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RELATION BETWEEN RBE AND LET TO INHIBIT NEURAL IMPULSE CONDUCTION

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Relation Between RBE and LET to Inhibit Neural Impulse Conduction

ABSTRACT

A dose of 285 krad (average) of 200-kV x rays absorbed by isolated, frog sciatic nerves promptly inactivated impulse transmission. Blockage of neural activity in this type of nerve required 600 krad (average) of 47.5-MeV protons. Protons of this energy have a relative biological effectiveness (RBE) of 0.48 with respect to 200-kV x rays, which have an assigned value of one. Other investigators exposed frog nerves to different types of radiation and reported inactivation doses. RBE values determined from such doses were 0.66 for 910-MeV α-particles, 0.87 for 260-kV x rays, and 30.60 for 5.3-MeV α-particles.

The RBE values to suppress neural activity depended on the linear energy transfer (LET) of the radiation particles. Radiations with high LET values had high RBE values. A plot of the logarithm of RBE versus the logarithm of LET yielded a linear relation for radiations with LET values between 1.3 and 110.0 keV/μ of tissue.

Frog sciatic nerves exposed to either 200-kV x rays or 47.5-MeV protons exhibited bioelectric changes prior to loss of excitability. Conduction velocity decreased and the detection period (stimulus-response interval) increased after nerves absorbed only one-fourth the inactivation dose. The present investigation failed to confirm the observation of others that the action potential amplitude increased with the onset of radiation. The action potential remained unaltered even after nerves absorbed one-fourth the inactivation dose of x rays and one-half the inactivation dose of protons.

Key words: irradiation; action potentials; nerves; RBE; LET.

INTRODUCTION

The purpose of this work was to determine the radiation dose to block impulse conduction in isolated frog sciatic nerves with 47.5-MeV protons ($^1_1$H) and 200-kV x rays. This information permitted the relative biological effectiveness (RBE) of protons to be established with respect to x rays. Since the radiation dose to suppress excitation in frog nerve was available for 5.3-MeV α-particles (1,2), 910-MeV α-particles (3) and 260 kV x rays (4,5), the RBE values of these radiations were considered.

Linear energy transfer (LET) expresses the loss of energy of a particle per unit of path travelled (6 to 10). The RBE of ionizing radiation on some biological systems depends not only on the amount of radiation energy absorbed, but on LET or how the energy is deposited along particle tracks (11 to 13). Since it is not known if an RBE-LET relation exists for nerves, this possibility was investigated. Current hypotheses about radiobiological mechanisms require information on whether RBE is or is not a function of LET.

The flux of low energy protons trapped in the inner Van Allen belt (14 to 16) have about the same degree of penetration and energy per nucleon as the cyclotron-accelerated protons administered to nerve preparations. Since eighty-five percent of the radiation encountered in interplanetary space are protons, information on their radiosensitivity has relevance to the galactic space environment and the radiation hazard there.

It has been asserted that the action potential amplitude and conduction velocity of rat caudal nerves were regularly increased with the onset of irradiation (17 to 19). Allen and Nicholls (20) repeated these experiments and could find no enhancement of neural activity. In the present study x rays and protons were used to irradiate frog nerve in order to determine if low doses of these radiations could provoke augmentation of conduction

velocity and action potential amplitude.

Action potential amplitude, conduction velocity, and detection period (stimulus-response interval) were studied as a function of the x-ray and proton dose absorbed by nerves to determine the relative radiosensitivity of these bioelectric parameters.

METHODS

Biological Procedure

Adult frogs (Rana pipiens) were employed in these experiments. The procedure and electrophysiologic equipment used to generate and record action potentials from isolated sciatic nerves was previously detailed (3,21).

Isolated nerves were maintained in frog Ringer's solution (22) at least sixty minutes before being tested for bioelectric activity. Some nerves were held in Ringer's solution for several hours before proton irradiations, because of the problems involved in obtaining and adjusting a cyclotron particle beam to a selected energy.

Sciatic nerves were individually placed inside a moist chamber on Ag-AgCl electrodes. This plastic chamber had a reservoir of water to maintain a high relative humidity. The stimulus strength delivered to the central end of each nerve was regulated to evoke a maximal action potential (MAP), which was displayed on an oscilloscope. MAP were generated at the rate of 20 per sec. Nerve preparations were subjected to either x rays or protons if the MAP amplitude demonstrated stability.

The moist chamber housing a sciatic nerve was positioned to intercept either a beam of protons or x rays prior to physiologic stimulation. The orientation and location of the moist chamber thereafter was never changed for a given nerve study. Action potentials were photographed before, during, and after irradiation.

X-irradiation Procedure

A moist chamber was positioned so that its 1.0 mil Mylar window was between the nerve chamber and the exit port of a therapeutic x-ray machine.

The x-ray machine was operated at 200-kV, 15-ma; the inherent filtration of the oil in the x-ray tube was equivalent to 1.0 mm Al; extra filtration consisted of 0.5 mm Al plate, an air path of 8.0 mm and the 1.0 mil Mylar window of the moist chamber. The focus-to-target distance was 13.6 cm.

The quality of the x-ray beam in terms of the half value layer (HVL) was determined by the procedure described by Johns (23). The HVL was found to be 0.83 mm Cu.

Isolated nerve preparations received 1,700 $\pm$ 9 R/min, as determined by a Victoreen condenser ionization chamber. This exposure rate included backscatter at the surface of nerves.

RBE values normally are presented in terms of the ratio of absorbed doses. If the air, x-ray exposure (roentgen units) is expressed by d, then the x-ray dose (rad units) absorbed by nerves (D_{nerve}) is

$$D_{nerve} = d \times \overline{f}$$

where $\overline{f}$ is the conversion factor from roentgens to rads (24). The $\overline{f}$ value for x rays is a function of the HVL. In these experiments x rays had a HVL of 0.83 mm Cu, which established the $\overline{f}$ value as 0.946 rad/R (24). The $\overline{f}$ value for muscle was taken as the closest approximation to nerve. In these studies x rays were absorbed by frog nerves at the rate of 1,615 $\pm$ 8 rad/min.

Proton Irradiation Procedure

The 88-inch sector-focused cyclotron at the Lawrence Radiation Laboratory in Berkeley served as a source of 47.5-MeV protons. Cyclotron-accelerated particles of $^{1}_{1}H$ were conducted in a four-inch diameter vacuum pipe from the cyclotron to a biophysical research cave. A quadrupole magnet

focused the cyclotron's beam and a particle absorbing collimator restricted the profile to a circular beam.

The proton dose was measured with a transmission ionization chamber (25) placed directly in the beam approximately 2 to 3 mm before the target nerve. As the proton beam passed through the ionization chamber, a charge was collected. This integrated charge was related to the average dose absorbed by the nerve (25). Faraday cup (26) and silicon diode (27) measurements of the proton dose rate yielded values in agreement with the transmission chamber.

The proton dose rate employed in these experiments was 50 $\pm$ 1.5 krad/min, except in six experiments in which the dose rate was specifically decreased to mimic x-ray dose rates used by others (18 to 19).

By tuning the rf system of the 88-inch cyclotron to 15 MHz, protons with an average energy of 47.5-MeV/nucleon were generated with a maximum beam current of 60 μA. The external beam power in this operating state was 2,850 watts (47.5-MeV x 60 μA). The range of 47.5-MeV protons was 2.31 g/cm^2 in air and 2.00 g/cm^2 in water. The stopping power of these proton particles was 11.488 MeV-cm^2/g or 1.1488 keV/μ in air. The linear energy transfer (LET) for 47.5-MeV protons in water was 13.149-MeV-cm^2/g or 1.3139-keV/μ.

High-energy protons were restricted by a collimator to a circular beam 6.2 mm in diameter. This proton beam could be turned on or off remotely by the removal or insertion of an absorbing plug. Nerve preparations housed in a moist chamber intercepted protons between the stimulating and recording electrodes.

RESULTS

Isolated sciatic nerves were placed on Ag-AgCl electrodes in a moist chamber and the chamber was sealed. Action potentials were generated by

10^{-5} sec rectangular pulses delivered to nerve preparations at 20 pulses/sec. The strength of the stimulus was regulated to evoke maximal action potentials (MAP).

Electrical stimulation of nerves was continuous, i.e., MAPs were generated during the preirradiation and irradiation period without interruption. About one minute before each action potential was photographed, the stimulus strength was adjusted to insure that the observed action potential was a MAP. If the MAP amplitude of a given nerve preparation remained unchanged during the preirradiation interval, the irradiation procedure was initiated with the nerve in place.

Nerves were maintained in moist chambers for about 3.5 hours during x-ray exposures. Nonirradiated nerves housed in moist chambers maintained their action potentials for over forty-eight hours while being continually stimulated.

Bioelectrical Effects of X rays

The radiation exposure to suppress neural excitability was determined by continually monitoring the MAP of nerves administered 200-kV x rays. In twenty experiments neural transmission was completely inhibited from 292 to 304 kR with an average of 300 kR (285 krad) for x rays.

Conduction velocity of neural impulses was studied as a function of the x-ray dose delivered to nerves. Conduction velocities were calculated from the interval between two action potential peaks, recorded with reversible electrodes at different distances along the length of nerves. Such conduction velocity determinations were free from the interference that could arise from variable latent period responses induced by radiation.

Fig. 1 is a composition of MAPs recorded from a nerve during a typical experiment. Before irradiation (0 kR in Fig. 1) the MAP amplitudes were

10.7 mV and conduction velocity was 28 M/sec. The distance between recording electrodes in this trial was 10 mm. MAP amplitudes were measured from the zero baseline to the peak of the negative wave. The detection period, defined as the interval between the stimulus shock and the rise of the action potential, was 0.3 msec for the first MAP and 0.8 msec for the second MAP prior to irradiation (0 kR in Fig. 1). During a 30 min preirradiation period, MAP amplitudes, conduction velocity, the detection periods, and the stimulus strength to evoke MAPs were not altered. Thereafter, x-irradiation was applied to the nerve preparation until its bioelectric responses ceased (300 kR or 285 krad in Fig. 1).

During the preirradiation period the conduction velocity, MAP amplitudes, and detection periods for any given nerve preparation remained stable, but variations between nerve preparations were found. The conduction velocity of twelve preirradiated nerves varied from 27 to 34 M/sec; MAP amplitudes ranged from 9 to 12 mV. The detection periods before irradiation ranged from 0.30 to 0.33 msec for the first MAP and from 0.60 to 0.80 msec for the second MAP.

The computed conduction velocity, relative MAP amplitude, and detection period of the action potentials in Fig. 1 were plotted as a function of the x-ray exposure in Fig. 2. The relative MAP amplitude of the ratio of the MAP amplitudes, A/A_o, where A was the MAP amplitude for a particular exposure of x rays and A_o was the MAP amplitude with zero roentgens of x rays. Conduction velocity and the detection period were plotted as percent changes from their preirradiated values.

Fig. 1 and 2 illustrate that nerve exposed to 75 kR (71.3 krad) or more of x rays suffered a retardation of conduction velocity and a lengthening of the detection period, even though the MAP amplitude remained unchanged.

Four times this amount of radiation was required to inactivate neural responses.

In some experiments 60 kR (57 krad) of x rays was sufficient to impair conduction velocity and increase the detection period. All nerve preparations demonstrated retarded conduction velocity and detection period changes after receiving 90 kR (85 krad) of x rays.

