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24 June 1960

A Difference between Biological Effects of Gamma Rays and Heavy Ions

Abstract. When irradiated with gamma rays, *Artemia* eggs show the typical sigmoidal survival curve of a multicellular organism, with little change at low doses and an abrupt decrease in survival above a threshold dose. On irradiation with 160-Mev oxygen ions, the threshold disappears and viability can be destroyed by passage of a single energetic ion.

Gamma-ray survival curves for many multicellular organisms are sigmoidal and show an initial insensitivity to low doses. Only above a threshold does the viability drop appreciably, and then it usually falls off quite rapidly. The ability of eggs of the common brine shrimp, *Artemia salina*, to hatch after gamma-ray exposure decreases in this way with increasing dose, as shown in Fig. 1. These eggs are moderately complex. The fertilized oöcyte divides to the blastula stage before becoming encysted and laid. In this stage the egg is about 200 μ in diameter, and it must dry before further development can take place. In all the experiments reported here the eggs were irradiated in high vacuum. There was no adverse effect from vacuum treatment alone. On immersion in sea water the egg develops rapidly, and at about 48 hours the shell cracks open and an embryo encased in a membrane is released. This process, called "emergence," is inhibited by radiation, as shown in Fig. 2. After another 6 to 8 hours a free-swimming larva comes out of the membrane; this step is called "hatching."

The long plateau which indicates the accumulation of gamma-ray damage is markedly reduced if the eggs are irradiated with 40-Mev helium ions and disappears entirely when 160-Mev oxygen ions are used. The particles were from the Yale heavy-ion linear accelerator, and dosimetry was carried out by methods previously described (*1*). The exponential decrease in survival indicates that the passage of a single energetic oxygen ion is responsible for the loss of activity of an egg. The result is qualitatively similar to that of Stapleton, Hollaender, and Martin with *Aspergillus* spores (*2*) but is more spectacular.

The three types of radiation differ from each other in the spacing between inactivating events, ranging from several thousand angstroms for gamma rays, to the order of tens of angstroms for 40-Mev helium ions (240 ev of energy loss per 100 angstroms of track), to angstroms for 160-Mev oxygen ions (3800 ev per 100 angstroms, or an average of 1.3 ion pairs, at 30 ev per ion pair, per angstrom of path). The effect is not caused by over-all dose rate, since (i) fast electrons given at a dose rate of 2 Mrad/min produced the same survival curve as gamma rays at 0.275 Mrad/hr, and (ii) the threshold was lower with helium ions and disappeared with oxygen ions, although the time required to deliver the total dose in the last two cases was about the same (tens of seconds).

Four possible explanations for the loss of a threshold with heavy ions are as follows.

1) The gamma-ray curve may be interpreted as showing that either a certain number (20 to 60) or a certain fraction of functioning units in the egg must be damaged to prevent development. It might be assumed that a single fast oxygen ion might do the necessary damage. However, converting the doses in Figs. 1 and 2 to particles per unit area shows that 24 and 9 oxygen ions per square micron are needed to suppress emergence and hatching, respectively, to 37 percent of that of controls. Each cell will have been traversed on the average by many ions before inactivation. Thus, the chance that any single ion will do all the necessary damage is small, and on this assumption the survival curves with oxygen ions would still show a cumulative effect, not the exponential form found.

2) The *simultaneous* inactivation of several widely separated areas by a heavy ion might be biologically more effective than consecutive inactivation by gamma rays. This is a process which might be very important in a metabolizing system, especially at low dose rates, but it is doubtful that it was important in dried eggs.

3) If the inactivating events took place close enough together in the densely ionizing track of an oxygen ion the resultant physicochemical events might be different, and conceivably more effective. There is some evidence for this process from experiments with heavy ions on dried enzymes (*3*), and it could be operative here.

