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# THE EFFECTS OF LOW DOSES OF IONIZING RADIATION UPON THE MICROMORPHOLOGY AND FUNCTIONAL STATE OF CELL SURFACE

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## Abstract

The cellular membrane as one of the targets of ionizing radiation might play an important role in the development and modification of radiation-induced alterations after low doses. The present paper reviews the micromorphological and functional changes of plasma membranes of irradiated blood and cultured cells with special emphasis on the surface conditions: lectin binding, negative surface charges. The review is completed by our own studies on distribution of positive surface charges and the bindings of two lectins, the Concanavalin A and the wheat germ agglutinin. It was found that the decrease of negative surface charges is unconcomitant with appearance of domains exposing positive ones, particularly on the surfaces of rufflings. The distribution of Concanavalin A binding sites turned from a uniform distribution to a polarized one, especially on apical regions where it appeared in large aggregates. The polarity in localization of wheat germ agglutinin on untreated fibroblasts observed in our experiments ceased shortly after irradiation.

Key words: ionizing radiation, surface charges, lectin binding, human fibroblast, cell membrane microdomains, surface morphology, scanning and transmission electron microscopy.

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## The plasma membrane as radiosensitive target

### Alterations of supramolecular organization of membrane

The structures and functions of biological membranes are organized in space and time, they are in interrelationship with the cellular environment and internal milieu /48/. The radiation-induced alterations in the supramolecular organization and coordinated functions of membranes contribute to the pathobiology of acute radiation injury /11, 27-29, 39, 64, 71/. The interphase death of cells /cytolysis/ after sublethal doses occurs through several membrane-bound processes /7, 26, 41, 69, 70/. Upon non-lethal doses of radiation the membranes become perturbed reversibly and this might be a direct or indirect cause of non-stochastic effects in tissues /11, 27, 29, 31/. A few examples on supramolecular and functional alterations characteristic of the radiation-induced membrane perturbation after sublethal doses are presented on Table 1.

Alterations in membrane fluidity upon irradiation and some other factors have been summarized recently /39/. Data are known concerning the fluidity changes of membranes following irradiation as well as on lipid peroxidation /Petkau et al, Alper et al. in ref. 11/. It is interesting to note, however, that recent observations point to the dose-dependent alterations both in quality and tendency. Namely, low doses /1 Gy/ produce an increase of fluidity of erythrocyte membranes through protein alterations without observable lipid peroxidation /46, 72/. Obviously, a lipid peroxidative

Table 1.

Membrane Effects of Low-level Irradiation

| Effect | Cell Type | Dose, Gy | Reference |
|---|---|---|---|
| I. Changes of supramolecular organization | | | |
| 1. Increase of cell membrane fluidity | erythrocytes | 1 | /72/ |
| 2. Decrease of negative charges | lymphocytes, erythrocytes, cultured cells | 0.25-2 | /51-53,58,59,61, 62,66/ |
| 3. Changes of amount and distribution of receptors and binding sites | | | |
| a/ increase of ConA$^+$ binding | lymphocytes, erythrocytes, platelets, fibroblasts, bladder endothel | 0.5-5 | /22,30,31,33,34, 36/ |
| b/ increase of WGA$^{++}$ binding | lymphocytes | 0.25 | /40/ |
| c/ loss of surface membrane immunoglobulins Fc receptors, decrease of capping membrane immunoglobulins | lymphocytes | 0.1-0.5 | /12,13,43/ |
| d/ increase of poliovirus binding and penetration | monkey kidney cells | 2.5 | /31/ |
| II. Membrane related cell activities | | | |
| 1. Decrease of rosetta formation | lymphocytes | 0.1-1 | /43,63/ |
| 2. Increase of phagocytic activity | alveolar macrophages J 774 cells | 7.5 | /15,50/ |
| 3. Decrease of IgG induced mast cell degranulation | mouse mast cells | 0.1 | /1/ |
| 4. Changes of enzymatic activity and transport | | | |
| a/ decrease of ATP-ase | rat liver | 8 | /47/ |
| b/ increase of adenylate cyclase | rat liver | 0.5 | /37/ |
| c/ decrease of K$^+$ transport | thymocytes | 2 | /9/ |
| d/ increase of amino acid uptake | C.albicans spheroplasts | 2.5 | /25/ |
| III. Micromorphological alterations | | | |
| 1. changes of cell shapes | lymphocytes | 0.5 | /8/ |
| | various tissues | 1-5 | /7,22/ |
| 2. increase of ruffling activity | human fibroblasts | 0.5 | /56/ |
| | rat glial tumor cells | 3 | /21/ |
| | J 774 cells | 7.5 | /15/ |
| | neuroblastoma cells | 8 | /45/ |
| | human glia cells* | 200 | /20/ |

*radioresistant cells surviving a rather excessive radiation dose for many days

$^+$Concanavalin A, $^{++}$wheat germ agglutinin

process concomitant with cross binding in proteins plays a role in fluidity decrease after high doses. The mechanism of fluidity increase after low doses has to be clarified.

