ABSTRACT

We have previously reported that, when 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer was used in the growth medium to control pH fluctuations during the 21-day expression period of our human cell hybrid (HeLa × skin fibroblast) transformation assay, the yield of radiation-induced neoplastically transformed foci after 7 Gy of gamma-irradiation was suppressed. We now demonstrate that the observed suppression is not related to the presence of the 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer per se but rather is a function of the growth medium pH. Detailed studies reveal that incubation of the irradiated cells during the entire 21-day expression period at pH 6.7-6.8 versus pH 7.0-7.2 significantly suppressed the transformation frequency after 7 Gy, from 4.4 × 10^-4 to 4.6 × 10^-5 (accumulated data). The endpoint fraction of flasks containing foci was also significantly reduced at the lower pH. Suppression was evident whether the growth medium pH was lowered from pH 7.0-7.2 to pH 6.7-6.8 by medium exchange on day 0, 1, or 9 even up to 15 days post-irradiation. Growth curves revealed that the population doubling time of the cells is extended and the unirradiated and irradiated plating efficiencies are lowered by long term low pH exposure. We discuss possible mechanisms for the observed suppression, in terms of the influence of low extracellular pH on cell turnover, repair of radiation damage, cell toxicity, and activity of cellular proteases.

INTRODUCTION

The effect of extracellular pH on both normal and malignant mammalian cell growth and its underlying mechanism of action has been the subject of extensive investigation (1-7). One aspect of this work which has been of interest to us is the observation that the extracellular pH of the growth medium can affect the radiation sensitivity of human and rodent cells in vitro (8-12). While there have been reports on the pH dependence of transformation of Syrian hamster cells by benzo[a]pyrene (13) and on the effect of pH shifts on mutant frequency at the thymidine kinase locus in mouse lymphoma cells (14), only one report to date has studied the possible relationship between extracellular pH and in vitro radiation-induced neoplastic transformation (15). In that study with C3H-10T1/2 cells, reduced extracellular pH (pH 6.8) from 1 to 24 h post-irradiation resulted in an increase in survival and a decrease in transformation frequency. The effects of radiation-induced malignant transformation of long term exposure to low pH, which might occur in high density in vitro transformation experiments that in general require lengthy post-irradiation expression periods, have not been addressed. We have recently developed a new quantitative assay for the study of radiation-induced neoplastic transformation in vitro, using human cell hybrids (HeLa × skin fibroblast) (16-18). The end-point measured is the expression of a cell surface protein (p75/150), detected by immunoperoxidase staining, which is closely correlated with tumorigenicity in this system for both spontaneous (19) and radiation-induced p75/150-positive cells (16).3 This system is useful for studying the transformation of cells from the preneoplastic to the neoplastic state, an important step in the multistep process of malignant transformation. As stated above, our system and other cell systems used for radiation-induced neoplastic transformation require long post-irradiation expression periods, during which frequent refeeding is required to maintain peak viability and pH. In an attempt to control pH fluctuations during refueling of the experimental flasks and to minimize acidification of the medium due to cell metabolism, we began to investigate the use of HEPES4 buffer in conjunction with sodium bicarbonate in the growth medium of our human hybrid cell transformation assay. A direct comparison revealed that the radiation-induced transformation frequency (calculated as foci/surviving cell) was substantially reduced when HEPES was used (18). It was also noted that the growth medium pH was lower at the HEPES concentration used in that study. Following these initial observations, we then considered whether this suppressive effect on the yield of radiation-induced transformants was due to the HEPES buffer per se or due to the pH reduction. We therefore began to study the effect of controlling extracellular pH, with and without HEPES buffer, during our 21-day expression period. We report that exposure of the irradiated cells to pH 6.8 medium alone also suppressed the yield of radiation-induced transformants significantly. Possible mechanisms and the general implications of this work to in vitro radiation-induced neoplastic transformation are addressed.

MATERIALS AND METHODS

Cells and Culture Conditions. The origin of the human hybrid (HeLa × skin fibroblast) cell line CGL1 used in this work and stock maintenance procedures have been previously described (16-18, 20). CGL1 does not express the cell surface protein p75/150 and is nontumorigenic when inoculated into nude mice. CGL3 is a p75/150-expressing tumorigenic segregant that arose spontaneously in a mass culture of the original fusion (ESH5) after more than 200 population doublings (20).

