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The Thermal Response of Mouse Adenocarcinoma Cells at Low pH*

KATHLEEN R. MEYER,† LARRY E. HOPWOOD‡ and EDWARD L. GILLETTE§

Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80523, *U.S.A.*

Abstract—*The response of MADCAP 37 tumor cells to* 42.0°C *and* 45.5°C *was determined at various* pH *levels. Cell viability, monitored by plating efficiency, did not vary in the* pH *range of* 6.50–8.00 *at* 37.0°C. *However, heating the cells to* 45.5°C *at* pH *values below* 7.00 *reduced survival* 10- *to* 40-*fold. Surviving fraction remained unchanged when the cells were heated at* pH *values greater than* 7.00. *The* $D\bar{o}$ *is approximately halved from* pH 7.00 *to* 6.50. *At* 42.0°C, *the sensitivity of the cells to heat at low* pH *was even more pronounced. The* $D\bar{o}$ *of the heat survival curve decreased* 7-*fold from* pH 7.30 *to* pH 6.50.

INTRODUCTION

UNDERSTANDING the response of cells to heat with respect to their microenvironment may enhance the usefulness of clinical trials now underway with hyperthermia. At least three components of the microenvironment have a demonstrable effect on hyperthermic response: oxygen tension [1–3], nutritional changes [4] and pH [5–7].

Gerweck and Rottinger [8] demonstrated that extra-cellular pH strongly influenced the response of exponential phase CHO cells to 41–44°C, while decreasing the pH from 7.6 to 6.7 did not affect cell viability at 37°C. Reduced pH tended to eliminate the large differential heat response between 42° and 43°C, the temperatures most often used in tumor studies, as well as to increase cell sensitivity at all temperatures examined [6]. At 45.5°C, Freeman *et al.* [7] saw the heat sensitivity of CHO cells increase sharply from pH 7.35 to 6.65, but remain constant from pH 7.35 to 7.85.

To further elucidate the role of pH in the thermal sensitivity of tumor cells, we have determined the heat response at various pH levels of an epithelial tumor cell line, MADCAP 37. This cell line was derived in our laboratory from a C3H mammary adenocarcinoma which has been used extensively in heat and radiation experiments. MADCAP 37 cells are similar to CHO cells in their increased sensitivity to hyperthermia at lowered pH.

MATERIALS AND METHODS

The MADCAP 37 cell line was maintained in 25 cm^2 plastic flasks with McCoy's medium supplemented with 10% fetal calf serum [9]. MADCAP 37 cells were harvested from exponentially growing cultures using 0.01 M EDTA for 4–6 min.

Survival was determined by colony formation 9–11 days after treatment. Cells were plated so that approximately 50 colonies per flask would result. However, the maximum number of cells plated per flask was 4×10^5 or 10^5/ml. This concentration did not alter the pH of the medium. The data points in the figures represent the means and standard errors of 4 flasks from an experiment. The means were corrected for plating efficiency (0.25–0.40) and multiplicity (1.4–1.6).

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†Present Address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, U.S.A.
‡Present Address: Radiology Department, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, U.S.A.
§Present Address: Comparative Oncology Unit, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

Heating

All heating was done by immersion of flasks into Precision-Freas waterbaths (precision Scientific Co.) capable of maintaining constant temperature within 0.05°C. Bath temperatures were monitored with thermometers calibrated against an NBS-certified mercury-in-glass thermometer with corrections made for the emergent stem factor.

For all experiments, asynchronous cells were plated 24 hr before heating in T-25 flasks containing 4 ml of McCoy's Medium [10]. At the start of the experiments, the T-25 flasks were sealed with paraffin wax and placed in wire test tube racks (4 8 per rack).

pH adjustment

The pH of the medium was adjusted by flushing 4 flasks with a mixture of CO_2 and air for 1 min at 37.0°C, similar to a procedure developed by Freeman *et al.* [7]. All flasks were then sealed and submerged in a 37.0°C waterbath. At designated times, groups of flasks were placed in either 42.0° or 45.5°C waterbaths. After heating, the flasks were returned to the 37.0°C waterbath. All flasks were maintained at the desired pH for the same length of time by adjusting the time at 37.0°C before and after heating. The pH of all flasks was readjusted to pH 7.30 before incubation. The pH was read with a Corning pH meter immediately after gassing to a particular pH or after being submerged in a 37.0°, 42.0° or 45.5°C waterbath.