When twenty nerves were individually exposed to x rays, there was no immediate increase in the MAP amplitude at the onset of irradiation, as reported by others (17 to 19). It was our finding that the MAP amplitudes remained unchanged, even when nerves were exposed to 115 kR (109 krad) of x rays. However, fourteen out of twenty nerves exhibited a small five to twelve percent increase in the MAP amplitude with 125 to 175 kR (119 to 166 krad) of x rays. These fourteen nerves demonstrated bioelectric changes with only 75 kR (71 krad), such as increases in the action potential duration (measured from the rise of the action potential until it recrosses the zero voltage baseline), reduction in conduction velocity and increases in the detection period. Six out of twenty nerves did not demonstrate any MAP amplitude increase with x-irradiation, only amplitude attenuation with x rays in excess of 175 kR (166 krad). Conduction velocity reduction, detection period lengthening, and MAP duration spreading were evidenced after these six nerves received 75 kR (71.3 krad) of x rays.

The stimulus strength to evoke MAPs was not significantly altered when nerves received 100 kR (95 krad) of x rays. In those instances in which the MAP amplitudes increased just prior to the rapid impulse attenuation, the stimulus strength decreased about ten percent. It was necessary to increase the stimulus strength to all nerve preparations during attenuation of the MAP amplitude.

Bioelectric Effects of Protons

If the MAP amplitude of a nerve remained stable for ten minutes, a 6.3 mm segment of the nerve was irradiated with 47.5-MeV protons. The MAP amplitudes of eighteen nerves were completely suppressed when they absorbed 580 to 620 krad of protons. The mean dose to block neural responses to electrical stimulation was 600 krad of 47.5-MeV protons.

Protons were absorbed by nerves at the rate of 50 krad/min. Thus, the mean exposure time for inactivation was twelve minutes. Since the MAP amplitude of frog sciatic nerves were not altered by a pure nitrogen atmosphere for 60 minutes in control experiments, it is unlikely that blockage of impulse conduction was due to oxygen depletion during proton irradiation. MAP amplitudes were reduced to sixteen percent of their original magnitude after 180 minutes in a pure nitrogen atmosphere.

Fig. 3 is a serial representation of on-the-line MAPs from a nerve that received proton irradiation. Before proton exposure the nerve required 0.1 msec for an impulse to pass between recording electrodes separated by 3.0 mm. Hence, the preirradiation conduction velocity in this preparation was 30 M/sec (0 krad in Fig. 3). The preirradiation MAP amplitudes were 10 mV (Fig. 3).

The variation of preirradiation electrophysiological parameters from nerve-to-nerve was similar to the variation mentioned for preirradiated x-ray nerve preparations.

Conduction velocity, relative MAP amplitude, and the detection period data obtained from oscillograms in Fig. 3 were related to the nerve's absorbed proton dose in Fig. 4. Conduction velocity slowed and the detection period increased with 150 to 200 krad of protons, while the relative MAP amplitude remained unaltered (Fig. 4). Even after the nerve absorbed 300 krad of protons, the MAP amplitude remained unchanged. The inactivation dose to

inhibit neural transmission in this nerve was 600 krad of protons (Fig. 3 and 4).

All nerves administered 240 krad of protons had retarded conduction velocities and prolonged detection periods. Radiation changes in conduction velocity and the detection period were provoked by 150 krad of protons in many nerve preparations.

In fifteen out of eighteen experiments there was no increase in the MAP amplitude of nerves administered 250 krad of protons. In general, MAP amplitudes were not increased with massive doses of protons just prior to rapid attenuation and final loss of the MAP, as was observed in some experiments with 200-kV x rays. Only three proton irradiated nerves revealed a four to twelve percent increase in the MAP amplitude. This increase in the MAP amplitude required 250 to 350 krad of protons.

Bachofer and Gautereaux (18) observed immediate increases in the action potentials amplitude of rat caudal nerve preparations with 280-kV x rays. They reported that 6 kR/min of x rays gave considerable enhancement of neural activity, while at higher dose rates the radiation-augmented activity was less, and at lower dose rates, irradiation-enhancement was greater, but less reproducible. The absorbed x-ray dose rate employed by Bachofer and Gautereaux (18) was equivalent to 5.7 krad/min.

The intensity of the 47.5-MeV proton beam was adjusted to 2.6, 5.6, and 6.8 krad/min to determine if these dose rates could increase conduction velocity and the MAP amplitude of frog nerve with the very onset of irradiation, as reported by Bachofer and Gautereaux (18). Two nerves were irradiated at each of these proton dose rates, but no enhancement was found in the MAP amplitude or conduction velocity, even after the nerves absorbed 200 krad. In these dose rate experiments the inactivation dose was not determined.

DISCUSSION

In this report the relative biological effectiveness (RBE) was defined as the ratio of D_x/D_t, where D_x was the absorbed dose of 200-kV x rays to suppress impulse conduction in frog nerve and D_t was the absorbed dose of a test radiation to produce the same effect. It was found that 285 krad (average) of 200-kV x rays blocked neural transmission. To produce this identical response with the same type of nerve required 600 krad (average) of 47.5-MeV protons. Hence, the RBE of protons was 0.48 with respect to x rays, which was assigned an RBE of 1.00 (Table I).

It was reported (4,5) that exposures greater than 300 and less than 390 kR (285 to 370 krad) of 260-kV x rays produced prompt abolishment of neural function in frog sciatic nerve. The average of these exposures, 345 kR (329 krad), was used as the neural blocking dose for 260-kV x rays in Table I. Thus, the RBE of 260-kV x rays was 0.87.

The apparent dose of 910-MeV α-particles to inhibit neural responses in frog sciatic nerves was reported to be 330 krad (3). The actual inactivation dose in these experiments was 430 krad of 910-MeV α-particles. This revision was based on dosimetric studies performed on the 910-MeV α-particle beam by J.T. Lyman (27). In 1970 sciatic nerves were irradiated with 910-MeV α-particles at the 184-inch cyclotron at the Lawrence Radiation Laboratory, Berkeley. It was confirmed by the author that 430 krad of 910-MeV α-particles promptly blocked neural transmission. The RBE for 910-MeV α-particles was 0.66.

According to Schmitz and Schaefer (29) 10 kR of x rays had no obvious effects on the bioelectric activity of frog sciatic nerves. Similarly, Janzen and Warren (30) found that the rat sciatic nerve was neither electro-physiologically nor histologically altered by 10 kR of 240-kV x rays.

Therefore, it was of interest that Bergstrom et al. (2) maintained that 10 krep (9.3 krad) of 5.3-MeV α-particles from ^{210}Po caused complete suppression of the action potential of frog nerve within a few minutes after irradiation, and that half this dose inhibited excitation at 30 minutes postirradiation. This was the lowest dose of radiation ever reported to cause the irreversible loss of neural responses from frog nerves. Radiobiologists accustomed to 8 to 10 krad as a threshold dose for neural damage (4,5,29 to 33) might regard the report of Bergstrom et al. (2) with suspicion. It is our contention that the low neural blocking dose of 5.3-MeV α-particles (2) can be accounted for on the basis of the high LET value of 5.3-MeV α-particles.

In Fig. 5 the logarithm of RBE values to promptly suppress neural responses in frog nerve were plotted against the logarithm of LET values of various radiations presented in Table I. RBE was found to increase as a function of LET for 47.5-MeV protons, 910-MeV α-particles, 260-kV x rays, 200-kV x rays, and 5.3-MeV α-particles, despite the fact that absorbed dose rates for these radiations varied considerably.

In Table I the LET determinations of 5.3-MeV α-particles from ^{210}Po, 47.4-MeV protons, and 910-MeV α-particles were based on the track segment method (6,7,8,34,35) and made use of table of energy losses of Barkas and Berger (36). The mean LET reported for 200-kV x rays was 2.5 keV/μ in air and 3.0 keV/μ in tissue (33). Since both the LET and track-length factors are not well defined for x rays, even mean LET values are subject to considerable uncertainty. A complicated total track length calculation based on the x-radiation spectrum (37,38) approximated LET values for x rays. The mean LET estimate for 260-kV x rays was 2.7 keV/μ in tissue.

The log (RBE versus LET) plot in Fig. 5 was based on experiments employing radiations with five different LET values. There is a serious lack

of RBE data corresponding to LET values between 3 and 110 keV/μ. This is because radiations within this LET range have very low penetration which dictate the use of single nerve fibers. Unfortunately, single nerve fibers have been scantily used in radiobiological studies.

It is unknown if the monotonic log (RBE versus LET) relation of Fig. 5 would go on indefinitely. In some biological systems RBE reaches a maximum with LET and with further increase of LET the RBE values decrease (11 to 13, 38,39). This behavior is explained on the basis that a critical volume for inactivation exists and with high-LET radiations more than one ion cluster is deposited in a critical volume. In such a case, the extra energy released is wasted. It remains to be determined if inactivation of nerve will follow this pattern.

The dose of various types of radiation to inactivate neural activity of frog nerve were presented in Table I. The actual inactivation dose of some jth species of radiation may be compared to a calculated inactivation dose, D_j, obtained by assuming:

$$D_j \times LET_j = D_{x\ ray} \times LET_{x\ ray} \qquad\qquad \text{Eq. (1)}$$

was valid over the LET range 1.3 to 100.0 keV/μ tissue. Since the actual inactivation dose ($D_{x\ ray}$) of 200 kV x rays is known experimentally, and the linear energy transfer of 200 kV x rays ($LET_{x\ ray}$) and the jth species of radiation (LET_j) is available, the value of D_j can be computed.

The calculated dose of 5.3-MeV α-particles to inactivate frog nerve was 7.8 krad by equation (1), which was sixteen percent lower than the actual inactivation dose listed in Table I. The α-particle dose rate error reported by Bergström et al. (40) was ± 30 percent. Even if the LET of α-particles from [210]Po (2,40) increased to 140 keV/μ in their passage through nerve, the calculated inactivation dose for 5.3-MeV α-particles would be in error by

thirty-four percent with respect to the real inactivation dose presented in Table I. In view of the large dose rate error in the Bergström et al. experiments (2,40), the calculated dose was sufficiently close to the actual inactivation dose for most purposes.

In Table I there is sufficient agreement between actual and calculated inactivation doses to suppress neural activity to warrant further inquiry into the relation between RBE and LET. There is a need to determine if neurologic effects of radiation can be made more meaningful as a function of LET.

RBE data for radiations with LET values less than 1.3 keV/μ of tissue were excluded from Table I. This table was also restricted to radiobiological information about frog nerves. Inquiry should not be prohibited which deals with the problems of the effects of low-LET radiations on neural tissue. Also, investigations of RBE-LET relations should not be restricted to frog nerve preparations. A few comments about the possible relevance of RBE to low-LET radiations is in order.

If frog nerves were irradiated with beta rays from ^{90}Sr with an LET of 0.2 keV/μ of tissue, the calculated inactivation dose would be 4.77×10^6 rad by equation (1). Recently, Kaack (41) reported that the neural responses of frog sciatic nerve were inhibited with 81.6 to 204 krad of beta (^{90}Sr) radiation. The RBE was 1.40 to 3.49 for this beta emitter. Since there is great disagreement between the real and calculated inactivation dose, it would appear that equation (1) is not relevant for beta (^{90}Sr) radiation. Although Kaack (42) found that beta rays from ^{90}Sr required 4.00×10^6 rad to block impulse transmission in the posttrunk of the turtle's superior ganglia, the use of equation (1) to predict beta ray inactivation doses for turtle nerve is questionable and requires further investigation.

