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RBE VALUES FOR LENS OPACIFICATION IN MICE
EXPOSED TO A 400 MeV NEUTRON BEAM

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A B S T R A C T

The effectiveness of fast neutrons in the induction of lens opacities has been the subject of a number of investigations because of its importance to radiation protection. The results obtained from mice show a high radiosensitivity to neutrons of medium energy at low doses but no data have so far been reported for neutrons with energies above 14 MeV.

A radiobiological collaborative experiment between CNEN and CERN has been undertaken in order to obtain information on the induction of lens opacification in mice exposed to a 400 MeV neutron beam. The 400 MeV neutron beam from the CERN 600 MeV Synchro-cyclotron was used for the exposures. The mice were exposed head on in the pure beam and behind an 18 g/cm^2 polythene attenuator where the maximum dose build-up occurred. The dose measurements were made with a tissue-equivalent parallel-plate ionization chamber.

Eight hundred and twenty-two mice, f1 hybrids between C57BL ♀ and C3H ♂ inbred strains were used. These were of an average age of 75 days at the time of exposure.

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After irradiation serial examinations with a slit-lamp microscope were performed. A particular method was used to quantitate the degree and the extent of the early opacities of the posterior subcapsular region of the lens.

Preliminary results will be presented of the observations from the first 20 months after exposure. By comparison with 250 kV X-rays these results give RBE values of about 5.5 for the pure beam and 2 for the attenuated beam.

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INTRODUCTION

The study of lens opacification was intensified after cataract was detected in American cyclotron workers and assumed to have been caused by high LET radiation¹⁾. The explanation for this was that cyclotron workers had the habit of holding fluorescent material in the path of the beam of 8-16 MeV deuterons or protons in order to locate beam position. Various materials were used and very few measurements were made of the particles emitted, but individual radiation exposures were substantial²⁾. Similar cases of cataracts in cyclotron workers were also observed in France³⁾. From information obtained at that time it was concluded that high LET radiation was more cataractogenetic than low LET radiation. A more recent review of this problem however shows rather clearly that the human eye is not specially sensitive to high LET radiation and the maximum permissible exposures have been changed accordingly⁴⁾.

From the experimental point of view lens opacification in mice has been the subject of extensive studies, and in particular the effect of neutrons has been investigated. In a recent study it was found that a dose of the order of 1 rad of neutrons from 0.43 to 1.8 MeV could produce definite opacification⁵⁾. Neutrons of an energy of 14 MeV have also been studied and are considerably less effective in producing opacity than the 0.43 MeV particles⁶⁾. The radiation induction of opacity also depends upon the species and a study by Upton and co-workers has proved a decreasing radiosensitivity of animals going from mouse to rat, guinea-pig and rabbit⁷⁾. A decrease in sensitivity is further established for still larger animals such as dogs, monkeys and goats⁴⁾.

In addition, effects of fractionation and of low doses have been subject to investigation due to their importance to radiation protection. In the light of existing evidence it has been

recommended recently that the human eye is not to be considered as a particularly radiosensitive organ and the dose limit has consequently been increased from 5 to 15 rem per year independent of the LET of the radiation in question⁸⁾.

So far no investigation has been made of the effectiveness for cataract formation caused by neutrons with energies above 14 MeV. Since for higher energies the interaction processes involved also include the nuclear interaction reactions, it was considered to be important to carry out a study of the relative radiobiological effectiveness for lens opacification in mice after exposure to 400 MeV neutrons. These particles have previously been used for studies of survival of spermatogonia type B in mice⁹⁾ as well as of weight loss of testes¹⁰⁾ in mice and are rather well known from a dosimetric point of view¹¹⁾.

THE IRRADIATION AND THE DOSIMETRY

The 400 MeV neutron beam used in this experiment was produced by the CERN 600 MeV Synchro-cyclotron. Its layout is shown in Fig. 1. Details of the beam, its composition and monitoring are reported elsewhere¹¹⁾. The internal intensity of the circulating proton beam was 1.2 μ A. The irradiation position was 3.1 m from the exit of the beam pipe and at this place the beam profile was measured with a 30 cm³ (diameter 23 mm) ionization chamber. The horizontal intensity variations observed are shown in Fig. 2. The diameter of the beam at this position was 26.5 cm with an intensity variation across the beam of less than 5% over an area of 20 cm in diameter. This variation was mainly due to non-uniformity of the window of the cyclotron through which the beam had to pass.

The depth-dose distribution of the 400 MeV neutron beam at the point of irradiation was measured with two ionization chambers under different experimental conditions. The first series of measurements were made with an air-filled, thin-walled, parallel-plate chamber 60 mm in diameter and 3 mm spacing with a 3.4 mg/cm² mylar entrance window. This chamber was used to measure the variation of dose with depth from 3.4 mg/cm² to 1 g/cm² using polythene as absorber. At

greater depths a 20 mm parallel-plate tissue-equivalent chamber filled with tissue-equivalent gas was used¹²⁾. This chamber with an entrance window of 570 mg/cm^2 was made to move along the central axis of the beam in a tissue-equivalent liquid (density 1.11 g/cm^3 , 56.9% water, 28.4% glycerol, 7.6% urea and 7.1% sucrose). The thin window chamber results were normalized to the 20 mm chamber readings at a depth of 570 mg/cm^2 . The depth-dose curve obtained for the tissue-equivalent liquid is shown in Fig. 3. The absorbed dose measurements for this beam were established using the same procedure as for the negative pion beam but without applying the geometry correction¹²⁾.

It was of interest to study lens opacity formation in the mice both caused by the pure 400 MeV neutron beam and at radiation equilibrium in tissue-equivalent material of this beam. The attenuation was established by a polythene absorber of 18 g/cm^2 and the animal exposures were carried out in front of and behind this block (Fig. 4). Absorbed dose was also measured behind the absorber and Fig. 5 shows the results.

The flux density of high-energy particles was also measured in front of and behind the polythene absorber using activation of ^{11}C in plastic scintillators. Using a cross-section of 20 mb, flux densities of $2.5 \cdot 10^5$ and $2.6 \cdot 10^5 \text{ n/cm}^2/\text{sec}$ were measured. The dose-rate to the eye of the mice was established at a depth of 100 mg/cm^2 which corresponds to the eye lens of the mice. At this place the dose-rate was 3.55 rad/h in front and 9.75 rad/h at the back of the polythene absorber. The errors involved in the dose estimate was about $\pm 10\%$.

The mice were irradiated individually head on in plastic containers, with walls of 120 mg/cm^2 . Twenty-five animals in front of and 25 behind the absorber were exposed simultaneously.

The X-ray irradiations were done with a 250 kVp X-ray set of 15 mA filtered by 2 mm Pb, 1 mm Cu, 1 mm Al (HVL 5 mm Cu) and which produced a dose-rate of 40.4 rad/h at a distance of 85 cm. The animals were exposed in groups of 25 in a plastic container to doses of 50, 100, 200 and 400 rad.

THE ANIMALS

Male mice hybrid between C57BL \times and C3H δ with an average age of 75 days were used. The animals were grouped in such a way that each litter had an unirradiated control animal. This was done in order to control a possible difference between the male litter-mates.

After irradiation the mice were kept 5 per cage in the normal standard conditions in the Casaccia animal house. In each cage an unirradiated control animal was placed together with 4 mice which had received radiation. The body weight of all the 822 mice used in this experiment were systematically recorded once every month.

METHODS OF EVALUATION

Structurally the eye lens is divided into the capsule, the epithelium, and the lens fibers. The lens epithelium is formed of a single layer of cells located on the anterior part of the capsule. The cells in the germinative epithelium zone proliferate and migrate to the posterior part of the lens while differentiating into fibers. The fibers form the posterior part of the lens. The older cells after having lost their nucleus and their walls form a part of the lens nucleus. The lens continues to grow at a decreasing rate throughout life and its weight increases accordingly.

There is a natural development of opacities with age in the posterior region of the lens which also depends on species and strains. The early stage of the opacity appears as the formation of small dots which increase in number and size with time and then tend to coalesce resulting in a possible reduction of the vision. The radiation influences this process by increasing the rate of opacity formation and decreasing the latent period. This is probably due to the radiation effects on the germinative epithelium of the lens.

The opacities of the eye lens were scored by periodically using a slit-lamp microscope (x28) after dilation of the pupils with 1% homathropin without using anesthesia. All the observations were made by the same examiner who had no prior knowledge of the identity

of the animal. The opacity scoring was done by examining the opacity of the nuclear and posterior subcapsular region of the lens. In order to judge the error from this observation a serial examination of the same group of animals was done. Good reproducibility was thus proved to have been achieved¹³⁾.

For observing the posterior lens opacification direct light was employed. The degree of lens opacification was classified by counting the opacities which were seen, without considering their size. The possibility to use this criterion was due to the relatively low degree of opacity, and when the opacity coalesced another criterion had to be applied. The data presented here are preliminary and include only 20 months of observation after exposure. Up to this time it was possible to use the counting method. However, for the largest doses, in particular for X-rays, the counting method was excluded. In order therefore to evaluate the larger opacity the classical methods for scoring which is referred to stages was applied. This method is characterized by a subjective qualitative analysis of the size of the lens that appears opaque^{5, 14)}.

RESULTS

The observation of the nuclear opacities 3, 5, 8 and 11 months after exposure is shown in Table 1. It is clearly seen that no difference between the irradiated and unirradiated animals were found. The nuclear opacities do not seem to be affected by the exposure to radiation.

The results from observing the posterior opacity are shown in Table 2 where the X-ray data, the data from the pure and attenuated 400 MeV neutron beam are shown together with the calculated standard deviations. These data are also presented in graphical form in Fig. 6, 7, 8, 9 and 10. The number of opacities reported are the sum of the number of opacities observed in the left and the right eye. No significant difference was found for the two eyes. The progression of lens opacities with time is reported for the different doses in

Fig. 11, 12 and 13. The progression seems to be linear for the irradiated as well as the unirradiated animals, increasing with the radiation dose.

From the results presented an evaluation of the RBE values from each of the regression lines (at the various times after irradiation) has been calculated. These values are shown in Table 3. It is clearly seen that each set of observations gives RBE values which are rather close: for the pure 400 neutron beam between 5 and 6 and for the attenuated neutron beam around 2.

It is possible, because of the consistency of these results, to establish an overall RBE value for the observed opacities of the eye lenses of mice independent of time after exposure. In order to achieve this a value of the RBE of 5.8 was chosen for the pure 400 MeV neutron beam and 2.2 for the attenuated beam. Modification of the dose scale by these factors allow a good superimposition of the X-ray and neutron data as shown in Fig. 14 and 15.

It can be concluded that RBE values of the order of 5.5 and 2.0 describe satisfactorily the relative biological effectiveness of the 400 MeV neutron beam in producing opacities in the lenses of the eyes of mice and further that these values do not depend substantially on time after exposure.

In addition a preliminary investigation of the progression of opacity in the anterior region of the lens has been carried out. The observed incidence of this opacity at 20 months after exposure is shown in Fig. 16 as a function of dose. In this case, RBE values of 5.4 and 1.9 have been found. In spite of the preliminary character of these results, the correspondence with those found for the posterior part of the lens seems to be good.

