EFFECT OF NEAR ULTRAVIOLET AND VISIBLE LIGHT ON AMOEBA

S. CHATTERJEE AND S. K. BHATTACHARJEE
Biology and Agriculture Division, Bhabha Atomic Research Centre,
Trombay, Bombay 400 085, India

SUMMARY

The near ultraviolet and visible light (VL) impinging at an intensity of 2.5 x 10^4 J s^{-1} m^{-2} for 2.5 h kills the mitotic and the early S-phase (0- to 15-min-old) amoebae. At the mid- and late S-period only a fraction of cells are killed by VL and the G_4 phase cells are quite resistant. Amoebae of all cell cycle stages show a delay in the first mitotic division. DNA synthesis, as measured by [3H]thymidine incorporation, is depressed in the VL-exposed early-S amoebae. A concurrent but temporary inhibition in [3H]leucine incorporation also occurs in these cells. However, no significant change in [3H]uridine incorporation has been found.

To localize the site of lethal damage, nuclear transplantation studies were undertaken between the control amoebae and the amoebae treated with VL. The nucleus of a VL-exposed early S-phase cell recovers when transplanted immediately after VL exposure into an enucleate G_4 cytoplasm but dies if grafted into an enucleate S-phase cytoplasm. The therapeutic effect of the G_4 cytoplasm, although at a lower level, is also evident even when the treated early S-phase nucleus is implanted 20 h later, but not after 48 h, into the G_4 cytoplasm. The amoeba cytoplasm shows resistance to VL-irradiation, can accept a control nucleus from any cell cycle stage, and function normally. The G_4 nucleus also remains apparently unaffected to VL exposure and can survive when it is transferred to the control cytoplasm of any cell-cycle phase. All these findings are discussed in the light of the possible existence of a repair system against VL-induced damage in the G_4-phase amoeba.

INTRODUCTION

Despite numerous studies on the biology of amoeba, little is known regarding its sensitivity to different kinds of radiations throughout the cell cycle. As a part of systematic studies designed to elucidate the action of various physical and chemical agents on this unicellular eukaryote, we have investigated the effects of near ultraviolet and visible light (VL) exposure during the different phases of its life cycle. The present report describes the cell cycle-dependent variations in sensitivity of amoeba to VL irradiation. Further, in an attempt to determine the site of VL-induced damage in the cell body of amoeba, nuclear transplantation experiments were undertaken between VL-exposed and control amoebae. The results of such cross-transfer studies are also presented in this paper.

MATERIAL AND METHODS

Culture conditions

Amoeba proteus were cultured according to the method described earlier (Chatterjee & Rao, 1974). They were fed with Tetrahymena pyriformis and maintained at 23 ± 1 °C. Under these conditions, generation time and S-period were found to be 36 ± 2 h and 6-7 h, respectively (Rao & Chatterjee, 1974). No G_4 stage was detected.
VL exposure

Light source used was a 400-W Philips white fluorescent lamp. The uncorrected spectrum of the source as seen by IP28 photomultiplier tube and the transmission characteristics of the glass used to cut off short wavelength ultraviolet light are shown in Fig. 1. Between 30 and 50 amoebae of known cell cycle stages (obtained by mechanical selection of dividers) were placed in syrupace watch glasses containing ca. 0.5 ml medium and were exposed at an intensity of $2.5 \times 10^3 \text{s}^{-1} \text{m}^{-2}$ of VL as measured by a Schwarz linear vacuum thermopile. The temperature of the culture medium during the exposure was maintained at about 21 °C by cooling a metal platform on which the syrupace watch glasses were placed, by means of a water-circulating thermostat (Lauda). Immediately after near ultraviolet and visible light (VL) exposure, the cells were thoroughly washed with medium and kept singly or in groups of 10 in syrupace watch glasses together with food organisms. Cells treated identically but without VL exposure were used as controls. No difference in response to VL exposure was found between cells kept in complete darkness and those kept in a 12 h light-12 h dark cycle (normal culture condition) after VL irradiation. Between 50 and 200 amoebae were exposed to VL for each experimental set unless otherwise stated.