Some of the activities of membrane-bound enzymes are also modified upon irradiation /Table 1/. The observations on the inhibiton of $K^+$ transport by low doses while its stimulation by high ones point to the inhibitory effect of low doses on active transport and to the increase of passive permeability of membranes after high doses /38/. The mechanism of radiation-induced increase of amino acid uptake is not known /25/. However, a possible relationship was found between the ruffling activity induced by insulin and the increase of uptake of amino acids /19/. Similar phenomenon might occur in cases of ruffling activity after irradiation.

Micromorphological alterations

There are many data available on the appearance of alterations of tissue surfaces like intestinal or vesicle epithels as well as of cell culture surfaces following low and sublethal doses. Several authors have reported on changes of certain tissue surfaces /7,22,64,71/, of erythrocytes /41/, of lymphocyte shapes /8/ after ionizing irradiation. Similar changes of shapes of lymphocytes were also observed after mixed neutron+gamma irradiation /35,58/. In cell cultures the surface changes called "ruffling activity" - is the morphological appearance of the cell movement - showed similarity

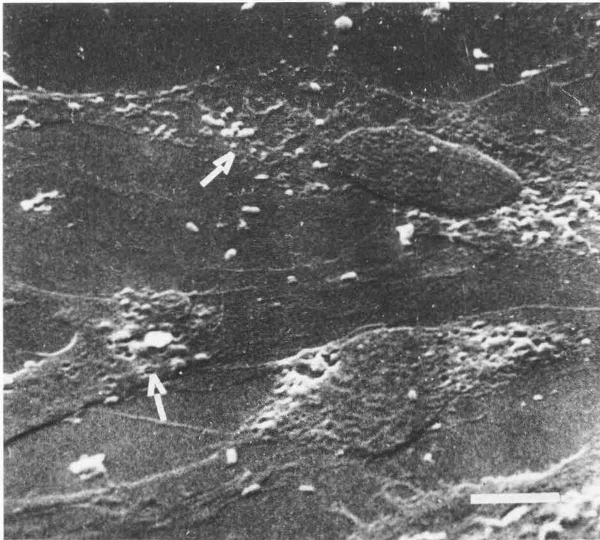
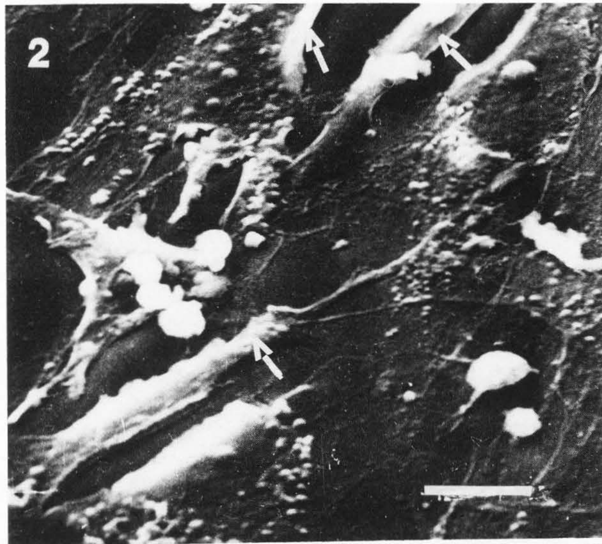


Figure 1. Semiconfluent culture of normal primary human fibroblasts. The cells and cell edges / ⟶ / are adhered to the substrate. Bar = 5 μm.



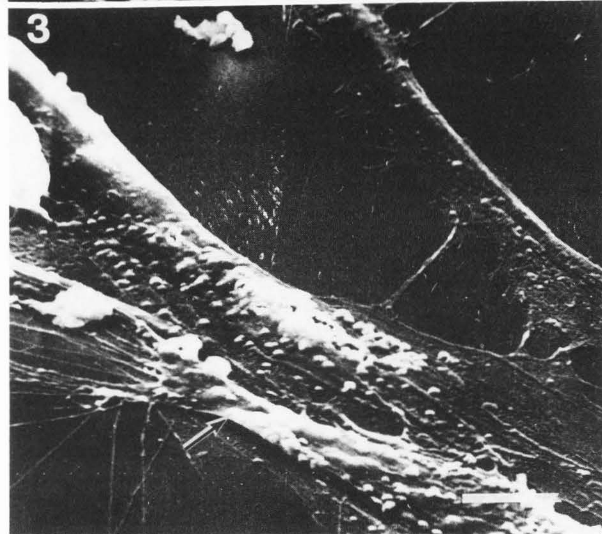




Figure 2. Primary human fibroblasts 30 minutes after 2.5 Gy X-irradiation. The elevated cell edges are well seen / ⟶ /. Bar = 5 μm.

