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THE EFFECT OF FRACTIONATED DOSES OF ELECTRONS AND PROTONS ON HAIR FOLLICLES IN THE ALBINO RAT

Masters Thesis

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DISCLAIMER

ABSTRACT

A study of the effect of single and fractionated doses of electrons and protons on Albino rat hair follicles has been undertaken. The survival and regrowth curves of hair follicles following irradiation are presented. Temporary hair suppression was evident from the delay in regrowth of new hair, which was about one day of delay per 100 rads for protons. It was found that permanent growth suppression occurred at 4800 rads for electrons and at a dose greater than 1200 rads for protons. The doses required to produce 50% follicle suppression at ten days post irradiation were 700 rads and 2100 rads for protons and electrons, respectively. A fractionation effect was found for both types of radiation with about 60% recovery for protons and 100% recovery for electrons.

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I would like to thank Francis Standish for helping with the experimental animals and express my appreciation to Mrs. Elsie Bilger for typing this thesis.

CHAPTER I

INTRODUCTION

Objective

It has been suggested by several studies undertaken in this laboratory[1,2,3] that the stem cells at the tips of the hair follicles are the critical skin components for the production of abnormal follicles and tumors by radiation. The purpose of this study is to obtain a more complete understanding of how the hair follicle cells recover from the effects of diverse types of radiation. The specific objective is to obtain hair follicle survival and regrowth curves in rat skin following irradiation with various single and fractionated doses of protons and electrons.

The Hair Follicle

Hair suppression is an important index of the biological effect of radiation on mammalian skin. Hair follicle development and the cyclic growth of hair in the rat have been studied extensively[4,5,6]. An in depth study of the radiosensitivity of the hair of the Albino rat to x-rays was performed by Geary[7]. It was found that radiosensitivity was very dependent on the hair cycle phase at the time of irradiation.

Maximum sensitivity occurred during the active phase (days 12-15), and minimum sensitivity occurred during the resting phase (days 25-28). Regrowth of hair subsequent to epilation was found to be more complete when the exposure occurred during the resting phase. The median effective dose of x-rays capable of producing complete epilation in 50% of the animals exposed was 1740 rads during the resting phase.

A procedure employed by Burlin, et al[8] in mice, involves plucking the hair in conjunction with irradiation. The extent of the damage to the hair follicles is exhibited either by 1) a delay in the regrowth of hair or 2) by the absence of regrowth.

It was found that the delay in regrowth (temporary damage) and the absence of regrowth (long-term damage) both increased with increasing exposures of x-rays over the range 500 to 5000 rads. A different and more enlightening indicator of damage to the hair follicle cells is the delay or absence of follicle elongation and enlargement prior to hair regrowth. Whether a delay in hair regrowth is caused by a slowing down of cell proliferation associated with follicle elongation or merely by a delay in initiation of the process would not be apparent if external hair protrusion were

the only indicator.

Fractionation of the Radiation

The fractionation effect has been documented for a variety of biological end points, including induction of ulceration and tumors in rat skin. Also hair suppression following x-irradiation in the mouse has been investigated by Burlin, et al[9]. It was found that the length of time between fractions was a significant factor in hair regrowth. The magnitude of the second dose, needed to produce the same degree of hair suppression as a single dose, increases with the length of the time interval between doses. The size of the second dose is indicative of the degree of recovery exhibited by the follicles. A maximum recovery occurred at a dose interval of four days and recovery at 24 hours was about 40%.

Recovery with respect to atrophic follicle induction in rats has been investigated by Burns, et al[10]. The difference (D_2-D_1) between the fractionated dose D_2 of electrons and the single dose D_1 for equal effects was studied. With an initial dose of 750 rads and a time interval of 31 days,

(D_2-D_1) was found to be 600 rads for incidence of atrophic follicles at 80 weeks, which is equivalent to 80% recovery. A similar result was obtained for a time interval of 24 hours (unpublished data).

Recovery of mammalian cells from radiation is generally divided into two components[11,12].

1) Intracellular (Elkind) recovery from sublethal injury which is complete in less than 24 hours.

2) Repopulation of cells by proliferation of the stem cell population retaining reproductive integrity. Repopulation time depends on cell cycle time and proportion of dividing and differentiating cells in the population but generally requires days or weeks for completion.

Intracellular recovery from sublethal injury occurring within 24 hours has been shown to decrease with increasing LET (linear energy transfer) of the radiation according to Fowler[13]. Thus a higher LET particle would be expected to exhibit a less prominent shoulder and consequently a smaller fractionation effect.

Stem Cell Population of the Follicles

The ability of the hair follicle to survive irradiation depends on the survival of the stem cell

population of the hair germ. According to Geary[7] temporary epilation is caused by damage to the epidermal elements of the follicle which anchor the hair shaft to the follicle base. Whereas permanent epilation is caused by killing or inactivation of the cells of the hair germ. Higher doses of radiation are required for permanent epilation than for temporary epilation because of repopulation of the stem cell population.

By assuming multicell follicle inactivation, and at the same time single target cell inactivation, an estimate of the number of germ cells can be made from the survival curve data[12]. The survival curve of the follicles would then be identical in form to that encountered in multi target survival theory[14]:

$$S=1 - (1 - e^{-kD})^n$$

where n=# of targets (stem cells) per follicle

D=dose

k=sensitivity constant

That is, each germ cell is equivalent to a single target and the entire follicle acts as a multi target system. The above equation simplifies to:

$$S= ne^{-kD}$$

for large D. Thus in a semilogarithmic presentation

of the survival curve, n is synonymous with the extrapolation number, and a rough estimate of the size of the stem cell population can be inferred.

CHAPTER II

MATERIALS AND METHODS

Experimental Animals

Male Albino CD strain rats from the Charles River Breeding Farms, Brookline, Massachusetts were the experimental animals in this experiment. The rats were delivered at 21 days of age and irradiated at 28 days, in the middle of the first hair resting phase[7]. The rats were shaved the day before irradiation to insure uniform penetration of the skin; and the hair in the irradiated skin was plucked following irradiation by means of a depilatory wax in order to initiate a new hair growth cycle simultaneously in all rats. Two rats out of 67 in the proton experiment and none of the 72 rats in the electron experiment exhibited premature hair growth, indicating an advanced hair cycle, and were removed from the experiment.

Before irradiation the rats were anesthetized by intraperitoneal injection of Nembutal (25 mg/kg) to insure absence of movement. Following irradiation the animals were housed two per cage and provided with food and water ad libitum.

Proton Beam and Irradiation System

The proton beam used in this experiment was

produced by the 15 MeV cyclotron at Sloan-Kettering Institute for Cancer Research in New York City. The cyclotron was capable of producing a beam of 10 MeV protons of strength 2.2×10^8 protons/cm^2sec, which was equivalent to a beam current of 0.4 nanoamps. The final dose rate to the animals was 138 rad/min. (For detailed dosimetry, see Appendix II). The beam was diffused and degraded in the beam tube by means of aluminum foils to obtain uniformity of beam spread and the desired energy characteristics (Figure 3).

The beam was monitored with an argon filled double ionization chamber which was calibrated by means of a removable beam stop (Faraday cup) in the beam tube. A 1.0 mm gap parallel plate ionization chamber was also used to determine depth-dose characteristics (see Appendix II).

Because the depth-dose curve for protons is the familiar Bragg curve and a linear depth-dose curve was desired, the energy characteristics of the beam were modified. A spinning disc absorber wheel was used which produced the necessary linear depth-dose by means of differential absorption in aluminum foils of various thicknesses. By superimposing different segments of the Bragg curve, one can adjust the

depth-dose curve, by means of the beam modifier, to any shape needed, (Figure 1). A beam reduction factor of 10% is introduced here because of the ribs in the spinning disc.

The depth-dose curves were determined by placing various thicknesses of aluminum absorber foils between the beam and ionization chamber. These curves and the beam spread were confirmed by exposing stacks of Kodak Translite film in the beam.

The rats were irradiated in small wooden boxes. The box lids were 1/4 inch plywood with an irradiation window 2 x 5 cm. The boxes were padded with foam to press the back of each rat against the window. In order to irradiate several rats per exposure, twelve rats were fastened to the rim of a revolving turntable, 94 cm in diameter, which rotated through the beam 18 times per minute. This exposes each animal to the beam 1/115 of the total exposure time.

Electron Beam and Irradiation System

A 1.00 MeV Van de Graaff accelerator located at the Union Carbide Research Laboratory in Tuxedo, New York was used to produce the electron beam used in this experiment. The beam scattered through 130 cm

of air and was partially absorbed by a 0.6 cm Lucite sheet containing a 0.6 cm hole, centered on the beam, in order to reduce beam strength. The energy of the electrons was 0.7 MeV and the initial beam current 220 uamps, yielding a dose rate of 460 ± 46 rads/min at the site of exposure. (See Appendix I for dosimetry calculations). The 1.0 mm gap parallel plate ionization chamber was used to determine dose rates and the depth-dose distribution, (Figure 2). This curve was confirmed by exposing Kodak Translite film stacks to the beam.

Because of the large beam spread several animals could be exposed simultaneously. An irradiation board was constructed which could hold 21 rats in three parallel rows, thereby simplifying the exposure considerably. A 2x5 cm window formed the exposure area on the backs of the rats. The boxes were constructed of cardboard with a 1/16 inch metal sheet covering the face of the system, with openings corresponding to the exposure windows.

New Hair Growth Observation

1) Photographic Method

In order to record the growth of new hair

subsequent to irradiation and plucking, the back of each rat was photographed at one week intervals up to four weeks after exposure at which time the growth cycle was completed. The rats were anesthetized with ether and photographs were taken through a microscope magnifying the skin 16 times. A high intensity dissecting lamp was used for illumination and Kodak Plus X Black and White Pan Film (ASA 125) was used in exposures of 0.5 sec. After development of the film, slides were made and the total number of hairs visible per unit area (0.09 cm^2) of skin was recorded for each rat. This method counts a regrown hair as any hair which protrudes through the epidermis and is visible on the surface.

2) Follicle Elongation Method

Prior to hair eruption, the follicle germ cells proliferate and the follicle elongates and enlarges[6]. This elongation can be observed and quantitated histologically, thus indicating what proportion of follicles have been inhibited from undergoing cell multiplication. Biopsies of skin were taken at one week intervals up to four weeks after irradiation and plucking. The biopsies were

then stained with Shiff's reagent, and cleared with a procedure involving alcohol, Xylene, and methyl salicylate. (See Appendix III for details).