Radioactive labelling

For measuring the incorporation of radioactive precursors of different macromolecules, cells were labelled directly with aqueous solutions of $[^{3}\text{H}]\text{thymidine}$ ($[^{3}\text{H}]\text{TdR}$, sp. act. 5.3 Ci/mM), $[^{3}\text{H}]\text{uridine}$ ($[^{3}\text{H}]\text{UdR}$, sp. act. 6.2 Ci/mM) or $L-[^{3}\text{H}]\text{leucine}$ (sp. act. 7.6 Ci/mM) for 2-5 h during or immediately after VL exposure. In case of RNA and protein synthesis, amoebae were labelled also on days 3, 5 and 7 after VL irradiation. After the labelling period, cells were thoroughly washed with an excess of non-radioactive precursor ($5 \times 10^{-3} \text{M}$).

The labelled cells were then placed on a gelatinized slide containing a drop of 45 % glacial acetic acid and squashed with a coverslip. The coverslip was flicked off after immersing the slide in liquid nitrogen for 1-2 min. The slides were then fixed in acetic acid/ethanol (1:3) and in the case of $[^{3}\text{H}]\text{TdR}$ and $[^{3}\text{H}]\text{UdR}$ labelling, further treated with ice-cold 5 % trichloroacetic acid for 10 min.

Enzymic digestions

A few cells labelled with $[^{3}\text{H}]\text{TdR}$ and $[^{3}\text{H}]\text{UdR}$ were extracted with deoxyribonuclease (DNase) and ribonuclease (RNase) respectively. DNase (0.1 mg/ml) and RNase (0.5 mg/ml) digestions were carried out at pH 6.9 for 6 h at 37 °C.
Authoradiography

The labelled and squashed cells were coated with Kodak NTB-2 or Ilford K-5 Nuclear Research Emulsion and kept in a dark, dry atmosphere at 4 °C for 2–6 weeks. After the exposure period, the slides were developed, stained with buffered toluidine blue and made permanent. The grain counts were made at a magnification of ca. 1250 using a squared grid in the ocular. The lateral grains were counted in cells labelled with I3H-TdR and I3H-UdR, whereas in the case of cells labelled with [3H]leucine, an area of ca. 3 × 10² μm² of cytoplasm in each cell was assayed. Appropriate background corrections were made.

Pinocytosis

Pinocytosis in amoeba was induced by immersing the cells in 0.125 M sodium chloride in 0.01 M phosphate buffer, pH 6.4. Pinocytosis was quantitated by the channel counting method (Chapman-Andresen, 1964).

Micrurgy

The nuclear transplantations and enucleations were carried out according to the procedure of Jeon & Lorch (1968). After the operation the cells were kept singly in Syracuse watch glasses together with the food organisms. All nuclear transplantations and enucleations of S-phase amoebae were performed when the cells were at least 1 h old because the nucleus is not fully reconstituted earlier than this. Clone formation, i.e. the production of 16 amoebae from one transplant, was taken as an index of survival. In both treated and control transfers the first mitosis was delayed between 24 and 48 h.

Notation and terminology used. TnCc, treated nucleus transplanted into enucleate control cytoplasm; CnTct, control nucleus transplanted into enucleate treated cytoplasm; CnCc, control nucleus transplanted into enucleate control cytoplasm; VL, near ultraviolet and visible light; ES, early S-phase, ca. between 0 and 15 min after mitosis.

Source of materials

All radioactive precursors were obtained from Bhabha Atomic Research Centre, Bombay, India. Unlabelled thymidine, uridine and leucine were purchased from Sigma Chemical, St Louis, Mo., U.S.A. DNase was purchased from Calbiochem, Los Angeles, Calif., U.S.A., and RNase from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