RESULTS

Varying the pH had no effect on plating efficiency of MADCAP 37 cells at 37.0°C. Figure 1 demonstrates the increase in sensitivity to 45.5°C which occured as the pH was lowered. From pH 7.00 to 8.06 the thermal response was similar, at least down to 10% survival. As the pH was lowered below 7.00, the slope increased slightly and, at pH 6.71 and 6.51, the shoulders of the heat survival curves also decreased.

As the pH of the medium was decreased, the sensitivity of MADCAP 37 cells to 45.5°C heating increased 10- to 40-fold depending upon the length of heating (Fig. 2). With only 5 minutes of heating at 45.5°C, lowering the pH had no effect on surviving fraction.

Ten minutes at 45.5°C at pH levels greater than pH 6.80 produced the same level of survival. At pH 6.51, however, survival dropped significantly to 2.7×10^{-2}. For

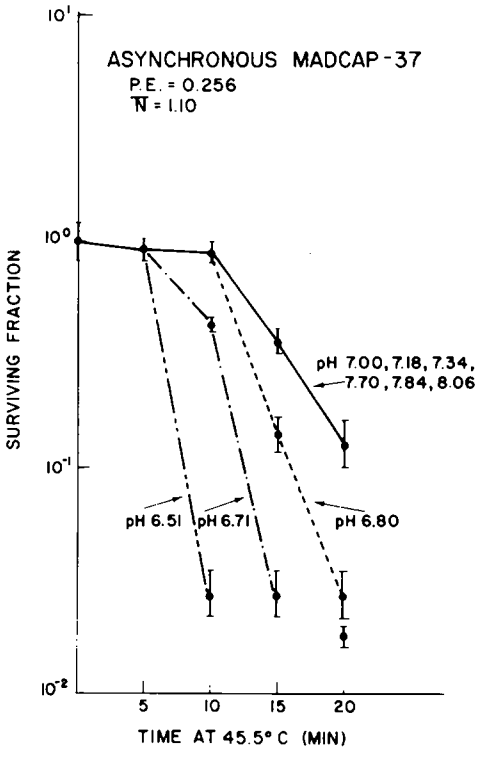

Fig. 1. Heat survival curves at various pH *values. The response to* 45.5°C *for up to* 20 min *was determined while the cells were maintained at the desired* pH. *The* pH *was achieved by gassing* 4 *T-30 flasks with a mixture of* CO_2 *and air for* 1 min, *and was maintained at* 37°C *for* 20–30 min *before and* 5 min *after the heating. The* pH *of all flasks was readjusted to* 7.30 *before placing in the cloning incubator. One curve represents the heat response for cells at* pH 7.00–8.06.

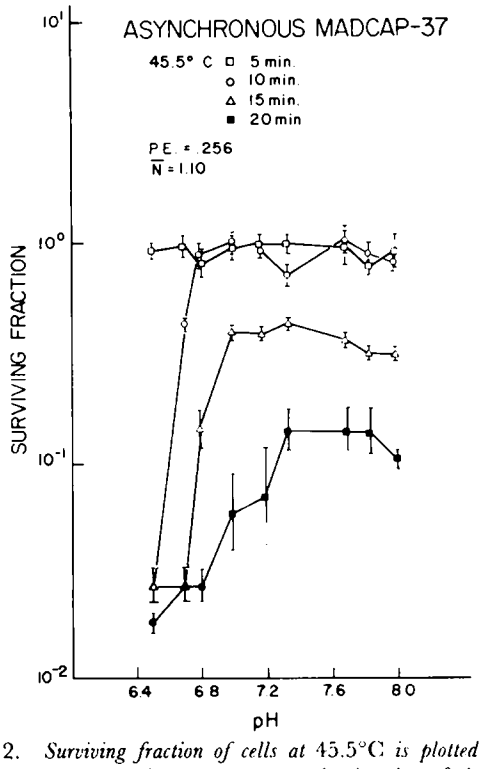

Fig. 2. Surviving fraction of cells at 45.5°C *is plotted as a function of* pH. *Each curve represents the duration of the heat treatment* (5, 10, 15 *or* 20 min). *See Fig.* 1 *legend for explanation of procedure.*

15 and 20 min of heating, survival remained unchanged down to pH 7.00 and 7.34, respectively. Below these values, survival dropped dramatically (Fig. 2).

In a similar set of experiments at 42.0°C, the sensitivity to the hyperthermic treatment was even more dramatic at lowered pH. Under control conditions at pH 7.30, the Dõ was 135 min. When the pH was decreased to 6.80 and 6.50, the Dõ values were 40 and 20 min, respectively (Fig. 3). The shoulder was also reduced at pH 6.50. In addition, at pH 6.80 thermal resistance was present by 8 hr of heating.