Elongated follicles were counted under microscope magnification of 80X. Only those follicles which were protruding <u>through</u> the collagen layer and exhibiting an obviously enlarged root bulb were counted. Total numbers of elongated follicles were recorded per unit area of field (0.07 cm^2).

CHAPTER III

RESULTS

Protocol

The doses administered and the size of the individual dose groups are shown in Table I. The last four groups in each experiment received fractionated doses with a twenty-four hour time interval. The proton doses were intended to be twice as large as those indicated in Table I, however technical problems involving dosimetry were encountered (see Appendix II).

TABLE I

Protons (Exp.91)		Electrons (Exp.98)	
Dose (rads)	# rats	Dose (rads)	# rats
0 (controls)	6	0 (controls)	6
75	6	600	6
150	5	1200	12
300	6	2400	6
450	6	3600	6
600	5	4800	6
750	6	6000	6
1250	6	1200+1200	6
150+150	4	1200+2400	6
150+300	5	1200+3600	6
150+450	6	1200+4800	6
150+600	6		72
	67		

Protons

1) New Hair Growth

One of the methods used to quantitate hair growth involved photographing the irradiated areas at weekly intervals. The total number of hairs visible per unit area (0.09 cm^2) is shown as a function of post irradiation time in Figure 4 for the proton experiment. The spread of the points at day ten and the subsequent grouping at day 17 indicates a delay of hair regrowth rather than long-term damage at these low doses. In Figure 5 the number of new hairs is plotted against time in order to show the kinetics of delay. The new hair appearance rate per unit area was calculated from the data in Figure 4 by subtracting the total hair count of the previous week from that of the week being calculated; in this way only the new hair erupting in the previous seven days is counted. It can be seen from this figure that as the dose increased, the periods of maximum new hair growth were delayed, with the largest delay in the 1250 rad group. Figure 3 shows the time to reach 50% normal hair density as a function of dose. The slope of the straight line through the points is about one day of delay per 100 rads of protons. The ten day total hair counts

(from Figure 4) are plotted as a function of dose in Figure 6, with error bars of plus or minus one standard error. The inhibition of growth is 50% at about 450 rads for the ten day time point.

2) Follicle Elongation

The elongation of follicles was quantitated in the proton experiment at weekly intervals up to one month. The ten day point again indicates temporary delay as shown in Figure 7, where the total number of growing follicles per unit area ($0.07cm^2$) is plotted as a function of dose. Temporary follicle growth inhibition was about 50% at 750 rads. The same data is plotted on semilogarithmic graph paper in Figure 8. The curve declines exponentially above 450 rads and exhibits an extrapolation number of 3.2 at ten days. Error bars are plus or minus one standard error.

3) Fractionation Effect for Follicles

A fractionation effect appears in the proton experiment data for temporary follicle suppression (Figure 8). The fractionated doses at 600 and 750 rads show slightly increased follicle counts suggesting that some recovery did occur between the initial 150 rad dose and the second doses. This difference

of 43 follicles at 600 rads is significant at a confidence level of 81%. The corresponding difference at 750 rads is significant at a confidence level of 87%.

Electrons

1) Follicle Elongation

The elongation of follicles was quantitated in the electron experiment at weekly intervals up to one month as in the proton experiment. The ten and twenty-four day post-irradiation graphs are shown in Figures 9 and 10 respectively, for follicle elongation after electron exposures. The ten day point exhibits temporary inhibition of 50% at about 2100 rads; whereas the 24 day point, which is indicative of long-term damage, reaches 50% inhibition at 3000 rads. The same data is plotted on a semilogarithmic scale in Figures 11 and 12. The shoulder of the curve appears to broaden with time (comparing Figures 11 and 12), showing some recovery over the two week interval between the two biopsies. Both graphs exhibit a sigmoid survival curve which has been observed previously[2] for hair follicles.

2) Fractionation Effect for Follicles

The fractionation effect for electrons on

follicle elongation is evident at both 10 and 24 day points. At the ten day point (Figure 9) the shift between the effect of fractionated and single electron doses is significant at the 67% confidence level in the 2400 rad dose group. At 3600 rads the difference is more obvious and significant at the 99.9% confidence level.

The 24 day data for electrons (Figure 10) again shows fractionation shifts in the 2400 and 3600 rad dose groups. The confidence levels of these differences are 98% at 2400 rads and 76% at 3600 rads. The 24 day post-irradiation point indicates long-term follicle suppression as compared to temporary suppression at 10 days. The differences between single and fractionated follicle counts and the corresponding significance levels of these shifts are summarized in Table II.

TABLE II

FRACTIONATION EFFECT

Radiation Particle	Day	Dose (rads)	Average Follicle Count/ Unit Area	Response Difference	Significance Level of Difference
Protons	10	150+450 600	219±33 176±17	43	81%
Protons	10	150+600 750	146±21 108±20	38	87%
Electrons	10	1200+1200 2400	128±48 103±27	25	67%
Electrons	10	1200+2400 3600	93.3±13.4 2.3±1.6	91	99.9%
Electrons	24	1200+1200 2400	149±15 82±21	67	98%
Electrons	24	1200+2400 3600	85±47 46±22	39	76%

Note: Day 10 = temporary suppression
Day 24 = long-term suppression

Stem Cell Population

The stem cell population of the resting hair follicle can theoretically be estimated from the extrapolation number of the permanent hair suppression curve. Because the doses administered in the proton experiment were lower than the 50% suppression dose, the regrowth of hair at 27 days was complete and the hair survival curve did not decline exponentially as expected (Figure 13). For this reason the proton experiment did not yield an estimate of the extrapolation number.

The electron data (Figures 11 and 12) is more informative than the proton data. An extrapolation number at 10 days (Figure 12) would vary considerably depending on how the extrapolation line is drawn. A maximum of about 1000 is obtained for the extrapolation number when the 3600 rad dose point is assumed to be the most reliable high dose point. A more conservative estimate of 100 results from the use of the 4800 rad dose point in the extrapolation line.

The long-term suppression of hair is exhibited in Figure 11, which is at 24 days post irradiation. The 6000 rad dose point has been rejected because the apparent increase in growing follicle count did

not result from hair regrowth within the irradiated area, but from the movement of adjacent hair and tissue into the irradiated area caused by contraction of the radiation scar. If the 4800 rad dose group is used, an extrapolation number of well over 10,000 is found.

CHAPTER IV

DISCUSSION

Both long-term and temporary hair suppression are evident in the regrowth curves. Long-term suppression is demonstrated in Figure 10 (day 24) for electrons, where the mean follicle count declines to 50% at about 2700 rads. This value is higher than Geary's estimate of 1740 rads for complete epilation in 50% of the animals following x-irradiation. A plateau region 1200 rads wide is also evident.

By comparison between Figures 9 and 10, it can be seen from the slopes of the two curves that some of the suppression at the ten day point was temporary. At the 24 day time point (Figure 10) the slope of the curve has declined considerably and the 50% suppression dose increased from 2100 to 2700 rads. Burlin[8] has found that 50% hair suppression in mice results from a 2000 rad dose of x-rays. Assuming that electrons and x-rays have the same relative biological effectiveness (RBE), it appears that rats are 35% more resistant to radiation induced hair suppression. Whether this difference in resistance to radiation is due to a penetration effect or difference in follicle

resistance is not known.

Long-term and temporary hair suppression resulting from proton irradiation can also be compared using Figures 8 and 13. At the ten day point, 50% hair suppression occurs at 700 rads. However at 24 days regrowth is complete at all doses up to and including 1200 rads; therefore, permanent 50% suppression occurs at a dose greater than 1200 rads. Table III summarizes the temporary and long-term 50% suppression doses observed in both the electron and proton experiments and the corresponding RBE's calculated from these values.

TABLE III

50% FOLLICLE SUPPRESSION

Time (Post Irradiation)	Dose (Rads)		R.B.E.
	Protons	Electrons	
10 Days	700	2100	3.00
24 Days	>1200	2700	<2.25

A fractionation effect for electrons was evident for both temporary and long-term follicle suppression. A fractionation effect was also observed for temporary suppression caused by protons. These results are summarized in Table II. The amount of recovery from the first fraction can be estimated from the shift in the exponentially declining portion of the curve by means of the $(D_2 - D_1)$ analysis described earlier. From Figure 8 the value of $D_2 - D_1$ is 90 rads which corresponds to 60% recovery for protons. It should be noted that this estimate is made on the basis of temporary suppression (day 10). The long-term data for electrons in Figure 10 shows complete recovery (100%) in the 2400 and 3600 rad dose groups. This estimate is somewhat higher than the 80% recovery observed by Burns, et al[10], however in that case the recovery was with respect to follicle survival at 80 weeks as compared to four weeks in this experiment.

The values of RBE calculated above indicate that protons are more effective in damaging hair follicle stem cells than electrons, suggesting a high linear energy transfer (LET) for the proton. According to Fowler's theory[13] of LET dependence, a low LET particle (electron) exhibits substantial recovery and a high

LET particle (proton) should exhibit little or no recovery. The recovery data presented here, 100% for electrons and 60% for protons, indicates that protons sometimes behave more like low LET particles than high. Thus the proton possesses the characteristics of low LET particles (60% recovery) and of high LET particles (RBE 2.25 - 3.00) simultaneously.

The size of the stem cell population could not be estimated from the proton data because the hair follicle response indicated recovery which is to be expected from low rather than high LET. The extrapolation numbers obtained from the electron data were very large and varied from 10^2 - 10^3 for temporary suppression to 10^4 for long-term suppression.

There is some question as to whether permanent hair and follicle growth suppression is dependent solely on inactivation of the stem cell population, and therefore whether the use of the extrapolation number as an estimate of the population size is justified. The possibility of damage to supporting tissue must also be considered. At high doses the skin in the irradiated area becomes completely ulcerated and thereby incapable of supporting hair growth. This could be the cause of temporary suppression

rather than damage to the epidermal elements of the follicle as suggested by Geary[7].

The shape of the regrowth curves in Figure 5 suggests that the hair development process has not slowed down but that its initiation has been delayed.

