CATION EXCHANGE IN MAMMALIAN ERYTHROCYTES

III. The Prolytic Effect of X-Rays on Human Cells*

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Although many types of radiobiological effects have been investigated on the cellular level, little attention has been given to the direct disturbance by ionizing radiation of the distribution of electrolytes between cells and their surrounding medium. Such disturbances are of considerable fundamental interest and the mammalian erythrocyte is a particularly advantageous material for observing them.

When erythrocytes are exposed to high dosage levels, radiation fixation (1) and the conversion of hemoglobin to methemoglobin (2, 3) are observed. At lower levels the production of radiation hemolysis is the most familiar feature. Early experiments indicated that this was secondary to permeability changes which cause the cells to swell to ultimate rupture after radiation exposure (4–6). Later work by Ting and Zirkle confirmed these changes in defibrinated human blood following exposure to 30,000 r of x-rays and subsequent low temperature storage (7–9). The subsequent work of Buhlmann, Liechti, and Wilbrandt (10, 11) showed that the effect was greatly magnified by low temperature storage, being considerably smaller when the cells were stored at room temperature or at 38°C.

In the course of recent studies of the direct x-ray injury of erythrocytes, we have observed that in freshly drawn heparinized human blood, irradiated and then maintained *in vitro* at 24 or 38°C. under a properly controlled atmosphere, potassium begins to leave the cells and is almost quantitatively replaced by sodium. Little if any cellular volume increase occurs for as long as 26 hours after irradiation. Radiation exposure thus produces a disturbance in the cells similar to the "prolytic" changes occurring in erythrocytes which are exposed to low concentrations of certain lysins (12–14). The results suggest that the first evidence of injury is the failure of specific potassium accumulation which precedes the later osmotic failure. The present communication contains observations of this effect and studies of the electrolyte disturbance using isotopically labeled sodium and potassium.

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EXPERIMENTAL

Whole venous blood was collected in a syringe wet with heparin (Upjohn) and 5 or 10 ml. aliquots were measured into 5 inch lusteroid tubes. The atmosphere in the tubes was replaced with alveolar air, and they were closed with paraffined corks, and irradiated. Controls were treated similarly without radiation exposure.

Since in previous work the radiation hemolysis effects were usually studied in blood stored at low temperature, preliminary experiments were done in which samples were stored in a cold room at about 5°C. or at room temperature (27 ± 2°C.) in the light or dark, and assayed after varying time intervals. In later experiments the blood was delivered to paraffin-lined flasks and equilibrated at 23.9 or 37.9°C. under an atmosphere of controlled composition as described in the first paper of this series (15). The gas contained about 5 per cent carbon dioxide and usually 5 per cent oxygen for approximate venous gas saturation although occasionally the blood was "arterialized" with 15 per cent oxygen. The remainder of the constituents were nitrogen and a saturation tension of water vapor. The flasks were opened from time to time and samples were removed for analysis. When analyses for both sodium and potassium in the same sample were required the top layer of centrifuged cells was washed as rapidly as possible with unchilled isotonic sucrose. Isotope studies were conducted in the manner previously described (15). Sodium radioactivity was usually measured in a 100 per cent geometry gamma ray ion chamber (16).

Tubes containing the blood were irradiated in lots of six, placed 27 cm. from the tungsten target of a General Electric "maximar 250" x-ray tube. The peak voltage was 215 to 225 kv. with a mean surface rate at the center of the field of 9 to 15r per second calibrated with a victoreen condenser r-meter. No external filter was used, the equivalent inherent filtration being about 3 mm. of aluminum. Although the energy distribution of the beam was quite inhomogeneous, its absorption in aluminum was roughly exponential with a half-value layer of about 8 mm.

RESULTS

Potassium Leakage at Various Radiation Exposures.—Fig. 1 shows the increase in potassium concentration in the plasma of human blood following various exposures. The cells were equilibrated without added dextrose at 23.9°C. under a controlled atmosphere to maintain essentially the venous condition of gas saturation. At the maximum dose (54,000 r) the plasma potassium concentration has increased to approximately eight times the normal value after 38 hours' equilibration. In the case of the lowest dose used (675 r), the potassium concentration is always above that of the controls, suggesting that a radiation effect exists even below 1000 r, but the differences approach the experimental uncertainty. At 6,750 r the effect is real.