Reagents and Medium. Cells were grown in minimum essential medium-Eagle modified (Flow Laboratories, Inc.) supplemented with 5% calf serum (GIBCO), 2 mm glutamine (GIBCO), nonessential amino acids (GIBCO), and 100 IU/ml penicillin (Irvine Scientific). Sodium bicarbonate (20 mM) was added to the medium so that, at 4.5% CO2 in humidified air at 37°C, the pH was maintained at pH 7.2. Low pH medium was prepared by titrating standard medium with 1.25% HCl so that incubation in humidified air at 4.5% CO2 at 37°C resulted in a medium pH of 6.8. HEPES buffer (0.01-5.9 g/liter) (GIBCO) was added only when specifically stated. Growth medium much more basic (pH >7.5) or much more acidic (pH <6.5) was found to be too toxic to the hybrid cells; therefore, it was not possible to extend these studies to a wider pH range.

Irradiation Protocol, Quantitative Assay, and Scoring. Three to 4 days before an experiment, CGL1 cells were plated into several T-75 flasks (Falcon) containing pH 7.2 medium, so that at the time of irradiation...
there were $4 \times 10^4$ cells/T-75 flasks. The flasks, with the cells still attached, were irradiated with a Shepherd and Associates Mark I self-shielded $^{177}\text{Cs}$ gamma-irradiator at room temperature (20°C), at a dose rate of 2.1 Gy/min. The standard dose in all experiments was 7 Gy. After a 6-h post-irradiation incubation at 37°C, the cells were trypsinized and counted and, depending on the experiment, 20–80 T-75 flasks, each containing 15 ml of pre-equilibrated medium (pH 7.2 or 6.8), were plated with $2.5 \times 10^4$ cells. After day 7 or 8, the cultures were fed 2 times/week, for the rest of the 21-day expression period, with the appropriate pH medium. The cultures were then fixed and stained for the presence of foci of cells expressing the p75/150 protein by an immunoperoxidase-staining technique (16, 17). These foci consist of densely packed, piled-up cells and are typically 1 to 2 mm in diameter. The intensity of immunoperoxidase staining is variable across the foci. For unirradiated controls, 20–80 T-75 flasks were plated with 6,000 cells/flask containing 15 ml of pre-equilibrated medium. This cell density was approximately the same as that of the surviving cells in the irradiated flasks of the experiment. The control cultures were incubated, fed, and stained for p75/150 by the immunoperoxidase method.

Data Presentation and Statistical Analysis. Data were scored by counting the total number of foci and their distribution within the flasks, as well as the total number of cells surviving the treatment as determined by cell number and plating efficiency determination. This reduced the number of T-75 flasks available for immunoperoxidase staining.

Influence of HEPES Buffer. Standard radiation transformation assays were carried out as stated above, with a duplicate experimental arm containing growth medium with HEPES buffered medium at 5.9 g/liter HEPES (18). For example, the accumulated transformation frequency was 4.6 $\times 10^{-5}$ at pH 7.0. As can be observed, while there appears to be no significant difference in the spontaneous transformation frequency between the two pHs (Table 1), there is a marked reduction in radiation-induced transformation frequency and fraction of flasks containing foci at reduced extracellular pH (Table 2), very similar to the original observation using bicarbonate buffer containing 5.9 g/liter HEPES (18). For example, the accumulated 7-Gy transformation yield was 281 positive foci out of 6.40 $\times 10^6$ surviving cells at pH 7.0–7.2, corresponding to a transformation frequency of $4.4 \times 10^{-4}$, while at pH 6.7–6.8 the yield was 20 positive foci out of 4.36 $\times 10^6$ surviving cells or a transformation frequency of $4.6 \times 10^{-5}$. The accumulated fraction of flasks containing foci after 7 Gy at pH 7.0–7.2 was 104 positive foci out of a total of 130 flasks, corresponding to a fraction of 0.80, while at pH 6.7–6.8 there were 18 positive foci out of a total of 172 flasks, corresponding to a fraction of 0.105. Statistical analyses ($\chi^2$ on accumulated data, $t$ test on the data sets) indicate that both the transformants/surviving

### RESULTS

In this study we began by examining the influence of HEPES concentration on the yield of radiation-induced transformants. The data obtained showed that this effect was maximized when the HEPES concentration was highest and that the pH of the medium was reduced, particularly at the highest HEPES concentration (Fig. 1). Titration of the pH of the bicarbonate-buffered medium at 0.01 g/liter HEPES down from pH 7.15 to 7.0 with HCl, or titration of the pH of the bicarbonate-buffered medium at 5.9 g/liter HEPES up from pH 6.8 to 7.0 with NaOH, resulted in the transformation frequencies shifting in the directions predicted if extracellular pH, as opposed to HEPES concentration per se, were the parameter influencing the transformation frequency (Fig. 1). Consequently, we began a more detailed study of this pH effect.

