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The Dose Response Curve for Tumor Induction with Single and Split Doses of 10 MeV Protons

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DISCLAIMER

Proton Paper

Introduction

Many of the biological effects produced by low Linear Energy Transfer (LET) radiations (<1 kev/μ) show a dependence not only on the total dose accumulated but the temporal pattern of exposure. In particular, splitting of a given dose into two fractions separated by a few hours reduces the effectiveness of radiation in cell killing (1, 2), in producing chromosome aberrations (3), and in the induction of skin tumors in rats (4, 5). This "split dose effect" has been interpreted as resulting from the recovery of "sublethal" or "suboncogenic" damage produced by the first radiation dose (1, 5).

When contrasting the biological effects of low and high LET radiation (>100 kev/μ) two differences are often noted. First, there is an increased effectiveness per unit dose of high LET radiation, characterized by Relative Biological Effect (RBE) values greater than one. Second, the temporal pattern of irradiation is of reduced significance in determining the final radiobiological effect. For example, in cell killing the split dose effect is substantially reduced (6, 7).

Particles with intermediate LET values may be expected to show either or both an increase in RBE or a reduction in split-dose effect relative to low LET particles. Previously we have induced skin tumors in rats using a beam of 8 mev protons having an average LET of about 10 kev/μ and observed

an RBE of about 3 (8). The purpose of the present study was to extend our dose-response curve into regions of lower dose, and measure the amount of recovery which occurs between two fractions spaced 24 hours apart. For induction of rat skin with electron irradiation, this interval is sufficient for essentially complete recovery to occur (4).

Materials and Methods

Ten cm^2 of dorsal rat skin were irradiated with either single doses or two doses separated by 24 hours of 10 mev protons produced by the cyclotron at the Sloan-Kettering Institute, Male Albino CD strain rats from Charles River Breeding Farm, Brookline, Massachusetts were irradiated at 27 and/or 28 days of age according to the protocol presented in Table 1. Groups 6 to 11 provided single dose-response data while groups 1 through 5 provided response data for animals receiving a conditioning dose of 150 rads. The animals were shaved before irradiation and no hair regrowth was observed in the subsequent 7 days indicating that the hair follicles were resting at the time of exposure. As in previous experiments (4), irradiations were carried out by anethesizing the animals (25 mg/kg of Sodium Pentobarbital) and placing them in boxes which were then mounted on a revolving table and rotated through the beam at 18 rpm.

As shown in Figure 1, the radiation geometry also included

a spinning disk of variable thickness aluminum absorbers. This disk moved the proton Bragg peak in and out of the skin so that the resulting dose curve decreased approximately linearly with tissue depth. The depth-dose curve, shown in Figure 2, was measured with both Kodak translite film and with a parallel plate ionization chamber covered with differing thicknesses of aluminum. A nearly linear depth-dose pattern was selected to facilitate comparisons between this experimenta and previous experiments in which rats were irradiated with monoenergetic electrons.

The surface dose rate to the rats of 138 rads/min. was determined by means of a monitoring ionization chamber which was calibrated against the current collected on a beam stop (Farriday cup).

The rats were observed weekly for the first four weeks and then every six weeks thereafter. The number of new tumors at each observation was recorded and at death all tumors were sectioned and pathological evaluations made. Also, at death, 2 cm^2 of full thickness skin were removed, whole mounts made and the follicle density determined using a dissecting microscope.

An additional five animals at each dose level were irradiated and the hair plucked using a depilitory wax immediately following the final irradiation. This procedure induced a uniform elongation of the follicles followed by erruption of hair.

Biopsies taken weekly for the first four week post irradiation were stained with a DNA specific schiff stain, dehydrated and cleared in Methylsalicylate. The elongated follicle density was then determined under a microscope.

Results

The acute radiation responses were mild, and confined to animals receiving more than 450 rads. There was a delay in the regrowth of new hair, and a blanching of the skin. No ulceration occurred, nor was the irradiated area discernable after 4 weeks.

The density of growing follicles 10 days after plucking is shown as a percentage of control in Figure 3. There is a shoulder of about 400 rads with a subsequent exponential decrease. Fractionation of total doses greater than the shoulder width showed a sparing of follicles relative to the corresponding single dose groups. Statistical variations preclude an accurate estimation of the amount of recovery, however.

Long term follicle survival, measured at death indicated similar results. Follicle survival was not different from controls for any dose group except at the highest dose. For a single 750 rad dose the follicle survival was 62 $\pm$ 15% of control, while for the fractionated exposure it was 79 $\pm$ 11% of control.