Previous observation has been made of the decrease in plasma potassium concentration of the sort occurring in the controls during the first 14 hours (15). The abnormally high initial concentration suggests that the cells were reversibly disturbed by the necessary manipulation of the blood during the initial phase of the experiment. Subsequently they were able to recover,

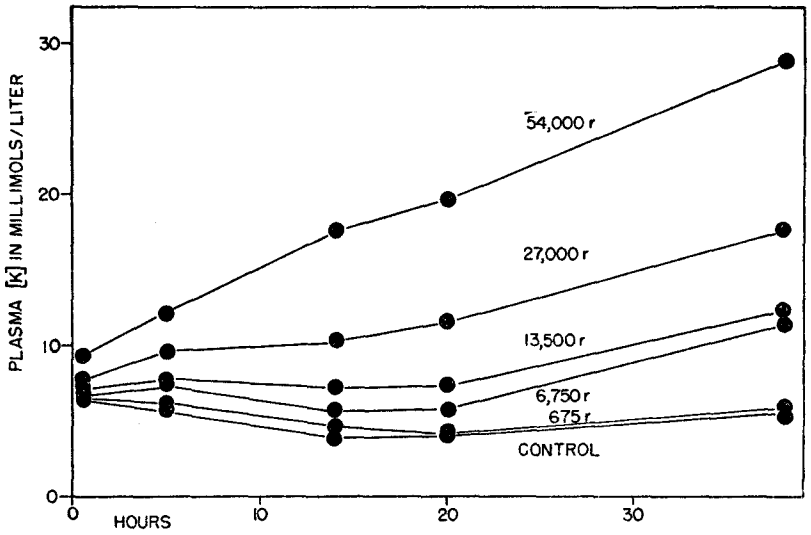

FIG. 1. Progressive increase of plasma potassium concentration (ordinates) in human blood following varying x-ray exposures (attached numbers are the surface exposures measured in the center of the field). The blood without added sugar was equilibrated at 23.9°C. under a controlled atmosphere following irradiation.

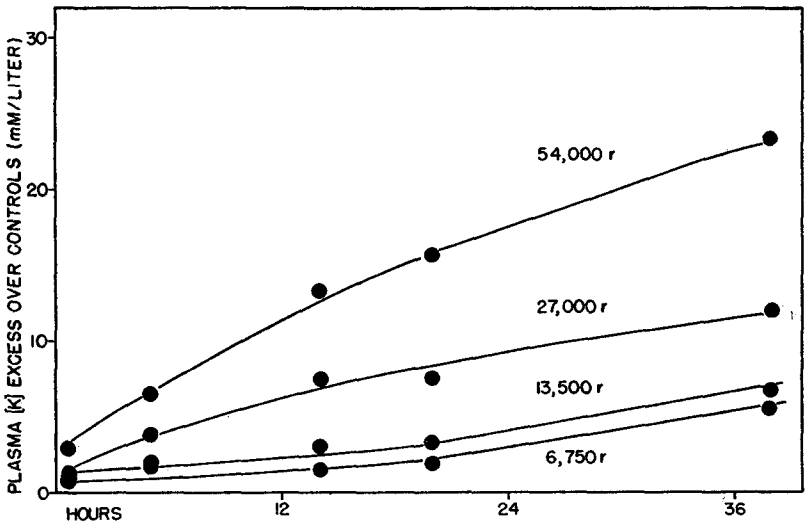

FIG. 2. Excess of plasma potassium concentration over the controls (see Fig. 1). The experimental points are fitted with second degree curves by the least squares method.

accumulating potassium in the process. The radiation effect is superimposed upon these changes and the two effects are equal at about 13,000 r. The result

of radiation alone is more clearly seen in Fig. 2 which shows the difference between the potassium concentration in the irradiated samples and the controls.