The mean LET value reported for gamma (^{60}Co) radiation was 0.3 keV/μ of tissue (23,43). Equation (1) predicts that 2.86×10^6 rad of gamma (^{60}Co) radiation would be the inactivation dose to suppress neural activity. Bachofer et al. (44) stated that it required 308 kR (296 krad) at pH 7.2 and 388 kR (373 krad) at pH 7.7 of gamma (^{60}Co) radiation to block neural activity of the frog sciatic nerve. Hence, the RBE of gamma rays was 0.96 (pH 7.2) and 0.76 (pH 7.7). Since the actual and calculated inactivation doses are not in agreement, it would appear that equation (1) cannot be employed when the source of radiation is gamma rays from ^{60}Co. Yet, Kaack (45) indicated that gamma rays from ^{60}Co required 1.44×10^6 rad to suppress neural conduction of the posttrunk of the turtle's superior cervical ganglia. This gamma inactivation dose is close to the calculated inactivation dose obtained by equation (1). It is possible that the agreement between actual and calculated inactivation for gamma (42) and beta (41) radiations is fortuitous for turtle's nerve.

The bioelectric responses of excitable neural membranes to radiation are intriguing, although perplexing. It was observed in this report that x-ray and proton irradiation could retard conduction velocity and increase the detection period at doses that were innocuous to the action potential amplitude. The radiobiologic mechanism for this differential action is not known.

Conduction velocity in rat caudal nerve (17 to 19) and earthworm fibers (46) have been reported to increase with the onset of x-irradiation. Some earthworm fibers had conduction velocities sixty-three percent faster during the preirradiation phase than at the start of irradiation (46); some rat caudal nerves exhibited conduction velocities twenty-one to fifty-seven percent faster 20 minutes before irradiation than at the initiation of irradiation

(18,19). The proportion of nerves with optimal physiological responses before irradiation was not specified (18,19). These preirradiation, optimal conduction velocity values make the effects of radiation difficult to interpret. Irradiation enhanced conduction velocity in both the rat and earthworm nerve preparations failed to achieve optimal, preirradiation conduction velocity.

Conduction velocity in the frog nerve preparations used in this research exhibited negligible variation during the preirradiation interval. With the onset of either x-ray or proton irradiation, no increase in conduction velocity was evident. When frog nerves absorbed about one-fourth of the inactivation dose, conduction velocity became retarded, not increased. The MAP amplitude remained unchanged at one-fourth the inactivation dose for x rays and protons, even though conduction velocity was diminished.

Gerstner et al. (4) reported that velocity of nerve conduction was unchanged until bullfrog nerves received approximately 100 R (95 rad) of x rays. The present findings are in concert with the observation of Gerstner et al. (4).

The amplitude of bioelectric responses from rat caudal nerves (17 to 19) and turtle's superior cervical ganglion (41,42) were reported to increase with the onset of radiation. Allen and Nicholls (20) irradiated rat caudal nerves and rat phrenic nerves and found no radiation enhancement of the MAP amplitude. Action potential amplitudes of frog nerves did not demonstrate a radiation augmentation when exposed to high-energy α-particles (3).

The findings in the present report indicate that neither 100 krad of x rays nor 250 krad of protons increased the MAP amplitude of frog sciatic nerves.

Beta irradiation of sciatic nerves were reported to cause no enhancement

of action potentials by Yamashita and Miyasaka (47) and variable responses

by Kaack (44).

Experimental evidence to explain why the action potential amplitude

appears to increase with radiation in some cases and not in others is lacking.

ACKNOWLEDGEMENT

REFERENCES

1. R.M. Bergström, The effect of 5.3 MeV Polonium[210] alpha-particles on the electrical activity of the frog sciatic nerve. Ann. Med. Exp. Fennicae **39**, 211-215 (1961).

2. R.E. Bergström, R.F. Blafield, and M.W. Brenner, The effect of α-particles from a ring-shaped Po[210] source on the action potential of single nerve fibers of the frog. Ann. Acad. Sci. Fennicae, Ser. A. V. **82**, 1-10 (1961).

3. C.T. Gaffey, Bioelectric effects of high energy irradiation on nerve. In _Response of the Nervous System to Ionizing Radiation_ (T.J. Haley and R.S. Snider, eds.), pp. 277-296, Academic Press, New York, 1962.

4. H.B. Gerstner, J.S. Orth, and E.O. Richey, Effect of high-intensity x-radiation on velocity of nerve conduction. Am. J. Physiol. **180**, 232-236 (1955).

5. H.B. Gerstner, Effect of high-intensity x-irradiation on the A group fibers of the frog's sciatic nerve. J. Am. Physiol. **184**, 333-337 (1956).

6. R.E. Zirkle, The radiobiological importance of the energy distribution along ionizing tracks. J. Cell. and Comp. Physiol. **16**, 221-235 (1940).

7. R.E. Zirkle, D.F. Marchbank, and K.D.Kuck, Exponential and sigmoidal survival curves resulting from α and x irradiation of Aspergillus spores. J. Cell. and Comp. Physiol. **39**, Suppl. 1, 75-85 (1952).

8. R.E. Zirkle, The radiobiological importance of linear energy transfer. In _Radiation Biology_ (A. Hollaender, ed.), Vol. 1, pp. 315-350, McGraw-Hill Book Company, New York, 1954.

9. H.H. Rossi, Spatial distribution of energy deposition by ionizing radiation. Radiation Res., Suppl. 2, 290-299 (1960).

10. H.H. Rossi, Energy distribution in the absorption of radiation. Adv. Biol. Med. Phys. 11, 27-85 (1967).

11. J.W. Boag, The relative biological effectiveness of different radiations. Natl. Bur. Standards (U.S.), Report No. 2946 (1953).

12. J.B. Storer, P.S. Harris, J.E. Furchner, and W.H. Langham, The relative biological effectiveness of various ionizing radiations in mammalian systems. Radiation Res. 6, 188-288 (1957).

13. H.H. Rossi, J.L. Bateman, V.P. Bond, L.J. Goodman, and E.E. Stickley, The dependence of RBE on the energy of fast neutrons. 1. Physical design and measurement of absorbed dose. Radiation Res. 13, 503-520 (1960).

14. S. Russak and K. Richardson, Proton flux, dosage, and damage estimates in the Van Allen belt. In Second Symposium on the Protection Against Radiation in Space (A. Reitz, ed.), pp. 251-256, Scientific and Technical Information Division, NASA SP-71, Washington, 1965.

15. C.A. Sondhaus and R.D. Evans, Dosimetry of radiation in space flight. In Radiation Dosimetry (F.H. Attix, E. Tochilin, eds.), Vol. 3, second edition, pp. 453-522, Academic Press, New York, 1969.

16. W. Langham (ed.), Radiobiological Factors in Manned Space Flight. National Academy of Sciences, Research Council, Washington, 1967.

17. C.S. Bachofer, Enhancement of activity of nerves by x-rays. Science 125, 1140-1141 (1957).

18. C.S. Bachofer and M.E. Gautereaux, Bioelectric activity of mammalian nerve during x-irradiation. Radiation Res. 12, 575-586 (1960).

19. C.S. Bachofer, Radiation effects on isolated nerves. In Response of the Nervous System to Ionizing Radiation (T.J. Haley and R.S. Snider, eds.), pp. 573-596, Academic Press, New York, 1962.

20. N. Allen and J.G. Nicholls, A study of the effects of x-rays on the electric properties of mammalian nerve and muscle. Proc. Roy. Soc. (London), Ser. B, 157, 536-561 (1963).

21. C.T. Gaffey, The response of maximal and submaximal action potentials from sciatic nerve to 200 kV x rays. Radiation Res. 45, 311-325 (1971).

22. P.H. Mitchell, A Textbook of General Physiology, 4th ed., McGraw-Hill Book Co., New York, 1948.

23. H. Johns, The Physics of Radiology, 2nd ed., Charles C. Thomas, Springfield, 1961.

24. Report of the International Commission on Radiological Units and Measurements (ICRU) 1959, National Bureau of Standards, Handbook 78, 1961.

25. A.C. Birge, H.O. Anger, and C.A. Tobias, Heavy charged-particle beams. In Radiation Dosimetry (C.J. Hine and G.L. Brownell, eds.), pp. 623-665, Academic Press, New York, 1956.

26. R.L. Fulton, L.R. Glasgow, and N.W. Yanni, Modification of the 88-inch and 184-inch cyclotron: biological research facility. Lawrence Radiation Laboratory Report UCRL-1128, University of California, Berkeley, 1963.

27. M.R. Raju, The use of miniature silicon diode as a radiation dosimeter. Phys. Med. Biol. 2, 371-376 (1966).

28. J.T. Lyman, Private communication.

29. W. Schmitz and H. Schaefer, Uber den Einfluss der Röentgenstrahlen auf den Nervenaktionsstrom. Strahlentherapie 46, 546-568 (1933).

30. A.M. Janzen and S. Warren, Effect of roentgen rays on the peripheral nerve of the rat. Radiology 38, 333-337 (1942).

31. J. Audiat, D. Auger, and A. Fessard, Etude des potentiels d'action des nerfs soumus su rayonment X. Comparison avec l'action des rayons ultraviolets. Compt. Rend. Soc. Biol. 116, 880-883 (1934).

32. E.L. Gasteiger, Effects of beta rays on mammalian nerve action potentials. Federation Proc. <u>10</u>, 48 (1951).

33. E. Linder, Uber das funktionelle und morphologische Verhalten peripherer Nerven längere Zeit nach Bestrahlung. Fortschr. Gebiete Röentgenstrahlen Nuklearmed. <u>90</u>, 618-624 (1959).

34. R.E. Zirkle and C.A. Tobias, Effects of ploidy and linear energy transfer on radiobiological survival curves. Arch. Biochem. Biophys. <u>47</u>, 282-306 (1953).

35. G.W. Barendsen, H.M.D. Walter, J.F. Fowler, and D.K. Bewley, Effects of different ionizing radiations on human cells in tissue culture. III. Experiments with cyclotron-accelerated alpha particles and deuterons. Radiation Res. <u>18</u>, 106-119 (1963).

36. W.H. Barkas and M.J. Berger, Tables of energy losses and ranges of heavy charged particles. In <u>Studies in Penetration of Charged Particles in Matter</u>. Scientific and Technical Information Division, NASA-NRC 1133, Washington, D.C., 1964.

37. D.V. Cormack and H.E. Johns, Electron energies and ion densities in water irradiated with 200-keV, 1-MeV and 25-MeV radiations. Brit. J. Radiol. <u>25</u>, 369-381 (1952).

38. D.E. Lea, <u>Actions of Radiations on Living Cells</u>, 2nd ed., Cambridge University Press, London, 1955.

39. G.W. Barendsen, The importance of microdosimetry for radiation biology and radiation protection. In <u>Proceedings of the Symposium on Microdosimetry</u> (H.G. Ebert, ed.), pp. 1-22, published by the European Communities, Brussels, 1968.

40. R.M. Bergström, R.F. Blafield, and M.W. Brenner, Irradiation of single nerve fibers with α-particles from a circular polonium[210] source. Acta

Physiol. Scandinav. 50, 24-25 Suppl. 175 (1960).

41. B. Kaack, Effect of beta irradiation on the oxygen consumption in the frog sciatic nerve. Radiation Res. 42, 405-412 (1970).

42. B. Kaack, Effect of beta irradiation on ganglionic bioelectric activity. Am. J. Physiol. 213, 1451-1458 (1967).

43. D.J. Rees, Health Physics, The M.I.T. Press, Butterworth and Co., Cambridge, 1967.

44. C.S. Bachofer, M.E. Gautereaux, and S.M. Kaack, Relative Sensitivity of isolated nerves to Co^{60} gamma rays. In Response of the Nervous System to Ionizing Radiation (T.J. Haley and R.S. Snider, eds.), pp. 221-242, Little, Brown and Co., Boston, 1964.

