Shrinking and Swelling after Alpha Irradiation of Various Parts of Large Erythrocytes.* (17320)

RALPH BUCHSBAUM AND RAYMOND E. ZIRKLE.
Institute of Radiobiology and Biophysics, University of Chicago, and Argonne National Laboratory, Chicago, Ill.

It is well known that ionizing radiations produce hemolysis of red blood cells *in vitro*, and this seems to be associated with swelling.[1] Nearly all investigators have studied these phenomena with large populations of erythrocytes so that variation in behavior of the individual cells can be smoothed out to get reproducible data. As far as we know, only one previous worker has used a microbeam to irradiate portions of a single cell. Tchakhotine[2] irradiated egg cells with ultraviolet rays focused to a 5 micron "point" and described local damage of the membrane and underlying cytoplasm. We are aware of no similar studies with ionizing radiation.

In this study we have sacrificed the many advantages of using massive populations of erythrocytes in order to direct our attention to the microscopic changes that can be observed in individual cells before, during and after irradiation. This method has revealed a striking effect that seems to have escaped attention. Using erythrocytes of Amphiuma and of man, we have observed that between the time of irradiation and the well-known swelling and hemolysis there occurs a shrinkage of the cells. After total alpha irradiation of a cell the whole cell shrinks. After irradiation of a portion of a cell only the irradiated portion shrinks.

Methods and materials. Two methods of irradiation were used, total cell irradiation in which cells in tissue cultures on coverslips were exposed, and microbeam or partial cell irradiation in which only portions of cells were exposed. Two sources of alpha particles were used.† For total cell irradiation the source was polonium deposited on a palladium disc 4 mm in diameter. This was mounted in a brass holder which simulated a microscope objective and could be screwed into the nosepiece of the microscope. In some experiments irradiation was administered from above by selecting a suitable field and then revolving the nosepiece to bring the sourceholder over it.[3] In other experiments the source-holder was mounted in the microscope condenser-holder so that cells could be irradiated from below. In the latter case, the cells were first observed with the condenser in place, then irradiated by substituting the source for the condenser, and finally observed with the condenser back in place. Alternatively, the cells could be observed continuously before, during and after irradiation by using vertical illumination without the exchanges of source and condenser.

For microbeam or partial cell irradiation a smaller source was used. The polonium was deposited on the end of a palladium wire, 0.3 mm in diameter, which was then inserted into a steel sheath made from hypodermic needle. One end of the sheath was covered with an aluminum cap, the top of which was perforated with a small hole for the exit of alpha particles. This small source was mounted on the condenser-holder. In use, a preparation was focused on the microscope stage with vertical illumination. The body tube was then racked down 20 μ, and the condenser-holder bearing the source racked up until the top of the aluminum cover with the

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1 Ting, T. P., and Zirkle, R. E., *J. Cell. and Comp. Physiol.*, 1940, **16**, 189.

2 Tchakhotine, S., *Compt. rend. soc. biol.*, 1935, **120**, 714.

† The sources were furnished by the Chemistry Division of Argonne National Laboratory. We are especially indebted to Dr. W. M. Manning and Mr. P. R. Fields for their courtesy and to Mrs. Sylvia Warshaw for her services in preparing the sources.

3 Zirkle, R. E., *J. Cell. and Comp. Physiol.*, 1932, **2**, 251.

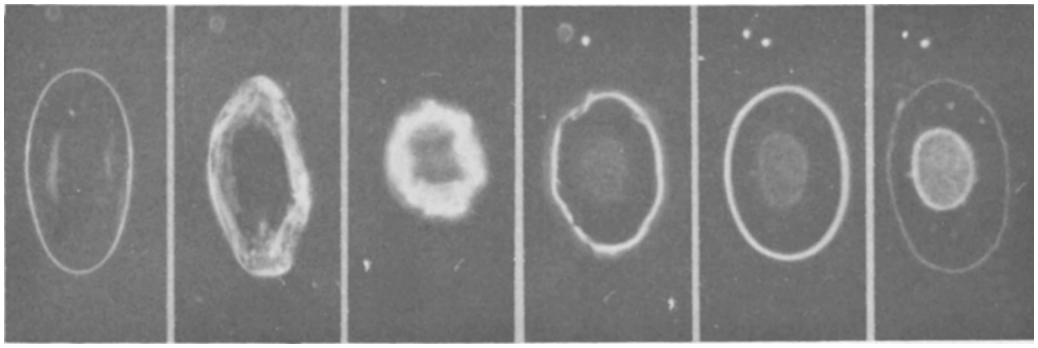

FIG. 1.
Photomicrographs of an Amphiuma erythrocyte showing normal cell, shrinkage, swelling, and hemolysis, after 5×10^5 rep of alpha radiation. Vertical illumination. Length of normal cell, 75 μ.

hole came into focus. By this procedure the source was brought as close as feasible to the cells. The hole was centered with respect to ocular cross hairs. To make an exposure a piece of coverslip was withdrawn and reinserted as a shutter in the space between the aluminum cap of the sheath and the 4 μ mica slip at the bottom of the preparation.

The radiation consisted exclusively of polonium alpha particles (energy 5.3 Mev). Since these particles have a total range of only about 35 μ in tissue, the path from the source to the cells was made as short as possible to permit maximal penetration into the cells. The 4 mm source had an initial activity of 790 μc. The distance between source and cell was 490 μ and the dose rate was calculated to be about 132,000 rep per minute. Exposures lasted from one to 50 minutes. The microbeam source had an activity of 275 μc at the start of these experiments. The total number of particles reaching the field of irradiation was determined by direct count of those passing through the small holes used. In the case of the 21.6 μ hole, which was used most extensively, 12,500 particles emerged per minute. The area covered by the particles was determined directly. An Eastman NTA plate was substituted for a cell preparation and bombarded for one minute. A photograph of a stage micrometer was also made at the same time. Superimposing these two images gave the area of bombardment. Exposures ranged from one to 90 minutes; the average dose rate was 84,000 rep per minute. Time of exposure was corrected for radioactive decay of the sources.