Figure 3. Primary human fibroblasts 1 h after 2.5 Gy X-irradiation appearing in spindle-shaped form and having cytoplasmic extensions. The contacts between cells and substrate are loosened at certain places / ⟶ /. Bar = 5 μm.

after various doses of irradiation /15, 20,21,45,56/ and other agents /6,68/. In such circumstances the originally well adhered fibroblasts become loose, the cell edges elevate from the substrate, several cells turn to spindle-shaped ones. These micromorphological changes are demonstrated on Figs. 1-3 /56/. The development of ruffling activity can also be observed after enzymatic

detachment of cells /6/ as well as after mechanical removal of glycoproteins attached loosely to cell surfaces /68/. A similar phenomenon was detected when the adsorptive pinocytosis of cationized ferritin was followed by transmission electron microscopy /17/. It has been published that the radiation-induced and insulin-induced ruffling activities were inhibited by dibutyryl-cyclicAMP /19,45/.

Alterations of cell surface

Membrane receptors and lectin-binding sites. The observed quantitative alterations of receptors and lectin-binding sites after relatively low doses of radiation suggest functional modifications of membranes. For instance, the decrease of binding of IgG on lymphocytes following 0.1 Gy /13/ was shown as well as the mast cell degranulation through IgG binding /1/. As shown on Table 1 data are available on radiation-induced decrease of surface immunoglobulins and Fc receptors of lymphocytes /12/, and an increase of binding of another ligand of biological importance, the polio virus and its facilitated penetration into a proper host cell /31/.

The quantitative changes of binding sites for lectins which bind specifically to appropriate chemical groups /5/ point to modification of membrane structure, the consequences of which are not as yet fully understood /22,30,33,34,36, 40/. Somewhat more details are already known concerning the time- and dose-dependences of ConA binding on various cell types /30,33,34,36/.

It was found that the ConA binding increased after low doses between 0.1 and 2 Gy, while the effect depended on cell type: out of blood cells of either human or animal origins the platelets proved to be the most sensitive elements, then followed by lymphocytes and finally by erythrocytes. Therefore, this phenomenon was even proposed as a biological indicator of radiation injury /33,34, 36/. The time course of radiation-induced membrane perturbation was followed on primary human fibroblasts after 2 Gy of X-irradiation and it was found that approximately 20-25 per cent increase occurred between 30 and 60 minutes after irradiation, while at about 3 h decreased to the control level. However, no increase of ConA binding was observed in a case of lymphoid leukemia cells /32/ after X-irradiation.

The increase of WGA binding was reported recently on lymphocytes /40/. The authors have found the increase up to 1 Gy which might be explained by an increase of number of binding sites, while between 2 and 7 Gy an opposite effect, i.e., the decrease of WGA binding was observed probably due to the removal of sialic acid from cell surface. Such effect was described earlier, too /2/.

Surface charges. The considerable decrease of negative surface charges is fairly known /Table 1/. The significance of this effect lies in that net negative surface charges characterize the eukaryotic cells /67/. It can be supposed that the distribution, amount and extent of these charged sites play a role in the performance and regulation of several membrane functions. According to biochemical investigations performed in recent years the chemical nature of materials responsible for the negatively charged sites on the cell surface are different in various cells. In lymphocytes, erythrocytes and platelets mainly the sialic acid provides the negative charges, while in cultures cells and fixed tissues this role is also attributed to the glucose-aminoglycans participating in the build-up of polysaccharide coat /10,42,53,59,60/.

The decrease of negative charges after irradiation is probably independent of the chemical nature of their carriers as the effect can be observed on various cell types /51-53,59/. The molecular background of this effect, however, is not fully known. It is a future task to elucidate whether a structural re-arrangement of the carrier molecules /52,53/ or their removal by desialylation /2/ is responsible. The decrease of negative charges proved to be reversible after low doses /52,53,59,66/. It is an interesting feature of dose-effect relationship that by increasing the dose the amount of negative charges decrease until a certain minimum then at higher doses the level of charges are again similar to the control /51-53,66/. The extent of decrease is dependent on the linear energy transfer /LET/ value of irradiations, the higher the LET, the more pronounced the decrease /66/.

Although the radiation-induced decrease of negative charges seems to be a general phenomenon as it could be observed on several cell types, there are data on exceptions, too. No such changes occurred in L5178Y murine leukemia cells /16/ and human lymphoid leukemia cells /32/.

Certain radioprotective or radiosensitizer compounds might influence the surface charges both in normal and in irradiated cells like SH-reagents, lectins, anaesthetics, microtubule poisons /11,51, 71/.

Some data accumulated in the literature and results of our laboratory show that the decrease of negative charges occurs together with the increased ruffling activity. As the enzymatic and mechanical treatments of cells induce ruffling activity /6,68/ and at the same time

decrease the net negative surface charges which play a role in maintaining the cell shape, it was thought that ionizing radiation provokes both effects. In fact the interrelationship between ruffling activity and changes of negative surface charges was demonstrated by our preliminary experiments. According to these findings the cationized ferritin in non-toxic concentrations shading the negatively charged sites induces an increase of cell movement of human primary embryo fibroblasts like ionizing radiation.

Beside the components of cell surfaces the extracellular matrix also contains a considerable amount of molecules carrying free negative charges. The chemical nature of these might be identical or similar to those found in glycocalyx. These substances also lose negative charges after irradiation /44,49/. Such alterations were considered as possible causes of late changes of ultrastructure and function of lungs.