45. B. Kaack, Augmentation of bioelectric activity by gamma irradiation. Am. J. Physiol. 213, 625-628 (1967).

46. C.S. Bachofer and M.E. Gautereaux, X-ray effects on single nerve fibers. J. Gen. Physiol. 42, 723-735 (1959).

47. H. Yamashita and T. Mikosaka, Effects of beta rays upon single nerve fiber. Proc. Soc. Exp. Biol. Med. 80, 375-377 (1962).

LEGENDS

Fig. 1 Maximal action potentials detected at different locations along the length of an isolated frog sciatic nerve. The white numbers in upper right corner of each dual-beam oscillogram indicated the kiloroentgens of x-rays delivered to the nerve.

Fig. 2 Relative values of the maximal action potential amplitude, conduction velocity, and detection period as a function of the amount of x-rays delivered to the nerve. The action potentials of Fig. 1 provided the information for this diagram.

Fig. 3 Dual-beam oscillograms of maximal action potentials recorded from a frog sciatic nerve at different sites along the length of the nerve. On each oscillogram was superimposed the proton dose in kilorads absorbed by the nerve.

Fig. 4 Relative values of maximal action potential amplitude, conduction velocity, and detection period as a function of the proton dose absorbed by a nerve. This figure was constructed from the action potentials in Fig. 2.

Fig. 5 In this study the relative biological effectiveness (RBE) refers to the ratio of the absorbed dose of 200 kV x rays required to promptly block impulse conduction by frog nerve to the absorbed dose of test radiations to produce the same effect. Linear energy transfer (LET) refers to the loss of energy of an ionizing particle per unit length of path traveled through tissue.

RBE VALUES OF RADIATIONS HAVING DIFFERING LET DISTRIBUTIONS

TO INHIBIT IMPULSE CONDUCTION IN FROG NERVE

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RBE Values of Radiations Having Differing LET Distributions to

Inhibit Impulse Conduction in Frog Nerve.

ABSTRACT

It was found that 285 krad (average) of 200-kV x rays promptly blocked neural transmission of frog sciatic nerves. To produce this same inhibition in the same type of nerve preparation required 600 krad (average) of 47.5-MeV protons. Hence, the relative biological effectiveness (RBE) of protons was 0.48 with respect to x rays, which was assigned an RBE of 1.00. From the reports of other investigators the RBE was computed to be 0.66 for 910-MeV α-particles, 0.87 for 260-kV x rays, and 30.60 for 5.3-MeV α-particles.

The RBE values to inhibit neural responses in frog nerves were found to increase as a function of the linear energy transfer (LET) for radiations with LET values between 1.3 and 110.00 keV/μ of tissue, despite the fact that the absorbed dose rates varied considerably with different species of radiation.

An equation to estimate the dose of radiation to block neural activity was provided. This equation was dependent on the RBE-LET relation described.

When sciatic nerves absorbed about one-fourth of the radiation dose to completely block neural responses, conduction velocity was impaired and the detection period was increased. The action potential amplitude was not altered with this amount of proton or x-irradiation.

Key words: action potentials; frog nerves; RBE; LET

INTRODUCTION

The purpose of this work was to determine the radiation dose to block impulse conduction in isolated frog sciatic nerves with 47.5-MeV protons (^1_1H) and 200-kV x rays. This information permitted the relative biological effectiveness (RBE) of protons to be established with respect to x rays. Since the radiation dose to suppress excitation in frog nerve was available for 5.3-MeV α-particles (1,2), 910-MeV α-particles (3) and 260 kV x rays (4,5), the RBE values of these radiations was considered.

The possibility of a dependency of RBE on the linear energy transfer (LET) to suppress neural activity was investigated. LET is usually expressed in terms of the loss of energy of a particle per unit of the path traveled (6,7,8,9,10). The RBE of some biological systems depend not only on the total amount of radiant energy absorbed, but also on the distribution of energy deposited along particle tracks, i.e. LET (11,12,13). It is not known if a RBE-LET relation exists for nerves subjected to radiation. Current hypotheses about radiobiological mechanisms require information on whether RBE is or is not a function of LET.

The flux of low energy protons trapped in the inner Van Allen belt (14,15,16) have about the same degree of penetration and energy per nucleon as the cyclotron-accelerated protons which are administered to nerve preparations in this study. Since eighty-five percent of the radiation encountered in interplanetary space are protons, information on the radiosensitivity of nerves to protons has relevance to the galactic space environment and the radiation hazard there.

It has been asserted that the action potential amplitude and conduction velocity of rat caudal nerves were regularly increased or enhanced with the onset of irradiation (17,18,19). Allen and Nicholls (20) repeated these experiments on rat caudal nerve preparations and rat phrenic nerves and could find no radiation enhancement of neural activity.

In the present studies, frog sciatic nerves were exposed to x-ray and proton irradiation while bioelectric responses were being measured. It was of interest to relate changes in action potential amplitude, conduction velocity, and detection period (stimulus-response interval) to the radiation dose administered to nerves.

METHODS

Biological Procedure:

Adult frogs (Rana pipiens) were employed in these experiments. The procedure and electrophysiologic equipment used to generate and record action potentials from isolated sciatic nerves was previously detailed (3,21).

Isolated nerves were maintained in frog Ringer's solution (22) at least sixty minutes before being tested for bioelectric activity. Some nerves were held in Ringer's solution for several hours before proton irradiations, because of the difficulties involved in obtaining and adjusting a cyclotron particle beam to a selected energy for protons.

Sciatic nerves were individually placed inside a moist chamber on Ag-AgCl electrodes. This plastic chamber had a reservoir of water to maintain a high relative humidity. The stimulus strength delivered to the central end of each nerve was regulated to evoke

a maximal action potential (MAP). MAPs were detected from different sites along the peripheral length of the nerve preparation and displayed on an oscilloscope. If the MAP amplitude remained unchanged for at least ten minutes while the nerve was stimulated at 20 p.p.s., the nerve preparation was considered physiologically stable and was subjected to either proton or x-irradiation.

The moist chamber housing a sciatic nerve was positioned to intercept either a beam of protons or x rays prior to recording MAPs. The orientation and location of the moist chamber thereafter was never changed for a given nerve study. Action potentials were photographed before, during, and after irradiation.

X-irradiation Procedure:

A moist chamber was positioned so that its 1.0-mil Mylar window was between the nerve chamber and the exit port of a therapeutic x-ray machine.

The x-ray machine was operated at 200-kV, 15-ma; the inherent filtration of the oil in the x-ray tube was equivalent to 1.0 mm Al; extra filtration consisted of 0.5 mm Al plate, an air path of 8.0 mm and the 1.0 mil Mylar window of the moist chamber. The focus-to-target distance was 13.6 cm.

The quality of the x-ray beam in terms of the half value layer (HVL) was determined by the procedure described by Johns (23). The HVL was found to be 0.83 mm Cu.

Isolated nerve preparations received 1,700 $\pm$ 9 R/min, as determined by a Victoreen condenser ionization chamber. This dose rate included backscatter at the surface of nerves.

Since it will be of interest to determine the RBE of different types of radiation, x-ray exposures need to be expressed in terms of the dose absorbed (rad) by nerves, D_{nerve}. The absorbed dose under electronic equilibrium conditions is proportional to exposure dose, d, according to the equation:

$$D_{nerve} = d \times \overline{f}$$

where $\overline{f}$ is the conversion factor from roentgens to rads (24). For practical purposes values for the conversion factor for water, muscle, and bone have reported as a function of the HVL for the radiation employed (24). The conversion factor, $\overline{f}$, was 0.946 for radiations with a HVL of 0.83 mm Cu. The $\overline{f}$ value for muscle was taken as the closest approximation to nerve.

Proton Irradiation Procedure:

An isolated sciatic nerve rested on five Ag-AgCl electrodes in a plastic, moist chamber prior to irradiation. Stimulation of the nerve and detection of MAPs was performed remotely.

The 88-inch sector-focused cyclotron at the Lawrence Radiation Laboratory in Berkeley served as a source of 47.5-MeV protons. Cyclotron-accelerated particles of $^{1}_{1}H$ were conducted in a four-inch diameter vacuum pipe from the cyclotron to a biophysical research cave. A quadrupole magnet focused the cyclotron's beam and a particle absorbing collimator restricted the profile to a circular beam.

The proton dose was measured with a transmission ionization chambers (25) placed directly in the beam approximately 2 to 3 mm before the target nerve. As the proton beam passed through the ionization chamber, a charge was collected. This integrated charge was related to the average dose absorbed by the nerve (25). Faraday

cup (26) and silicon diode (27) measurements of the proton dose rate yielded values in agreement with the transmission chamber.

The proton dose rate employed in these experiments was $50 \pm$ 1.5 krad/min, except in studies in which the dose rate was specifically decreased.

By tuning the rf system of the 88-inch cyclotron to 15 MHz, protons with an average energy of 47.5-MeV/nucleon were generated with a maximum beam current of 60μA. The external beam power in this operating state was 2,850 watts (47.5-MeV x 60μA). The range of 47.5-MeV protons was 2.31 g/cm^2 in air and 2.00 g/cm^2 in water. The stopping power of these proton particles was 11.488 MeV-cm^2/g or 1.1488 keV/μ in air. The linear energy transfer (LET) for 47.5-MeV protons in water was 13,139-MeV-cm^2/g or 1.3139-keV/μ.

Cables connected the nerve in the moist chamber to appropriate electrophysiologic equipment in a shielded cave. Action potentials from nerve preparations were viewed continuously on an oscilloscope, before, during, and after irradiation.

In these experiments protons were restricted by a collimator to a circular beam 6.2 mm in diameter. This proton beam intercepted the nerve between the stimulating electrodes and the recording electrodes.

The effects of irradiating the whole sciatic nerve preparation with protons was tested and gave results similar to those to be reported.

RESULTS

Isolated sciatic nerves were placed on Ag-AgCl electrodes in a moist chamber and the chamber was sealed. Action potentials were

generated by 10^{-5} sec rectangular pulses delivered to nerve preparations at 20 p.p.s. The strength of the stimulus was regulated to evoke maximal action potentials (MAP).

Electrical stimulation of nerves was continuous, i.e. MAPs were generated during the preirradiation and irradiation period without interruption. About one minute before each action potential was photographed, the stimulus strength was adjusted to insure that the observed action potential was a MAP. If the MAP amplitude of a given nerve preparation remained unchanged during the preirradiation interval, the irradiation procedure was initiated with the nerve in place.

Nerves were maintained in moist chambers for about 3.5 hours during x-ray exposures. Nonirradiated nerves housed in moist chambers maintained their action potentials for thirty-two hours while being continually stimulated.

Bioelectrical Effects of X rays:

The radiation exposure to immediately suppress neural excitability was determined by continually monitoring the MAP of nerves administered 200-kV x rays. In twenty experiments neural transmission was completely inhibited from 292 to 304 kR with an average of 300 kR for x rays. An exposure dose of 300 kR of x rays is equivalent to an absorbed dose of 285 krad (300 kR x 0.95 krad/kr).

Conduction velocity of neural impulses was studied as a function of the x-ray dose delivered to nerves. Conduction velocities were calculated from the interval between two action potential peaks, recorded with reversible electrodes at different distances

along the length of nerves. Such conduction velocity determinations were free from the interference that could arise from variable latent period responses induced by radiation.