Tables 1 and 2 contain the data from several experiments comparing the transformation frequency and fraction of flasks containing foci for unirradiated and irradiated (7 Gy) cells held at either pH 6.7–6.8 or pH 7.0–7.2 for the entire expression period (21 days). As can be observed, while there appears to be no significant difference in the spontaneous transformation frequency at the two pHs (Table 1), there is a marked reduction in radiation-induced transformation frequency and fraction of flasks containing foci at reduced extracellular pH (Table 2), very similar to the original observation using bicarbonate buffer containing 5.9 g/liter HEPES (18). For example, the accumulated 7-Gy transformation yield was 281 positive foci out of 6.40 $\times 10^6$ surviving cells at pH 7.0–7.2, corresponding to a transformation frequency of $4.4 \times 10^{-4}$, while at pH 6.7–6.8 the yield was 20 positive foci out of 4.36 $\times 10^6$ surviving cells or a transformation frequency of $4.6 \times 10^{-5}$. The accumulated fraction of flasks containing foci after 7 Gy at pH 7.0–7.2 was 104 positive foci out of a total of 130 flasks, corresponding to a fraction of 0.80, while at pH 6.7–6.8 there were 18 positive foci out of a total of 172 flasks, corresponding to a fraction of 0.105. Statistical analyses ($\chi^2$ on accumulated data, $t$ test on the data sets) indicate that both the transformants/surviving
Table 1  Effect of reduced extracellular pH on spontaneous transformation frequency
Accumulated plating efficiency at pH 7.0-7.2 was 0.72 ± 0.08 and at pH 6.7-6.8 was 0.44 ± 0.14.

<table>
<thead>
<tr>
<th>pH</th>
<th>Total no. of viable cells</th>
<th>No. of foci</th>
<th>Fraction of flasks containing foci</th>
<th>TF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.17 ± 0.04</td>
<td>1.93 x 10⁵</td>
<td>1</td>
<td>1/30 = 0.033</td>
<td>5.2 x 10⁻⁴</td>
</tr>
<tr>
<td>7.16 ± 0.06</td>
<td>4.23 x 10⁵</td>
<td>0</td>
<td>0/20</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>7.13 ± 0.05</td>
<td>1.07 x 10⁵</td>
<td>0</td>
<td>0/27</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>7.02 ± 0.03</td>
<td>1.16 x 10⁵</td>
<td>0</td>
<td>0/27</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>7.12 ± 0.09</td>
<td>1.05 x 10⁵</td>
<td>0</td>
<td>0/28</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>7.05 ± 0.06</td>
<td>1.19 x 10⁵</td>
<td>0</td>
<td>0/28</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>Accumulated</td>
<td>6.82 x 10⁵</td>
<td>2</td>
<td>2/161 = 0.012</td>
<td>2.9 x 10⁻⁴</td>
</tr>
<tr>
<td>6.76 ± 0.07</td>
<td>7.70 x 10⁵</td>
<td>5</td>
<td>4/30 = 0.13</td>
<td>6.5 x 10⁻⁵</td>
</tr>
<tr>
<td>6.80 ± 0.04</td>
<td>1.09 x 10⁵</td>
<td>0</td>
<td>0/20</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>6.79 ± 0.05</td>
<td>1.80 x 10⁵</td>
<td>1</td>
<td>1/58 = 0.017</td>
<td>5.9 x 10⁻⁴</td>
</tr>
<tr>
<td>6.75 ± 0.05</td>
<td>6.72 x 10⁵</td>
<td>0</td>
<td>0/28</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>6.78 ± 0.03</td>
<td>1.24 x 10⁵</td>
<td>0</td>
<td>0/48</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>6.76 ± 0.06</td>
<td>8.82 x 10⁵</td>
<td>0</td>
<td>0/49</td>
<td>7.0 x 10⁻⁴</td>
</tr>
<tr>
<td>Accumulated</td>
<td>6.46 x 10⁵</td>
<td>6</td>
<td>5/233 = 0.021</td>
<td>9.3 x 10⁻⁴</td>
</tr>
</tbody>
</table>

* Statistical analyses (2 x 2 x 3 on accumulated fraction of flasks containing foci) indicate the difference at the two pH values is not significant (P > 0.5).