A comparison of the control growth curve with that of the 750 rad dose group shows that the shapes of the individual curves are similar except that the 750 rad curve is delayed by seven days. If the entire elongation process had slowed down, the initial slope of the 750 rad curve would be less than that of the control group. The fact that the initial (increasing) slopes of the growth curves in the 750 and 1250 rad dose groups are similar to the control group indicates that the follicles are still synchronized with respect to growth, in spite of the observed delay. The question of whether the delay is caused by the proliferation of the stem cell population or by supporting tissue damage cannot be ascertained on the basis of the data presented.

A distinction has been made between follicle elongation and actual hair growth. The inhibition of these two processes must be considered separately because of the implications inherent in each effect.

Inhibition of follicle elongation indicates that the ability of the stem cell population to proliferate has been limited. Whereas inhibition of hair growth indicates only that the ability of the follicle to produce hair has decreased; the existence or absence of cellular proliferation cannot be inferred from this data.

SUMMARY

Temporary hair growth suppression and follicle elongation suppression has been observed in Albino rats following proton irradiation. Both temporary and long-term (24 days) follicle suppression were observed following electron irradiation. The delay in regrowth of new hair was about one day of delay per 100 rads of protons. Permanent total suppression occurred at 4800 rads for electrons and at a dose greater than 1200 rads for protons. The doses required to produce 50% follicle suppression at ten days post irradiation were 700 rads and 2100 rads for protons and electrons, respectively. The RBE between protons and electrons calculated from these values was < 2.25 for long-term suppression and 3.00 for temporary suppression. A fractionation effect was observed for both types of radiation with 60% recovery for protons and 100% recovery for electrons. A parallel experiment is under way to study the effectiveness of single and fractionated doses of protons and electrons with respect to tumor induction in rat skin.

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FIGURES

FIGURE 1

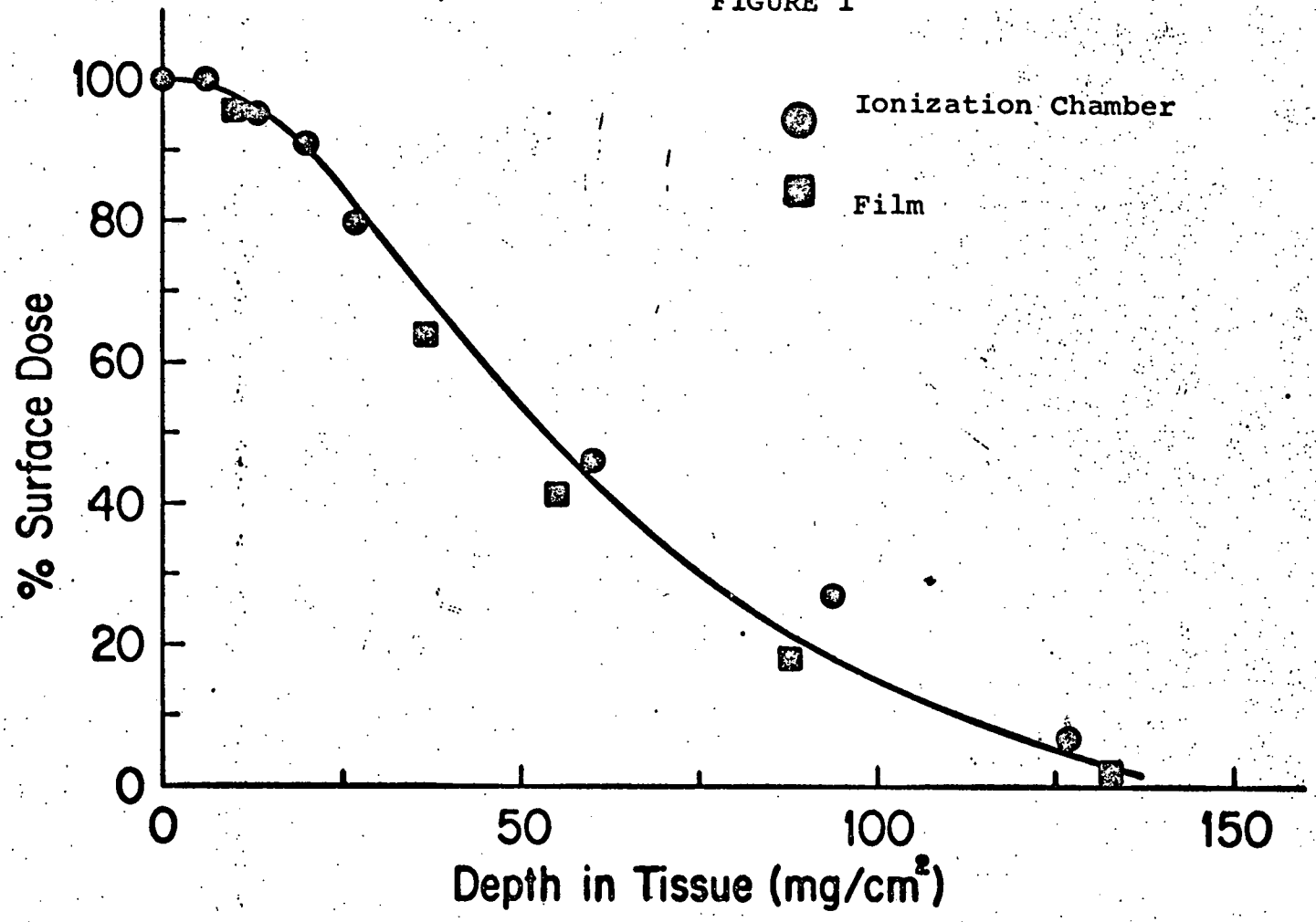
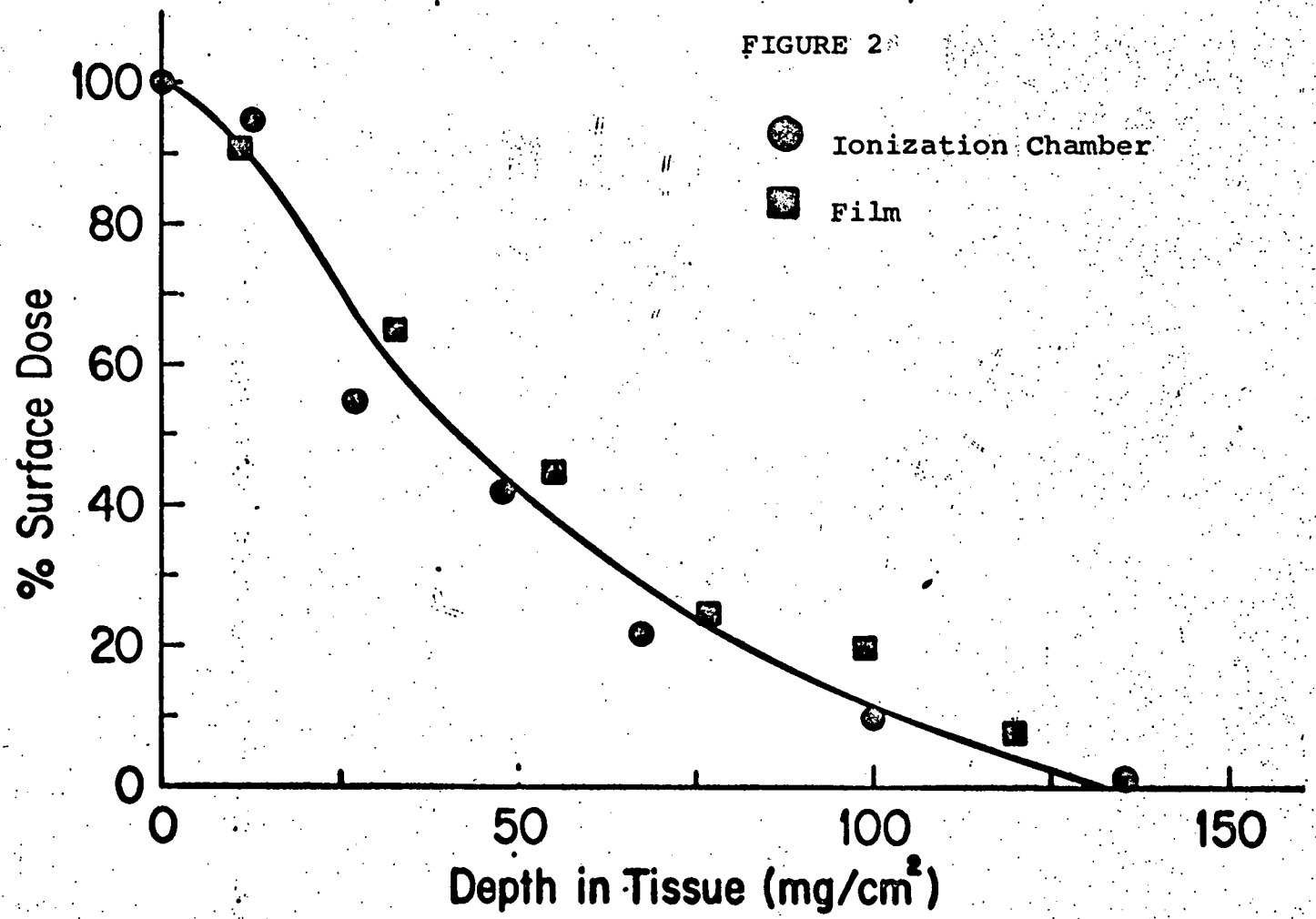

Ionization Chamber
Film
% Surface Dose
100
80
60
40
20
0
0
50
100
150
Depth in Tissue (mg/cm²)

FIGURE 2


Ionization Chamber
Film
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80
60
40
20
0
% Surface Dose
0
50
100
150
Depth in Tissue (mg/cm²)