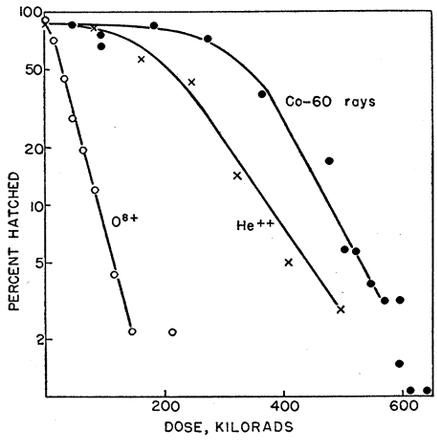

Fig. 1. Plot of the percentage of *Artemia* eggs hatched (semi-log scale) against dose for three different radiations.

4) The most likely possibility is that if enough damage is done within some limited volume the egg will not develop. This damage can be cumulated through many "hits" from gamma rays or caused by a single oxygen ion. Presumably the dimensions of this volume are less than 1 μ. There may be several such volumes per egg.

An extrapolation of this result leads one to consider the possibility that heavy ions in the primary cosmic rays which are met with above the earth's atmosphere may produce radiological effects at low total dose levels which would not be expected from x-ray data because of threshold effects. This suggestion is contrary to the conclusion advanced by Zeman, Curtis, Gebhard, and Haymaker (*4*) from their work with microbeams of deuterons on mouse-brain tissue. *Artemia* is an unusual material, as shown by its extreme

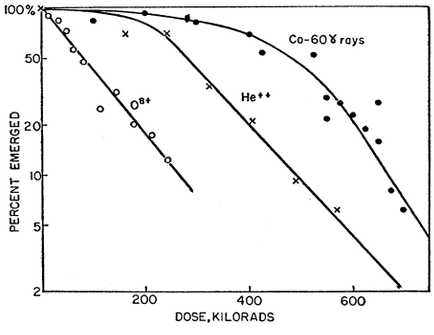

Fig. 2. Plot of ability of the *Artemia* larva to emerge from the egg (the first stage in development) against dose for three different radiations.

resistance to radiation, but the vanishing of the threshold shown by our data is a sufficiently significant phenomenon to warrant looking for it in other systems which are affected by lower dosages (*5*).

FRANKLIN HUTCHINSON
STEPHEN S. EASTER, JR.
Biophysics Department, Yale University, New Haven, Connecticut

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27 June 1960

Nerve-End Recording in Conducting Volume

Abstract. When the end of a freshly cut nerve is drawn into a tube by means of a hydraulic device that serves as a holder and as an electrode, monophasic positive records of action potentials are recorded. A trailing positive phase develops, with time, after the cut. After-potentials can also be recorded by this method.

Potentials comparable in size to those recorded conventionally in oil or air may be obtained from the end of a nerve which has been drawn into a small glass tube. In this method the entire nerve is at all times completely immersed in Ringer's solution, and the potential drop occurs between a wire inserted inside the tube and an indifferent lead in the surrounding medium. This method has been used, in principle, for stimulation but apparently not for recording from nerve (*1*).

The device shown in Fig. 1 provides precise control of the position of the nerve end in its holder. The tubing, completely filled with Ringer's solution, constitutes a hydraulic pressure system. The contained fluid can be forced in or out, and the nerve moving along with it may be fixed at any point by means of screw controls *A* and *B*. These coarse and fine controls apply pressure through inserts in the Plexiglas block holding the control bulb.

Electrodes were constructed of silver wire (Birmingham and Stubs gauge 22) inserted through a length of about 15 cm of polyethylene tubing (inside diameter, 0.034 in.; outside diameter, 0.050 in.). At one end the wire extended about 1 cm beyond the tube. At the other, the wire was bent upon itself and sealed to the edge of the Plexiglas stopper *C*, which was inserted into the control bulb constructed from 10 cm of amber latex tubing (inside diameter, 0.125 in.). A second wire attached outside the end of the tube served as the indifferent lead.