RESULTS AND OBSERVATIONS

Effects of VL-irradiation on whole amoebae

Lethality and mitotic delay in VL-exposed amoebae. In order to determine the sensitivity of different phases of the cell cycle of amoeba to VL exposure, cells of known ages were exposed to VL and the results are depicted in Fig. 2. The mitotic and the early S-phase (0–15 min old) cells were killed within 3–5 weeks after the cells were exposed to 2.5 × 10² J s⁻¹ m⁻² of visible light for 2.5 h. In control cells the survival rate was > 99%. About 8% of the mitotic cells were able to divide, producing unequal daughter cells, whereas the rest died without any division. Among the early S-phase cells, less than 3% of cells underwent mitotic division after a delay of 1–2 weeks, but the daughter cells died without any further division. Many VL-treated cells became giant, dark in colour and showed multinucleate form. They also displayed sluggish movement and inability to capture food organisms. About a week after VL exposure most of the cells failed to attach to the substratum and became spherical.
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In contrast to VL-induced lethality of the mitotic and the early-\(S\) amoebae, later phases of the \(S\)-period showed decreased sensitivity and the \(G_2\) cells were found to be quite resistant to the highest dose employed in the present study. The early (8–9 h), mid- (20–24 h) and late (36–38 h) \(G_2\) cells were arbitrarily chosen for VL irradiation and no cell death was observed in any of these stages. Moreover, unlike the \(S\) phase, no detectable morphological changes were found to occur in the \(G_2\) amoebae after VL treatment. However, a delay in the first mitosis (between 2–4 days) was noted in all these phases of the VL-exposed amoebae.

![Graph showing percent survival of amoebae exposed to a dose of \(2.3 \times 10^6\) J m\(^{-2}\) of light as a function of age of the cells. \(M\) denotes early mitotic stage.]

**Dose dependence of early-\(S\) cells.** In an attempt to find out the minimum dose needed to bring about 100\% lethality in the early \(S\)-phase amoebae, the cells were irradiated with various doses of white light. The results are given in Table 1.

**Macromolecular synthesis in VL-irradiated amoebae.** The ability of the VL-exposed early-\(S\) cells to synthesize nuclear DNA, RNA and protein was tested by labelling the cells with appropriate radioactive precursors prior to VL exposure (pre-labelled). In another set of experiments, the amoebae were labelled immediately after VL-irradiation (post-labelled). The grain count data of pre- and post-labelling experiments are summarized in Table 2. In the case of \(^3\)H-TdR label, a few treated cells were also labelled at different \(G_2\) periods to see whether there was any significant tracer incorporation beyond the \(S\)-period. None was found. Further, VL-exposed early-\(S\) cells were labelled periodically with \(^3\)H-UdR and \([\textbf{3H}]\)leucine in order to check their incorporation activity when compared to the randomly selected interphase amoebae which served as controls. No significant difference in grain density between the control and the treated cells was found in these experiments.

**Pinocytic activity in VL-treated amoebae.** In an attempt to find out whether VL-irradiation can interfere with the membrane activity, the \(S\)-phase cells were induced
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to form pinocytic channels immediately after VL exposure and also at periodic
intervals thereafter. The number of pinocytic channels formed at any given time
after induction in the treated and the control cells was taken as an index of relative
membrane activity. No significant difference was found between the treated and the
control amoebae in number, duration or shape of the channels (as visualized in light
microscope) within a 7-day period studied after VL-irradiation.

Table 1. Dose dependence of early S-phase amoeba

<table>
<thead>
<tr>
<th>Dose in units of 10^6 J m^-2</th>
<th>No. of cells exposed</th>
<th>Mitotic delay, in days</th>
<th>Lethality, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>32</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>9.0</td>
<td>32</td>
<td>7-10</td>
<td>25</td>
</tr>
<tr>
<td>13.5</td>
<td>28</td>
<td>7-10</td>
<td>46</td>
</tr>
<tr>
<td>18.0</td>
<td>33</td>
<td>7-10</td>
<td>48</td>
</tr>
<tr>
<td>22.5</td>
<td>32</td>
<td>7-10</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Macromolecular synthesis in VL-irradiated early S-phase amoeba

<table>
<thead>
<tr>
<th>Labelling condition</th>
<th>Radioactive precursor</th>
<th>No. of cells</th>
<th>No. of grains</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Pre-labelled</td>
<td>3H-TdR</td>
<td>23</td>
<td>17</td>
<td>300 ± 19</td>
</tr>
<tr>
<td></td>
<td>3H-UdR</td>
<td>20</td>
<td>19</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>Post-labelled</td>
<td>[3H]leucine</td>
<td>22</td>
<td>20</td>
<td>268 ± 12</td>
</tr>
<tr>
<td></td>
<td>3H-TdR</td>
<td>25</td>
<td>20</td>
<td>65 ± 6</td>
</tr>
<tr>
<td></td>
<td>3H-UdR</td>
<td>20</td>
<td>20</td>
<td>80 ± 10</td>
</tr>
<tr>
<td></td>
<td>[3H]leucine</td>
<td>20</td>
<td>23</td>
<td>156 ± 13</td>
</tr>
</tbody>
</table>

* Student's t-test: values greater than 0.05 not significant.