DISCUSSION

As already mentioned, a high degree of lens opacification cannot be evaluated by the counting method used in this work, but has to be scored according to the classical method of dividing the degree of opacity into stages 1, 2, 3 and 4. An attempt was made to correlate these two methods also for the lower degree of opacity and compare the results. Examples of this are shown in Fig. 17 and 18.

It seems possible from this correlation that the RBE values for lens opacification in mice obtained by the counting method might be compared to those obtained from the classical method of scoring. Such comparison is of interest in order to consider the RBE values for neutron radiation of different energies. 0.43 and 1.8 MeV neutrons have been extensively studied and although depending upon stages of opacity, RBE values of 8.9 and 6.6 from stage 2 have been reported. In the case of the 400 MeV pure neutron beam some variation of the RBE values has been found but this variation is considered to be within the precision of the measurements made.

More recently a fleck-counting method⁶⁾ for opacity determination has yielded an RBE value for the 0.43 MeV neutrons of 55 for a dose of 0.53 rad, and for 14 MeV neutrons an RBE of 6 for a dose of 4.8 rad. The fleck-counting method appears very similar to the one used in this work.

The lower RBE value found for irradiation behind the attenuator may be explained by the larger percentage of the dose being due to high energy minimum ionizing proton radiation produced in the attenuator. At this place the dose from nuclear spallation products represents considerably less than half of the total dose.

In our experiment the method of counting the individual opacities in the posterior region of the lens of the eye provides good reproducibility and can be used for the evaluation of the effects of low doses on the eye lens. It is also evident that the opacity in the nuclear region of the lens is not influenced by the radiation. The progression of opacity in the unirradiated animals as well as in the irradiated ones is linear with time. For the dose range studied the RBE values seem to be constant. This does not exclude the possibility that the RBE values at smaller doses might differ. In addition the RBE values found are constant as a function of time after exposure both for the 400 MeV pure neutron beam and the attenuated beam.

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FIGURE CAPTIONS

- Fig. 1. Layout of the 400 MeV neutron beam and irradiation area.
- Fig. 2. Horizontal beam profile.
- Fig. 3. Depth-dose distribution for the 400 MeV neutron beam.
- Fig. 4. Experimental arrangement for animal exposures.
- Fig. 5. Dose distribution behind a polythene block (18 g/cm^2)
- Fig. 6. Number of opacities as a function of dose for mice exposed to X-rays and in the 400 MeV neutron beam 8 months after exposure.
- Fig. 7. Number of opacities as a function of dose for mice exposed to X-rays and in the 400 MeV neutron beam 11 months after exposure.
- Fig. 8. Number of opacities as a function of dose for mice exposed to X-rays and in the 400 MeV neutron beam 13 months after exposure.
- Fig. 9. Number of opacities as a function of dose for mice exposed to X-rays and in the 400 MeV neutron beam 18 months after exposure.
- Fig. 10. Number of opacities as a function of dose for mice exposed to X-rays and in the 400 MeV neutron beam 20 months after exposure.
- Fig. 11. Progression of lens opacity after irradiation for the mice exposed to 250 kVp X-rays.
- Fig. 12. Progression of lens opacity after irradiation for the mice exposed to the 400 MeV neutron beam.
- Fig. 13. Progression of lens opacity after irradiation for the mice exposed to the attenuated 400 MeV neutron beam.
- Fig. 14. The observed results at various times after exposure as a function of dose of X-rays and of the pure 400 MeV neutron beam assuming an RBE of 5.8.
- Fig. 15. The observed results at various times after exposure as a function of dose of X-rays and of attenuated 400 MeV neutrons assuming an RBE of 2.2.

Fig. 16. Incidence of opacity in the anterior region of the lens 20 months after irradiation.

Fig. 17. Average degree of opacity at 13 and 20 months after exposure for the 400 MeV neutron beam and 250 kVp X-rays.

Fig. 18. Average degree of opacity at 13 and 20 months after exposure for the 400 MeV attenuated neutron beam and 250 kVp X-rays.

Table 1

Number of opacities in the nuclear region of the lens

250 kVp X rays	3 months after irradiation		5 months after irradiation		8 months after irradiation		11 months after irradiation	
Dose (rad)	No. of mice	Mean No. of op.	No. of mice	Mean No. of op.	No. of mice	Mean No. of op.	No. of mice	Mean No. of op.
Controls	61	3.9	61	4.0	61	4.3	30	4.1
50	52	3.3	52	3.4	52	3.7	25	4.1
100	52	4.6	52	4.7	52	4.8	24	3.9
200	54	3.5	54	3.6	53	3.7	24	4.1
300	50	3.3	50	3.6	50	4.0	25	3.8
400	50	3.6	50	3.6	50	3.9	25	3.4
400 MeV neutron beam.								
Controls	51	3.7	51	4.2	51	4.7	21	4.8
5	47	3.7	47	3.9	47	4.2	23	4.7
9	51	4.0	50	4.4	50	4.5	20	4.6
19	50	3.7	48	4.0	48	4.4	22	5.1
38	48	3.9	47	4.1	47	4.4	22	4.9
400 MeV attenuated neutron beam								
Controls	48	3.5	48	3.7	48	3.9	22	5.0
7	49	4.0	49	4.2	48	4.4	21	4.6
15	49	3.4	49	3.7	49	3.8	25	4.5
27	50	4.1	49	4.4	49	4.6	24	4.8
58	48	4.5	48	4.9	48	5.1	21	4.9

Table 2

Number of opacities in the posterior region of the lens

250 kVp X-rays	8 months after irradiation		11 months after irradiation		13 months after irradiation		18 months after irradiation		20 months after irradiation	
Dose (rad)	No. of mice	No. of opacities Mean \pm σ	No. of mice	No. of opacities Mean \pm σ	No. of mice	No. of opacities Mean \pm σ	No. of mice	No. of opacities Mean \pm σ	No. of mice	No. of opacities Mean \pm σ
Controls	61	13.0 \pm 3.5	30	24.4 \pm 4.3	30	32.5 \pm 5.4	29	54.2 \pm 5.0	29	63.8 \pm 6.1
50	52	20.0 \pm 5.0	25	35.0 \pm 7.6	24	44.6 \pm 8.2	22	72.5 \pm 7.8	23	79.2 \pm 6.9
100	52	27.2 \pm 6.3	24	44.9 \pm 6.0	25	58.8 \pm 12.1	23	82.3 \pm 17.4	24	101.4 \pm 11.8
200	53	46.6 \pm 9.0	24	63.2 \pm 10.8	29	86.6 \pm 13.3	22	117.0 \pm 20.4	27	135.2 \pm 18.5
300	50	64.0 \pm 10.6	25	89.1 \pm 12.2	24	117.5 \pm 20.0	23	150.0 \pm 13.9	17	-
400	50	82.9 \pm 11.5	25	121.8 \pm 13.2	23	147.7 \pm 12.1	23	-	19	-
400 MeV neutron pure beam										
Controls	51	12.6 \pm 4.4	21	23.3 \pm 6.3	26	30.6 \pm 5.2	22	50.3 \pm 6.0	22	64.8 \pm 6.7
5	47	16.6 \pm 5.4	23	30.5 \pm 6.0	24	38.1 \pm 5.8	21	62.6 \pm 7.8	22	74.7 \pm 9.1
9	50	24.6 \pm 6.3	20	38.9 \pm 6.5	29	50.5 \pm 9.5	19	77.0 \pm 13.1	24	86.7 \pm 12.0
19	48	30.3 \pm 6.5	22	47.8 \pm 7.6	26	62.2 \pm 11.4	22	88.1 \pm 11.4	20	105.7 \pm 14.7
38	47	48.8 \pm 10.5	22	78.2 \pm 9.8	23	95.9 \pm 18.1	20	120.6 \pm 8.1	21	138.7 \pm 13.8
400 MeV neutron attenuated beam										
Controls	48	13.7 \pm 3.8	22	24.5 \pm 4.7	25	35.0 \pm 6.8	20	55.1 \pm 6.6	19	62.5 \pm 4.9
7	48	16.7 \pm 5.2	21	31.2 \pm 9.2	26	39.8 \pm 6.4	20	66.3 \pm 10.2	21	77.1 \pm 8.2
15	49	17.4 \pm 5.7	25	29.8 \pm 5.8	22	38.0 \pm 7.7	22	64.2 \pm 7.0	20	80.1 \pm 10.1
27	49	24.5 \pm 5.7	24	41.8 \pm 6.9	25	52.7 \pm 11.3	21	79.4 \pm 10.0	20	89.7 \pm 10.7
58	48	32.1 \pm 9.9	21	54.2 \pm 8.5	26	70.4 \pm 10.5	21	95.2 \pm 8.5	23	114.4 \pm 9.7

Table 3

RBE values from regression lines
at various times after exposure
to the 400 MeV neutron beam

Time after exposure (months)	RBE pure beam	RBE attenuated beam
8	5.2	1.7
11	5.9	2.1
13	5.7	2.2
18	5.3	2.0
20	5.2	2.1

