729518

BNL 11477

REPOSITORY PNL
COLLECTION Prisoner Study
BOX No. 3156
FOLDER N/A

LENS OPACIFICATION IN THE MOUSE; IMPLICATIONS FOR RBE AND QF*

V. P. BOND

Brookhaven National Laboratory
Medical Research Center
Upton, L.I., N.Y.

BEST COPY AVAILABLE

Presented at the Second Panel on the Biophysical Aspects of Radiation Quality, Vienna, Austria, April 10-14, 1967

*Research supported by the U.S. Atomic Energy Commission

1260954

Lens Opacification in the Mouse; Implications for RBE and QF*

V. P. Bond

Extensive studies on the effectiveness of monoenergetic fast neutrons in the development of lens opacities in the mouse have been under way for some time,[1] and recent studies have indicated that the relative biological effectiveness (RBE) of low energy fast neutrons for the biological effect might be quite high at low doses and low dose rates.[2] The purpose of the current paper is to present some of these data in summary form and then to discuss the possible pertinence of such findings for man.

In the presentation, the following approach will be used: The process of lens opacification following exposure to radiation will be discussed in general, and the experimental techniques and scoring method employed will be described. Results on lens opacification following x-ray exposure as a function of dose and dose rate or fractionation will be outlined, as well as the effects of hypoxia and hyperbaraic oxygen. The effects of monoenergetic fast neutrons on lens opacification as a function of dose and of dose rate or fractionation will be summarized. The relative biological effectiveness (RBE) of neutrons will then be discussed in terms of how it is determined, and how it varies as a function of dose, of dose rate and of oxygen tension. Finally, the possible pertinence of these data for man will be discussed.

In the discussions, both RBE and QF (quality factor) will be dealt with. The RBE indicates the ratio, as determined by experimentation, of the

*Presented at the Second Panel on the Biophysical Aspects of Radiation Quality, Vienna, Austria, April 10-14, 1967.

1260955

dose of the standard X or gamma radiation required to produce a given degree of biological effect, to the dose of the unknown (usually high LET) radiation required to give an equal degree of the same biological effect. QF represents the same ratio, but is a "legislated" value established by a properly constituted group such as the International Commission on Radiation Protection, for use in routine radiation protection work. The numerical values for RBE and QF will be dealt with; however it should be clear that these are not intended as recommendations for QF values. Nonetheless, QF values are ultimately based on the best data available on RBE values, and thus it is pertinent to consider radiobiologically determined RBE's and the relationship which these values might have to QF.

The data summarized represents a joint effort between the Medical Department of the Brookhaven National Laboratory, and the Radiological Laboratories at Columbia University. The biological data were obtained largely by Dr. John Bateman at Brookhaven, and the dosimetric data were obtained chiefly by Dr. Harald Rossi and his group at Columbia University. The experimental data will be published in detail elsewhere.[2]

LENS OPACIFICATION

The ocular lens is enclosed in a capsule, and can be divided into an equatorial region, and anterior pole and a posterior pole. The lens consists largely of fiber cells, and is covered with an epithelium anteriorly. Dividing cells are limited largely to the anterior equatorial region, and the progeny of these dividing cells migrate posteriorly and then centrally to form the lens fibers. Cell division apparently continues throughout life

1260956

and thus the lens can be regarded as one of the "renewal tissues". However, unlike other renewal systems such as the granulocytic cell series or the epithelium of the bowel in which mature cells are normally lost and replaced at a rapid rate, there appears to be no clear-cut mechanism for loss of cells from the lens. Thus, if a dividing cell is injured and does not die and become resorbed, this abnormal cell will remain within the substance of the lens.

Immediately following exposure to radiation, damage probably is confined to the dividing cells in the anterior equatorial region. Opacification appears to result from a disturbance of cell maturation at two stages.[3] The lens fiber cells fail, especially at higher doses, to differentiate and this results in an accumulation posteriorly of atypical epithelial cell nuclei. Of more importance in the formation of opacity, with even very low doses, is the failure of transition from nucleated fiber cells to the non-nucleated prismatic cell. The prismatic cells show a high degree of ordering both at the histological level and at the molecular level as evidenced by a strong bi-refringence pattern. The young fibers fail to achieve this degree of differentiation after radiation exposure, and undergo cytoplasmic condensation, together with the appearance of abnormal cytoplasmic bodies. Distal retraction of the cytoplasm away from the cell membrane occurs, and there is a broadening of the central portion of the fiber. Thus the process appears to be related not primarily to cell death, but to cell injury. The injured cells are unable to mature normally, and the presence of the injured cells would seem to be the basis for the observed progress of opacification.