The effects of VL-irradiation on nucleus and cytoplasm of amoebae

Experiments to show that the ES cytoplasm of VL-exposed amoebae is not lethally
damaged. The ES cells were irradiated with VL at an intensity of 2.5 x 10^2 J s^-1 m^-2
for 2.5 h. These cells, if left to themselves, would die within 3-5 weeks. Immediately
after VL-irradiation the cells were enucleated and the nuclei from the unirradiated
(control) S-phase (1-2 h old) and G2-phase (ca. 20 h old) cells were implanted into
them. The controls consisted of unirradiated, enucleate ES cells into which nuclei
from the unirradiated S- and G2-phase cells were transplanted. The results of these
experiments are shown in Table 3.

Proof that the ES nucleus is lethally damaged due to VL exposure and that the G2
cytoplasm might contain a recovery factor. In order to assess the sensitivity of the
treated ES nuclei, they were grafted immediately after VL-irradiation into the
enucleate control cytoplasm from different cell-cycle stages. When the treated ES
nucleus was transferred to unirradiated S-phase (1-2 h old) cytoplasm, all transplants
died within a week. However, a dramatic reversal from lethality was observed when
these nuclei were transplanted into the G2-phase (20 h old) cytoplasm. In this case ca. 90% of cells survived and produced clones. The results of these transplantation experiments are also summarized in Table 3.

Table 3. Results of nuclear transplantations between: VL-treated and enucleate early S-phase cytoplasm and the control nucleus (I); and VL-treated early S-phase nucleus and control cytoplasm (II)

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>Age of control cytoplasm, h</th>
<th>Age of control nucleus, h</th>
<th>No. of transplants</th>
<th>No. of cell deaths</th>
<th>Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1T1</td>
<td>1-2</td>
<td>-</td>
<td>24</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>C2C1</td>
<td>1-2</td>
<td>-</td>
<td>20</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1T1</td>
<td>-</td>
<td>24</td>
<td>24</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>C2C1</td>
<td>-</td>
<td>22</td>
<td>20</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>II.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1C1</td>
<td>1-2</td>
<td>-</td>
<td>34</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>C1C2</td>
<td>1-2</td>
<td>-</td>
<td>20</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1C1</td>
<td>20</td>
<td>-</td>
<td>50</td>
<td>6</td>
<td>88</td>
</tr>
<tr>
<td>C1C2</td>
<td>20</td>
<td>-</td>
<td>22</td>
<td>2</td>
<td>91</td>
</tr>
</tbody>
</table>

Table 4. Results of delayed transfer experiments of VL-exposed early S-phase amoeba. The treated nucleus was implanted 20 h later into control cytoplasm of different cell-cycle phases

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>Age of control cytoplasm, h</th>
<th>No. of transplants</th>
<th>No. of cell deaths</th>
<th>Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1C1</td>
<td>1-2</td>
<td>28</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>C1C2</td>
<td>1-2</td>
<td>24</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1C1</td>
<td>20</td>
<td>32</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>C1C2</td>
<td>20</td>
<td>18</td>
<td>2</td>
<td>89</td>
</tr>
</tbody>
</table>

Experiments were next carried out to ascertain the effects of delayed implantation of the VL-exposed ES nuclei into the control cytoplasm at different intervals of time. In these experiments the treated nuclei were kept in their original cytoplasm for ca. 20 and 48 h before they were combined with the unirradiated S- or G2-phase cytoplasm. When the VL-exposed ES nuclei were transferred 20 h later into the enucleate G2 cells, the survival rate was lower (ca. 63%) when compared to those nuclei which were transplanted immediately after VL administration. Surprisingly, 36% of the transplants survived and produced clones when instead of the G2 cytoplasm the treated nuclei were grafted into the S-phase cytoplasm 20 h after VL treat-
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Evidence that the G2 nucleus is not lethally sensitized by VL exposure. Since it has been shown that G2 cytoplasm is resistant to VL irradiation, experiments were made in order to assay the sensitivity of the VL-treated G2 nucleus. When the nuclei from VL-exposed G2 (20-24 h old) cells were grafted immediately into either S-phase (1-2 h old) or G2-phase (20-24 h old) cytoplasm, more than 80 and 90% of transplants survived, respectively, and formed clones. These results are depicted in Table 5.