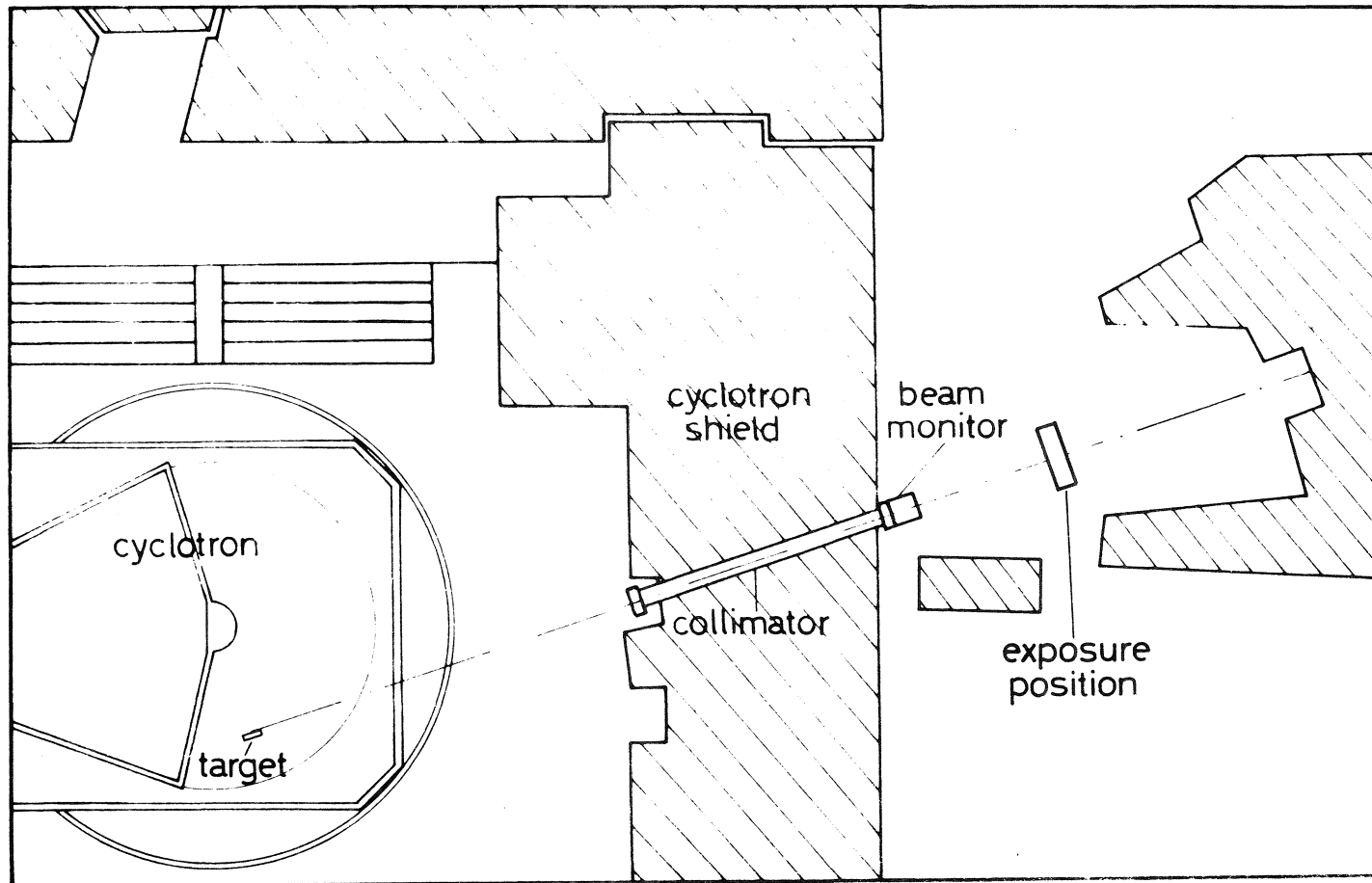


fig. 1

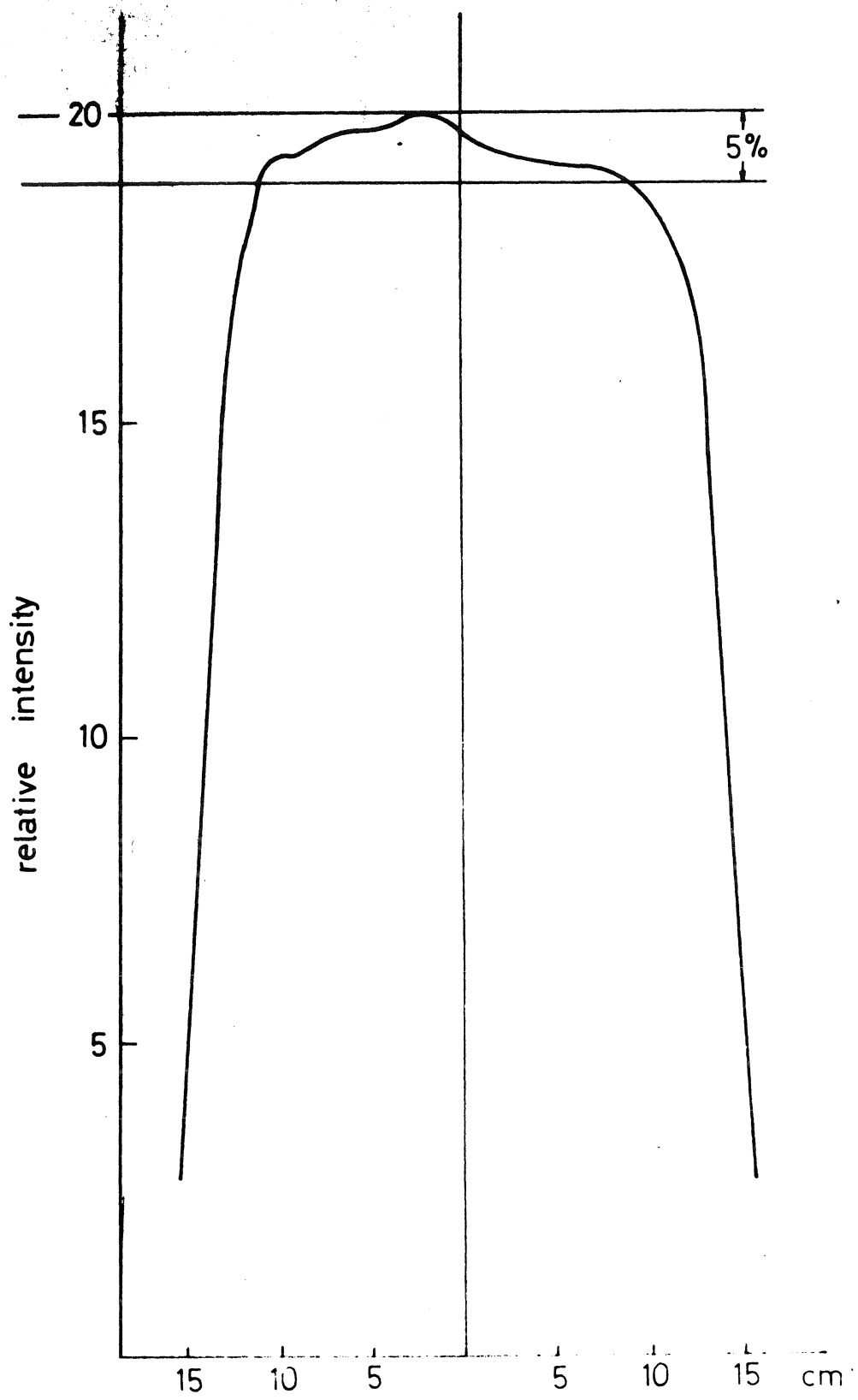


fig. 2

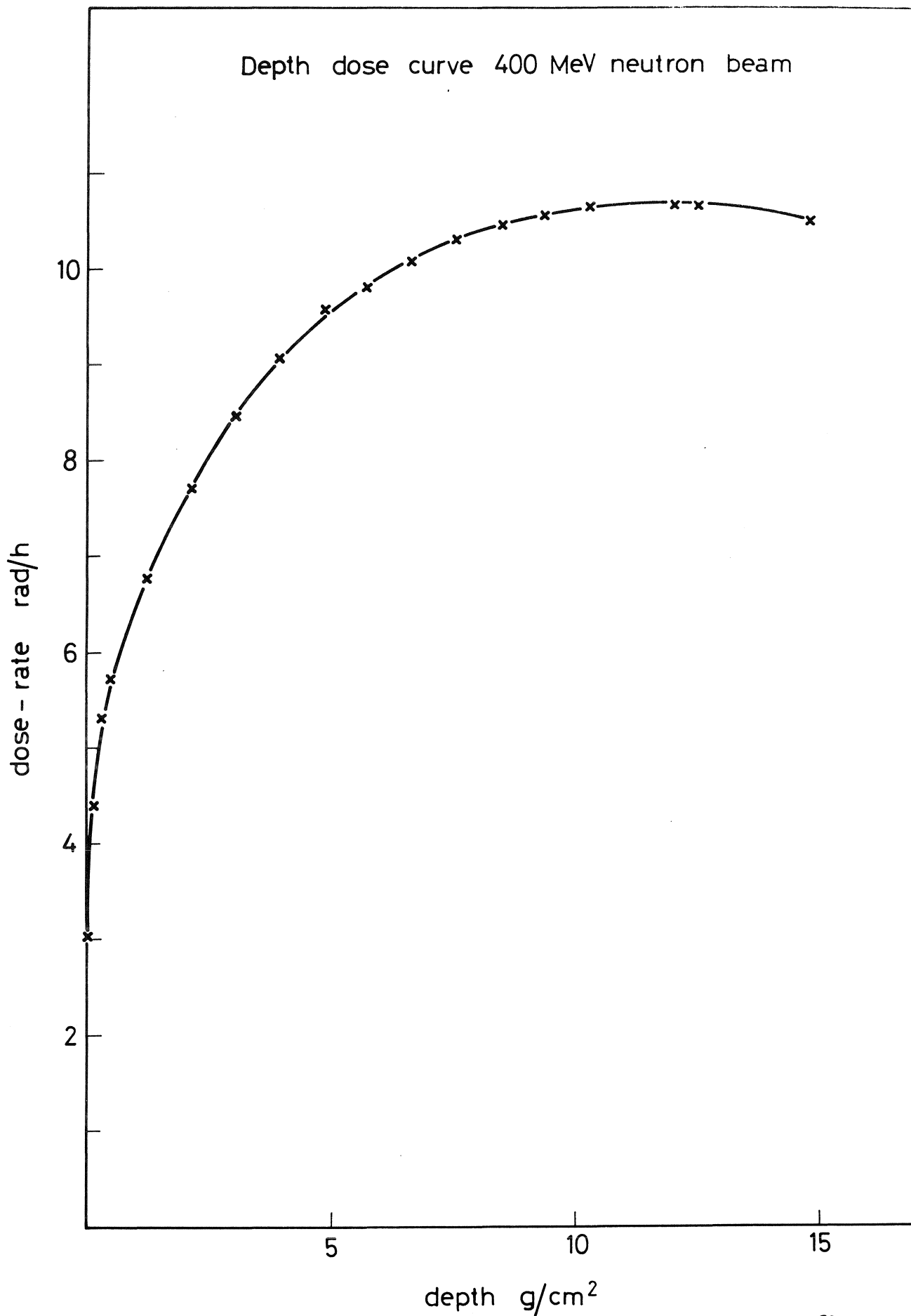


fig. 3

polythene block 18g/cm^2

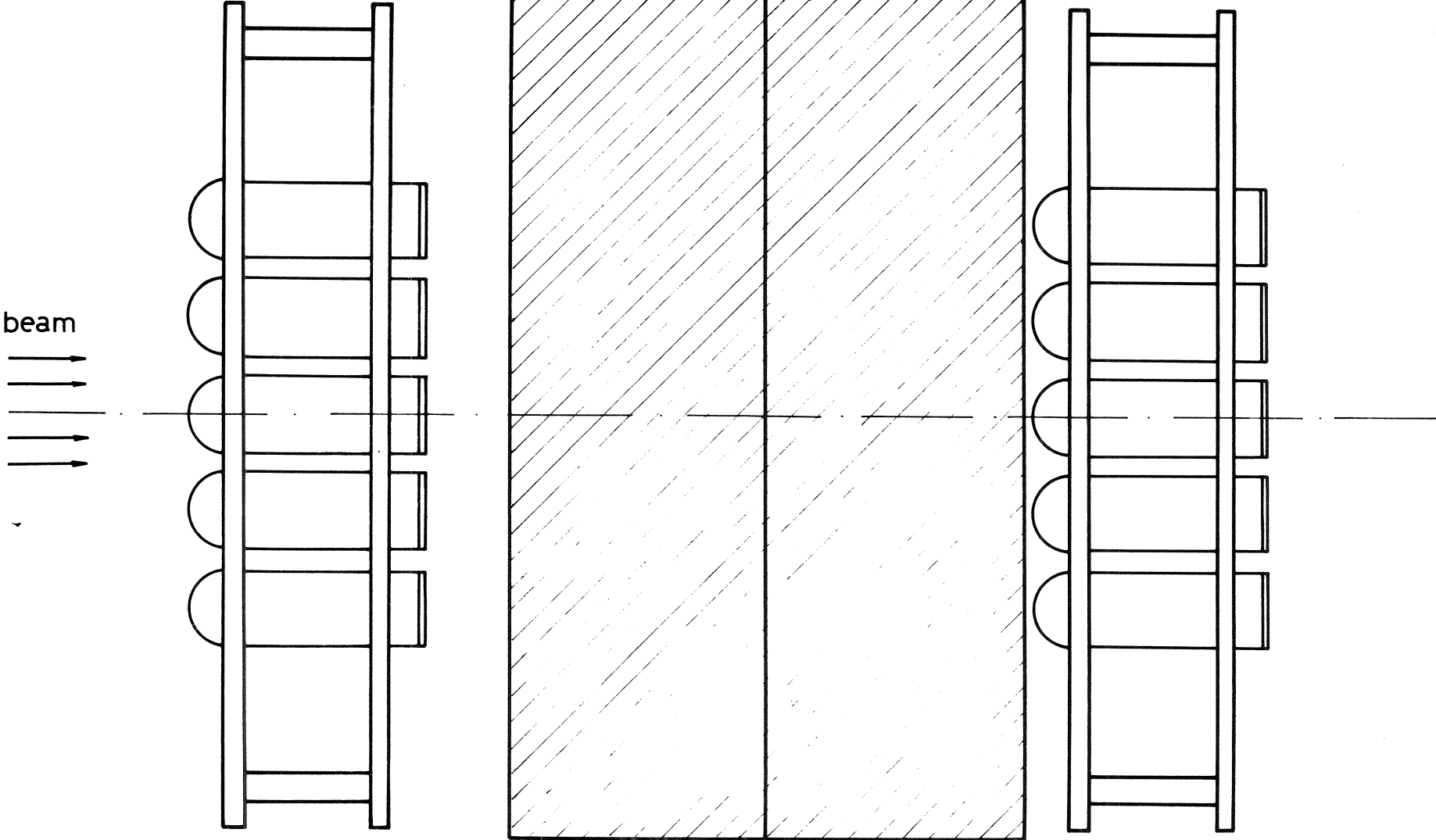