Reciprocity of Potassium Loss and Sodium Penetration.—Table I shows that no significant increase in mean cell volume is produced by radiation since the slight increase in the controls exceeds that in the irradiated cells. The blood received 43,000 r, and was subsequently maintained as in the previous experiment. In this case the potassium which leaves the irradiated cells is almost

TABLE I

Equivalence of Potassium Leakage and Sodium Penetration

Time	Hematocrit	Na concentration		K concentration		Total [Na] + [K]	
		Cells*	Plasma	Cells*	Plasma	In cells	In plasma
hrs.	*per cent*	*mM/liter*	*mM/liter*	*mM/liter*	*mM/liter*	*mM/liter blood*	*mM/liter blood*
Irradiated samples (43,000 r)							
1.25	34.8	18.5	145	126	6.7	78	99
2.50	35.7	21.8	138	122	8.1	79	94
19	35.4	41.5	129	105	19.0	80	96
25.6	35.1	46.0	127	103	20.2	81	95
42	—	—	—	—	—		
Controls							
1.25	34.2	15.8	149	140	5.5		
2.50	35.2	12.8	153	143	5.2		
19	37.0	15.6	152	140	5.7		
25.6	—	—	—	—	—		
42	36.0	19.1	150	142	5.1		

* Concentrations per liter of cell water assuming this to be 65 per cent of the total cell volume.

exactly compensated stoichiometrically by the penetration of sodium, the net gain in total cellular cations being less than 4 per cent.

Effect of Storage Temperature.—Earlier studies have shown that the osmotic fragility changes which occur following the exposure of blood to x-rays are definitely accentuated by subsequent low temperature storage. The failure of specific potassium accumulation is also affected by temperature. Fig. 3 shows the increase in potassium leakage and sodium penetration produced by low temperature storage of human cells irradiated at room temperature. The blood was stored in lusteroid tubes without addition of sugar.

Penetration of K^{42}.—In blood under normal physiological conditions potassium and sodium are constantly moving into and out of the cells. Exposure to radiation could cause a net decrease of cellular potassium (*a*) by reducing the

penetration rate, (*b*) by accelerating the rate of loss from cells to plasma, or by both. It is now a familiar principle (15) that in a closed system of two compartments, the amount of a given cation which goes from the first to the second compartment and *vice versa* may be determined in a single experiment if determinations of specific activity are made in both compartments. Required also are determinations of change in radioactivity and in total cation made in one compartment only. It thus becomes possible to choose among the possible

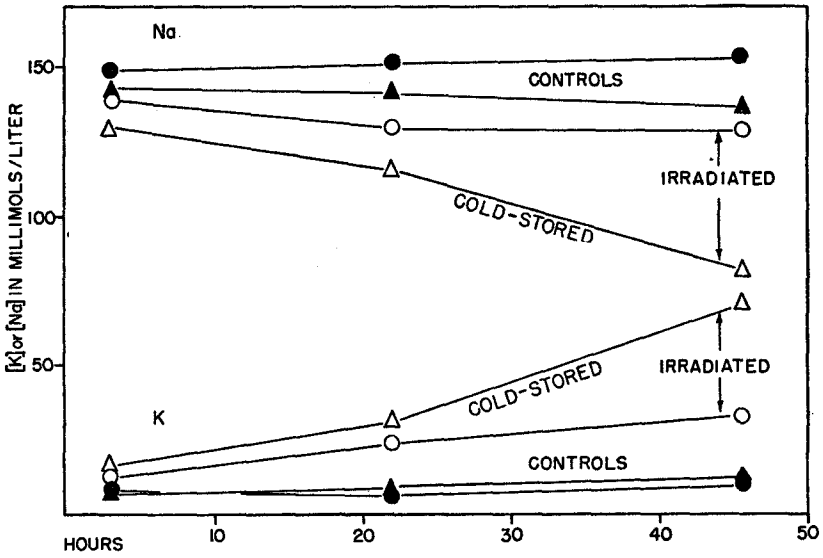

FIG. 3. Effect of storage at low temperature on erythrocyte potassium leakage and sodium penetration in irradiated (43,000 r surface exposure) and control blood stored in lusteroid tubes (no sugar added). Plasma potassium and sodium concentrations are shown for irradiated blood at 27°C. (○) and 5°C. (△), also controls at 27°C. (●) and 5°C. (▲).

ways in which the cellular potassium decrease occurs. The effect of radiation on sodium transport can also be studied by isotope methods.

Since for potassium the cells and plasma of normal human blood may be considered as a two-compartment system (15), observations of the initial changes immediately following the labeling of the plasma will be easily interpreted. During the initial period, the fraction of plasma radioactivity penetrating per hour is equal to the fraction of potassium ions which penetrate. In irradiated blood, the hourly rate of increase in plasma potassium is the amount by which the mean loss from the cells exceeds the uptake. The rate from cells to plasma is thus obtained by difference.