Fig. 1 is a composition of MAPs recorded from a nerve during a typical experiment. Before irradiation (0 kR in Fig. 1) the MAP amplitudes were 10.7 mV and conduction velocity was 28 M/sec. The distance between recording electrodes in this trial was 10 mm. MAP amplitudes were measured from the zero baseline to the peak of the negative wave. The detection period, defined as the interval between the stimulus shock and the rise of the action potential, was 0.3 msec for the first MAP and 0.8 msec for the second MAP prior to irradiation (0. kR in Fig. 1). During a 30 min preirradiation period, MAP amplitudes, conduction velocity, the detection periods, and the stimulus strength to evoke MAPs were not altered. Thereafter, x-irradiation was applied to the nerve preparation until its bioelectric responses ceased.

During the preirradiation period the conduction velocity, MAP amplitudes, and detection periods for any given nerve preparation remained stable, but variations between nerve preparations were found. The conduction velocity of twelve preirradiated nerves varied from 27 to 34 M/sec; MAP amplitudes ranged from 9 to 12 mV. The detection periods before irradiation ranged from 0.30 to 0.33 msec for the first MAP and from 0.60 to 0.80 msec for the second MAP.

The computed conduction velocity, relative MAP amplitude, and detection period of the action potentials in Fig. 1 were plotted as a function of the x-ray exposure in Fig. 2. The relative MAP

amplitude was the ratio of the MAP amplitudes, A/A_o, where A was the MAP amplitude for a particular exposure of x rays and A_o was the MAP amplitude with zero R of x rays. Conduction velocity and the detection period were plotted as percent changes from their preirradiated values.

From Fig. 1 and 2 it can be seen that nerve exposed to 75 kR (71.3 krad) of x rays suffered a retardation of conduction velocity and a lengthening of the detection period, even though the MAP amplitude remained unchanged. Four times this amount of radiation was required to inhibit neural responses.

In some experiments 60 kR (57 krad) was sufficient to impair conduction velocity and increase the detection period. All nerve preparations demonstrated harmful conduction velocity and detection period changes after receiving 90 kR (85 krad) of x rays.

When twenty nerves were administered x rays, there was no immediate increase in the MAP amplitude at the onset of irradiation, as was reported elsewhere (17,18,19). It was our finding that MAP amplitudes remained unchanged, even when nerves were exposed to 100 kR (95 krad) of x rays. However, fourteen out of twenty nerve preparations exhibited a five to twelve percent increase in MAP amplitude with 125 to 175 kR (118.8 to 166.3 krad) of x rays. These same nerves revealed increases in the duration (measured from the rise of the action potential until it reached the zero voltage baseline) of MAPs, reduction in conduction velocity, and increases in the detection period preceding increases in MAP amplitudes. Six x-irradiated nerves did not demonstrate heightened MAP amplitudes, only attenuation; conduction velocity

reduction, detection period lengthening, and MAP duration spreading were evidenced after these six nerves received about 75 kR (71.3 krad) of x rays.

The stimulus strength to evoke MAPs was not significantly altered when nerves were exposed to 100 kR (95 krad). In those instances in which MAP amplitudes increased, the stimulus strength decreased. It was necessary to increase the stimulus strength to all nerve preparations during the attenuation of the MAP amplitude preceding the loss of excitation.

Bioelectric Effects of Protons:

Individual nerves were sealed in a moist chamber and stimulated to generate MAPs. If the MAP amplitude of a nerve remained stable for ten minutes, the segment of the nerve between the stimulating and recording electrodes was irradiated with a circular beam (6.2 mm diameter) of 47.5-MeV protons.

A dose rate of 50 ± 1.5 krad of protons/min was employed to irradiate nerve preparations, except where the dose rate was specifically mentioned as being otherwise.

The MAPs of eighteen nerves were completely suppressed when they absorbed 580 to 620 krad of protons. The mean dose to block neural responses to electrical stimulation was 600 krad of 47.5-MeV protons.

Fig. 3 is a serial representation of on-the-line MAPs from a nerve that received proton irradiation. Before proton exposure the nerve required 0.1 msec for an impulse to pass between recording electrodes separated by 3.0 mm. Hence, the preirradiation

conduction velocity in this preparation was 30 M/sec (0 krad in Fig. 3). The preirradiation MAP amplitudes were 10 mV (Fig. 3).

The variation of preirradiation electrophysiological parameters from nerve-to-nerve was similar to the variation mentioned for pre-irradiated x-ray nerve preparations.

Conduction velocity, relative MAP amplitude, and the detection period data obtained from oscillograms in Fig. 3 were related to the nerve's absorbed proton dose in Fig. 4. Conduction velocity slowed and the detection period increased with 150 to 200 krad of protons, while the relative MAP amplitude remained unaltered (Fig. 4). Even after the nerve absorbed 300 krad of protons, the MAP amplitude remained unchanged. The inactivation dose to inhibit neural transmission in this nerve was 600 krad of protons (Fig. 3 and 4).

All nerves administered 240 krad of protons had retarded conduction velocities and prolonged detection periods. Radiation changes in conduction velocity and the detection period were pro-voked by 150 krad of protons in many nerve preparations.

In fifteen experiments there was no increase in the MAP ampli-tude of nerves administered 250 krad of protons. In general, MAP amplitudes were not increased with massive doses of protons just prior to rapid attenuation and final loss of the MAP, as was ob-served in most experiments with 200-kV x rays. Only three proton irradiated nerves in eighteen experiments revealed a four to twelve percent increase in the MAP amplitude. This increase in the MAP amplitude required about 250 to 350 krad of protons.

Bachofer and Gautereaux (18) observed immediate increases in

the action potentials amplitude of rat caudal nerve preparations with 280-kV x rays. They reported that 6 kR/min of x rays gave considerable enhancement of neural activity, while at higher dose rates the radiation augmented activity was less, and at lower dose rates, irradiation enhancement was greater, but less reproducible. The absorbed x-ray dose rate employed by Bachofer and Gautereaux (18) was equivalent to 5.7 krad/min.

To test the effects of dose rates on MAP amplitude of sciatic nerves, the intensity of the 47.5-MeV proton beam was adjusted to 2.6, 5.6, and 6.8 krad of protons/min. Two nerves were individually irradiated at each of these dose rates. The MAP amplitudes of these six nerves were not augmented at the onset of irradiation. Even after each nerve preparation had absorbed about 200 krad of protons, MAP amplitudes were not increased.

DISCUSSION

In this report the relative biological effectiveness (RBE) was defined as the ratio of D_x/D_t, where D_x was the absorbed dose of 200-kV x rays to suppress impulse conduction in frog nerve and D_t was the absorbed dose of a test radiation to produce the same effect. It was found that 285 krad (average) of 200-kV x rays blocked neural transmission. To produce this identical response with the same type of nerve required 600 krad (average) of 47.5-MeV protons. Hence, the RBE of protons was 0.48 with respect to x rays, which was assigned an RBE of 1.00 (Table I).

It was reported (4,5) that exposures greater than 300 and less than 390 kR (285 to 370 krad) of 260-kV x rays produced prompt

abolishment of neural function in frog sciatic nerve. The average
of these exposures, 345 kR (329 krad), was used as the neural block-
ing dose for 260-kV x rays in Table I. Thus, the RBE of 260-kV
x rays was 0.87.

The apparent dose of 910-MeV α-particles to inhibit neural
responses in frog sciatic nerves was reported to be 330 krad (3).
The actual inactivation dose in these experiments was 430 krad
of 910-MeV α-particles. This revision was based on dosimetric
studies performed on the 910-MeV α-particle beam by J. T. Lyman
(27). In 1970 sciatic nerves were irradiated with 910-MeV α-par-
ticles at the 184-inch cyclotron at the Lawrence Radiation Labora-
tory, Berkeley. It was confirmed by the author that 430 krad of
910-MeV α-particles promptly blocked neural transmission. The RBE
for 910-MeV α-particles was 0.66.

According to Schmitz and Schaefer (29) 10 kR of x rays had
no obvious effects on the bioelectric activity of frog sciatic
nerves. Similarly, Janzen and Warren (30) found that the rat sciatic
nerve was neither electrophysiologically nor histologically altered
by 10 kR of 240-kV x rays. Therefore, it was of interest that
Bergstrom et al. (2) maintained that 10 krep (9.3 krad) of 5.3-
MeV α-particles from ^{210}Po caused complete suppression of the action
potential of frog nerve within a few minutes after irradiation, and
that half this dose inhibited excitation at 30 minutes postirradia-
tion. This was the lowest dose of radiation ever reported to cause
the irreversible loss of neural responses from frog nerves. Radio-
biologists accustomed to 8 to 10 krad as a threshold dose for neural
damage (4,5, 29 to 33) might regard the report of Bergstrom et al. (2)

with suspicion. It is our contention that the low neural blocking
dose of 5.3-MeV α-particles (2) can be accounted for on the basis
of the high-LET of 5.3-MeV α-particles.

In Fig. 5 the logarithm of RBE values to promptly suppress
neural responses in frog nerve were plotted against the logarithm
of LET values of various radiations presented in Table I. RBE was
found to increase as a function of LET for 47.5-MeV protons, 910-
MeV α-particles, 260-kV x rays, 200-kV x rays, and 5.3-MeV α-par-
ticles, despite the fact that absorbed dose rates for these radia-
tions varied considerably.

It should be stated that LET determinations of 5.3-MeV α-par-
ticles from ^{210}Po, 47.4-MeV protons, and 910-MeV α-particles were
based on the track segment method (6,7,8,34,35) and made use of
table of energy losses of Barkas and Berger (36). The mean LET
reported for 200-kV x rays was 2.5 keV/μ in air and 3.0 keV/μ in
tissue (33). Since both the LET and track-length factors are not
well defined for x rays, even mean LET values are subject to consid-
erable uncertainty.. A complicated total track length calculation
based on the x-radiation spectrum (37,38) approximated LET values
for x rays. The mean LET estimate for 260-kV x rays was 2.7 keV/μ
in tissue.

The log (RBE versus LET) plot in Fig. 5 was based on experi-
ments employing radiations with only five different LET values.
There is a serious lack of RBE data corresponding to LET values
between 3 and 110 keV/μ. This is because radiations within this
LET range have very low penetration which dictate the use of single
nerve fibers. Unfortunately, single nerve fibers have been rarely

used in radiobiologic studies.

It would be expected that the monotonic log (RBE versus LET) relation of Fig. 5 would not go on indefinitely. With high-LET radiations a physical state would be reached in which more energy would be deposited than is required for neural inhibition. The log (RBE versus LET) relation would then exhibit saturation and perhaps, decrease if the pattern in other biological systems is followed (11 to 13, 38,39).

In determining the relative biological effectiveness of a test radiation (RBE_t), it was assumed:

$$D_t \times RBE_t = D_x \times RBE_x \qquad \text{Eq. 1}$$

where D_x was the absorbed dose of 200-kV x rays to block neural activity, D_t was the absorbed dose of a test radiation to produce the same radiobiologic effect, and RBE_x was 1.0 by definition for 200-kV x rays. The absorbed dose of various radiations required to inhibit neural activity was found to be inversely proportional to the relative biological effectiveness (Table I).