Table 5. Summary of experiments involving transplantations of VL-irradiated G2 nucleus into (I) control S-phase cytoplasm; and (II) control G2-phase cytoplasm

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>Age of control cytoplasm, h</th>
<th>No. of transplants</th>
<th>No. of cell deaths</th>
<th>Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_sC_1$</td>
<td>1-2</td>
<td>28</td>
<td>5</td>
<td>82</td>
</tr>
<tr>
<td>$C_0C_1$</td>
<td>1-2</td>
<td>24</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>II.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_sC_0$</td>
<td>20-24</td>
<td>21</td>
<td>3</td>
<td>86</td>
</tr>
<tr>
<td>$C_0C_1$</td>
<td>20-24</td>
<td>17</td>
<td>1</td>
<td>94</td>
</tr>
</tbody>
</table>

DISCUSSION

There have been several reports of photosensitization by VL in different eukaryotic organisms (see Krinsky, 1968). Although killing has been obtained after high-intensity VL exposure, unlike bacteria (Webb & Malina, 1967), no mutagenic effect is usually seen with amoebae.

The responses of amoebae to various kinds of radiant energy have been recently reviewed by Ord (1973). However, the studies on the effects of VL on amoeba are restricted mainly to its motility (Kühne, 1864; Harrington & Leaning, 1900; Mast, 1931). All these works have shown the photic suppression of amoeboid movement by hue variations. Hitchcock (1961) has demonstrated that the maximum sensitivity of amoeba lies in the green region (515 nm) as measured by the suppression of plasmasol movement. Prescott (1956) also reported the production of unequal daughter cells by exposing dividing *Amoeba proteus* to strong illumination.

Our studies demonstrate the response of amoeba to VL throughout the cell cycle. All phases of the cell cycle of amoeba show delay in the first mitosis even when quite a low dose of VL is administered. The division delay has also been shown to occur in *Amoeba proteus* exposed to other kinds of radiation such as ultraviolet, X- and γ-rays (Mazia & Hirshfield, 1951; Ord & Danielli, 1956; Škreb & Horvat, 1967).

In contrast to mitotic delay, death of *Amoeba proteus* due to VL-exposure is dependent on the precise position of the cells in the cell cycle during irradiation. Only the mitotic and 0-15 min-old cells in the early S-phase are extremely sensitive to VL-irradiation. Cells in later stages of S gradually acquire resistance to VL, and in
G2 amoebae no lethality has been observed even with the maximum dose employed in the present study.

Next, it is interesting to consider whether any change in the membrane activity could be detected in the VL-treated amoebae at early S-phase. Pinocytosis has been taken as an index of membrane activity. In an earlier report, Rinaldi (1959) has shown that ultraviolet irradiation itself can induce pinocytosis in *Amoeba proteus*. However, we have not found any such induction of pinocytosis in VL-exposed amoeba nor could any change be detected in their pinocytic channel-forming ability after induction. It appears, therefore, that VL exposure may not lead to any severe damage and functional alteration in the plasma membrane of amoebae.

We have examined the question of whether VL-exposed early-S amoebae show any drastic change in their nuclear DNA, RNA and protein-synthetic capacity which can be directly correlated with their lethality. The data on the effect of VL on these macromolecular syntheses are inconclusive. The DNA, RNA and protein syntheses, as judged by incorporation of the respective labelled precursors, remain apparently unaffected in amoebae during the period of VL-exposure. There is a partial but significant inhibition in 3H-TdR incorporation in the post-labelling experiments, but no prolongation of DNA synthesis has been observed beyond the S-period. Also, no repair replication (unscheduled DNA synthesis) in the VL-exposed amoebae could be detected by autoradiography. Concurrent to this suppression of 3H-TdR incorporation, a decline in [3H]leucine incorporation has also been found. It is not certain whether or not this indicates any interrelationship between DNA and protein synthesis in the VL-treated amoeba. RNA synthesis, as measured by 3H-UdR incorporation, does not show any appreciable change after VL-irradiation. All these findings imply that any deleterious effect on nuclear DNA, RNA synthesis and protein synthesis that might be caused by VL exposure may not be the primary lesion responsible for the lethality in amoebae.