Although a number of systems for scoring the degree of lens opacification have been developed, the method developed by Bateman[1] appears to

1260957

be the one which is most sensitive and hence best able to detect minimal degrees of injury. Examination is undertaken using a single "blind" system, the detailed study of the lens being made by means of a slit-lamp microscope. Anterior polar and posterior subcapsular opacifications are scored separately. An arbitrary scale of 0 to 100 is employed to indicate increasing degrees of opacification. The method has been found to yield remarkably reproducable results in the hands of a single observer.

EXPERIMENTAL DESIGN

The apparatus and procedures used for exposure and examination have been described in detail previously[1] and will be summarized only briefly here. Exposure to X-rays and to gamma rays from Cobalt-60 are accomplished in the usual fashion, and the doses accurately determined. Monoenergetic neutrons are obtained by use of a Van de Graaff generator, principally by causing 2.8 MeV protons to impinge on a tritium target. With the p,T reaction, the energy of the neutrons emitted is a strong function of angle with respect to the beam axis, and takes on values from close to 2 MeV in the forward direction to 0.43 MeV at an angle of 130° to the beam axis. Exposures are carried out at a distance of 10 cm. from the target with 0.43 MeV neutrons, and at a distance of 20 cm. from the target with 1.80 MeV neutrons. The present discussion will deal mainly with exposures to 0.43 MeV neutrons, which deliver radiations with the highest LET used in these studies.

Dosimetry was carried out with high precision, using a combination phantom-dosimeter designed to allow accurate measurement of the dose actually delivered to the lens.

1260958

RESULTS; X-RAY EXPOSURE ONLY

Some of the results obtained, showing cumulative incidence of posterior opacifications scored at the 5% or higher level, are given in Fig. 1. Note that an appreciable percentage of normal animals develop such opacifications as they become older. With x-ray (or fast neutron) exposure a curve similar to that obtained in the normal is seen, but displaced forward in time. The opacifications noted after radiation exposure are identical to those which are observed normally at a later date. Radiation could be thought of as advancing in time, a process that occurs normally. The overall response following radiation exposure can be characterized in a variety of ways; a convenient and adequate representation can be made in terms of the "latent period" defined as the time required to reach a 50% incidence of opacification at a given level of severity[5].

As the dose of radiation is increased, the lens opacification curve is displaced successively to the left. Fractionation studies have been carried out[1] with equal doses of x-radiation delivered 12 hours apart. The fractionated exposure was less effective than a single exposure of the same total dose; the fractionated exposure required a total dose approximately 1.5 times higher to yield the same degree of biological effect.

Further exposures to x-ray were carried out in such a way that the animals were rendered hypoxic by being kept in a mixture of 94% nitrogen and 6% oxygen; exposures were also carried out under hyperbaric conditions i.e. the mice were exposed while under three atmospheres of pure oxygen[6]. In addition, dose rate studies were carried out under either normal or hyperbaric conditions, and total doses were delivered at the rates of 2 and 100 rads/min.

1260959

There were no detectable effects of hypoxia; the degree of effect from a given dose was identical whether the exposure was accomplished under hypoxic or normal atmospheric conditions. Hyperbaric oxygen, on the other hand, enhanced the degree of effect. Thus, the same degree of effect was obtained with approximately one-half the dose required under atmospheric conditions. This factor of two in increased effectiveness may be compared with an enhancement factor of only approximately 1.3 for organs such as the skin, in studies in which human beings have been exposed to x-radiation under hyperbaric conditions[7,8].

No dose rate effect was noted for exposures in normal atmosphere. Thus 100 rads yielded approximately the same degree of effect whether delivered in 1 or 50 minutes. A small and possibly significant dose rate effect was noted under hyperbaric conditions. From these studies it would appear that the time for appreciable recovery from the sublethal injury following x-ray exposure of the cells of the lens is greater than 4 hours (dose rate studies), but less than 12 hours (fractionation studies).

RESULTS; NEUTRON EXPOSURES

The effects of neutron exposure are qualitatively indistinguishable from those obtained following x-ray exposure, and are illustrated in Fig. 1. Thus the response obtained with x-rays can be duplicated with neutrons, but at a much lower dose level. By adjustment of the x-ray or neutron dose, response curves that essentially superimpose can be obtained.

Fractionation studies were carried out with neutrons, in which equal doses of neutrons were given with a 22 hour interval between exposures. With

1260960

either 0.43 MeV or 1.80 MeV neutrons, the results were identical with those for a single exposure i.e. fractionation did not affect the degree of response as it did with x-ray exposure.

No data have been obtained with exposure to neutrons under either hypoxic or hyperbaric conditions.

