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DISCLAIMER

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Running Title:

ABSTRACT

Frog sciatic nerves *in vitro* are irradiated with focused, cyclotron-accelerated heavy ions. Four different charged particle beams are employed: 43-MeV protons, 60-MeV, 76-MeV, and 110 MeV helium ions The average dose absorbed by isolated nerves to completely suppress propagation of nerve impulses is 680 krad for 43-MeV protons, 300 krad for 110-MeV helium ions, 230 krad for 76-MeV helium ions, and 210 krad for 60-MeV helium ions. The experimental relative biological effectiveness (RBE_{exp}) to inhibit transmission of action potentials is 1.2 for 43-MeV protons, 2.9 for 110-MeV helium ions, 4.0 for 76-MeV helium ions, and 4.4 for 60-MeV helium ions. RBE_{exp} is established with respect to the absorbed dose from 200-kV x rays.

We find that the RBE_{exp} for neural conduction failure in frog nerve is a function of the linear energy transfer (LET). The RBE/LET linear relationship detailed in this report also supports data available from other investigators.

Values for a theoretical relative biological effectiveness (RBE_{theor}) based on an elemental equation are in fair agreement with RBE_{exp} values.

We found that about 2.5×10^4 rad-ion pairs/micron of nerve are critical to induce conduction failure in frog sciatic nerve.

INTRODUCTION

The first description of impaired excitability in peripheral nerve from an exposure to ionizing radiation was given by Lazarus-Barlow (1913). The radiation dose to fully abolish peripheral nerve action potentials remained unknown for the next twenty years until the work of Audiat, Auger and Fessard (1934). We still do not fully understand how radiation halts neural impulse transmission, however, research investigators have provided information about the fundamental changes ionizing radiations can cause in nerves. Biological indices, such as conduction velocity (Gerstner, Orth, and Richey, 1955), the refractory period (Makarov, 1934), and ionic membrane permeability (Rothenberg, 1950; Gaffey, 1962) have been used to study how the nerve interacts with ionizing radiation fields.

The minimum dose to completely suppress action potentials in frog sciatic nerve is estimated in this report The heavy ions used were 43-MeV protons, and 60-MeV, 76-MeV, and 110-MeV helium ions; the relative biological effectiveness (RBE) of these radiations with respect to x rays has also been evaluated The RBE values for other radiation particles has been determined from information in the literature. Finally, the potential relationship between the RBE for nerves and the linear energy transfer of various radiations has been considered.

If the loss of neural excitation depends only on the production of a certain number of ion pairs, then the distribution of ion pairs should not be a relevant factor. If the loss of excitation occurs only in the critical volume of the membrane of nerve, then an appreciable number of ion pairs must be produced close to one another to suppress excitation. The distribution of ions along the track of an ionizing particle, or linear energy transfer (LET), is defined as the loss of energy of a particle per unit of path travelled

(Zirkle, 1940, 1954; Zirkle et al., 1952; Rossi, 1960, 1967). The consideration arises as to whether the RBE to block neural excitation is related to the LET of various radiations used to halt neural impulse transmission. Modern hypotheses dealing with the mode of action of ionizing radiation seek information that verifies either the absence or the existence of RBE/LET correlations. The present study supports an earlier view (Gaffey, 1971b) that the greater the LET of a radiation the greater the RBE to block neural excitation.

METHODS

Biological Procedure

Alert, drug-free, adult frogs (Rana pipiens), weighing about 35 g each, were decapitated and their spinal cords pithed. Sciatic nerves were carefully removed to avoid trauma to the nerves and their intimately associated blood vessels. Each nerve was tied with surgical thread at its central and peripheral terminal. Nerves were separately stored in labelled vials containing a small volume of Ringer's solution (Mitchell, 1948). Nerve preparations were bathed in a balanced isotonic solution for one hour (minimum) to permit the trimmed side branches to heal and to allow enough time for each nerve to come into dynamic equilibrium with the salt solution.