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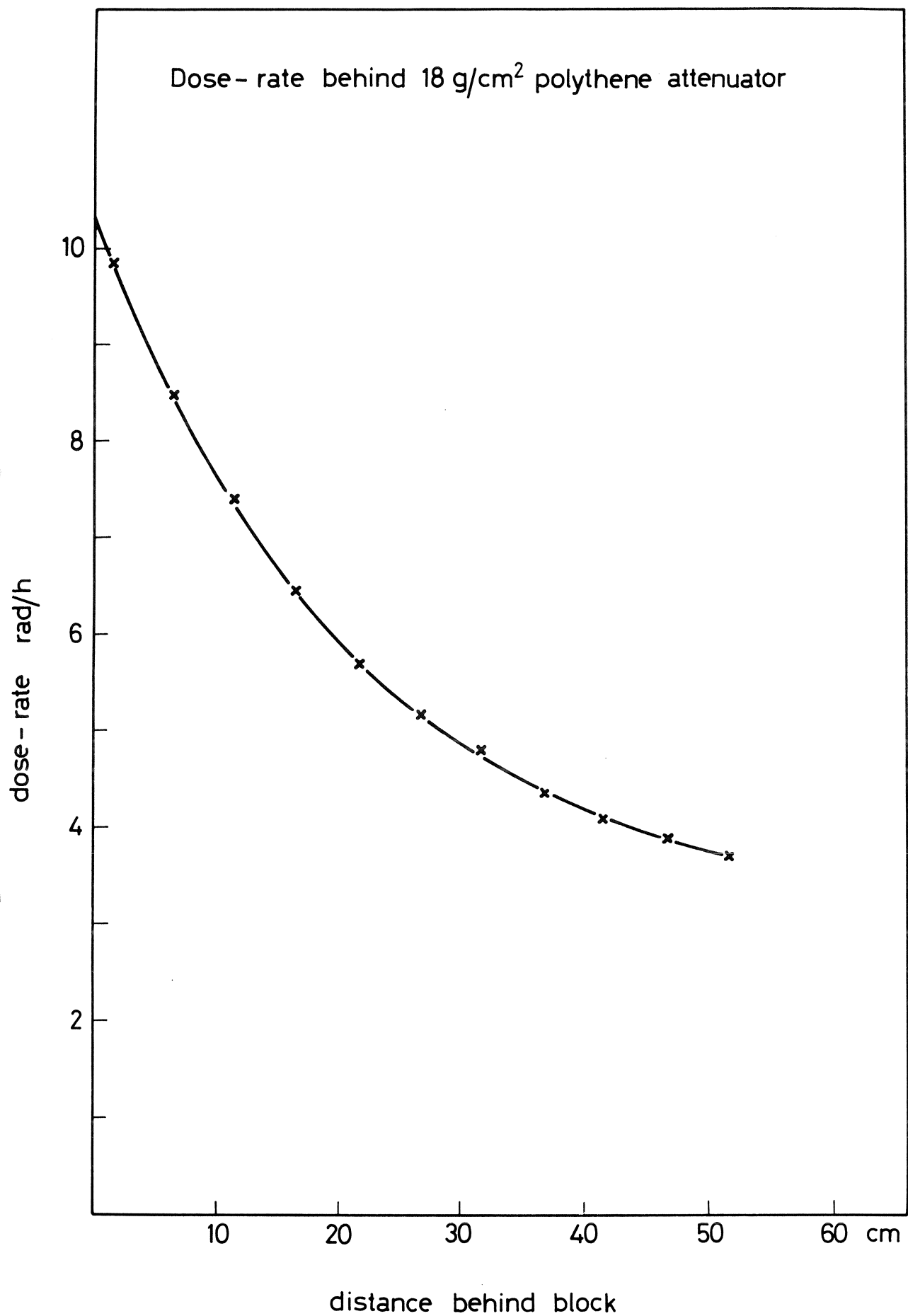


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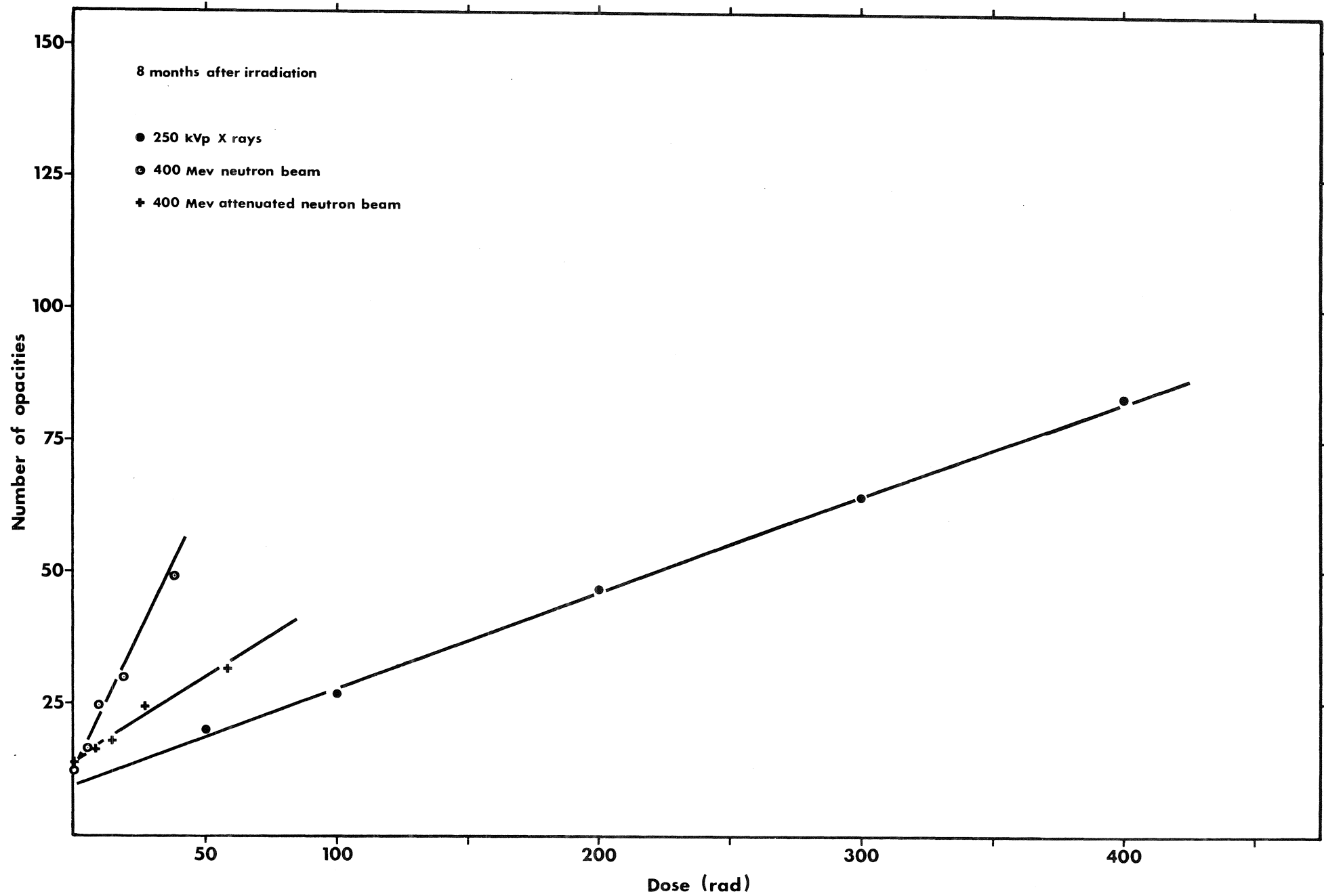