A typical result for the penetration of K^{42} at 23.9°C. is shown in Fig. 4.

The penetration rate for both the irradiated samples and the controls is 4.2 per cent of the plasma potassium per hour. In a second experiment the rate changed from 5.3 to 4.0 per cent per hour, representing only a slight decrease. Fig. 5 shows the over-all loss of potassium which occurred from the irradiated cells at a rate of about 12 per cent per hour (of the control plasma K). Thus the exposure has accelerated the outgo rate from 4.2 to 16 per cent per hour. We conclude that the potassium loss from the cells, as shown by the rate of increase in plasma potassium, is due primarily to an accelerated outgo from the cells

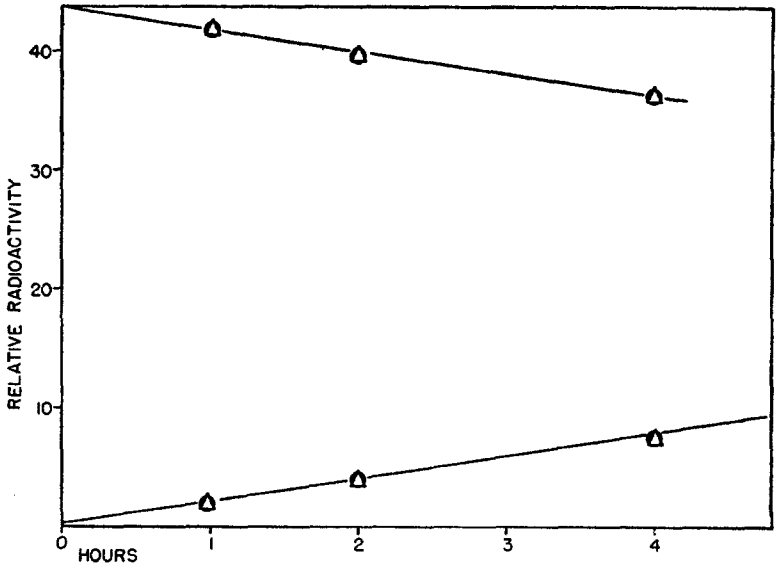

FIG. 4. Changes in the K^{42} radioactivity of cells (lower curve) and plasma (upper curve) of control samples (△) and irradiated samples (●) (35,000 r surface exposure) equilibrated under a controlled atmosphere at 23.9°C. (with added sugar).

rather than to an impairment of uptake. The conditions of equilibration under a controlled atmosphere were the same as in the previous experiments.

Equations for the Penetration of Na^{24}.—The specific activity changes in experiments with radioactive sodium in normal human blood were reported in a previous communication (17) in which it was observed that the intracellular sodium was not homogeneous, about half being exchanged very slowly. Because of this and the complicating effect of the progressive net increase in intracellular sodium in irradiated blood, the simple analysis used in the potassium experiments will not apply and a more complete treatment of the problem is required. Fortunately the slowly exchanging fraction in the cells moves at such a slow rate that a satisfactory approximation may be obtained by assuming that the rapid fraction forms a two-compartment system with the plasma.

The familiar equations used in interpreting experiments in such a system (15) apply only under steady state conditions under which the two opposing transports are equal and opposite. In the present case considerable information can be obtained by applying a new set of equations derived for the case in which the two transports are unequal.

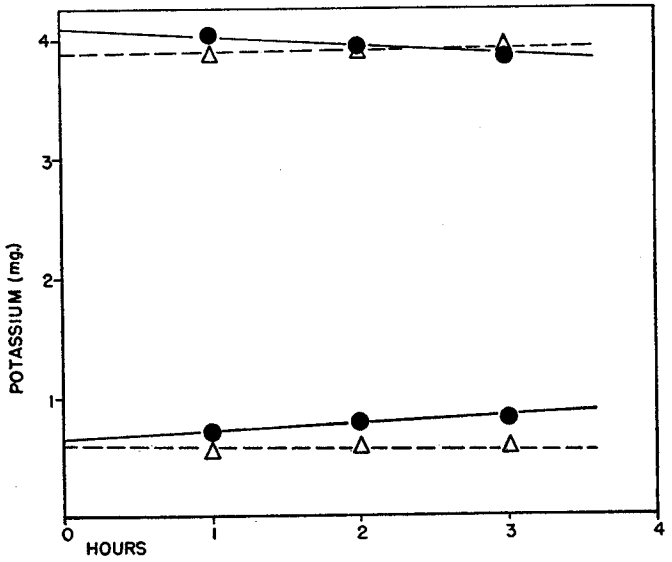

FIG. 5. Total potassium (milligrams) in cells (upper curve) and plasma (lower) of control (△) and irradiated (●) blood samples at 23.9°C. (see Fig. 4).