We have tried to pinpoint the site of VL-induced lethal damage and also assayed the nuclear and the cytoplasmic sensitivities separately by reciprocal nuclear transfers between the treated and the control amoebae. Earlier, a similar kind of study was undertaken by Ord & Danielli (1956) in the case of X-ray treated *Amoeba proteus*. They have shown that the nucleus was 2-4 times more sensitive to X-rays than the cytoplasm. In the case of ultraviolet-exposed amoeba, Iverson (1958) has shown that ultraviolet damage to the cytoplasm could be reversed by substitution of the irradiated nucleus with an unexposed nucleus, but that control cytoplasm was incapable of reversing ultraviolet injury to the nucleus. From their ultraviolet-microbeam studies on *Amoeba proteus*, Jagger, Prescott & Gaulden (1969) have concluded that the killing of a flattened amoeba is caused equally by damage to the cytoplasm and to the nucleus. However, no information is available from these studies regarding the age-dependent sensitivity of the amoebae to irradiation with ultraviolet or X-rays.

Our studies clearly show that the cytoplasm of S- and G2-phase amoebae is not lethally photosensitized by the maximum dose of VL administered in our experiments. It can accept an unirradiated nucleus from any stage of the cell cycle and function normally. On the other hand, the VL-exposed ES nucleus shows differential sensi-
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tivity according to the age of the recipient cytoplasm into which it is implanted. When
the ES nucleus is transplanted immediately after VL exposure into 1–2 h-old S-phase
cytoplasm (the earliest time such transplantation is possible, see Material and methods)
all the transplants die within a week. But about 90% of the transplants survive when
ES nuclei are transferred immediately after VL exposure into G2 cytoplasm. When
the treated ES nucleus is grafted into G2 cytoplasm 20 h later, a similar, albeit
decreased, therapeutic effect has been noted. However, 100% of the transplants die
if the above mentioned transfers are performed 48 h after VL irradiation irrespective
of the age of the host cytoplasm. These findings suggest a gradual deterioration and
later an irreversible damage of the amoeba nucleus between 20 and 48 h after VL

treatment.

All these findings strongly suggest that the primary site of VL-induced lethal
damage in amoeba is the nucleus and that the cytoplasm seems to be capable of
recovery from whatever injury it might have sustained due to VL exposure, when its
original nucleus is replaced by an unirradiated one.

It is interesting to consider the age-dependent sensitivity of the amoeba nucleus
to VL which can be modified according to the state of the cytoplasm into which it is
grafted. Our observations argue for the existence of a recovery mechanism against
VL-induced damage in the G2-phase amoeba. Even when a VL-treated ES nucleus
is kept in its original cytoplasm for 20 h before transference to the control G2 cyto-
plasm, remarkable recovery has been found to occur and more than 60% of such
transplants are able to produce clones. Also consistent with the idea for the existence
of a recovery mechanism in the G2 amoeba is the observation that when a VL-exposed
G2 nucleus is transplanted into S-phase control cytoplasm, most of the transplants
survive and produce clones. Such observation encourages speculation that the G2
nucleus may possess a recovery factor(s) which might be lacking or non-functional in
the early-S nucleus.

The cell-cycle phase-specific sensitivity of amoeba to VL is similar to ultraviolet
effects produced in other eukaryotes (Frindel & Tubiana, 1971), where the cells are
resistant in G1, sensitive through S, and resistant in G2. The progressive resistance of
an amoeba to VL exposure, as it moves through the cell cycle, could be due to gradual
build-up of a recovery factor(s), e.g. repair enzyme. The mitotic and the early-S cells
may lack this recovery factor, making the cells vulnerable to VL irradiation during
these stages of the life cycle. It has been demonstrated that both prokaryotes and
eukaryotes are capable of repairing the damage caused by ultraviolet irradiation. This
is mediated through a repair enzyme system in them (Hanawalt, 1968; Cleaver, 1974).
One is tempted to invoke a similar kind of mechanism which might be operating in
the G2-phase amoebae. Elucidation of such a mechanism would have to await the
identification of a repair enzyme system against VL-induced damage in amoeba.

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