fig. 6

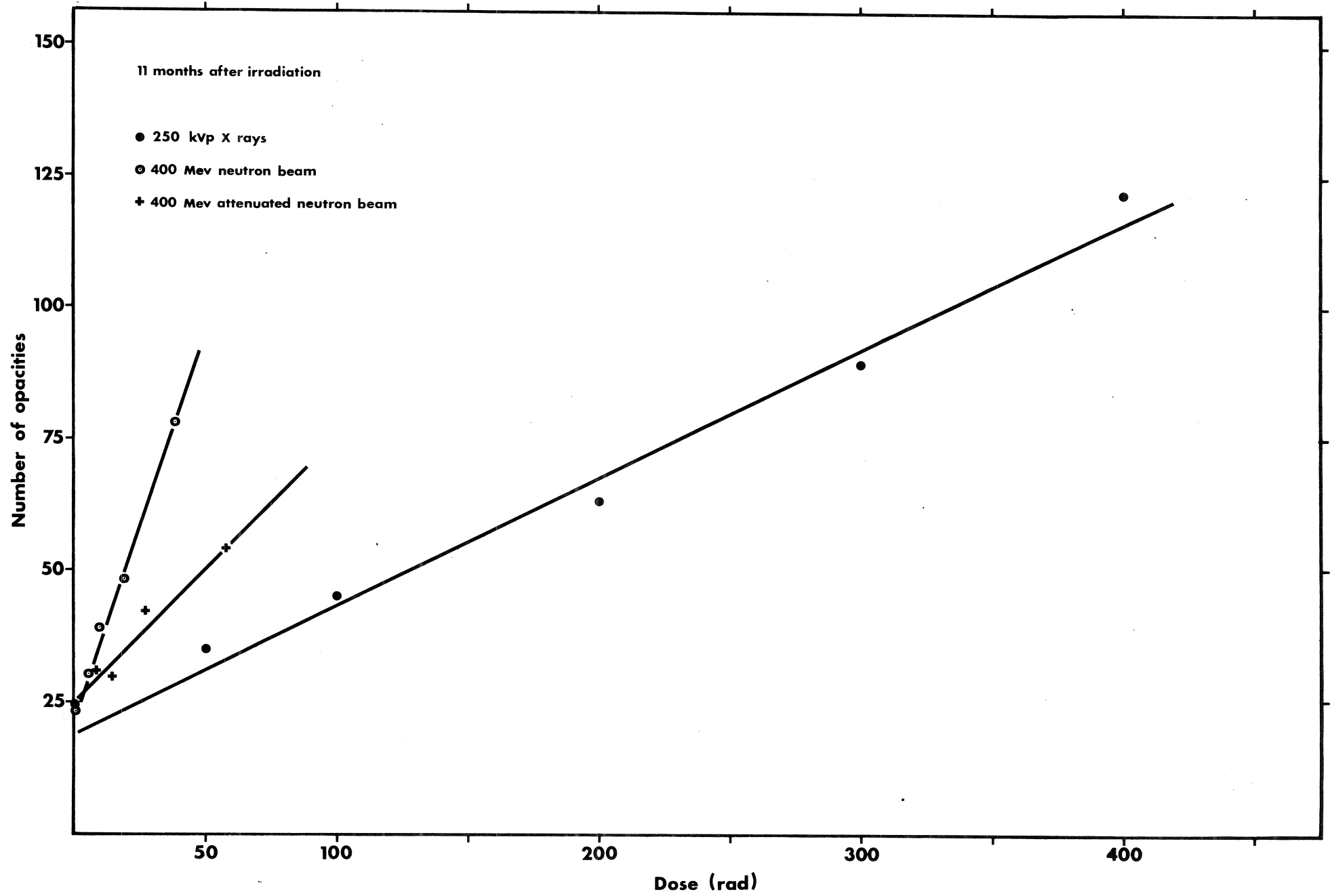


fig. 7

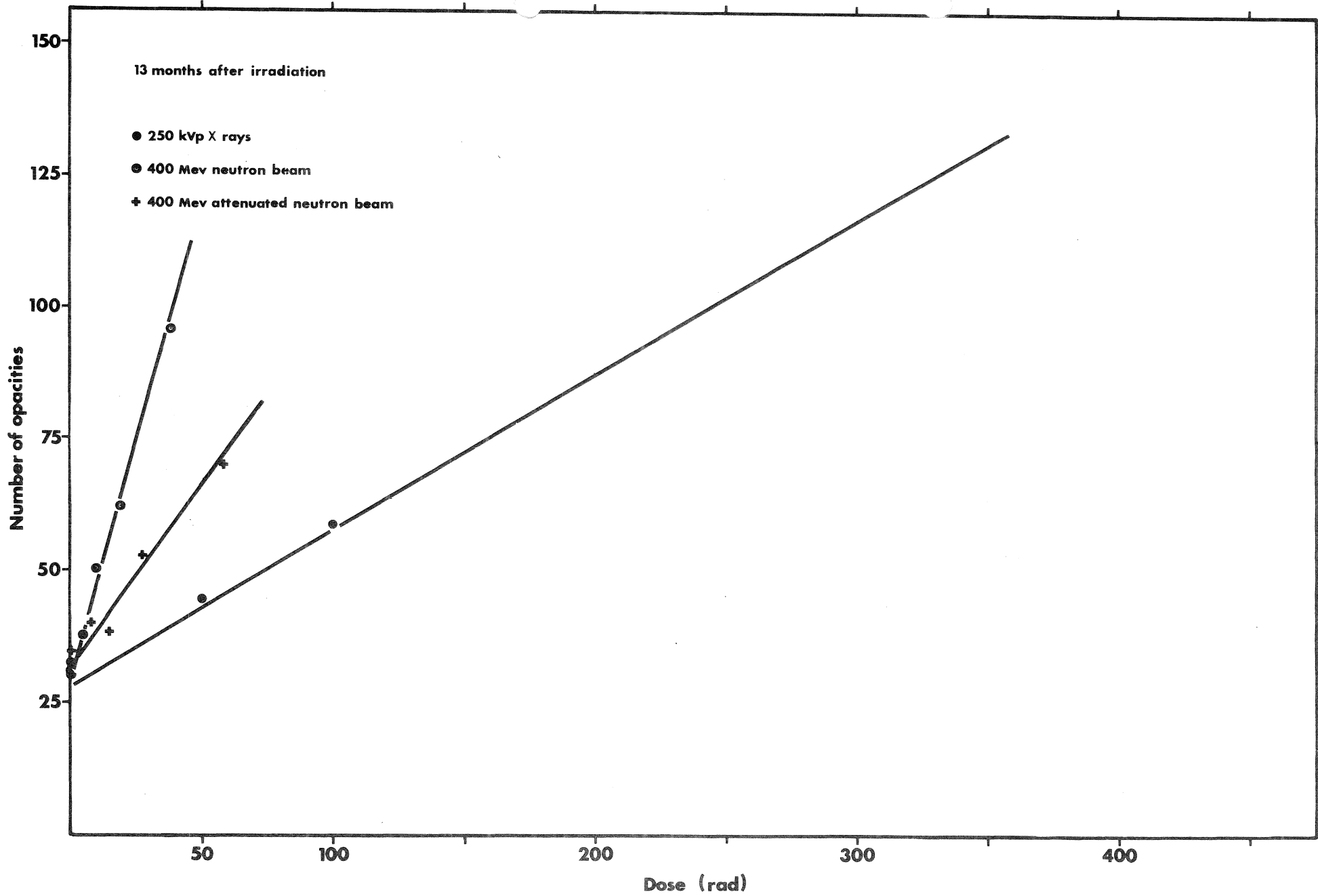


fig. 8