The RBE of neutrons for lens opacification can be determined, even though this effect is in many ways different from the more usual criteria used to determine RBE. Many criteria of effect, such as 30 day lethality, represent all-or-none phenomena which can be easily scored at a definite time following exposure. Lens opacification, on the other hand, represents a continuing process with the severity of opacification in a given eye continuing to increase with time, and the effect is seen even in unexposed animals of sufficient age. It is clear from Fig. 1 that one can nevertheless duplicate precisely the total response to a given dose of x-rays, by administering a smaller dose of neutrons, i.e., the same rate of progression of the same biological effect can be obtained by either means. The ratio of the doses required to produce this response can then be compared and is by definition the RBE of the neutrons relative to x-rays. The latent period, i.e. the time to 50% incidence at a given level of opacification, is a measure of rate of progression and has been found to be a most useful parameter for purposes of comparison[5].

The RBE of 0.43 MeV neutrons as a function of dose when each radiation is delivered in a single exposure over a short period of time, is indicated in Table 1. The values marked with an asterisk are extrapolated values; the lowest dose of neutrons at which actual data are available is 0.50 rads. Note

1260961

that the RBE is highly dependent on dose, and rises from a value of approximately 10 at 50 rads of neutrons, to a value of approximately 60 at 0.50 rads of neutrons. Extrapolating, one would expect the RBE to approach a value of 100 at doses of the order of 1/10 of a rad of neutrons.

The RBE is also a function of dose rate or of fractionation. This is apparent since the response to neutrons appears to be essentially independent of dose rate or fractionation pattern, while with low dose rates or fractionation the dose of x-rays required to give the same degree of biological effect is higher. With the fractionation pattern studied, i.e., equal doses delivered 12 hours apart, the factor indicating the decreased effectiveness of x-rays is approximately 1.5; thus the RBE values at low dose rates would increase by a factor of at least 1.5, and perhaps more if the exposures are delivered in 3 or more sessions, separated by 12 hours or more.

The degree of oxygenation may also be expected to affect the RBE, since the response to neutrons is expected to be essentially independent of oxygen tension while that to x-rays is markedly enhanced under hyperbaric oxygenation. The lack of effect of hypoxia with x-ray exposure may indicate that the lens is normally hypoxic as compared to other tissues. This is also indicated by the fact that hyperbaric oxygen enhances the effect of x-radiation on the lens by a factor of 2, as opposed to a factor of approximately 1.3 for other tissues such as the skin. The circumstance may well be the principal reason for the relatively high RBE noted for the lens of the eye exposed under normal atmospheric conditions. The normal hypoxic condition of the lens increases the dose necessary to produce a given effect selectively for x-rays but not for neutrons. Thus the RBE is higher than might be expected if the lens were oxygenated to the same degree as other tissues.

1260962

POSSIBLE EXTRAPOLATION TO MAN

It is clear from a number of studies[9] that the lens of the eye of the mouse is quite sensitive to radiation as compared to that of most other species, including man. The reasons for the observed differences in sensitivity are not known. It is thus clear that it would not be advisable to use data obtained in the mouse to establish absolute doses at which a given degree of effect would be expected. There are better grounds, on the other hand, for accepting the ratio of the doses of x-rays and neutrons required to produce a given degree of effect in one species for extrapolation to another. It appears to be worthwhile therefore to examine the problems involved in attempting to extrapolate from mouse data to man. Before attempting to do this, it is useful to review the maximum permissable doses for x-rays and neutrons that are presently used in radiation protection. In Fig. 2 is shown schematically the present MPD levels for low LET (x or gamma radiations) and high LET (fast neutrons in the KeV range). The accumulated dose vs. age in years is plotted. With low LET radiation the maximum accumulation of dose is at the rate of 5 rads per year starting at age 18, so that the maximum allowable exposure at any given age is 5 (N - 18) rads, where N is the age in years. The lower curve shows the allowed maximum rate of accumulation of high LET radiation, when all exposure is assumed to be from high LET radiation only. A QF value of 10 is used in arriving at the slope of this curve. The broken line is intended to indicate that, for brief periods of time one can accumulate exposure at the rate of 12 rads per year, provided the total exposure remains below the 5 (N-18) value. Note that, in a working lifetime of 40 years, a maximum of 200 rads of low LET radiation, or of 20 rads

1260963

of high LET radiation alone, may be accumulated within these MPD limits.