Table 2  Effect of reduced extracellular pH on radiation-induced transformation frequency after 7 Gy of gamma-irradiation
Accumulated irradiated plating efficiency at pH 7.0-7.2 was 0.16 ± 0.05 and at pH 6.7-6.8 was 0.08 ± 0.03. Statistical analyses on the endpoints of fraction of flasks containing foci and foci per surviving cell (transformation frequency) indicate the difference at the two pH values is not significant (P > 0.5). The results from three separate experiments are shown (×, Δ, □). The data from one experiment (0, 9, and 21 days) have been connected by a dashed line to indicate the trend.

<table>
<thead>
<tr>
<th>pH</th>
<th>Total no. of surviving cells</th>
<th>No. of foci</th>
<th>Fraction of flasks containing foci</th>
<th>TF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.17 ± 0.04</td>
<td>2.02 x 10⁵</td>
<td>136</td>
<td>28/28 = 1.00</td>
<td>6.7 x 10⁻⁴</td>
</tr>
<tr>
<td>7.16 ± 0.06</td>
<td>4.70 x 10⁵</td>
<td>11</td>
<td>11/20 = 0.55</td>
<td>2.3 x 10⁻⁴</td>
</tr>
<tr>
<td>7.00 ± 0.08</td>
<td>1.00 x 10⁵</td>
<td>29</td>
<td>21/28 = 0.75</td>
<td>2.9 x 10⁻⁴</td>
</tr>
<tr>
<td>7.11 ± 0.09</td>
<td>1.30 x 10⁵</td>
<td>43</td>
<td>22/28 = 0.85</td>
<td>3.3 x 10⁻⁴</td>
</tr>
<tr>
<td>7.05 ± 0.06</td>
<td>1.61 x 10⁵</td>
<td>62</td>
<td>22/28 = 0.78</td>
<td>3.8 x 10⁻⁴</td>
</tr>
<tr>
<td>Accumulated</td>
<td>6.40 x 10⁵</td>
<td>281</td>
<td>104/130 = 0.80</td>
<td>4.4 x 10⁻⁴</td>
</tr>
<tr>
<td>6.76 ± 0.07</td>
<td>6.30 x 10⁵</td>
<td>4</td>
<td>4/28 = 0.143</td>
<td>6.3 x 10⁻⁴</td>
</tr>
<tr>
<td>6.80 ± 0.04</td>
<td>1.56 x 10⁵</td>
<td>4</td>
<td>4/20 = 0.200</td>
<td>2.6 x 10⁻⁴</td>
</tr>
<tr>
<td>6.69 ± 0.06</td>
<td>2.40 x 10⁵</td>
<td>2</td>
<td>2/24 = 0.083</td>
<td>8.3 x 10⁻⁴</td>
</tr>
<tr>
<td>6.78 ± 0.03</td>
<td>1.22 x 10⁵</td>
<td>6</td>
<td>5/50 = 0.100</td>
<td>4.9 x 10⁻⁴</td>
</tr>
<tr>
<td>6.76 ± 0.06</td>
<td>7.13 x 10⁵</td>
<td>4</td>
<td>3/50 = 0.060</td>
<td>5.6 x 10⁻⁴</td>
</tr>
<tr>
<td>Accumulated</td>
<td>4.36 x 10⁵</td>
<td>20</td>
<td>18/172 = 0.105</td>
<td>4.6 x 10⁻⁴</td>
</tr>
</tbody>
</table>

* TF, transformation frequency calculated per viable cell.

cells and the fraction of flasks containing foci are significantly reduced at the lower pH (P < 0.01). The absolute values for the transformation frequencies are not the same in the HEPES (Fig. 1) and pH (Table 2) studies, due to the fact that these experiments were completed over a 1-year period and different batches of serum were used for these two sets of experiments. Serum batch is known to affect radiation-induced transformation frequency in these cells (17).