The tumor incidence as a function of time is shown in Figures 4 and 5 for the single and fractionated exposure groups respectively. For the single dose groups there was a tumor

free interval of about 20 weeks for all groups except the 150 r group in which one tumor appeared at 53 weeks and the 75 r group in which no tumors appeared. The 750 r group was terminated at 80 weeks post irradiation because of the large number of tumors per animal. The cumulative tumor incidence function for this group is nearly linear in time and may be projected to a value of 4.3 tumors/rat at 100 weeks for comparison with the other groups at this time.

For the fractionated exposures, the first tumor appeared between 27 and 40 weeks post irradiation for the 3 highest dose groups, and at 71 weeks for the lowest dose group. The error bars shown for all groups represent standard deviations calculated from the square root of the number of tumors observed.

Discussion

Two endpoints relating to the survival of irradiated tissue were measured: the capacity of follicles to elongate after the proliferative stimulus of plucking, and the long term survival of hair follicles. Both endpoints showed similar dose response patterns consisting of a broad shoulder followed by a decrease to 40% of control at 750 rads for follicle elongation or 62% at 750 rads for long term follicle survival. Fractionation effects can only be observed for doses greater than the shoulder width, i.e., the two highest fractionation points assayed for follicle elongation and the highest dose point for long term survival. At these doses, fractionation had a sparing effect, which is similar to that observed for fractionation of low LET electron

radiation in rat skin (4).

It has been proposed that tumor dose-response curves are the product of two functions, one for the induction of transformed events and the other for survival (9) of oncogenic targets. In order to analyse the production of transformed events as a function of dose one must correct the observed tumor yield for lethality. Ideally this should be done using the survival function for the target at risk for transformation, but since this population is unknown one must assume a function. In previous studies we have observed that the long term survival of hair follicles is a reasonable index of tissue survival as a whole and assumed that the survival of oncogenic targets may be approximated by the follicle survival (4). Dividing the tumor incidence by the fractional follicle survival gives an estimate of the tumor yield in the absence of killing.

Figure 3 shows the results of such calculations. The response curve rises steeply with increasing dose. The tumor response in this experiment was more vigorous than that seen in other experiments in which rats were irradiated with either electrons or protons. When compared with electron irradiation of 28 day old rats (4, 5) the protons in this study have a Relative Biological Effectiveness (RBE) between 2 and 3. When compared with rats irradiated with protons at 58 days of age (8), the animals in this experiment are more sensitive, suggesting

that age at irradiation may be an important factor in determining the tumor response. Similar age effects have been noted by others for various radiation induced tumors (11).

There is a displacment between the single and fractionated dose response curves shown in Figures 6 which may be interpreted as resulting from the recovery of suboncogenic damage (10). The errors associated with the measured tumor yields in the 300 and 450 rad total dose groups precludes their use in evaluating the amount of recovery. There is, however, sufficient difference between the fractionated and single exposure groups at 600 and 750 rads to enable estimation of the amount of recovery which occurred during the 24 hour fractionation interval.

We have previously defined the amount of unrecovered damage remaining in the split dose groups, p, by formula (10):

$$P = \frac{I(D_1, D_2, T) - \left(I(D_1) + I(D_2)\right)}{I(D_1 + D_2) - \left(I(D_1) + I(D_2)\right)}$$

where $I(D_i)$ is the tumor yield for dose fraction i, and $I(D_1, D_2^T)$ is the yield for two dose fractions, D_1 and D_2, separated by T hours. The above equation may be rearranged to yield an expression for the expected tumor yield in the fractionated groups as a function of the observed single dose responses and

$$I(D_1, D_2, T)\} = P\, I(D_1 + D_2) + (1-P)\left(I(D_1) + I(D_2)\right)$$

For 600 rads total dose, the observed yield is .62

tumors/rat/follicle, which is less than the expected yield for complete recovery, 1.04. The expected yield is within the 95% confidence interval of the observed response, however. P values greater than 0, indicating less recovery, predict expected incidence values greater than 1.04. There is less than a 5% probability that the observed tumor yield would have occurred if the true P value were greater than zero.

For 750 rads total dose the observed yield, 2.04 exceeds the expected yield for complete recovery, 1.7 and leads to a best estimate of P = 0.05. The upper 95% confidence limit for the observed yield is 2.86, which corresponds to a P value of .22, i.e., the recovery was 78% complete. Thus while the observed yield suggests essentially complete recovery, estimates of 22% unrecovered damage are consistent at the 5% level of significance.