A "threshold" dose for lens opacification for low LET radiation is shown at the 600 rad level. A corresponding level for high LET radiation with a QF of 10 would, of course, be 60 rads (assuming that the QF for lens opacification is numerically equal to the RBE for this effect in man). The value of 600 for low LET radiation delivered at a relatively low dose rate appears to have been derived principally from the data of Merriam and Focht[4] obtained on human beings exposed to radiations for therapeutic purposes. Note that a safety factor of 3 exists, i.e. the maximum allowable dose of either high or low LET radiation that one can accumulate in an entire working lifetime is one-third the dose thought to be necessary to produce lens opacification.

In Fig. 3 are shown schematically the data that would make it possible to derive a QF for neutron exposures of man, from data obtained on the mouse. On the ordinate is plotted the degree of radiation-induced lens damage, the scale of which has been chosen so as to make the regression of mouse opacification on dose of neutrons linear. The reasons for this choice of scale are as follows: although it is relatively easy to establish a scale of opacification in terms of which general agreement can be reached on which of two effects is greater, there is no commonly accepted scale in terms of which their ratio may be expressed. A scale must, of course, be chosen if one is to plot effect vs. dose, and this choice is arbitrary except for the requirement that the scale must preserve the accepted order of degrees of effect. The scale employed (Fig. 3) satisfies this requirement and is, furthermore, convenient for present purposes in that the regression of effect on dose of neutrons is linear. Using this measure of effect gives the desired

1260964

line for neutrons (assuming linear effect and dose scales), and at the same time, fixes the relative position and shape of the x-ray curve. On a horizontal line corresponding to any given level, the RBE for that level is the ratio of segments cut by the two curves.

The basic curves in Fig. 1 are those depicting neutron and x-irradiations of mice, carried out in a single exposure. These curves represent experimental findings. The curve for mouse, x-rays, protracted exposure, was derived from the curve for single exposure, by introducing a factor of 2 to indicate decreasing effectiveness with protraction.

In attempting to extrapolate to man, it is necessary to specify a level of damage that will be considered "significant" in man, and attempt to estimate what level of damage in the mouse corresponds to that specified for man. A significant level for man is taken as that which is expected to follow exposure to 600 rads of low LET radiation; the corresponding level in the mouse was taken arbitrarily as a doubling of the incidence of 2.5% posterior lens opacifications, occurring at the time that 70% of the life span of the animal has elapsed (12 months after exposure of a mouse when 4 months old). Incidence of this degree of opacification in unirradiated control animals is 35% at this time; the acceptable level following radiation is taken to be 70%. The horizontal dotted line represents this level of damage.

With these assumptions one can then attempt to derive a dose of neutrons that will be expected to result in the same level of damage in the same time interval, from which the QF value for man can be derived. In so doing the question arises as to whether one should compare between species at the same level of physical dose of low LET radiation to produce the given level of damage in the human being, or whether one should

compare at the same level of effect. In other words does one take the RBE from the mouse data that corresponds to the effect produced in the mouse by 600 rads of x-rays, or does one use the considerably higher RBE that corresponds to presumably the same level of damage, but at a dose level much lower than the 600 rads used for man. There appears to be no basis, in the absence of some data on lens opacification following neutron exposure in man to decide between these two alternatives. One is inclined to compare at the same level of effect, although such inclinations are more on the basis of intuition than logic. There is some evidence, although the dose estimates admittedly could be in error by a large factor, that opacification may occur in man following exposures to neutrons of the order of 50 rads or less[9]. These data, if true, would indicate that the ratio at the same level of effect might be the more appropriate choice. If comparison is made at the same level of effect, then one can apply the RBE obtained for the mouse at that level of effect, to derive from the 600 R taken as the dose of x-rays that will produce opacification in man, the corresponding dose of neutrons that should produce the same degree of effect in the mouse. A dose thus derived is indicated on the dotted line in Fig. 3, labeled 0.43 MeV neutrons, MAN.

The process of extrapolation and the derived numerical values are summarized below:

Dose for man to produce significant opacification, x-rays, protracted exposure ----	600 rads
Dose to mouse for same degree of effect, x-rays, single exposure----	70 rads
Dose to mouse to give similar degree of effect, x-rays, protracted exposure----	140 rads
Dose to mouse to give same degree of effect, neutrons, single or protracted exposure----	2 rads
Ratio of x-ray doses to produce acceptable level of damage, man to mouse; $\frac{600}{140} = 4.3$	

1260966

Dose of neutrons to produce the comparable level of damage in man, single or protracted exposure ----2 rads x 4.3 or 8.6 rads of neutrons.

One might also examine what the values would have to be in order to retain the same factor of 3 safety, derived from the ratio of the dose of x-rays delivered in a protracted fashion believed to be necessary to produce significant lens opacification in man (600 R), to the maximum dose of x-radiation that an individual is allowed to accumulate in a working lifetime of 40 years, (200 R). The value for exposure to high LET radiations, retaining this same factor of safety, would be $\frac{8.6}{3}$ or 2.9 rads.