Radiation Procedure

The 88-inch cyclotron at Lawrence Berkeley Laboratory supplied 43-MeV protons, 60-MeV, 76-MeV, and 110-MeV helium ions. The cyclotron's exit port delivered a 4-in circular beam of accelerated heavy ions to a shielded biophysical cave. Magnetic focusing restricted these heavy-ion beams to a 1-in circular beam, and they were further limited to a 6.0 x 25.4 mm gap by a tantalium

absorbing collimator. A transmission ionization chamber (Birge et al., 1956) inserted in the cyclotron's beam collected a charge as nuclear particles passed through it. Charge was measured with precision condensers and electrometers. The average charge collected over a given time was a function of the average dose absorbed by the test nerve. Although each nerve preparation was approximately 45-mm long, only 6 mm of nerve near the middle was irradiated.

The dose values from the ionizing chamber were in good agreement with Faraday cup measurements. The dose rates we used ranged from 6 to 10 krad/min to mimic x-ray doses used by others (Bachofer and Gautereaux, 1960; Bachofer, 1962).

Action Potential Measurements

A moist chamber housing a sciatic nerve was locked into a precision alignment apparatus in preparation for intercepting a heavy-ion beam from the 88-in cyclotron. An isolated nerve rested on Ag-AgCl electrodes. The nerve chamber was sealed with a 0.5 mil Mylar window and positioned immediately downstream from the ionizing chamber. In this manner the path between the dose monitoring device and the test nerve was reduced to 2 to 3 mm.

One pair of electrodes in the nerve chamber sent electrical pulses (0.1 msec in duration at 10 pps) to the central portion of the sciatic nerve. The voltage strength from a stimulator (Grass, Model S-4 and isolation unit) was regulated to evoke maximum action potentials. Another pair of electrodes in contact with the peripheral segment of the nerve detected action potentials that had been transmitted. These recording electrodes ran leads to a preamplifier (Grass, Model 532) which displayed its signal on an oscilloscope with a high-gain differential input amplifier (Tektronix Model 532 with type 53/54 plug-in).

Action potentials were photographed as oscillograms using a polaroid oscilloscope camera. Once a test nerve was positioned it was never altered with respect to the beam. Nerves were stimulated and action potentials recorded in a shielded area removed from the irradiation site. Heavy-ion beams were also regulated by remote-control.

The linear energy values of the four heavy ions employed in this nerve study are presented in Table 1.

RESULTS

Inactivation Dose

A pre-irradiation period of electrical stimulation confirmed the fidelity and stability of each nerve's propagated response. Thereafter, heavy-ion irradiation was initiated and the time to suppress neural exictability was determined by monitoring action potentials until supramaximal stimuli failed to evoke a detectable neural response. The absorbed dose of heavy ions that completely blocked action potentials was calculated from the observed exposure time and the measured dose rate.

An example of the amplitude of neural action potentials as a function of the accumulated dose of 110-MeV helium ions is shown in Figure 1. The maximum action potential amplitude is resistant to radiation induced change until the nerve absorbed about 200 krad. Additional radiation provoked a rapid attenuation and ultimate loss of the nerve's signal. The heavy-ion dose to block neural activity is readily obtained from individual dose-response curves, as illustrated in Figure 1.

We irradiated twelve nerves and found that the average dose that would inhibit impulse conduction was 210 krad (60-MeV helium ions), 230 krad (76-MeV

helium ions), 300 krad (110-MeV helium ions), and 680 krad (43-MeV protons). These inactivation doses were subsequently validated in other experiments dealing with narrow-field, heavy-ion effects on small segments of frog sciatic nerves (to be reported).

We estimate from Figure 1 that the nerve absorbed 200 krad of 110-MeV helium ions to attenuate the action potential's amplitude by only 5 percent. The measured dose to provoke a 5 percent radiobiological increase is 180 krad for the action potential's latent period, 175 krad for conduction velocity, 130 krad for the duration of the action potential, and 20 krad for the peak time. Apparently the action potential is the least radiosensitive of the parameters considered, whereas the shift in the peak time of the action potential is the most radiolabile index of physiological change. Nonetheless, neural-blocking doses are valuable radiation measures because RBE values are normally based on this radiobiological end point.