The calculated doses of radiation to block conduction in frog nerve were listed in Table I. These calculated inactivation doses (D_t) were determined by assuming:

$$D_t \times LET_t = D_x \times LET_x \qquad \text{Eq. 2}$$

was valid over the LET range 1.3 to 110 keV/μ tissue. If the product of the linear energy transfer (LET_x) and actual inactivation dose (D_x) for 200-kV is known, and the linear energy transfer of the test radiation (LET_t) is available, the D_t can be computed.

The calculated dose of 5.3-MeV α-particles to inactivate frog nerve was 7.8 krad, which was 16% lower than the actual inactivation

dose. The α-particle dose rate error reported by Bergstrom et al. (40) was ± 30%. If it is assumed that the LET of α-particles from ^{210}Po was increased to 140-keV/μ in the nerves irradiated (2), the calculated dose would be in error by 34% with respect to the actual inactivation dose. In view of the large dose rate error in the Bergstrom et al. experiments (2,40), the calculated inactivation dose in Table I was close enough to the real inactivation dose for most purposes.

There is sufficient agreement between actual and calculated inactivation doses to suppress neural activity with various species of radiation (Table I) to warrant further inquiry into the relation between RBE and LET. There is a need to determine if neurologic effects of radiation can be made more meaningful as a function of LET.

Kaack irradiated the posttrunk of the turtle's superior cervical ganglia with beta rays from $^{90}Sr.$(41) and gamma rays from ^{60}Co (42). The mean LET estimate for beta (^{90}Sr) irradiation was 0.2 keV/μ of tissue; the mean LET reported for gamma (^{60}Co) was 0.3 keV/μ of tissue (23,43). If the radiosensitivity of frog nerve and turtle excitable membranes are assumed to be the same, then equation (2) would predict that it would require 4.27×10^6 rad of beta (^{90}Sr) and 2.86×10^6 rad of gamma (^{60}Co) radiation to suppress bioelectric activity. The actual inactivation doses reported were 4.00×10^6 rad for beta irradiation (41) and 1.50×10^6 R or 1.44×10^6 rad of gamma irradiation (42). Considering the assumptions involved in the use of equation (2) here, the agreement between actual and calculated inactivation doses is acceptable.

Recently, Kaack (44) reported that neural responses of frog sciatic nerve were inhibited with 81.6 to 204 krad of beta (^{90}Sr) radiation. The RBE of beta (^{90}Sr) radiation was 1.40 to 3.49. Since equation (2) predicted an inactivation dose greater than the observed dose, equation (2) is not valid in this instance.

Bachofer et al. (45) found that it required 308 kR (296 krad) at pH 7.2 and 388 kR (373 krad) at pH 7.7 of gamma (^{60}Co) radiation to block neural activity in frog sciatic nerves. Hence, the RBE of gamma (^{60}Co) radiation was 0.96 (pH 7.2) and 0.77 (pH 7.7). Equation (2) is not applicable in this case since the disagreement between real and calculated inactivation doses were large.

The bioelectric responses of excitable neural membranes to radiation are intriguing, although perplexing. It was observed in this report that x-ray and proton irradiation could retard conduction velocity and increase the detection period at doses that were innocuous to the action potential amplitude. The radiobiologic mechanism for this differential action is not known.

Conduction velocity in rat caudal nerve (17 to 19) and earthworm fibers (46) have been reported to increase with the onset of x-irradiation. Some earthworm fibers had conduction velocities 63% faster during the preirradiation phase than at the start of irradiation (46); some rat caudal nerves exhibited conduction velocities 21 to 57% faster 20 minutes before irradiation than at the initiation of irradiation (18,19). The proportion of nerves with optimal physiological responses before irradiation was not specified (18,19). These preirradiation, optimal conduction velocity values make the effects of radiation difficult to interpret.

Irradiation enhanced conduction velocity in both the rat and earth-worm nerve preparations failed to achieve optimal, preirradiation conduction velocity.

Conduction velocity in the frog nerve preparations used in this research exhibited negligible variation during the preirradiation interval. With the onset of x-ray or proton irradiation, no increase in conduction velocity was manifest. Only after frog nerves absorbed about one-fourth of the radiation dose to fully block conduction with x rays and protons was conduction velocity altered, and this change was a decrease in conduction velocity. Conduction velocity slowing in frog nerve preparations with x rays and protons took place while the action potential remained unchanged.

Gerstner et al. (4) reported that velocity of nerve conduction was unchanged until bullfrog nerves received approximately 100 R (95 rad) of x rays. The present findings are in concert with the observation of Gerstner et al. (4).

The amplitude of bioelectric responses from rat caudal nerves (17 to 19), turtle's superior cervical ganglion (41,42) were reported to increase with the onset of radiation. Allen and Nicholls (20) irradiated rat caudal nerves and rat phrenic nerves and found no radiation enhancement of the MAP amplitude. Action potential amplitudes of frog nerves did not demonstrate a radiation augmentation when exposed to high-energy α-particles (3).

The findings in the present report indicate that neither 100 krad of x rays nor 250 krad of protons increased the MAP amplitude of frog sciatic nerves.

Beta irradiation of sciatic nerves were reported to cause no

enhancement of action potentials by Yamashita and Miyasaka (47) and variable responses by Kaack (44).

Experimental evidence to explain why the action potential amplitude appears to increase with radiation in some cases and not in others is lacking.

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REFERENCES

1. R. M. Bergström, The effect of 5.3 MeV Polonium^{210} alpha-particles on the electrical activity of the frog sciatic nerve. Ann.Med. Exp. Fennicae $\underline{39}$, 211-215 (1961).

2. R. E. Bergström, R. F. Blafield, and M. W. Brenner, The effect of α-particles from a ring-shaped Po^{210} source on the action potential of single nerve fibers of the frog. Ann.Acad.Sci. Fennicae, Ser. A. V. $\underline{82}$, 1-10 (1961).

3. C. T. Gaffey, Bioelectric effects of high energy irradiation on nerve. In Response of the Nervous System to Ionizing Radiation (T. J. Haley and R. S. Snider, eds.), pp. 277-296, Academic Press, New York, 1962.

4. H. B. Gerstner, J. S. Orth, and E. O. Richey, Effect of high-intensity x-radiation on velocity of nerve conduction. Am.J. Physiol. $\underline{180}$, 232-236 (1955).

5. H. B. Gerstner, Effect of high-intensity x-irradiation on the A group fibers of the frog's sciatic nerve. J.Am.Physiol. $\underline{184}$, 333-337 (1956).

6. R. E. Zirkle, The radiobiological importance of the energy distribution along ionizing tracks. J.Cell. and Comp.Physiol. $\underline{16}$, 221-235 (1940).

7. R. E. Zirkle, D. F. Marchbank, and K. D. Kuck, Exponential and sigmoidal survival curves resulting from α and x irradiation of Aspergillus spores. J. Cell. and Coomp. Physiol. $\underline{39}$, Suppl. 1, 75-85 (1952).

8. R. E. Zirkle, The radiobiological importance of linear energy transfer. In Radiation Biology (A. Hollaender, ed.), Vol. 1, pp. 315-350, McGraw-Hill Book Company, New York, 1954.

9. H. H. Rossi, Spatial distribution of energy deposition by ionizing radiation. Radiation Res., Suppl. 2, 290-299 (1960).

10. H. H. Rossi, Energy distribution in the absorption of radiation. Adv. Biol. Med. Phys. 11, 27-85 (1967).

11. J. W. Boag, The relative biological effectiveness of different radiations. Natl. Bur. Standards (U.S.), Report No. 2946 (1953).

12. J. B. Storer, P. S. Harris, J. E. Furchner, and W. H. Langham, The relative biological effectiveness of various ionizing radiations in mammalian systems. Radiation Res. 6, 188-288 (1957).

13. H. H. Rossi, J. L. Bateman, V. P. Bond, L. J. Goodman, and E. E. Stickley, The dependence of RBE on the energy of fast neutrons. 1. Physical design and measurement of absorbed dose. Radiation Res. 13, 503-520 (1960).

14. S. Russak and K. Richardson, Proton flux, dosage, and damage estimates in the Van Allen belt. In Second Symposium on the Protection Against Radiation in Space (A. Reitz, ed.), pp. 251-256, Scientific and Technical Information Division, NASA SP-71, Washington, 1965.

15. C. A. Sondhaus and R. D. Evans, Dosimetry of radiation in space flight. In Radiation Dosimetry (F. H. Attix, E. Tochilin, eds.), Vol. 3, second edition, pp. 453-522, Academic Press, New York, 1969.

16. W. Langham (ed.), Radiobiological Factors in Manned Space Flight. National Academy of Sciences, Research Council, Washington, 1967.

17. C. S. Bachofer, Enhancement of activity of nerves by x-rays. Science 125, 1140-1141 (1957).

18. C. S. Bachofer and M. E. Gautereaux, Bioelectric activity of

mammalian nerve during x-irradiation. Radiation Res. _12_, 575-586 (1960).

19. C. S. Bachofer, Radiation effects on isolated nerves, In Response of the Nervous System to Ionizing Radiation (T. J. Haley and R.S. Snider, eds.), pp. 573-596, Academic Press, New York, 1962.

20. N. Allen and J. G. Nicholls, A study of the effects of x-rays on the electric properties of mammalian nerve and muscle. Proc. Roy.Soc. (London), Ser. B, _157_, 536-561 (1963).

21. C. T. Gaffey, The response of maximal and submaximal action potentials from sciatic nerve to 200 kV x-rays. Radiation Res., to be published.

22. P. H. Mitchell, A Textbook of General Physiology, 4th ed., McGraw Hill Book Co., New York, 1948.

23. H. Johns, The Physics of Radiology, 2nd ed., Charles C. Thomas, Springfield, 1961.

24. Report of the International Commission on Radiological Units and Measurements (ICRU) 1959, National Bureau of Standards, Handbook 78, 1961.

25. A. C. Birge, H. O. Anger, and C. A. Tobias, Heavy charged-particle beams. In Radiation Dosimetry (C. J. Hine and G. L. Brownell, eds.) pp. 623-665, Academic Press, New York, 1956.

26. R. L. Fulton, L. R. Glasgow, and N. W. Yanni, Modification of the 88-inch and 184-inch cyclotron: biological research facility. Lawrence Radiation Report UCRL-11128, University of California, Berkeley, 1963.

27. M. R. Raju, The use of miniature silicon diode as a radiation dosimeter. Phys.Med.Biol. _2_, 371-376 (1966).

28. J. T. Lyman, Private communication.

29. W. Schmitz and H. Schaefer, Uber den Einfluss der Roetgegstrahlen auf den Nervenaktionsstrom. Strahlentherapie 46, 546-568 (1933).

30. A. M. Janzen and S. Warren, Effect of roentgen rays on the peripheral nerve of the rat. Radiology 38, 333-337 (1942).

31. J. Audiat, D. Auger, and A. Fessard, Etude des potentiels d'action des nerfs soumus su rayonment X. Comparison avec l'action des rayons ultraviolets. Compt. Rend. Soc. Biol. 116, 880-883 (1934).

32. E. L. Gasteiger, Effects of beta rays on mammalian nerve action potentials. Federation Proc. 10, 48 (1951).

33. E. Linder, Uber das funktionelle und morphologische Verhalten peripherer Nerven längere Zeit nach Bestrahlung. Fortschr. Gebiete Roentgenstrahlen Nuklearmed 90, 618-624 (1959).

34. R. E. Zirkle and C. A. Tobias, Effects of ploidy and linear energy transfer on radiobiological survival curves. Arch. Biochem. Biophys. 47, 282-306 (1953).

