Shape and Volume Changes in Frog Erythrocytes Following Ultraviolet Radiation

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ABSTRACT Frog erythrocytes in Ringer's solution were exposed to ultraviolet radiation and then followed in camera lucida drawings for changes in shape and dimension. Cell thickness was found to increase while cell width remained constant throughout the period prior to hemolysis. The cell shortened and bulged at the ends during the middle third of the prolytic period while a region around the cell center remained constricted. When this constricted region gave way, the cell became spherical and hemolyzed. Cell volume as calculated from the cell's dimensions increased linearly with time throughout the prolytic period to hemolysis then dropped rapidly to a constant value somewhat higher than the original cell volume. These changes in shape and volume are consistent with a colloid osmotic type of hemolysis but with other factors acting to limit the rate of swelling and the forms assumed during the swelling process. The relationship between the time of hemolysis and the cell surface area exposed to the ultraviolet is discussed as it applies to the site of ultraviolet damage.

INTRODUCTION

The responses of mammalian erythrocytes to ultraviolet radiation are consistent with the hypothesis that swelling and hemolysis depend upon the colloid osmotic pressure of the cell (1, 6). This is supported by the effects of this radiation upon erythrocyte cation permeability (2). However, the failure to note similar changes in ultraviolet-irradiated nucleate cells (12), although not necessarily negating such changes, makes further study of this phenomenon in a less specialized cell desirable. A possible compromise may be found in the nucleate erythrocyte of the lower vertebrate forms, a cell which like the mammalian erythrocyte has the advantage of a sharp end point for cell swelling; *i.e.*, hemolysis. Unfortunately, little is known of the shape and volume changes in these cells as they hemolyze (10). It is the purpose of the present study to follow the changes in outline and dimension of frog erythrocytes exposed to ultraviolet radiation.

METHODS

Blood was drawn from the aorta of a pithed frog (*Rana pipiens*) and added to a few crystals of heparin to prevent clotting. The cells were washed three times in amphibian Ringer's solution (3) and finally suspended in Ringer's at a concentration of 1:200 by volume. Samples of this final suspension were added to quartz slides under vaseline-edged coverslips. Two or three adjacent sites containing four to six cells each (some cells flat on the slide, others on edge) were chosen and the cells outlined under phase contrast oil immersion using a camera lucida. The cells were then exposed to ultraviolet radiation (4 to 6 minutes) from a General Electric 15 watt germicidal lamp set 5 cm. from the slide. These same cells were drawn at 5 or 10 minute intervals following irradiation to and beyond hemolysis.

Measurements of cell length ($2a$), cell width ($2b$), and cell thickness ($2c$) were recorded for each of fifty-four cells in four experiments (four frogs) and plotted as a function of time after irradiation. In order to eliminate the discrepancies in hemolysis times, the prolytic period for each cell following irradiation was divided into ten parts. This permitted zero to represent dimensions before radiation and one the dimensions at hemolysis. The intervening values were taken from the plots of the dimensions of individual cells as a function of time after radiation.

Volume calculations were made from the mean values of the cell dimensions using the formula for the volume of a spheroid, $V = \frac{4}{3} \pi abc$, where a, b, and c were the semiaxes of the spheroid. Area measurements of the cells on edge were made with an optical planimeter.

RESULTS

The frog erythrocyte has been described as an ovate, biconcave plate or disc (9) and indeed the elliptical nature is apparent when the cells are viewed flat upon the slide. An analysis of the cells on edge suggested that they might appropriately be considered flat ovate spheroids with three different axes. Table I records the areas of camera lucida drawings of cells viewed on edge as measured with a planimeter, and as calculated for an ellipse of the measured dimensions. As the cell swelled, the shape changed in various ways as will be reviewed below. Throughout this swelling, the area of the section viewed on edge as calculated for an ellipse with the cell's dimensions was by inspection not significantly different from the area measured with the planimeter. Were the cells plate-like, the area of cells viewed on edge should more aptly be calculated as a rectangle. Rectangular areas averaged 21 per cent larger (range 17 to 25 per cent) than the measured areas.

Shape Changes following Radiation

The changes in cell shape and dimensions following ultraviolet radiation are presented in Fig. 1 *a* and Fig. 2. The cell thickness (measured at the thickest point which in nearly all cases was the middle of the cell) began to increase

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immediately following irradiation and continued to increase at an accelerated rate to 2.6 times the initial thickness at the time of hemolysis. Cell length remained constant for the first third of the prolytic period and then decreased at an accelerated rate to 0.75 times the original length at the moment of hemolysis. Cell width remained essentially constant throughout the prolytic period (0.97 times the original width at hemolysis). These changes represent the change of a normally flattened spheroid cell to one which is nearly a perfect sphere at the moment of hemolysis. Cell length and cell width de-

TABLE I

SUMMARY OF THE AREAS OF CELL OUTLINES DRAWN IN EDGE VIEW

A_1, area of cell outlines in square centimeters as measured by a planimeter.