The notation is the same as previously used with the following additions:

R_1, R_2 = the total radioactivity in compartments 1 and 2 respectively;
S_1 (t), S_2 (t) = variable amounts of S in compartments 1 and 2;
S_1 (0), S_2 (0) = initial amounts;
$\bar{S}_1$, $\bar{S}_2$ = average values of S_1, S_2;
ρ_{21} = rate of flow from S from compartment 1 to compartment 2;
ρ_{12} = opposing rate of flow;
Δ = difference in rates.

It has been shown (18) that the differential equations describing the specific activity changes when the two transport rates differ are

$$da_1/dt = (\rho_{12}/S_1)(a_2 - a_1) \tag{1}$$

and

$$da_2/dt = (\rho_{21}/S_2)(a_1 - a_2).$$

For the initial conditions $a_1 = a_1(0)$ and $a_2 = 0$, the solutions are

$$\begin{aligned} a_1/a_1(0) &= S_1(0)/S + [S_2(0)/S] \exp\left[-S\int_0^t (\rho_{12}/S_1S_2)\,dt\right]^1 \\ &= [S_1(0)/S]\left\{1 + (S_2/S_1)\exp\left[-S\int_0^t (\rho_{21}/S_1S_2)\,dt\right]\right\} \end{aligned} \tag{2}$$

$$a_2/a_1(0) = [S_1(0)/S]\left\{1 - \exp\left[-S\int_0^t (\rho_{21}/S_1S_2)\,dt\right]\right\} \tag{3}$$

Na^{24} Experiments.—In a series of experiments with radioactive sodium the plasma was initially labeled and the disappearance of activity was followed. The radioactivity variations are best employed in interpreting the results because of the precision of the determinations, particularly when the ion chamber was used. Since the rapidly exchanging sodium moves in and out so much faster than the over-all mass rate of movement into the cells, Δ, which is roughly constant, Equation 2 becomes

$$R_1/R_1(0) = S_1(t)/S + [S_2(t)/S] \exp\left[-(\rho + \Delta)(1/\bar{S}_1 + 1/\bar{S}_2)t\right] \tag{4}$$

where ρ is the outward rate, and $\rho + \Delta$ the rate inward. This expression predicts that the plasma radioactivity curve will consist of a rapidly varying component, which is roughly exponential in form, superimposed on a uniformly declining base line whose fractional rate of decrease is the same as the fractional rate of disappearance of total sodium from the plasma. By extrapolating the linear portion back to its intersection with the axis of ordinates, the base line slope $(dR_1/dt)_{eq}$ and the ratio $S_2(0)/S$ may be obtained; and from the latter, the initial size $S_2(0)$ of the rapidly exchanging intracellular compartment.

Fig. 6 shows three representative results in which the penetration of radioactivity was observed both in control blood and after exposure to radiation followed by different postirradiation periods of sodium accumulation. The curves for these and other experiments follow the general form of Equation 4 quite closely, considering the relative smallness of the effect which places a rather stringent requirement on the accuracy of the radioactivity determinations. Table II shows the correlation observed between the base line slope (column 4) and Δ the measured rate of replacement of intracellular potassium with sodium (column 3). In each case the small but significant amount (column 5) by which the base line slope exceeds the rate of increase of cell sodium represents the exceedingly slow rate of exchange with the bound fraction of intracellular sodium. If the latter rate be depressed to any extent by radiation the effect is insignificant. The size of the rapidly exchanging compartment (column 9) as determined from the intercept, increases with radiation exposure

[1] For typographical simplicity we employ the convention $exp\ x = e^x$.

and postirradiation storage, both of which contribute to the enhanced replacement of intracellular potassium with sodium. The effect is best seen in the last two lines (Experiments 6 and 7). The variation in the magnitude of the slowly exchanging sodium component is not sufficiently great to be significant.