It is emphasized that the RBE values determined in the mouse is a strong function of dose; thus, numbers derived from these RBE values will be a strong function of dose level chosen.

The fact that exposure to x-rays under hyperbaric conditions enhances the effect of x-radiation does not alter the situation, provided one assumes the same degree of hypoxia in the lens of the mouse and of the man. The value of 600 R used as that necessary to produce lens opacification in man was derived from data obtained on the normal, presumably hypoxic human lens.

As pointed out above, the high RBE values obtained for lens damage in the mouse may be partly on the basis that the lens is normally hypoxic and therefore resistant to the effects of low LET radiation. The high RBE's at low dose levels might well be expected from the survival curves obtained with low and high LET radiations in other biological systems. In many systems studied the dose survival curve for high LET radiations is characteristically exponential, while the curve for low LET radiations exhibits a "shoulder" before apparently becoming exponential. From the nature of these two curves, the RBE increases with decreasing dose. Effects can be observed in the lens presumably at much lower levels of damage and lower doses than in other systems in

1260967

which one is dealing usually with cell survival. In the lens the primary damage appears to be cell injury, and the injured cells are retained and can be observed. Thus it becomes possible to actually measure RBE's in the low dose range where the values would be expected to be high.

It is emphasized that all numbers used in the above approach for extrapolating from mouse to man are intended to be illustrative only; no recommendations are made with respect to QF value for neutrons for use in radiation protection.

SUMMARY

Extensive data collected on lens opacification in the mouse exposed to x-rays have established the following:

1) An increased incidence of lens opacities is seen following even small doses.
2) Fractionation with a 12 hour interval between exposures decreases the degree of effect.
3) The same total doses given at the rates of 2 or 100 rads/min are equally effective.
4) Hypoxia does not significantly alter radiosensitivity.
5) Hyperbaric oxygen appreciably increases radiosensitivity.

Exposures to monoenergetic neutrons have demonstrated additionally that:

1) RBE values are relatively high
2) The values increase with decreasing neutron energy
3) The RBE increases appreciably with decreasing dose
4) The values increase with decreasing "dose rate", due to a rate dependence of effect with x-rays.

1260968

The difficulties of extrapolating these data to QF values applicable for radiation protection are discussed, and a possible approach to evaluating the significance of the data in this respect is presented.

ACKNOWLEDGMENT

Thanks are expressed to Drs. J. L. Bateman, C. V. Robinson and H. H. Rossi for their helpful discussions and suggestions during the preparation of this paper.

1260969

TABLE 1

RBE Values for 0.43 MeV Neutrons Relative to 250 KVP X-Rays as a Function of Dose

(Both irradiations were administered in a single session. The values refer to posterior lens opacification in the mouse).

Absorbed Dose in Rads		RBE
0.43 MeV Neutrons	250 KVP X-rays	
0.15*	15.0*	100*
0.5	31.0	62
0.75	40	53
1.0	47	47
2.0	71	36
3	90	30
5	123	25
7	150	21
10	190	19
15	240	16
20	290	15
30	365	12
40	440	11
50	500	10
60	550	9