Thus, the response of rat skin to proton irradiation shows a rapid form of recovery of both sublethal and suboncogenic injury and RBE values between 2 and 3. The combination of RBE values greater than one, and recovery indicates that while protons induce injury more efficiently per unit absorbed dose than electrons, there is still a reversible component to the injury process.

Figure Legends

Figure 1. Irradiation geometry. Anesthetized rats were placed inside wooden animal boxes on a revolving table and rotated through the beam at 18 rpm. The beam was degraded by 10 mils of aluminum in the beam tube and a spinning absorber disk of varying thicknesses of aluminum which resulted in a nearly linear depth dose curve in the rat skin. The dose rate was determined using both the beam stop and the parallel plate ionization chamber.

Figure 2. Depth-dose curve in tissue determined by exposure of X-ray film (▬) and by placing aluminum absorbers over the ionization chamber (●).

Figure 3. Growing follicles per unit area expressed as a percentage of controls 10 days after irradiation and hair plucking.

Figure 4. Cumulative tumors per rat versus time post irradiation for groups receiving single proton exposures.

Figure 5. Cumulative tumors per rat versus time post irradiation for groups receiving fractionated proton exposures.

Figure 6. Tumors per surviving follicle 100 weeks post irradiation as a function of dose. The response curve for both single and fractionated doses rises sharply with dose and the fractionated curve is displaced from the single dose curve.