The experimental data also permit an estimate of the effect of radiation on the rate of transport of the rapidly exchanging fraction. We consider only the rate

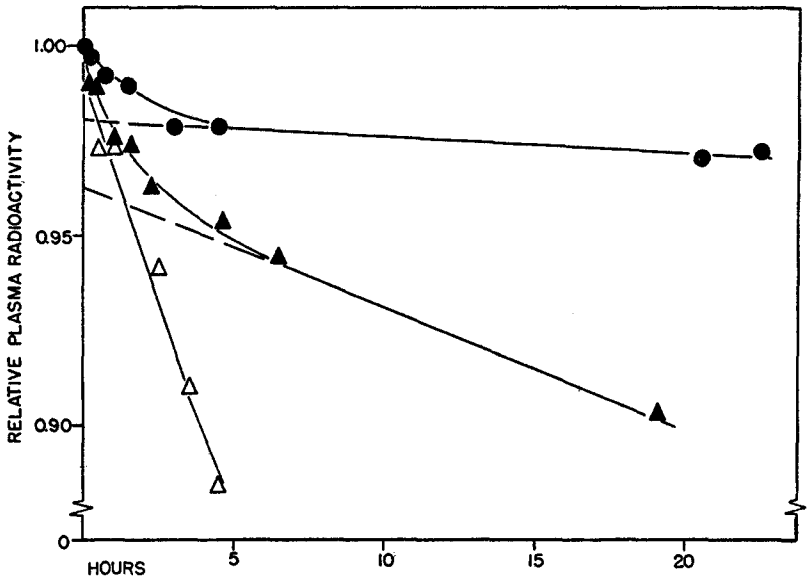

FIG. 6. Disappearance of Na^{24} from the plasma of control and irradiated blood. The ordinate scale is interrupted, showing only the greatly magnified upper portion. The control points (●) are those from a typical experiment on non-irradiated blood. The irradiated samples were exposed to 42,000 r, one group being kept at 38°C. for 6 hours (▲) and a second for 17½ hours (△) before introducing the isotope. The broken lines indicate the method of drawing the tangent to the curve and determining the zero intercept.

of transport, ρ, from plasma to cells. The reverse rate is obtained by adding the small quantity, Δ. By determining the effective exponential constants for the various experiments, using Equation 4, estimates are made of the ρ values which are given in the last column. The mean value for irradiated cells shows no significant change compared to the mean for the controls. In an attempt to obtain further information on this point, two experiments were done in which the cells were labeled with Na^{24} by overnight equilibration. On the following day portions of this blood were irradiated, and the cells, together with a set of controls, were resuspended in non-radioactive plasma, and the loss of activ-

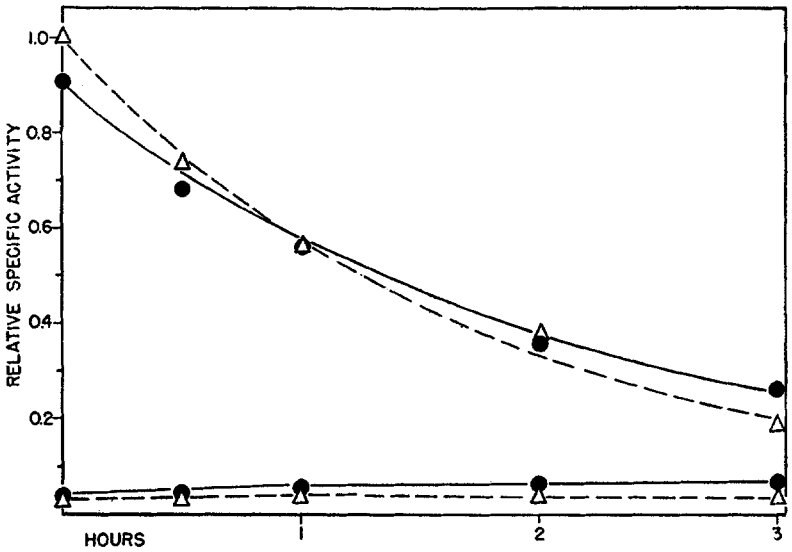

FIG. 7. Relative specific activity variations of plasma and rapidly exchanging cell sodium in control (△ and - - -) and blood exposed to 42,000 r (● and ——). The cells were initially labeled and resuspended in non-radioactive plasma. The lower curves represent the plasma and the upper curves (see text) the rapidly exchanging fraction of cell sodium. The ordinates represent the ratios of the specific activities to that of the rapidly exchanging cell fraction at the moment of resuspension.