Cell preparations were made as follows. After partial caudal amputation, a drop of Amphiuma blood was allowed to fall upon a coverslip and was covered immediately with a mica coverslip 3.5 to 4.5 μ thick (0.7 to 0.8 mg per cm^2). Blood from man was drawn by skin puncture. The preparations were sealed with vaseline. Cell changes were recorded both by fast-motion cine-photomicrography and by still photomicrography. Cytological details not visible by vertical illumination were studied with phase microscopy.

Results of Total Cell Irradiation. Groups of irradiated erythrocytes from Amphiuma or man generally show the same type of response with respect to hemolysis. A typical response is shown in Fig. 1. After irradiation the cells shrink, becoming very wrinkled. Later they swell until they reach or exceed their initial size, after which they hemolyze. Cells which shrink the earliest and to the greatest extent take the longest to swell and to hemolyze.

Results of partial cell irradiation. Individual Amphiuma erythrocytes, after irradiation as described above, shrink in the irradiated portion only. However, the subsequent swelling involves the entire cell and results in hemolysis (Fig. 2).

Cells irradiated over their central portions shrink less and hemolyze significantly sooner than those irradiated at their ends. The ends of the Amphiuma erythrocyte are thinner than the center, which contains the nucleus. Since

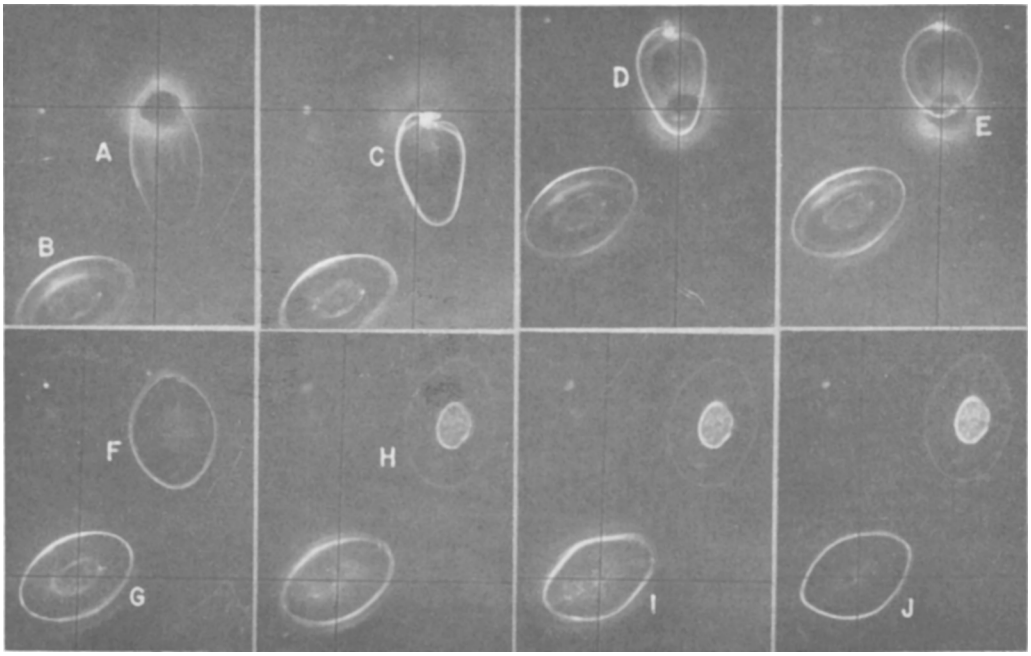

FIG. 2.

Photomicrographs of 2 Amphiuma erythrocytes exposed to partial cell irradiation with polonium alpha particles through a 21.6 μ hole. Each exposure lasted 90 minutes and corresponded to a dose of 7.5×10^6 rep. (A) Cell being irradiated at one end; outline of opening through aluminum cap is also shown by focusing down 20 μ and making a second exposure; (B) normal unirradiated control. (C) 15 minutes later, one end of irradiated cell is severely shrunken; (D) the other end of the same cell is being irradiated. (E) The second end is shrinking; body of cell is swelling. (F) Swelling continues; nuclear change is evident; (G) Control cell is now being irradiated at its center. (H) First cell has hemolyzed 143 minutes after beginning of irradiation. (I) Second cell beginning to shrink, particularly on the lower side 48 minutes after beginning of irradiation; (J) Second cell begins to hemolyze 94 minutes after beginning of irradiation, (complete hemolysis, not shown in figure, occurred 120 minutes after beginning of irradiation).

it is likely that the alpha particles pass completely through the ends of the cells, whereas they do not pass completely through the cell at its center, irradiation at either end of the cell involves twice as much membrane area as is the case for irradiation at the center. It is not clear whether the longer hemolysis time of the cells irradiated at their ends is due to greater shrinkage or to the fact that the nucleus was not irradiated. Experiments are in progress to determine this point.

Summary. Erythrocytes from Amphiuma and from man were irradiated *in vitro* with polonium alpha particles (a) from an extended source (4 mm in diameter) and (b) with a small beam (20 μ in diameter). The cells first shrank, then swelled and hemolyzed. Local irradiation of a part of a single cell resulted in shrinkage only in the irradiated portion. This was followed by swelling of the whole cell and hemolysis.

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