A_2, area in square centimeters of an ellipse with the cell outline's dimensions calculated as $\pi\ a \times c$ where a and c are the semiaxes for length and thickness of the cell outline.

n, number of cells measured.

A_1/A_2, correction factor mentioned in text and in Fig. 1 *b*.

t/t_h, time of measurement/time of hemolysis.

t/t_h	n	A_1	A_2	A_1/A_2
0	16	3.58	3.80	0.94
0.1	13	3.76	4.00	0.94
0.2	15	3.80	3.97	0.96
0.3	13	4.23	4.25	0.99
0.4	14	4.42	4.45	0.99
0.5	11	5.25	5.09	1.03
0.6	14	5.50	5.25	1.05
0.7	14	6.07	5.77	1.05
0.8	15	6.39	6.03	1.06
0.9	12	7.44	7.09	1.05
1.0	16	7.47	7.38	1.01

creased only slightly following hemolysis while cell thickness dropped, sharply at first, to a constant value 1.8 times the initial thickness.

The shape changes of the cell were somewhat complex. Fig. 2 depicts some individual cells viewed on edge at various intervals following ultraviolet radiation. It should be noted that the cell increased in thickness first. Then as the cell shortened, the ends began to bulge, in some cases bulging more than at the cell center. An oval region around the cell center remained constricted until the latter part of the prolytic period. When this region gave way, the cell swelled to a sphere and hemolyzed.

During these cell changes, the nucleus was also observed to change in shape. The nuclear thickness averaged 3.9 μ before irradiation (in the view on edge where it could be observed) but increased during the first half of the prolytic period and leveled off at about 6.0 μ. Nuclear width remained constant at

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about 6.0 μ so that width and thickness were the same throughout the second half of the prolytic period. The nuclear length (average 10.1 μ before irradiation) decreased steadily to a constant value of 7.5 μ at 0.8 of the prolytic period. The nucleus was quite obviously altered after radiation, its appearance being highly refractile.

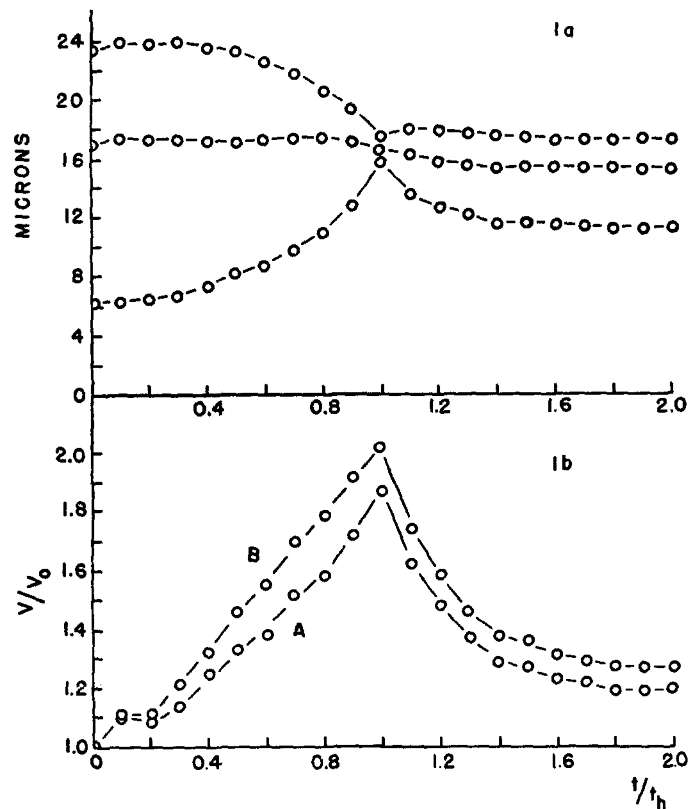

FIGURE 1 *a*. Changes in cell dimensions as a function of time after ultraviolet radiation. The upper line is cell length; the middle line is cell width; the lower line is cell thickness. The standard deviations for cell length ranged from 1.74 μ to 2.41 μ; for cell width, 1.01 μ to 1.33 μ; for cell thickness, 0.69 μ to 2.75 μ.