Relative Biological Effectiveness

Relative biological effectiveness is defined here as the ratio of the absorbed dose of 200-kV x rays to the absorbed dose of a test species of radiation which will fully inhibit the propagation of action potentials in frog sciatic nerve. We previously reported that it took 285 krad of 200-kV x rays to block neural activity of frog nerve (Gaffey, 1971a). The RBE values for the heavy-ion beams we used are listed in Table 1. Each species of radiation has been compared to 200-kV x rays, which has been assigned an RBE of one by convention. We found that the RBE to halt neural activity increased with increasing LET values. Other investigators have reported doses that will block conduction in frog sciatic nerve (Table 1). These data, when added to the information in this report, support the view that RBE is dependent on LET.

A plot of the relationship between RBE and LET is given in Figure 2. The data points in this graph were obtained from the information given in Table 1.

LET

In Table 1, LET determinations for heavy ions are based on the track segment method (Zirkle, 1940, 1954; Zirkle et al., 1952; Zirkle and Tobias, 1953; Barendsen et al., 1963; Fowler, 1975) and the table of energy losses of Barkas and Berger (1964). Linder (1959) reported that the mean LET for 200-kV x rays was 2.5 keV/micron in air and 3.0 keV/micron in tissue. Track length factors and LET values are not as well defined for x rays as they are for heavy ions. Cormack and John (1952) and Lea (1955) gave a useful track-length calculation for LET estimates which depended on the x-ray spectrum. The mean LET value was determined to be 2.7 keV/micron in tissue for 260-kV x rays for the frog nerve irradiations of Gerstner et al. (1955, 1956).

DISCUSSION

RBE/LET Relationship

In Figure 2 the experimental RBE to block neural transmission is presented as a function of LET for heavy-ion and x-ray irradiations. LET ranges from 1.2 to 4.4 keV/micron of water; experimental RBE ranges from 0.41 to 1.35. Although these ranges are small and the number of radiations is limited, we are able to describe the RBE/LET relationship in Figure 2 with the linear equation:

$$y = mx + b, \qquad (1)$$

where y represents the theoretical RBE, x represents LET, m is 0.31 or the slope, and b is 0.07 or the ordinate intercept when LET is zero.

If equation (1) is valid, then it should prove to be a useful tool for calculating theoretical RBE values for radiations from their known LET values. Consider the following case. Bergström et al. (1961) reported that 10 krep (9.3 krad) of 5.3-MeV helium ions from ^{210}Po caused a complete loss of the action potential of frog nerve within a few minutes after irradiation. The 5.3-Mev helium ions have an LET of 110.0 keV/micron of water. Equation (1) predicts that the theoretical RBE (y) for 5.3-MeV helium ions will be:

$$y = (0.31)\ (110.0) + 0.07$$

$$y = 34.17\ (RBE_{theor}).$$

The experimental RBE obtained from the measured inactivation dose is 30.67 (Bergström et al., 1961). Thus, the experimental RBE and the theoretical RBE differ by 11.4 percent. It is remarkable that the theoretical RBE is so close to the measured RBE since equation (1) makes use of an LET value that is two orders of magnitude greater than any appearing in Figure 2. The ordinate and abscissa of Figure 2 would have to be extended considerably to include the RBE/LET data obtained from the report of Bergström et al. (1961).

Table 1 contains experimental RBE values and theoretical RBE values (equation 1) for nine radiations. If we accept that the experimental RBE is approximately equal to the theoretical RBE, then we can obtain a theoretical inactivation dose (D_{theor}) to block impulse transmission in frog nerve for a test species of radiation. The basic definition of the experimental RBE that will completely suppress neural excititation is:

$$RBE_{exp} = \frac{\text{Dose of 200-kV x ray}}{\text{Dose of test radiation}} . \qquad (2)$$

Since 285 krad is the measured inactivation dose to block frog nerve with 200-kv x rays (Gaffey, 1971a), then

$$RBE_{exp} = \frac{285 \text{ krad}}{D_{theor}} = RBE_{theor}. \quad (3)$$

The RBE_{theor} for a test radiation can be obtained by equation (1). For example, the 5.3-MeV helium beam used by Bergström et al. (1961) had a theoretical RBE of 34.17, which was obtained from inserting the respective LET value for 5.3-MeV helium ions from equation (1). The theoretical inactivation dose for 5.3-MeV helium ions from equation (3) is 8.34 krad. Bergström et al. (1961) reported 9.3 krad as the experimental inactivation dose, or 10.8 percent difference between the measured and the theoretical dose.