FIGURE 3

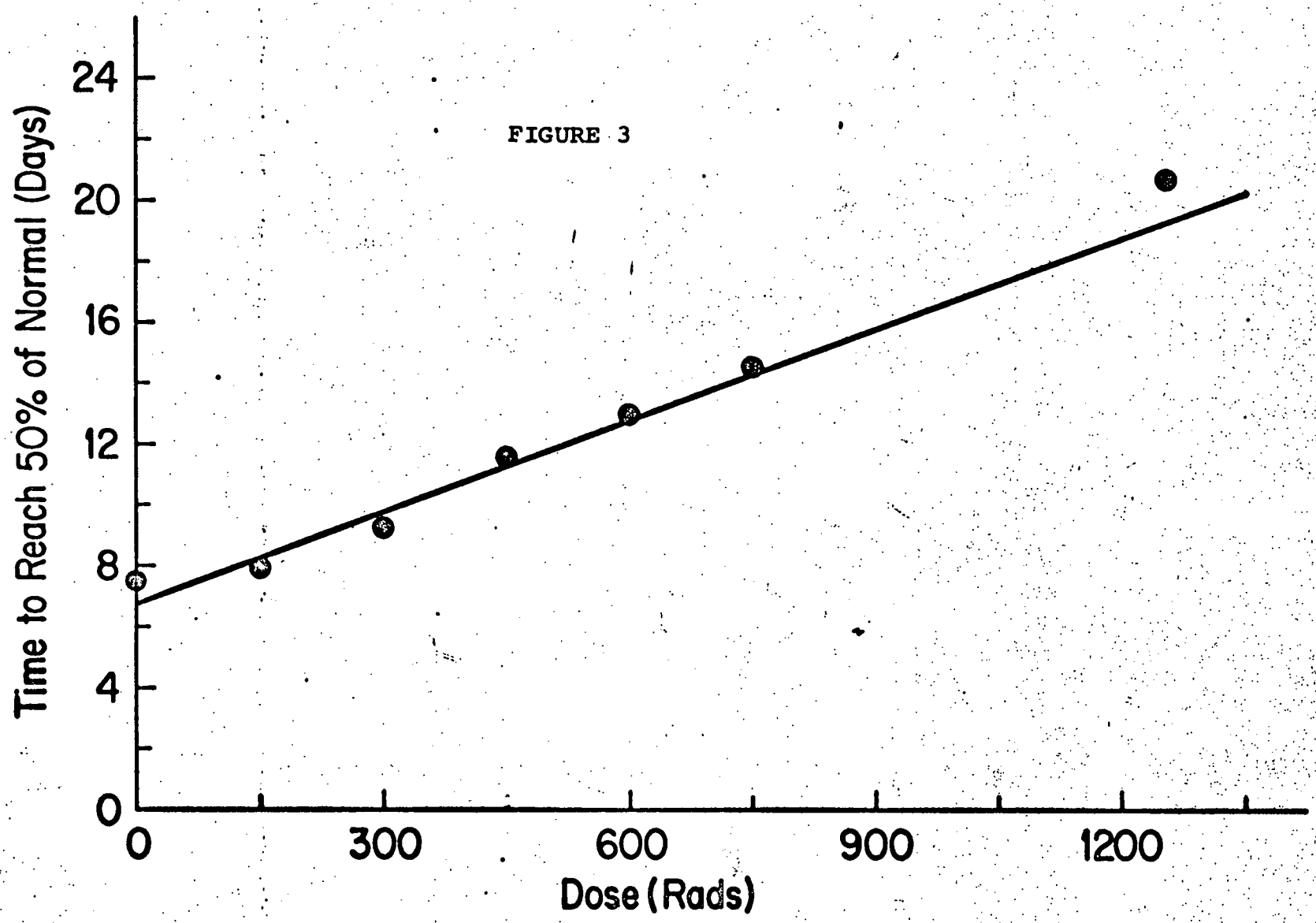

Time to Reach 50% of Normal (Days)
24
20
16
12
8
4
0
0
300
600
900
1200
Dose (Rads)

FIGURE 4

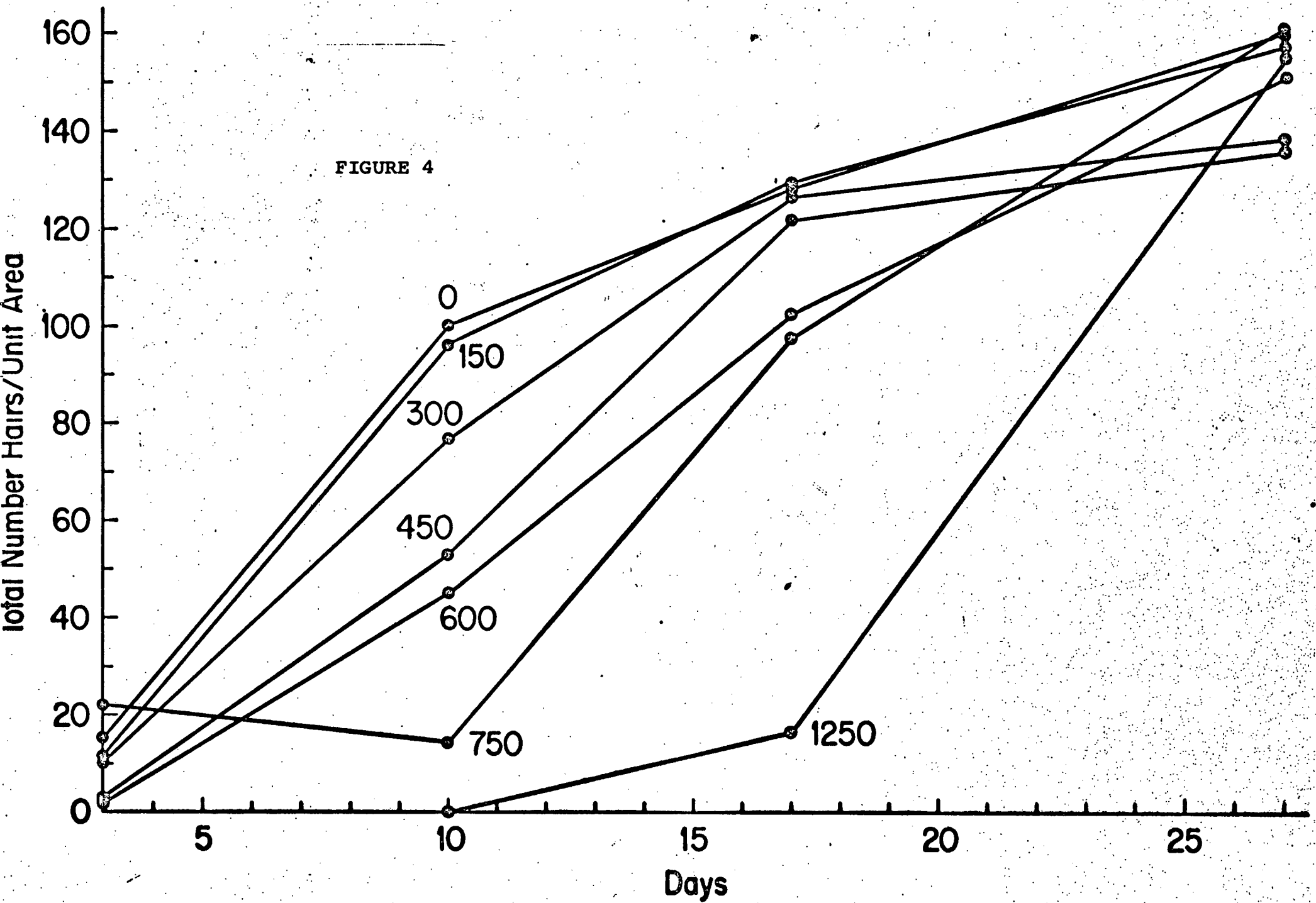
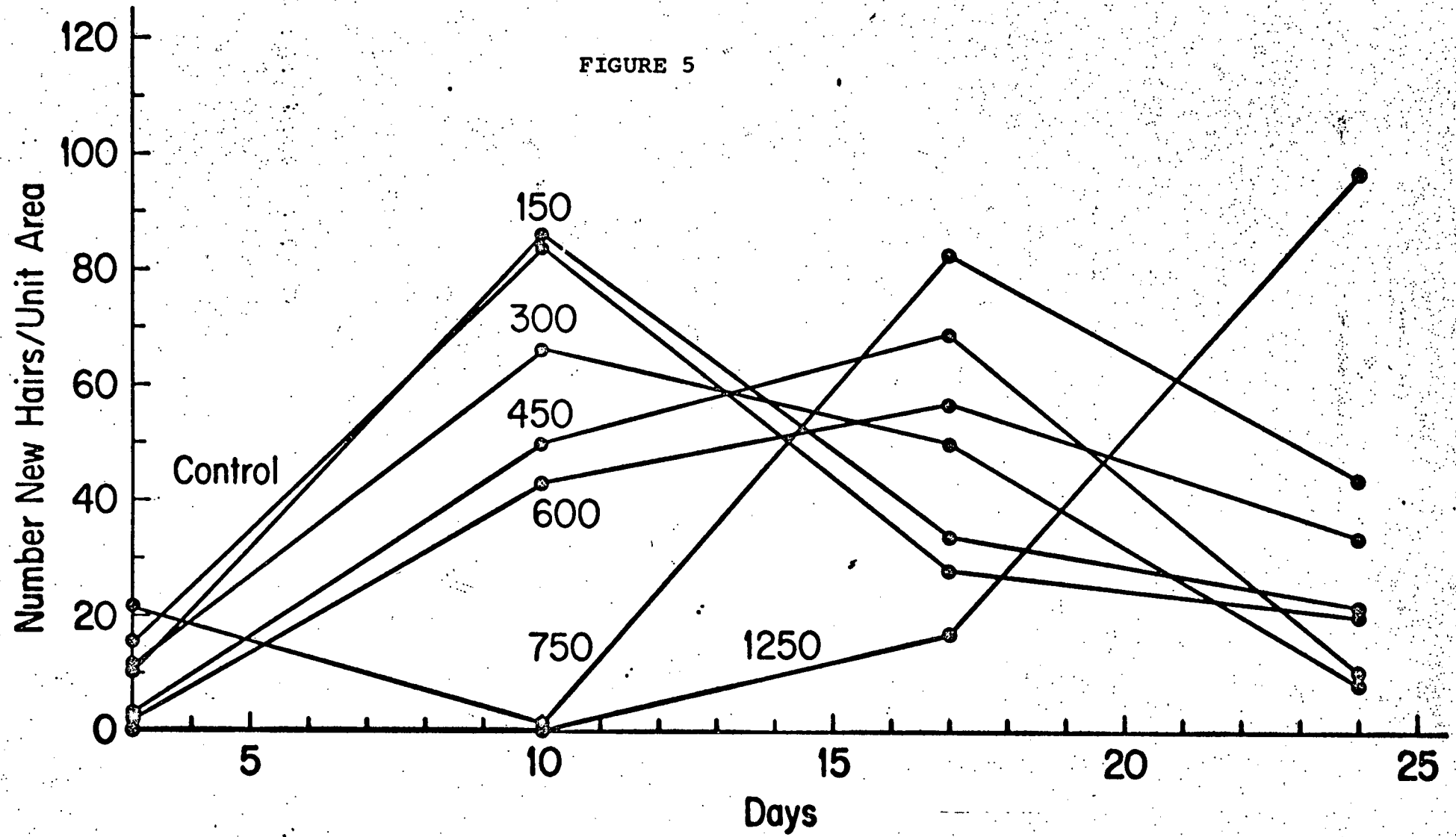