Regional changes of membranes. Recent findings point to the relationships between certain membrane regions and their functions. The distribution of certain membrane components, enzymes, receptors depend on the cell type and/or their functional state. One extreme case for the inhomogeneous distribution of membrane components is when they are localized only on one side of the cell, this situation is called "polarity" /18,48,55/. Such cases for epithelial cells were recently summarized /55/ and described for fibroblasts /57/.

Some data are already available concerning the effect of low doses upon the distribution of surface domains. Thus, the amount of Concanavalin A bound to urothelial cells was increased as well as its distribution became uniform /22/. Our own observations on another ligand support the information: the decrease of cationized ferritin binding on fibroblasts run parallel with the change of distribution /59/. The binding sites for cationized ferritin on fibroblasts /Fig. 4A/ are localized on the apical surface in clusters close to each other /57,59/. Upon irradiation there were found both decreased number of domains with negative charges and the number of bound ferritin particles within a binding domain /Fig. 4B/. The altered surface conditions were normalized within approximately 1 h /Fig. 4C/. Data of other authors applying colloid iron binding support these observations /61/.

## Radiation-induced alterations on the binding and distribution of ligands

As relatively few publications reviewed above deal with regional changes

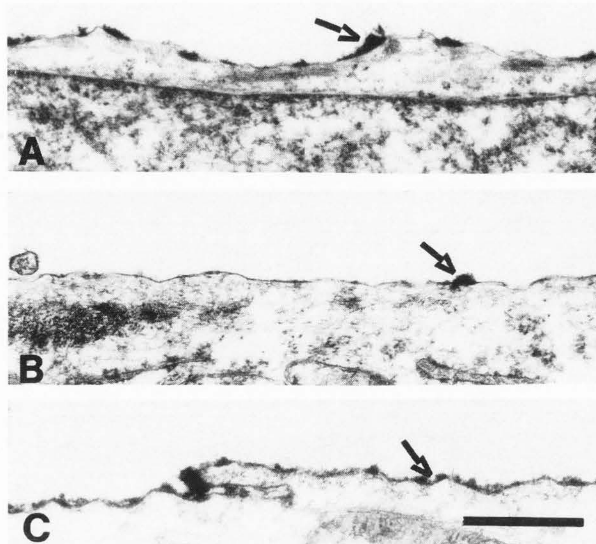



Figure 4. Binding of cationized ferritin /CF/ on non-exposed primary human fibroblasts /A/, and 10 min /B/, 1 h /C/ after 2.5 Gy X-irradiation. CF binding takes place in clusters / ⟶ /. Bar = 0.5 μm.

of cell surfaces after irradiation, the aim of our recent investigations was to follow radiation-induced alterations in the range of 0.5 to 2.5 Gy of binding sites for various ligands. For these purposes an anionic marker, the native ferritine /18/ and two lectins, the Concanavalin A and wheat germ agglutinin were applied on primary human fibroblasts.

## Materials and methods

Cell culture. The cells were collected after slight trypsinization of human embryo tissues obtained from interrupted healthy pregnancy of 2-3 months. The cell suspension was filtered through a gauze, centrifuged and resuspended in Parker's medium containing 10% foetal calf serum and antibiotics. The concentration of cells was approximately $2\text{-}3 \times 10^5$ cells per ml. After 30 minutes - 1 h incubation in Bellco tubes, mainly the fibroblasts adhered stongly to the glass surfaces. The cell types of non-fibroblast character were washed out by vigorous shaking. The growing cells were examined in a light microscope. According to our observation the morphology of cells and the morphological appearance of the monolayer showed fibroblast features.

Irradiation. X-irradiation was performed with a THX-250 machine. Conditions: 200 kV, half value layer /h.v.l./ 1.0 mm Cu, source-surface distance /S.S.D./ 90 cm, dose-rate 0.317 $Gy_{water} \cdot min^{-1}$.The

exposures were measured by a calibrated ionization chamber /Farmer dose meter Type 250/3,0,6 $cm^3$ ionization chamber/. For calculation of the absorbed dose /in water/ from the exposure, the rad/R conversion factor and the value of h.v.l. were used /23,24/.

Cytochemistry. For the detection of Concanavalin A bound to glucose and mannose side-chains /5/ the Concanavalin A-peroxidase reaction was used /4/. The sialic acid and N-acetyl-glucosamine side-chains connected with WGA were visualized by glucose oxidase /14/. Previously the cells were fixed for 30 minutes by 0.025 per cent glutaraldehyde in phosphate buffer. The positive surface charges were labelled and visualized by native ferritin. The prefixed cells were incubated in native ferritin /1 mg per ml in phosphate buffer/ for 1 minute. For controlling the specificity of ConA binding alpha-D-mannose, of WGA binding N-acetyl-glucosamine were used. The materials mentioned were obtained from Sigma Co. All inhibitors were used in the concentration of 0.05 M in PBS before incubation of lectins with cells.