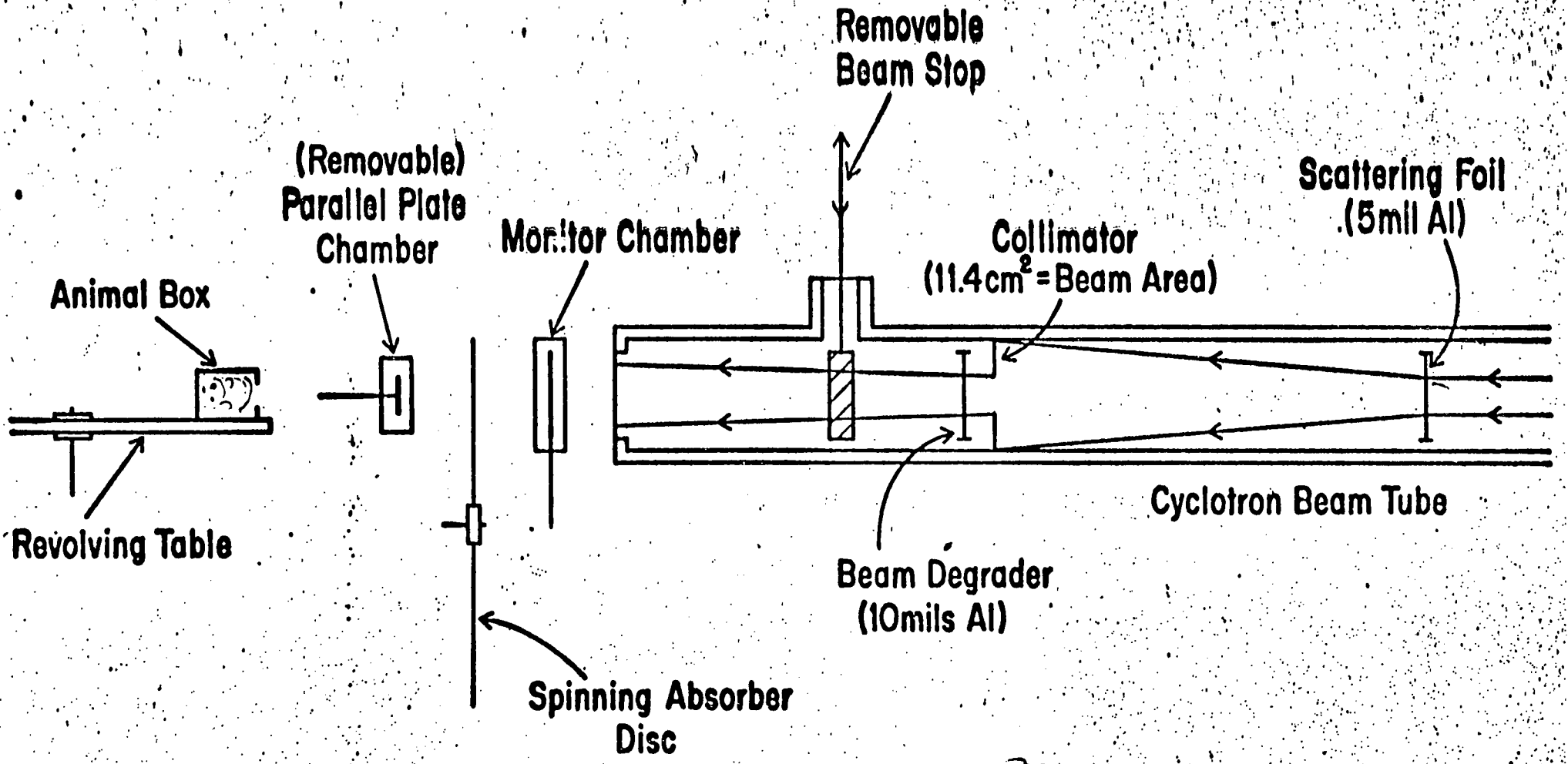


FIGURE 14

figure 1

FIGURE 1