160
140
120
100
80
60
40
20
0
Total Number Hairs/Unit Area
5
10
15
20
25
Days
0
150
300
450
600
750
1250

FIGURE 5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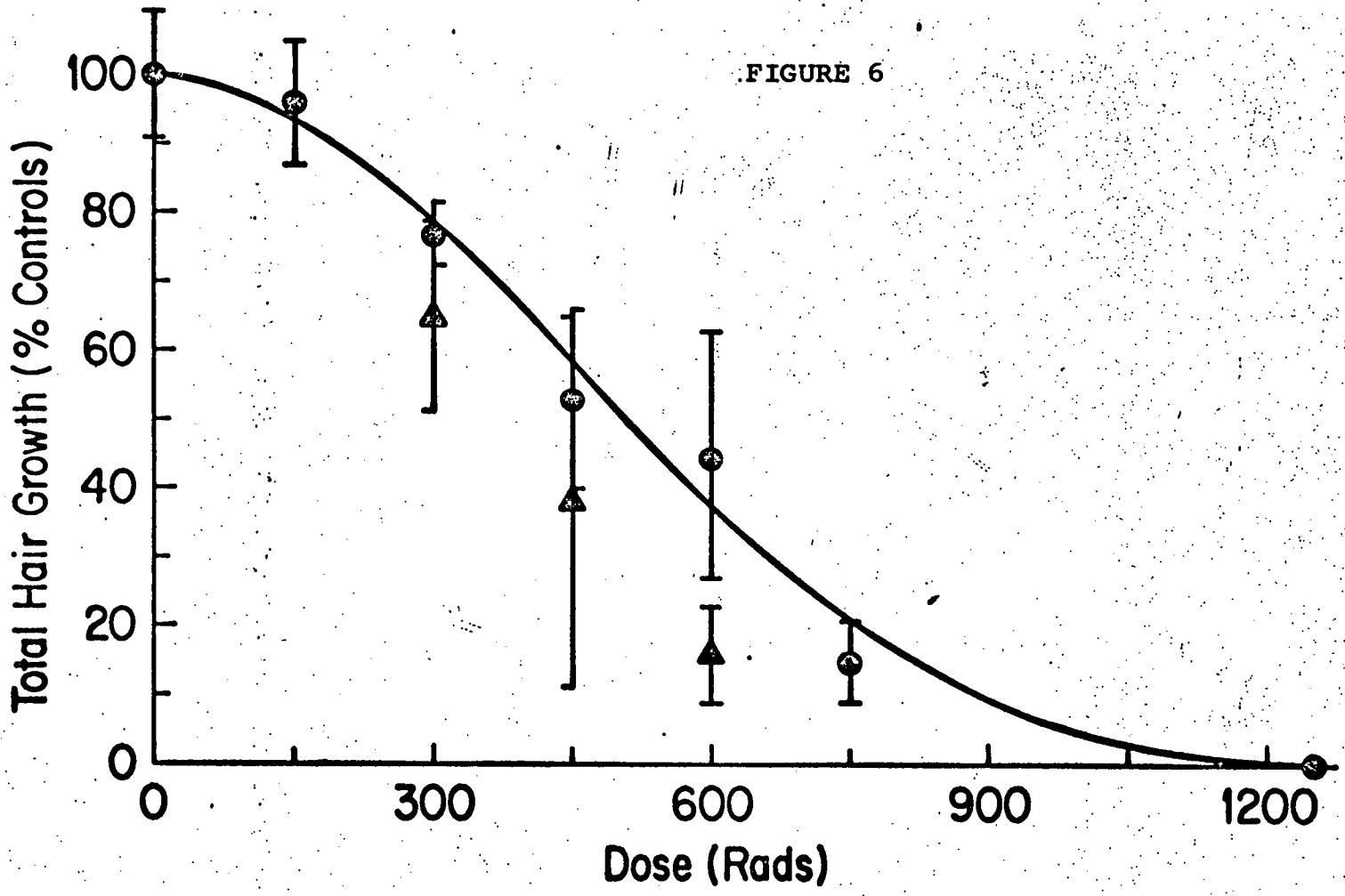
100
80
60
40
20
0
Number New Hairs/Unit Area
150
300
450
600
750
1250
Control
5
10
15
20
25
Days

FIGURE 6


Total Hair Growth (% Controls)
100
80
60
40
20
0
0
300
600
900
1200
Dose (Rads)

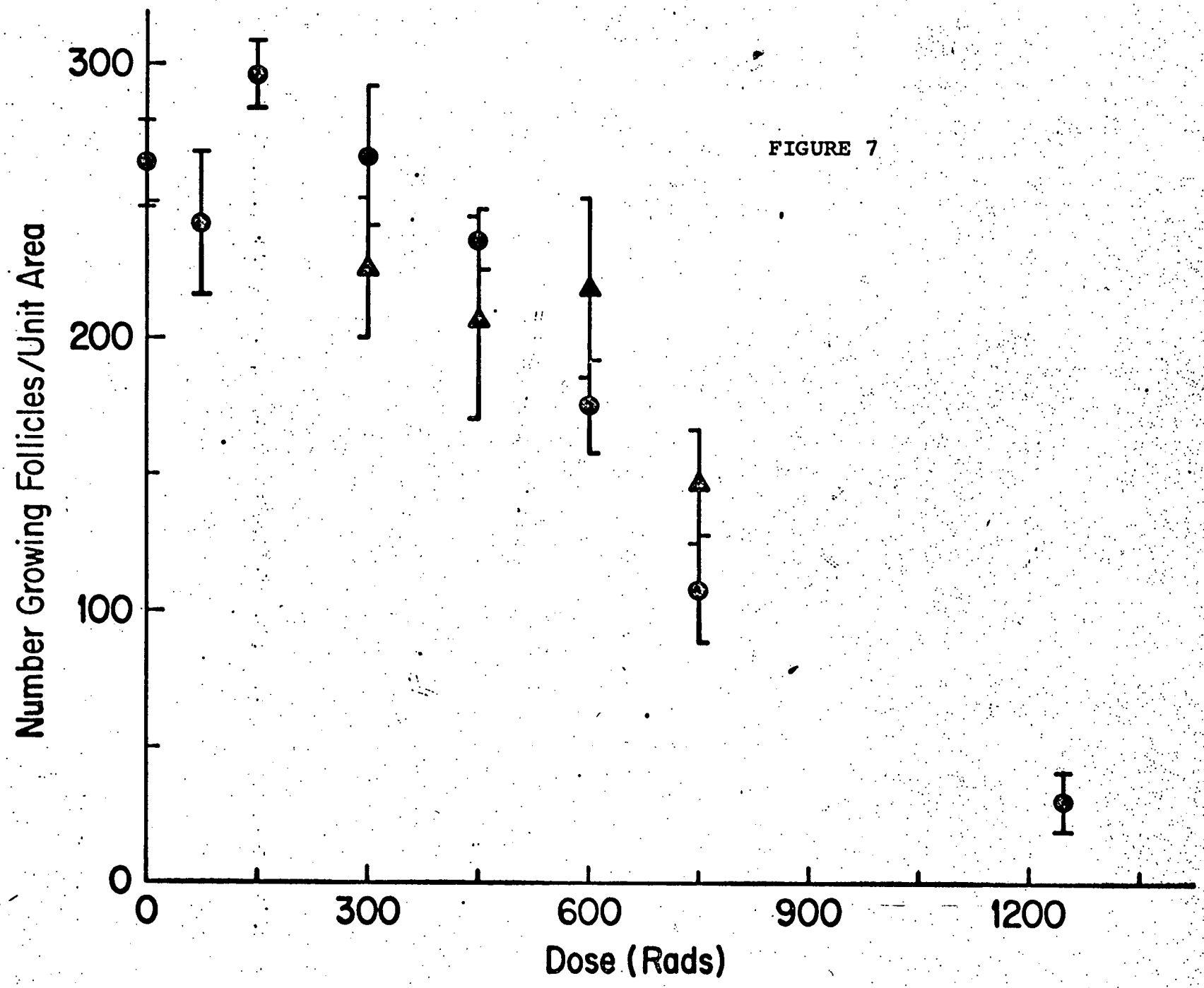

300
200
100
0
Number Growing Follicles/Unit Area
0
300
600
900
1200
Dose (Rads)