TABLE II

Penetration of Na^{24} into Control and Irradiated Erythrocytes at 38°C.

Experiment	Radiation	Decrease in plasma Na per hour = Δ*	$\left(\frac{dR_1}{dt}\right)_{eq.}$ ‡	$\left(\frac{dR_1}{dt}\right)_{eq.} - \Delta$	Mean storage interval after irradiation	Initial cell Na			Fast component Na transport rate from cells to plasma = ρ
						Total	Slowly exchanging	Rapidly exchanging	
	r	*per cent plasma Na/hr.*	*per cent/hr.*	*per cent/hr.*	*hrs.*	*mM/liter of cells*			*mM/liter of cells/hr.*
1	15,000	0.06	0.11	0.05	2	13	7.5	5.5	5.1
	0	0.07	0.07	0.00	—	12.5	7	5.5	3.0
2	35,000	0.25	0.19	−0.06	1½	13	8	5	3.1
	0	—	—	—	—	9.5	5	4.5	3.3
3	48,000	0.24	0.26	0.02	3	—	—	—	—
	0	0.01	0.02	0.01	—	—	—	—	—
4	0	0.014	0.07	0.056	—	11.5	7	4.5	3.4
5	0	0.017	0.03	0.013	—	14.5	9	5.5	2.5
6	42,000	0.18	0.33	0.15	6	14	7.5	6.5	3.1
7	38,000	0.19	—	—	10	20	6.5	13.5	3.3

* The chemical data were obtained by fitting a linear function to the serial Na analyses by the method of least squares.

‡ Per cent rate of decrease of plasma radioactivity per hour obtained from the slope of the tangent to the curve (see Fig. 6).

ity followed. The interpretation of these experiments was complicated by the presence of some activity which entered the slowly exchanging compartment during the equilibration period. From the several earlier observations of the penetration of the isotope in non-irradiated blood, it was estimated that after 15 hours' equilibration the rapidly exchanging compartment contained about 87 per cent of the total cellular activity. Similarly, it was estimated that about 41 per cent of the intracellular sodium was in the rapidly exchanging fraction. Using these data the relative specific activities were calculated for one of the experiments shown in Fig. 7. The curves shown in the figure were obtained by constructing semilog plots and fitting straight lines to the points. The resulting functions were then replotted on a linear ordinate scale. The close similarity for irradiated and control blood indicates that the kinetics of sodium transport are not significantly altered by radiation. The mean value of ρ for the irradiated and control samples is 3.2 per liter of cells per hour in satisfactory agreement with 3.3 which is the mean of the values given in Table II.

DISCUSSION

Comparison of Dose with Effect.—A precise comparison of the biological effect with the radiation dose is limited by the spatial non-uniformity of the radiation. As shown in Fig. 1, even after a $2\frac{1}{4}$ hour period of radiation exposure the unexposed controls showed a definite reversible disturbance of the potassium equilibrium. In order to get sufficiently high x-ray exposures within the necessarily short time for minimal disturbance of the controls, it was necessary to place the material close to the port of the x-ray tube and to use an unfiltered beam. Under these conditions, the radiation intensity varied throughout the irradiated specimen and a considerable correction must be made in order to relate the mean dose received by the blood to the surface exposure in the center of the field. Since the surface exposure was measured, this quantity is given in Figs. 1 to 7 and in Table I. By a graphical integration of the dose over the known field variation and over the known absorption of the beam in the irradiated material, the average dose was found to be about 75 per cent of the surface exposure. This quantity is used in the curve of effect *versus* dose (Fig. 8).

The decrease in cell potassium following radiation exposure was not entirely regular. To obtain a semiquantitative objective evaluation of radiobiological effect, the experimental points in Fig. 2 were fitted with second degree curves by the least squares procedure. From the coefficients the average slopes were determined, and also the maximum and minimum slopes. The results are plotted in Fig. 8 as a function of the mean radiation dose (0.75 $\times$ surface exposure). The figure also includes a straight line representing a least squares fit to the data. At 23.9°C. the curve passes close to the origin, suggesting little if any threshold effect. The mean rate of decrease of cell potassium at this temperature is about 0.4 per cent per hour at 20,000 r and approximately doubles for a 20,000 r increase.