*Extrapolated values

1260970

FIGURES

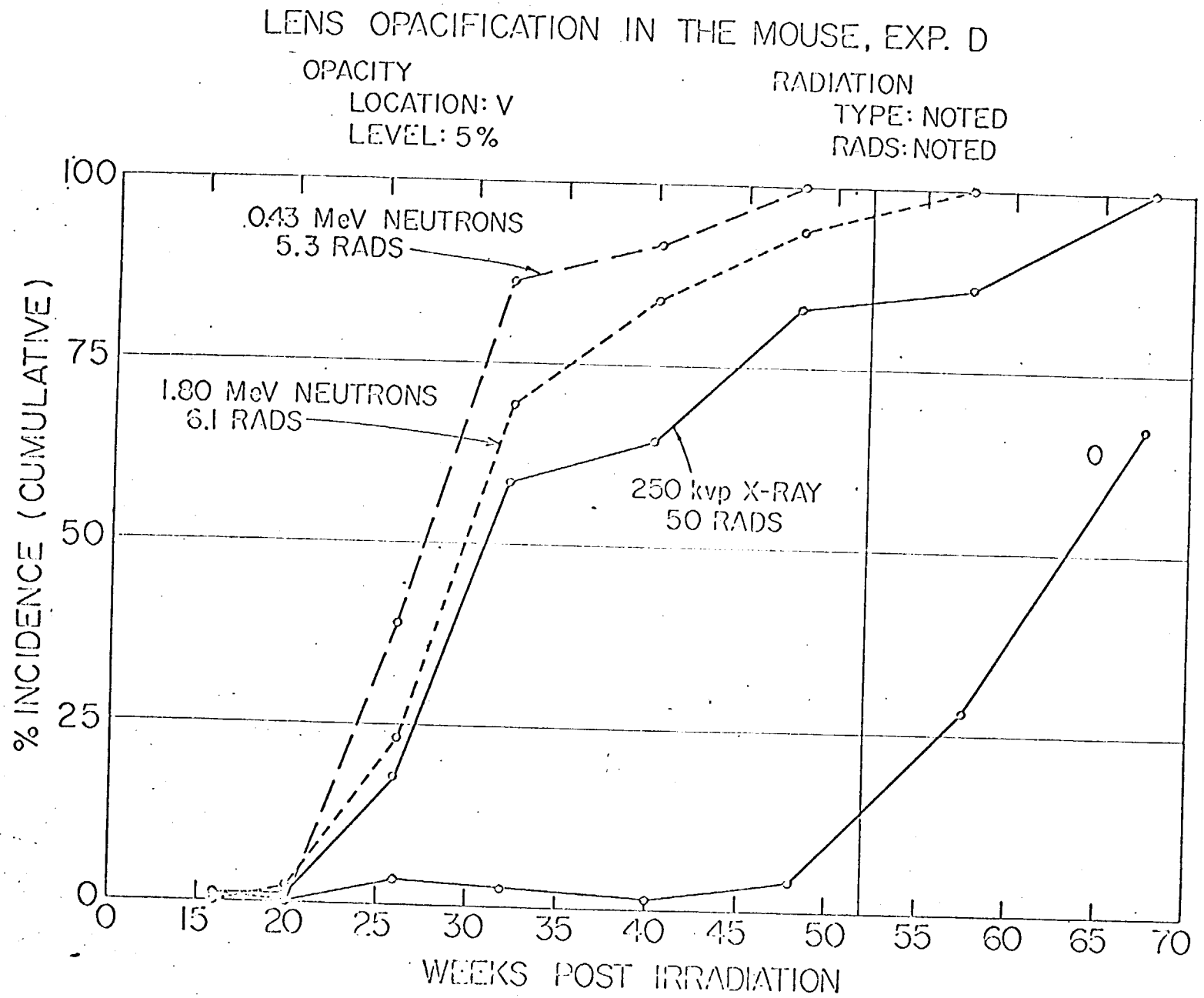
Figure 1 Cumulative incidence of lens opacifications in the mouse, as a function of time following exposure.