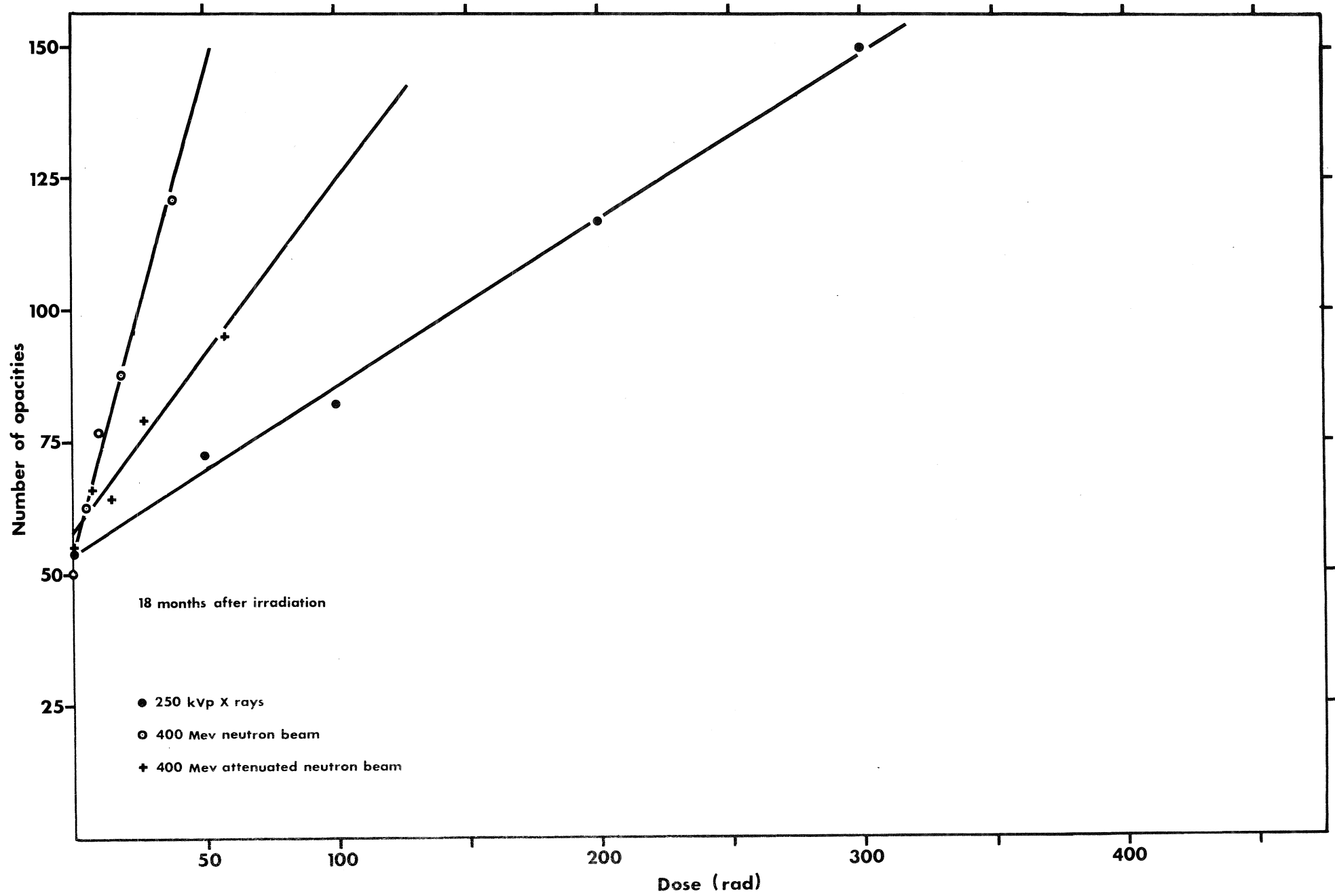


fig. 9

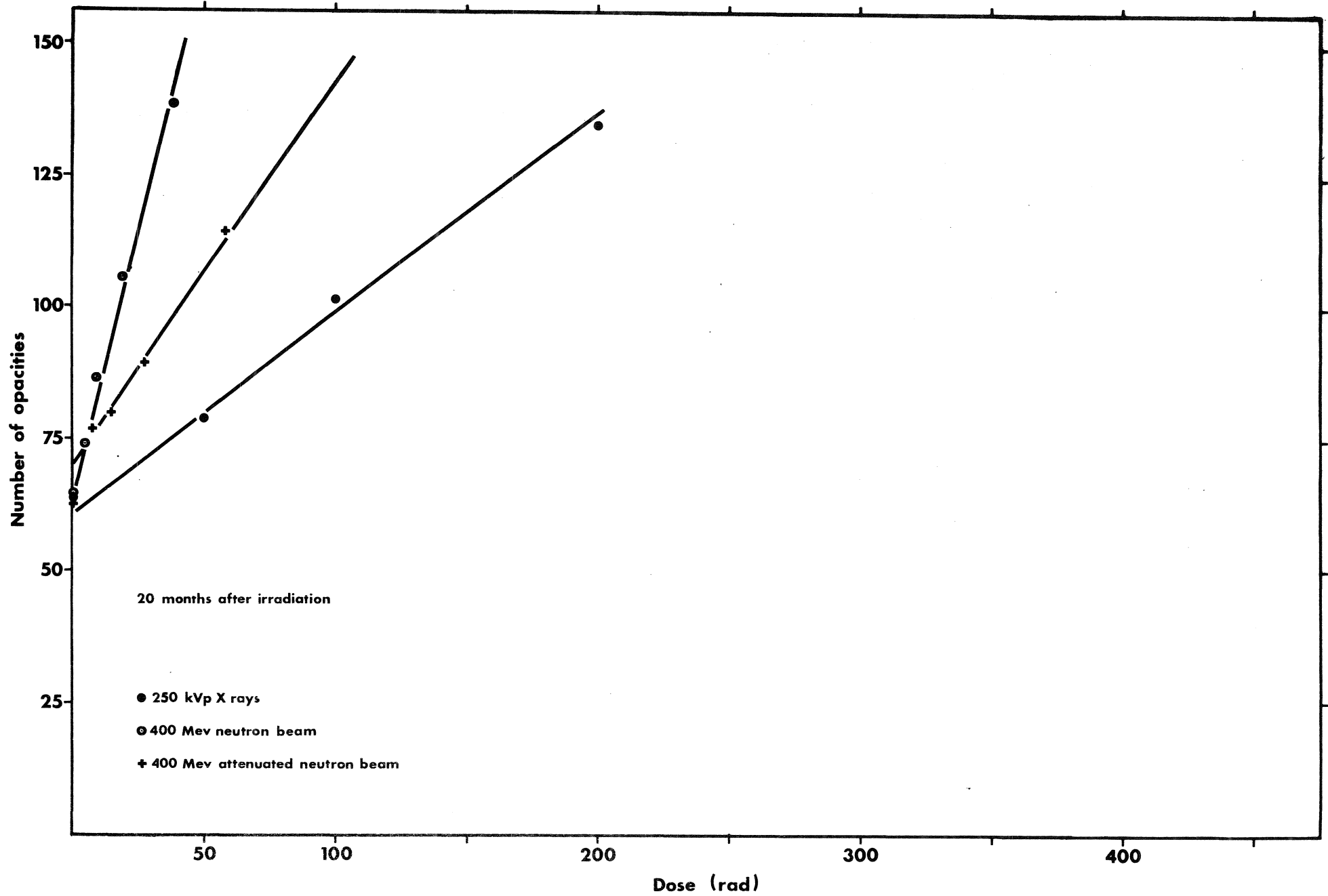
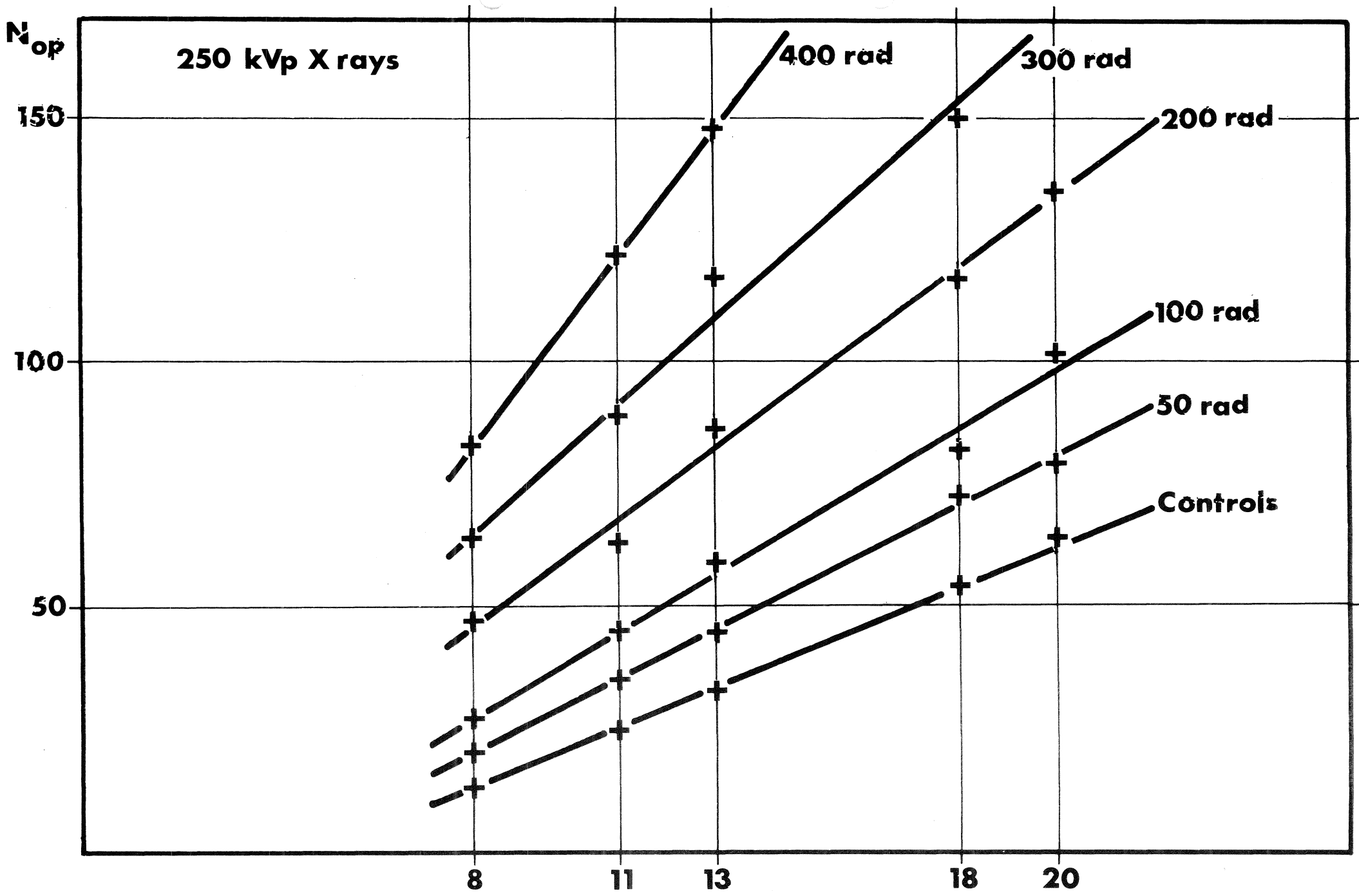