Ion Density

A charged particle leaves a track of excited and ionized atoms and molecules as it passes through matter. The spacing of the energy released along the track is described by LET, usually measured in keV/micron of track. An LET value for the electron tracks made by 200-kV x rays is 3.0 keV/micron of water. If we assume that 34 eV is required to produce an ion pair in neural tissue, then the ion density is obtained by dividing the LET (keV/micron) by 0.034 keV/ion pair. The LET of 200-kV x rays is equivalent to an ion density of 3.0/0.034 or 88.2 ion pairs/micron of nerve. In this report ion density (number of ion pairs/micron) is used to describe energy releases from heavy ions and x rays passing through nerve as discrete events. In Table 2 ion density is listed for nine radiations with their corresponding frog nerve inactivation dose. The product of ion density and inactivation dose has units of rad-ion pairs/micron of nerve. We found that the energy required to inhibit

neural excitation is almost constant when expressed in these units. Our value averaged 2.53×10^4 rad-ion pairs/micron of nerve for the radiations employed, which suggests there is a critical and fixed amount of neural membrane damage that must occur before impulses can no longer be conducted. Equivalent membrane suppression can be initiated with high doses of low-LET (low-ion density) radiations, or with small doses of high-LET (high-ion density) radiations. On the molecular level, perhaps the membrane structures essential to maintain ionic permeability are irreversibly denatured by 2.53×10^4 rad-ion pairs/micron of nerve. Scientists who seek to develop concepts of how radiation acts on excitable neural membranes should consider not only biological factors as functions of radiation, but physical factors, such as the molecular effects of different LET radiations on lipid-protein membranes, as well. These studies were supported by the Biomedical and Environmental Research Division of the Department of Energy, and NASA.

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Table 1. Experimental Doses of Radiation to Block Action Potentials in Frog Sciatic Nerves, Relative Biological Effectiveness (RBE), and Linear Energy Transfer (LET)

Radiation	Dose (krad)	Reference	LET (keV/μ)	Experimental RBE	Theoretical RBE
43-MeV protons	680.0	This report	1.2	0.41	0.44
48-MeV protons	600.0	Gaffey, 1971a	1.3	0.47	0.47
910-MeV helium ions	430.0	Gaffey, 1971a	1.6	0.66	0.58
260 kV x rays	329.0	Gerstner et al., 1955, 1956	2.7	0.86	0.90
110-MeV helium ions	300.0	This report	2.9	0.95	0.96
200-kV x rays	285.0	Gaffey, 1971a	3.0	1.00	1.00
76-Mev helium ions	230.0	This report	4.0	1.23	1.31
60-MeV helium ions	210.0	This report	4.4	1.35	1.43
5.3-MeV helium ions	9.3	Bergström et al., 1961	110.0	30.64	34.17

Table 2. Ion Density of Radiations and their Corresponding Experimental Doses that Will Block Action Potentials in Frog Sciatic Nerve

Radiation	Ion Density (ion pairs/)	Dose (krad)	Ion Density x Dose (rad-ion pair/) x 10^4
43-MeV protons	35.3	680	2.40
48-MeV protons	38.2	600	2.29
910-MeV helium ions	47.1	430	2.03
260-kV x rays	79.4	329	2.61
110-MeV helium ions	85.3	300	2.56
200-kV x rays	88.2	285	2.51
76-MeV helium ions	117.6	230	2.70
60-MeV helium ions	129.4	210	2.72
5.3-MeV helium ions	3235.3	9.3	3.00

FIGURE LEGENDS

Figure 1: Ratio of the values of maximum action potential amplitudes before (V_o) and during (V) irradiation as a function of the absorbed heavy-ion dose. This radiation-response relationship allows the inactivation dose for the failure of neural activity to be estimated at 300 krad for 110-MeV helium ions.