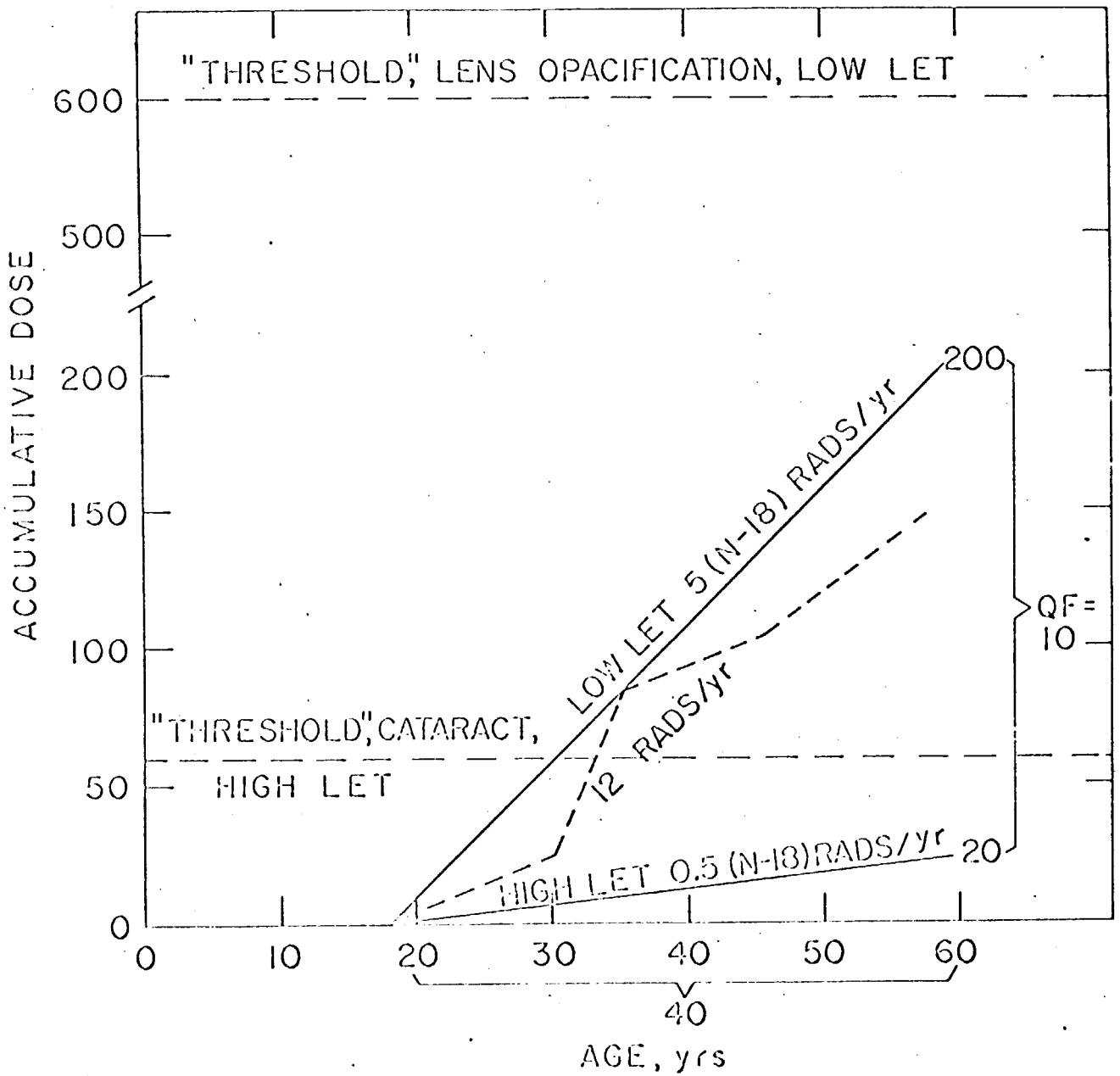
Figure 2 Schematic representation of maximum permissable doses (MPD) used in radiation protection.

Figure 3 Schematic representation of differences in dose effect relationships with respect to radiation induced lens opacification in the mouse vs. man. The degree of radiation induced lens damage is plotted against the dose in rads.