35. G. W. Barendsen, H. M. D. Walter, J. F. Fowler, and D. K. Bewley, Effects of different ionizing radiations on human cells in tissue culture. III Experiments with cyclotron-accelerated alpha particles and deuterons. Radiation Res. 18, 106-119 (1963).

36. W. H. Barkas and M. J. Berger, Tables of energy losses and ranges of heavy charged particles. In Studies in Penetration of Charged Particles in Matter. Scientific and Technical Information Division, NASA-NRC 1133, Washington, D. C., 1964.

37. D. V. Cormack and H. E. Johns, Electron energies and ion densities in water irradiated with 200-keV, 1-MeV and 25-MeV radiations. Brit. J. Radiol. 25, 369-381 (1952).

38. D. E. Lea, _Actions of Radiations on Living Cells_, 2nd ed., Cambridge University Press, London, 1955.

39. G. W. Barendsen, The importance of microdosimetry for radiation biology and radiation protection. In _Proceedings of the Symposium on Microdosimetry_ (H. G. Ebert, ed.) pp. 1-22, Published by the European Communities, Brussels, 1968.

40. R. M. Bergström, R. F. Blafield, and M. W. Brenner, Irradiation of single nerve fibers with α-particles from a circular polonium^{210} source. Acta physiol. Scandinav. $\underline{50}$, 24-25 Suppl. 175 (1960).

41. B. Kaack, Effect of beta irradiation on ganglionic bioelectric activity. Am.J.Physiol. $\underline{213}$, 1451-1458 (1967).

42. B. Kaack, Augmentation of bioelectric activity by gamma irradiation. Am.J.Physiol. $\underline{213}$, 625-628 (1967).

43. D. J. Rees, _Health Physics_, The M.I.T. Press, Butterworth and Co., Cambridge, 1967.

44. B. Kaack, Effect of beta irradiation on the oxygen consumption in the frog sciatic nerve. Radiation Res. $\underline{42}$, 405-412 (1970).

45. C. S. Bachofer, M. E. Gautereaux, and S. M. Kaack, Relative sensitivity of isolated nerves to Co^{60} gamma rays. In _Response of the Nervous System to Ionizing Radiation_ (T. J. Haley and R. S. Snider, eds.) pp. 221-242, Little, Brown and Co., Boston, 1964.

46. C. S. Bachofer and M. E. Gautereaux, X-ray effects on single nerve fibers. J.Gen.Physiol. $\underline{42}$, 723-735 (1959).

47. H. Yamashita and T. Mikosaka, Effects of beta rays upon single nerve fiber. Proc. Soc. Exp. Biol. Med. $\underline{80}$, 375-377 (1962).

LEGENDS

Fig. 1 Maximal action potentials detected at different locations along the length of an isolated frog sciatic nerve. The white numbers in upper right corner of each dual-beam oscillogram indicated the kiloroentgens of x-rays delivered to the nerve.

Fig. 2 Relative values of the maximal action potential amplitude, conduction velocity, and detection period as a function of the amount of x-rays delivered to the nerve. The action potentials of Fig. 1 provided the information for this diagram.

Fig. 3 Dual-beam oscillograms of maximal action potentials recorded from a frog sciatic nerve at different sites along the length of the nerve. On each oscillogram was superimposed the proton dose in kilorads absorbed by the nerve.

Fig. 4 Relative values of maximal action potential amplitude, conduction velocity, and detection period as a function of the proton dose absorbed by a nerve. This figure was constructed from the action potentials in Fig. 2.

Fig. 5 In this study the relative biological effectiveness (RBE) refers to the ratio of the absorbed dose of 200 kV x-rays required to promptly block impulse conduction by frog nerve to the absorbed dose of test radiations to produce the same effect. Linear energy transfer (LET) refers to the loss of energy of an ionizing particle per unit length of path traveled through matter. For certain species of radiation, RBE was a function of LET.
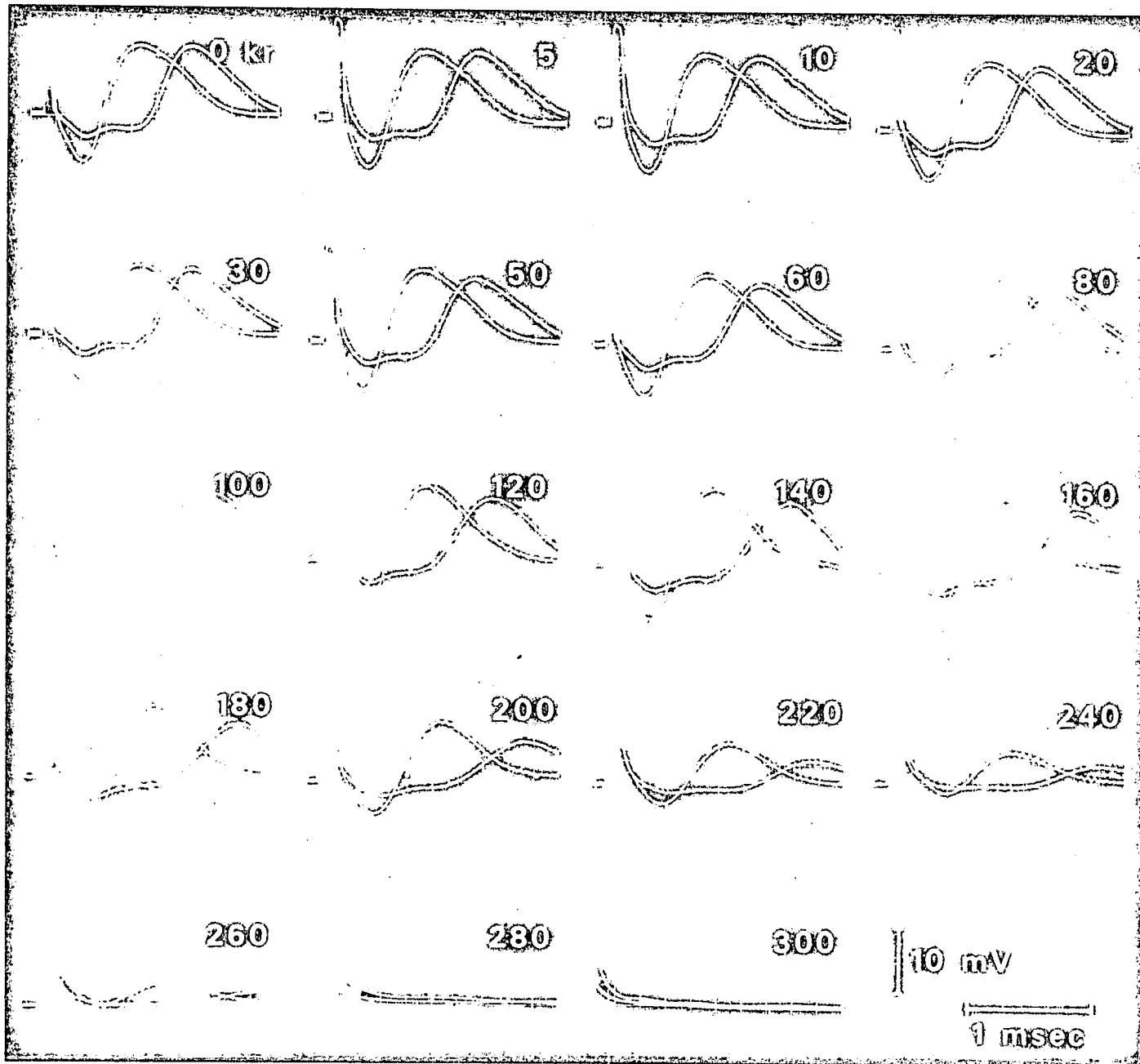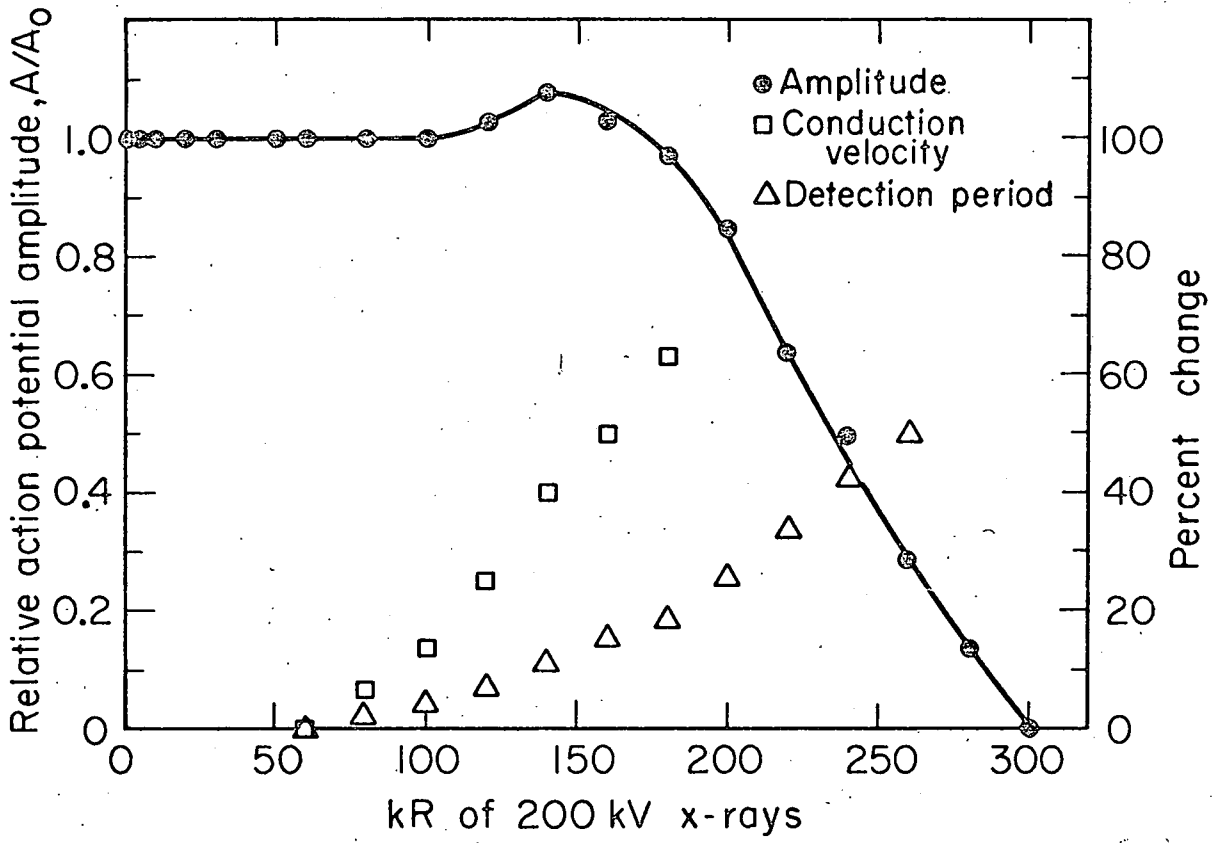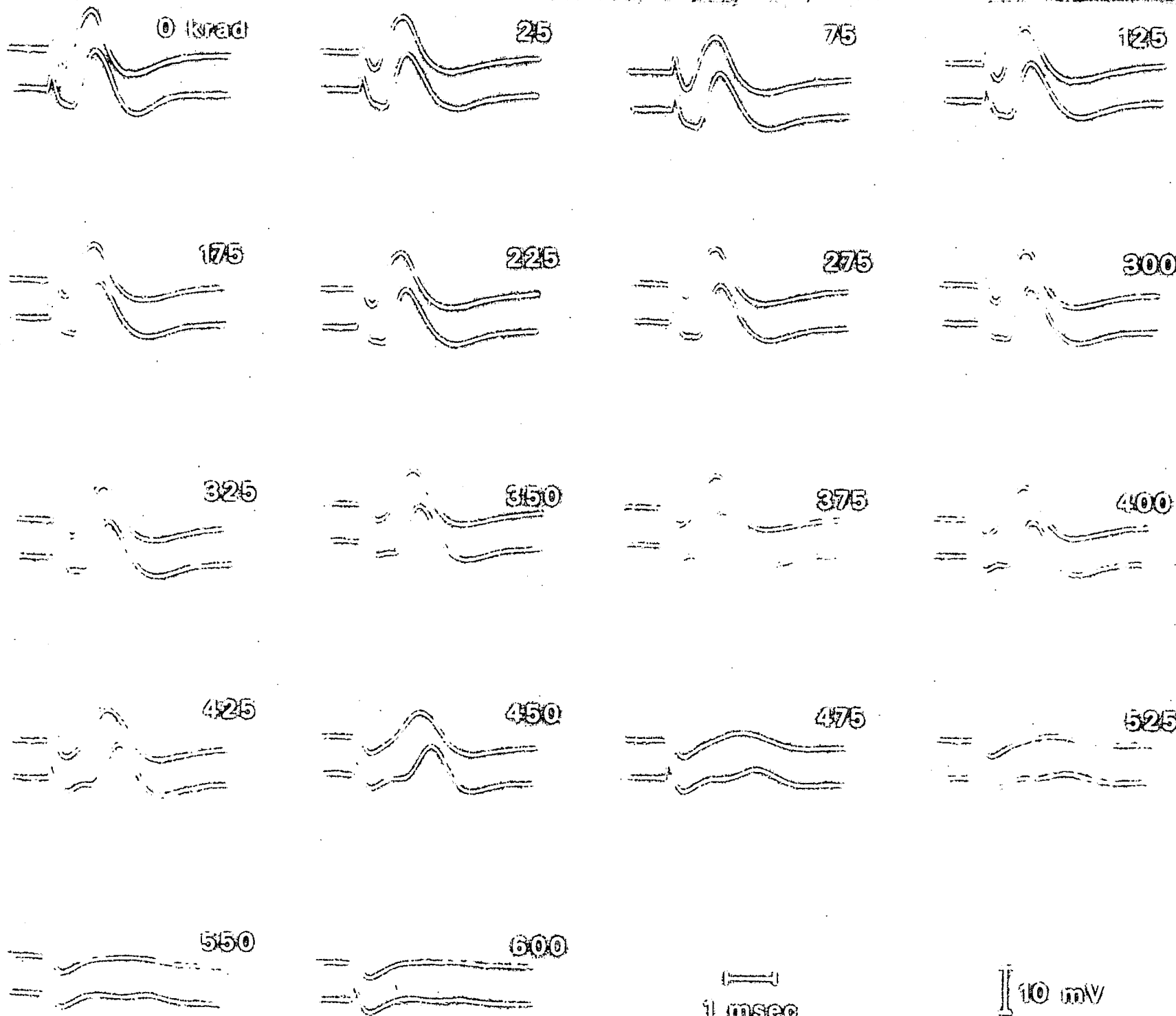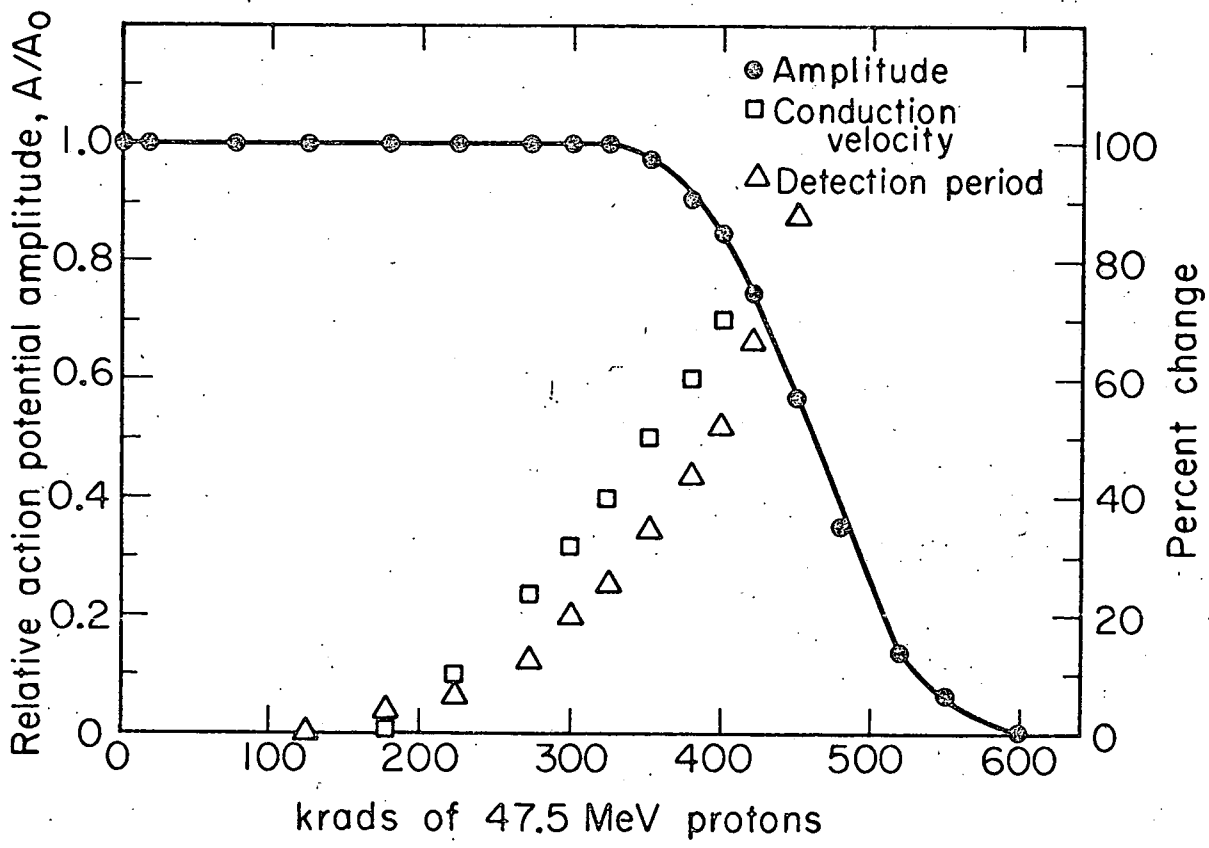
Fig. 2 1