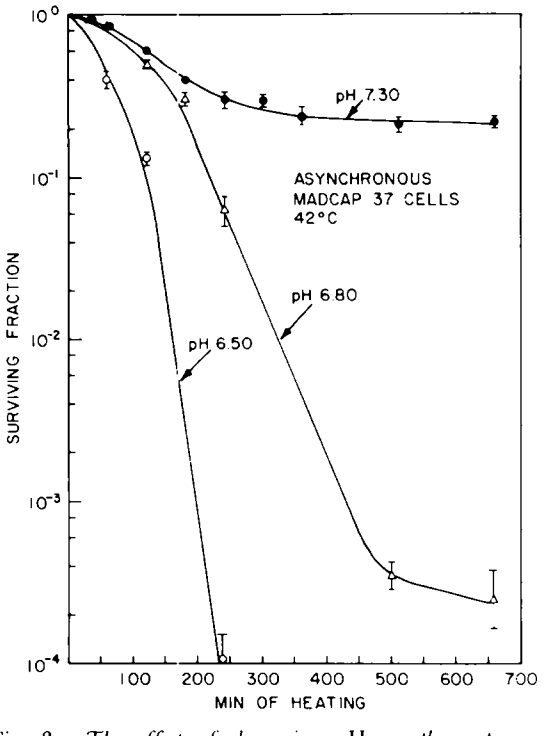

Fig. 3. The effect of decreasing pH *on the response of asynchronous MADCAP 37 cells to* 42°C. *The procedure followed was similar to that described in Fig. 1 except only* 3 pH *levels were used.*

DISCUSSION

The response of MADCAP 37 cells to hyperthermia is pH dependent and a similar response has been reported for other cell lines [5–8]. After 4 hr of heating at 42.0°C, Gerweck and Rottinger [8] reported a 500-fold decrease in survival at pH 6.7 relative to pH 7.4. After the same heat dose, MADCAP 37 cells exhibited a 5-fold decrease at pH 6.80 and a 3000-fold decrease at pH 6.50. At 45.5°C, the sensitivity of the cells at pH values less than 7.00 increased 10- to 40-fold depending upon the length of heating (Fig. 2). These data agree well with those obtained by Freeman *et al.* [7] for CHO cells at 45.5°C where the method of adjusting the pH was similar.

This increased heat sensitivity was reflected both in the decrease in Dõ's (Figs. 1 and 3) and, at low pH, by the decrease in the shoulder of the heat survival curve indicating a modification of repair capabilities. The heat response of MADCAP 37 cells under normal pH conditions was similar to other cell lines, and has been reported in a separate publication [10]. Comparing the change in Dõ's for the 42.0° and 45.5°C heat treatment indicates that there is a greater sensitivity to low pH when the cells were heated at the lower temperature. Since most therapeutic heating is done at 41°–43°C, low pH could enhance the effectiveness of heat. The pH sensitivity was also most pronounced at 42°C in CHO cells [6].

Furthermore, the heat survival curve plateau at lower temperatures (Fig. 3), which has been referred to as thermal resistance [11], can be affected by culture conditions such as pH. When the pH at which cells were heated shifted from 7.30 to 6.80, thermal resistance was still observed at 42.0°C but the plateau was almost a 1000-fold lower (Fig. 3). The resistant fraction generally seen at temperatures less than 43.0°C after 4–5 hr of heating is not completely eliminated but rather decreased by low pH. A resistant fraction of CHO cells also remained when the pH was lowered from pH 7.4 [6, 8].

These studies support the finding that low pH greatly sensitizes cells to hyperthermia, and clearly show the necessity of monitoring pH during *in vitro* experiments, both at low and high temperatures. Clinically, pH could be utilized to accentuate the differential heat response between normal and tumor tissue since tumors may have areas of low pH [12, 13]. Recently, Gerweck [6] discussed the clinical ramifications of the pH effect quite thoroughly. von Ardenne *et al.* [14] have utilized heat and glucose infusions to produce "overacidification" in rodent and human tumors. They reported that lowered pH and heat labilized the lysosomal membranes. Overgaard [5] reported that low pH and heat caused an increase in lysosomal activity resulting in cell death in L1A2 ascites cells. While pH is clearly affecting the heat response of both cells in culture and tumors *in situ*, the mechanism of this phenomenon is not understood and should be investigated further.

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