Transmission and scanning electron microscopy. Cells were fixed on coverslips with 2.5 per cent glutaraldehyde dissolved in 0.1 M phosphate buffer pH 7.3 at 4°C for 1 h. After washing with the same buffer, further fixation of cells with 1 per cent $OsO_4$ was carried out at 4°C for 1 h. For transmission electron microscopy the material was dehydrated in a graded series of acetone and embedded in Durcupan AC /Fluka/. After 24 h polymerization at 56°C the specimens were immersed in liquid nitrogen for 10-15 seconds. This resulted in a clean separation of the Durcupan block from the coverslip. Ultrathin sections were cut with a glass knife on an LKB ultramicrotome in orientations perpendicular to and parallel with the plane of the cell culture. The sections were stained with uranyl acetate and lead citrate, and examined with a JEM 6C electron microscope. For scanning electron microscopy the cells were dehydrated in ethanol and amyl acetate series, critical point dried /Sorvall critical point drying system/ in $CO_2$ and coated with gold. Specimens were viewed and photographed using JSM 50 A SEM at accelerating voltage of 20 kV at tilt angle of 45°.

## Results

Binding of native ferritin. The native ferritin /NF/ as an anionic marker is localized of fibroblast plasma membranes both on apical and basal surfaces /Fig. 5/. There was an increase of NF experienced 10-30 minutes after 0.5 to 2.5 Gy X-irradiation of cells /Fig. 6/.

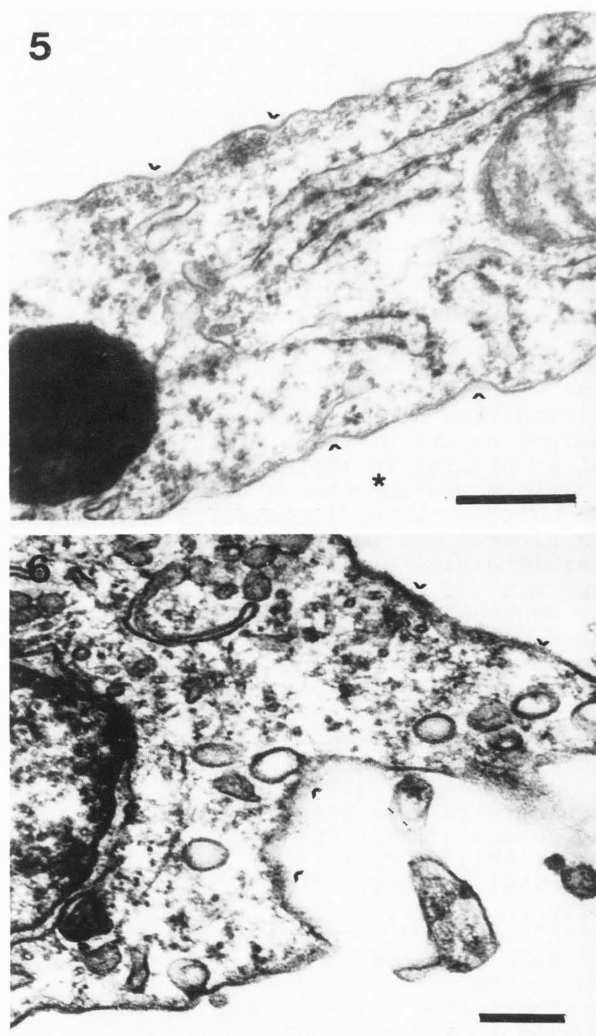
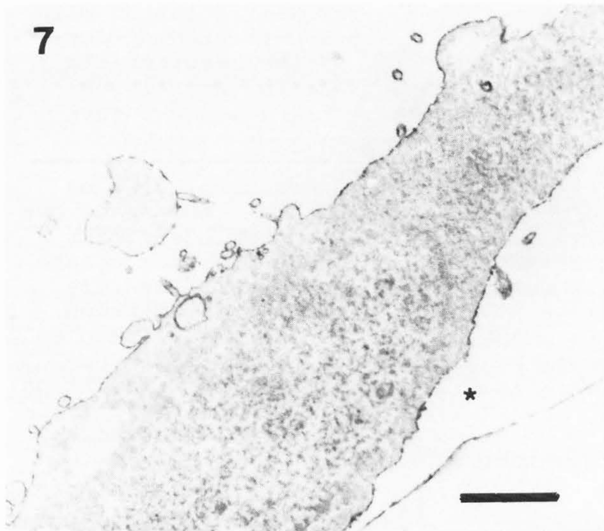



Figure 5. Native ferritin /NF/ binding on primary human fibroblasts. The NF is localized equally on membrane /›/ of apical and basal surfaces. The asterisk indicates the basal surface of cells. Bar = 0.5 µm.
Figure 6. Native ferritin /NF/ binding on primary human fibroblasts 30 minutes after 2.5 Gy X-irradiation. The bound amount of NF is increased on membrane /›/. Bar = 0.25 µm.

This figure demonstrates that the NF bound mostly to the membrane and elevated cell edges.

Detection of Concanavalin A binding sites. The reaction indicating the Concanavalin A /ConA/ binding was uniform on the whole cell surface, it appeared as a continuous line /Fig. 7/. Thirty minutes to 1 h after irradiation with 2.5 Gy several phenomena could be observed.