FIGURE 1 *b*. Changes in relative volume as a function of time after ultraviolet radiation. Line A represents the changes in volume calculated for the cell as an ovate spheroid ($V = \frac{4}{3}\pi abc$). Line B represents cell volume calculated as in A but with the corrections applied from the last column in Table I. V/Vo = volume at time t/volume at zero time. t/t_h = time of measurement/time of hemolysis.

On the basis of the dimensions in Fig. 1 *a*, cell volumes were calculated and are presented in Fig. 1 *b* (curve A). The cell volume increased as a function of time from an initial volume of 1290 μ^3 to a critical volume of 2415 μ^3 when hemolysis occurred (V/Vo = 1.87). The generated curve is approximately logarithmic but for the purposes of interpretation has been resolved into three

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straight lines denoting increasing rates of swelling and with breaks at t/t_h = 0.3 and 0.8. Following hemolysis the volume dropped rapidly to a constant volume of about 1.2 times the original volume of the cell. This curve was calculated without correcting for the slight differences between the measured area of the cell cross-section and the area calculated for an ellipse of the

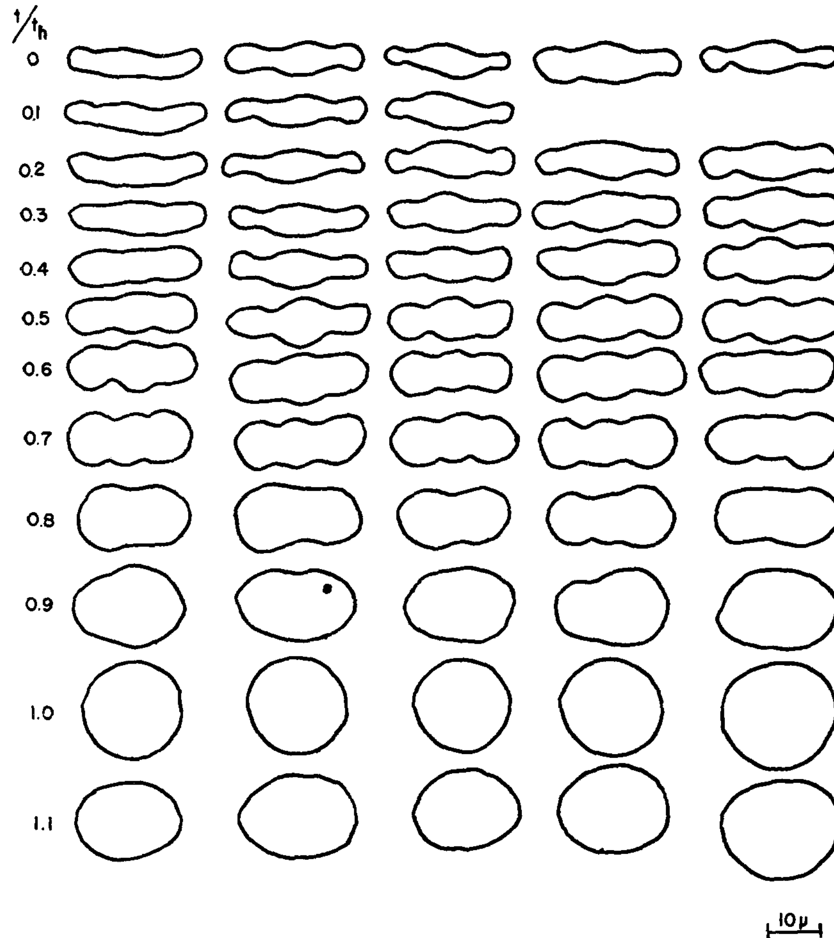

FIGURE 2. Individual cells viewed on edge showing the manner of shape change for the frog erythrocyte following ultraviolet radiation. t/t_h = time of outline/time of hemolysis.

measured dimensions (see Table I). These differences were consistent however, even if not significantly different. If the corrections in area are carried over to volume corrections (this is justified because the cell width remains constant until hemolysis, *i.e.* $A = \pi ac$ and $V = \frac{4}{3} b\pi ac$ where b remains constant), then the volume increase was linear with time to hemolysis (Fig. 1 *b*,

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curve B). Following hemolysis curve B was similar to curve A but leveled off at $V/Vo = 1.27$.

The cell volume could be followed for individual cells viewed on edge when advantage was taken of the fact that cell width remained constant throughout the prolytic period (see Fig. 1 *a*). Volumes of some cells viewed on edge are presented in Fig. 3 using an average cell width of 17.2 μ. Note that the increases in volume followed the same pattern as that shown in Fig. 1 *b* but with some variation among the individual cells.