fig. 10



Months after irradiation

fig. 11

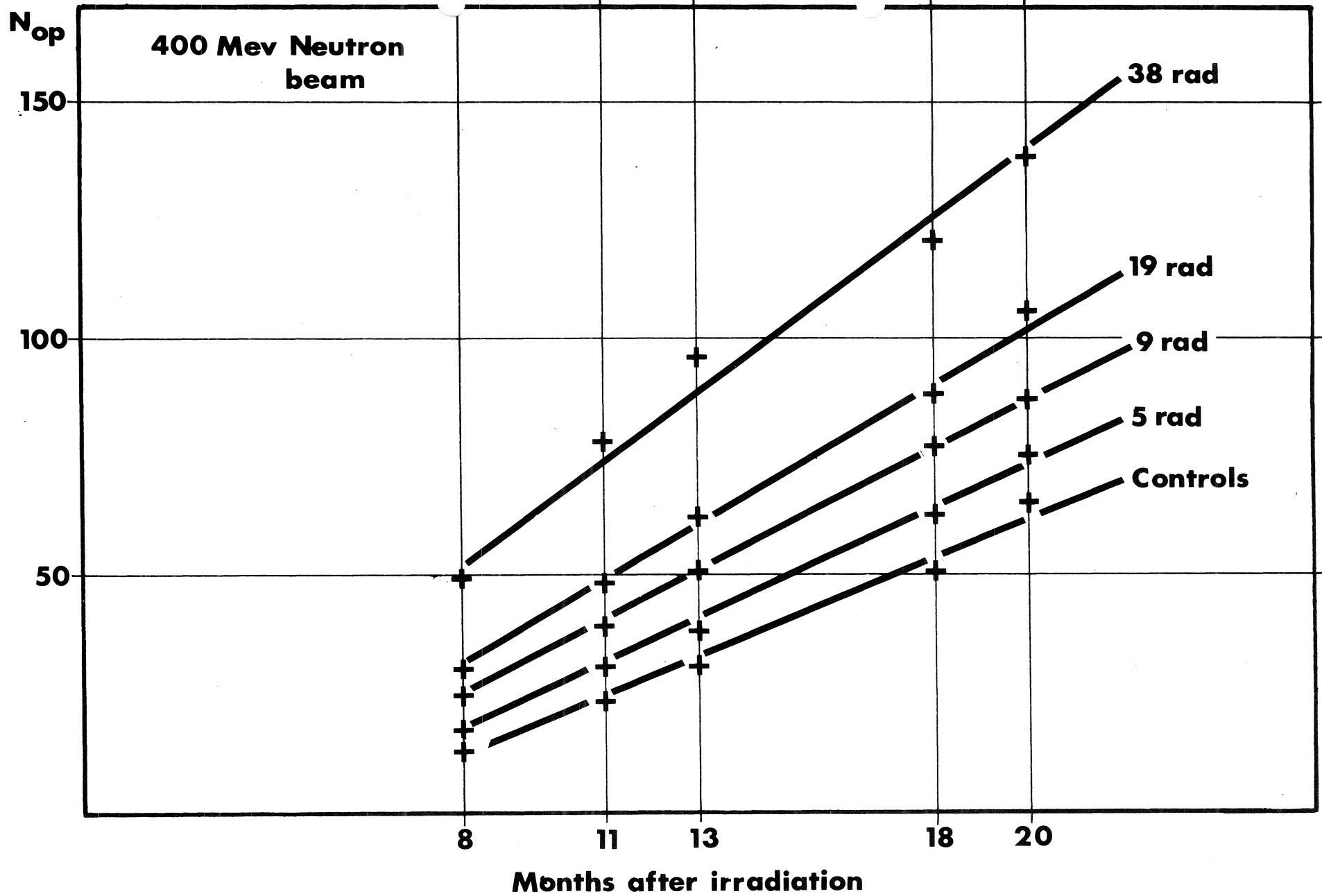


fig. 12

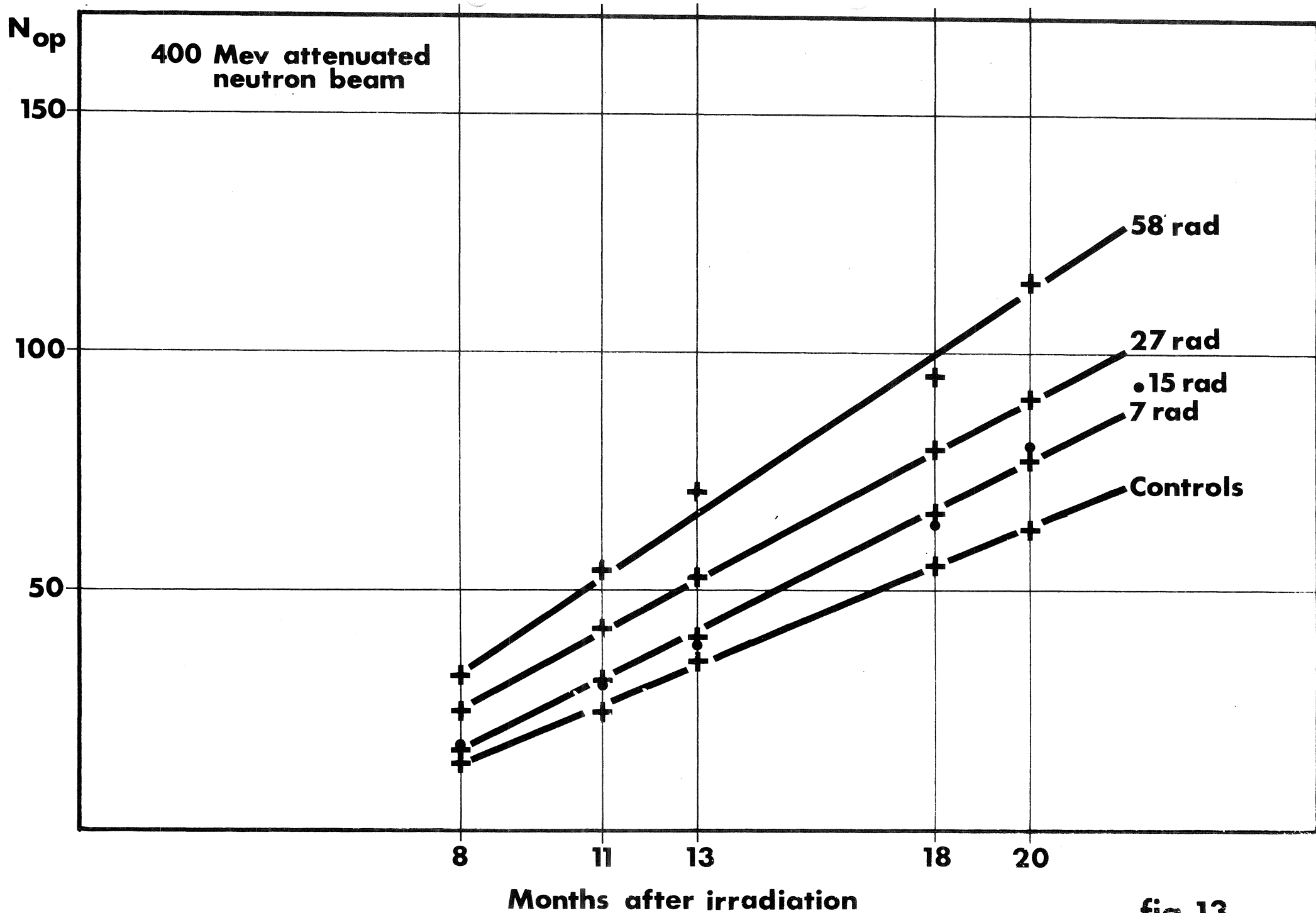


fig. 13

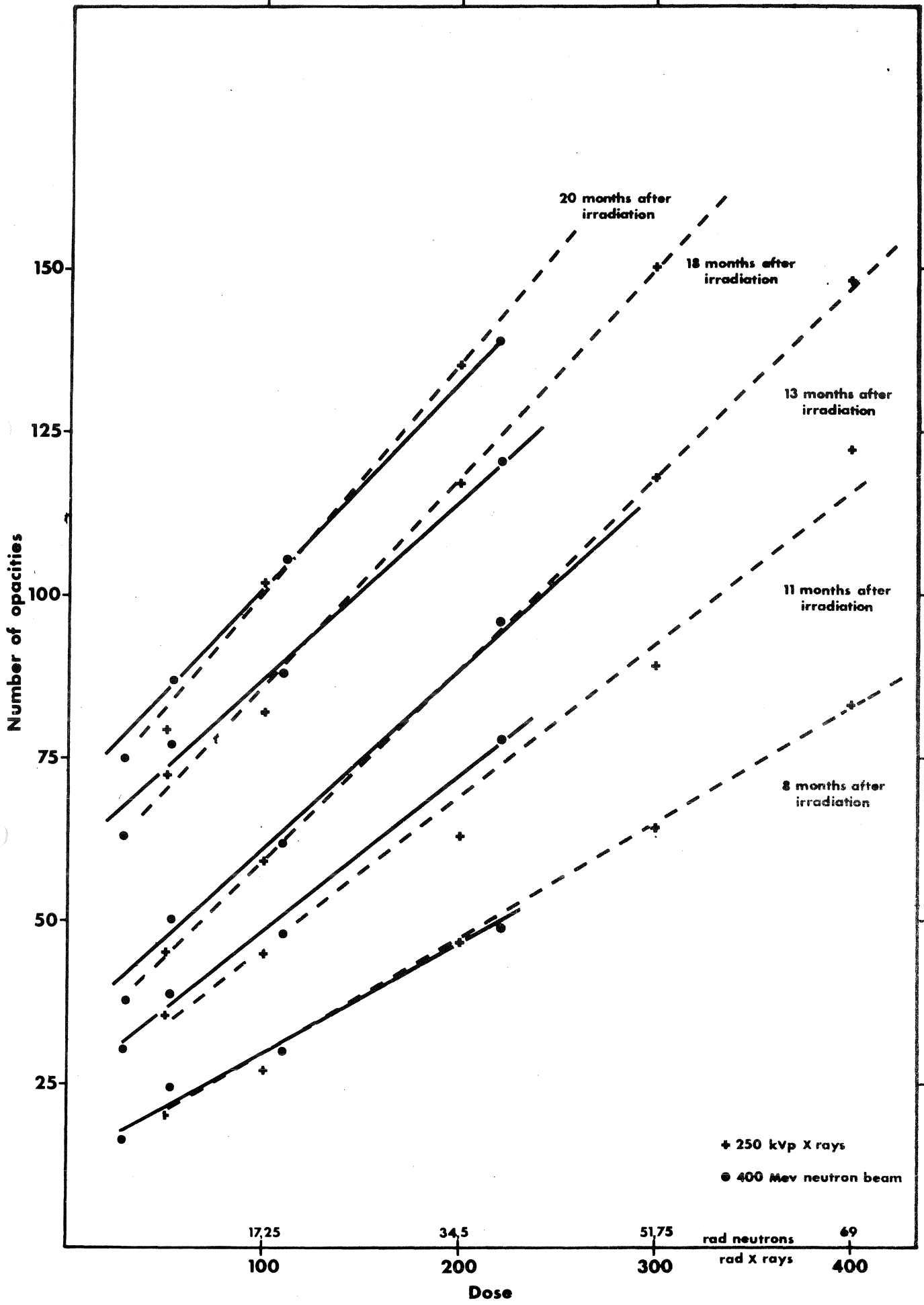


fig. 14