The even binding was broken, on some places there was no binding at all, while on others, particularly on the apical surfaces and elevated edges the ConA was bound in large aggregates /Fig. 8/. Three h after irradiation, however, the ConA binding was again similar to the unirradiated control pointing to the regeneration of perturbed membrane surface /Fig. 9/.



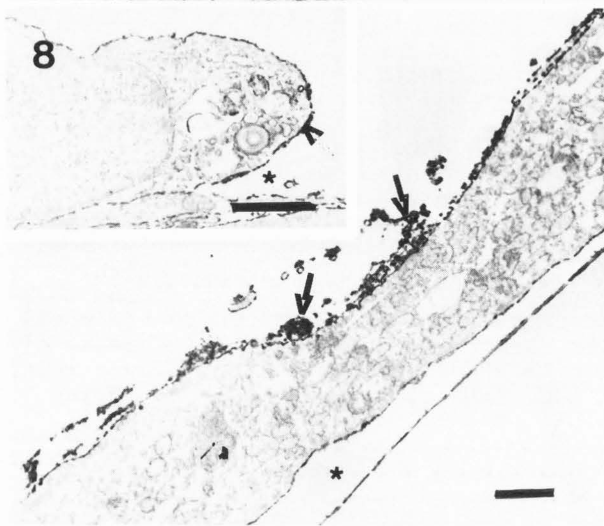




Figure 7. ConA binding on primary human fibroblasts. The reaction is uniform all over the surface. The asterisk indicates the basal surface of cells. Bar = 0.5 μm.
Figure 8. ConA binding on primary human fibroblasts 1 h after 2.5 Gy X-irradiation. At the apical surface an increase of ConA binding can be seen in aggregates /—>/. The ConA was also found in aggregates on elevated edges of cells /> /. The asterisks indicate the basal region of the cells. Bar = 0.25 μm, in insert 0.5 μm.

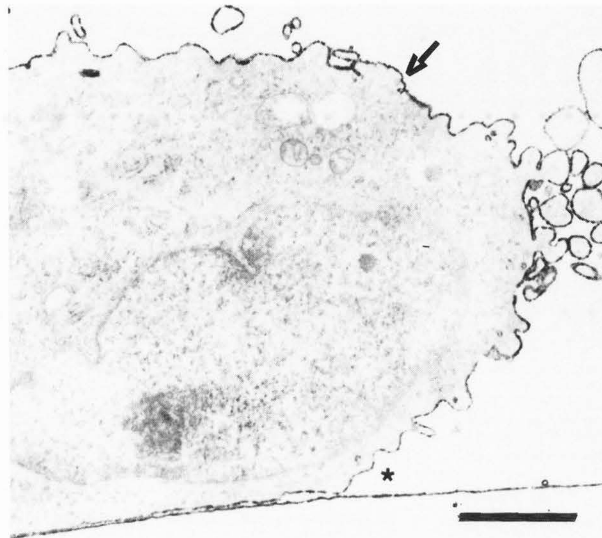


Figure 9. ConA binding on primary human fibroblasts 3 h after 2.5 Gy X-irradiation. The appearance of cells again became similar to unirradiated cells /—>/. The asterisk indicates the basal region of cells. Bar = 0.5 μm.

Detection of wheat germ agglutinin binding sites. The wheat germ agglutinin /WGA/ on unirradiated fibroblasts appeared mainly on the apical surfaces as a continuous line and it was almost completely absent on the basal one /Fig.10/.

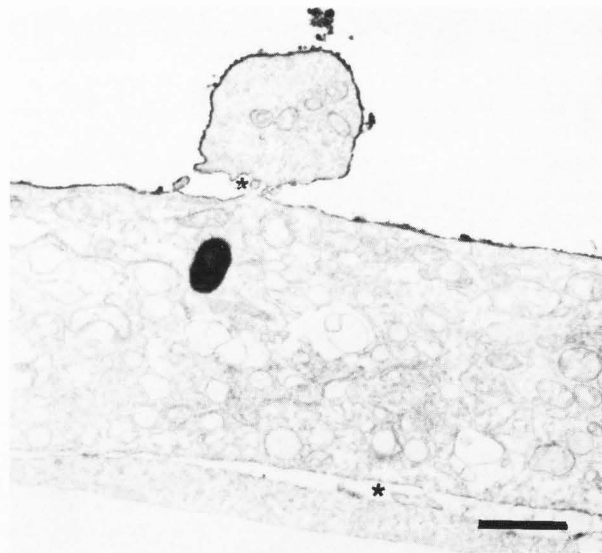


Figure 10. WGA binding on primary human fibroblasts. Bindings are seen exclusively on apical regions. The asterisks indicate the basal region. Bar = 0.5 μm.