FIGURE 7

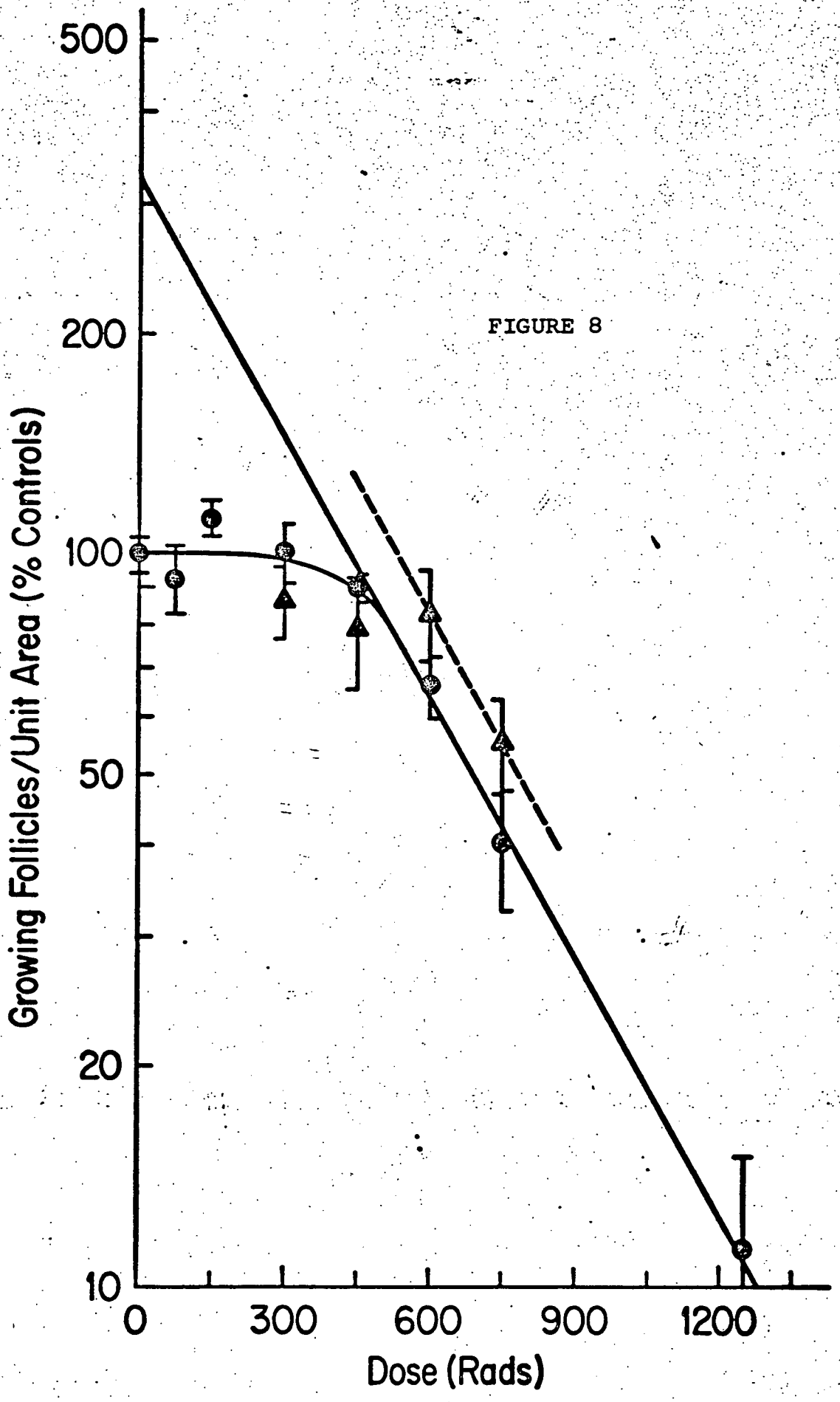

500
200
100
50
20
10
0
300
600
900
1200
Growing Follicles/Unit Area (% Controls)
Dose (Rads)

FIGURE 8

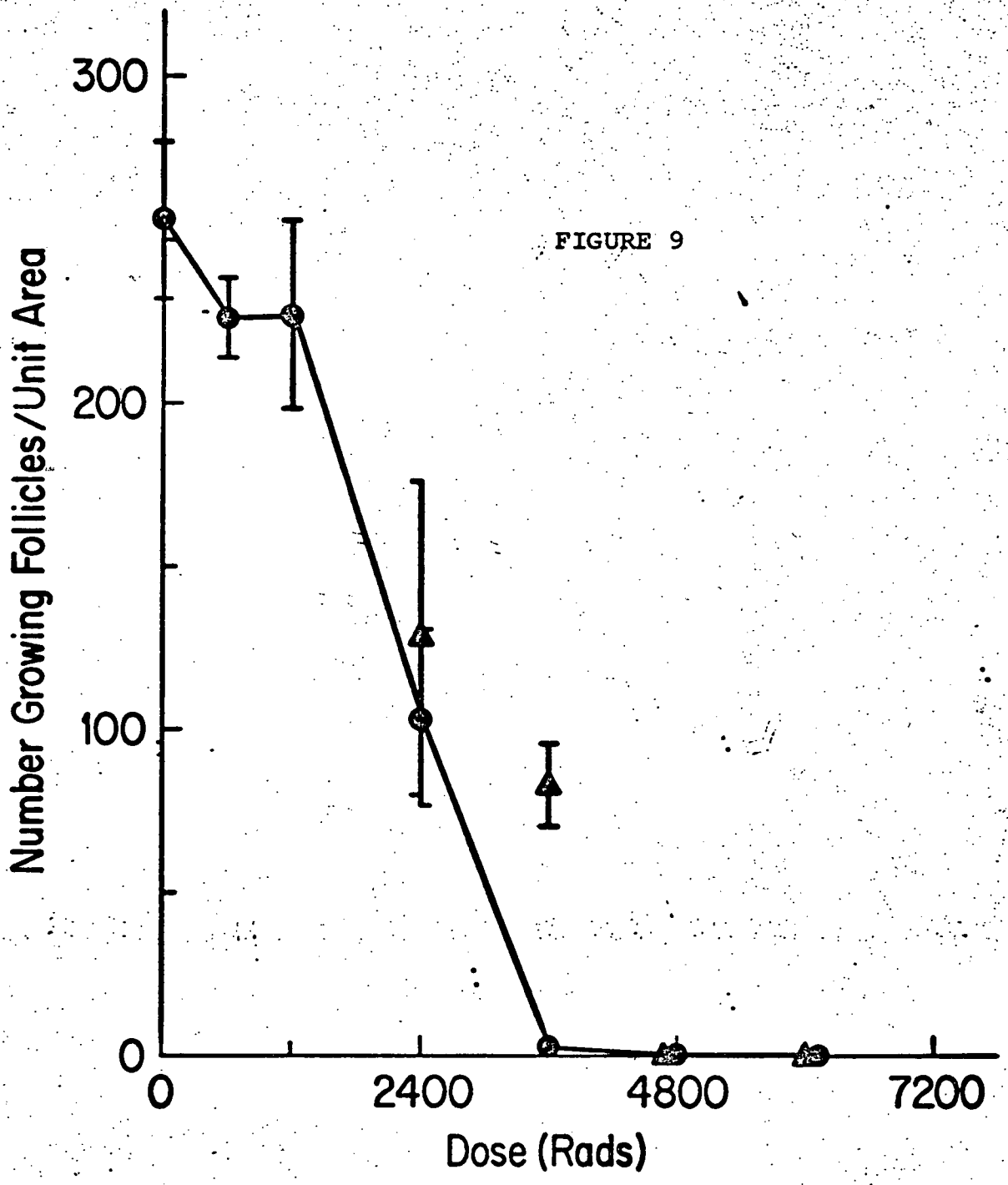

300
200
100
0
0
2400
4800
7200
Number Growing Follicles/Unit Area
Dose (Rads)

FIGURE 9

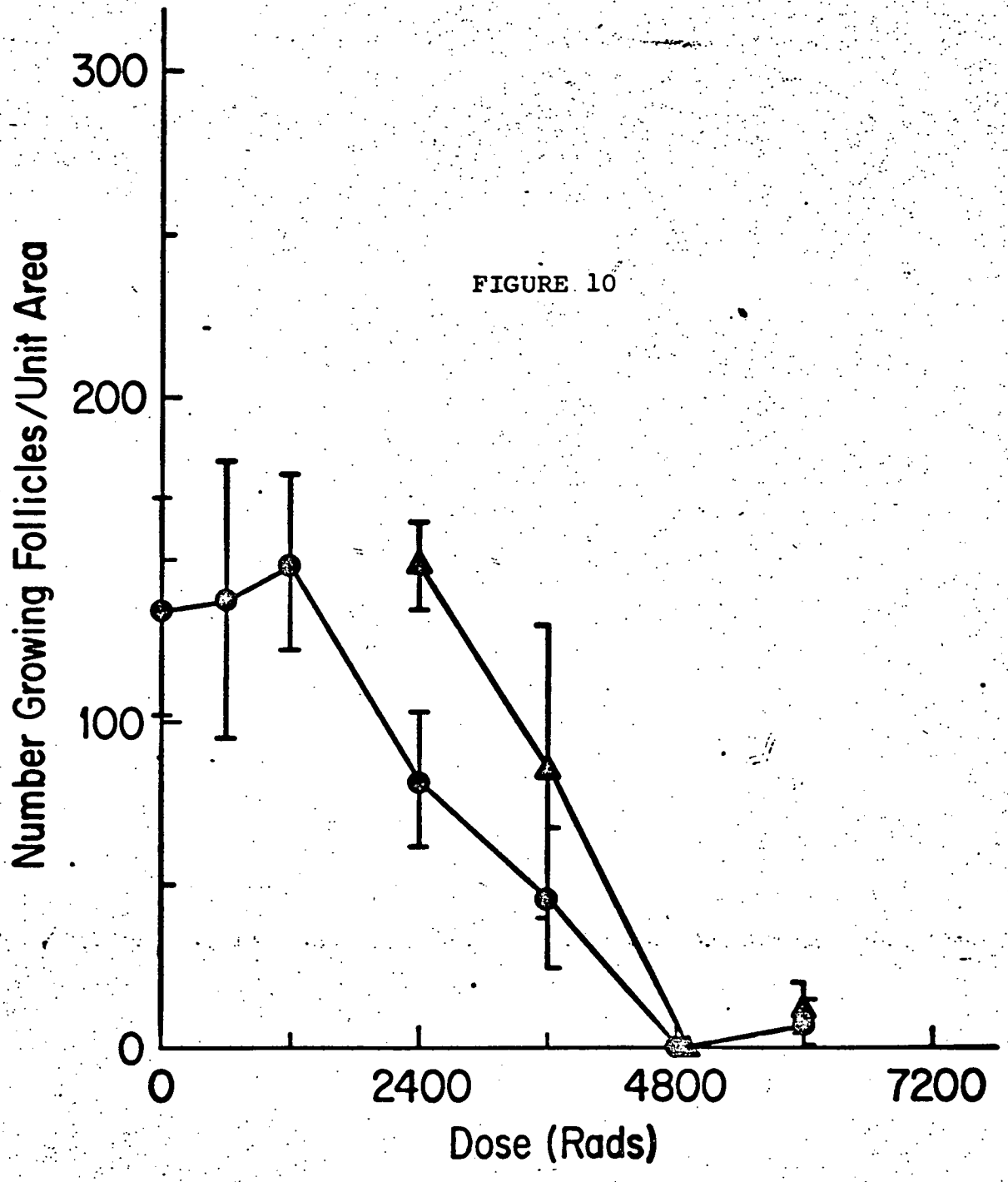

300
200
100
0
Number Growing Follicles/Unit Area
0
2400
4800
7200
Dose (Rads)