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Figure 2: Relative biological effectiveness is the ratio of the absorbed dose of 200-kV x rays necessary to suppress action potentials in frog nerve to the absorbed dose of test radiations to produce the same effect. Linear energy transfer is the loss of energy of an ionizing particle per unit length of the path travelled through tissue.

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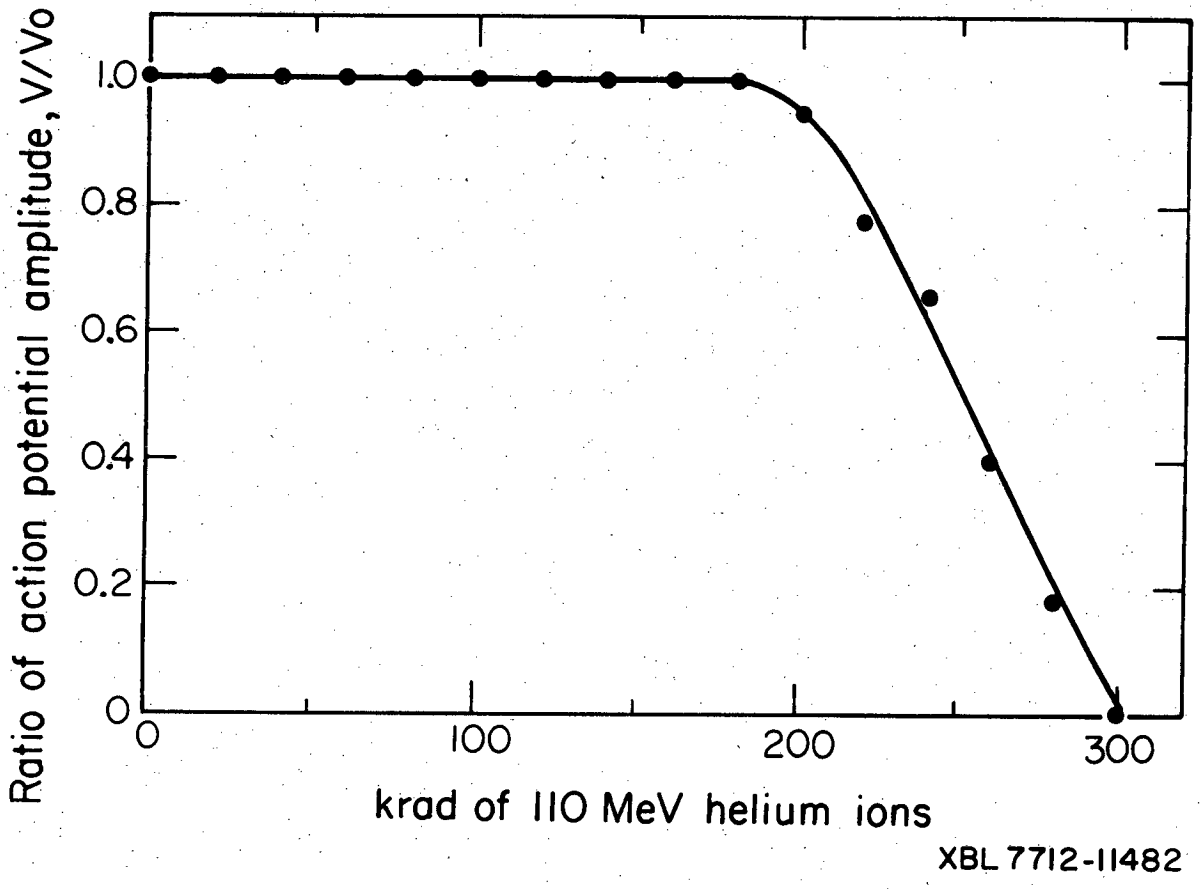
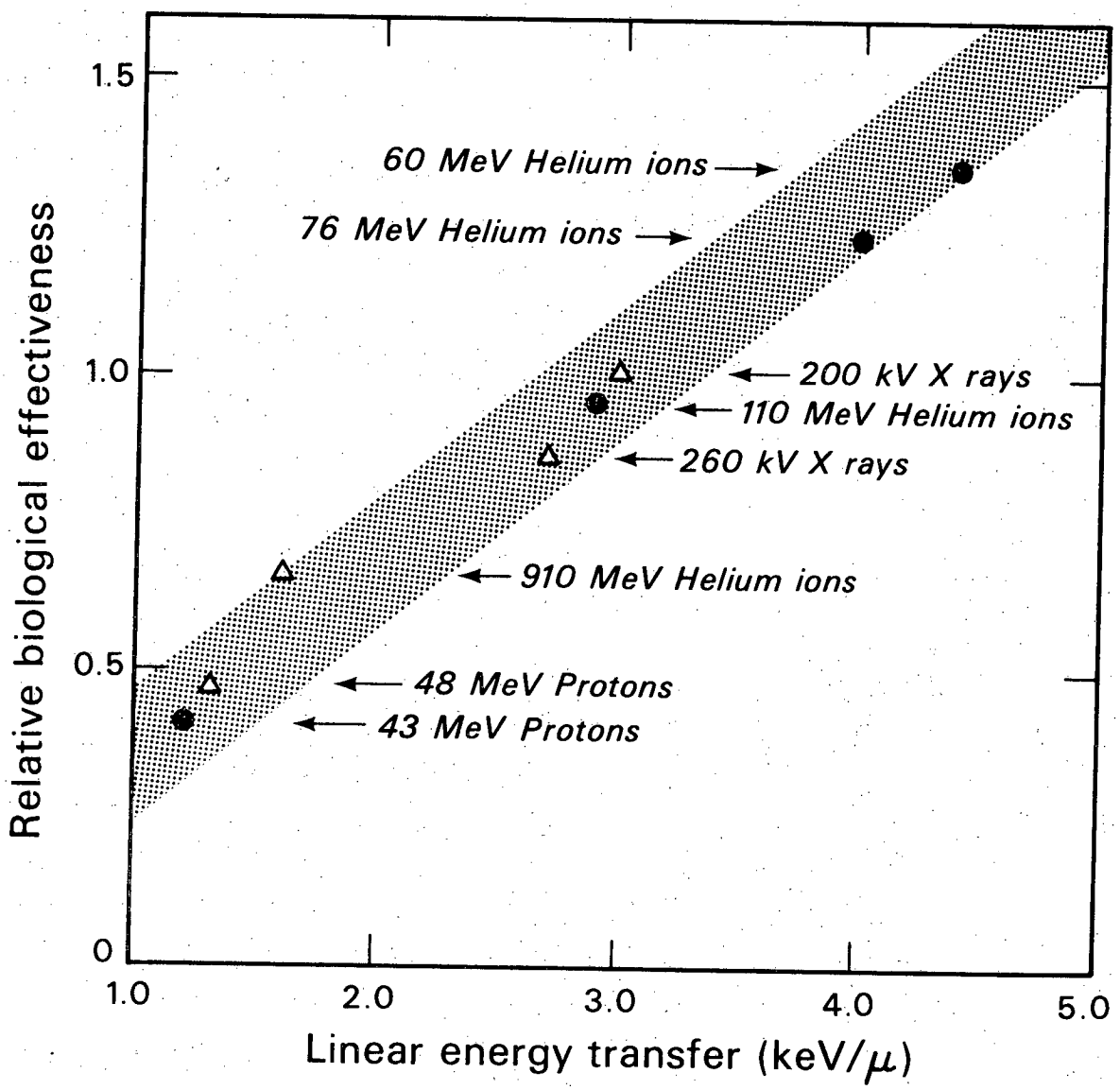

Ratio of action potential amplitude, V/Vo
1.0
0.8
0.6
0.4
0.2
0
0
100
200
300
krad of 110 MeV helium ions
XBL 7712-11482



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