XBL7010-4010

Fig. 2

0 krad   25   75   125

175   225   275   300

325   350   375   400

425   450   475   525
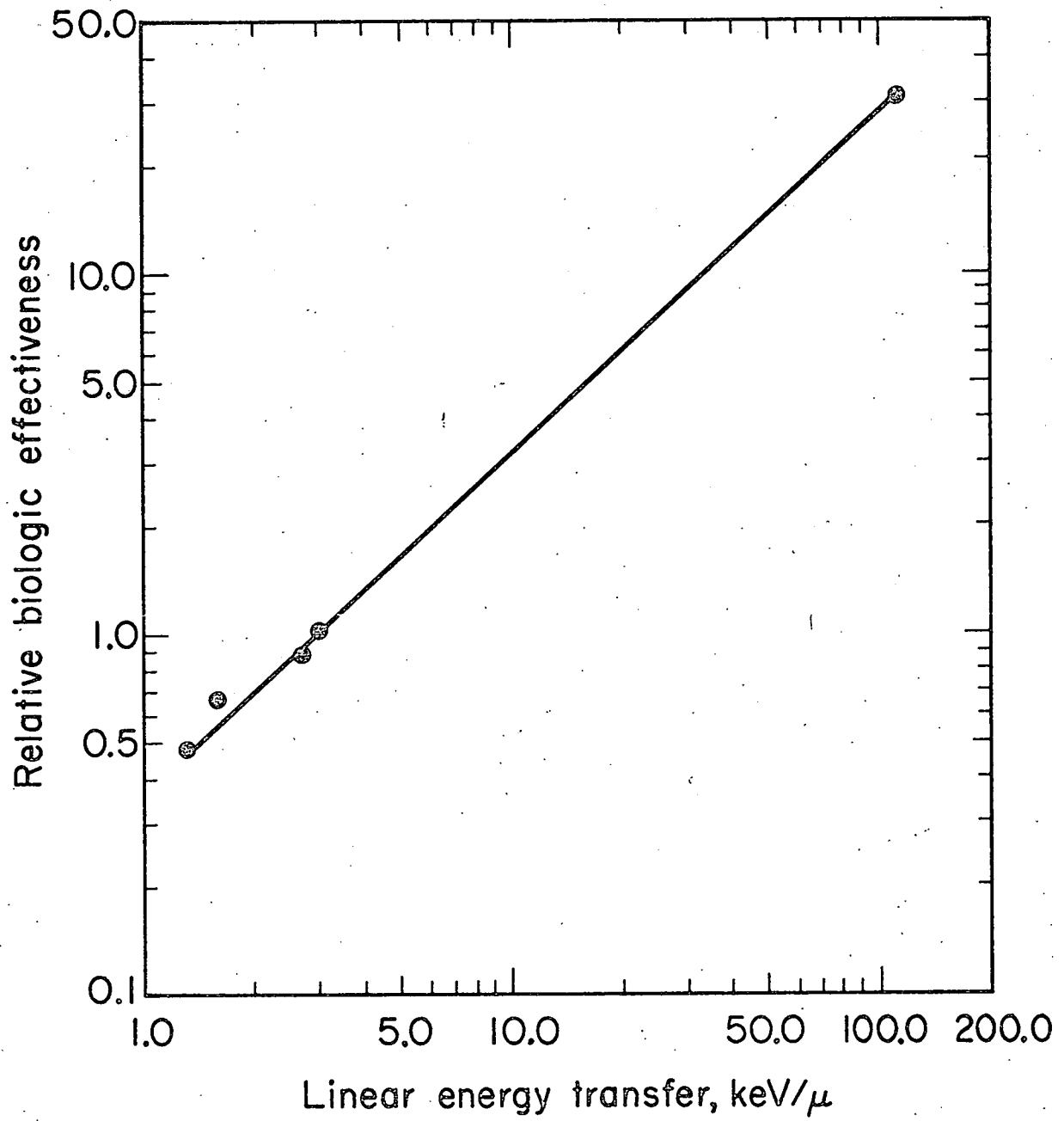
550   600

1 msec   10 mV

Fig. 3

XBL7010-4011

Fig. 4

XBL7012-4291

Fig. 5

TABLE I

The Absorbed Doses of Radiation to Inhibit Neural Responses in Frog Nerves,

Relative Biological Effectiveness (RBE), and Linear Energy Transfer (LET)

Radiations	Actual inactivation dose (krad)	RBE	LET (keV/μ)	Calculated inactivation dose (krad)
α-particles, 5.3 MeV	9.3 (a)	30.60	110.0	7.8
x-rays, 200 kV	285.0 (b)	1.00	3.0	(reference dose)
x-rays, 260 kV	329.0 (c)	0.87	2.7	316.0
α-particles, 910 MeV	430.0 (d)	0.66	1.6	535.0
protons, 47.5 MeV	600.0 (b)	0.48	1.3	657.0

(a) Bergström et al. (2), (b) this report, (c) Gerstner et al. (4) and Gerstner (5),

(d) Gaffey (3)