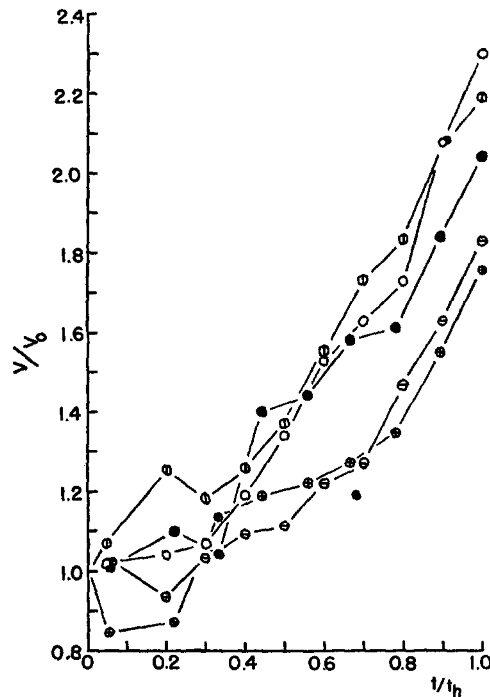

FIGURE 3. Relative volumes of individual cells viewed on edge calculated as $V = \frac{4}{3}\pi abc$ where *b* was the semiaxis which remained constant throughout the prolytic period (see Fig. 1*a*). $b = 8.6\ \mu$, *a* and *c* were measured for each cell viewed on edge. V/Vo = volume at time *t*/volume at zero time. t/t_h = time of measurement/time of hemolysis.

Surface Area and Radiation

It should be noted that the cells irradiated while flat on the slide presented a larger surface area normal to the incident radiation than cells irradiated while on edge. A consideration of the hemolysis times for cells receiving ultraviolet over a broad surface and those irradiated over a restricted surface is presented in Table II with an approximation of the surface areas exposed. There was considerable variation as indicated by the standard deviation, yet

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there was a highly significant difference in the times of hemolysis between the cells treated in these two ways.

TABLE II

SUMMARY OF THE HEMOLYSIS TIMES FOR CELLS IRRADIATED FLAT UPON THE SLIDE AND CELLS IRRADIATED ON EDGE

Cell areas (flat cells, $A = \pi ab$; cells viewed on edge, $A = \pi ac$) were calculated from the data in Fig. 1 *a*. *P* was calculated by the "Student" *t* method; *n* represents the number of cells in the calculation. Line 3 represents the ratio of flat cells to cells viewed on edge for surface area and for ultraviolet effect.

	n	Hemolysis time with standard deviation	*P*	Cell area
		min.		*square microns*
Flat cells	40	58.8±20.9	<0.01	321
Cells viewed on edge	18	85.6±38.9		112
Flat:edge view		1.45		2.86

DISCUSSION

A change in cell shape and volume occurred immediately following irradiation as a result of an increase in cell thickness and in a manner which would be expected of a flattened spheroid type cell. Evidence of a heterogeneous cell anatomy became apparent during the middle third of the prolytic period as the cell began to shorten, the center continued to increase in thickness, and in addition, the ends began to bulge; *i.e.*, the ends of the cell now increased in thickness more rapidly than the center of the cell. An oval region around the center third of the cell resisted change throughout the middle third but disappeared during the final quarter of the prolytic period. When this constriction gave way, the cell became a spheroid, and at the time of hemolysis was very nearly a sphere. Following hemolysis, the cells rapidly became spheroids again with all the dimensions falling below the values at the critical volume. However, the constricted oval around the center of the cell did not reappear, so that the cell never returned to its original shape.

The current hypothesis for the swelling and hemolysis of erythrocytes in an isotonic medium following treatment with certain hemolysins (including ultraviolet irradiation) relies upon the influx of water under the influence of the colloid osmotic pressure of the cell (1, 6, 13, etc.). For the osmotic pressure of the intracellular colloid to be effective in causing water to enter the cell, the cell membrane must be made permeable to cations by the hemolysin. Once cations are permitted to move freely across the cell membrane, the cell swells, rapidly at first because of the high intracellular colloid osmotic pres-