The next phase of this study was to vary the time during the post-irradiation expression period for which the cells were held at reduced pH. Fig. 2 shows data from three experiments where the cells were treated, plated at pH 7.0-7.2, and then transferred to pH 6.7-6.8 either immediately or after 1, 9, or 15 days, compared to cells which were held at pH 7.0-7.2 for the complete expression period of 21 days. As can be seen from the data, the transformation frequency increases as the time spent at pH 7.0-7.2 before the switch to the low pH medium increases.

Since the population-doubling time of mammalian cells is known to be quite pH dependent (1) and cell turnover is thought to be an important factor in radiation-induced cell transformation (21, 22), we examined the effect of reduced pH on the doubling time and the plating efficiency of our cells under the conditions used for the transformation experiments. Fig. 3 shows complete growth curves of irradiated (7 Gy) cells at pH 7.0-7.2 and pH 6.7-6.8. The population-doubling time was calculated from the data between days 4 and 7, where no refeddings and subsequent pH changes have occurred. At pH 7.0-7.2 the doubling time is 20-21 h, while at pH 6.7-6.8 it is 30-32 h. The cells at pH 7.0-7.2 reach a steady state plateau level at 8 to 11.0 x 10⁶ cells/T-75 flask  by day 10. The cells at pH 6.7-6.8 initially reach a lower steady state plateau level by...
Fig. 4 shows the plating efficiency of irradiated cells as a function of the age of the culture. It can be seen that the cells at reduced pH initially have a lower plating efficiency than those held at pH 7.2. Furthermore, there is a lag in the increase in plating efficiency as a function of time after plating of 2 to 3 days. In addition, there is a decrease in plating efficiency of the irradiated cells held at pH 7.2 at times beyond 8 days. This decrease is not as marked (if it occurs at all) for the cells held at reduced pH. We have addressed the possible implications of this delayed decrease in irradiated plating efficiency at pH 7.2 in a separate manuscript (23).

DISCUSSION

We have shown that the suppression of the radiation-induced neoplastic transformation of human hybrid cells in vitro by long term incubation in medium containing HEPES is the result of medium acidification rather than the presence of the HEPES chemical itself (Fig. 1, Table 2). The individual and accumulated transformation frequency and fraction of flasks containing foci data indicate that the suppression at low pH is significant (Table 2). The longer the time that the irradiated cells are kept at the control pH (7.0–7.2) during the post-irradiation expression period, before being switched to low pH medium, the less evident the suppression and the higher the transformation frequency (Fig. 2).

Growth curve data show that the doubling time of the irradiated cells is extended at pH 6.7–6.8, compared to pH 7.0–7.2 (Fig. 3). Control growth curves of unirradiated CGL1 cells (data not shown) show the same pH dependence on doubling time, indicating that this effect is not radiation specific. The extended doubling time is also apparent in the shift in recovery of the irradiated plating efficiency at low pH, a reflection of the repopulation of viable cells after irradiation (Fig. 4). The observation of an optimal growth medium pH for a specific cell type is in general agreement with the literature (1–4). The rate of cell turnover is thought to be an important factor in determining transformation frequency in vitro (22). This conclusion has principally come from experiments which deal with the dependence of transformation frequency on density of cells plated post-irradiation, for cell systems which exhibit contact inhibition of cell growth. Such a cell density dependence has been also observed in this system (17), which, however, does not exhibit density-dependent inhibition of growth. Therefore, implication of reduced amount of cell turnover as the reason for the reduction in transformation frequency at low pH has to be made with caution in this system. Indeed, we have done an experiment1 where the low pH cultures were allowed to incubate for an equivalent number of cell doublings as the normal pH controls, by extending the expression time by several days (25 versus 18 days), and the reduction in transformation yield persisted. Therefore, while we would not rule out lower rate of cell turnover as a possible contributing factor at the lower pH, it is important to consider other phenomena which may be underlying our observation.

