METHODS

IONIZING RADIATION INJURIES OF ERYTHROCYTE MEMBRANES

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The view is now accepted in radiobiology that injury not only to DNA, but also to biological membranes, plays an important role in radiation damage to cells [2]. The severity of the damage to the body as a whole resulting from irradiation increases with an increase in the dose of radiation. To determine the contribution of membrane injuries to the development of radiation pathology of cells, and also to develop methods of indication of radiation damage it is important to study the degree of injury caused to membrane structures by different doses of ionizing radiation. Various methods have been used to study this problem [3, 5, 8]; increased damage to erythrocyte membranes, dependent on high radiation doses, has been demonstrated as a result of irradiation *in vitro* [8]. Erythrocytes contained in peripheral blood are a good material with which to study membrane injuries because of the large numbers of them in the blood and because they can be obtained without any significant trauma to the human body.

The objectives of this investigation were to develop a method of studying the degree of injury to erythrocyte membranes and to study injuries to erythrocyte membranes in animals after irradiation.

EXPERIMENTAL METHOD

To assess the degree of injury to cells after irradiation the character of distribution of erythrocytes in urografin during sedimentation was studied (urografin is an x-ray contrast substance which can also be used for the fractionation of blood cells [1]). To determine the qualitative characteristics of injury to cells separated by centrifugation in urografin the distribution of erythrocytes was studied after the addition of chemical substances whose harmful action on the various components of membranes had been adequately studied. Rats weighing 180-220 g were used in the experiments. Some of the rats were exposed to whole-body irradiation in doses of 100 to 600 R (EGO-2 apparatus, 112 R/min). Blood was collected from each animal 3 h after irradiation, and heparin was used as the anticoagulant. The samples were incubated at 37°C for 1 h to sediment the erythrocytes. The erythrocyte fraction was then separated and 0.5 ml of it was treated with 0.25 ml of solutions of one of the following substances which damage membranes: Triton X-100, ethanol, Tween-80, acetic acid. The samples were kept in an air incubator for between 30 and 105 min at 37°C or 64°C. Next, 0.5 ml of the samples, containing 0.8 billion cells, was poured on the surface of 4 ml of 36% urografin and centrifuged at 15,000 rpm for 20 min on the K-24 centrifuge. A sample of 0.2 ml was then taken from the top zone, and the residue also was collected separately. Each fraction was dissolved in 5 ml H_2O and its optical density measured at 380 nm. The number of cells forming each zone was then determined from a calibration curve, plotted by comparing the number of erythrocytes counted in a Goryaev's chamber and the corresponding optical density of the same samples after hemolysis. The experimental results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

After centrifugation of the erythrocyte samples taken from the control animals, and not exposed to the action of physical or chemical factors, in urografin several zones were formed (Fig. 1). Intact erythrocytes do not penetrate through urografin and remain on its surface (Fig. 1, zone 2). A small proportion of erythrocytes disintegrated during isolation. The disintegration products, including those absorbing light at 380 nm, were distributed above

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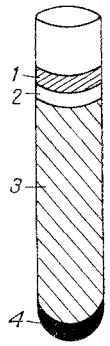

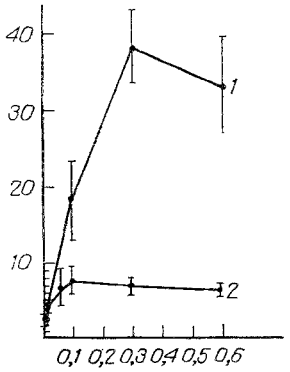

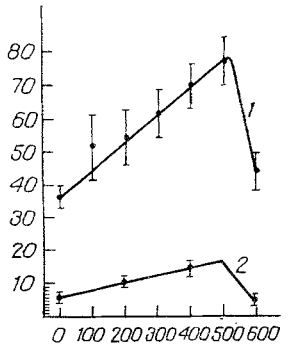

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Distribution of erythrocytes and their disintegration products after centrifugation in urografin. Zone 1) erythrocyte disintegration products; zone 2) erythrocytes; zone 3) urografin; zone 4) residue of injured erythrocytes.