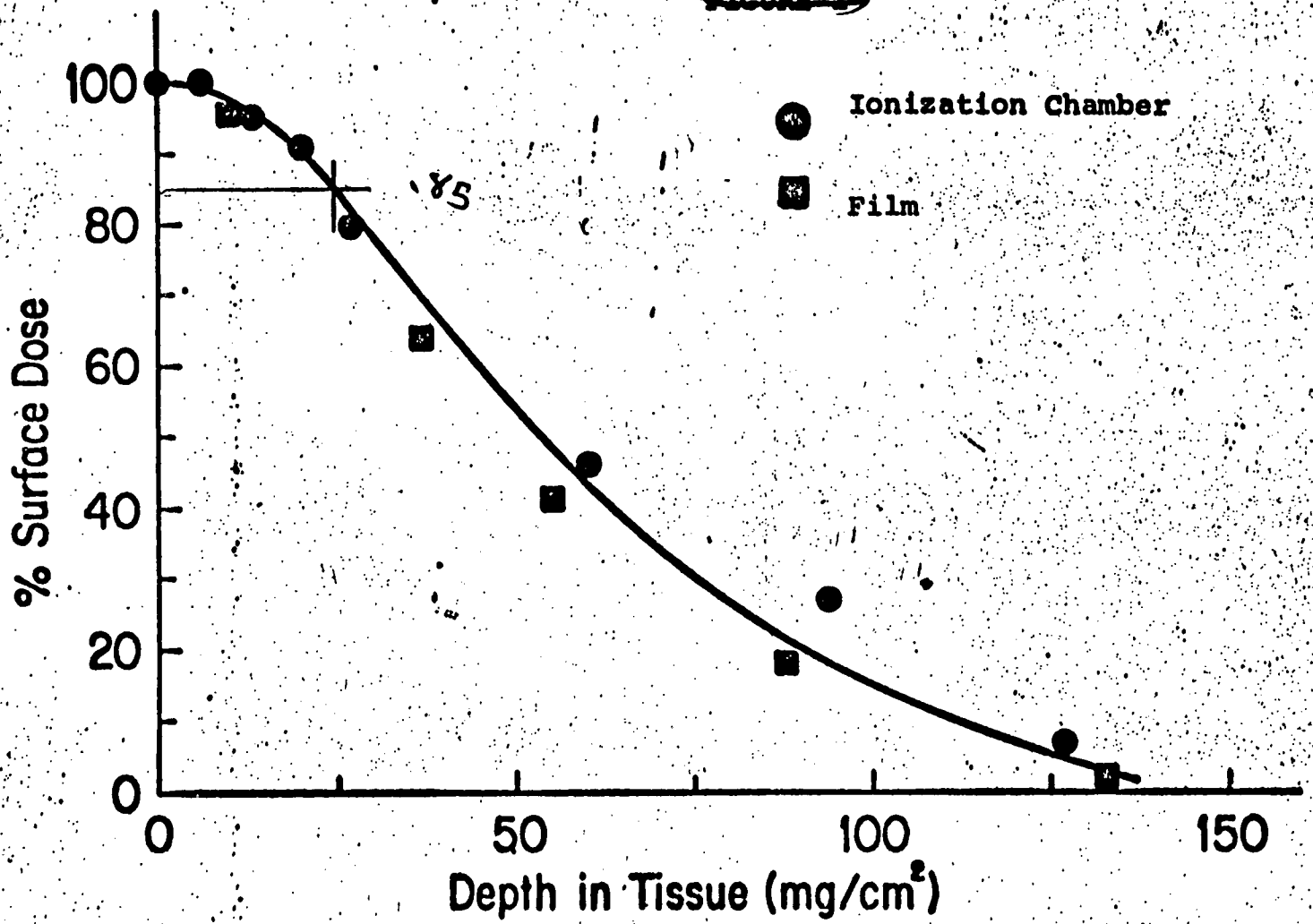
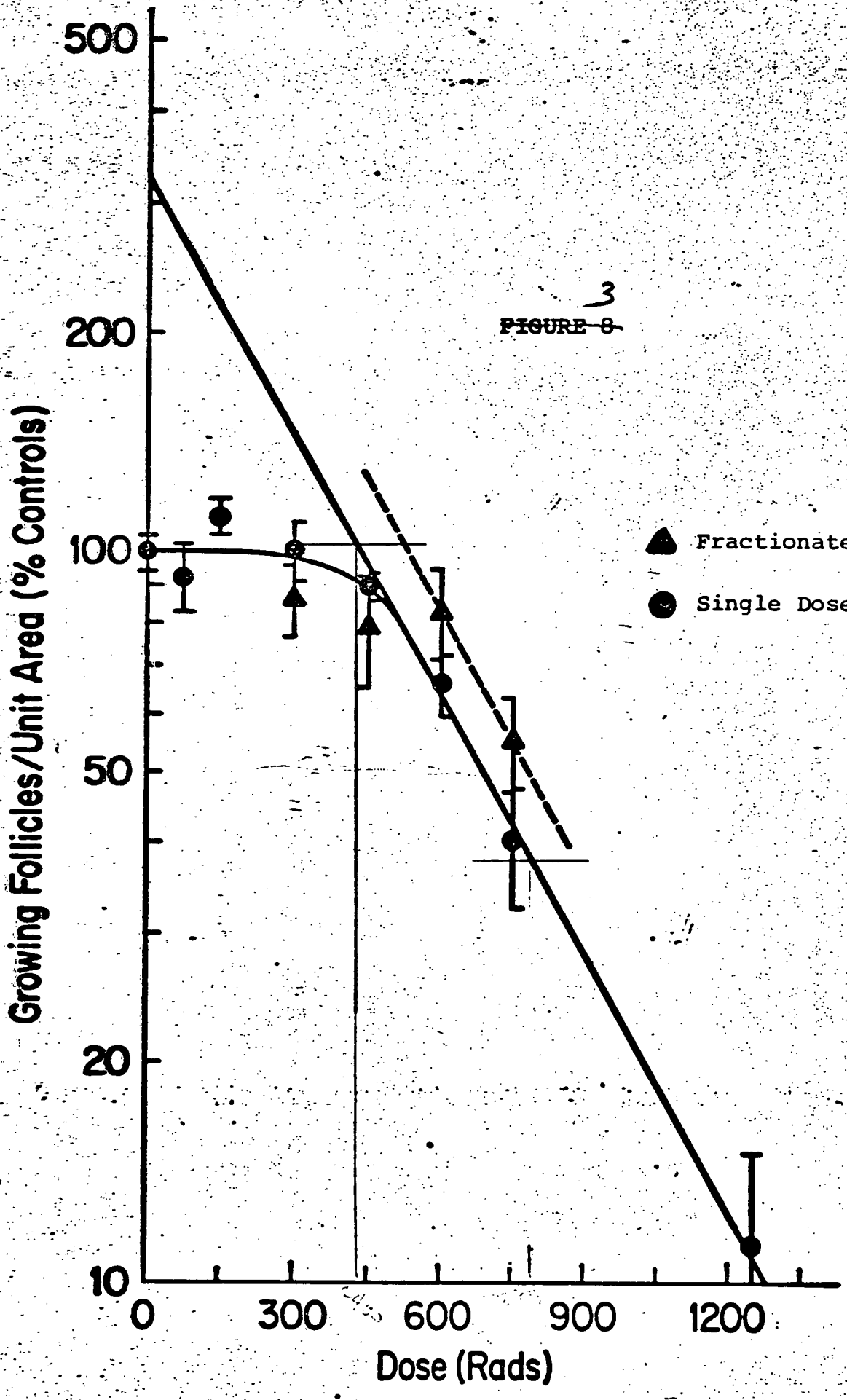