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sure, then more slowly as the colloid is diluted and equilibrium is approached. Such a curve was not obtained in the swelling of frog erythrocytes following ultraviolet radiation, however, for the swelling curve was convex to the abscissa and not concave to the abscissa as might be expected. If this curve is resolved into three straight lines, then the breaks come at $t/t_h = 0.3$ and 0.8. It is at these points that the changes in the manner of swelling were noted, *i.e.* at 0.3 the cell began to shorten and bulge at the ends; at 0.8 the central constricted oval gave way. It must be remembered that these volumes were calculated as if the cells were regular ovate spheroids (see Results) and changes in the *rate* of swelling would be expected at points where an alteration in the *manner* of swelling leads to a change in the measured dimensions (length, width, and thickness) toward a spherical shape. When corrections were made to bring the cell volume into line with the cross-sectional area, a linear swelling curve resulted. This probably represents the actual course of cell swelling, since the corrections amend the slight deviations from the ovate shape associated with the bulging cell ends and the constricted central oval. Thus the cell swells, *i.e.* water moves across the cell membrane, at a constant rate throughout most of the prolytic period despite the varying shape changes which attend this swelling. The cell's ultrastructure does not appear rigid enough to oppose the colloid osmotic pressure of the cell's hemoglobin.

Accepting the linear swelling curve, it is still necessary to account for the failure to obtain a curve concave to the abscissa anticipated on the basis of colloid osmotic swelling. A fixed rate of swelling would be expected if the cell permeability to water were low. This is not supported by experiments with frog erythrocytes in distilled water, where 75 per cent hemolysis is reached in about a minute (5). More likely a limited permeability of the cell to ions regulates the rate of swelling, since in colloid swelling water entering the cell would be opposed by and thus limited by extracellular ions. A similar phenomenon was noted by Parpart and Green (8) in butyl alcohol–treated rabbit erythrocytes, although it was suggested that the rate of cation exchange regulated swelling. Their swelling curves were also essentially linear.

Following hemolysis, the cell rapidly returned to an ovate spheroid in a manner which suggests an elasticity for the cell ultrastructure. Such elasticity may be related to a high degree of organization in the cell periphery similar to that described by Ponder (11) for the mammalian erythrocyte. However, the frog erythrocyte volume remains above rather than falling below normal following hemolysis because of the irreversible loss of the oval around the cell center. This structural feature seems important in the maintenance of normal cell form and merits further study.

The suggested site of the initial and presumably definitive effect of radiation in the cell which subsequently swells and hemolyzes is the cell membrane (1). That the cell can hemolyze following ultraviolet irradiation without alteration

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of the cell metabolism has been demonstrated (2, 4). The experiments in which the time of hemolysis was found to be significantly shorter for cells irradiated over a broad membrane surface (flat cells) than for cells irradiated over a restricted surface (cells on edge) point to the cell membrane as the site of radiation damage although the failure to note a proportionate increase in effect with increased surface area detracts somewhat from this idea.

In summary it may be said that the fact of cell swelling and hemolysis in frog erythrocytes following ultraviolet radiation is most likely a colloid osmotic phenomenon. It seems also likely that damage to the cell membrane (see Table II) and perhaps membrane lipoproteins (see references 1 and 7) initiates this process. Nonetheless other factors, *e.g.* cell ultrastructure and perhaps the rates of cation movement alter the rate of cell swelling and determine the various forms of the cell during the prolytic period and following hemolysis.

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REFERENCES

1. COOK, J. S., *J. Cell. and Comp. Physiol.*, 1956, **47**, 55.
2. GREEN, J. W., *J. Cell. and Comp. Physiol.*, 1956, **47**, 125.
3. HAMBURGER, V., A Manual of Experimental Embryology, Chicago, University of of Chicago Press, 1950.
4. HARVIE, L., and MARONEY, S. P., JR. *Virginia J. Sc.*, 1959, **10**, 263.
5. JACOBS, M. H., GLASSMAN, H. N., and PARPART, A. K., *J. Exp. Zool.*, 1950, **113**, 277.
6. LEU, J., WILBRANDT, W., and LIECHTI, A., *Strahlentherapie*, 1942, **71**, 487.
7. MARONEY, S. P., JR., data to be published.
8. PARPART, A. K., and GREEN, J. W., *J. Cell. and Comp. Physiol.*, 1951, **38**, 347.
9. PONDER, E., *J. Exp. Biol.*, 1942, **19**, 215.
10. PONDER, E., Hemolysis and Related Phenomena, New York, Grune & Stratton, 1948.
11. PONDER, E., Protoplasmatologia, Handbuch der Protoplasmaforschung, Vienna, Springer Verlag, 1955, **10** (2).
12. REED, E. A., *J. Cell. and Comp. Physiol.*, 1948, **31**, 261.
13. WILBRANDT, W., *Arch. ges. Physiol.*, 1941, **245**, 22.

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