FIGURE 10

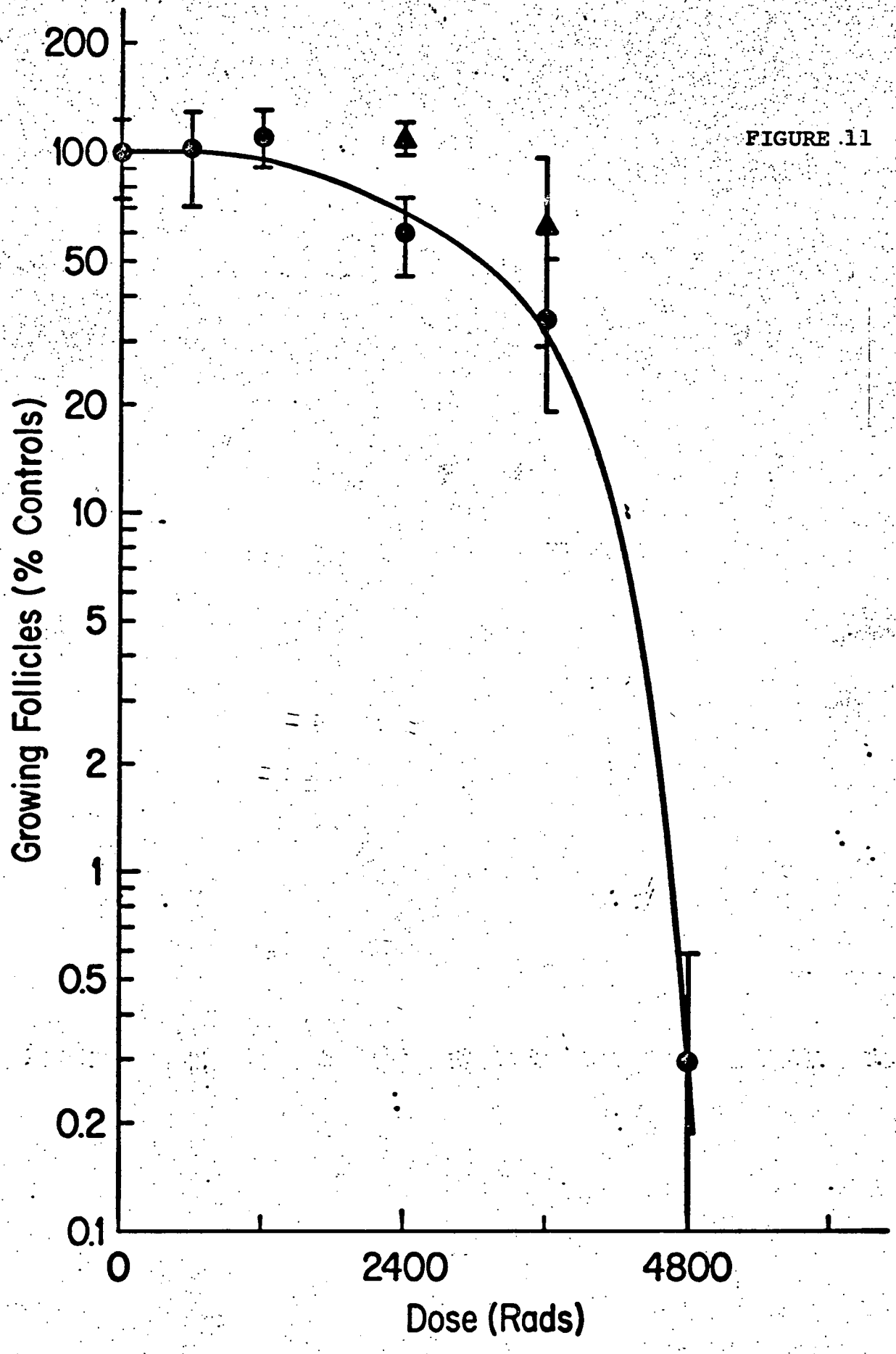

200
100
50
20
10
5
2
1
0.5
0.2
0.1
Growing Follicles (% Controls)
0
2400
4800
Dose (Rads)

FIGURE .11

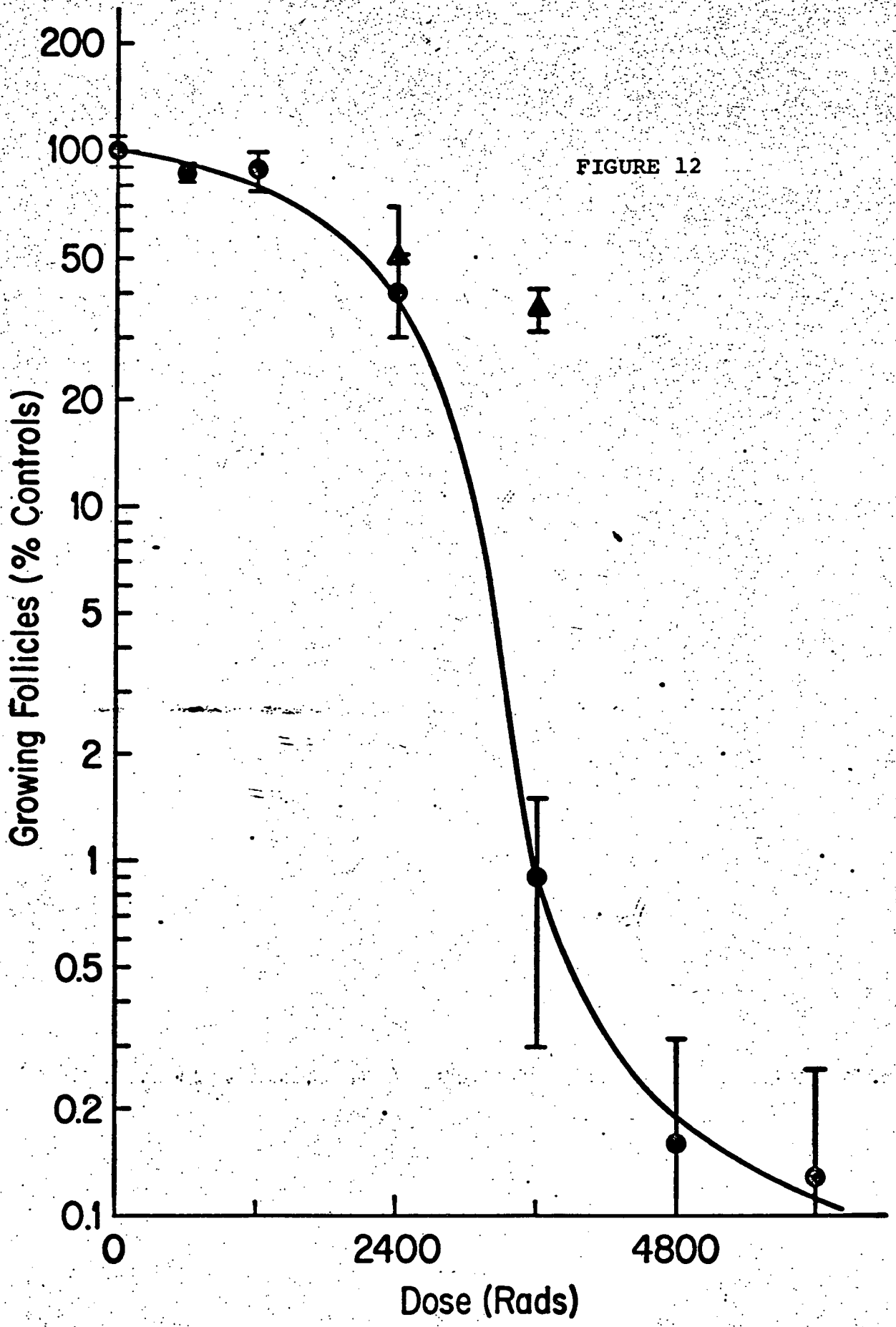
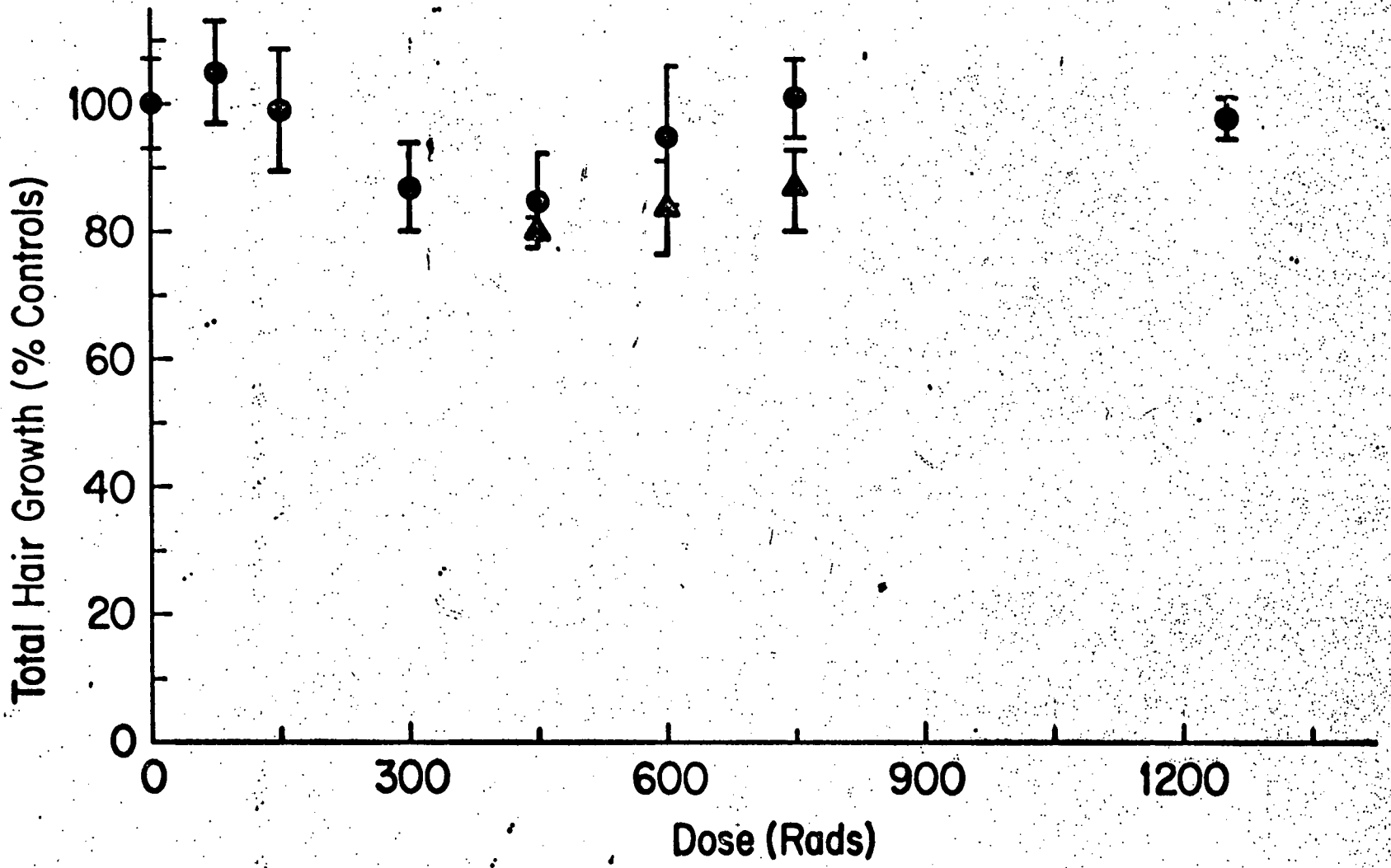