Figure 2


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

~~FIGURE 8~~ 3

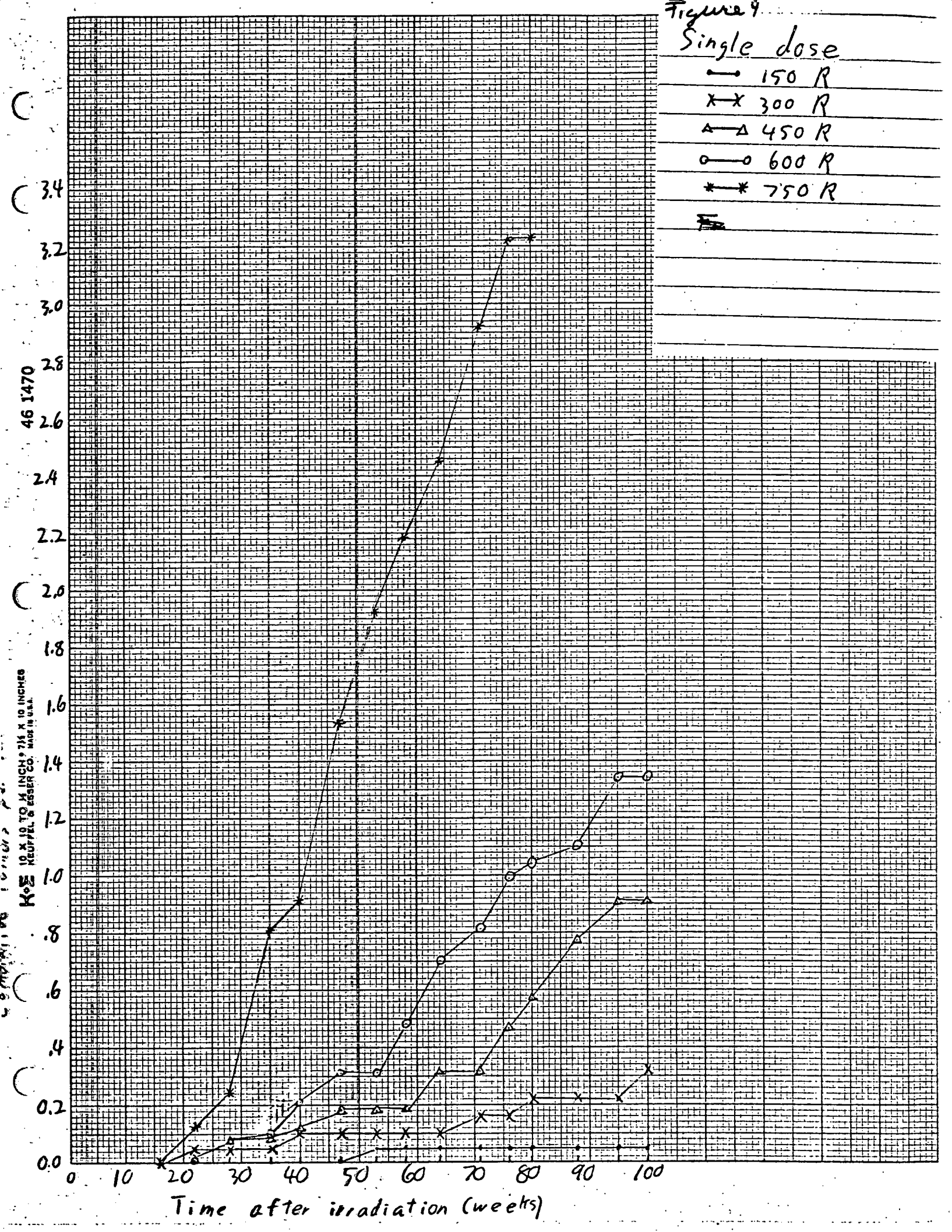


Figure 4
Single dose
150 R
300 R
450 R
600 R
750 R
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
100
Time after irradiation (weeks)
46 1470
KOE 10 X 10 TO 1/4 INCH • 7 1/4 X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