Fig. 2. Harmful action of different concentrations of Triton X-100 (1) and ethanol (2) on erythrocyte membranes. Abscissa, concentration of Triton X-100 (in %) and ethanol (in M) in samples; ordinate, number of erythrocytes in zone 4 (in millions).

Fig. 3. Harmful action of 0.3% Triton X-100 (1) and of 0.1 M ethanol (2) on erythrocytes in blood of rats irradiated in various doses. Abscissa, dose of irradiation (in R); ordinate, number of erythrocytes in zone 4 (in millions).

the intact cells (Fig. 1, zone 1). Some erythrocytes (0.1-0.2% of the total number of cells applied) penetrated into the urografin (Fig. 1, zones 3 and 4). In urografin the erythrocytes either disintegrated, in which case the products of urografin-induced disintegration were distributed in zone 3, or they reached the bottom of the tube (Fig. 1, zone 4). Attempts were made to determine the causes of formation of the cell residue, on the assumption that erythrocytes penetrated into urografin after injury to their membranes, but while still preserving their cellular integrity. Experiments accordingly were carried out in which the erythrocytes were exposed to certain chemical and physical factors injuring membranes.

1. Action of Chemical Factors. The effect of Triton X-100 on the number of erythrocytes capable of passing through urografin with their integrity preserved is shown in Fig. 2. The number of these cells increased with an increase in the concentration of Triton X-100 to 0.3%. Triton X-100 is known to injure erythrocyte membranes; by eluting up to 50-60% of high- and low-molecular-weight proteins [7], it is firmly bound with the membrane, and is adsorbed on hydrophobic regions. An increase in the number of cells in the residue with an increase in the concentration of Triton X-100 indicates an increase in the number of cells with membranes injured by the detergent. When the concentration of detergent exceeded 0.3%, an ever-increasing proportion of the damaged cells disintegrated, so that the residue did not increase but decreased. Almost total solubilization of the erythrocyte membranes took place in a 1% solution of Triton X-100 [4].

Under the influence of ethanol (Fig. 2) an increase also was observed in the number of sedimenting uninjured cells, but they were fewer in number than after treatment with Triton X-100. Similar data also were obtained when intact erythrocytes were treated with acetic acid, Tween-80, and water.

2. Action of Physical Factors. Since an increase in the temperature of the incubation medium above 37°C is known to increase damage to cells, the effect of various temperatures on disintegration of erythrocytes and on damage to their membranes was investigated in the presence of harmful chemical factors (Table 1).

TABLE 1. Effect of Temperature and Chemical Agents on Disintegration of Erythrocytes and Damage to Their Membranes

Chemical agent	37 °C		64 °C	
	disintegrating cells (zone 1), millions	cells in residue (zone 4), millions	disintegrating cells (zone 1), millions	cells in residue (zone 4), millions
Distilled water	18,17±0,48	1,91±0,52	103,80±24,45	87,00±9,78
0,3% Triton X-100	63,10±8,00	12,76±4,18	153,25±6,15	116,92±10,96
	141,30±5,48	54,52±9,56	169,50±5,71	45,90±6,50

TABLE 2. Effect of Duration of Incubation of Erythrocytes at 37°C on Damage to Erythrocyte Membranes and Cell Disintegration

Chemical agent	30 min		60 min		105 min	
	disintegrating cells, millions	cells in residue, millions	desintegrating cells, millions	cells in residue, millions	disintegrating cells, millions	cells in residue, millions
—	18,17±0,48	1,91±0,52	13,20±3,34	1,51±0,37	44,30±13,75	2,25±0,54
Distilled water	63,10±8,00	12,76±4,18	—	—	76,80±4,42	7,10±1,74
0,3% Triton X-100	141,30±5,48	54,32±9,56	143,32±13,05	47,70±5,00	129,25±5,38	44,32±6,78
0,1 M Ethanol	21,15±5,38	4,41±0,82	24,00±2,75	3,82±0,44	—	—

TABLE 3. Effect of Gamma-Irradiation *in vivo* on Disintegration of Erythrocytes and Damage to Their Membranes