1260971

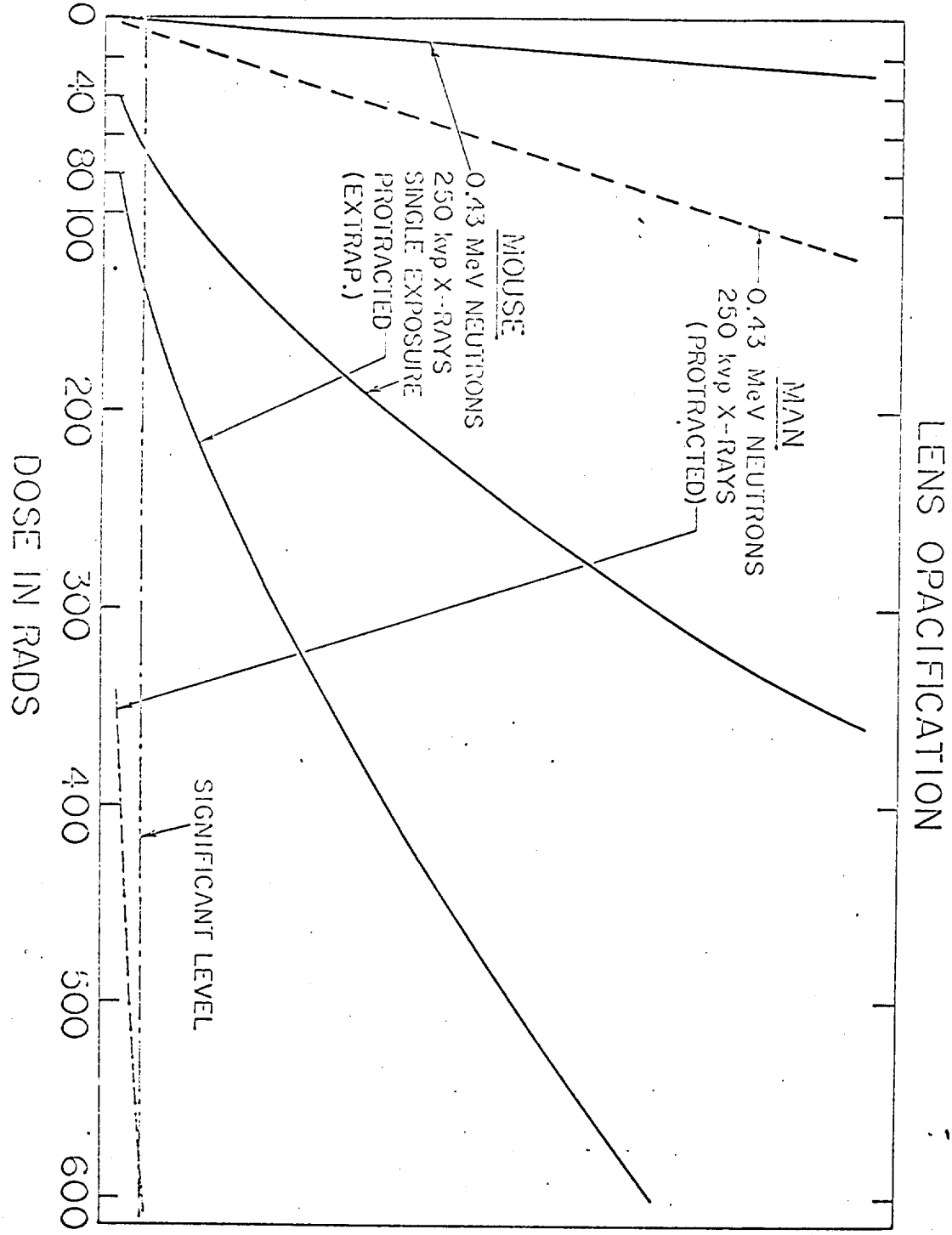
1260912


LENS OPACIFICATION IN THE MOUSE, EXP. D
OPACITY
LOCATION: V
LEVEL: 5%
RADIATION
TYPE: NOTED
RADS: NOTED
.043 MeV NEUTRONS
5.3 RADS
1.80 MeV NEUTRONS
6.1 RADS
250 kvp X-RAY
50 RADS
0
% INCIDENCE (CUMULATIVE)
100
75
50
25
0
0
15
20
25
30
35
40
45
50
55
60
65
70
WEEKS POST IRRADIATION


"THRESHOLD," LENS OPACIFICATION, LOW LET
600
500
200
150
100
50
0
ACCUMULATIVE DOSE
"THRESHOLD", CATARACT, HIGH LET
LOW LET 5 (N-18) RADS/yr
12 RADS/yr
HIGH LET 0.5 (N-18) RADS/yr
200
20
QF = 10
0
10
20
30
40
50
60
40
AGE, yrs

1260973

DEGREE OF RADIATION-INDUCED LENS DAMAGE
(SCALE ADJUSTED TO MAKE REGRESSION LINEAR ON NEUTRON DOSE, MOUSE)



1260974

REFERENCES

1. Bateman, J. L., Bond, V. P. and Rossi, H. H. Lens Opacification in Mice exposed to Monoenergetic Fast Neutrons. Presented at Symp. on Biological Effects of Neutron and Protection in Irradiation, sponsored by the IAEA, BNL, Upton, N. Y., Oct. 7-11, 1963.

2. Bateman, J. L. et al, in preparation.

3. Johnson, H. A., Bateman, J. L. and Amendola, F. Pathogenesis of the Radiation Cataract in the Mouse. Presented at the Radiation Research Society Meeting, Puerto Rico, May 7-11, 1967.

4. Merriam, G. R. and Focht, E. F. A Clinical and Experimental Study of the Effect of Single and Divided Doses of Radiation on Cataract Production. Amer. Ophth. Soc. 60, 35-52, 1962.

5. Rossi, H. H. The Role of Associated Absorption Events in Lenticular Radiation Injury. Proc. of Second Panel on the Biophysical Aspects of Radiation Quality, Vienna, Austria, April 10-14, 1967. To be published.

6. Bateman, J. L., Bond, V. P., Rossi, H. H. Lens Opacity Induction as a Function of Environmental Oxygen Concentration During X-Irradiation. Presented at the Radiation Research Society Meeting, San Juan, Puerto Rico, May 7-11, 1967.

7. van den Brenk, H.A.S., Kerr, R. C., Richter, W. and Papworth, M.P. Enhancement of Radiosensitivity of Skin of Patients by High-Pressure Oxygen. Brit. J. Radiol. 38, 857-864, Nov. 1965.

8. Atkins, H. L., Seaman, W. B., Jacox, H. W. and Matteo, R. S. Experience with Hyperbaric Oxygenation in Clinical Radiotherapy. Am. J. Roentgenol. 63, 651-663, 1965.

1260975

9. Report of the United National Scientific Committee on the Effects of Radiation. General Assembly Official Records: 17th Session, Supplement No. 60 (A/5216), United Nations, New York, 1962.

1260976