Fractionated dose

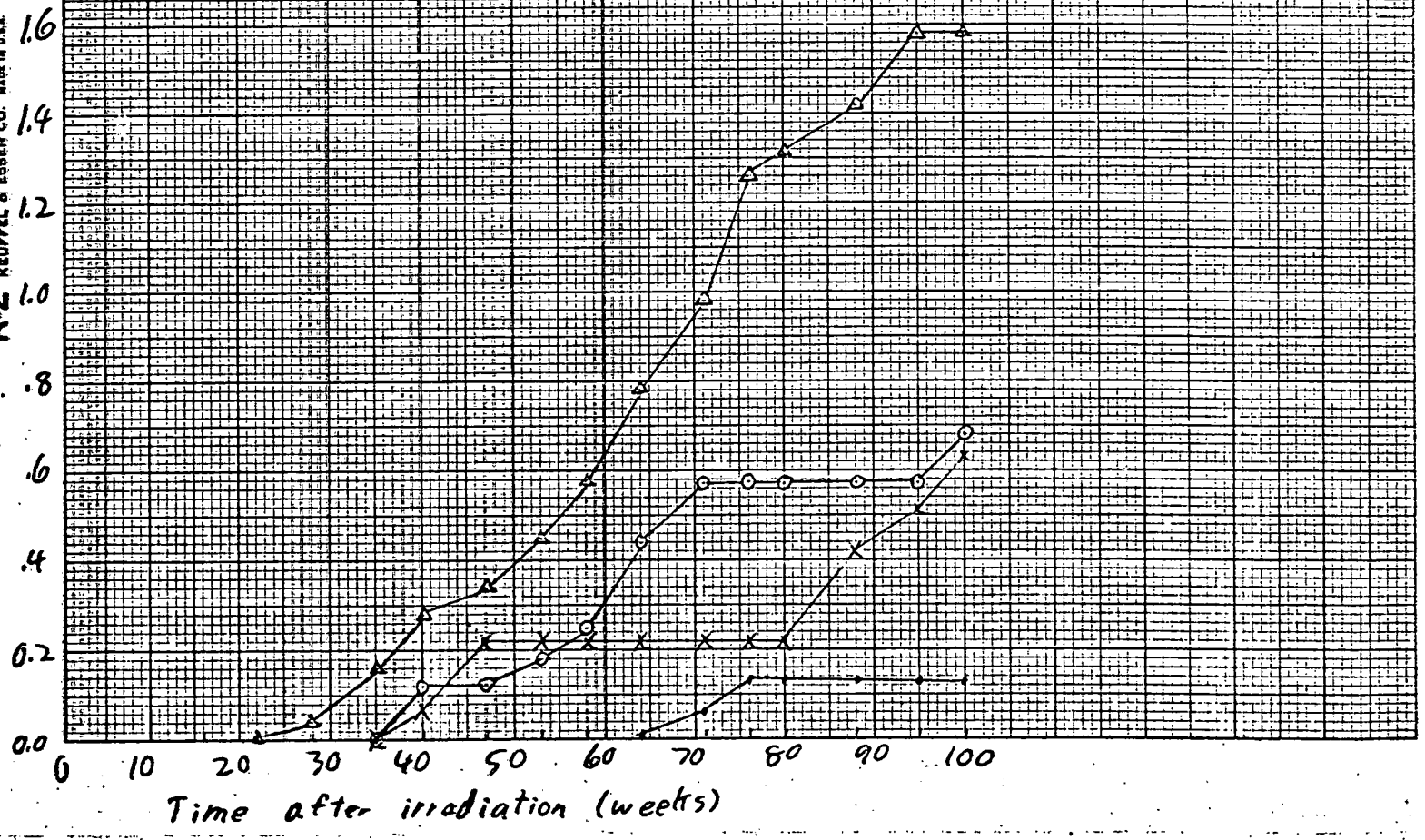
—●— 150 + 150 R

—○— 150 + 300 R

—×— 150 + 450 R

—△— 150 + 600 R

Figure 5



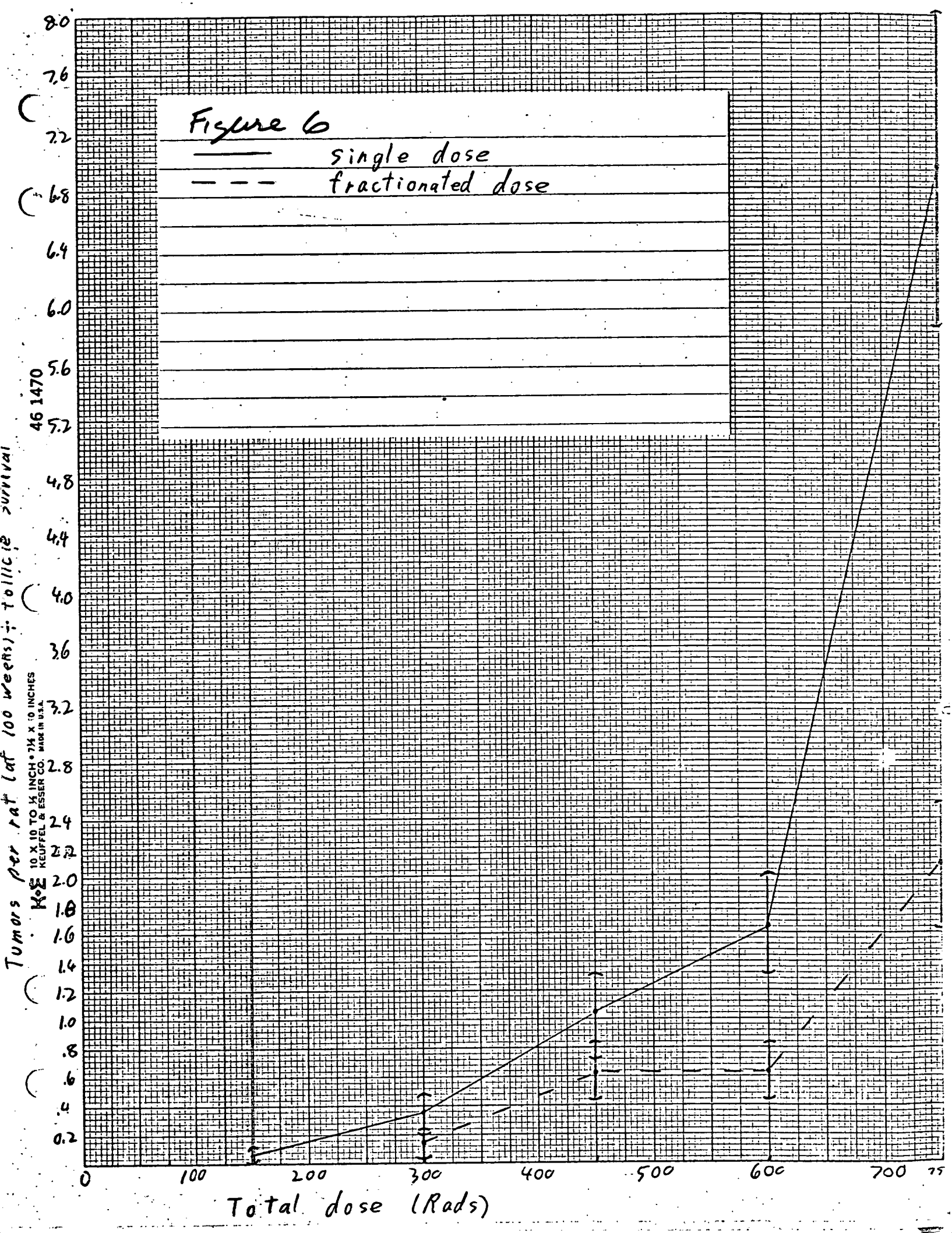


Figure 6
Single dose
fractionated dose
Tumors per rat at 100 weeks ÷ follicle survival
Total dose (Rads)
0
100
200
300
400
500
600
700
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
0.2
46 1470
10 X 10 TO ½ INCH • 7½ X 10 INCHES
KEUFFEL & ESSER CO. MADE IN U.S.A.

REFERENCES

1. Elkind,M.M. and G.F.Whitmore, The Radiobiology of Cultured Mammalian Cells, Gordon and Breach, New York, 1967.

2. Withers,H.R. Recovery and Repopulation in vivo by Mouse Skin Epithelial Cells during Fractionated Irradiation. Rad. Res. 32:227-239, 1967.

3. Okada,S. Radiation Biochemistry Vol.I: Cells Academic Press New York, 1970.

4. Burns,F.J., R.E.Albert, I.P.Sinclair and M.Vanderlaan, The Effect of a 24-Hour Fractionation Interval on the Induction of Rat Skin Tumors by Electron Radiation. Rad. Res. 62:478,1975.