Air bubbles were eliminated from the system by drawing Ringer's solution through the rubber tube and then replacing the terminal plug *D*. The polyethylene tube *E* was mounted on a manipulator constructed from rod-end bearings. Holding tubes *F* were constructed of melting-point glass tubing (inside diameter, 0.8 to 1.0 mm; outside diameter, 1.0 to 1.5 mm); the smooth cut ends were fire-polished to provide the desired size of aperture. The tubes were sorted by means of a series of brass wires of standard gauge (B & S 18 to 36). A tube with opening of appropriate size could be quickly selected by comparing the nerve diameter with the standard wires. The glass tubes were readily slipped over the silver wire and into the polyethylene tubing as needed. The silver wire was chloridized for about 2 cm at the tip before the glass tube was attached.

Records were obtained from exsected sciatic nerve or spinal root of *Rana pipiens* and *R. catesbeiana*. The preparation was grounded via the metal tubes used for circulating the water that maintained a temperature of 15°C in the bath. The end of the nerve was usually about 1 cm from the end of the wire in the tube, but changes in this distance had no observable effect on the record.

Several electrodes for stimulation and recording could be used simultaneously. A switching arrangement allowed any combination to be selected. The use of multiple indifferent leads did not seem to complicate the records. Each indifferent lead was placed near the glass tube of its companion electrode. The nerve end, oriented close to the tube opening, was drawn into the tube by release of pressure, by the fingers directly or via one of the screw controls.

Injury current was maximal immediately after the nerve had been cut with sharp scissors. The current declined with time, presumably due to narrowing of the cut end during outflow of axoplasm and the spreading of myelin over the cut end (*2*). Action currents at the distal, "healed" end and at the freshly cut proximal end of the sciatic nerve of a frog are shown in Fig. 2. Action currents were monophasic positive immediately after the cut, whether the nerve was pulled into the tube for a few hundred microns or for several millimeters (*3*). With time, after the nerve was cut, a trailing negative phase developed unless the end was left in the tube, in which case the monophasic positive record persisted (*4*). After-potentials lasted about 0.5 second and were initially positive-going, while a later negative phase developed during a train. These positive and negative phases appear to correspond, respectively, to the negative and positive after-potentials conventionally recorded with external electrodes. With passage of

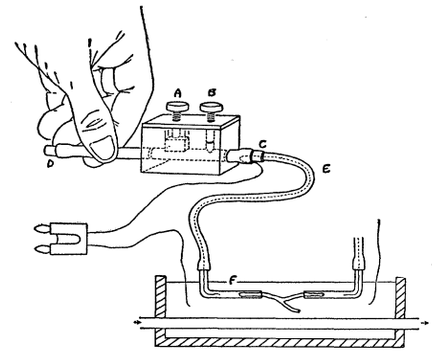

Fig. 1. Hydraulic holding and recording device for nerve end.

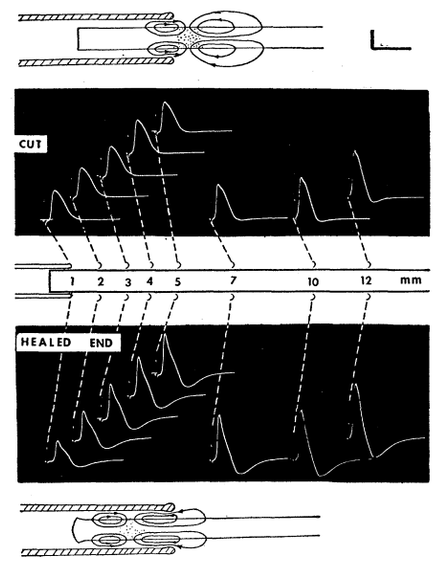

Fig. 2. Action currents recorded by means of the hydraulic holding device. The sciatic nerve of a bullfrog was prepared 8 hours before the recording was made. Upper records are from the proximal end of the freshly cut nerve, which had been drawn into the tube for the distances indicated. Diagram (top) shows the net direction of current flow outward near the nerve end. Lower records are from the distal (healed) end of the nerve, which had not been touched since the cut was made, 8 hours previously. Diagram (bottom) shows the net current flow inward during the negative phase. (Top, right) Calibration: 2 mv, 2 msec.