The Prolytic Effect.—The production by low concentrations of hemolysins of a similar effect in human erythrocytes has been described by several workers (12–14). Its verification for ionizing radiation again stresses the importance of specific potassium accumulation in the physiology of the red cell. It is of interest that the ion specificity failure occurs considerably in advance of the osmotic disturbances. The fact that both effects are disturbances of ionic stability

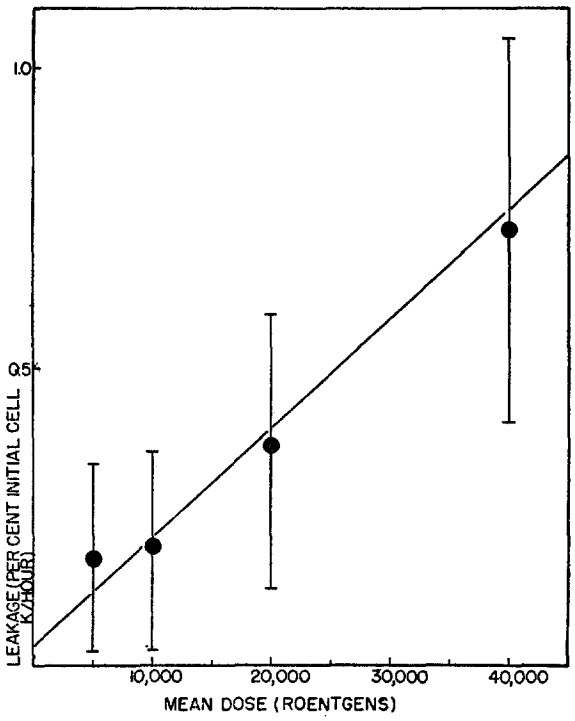

FIG. 8. Potassium leakage rate in per cent initial cell K per hour *versus* mean radiation dose. The vertical bars extend from the minimum to the maximum rates. A straight line function fitted by the least squares method is included.

suggests that they are closely related. The low temperature accentuation phenomenon is common to both. Recently Ting (19), using K^{42}, has observed potassium disturbances in human cells at as little as 400 r when they are equilibrated at 6°C. following irradiation at room temperature.

The extent to which the results of x-ray exposure are similar to the effects produced by lytic agents is not yet established. A clear point of difference is that the x-ray effects occur in the presence of the plasma proteins which exert a definite protecting action in the experiments with lysins. A point in common is the inability of these investigations to distinguish between the complete disturbance of part of the cells and the partial disturbance of all of them.

The concept of two intracellular sodium compartments provides a convenient approximation for describing the effects of radiation exposure and post-irradiation leakage. The increase in the size of the rapidly exchanging compartment concomitant with sodium penetration following exposure is of considerable interest. If it be admitted that sodium penetrates as free ions then the observation identifies the rapidly exchanging compartment as being essentially free ionic sodium. The slowly exchanging sodium would thus be bound in some way, the amount being quite small in terms of the total cation content of the cell but relatively large in terms of the small total intracellular sodium.

Since at all but the lowest exposures the rate of potassium leakage produced by x-rays is greater than the rate of penetration of potassium in normal cells, the effect cannot be explained simply as an impairment of the uptake of potassium. The isotope experiments confirm this but also show that the uptake is not accelerated as might be the case, say, in a general increase of membrane permeability. X-ray exposure has seemingly disturbed a mechanism of potassium accumulation which is based on a selective retention of the intracellular cation.

SUMMARY

Freshly drawn heparinized human whole blood is exposed to x-rays in amounts up to 54,000 r *in vitro* and then equilibrated under a controlled atmosphere at 24 or 38°C. For as long as 26 hours following exposure, potassium is progressively lost from the cells and quantitatively replaced by sodium with little, if any, osmotic disturbance. The mean rate of loss at 20,000 r and 24°C. is about 0.4 per cent of the initial cell potassium per hour and approximately doubles for a 20,000 r increase. It is accentuated if blood is stored at low temperature (5°C.) following radiation exposure. Isotope experiments show that the rate of entrance of potassium into the cells is practically unaltered, the principal effect being an acceleration of the rate from cells to plasma. This suggests that radiation may have interfered with a mechanism of selective potassium accumulation based on preferential retention of the element. The sodium which enters the cells following irradiation contributes to the rapidly exchanging portion of the cellular sodium, suggesting that this fraction is ionic sodium.

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