5. Vanderlaan,M. and F.J.Burns, Split Dose Recovery for Radiation Induced Tumors in Rat Skin ,(for submission to Inter. J. Rad. Bio.)

6. Barendsen,G.W., H.M.D.Walter, J.F.Fowler, and D.K.Bewley, Effects of Different Ionizing Radiations on Human Cells in Tissue Culture, Rad. Res. 18:106-119, 1963.

7. Todd,P. Heavy Ion Irradiation of Cultured Human Cells, Rad. Res. Suppl. 7 196-207, 1967.

8. Burns,F.J., R.E.Albert, P.Bennett and I.P.Sinclair, Tumor Induction in Rat Skin after Proton Irradiation in a Sieve Pattern, Rad. Res. 50:181-190

9. Mole,R.H. Ionizing Radiation as a Carcinogen: Practical Questions and Academic Pursuits, Brit. J. Radiology 48:157-169, 1975.

10. Vanderlaan,M., F.J.Burns and R.E.Albert, IAEA Symposium on Biological Effects of Low Level Radiation Pertinent to Protection of Man and his Environment. Proceedings in press.

11. Coustanera,T.J., D.C.Jones, D.J.Kimeldorf and V.J.Rosen, The Effect of Age at Exposure to a Sublethal Dose of Fast Neutrons on Tumorigenesis in the Male Rat. Cancer Research 31:1543-1549 1971.