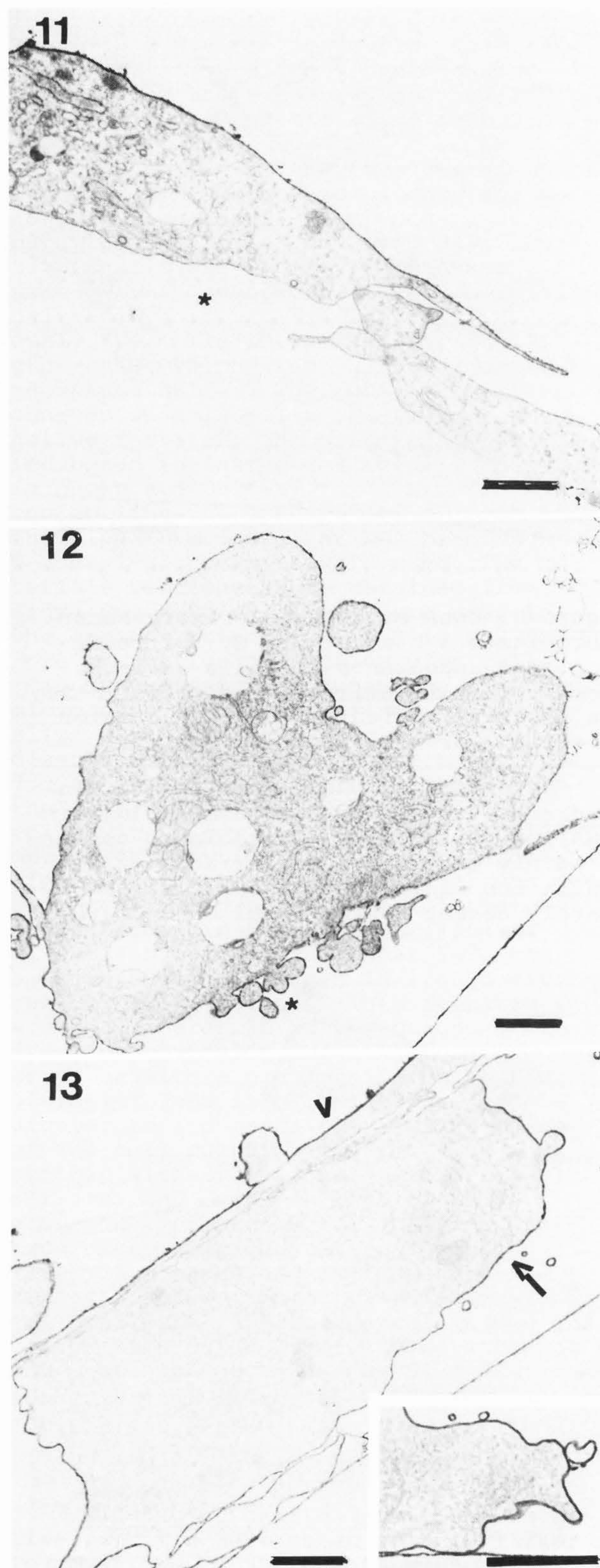



Figure 11. WGA binding on primary human fibroblasts 30 minutes after irradiation with 2.5 Gy. Polarized binding of lectin did not change. The asterisk indicates the basal region of cell. Bar = 0.5 μm.
Figure 12. WGA binding on primary human fibroblasts 1 h after X-irradiation with 2.5 Gy. The reaction can be seen on many parts of basal surface /*/, too. Bar = 0.5 μm.

Figure 13. WGA binding of primary human fibroblasts 3 h after X-irradiation with 2.5 Gy. Polarity of lectin binding disappeared /insert/, or the reaction can be seen on basal /—>/ or apical surfaces / > /. Bar = 0.5 μm, in insert = 1 μm.

There was a more pronounced binding on the cell protrusions and in the space between cell body and protrusions. This polarized binding of WGA does not change in 30 minutes after X-irradiation /Fig. 11/. Sixty minutes after X-irradiation with 2.5 Gy WGA binding was seen also on the basal surfaces in uneven distribution /Fig. 12/. Three h after irradiation the whole cell surface contained WGA binding sites in uniform distribution /Fig. 13/.

## Discussion and Conclusions

The data presented above contribute to the understanding of membrane changes upon ionizing radiation effect. It was shown that the irradiation resulted in the change of distribution of membrane domains. Evidence was given to the increase of amount of surface positive charges which was more pronounced on elevated cell edges also induced by irradiation. The biological importance of positively charged domains are not known, though there are a few data pointing to the role of coated pits - where the positive charges are localized - in uptake of materials /54,65/. They might play a role in ruffling activity as it is connected with increased material uptake /15,20/.

The radiation-induced increase of $^{3}$H-labelled ConA binding to cell surfaces was demonstrated earlier in our laboratory /30,33,34/. Our present data support the former observations. The mechanism of regional changes of ConA binding domains and its importance in the cell physiology is not known yet. These are the tasks of our further investigations.

The distribution of WGA binding sites also raise several further problems. Polarity of WGA binding was described for thyroid cells /3/. As we have found that its polarity disappeared in irradiated cells, it might mean either that the former cryptic binding sites appear at the basal surfaces or the barriers maintaining the former polarity

were broken.

The studies on cell surface phenomena after irradiation need further investigations. The presently available techniques are fairly exploited and the results obtained by them are summarized above. It is obvious that further developments are expected by applying ligands like hormones or monoclonal antibodies against cell surface antigens.