Dose of irradiation, R	Disintegrating cells, millions	Cells in residue, millions
—	3,41±0,61	1,62±0,24
200	4,50±0,97	2,47±0,23
400	2,65±0,73	1,83±0,31
600	3,62±0,26	1,39±0,22

It will be clear from Table 1 that an increase in temperature led to an approximately fivefold increase in the intensity of erythrocyte disintegration and a 10- to 20-fold increase in the number of injured cells (Fig. 1, zone 4), except in the case of Triton X-100, for which injury and disintegration reached a maximum at 37°C. These results support the hypothesis that erythrocytes in the residue after centrifugation in urografin are formed by cumulation of membrane injuries. Consequently, the degree of injury to the membranes can be judged by the number of cells in that zone.

To test the validity of this conclusion it was interesting to study the distribution of erythrocytes and their disintegration products during centrifugation in urografin under conditions not significantly affecting injury to the cells. Table 2 gives data showing the effect of duration of incubation of the erythrocytes at 37°C with chemical agents on damage to the erythrocyte membranes and disintegration of the cells.

It will be clear from Table 2 that the duration of incubation at 37°C does not play a significant role in disintegration of the cells and the formation of injuries to the erythrocyte membranes. Incubation for 30 min led to results similar to those of incubation for 60 and 105 min, although it must be admitted that during incubation of erythrocytes for 105 min without the addition of chemical agents, the number of disintegrating cells was increased.

3. Determination of Damage to Erythrocyte Membranes following Irradiation in Experiments *in vivo*. By the use of the technical approach described above the harmful effect of whole-body γ-ray irradiation of rats in a dose of up to 600 R on erythrocyte membranes was investigated (Table 3).

No dose-dependent increase in the number of cells in the residue or in the number of

disintegrating cells (Table 3) could be found 3 h after irradiation by the direct method (incubation of erythrocytes at 37°C for 30 min without exposure to additional chemical factors). By contrast with this, after incubation of erythrocytes from control and irradiated animals with Triton X-100 or with ethanol a significant increase was observed in the number of cells with altered membranes after irradiation in doses of between 200 and 500 R (Fig. 3). The degree of increase in the number of damaged cells depended on the dose of irradiation. Changes after irradiation in doses of 200 and 500 R, revealed after treatment of the cells with Triton X-100, differed significantly ($P < 0.05$) from the normal level and from one another. A similar result could be obtained by treatment of the erythrocytes with other chemical agents. For example, after treatment of the erythrocytes of intact rats with 0.3% Tween at 37°C for 30 min the number of erythrocytes with damaged membranes was 44.96 ± 4.20 million cells, compared with 126.86 ± 30.80 million cells in rats irradiated in a dose of 400 R. The number of irradiated erythrocytes in zone 1, disintegrating under the influence of the chemical agents, did not depend on the dose of irradiation.

The degree of damage to the cells following exposure to ionizing radiation in doses of up to 500 R can thus be assessed by the number of erythrocytes passing through urografin during centrifugation.

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STUDY OF IMMUNOLOGIC REACTIONS ON A SILICON SURFACE BY ELLIPSOMETRY

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Ellipsometry is an optical method whereby changes in the state of polarization of light reflected from the phase boundary between two media can be used to measure the thickness and optical constants of thin films located on the boundary and also to study the kinetics of surface processes leading to film formation (such as adsorption, electrochemical reactions on electrodes, and so on) [1].

Rothen [7] showed by the use of ellipsometry that immersion of glass and metal plates covered with a layer of antigen in homologous antiserum leads to specific adsorption of antibodies on them. The thickness of the adsorbing layer under these circumstances is measured in tens or even hundreds of Angström units, whereas during nonspecific adsorption from heterologous antiserum it measures about 5-20 Å. The reason why these processes can take place is because determinant groups of the adsorbed antigen remain accessible for the active centers of the antibodies. The sensitivity of this method, according to our own observations and data obtained in other countries [9], is about 10^{-6} g antibodies/ml.

In 1969 Rothen and Mathot [8] developed an even more sensitive (down to 10^{-9} g/ml) variant of this method, known as immunoelectroadsorption (IEA). An appropriate electron po-

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