Radiation survival studies of human and rodent cells have shown that immediate post-irradiation incubation of cells at low pH increases survival significantly (8–12). This increase in survival has been attributed to the repair of PLD (10–12). Raaphorst et al. (15) have shown that incubation of the irradiated C3H-10T1/2 cells in pH 6.8 medium for 1–24 h after irradiation resulted in PLD repair, increased survival, and a decrease in transformation frequency. Repair of PLD after irradiation is usually complete within 6 h, although small additional amounts of recovery can be evident up to 24 h later (24, 25). In our experiments the cells were allowed to repair PLD for 6 h at pH 7.2 (e.g., data in Table 2) before transfer to pH 6.8 medium. It would, therefore, seem clear that pH effects on PLD repair are not underlying our observations. Furthermore, significant reduction in transformation frequency is seen when the cells are exposed to low pH medium after 1, 9, and even 15 days (Fig. 2). This suggests that, whatever the nature of the transforming event/events in our system which lead to expression of the p75/150 cell surface protein and malignancy, these events can still be modified by reduction of extracellular pH several days after radiation exposure, long after repair of radiation damage is complete.

We therefore considered the possibility that the post-irradiation low pH treatment might be toxic to p75/150-expressing cells; we attempted to test this by incubating a spontaneously arising p75/150-positive mutant (CGL3) for 9 days at pH 6.7–6.8 and then staining for plating efficiency determination and the expression of the p75/150 cell surface marker. All colonies were still positive for p75/150, although the plating efficiency was lowered from 0.73 ± 0.08 at pH 7.0–7.2 to 0.35 ± 0.06 at pH 6.7–6.8, similar to the response of the parental nonmalignant hybrids (CGL1) (see Tables 1 and 2). These data suggest that a straightforward explanation of the reduction in the yield of radiation-induced transformed cells by low pH in terms of selective toxicity is not indicated.

This led us to propose another mechanism, in which low extracellular pH somehow reduces the probability of or inhibits a biochemical event/events that leads to p75/150 expression and malignant transformation. Intracellular pH, although buffered quite effectively in general, is sensitive to lowering of the extracellular pH below neutrality (26–29). In the acidic range the intracellular pH seems to reflect more closely the extracellular pH value (26–29). While there are many enzymes and molecules which will be affected by the lowering of intracellular pH (30), there is a class of enzymes, the proteases, whose

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1. Experiment refers to a study or test conducted to establish a fact or to determine a condition or characteristic.

---

2. C3H-10T1/2 cells are rodent cells used in radiation survival studies.

---

3. PLD: Post-Linear Damage, a term used in radiation biology to describe the repair of damage to the cell that occurs after the initial radiation exposure.

---

4. CGL1 is a human hybrid cell line used in these experiments.

---

5. HEPES: A buffering agent commonly used in cell culture media to maintain pH stability.

---

6. Long-term incubation refers to the duration of cell culture under specific conditions, in this case, pH 6.7–6.8.

---

7. Cell turnover is a measure of the rate at which cells are produced and replaced within a tissue or population of cells.

---

8. Transformation frequency measures the rate at which cells undergo transformation in vitro.

---

9. Selective toxicity refers to the ability of a treatment to selectively alter the growth or survival of cells, often leading to the reduction of malignant transformation.

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10. pH: A measure of the acidity or basicity of a solution, expressed on a scale from 0 to 14, with 7 being neutral, values below 7 being acidic, and values above 7 being basic.

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activity has been implicated in radiation-induced malignant transformation (31, 32). Recently, a M, 70,000 serine protease has been isolated from C3H10T1/2 cells (33). It is located in the cytosol and studies of protease activity versus pH in vitro revealed that it has a very narrow range of activity, with a sharp maximum at pH 7.0 (33, 34). While the exact mechanism by which proteases are involved in radiation-induced malignant transformation is unknown, their inhibition by various protease inhibitors has been found to correlate very well with suppression of malignant transformation (31, 32, 34). We have completed some studies with the protease inhibitor antipain in our cell hybrid system and have found that addition of antipain during, shortly after, and even up to 10 days after irradiation suppresses the yield of radiation-induced p75/150-positive foci (35). It is, therefore, interesting to postulate a potential link between the lowered activity of cellular proteases at low intracellular pH (possible when the extracellular pH is lowered) and the reduced yield of radiation-induced p75/150-positive foci in our system.

The effect of pH on other transformation systems has not been systematically investigated and these studies indicate that extracellular pH may be an important parameter to carefully control in quantitative studies of in vitro neoplastic transformation.

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REFERENCES

Suppression of Radiation-induced Neoplastic Transformation of Human Cell Hybrids by Long Term Incubation at Low Extracellular pH

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