200
100
50
20
10
5
2
1
0.5
0.2
0.1
Growing Follicles (% Controls)
0
2400
4800
Dose (Rads)

FIGURE 12

FIGURE 13


Total Hair Growth (% Controls)
100
80
60
40
20
0
0
300
600
900
1200
Dose (Rads)

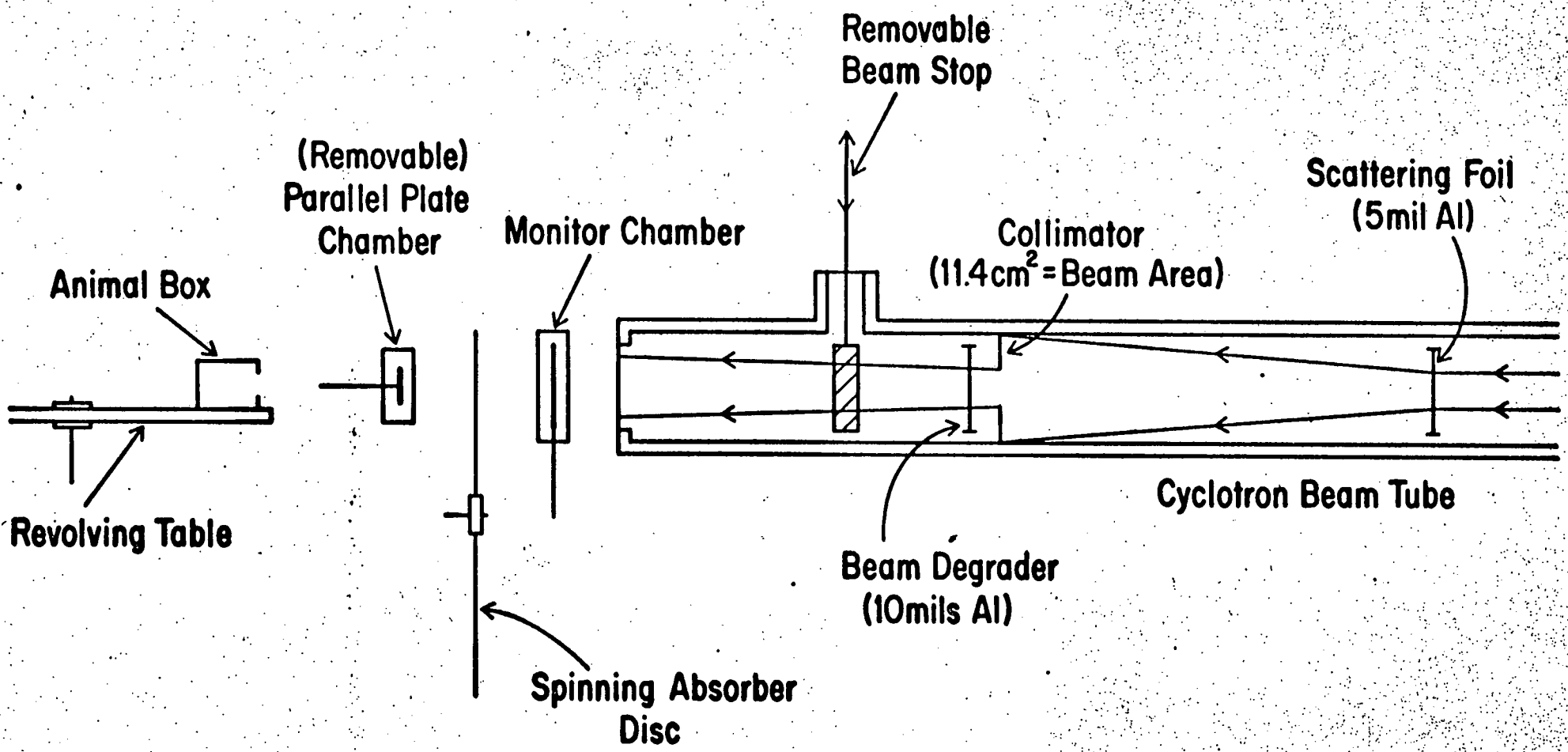

Removable
Beam Stop
(Removable)
Parallel Plate
Chamber
Monitor Chamber
Collimator
(11.4cm² = Beam Area)
Scattering Foil
(5mil Al)
Animal Box
Revolving Table
Cyclotron Beam Tube
Beam Degrader
(10mils Al)
Spinning Absorber
Disc

FIGURE 14

APPENDIX I

ELECTRON DOSIMETRY

The Bragg-Gray principle can be used as a means of relating the dose rate at a small air cavity, in an irradiated medium, to the characteristics of the cavity and air. The relationship is as follows:[15]

$$\text{Dose Rate} = \frac{dD}{dt} = \frac{I\ W\ p}{V\ d}\ 10^5\ \frac{\text{rads}}{\text{sec}}$$

where: - I = saturation current (amps)

W = energy expended per ion pair (34 eV/ion pair)

V = volume of collecting cavity (.143 cm^2)

d = density of air (1.29×10^{-3} gm/cm^3)

p = relative mass stopping power of tissue to air (1.135)

Upon substituting the appropriate values we obtain for the dose rate:

$$\frac{dD}{dt} = 2.08 \times 10^{10}\ I(\text{amps})\ \ \text{rad/sec}$$

$$= 1.26 \times 10^{12}\ I(\text{amps})\ \ \text{rad/min}$$

The chamber current used in the experiment was 3.65×10^{-10} ±10% amps, corresponding to a dose rate of:

$$\frac{dD}{dt} = 1.26 \times 10^{12}(3.65 \times 10^{-10}) = 460 \pm 46\ \text{rad/min}$$

Thus a surface dose rate of 460 rads was used with exposure times calculated accordingly.

APPENDIX II

PROTON DOSIMETRY

In order to calculate the proton beam dose, the beam flux was measured and converted to dose rate. The beam flux was monitored continuously during the experiment by means of an argon filled double ionization chamber with 205 volts applied to the central foil. The chamber current was integrated to compensate for beam fluctuations (which were small).

The monitor chamber was calibrated by means of a removable beam stop (faraday cup) in the beam tube of the cyclotron (see Figure 15). The ratio of the beam stop current to the monitor chamber current was found to be 4.2×10^{-4} up to a beam current of 1.0 nanoamp. The beam was operated at 0.4 nanoamps, or a monitor chamber reading of 0.95 uamps.

The dose rate to the animals was calculated as follows:

$$\text{Beam current} = I_b = (0.95 \times 10^{-6})\ 4.2 \times 10^{-4}$$
$$= 0.4 \text{ nanoamp}$$

$$I_b = F\ q\ A$$

where: F = beam flux (protons/cm^2sec)

q = proton charge (1.6×10^{-19} coulomb/proton)

A = area of beam (11.4 cm^2)

Solving for the flux, we obtain:

$$F = I_b / q A$$

$$F = \frac{4.0 \times 10^{-10}}{11.4 \ (1/6 \times 10^{-19})} \text{ protons/cm}^2\text{sec}$$

$$F = 2.2 \times 10^8 \text{ protons/cm}^2\text{sec}$$

We can now use the relation:

$$\frac{dD}{dt} = F \frac{dE}{dR}$$

to calculate the dose rate from the flux and the average energy loss per unit distance. The energy of the protons was 10 MeV for which the range in tissue is 1.2 mm[16] or 0.12 gm/cm^2. The average value of dE/dR is then 83 MeV cm^2/gm, which yields a dose rate of:

$$\frac{dD}{dt} = 2.2 \times 10^8 \ (83) = 1.83 \times 10^{10} \text{ MeV/gm sec}$$

when substituted into the previous equation. Converting to ergs and then rads, we have:

$$\frac{dD}{dt} = 2.93 \times 10^4 \text{ erg/ gm sec}$$

$$= 2.93 \times 10^2 \text{ rad/ sec}$$

$$= 1.76 \times 10^4 \text{ rad/ min}$$

By using the table rotation factor (1/115 = .0087) and the rib reduction factor of the spinning disc (.90), we obtain a final dose rate to the animals of:

$$\frac{dD}{dt} = 138 \text{ rad/min}$$

It was later found that during the original monitor chamber calibration, the Keithley electrometer used to measure the faraday cup current was in error by a factor of two. As a result, the observed dose rate of 138 rad/min was actually 50% of the intended dose rate. However this error was subsequently found and the final doses to the animals were corrected.

The dose rate was checked with the parallel plate chamber described earlier in the electron section. This chamber yielded a dose rate of 162 rad/min which is about 17% higher than the monitor chamber reading. The corrected monitor chamber reading was considered to be more accurate and was used in the experiment.

APPENDIX III

BIOPSY STAINING PROCEDURE

The staining and clearing procedures used to prepare the biopsies for counting are listed below:

Staining:

1) Carnoy solution - 2 to 3 hours
2) 70% alcohol (can store up to one month)
3) H_2O - ten minutes
4) HCl (60°) - 5 minutes
5) H_2O - 5 minutes
6) Remove muscle layer
7) Schiff's solution - 5 minutes
8) $NaHCO_3$ - 5 minutes
9) Acetic acid (45%) - 10 minutes
10) 70% alcohol - several days (change once)

Clearing:

1) 95% alcohol - 2 hours
2) 100% alcohol - 4 hours (change once)
3) Xylene - 2 hours
4) methyl salicylate - 2 hours (storage)

After the biopsies were counted, they were mounted on slides for storage.