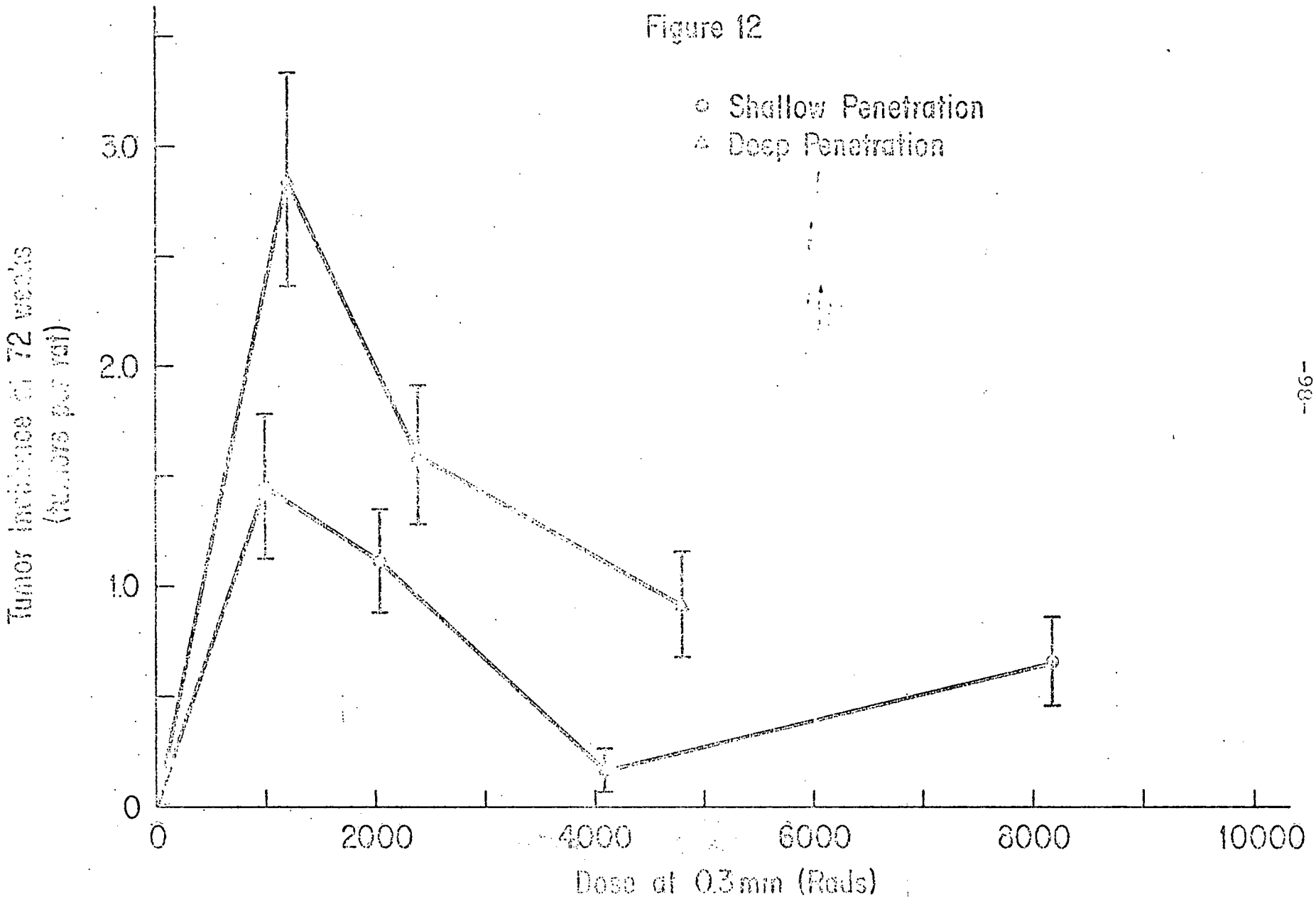
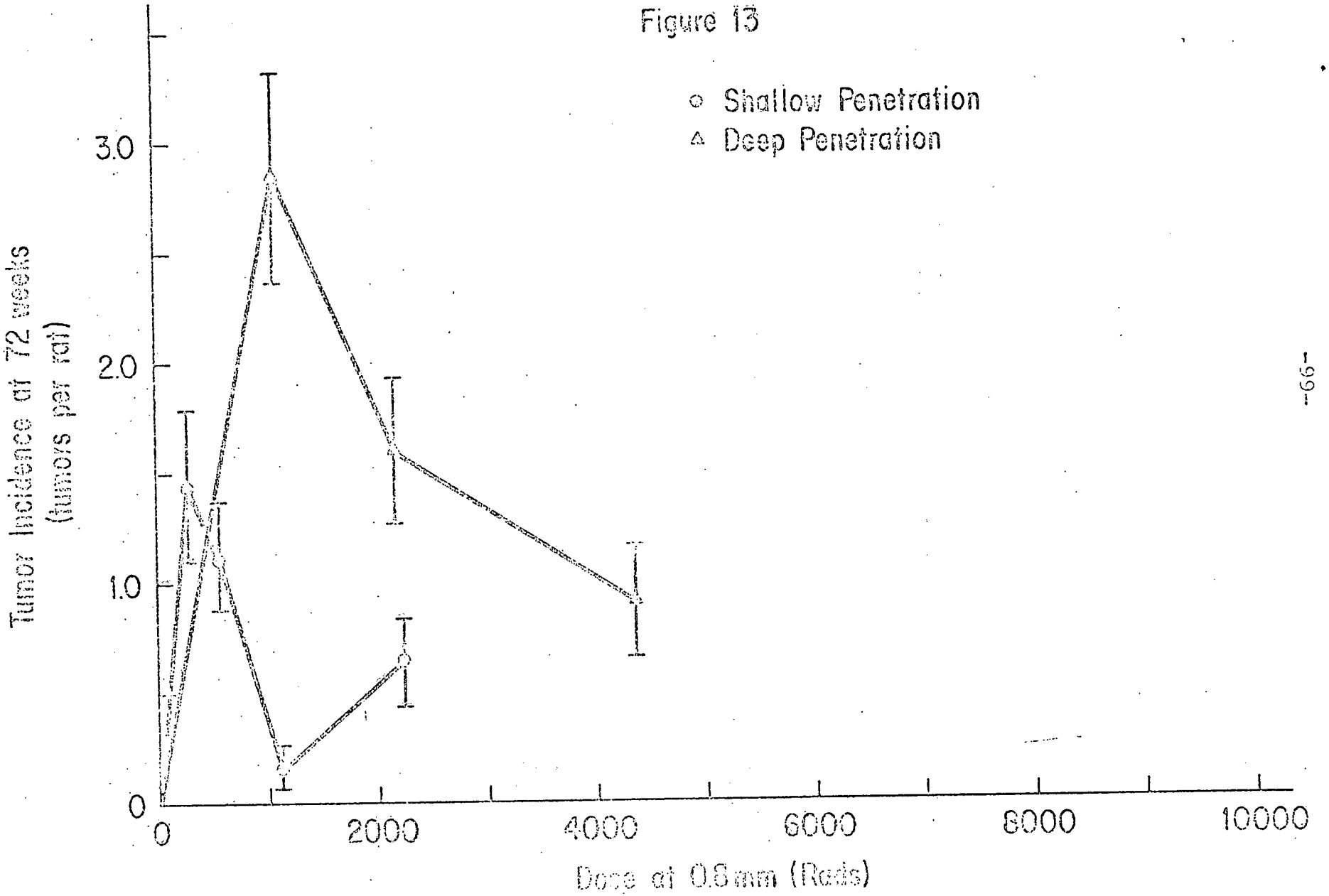