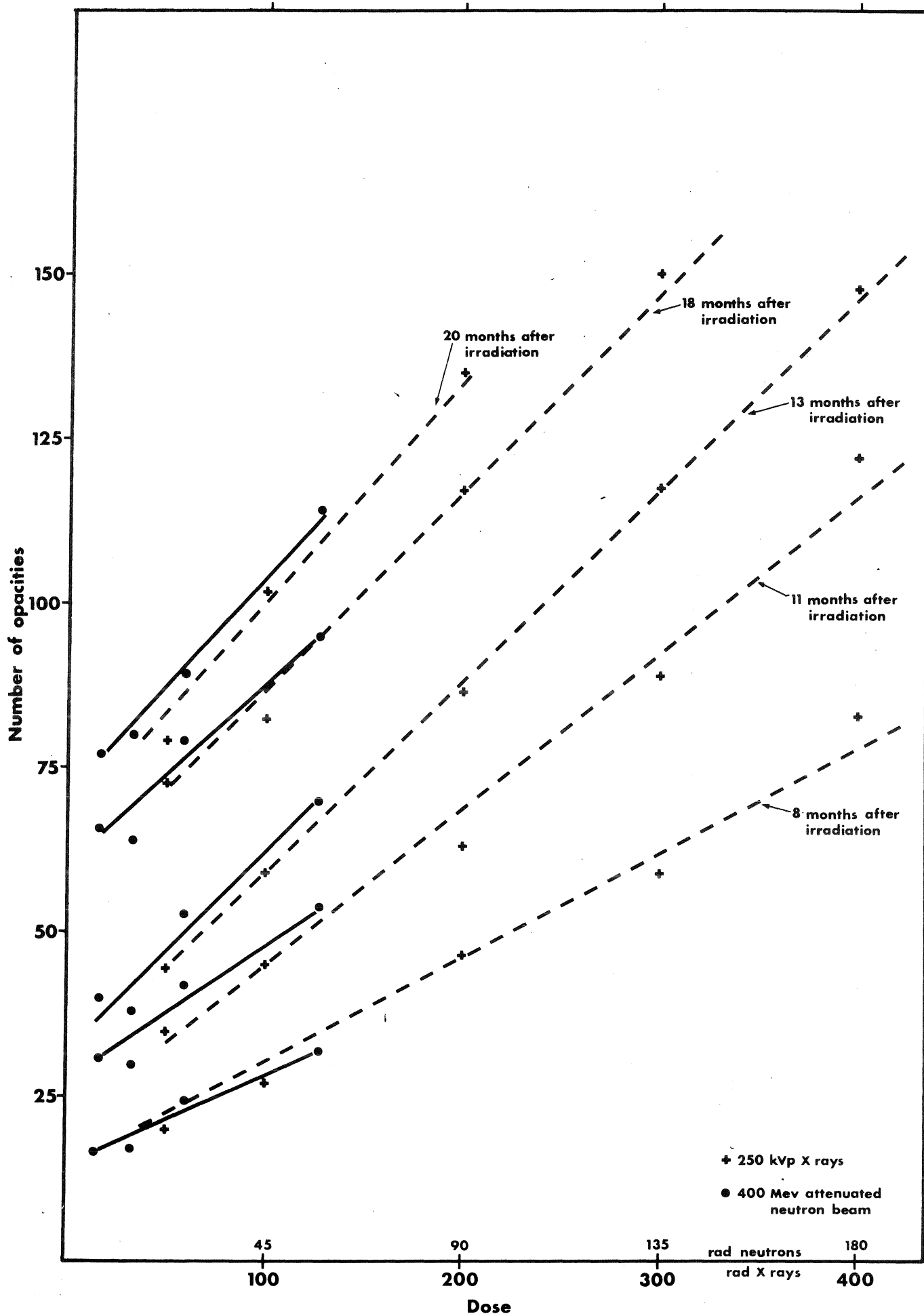


fig. 15

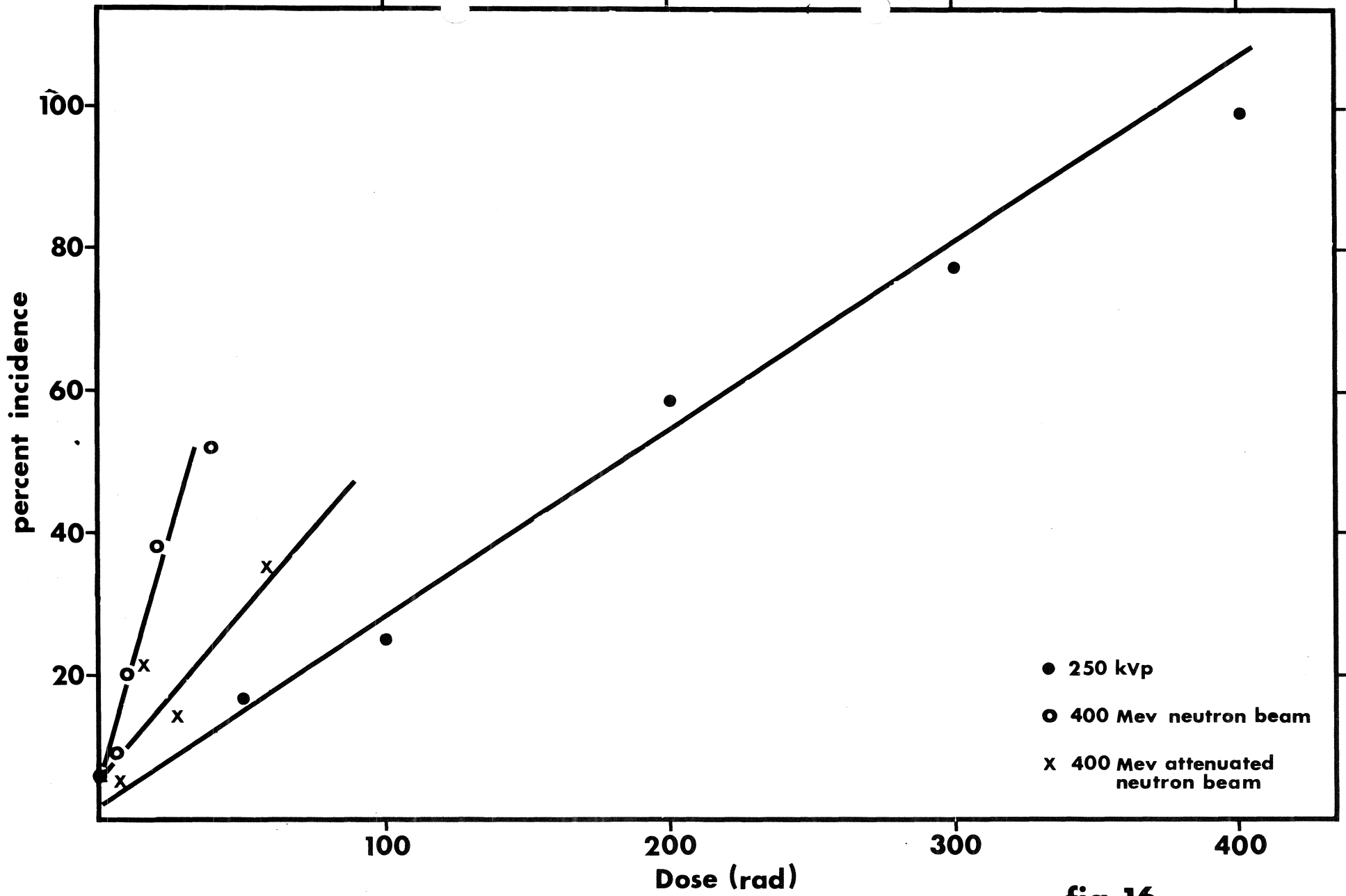


fig.16

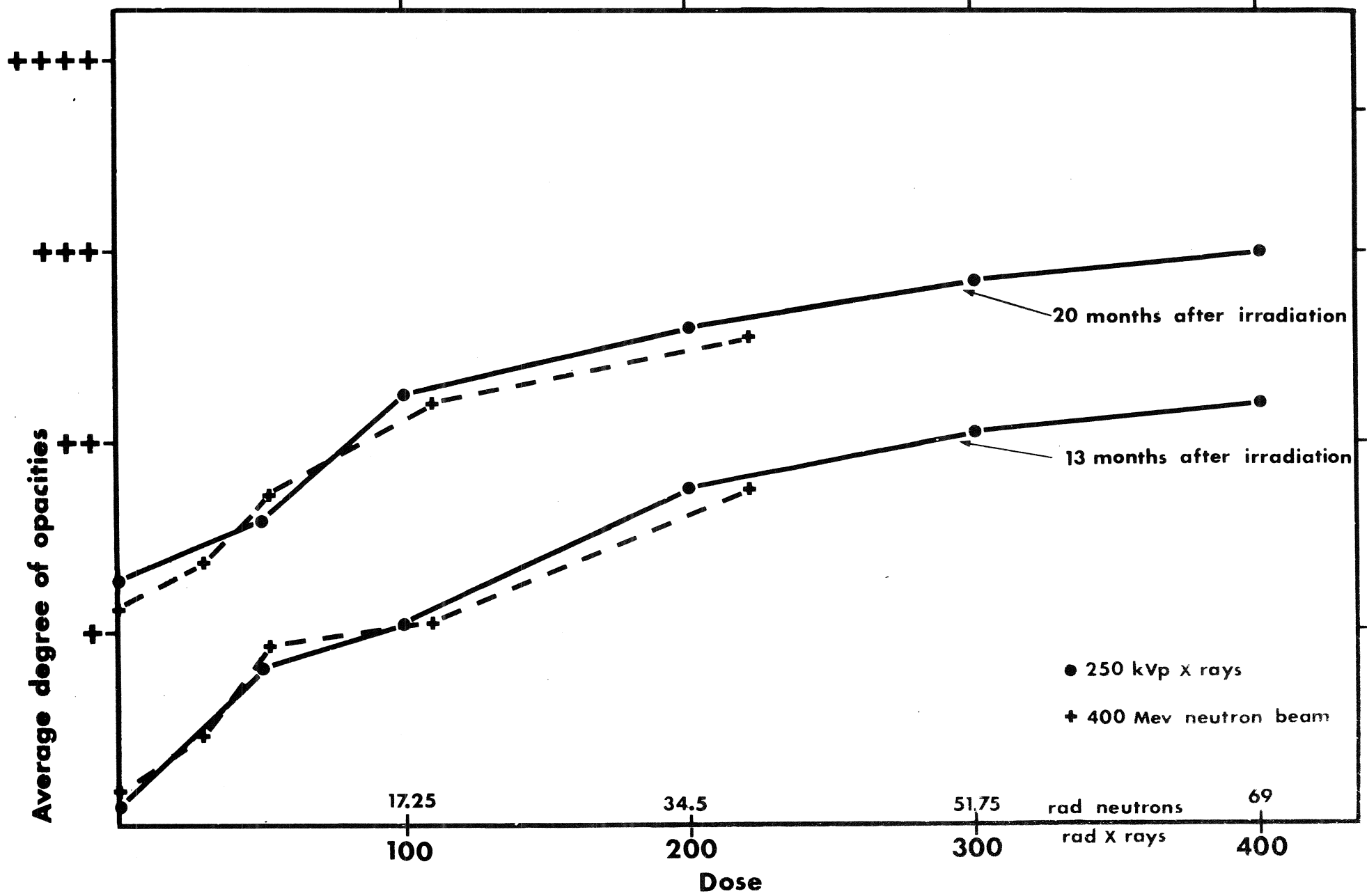


fig. 17

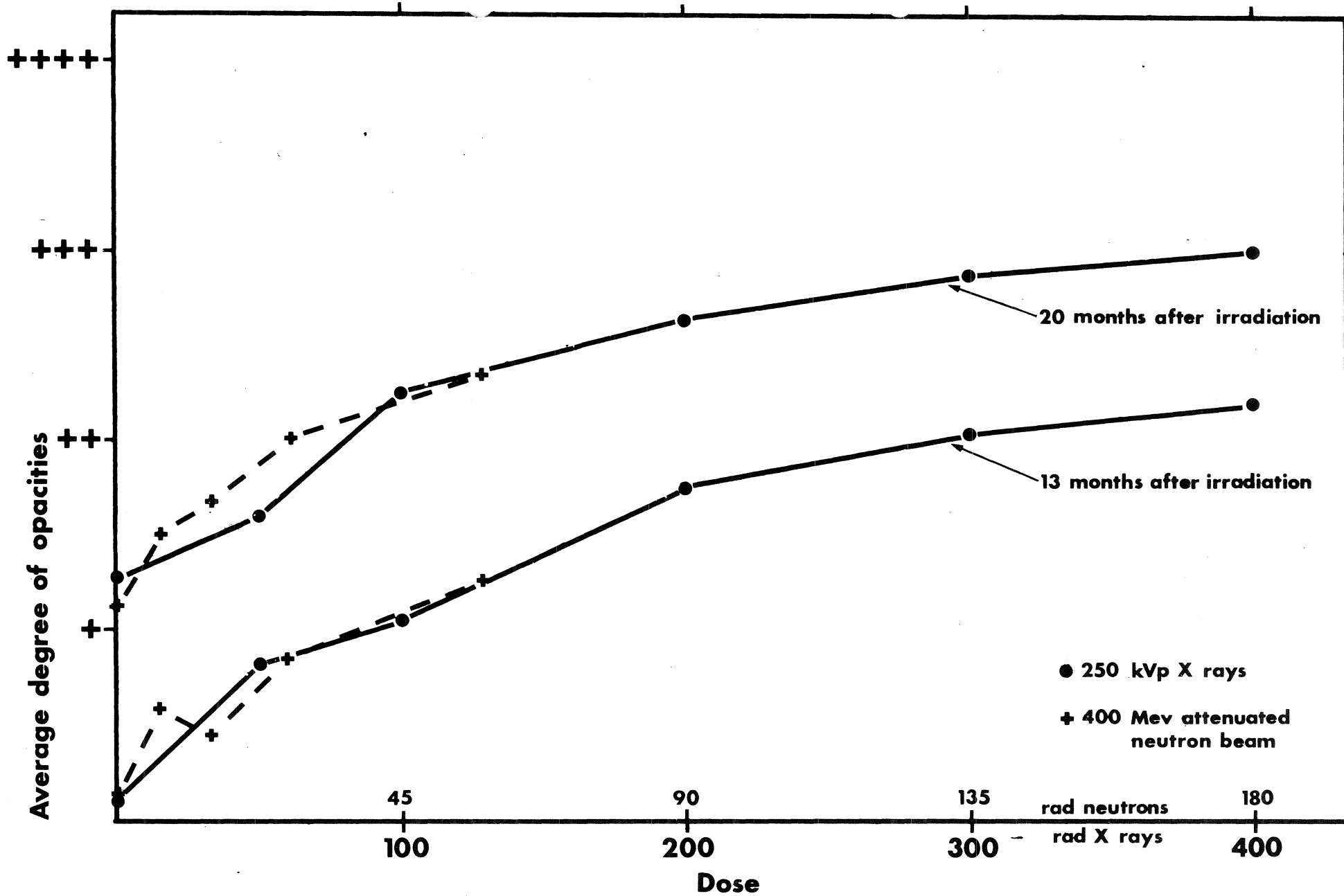


fig. 18