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## Discussion with Reviewers

T.M. Seed: How do the authors define "low dose" and "high dose" irradiation- physical or biological descriptions, or both? In regard to the latter, can the very interesting differential responses of plasmalemmal domains be used to define the "low dose" region?

J.G.Szekely: Definition is needed for low dose.

Authors: We admit that the term "low dose" is rather loosely used in the literature. The definitions are different when the expression is used in radiation protection or in cellular radiation biology. In the latter, the microdosimetric aproach considers the dose as low when only a small fraction of cells are hit. Obviously, this can not be used for cellular somatic studies like membrane effects. Another approach can be based on survival data taking mitotic /and not interphase/ death as end-point. In that case values corresponding to $D_q$ or less than $D_o$ can be considered as low dose. For human fibroblasts values are in the ranges of 0.1-0.5 and 1.1-2.5 Gy, respectively. Similarly, for mutagenic and cytogenetic end-points the dose-range is considered as low where the dose-response is linear - i.e., no intertrack interactions can be expected. Thus, in cellular effects a range of 0.1 and 1 or even 3 Gy can be considered as low dose. Studying the membrane effects we think that low doses are after which the membrane structure and function are

regenerated, the radiation doses do not lead to destruction of membrane. In this respect, for fibroblasts 2.5 Gy, a value close to $D_0$ can be taken as low when the effects are studied in a few hours after irradiation. Nevertheless, we emphasize that many features presented appear already in the range of 0.25 to 0.5 Gy. In our presentation the pictures after 2.5 Gy were shown just to demonstrate well-observable effects. We admit, however, that the term "low dose" has to be avoided or used only when comparative data are available and presented on a certain biological effect in a rather wide dose range. Concerning the radiation induced increase of lectin-binding in lymphocytes we have got a linear increase up to 1 Gy, in case of fibroblasts the increase was experienced up to 2.5 Gy then the larger doses lead to disintegration of membranes.

J.G.Szekely: Definition is needed for "sublethal" and "nonlethal" doses.
Authors: The terms "sublethal" and "nonlethal" doses were used according to the ICRU Report 30 /International Commission on Radiation Units and Measurements, Washington, D.C., 1979/ entitled "Quantitative Concepts and Dosimetry in Radiobiology". Accordingly, "...sublethal damage, the accumulation of which may result in cell lethality, and nonlethal damage, which is damage that does not lead to cell death,...".

G.P. Raaphorst: Since the investigators used human embryo tissue as a source for cells, there would be a heterogeneity in cell types in cultured samples. The authors refer to their results using ConA and WGA binding on fibroblasts even though one expects a mixed population. Please, explain whether you identify and analyze fibroblasts only and whether there is a heterogeneous response in ConA and WGA binding in such a mixed cell population?
Authors: For our experiments we used pure fibroblast cultures obtained as described in Materials and Methods. The basis of selecting out the fibroblasts was their quick adherence to the substrate as shaking the cultures and changing the medium 30 min - 1 hour after the beginning of incubation results in pure fibroblasts cultures /Polinger IS, Exp.Cell Res. 1970 63, 78, Chen WT, J. Cell Sci. 1981 48, 1/.

G.P. Raaphorst: Do cell cycle effects modify the ConA and WGA binding effects in the presence and absence of radiation. If so, it may well be that radiation induced division delay may account for the dynamic changes in ConA and WGA binding after irradiation.
Authors: The changes of lectin binding by cells in the course of cell cycle are known from the literature. We do not know data concerning the distribution of bound lectin in the various phases of cell cycle /G1, S, G2/. Such possible alterations would not influence the evaluation of our experiments because we always used a confluent monolayer when a contact inhibition had developed, i.e., most of cells were in $G_0$ phase. Thus, it was not surprising that the distribution of lectin on cell surfaces was uniform. Our earlier results on radiation-induced changes in lectin binding were also obtained on non-dividing cells, i.e., non-stimulated lymphocytes, erythrocytes and platelets /33,34/.

G.P. Raaphorst: The temporal responses of ConA and WGA binding are quite different after irradiation. Could the authors offer any explanation for this?
Authors: It is known that the chemical structures of ConA and WGA binding sites are different as well as their distribution on a cell surface. Consequently, the radiation-induced changes in distribution of ConA and WGA binding sites occur independently from each other. The increased ConA binding upon ionizing radiation could be observed mainly on apical surfaces. We do not know the explanation of this phenomenon. It may correlate with the decrease of negative charges of these regions, or the expression of cryptic binding sites may take place. As for WGA binding, we experienced long-lasting changes of polarity. In the paper we discuss some possibilities to explain it.

G.P. Raaphorst: The authors indicate polarity in WGA binding. Does such polarity occur only in cells attached to a glass or plastic substrate or also for cells in suspension? Is such binding related or dependent on position in the cell cycle?
Authors: We did not investigate the WGA binding to the cells in suspension, but we have included this kind of experiment into our plans. According to our results, however, the polarity of negative charges as detected by binding of cationized ferritin disappeared when the cells were brought into suspension. The changes are similar to those of epithelial polarities and point to the regulation of cell - substrate contact.