o Shallow Penetration
Δ Deep Penetration
3.0
2.0
1.0
0
0
2000
4000
6000
8000
10000
Dose at 0.3mm (Rads)

Figure 13


o Shallow Penetration
Δ Deep Penetration
Tumor Incidence at 72 weeks
(tumors per rat)
3.0
2.0
1.0
0
0
2000
4000
6000
8000
10000
Dose at 0.6 mm (Rads)

9.0 Appendix 1

9.1 Electron Beam Dosimetry

According to the Bragg-Gray principle, the dose rate, dD/dt, as measured by the ionization in a small air cavity embedded in the absorbing medium, is given by (Birge, Anger, and Tobias, 1956):

$$dD/dt \text{ (rads/sec)} = 1.6 \times 10^{-14} \, S_m W J \qquad (1)$$

where, J = number of ion pairs produced in the air per gram per second

W = the amount of energy in electron volts expended per ion pair

S_m = the relative mass stopping power of tissue

J is given by the expression:

$$J = I / r_{air} V_{ch} 1.6 \times 10^{-7}$$

where, I = the saturation current in μμamps

r_{air} = density of air at operating temperature and pressure = 1.18×10^{-3} gm/cm^3

V_{ch} = the chamber collecting volume = .143 cm^3

Substitution of the expression for J and the other numerical factors into equation 1 gives:

$$dD/dt \text{ (rads/sec)} = 2.08\ 10^{-2} I$$

or

$$dD/dt \text{ (rads/min)} = 1.26 I$$

The fixed gap chamber was calibrated with respect to an extrapolation chamber. It was found that the saturation current was proportional to the chamber volume, even for a 5.0 mm gap. Therefore the 1.0 mm fixed gap was well within the region of linearity.

The average dose rate for a complete revolution can be calculated from the product of the dose per revolution and the number of revolutions per second. The dose per revolution is given by the expression:

$$\int_0^T (dD/dt)_{inst} dt$$

10.0 Appendix 2

10.1 DNA Extraction Procedure

1. Pinch up area of rat skin with square ended forceps. Cut around edge of forceps with a scapel removing 1 cm^2 of tissue.

2. Weigh and freeze.

3. Mince with scapel.

4. Place in glass homogenizer tube, add 2 ml of 1M NaOH and digest for 1 hr at 37°C.

5. Grind with glass homogenizer and allow to digest 1/2 hr longer.

6. Cool then neutralize with 1 ml cold 2M HCl.

7. Precipitate with .5 ml 2M $HClO_4$.

8. Agitate and pore through filter washing with cold 0.4M $HClO_4$.

9. Wash precipitate with alcohol.

10. Wash precipitate with ether.

11. Wash precipitate with 0.4 $HClO_4$.

12. Remove filter paper, trim and cut into 10 pieces and place in test tube.

13. Add 2.5 ml 0.5M $HClO_4$.

14. Hydrolize the DNA for 20 mins at 90°C.

15. Take 0.5 ml for Burton's analysis.

16. Take 0.8 ml for scintillation counting.

This procedure is a modification of that of Schmidt and Thannhauser (1945).