HYBRIDOMA GROWTH, METABOLISM, AND PRODUCT FORMATION IN 
HEPES-BUFFERED MEDIUM: II. EFFECT OF pH

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ABSTRACT

The effects of pH on cell metabolism during the batch growth of hybridomas in flasks were evaluated. Maintaining the pH at 7.2 resulted in a reduction of the maximum antibody and viable cell concentrations by ca. 40% and increased glucose and amino acid metabolic quotients. When the pH was allowed to fall to 6.7 there was further growth and antibody production after glutamine was exhausted using branched-chain amino acids as substrates. Y'[lac/gluc] increased from 1.32 at pH 6.7 to 1.50 at pH 7.2.

INTRODUCTION

The optimum pH values for cell growth and product formation by recombinant cell lines and by hybridomas may or may not be identical. Eagle (1973) reported that for mammalian cell growth there is often a relatively broad optimum pH range, although this optimum range varies with the species and cell lines tested. In batch culture experiments Birch and Edwards (1980), Barton (1971), and Harbour et al. (1989) found specific growth rates and maximum cell densities for human lymphoblastoid, HeLa, and hybridoma cells to be a function of the initial pH of the culture medium. Consequently, Harbour et al. (1989) observed much reduced final MAB concentrations at low culture pH values. On the other hand, Miller et al. (1988) demonstrated that while cell growth and viability of AB2-143.2 hybridoma cells grown in continuous culture is optimal between pH 7.1 and 7.4, antibody metabolic quotients are higher during periods of stress [i.e., pH 6.8].

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In this paper we report on two experiments with a mouse hybridoma cell line in small-scale batch cultures in which the medium pH-value is either maintained at pH 7.1-7.2 or allowed to decrease to pH 6.7 during batch cultivation. We monitor the resulting differences in cell growth, metabolism and product formation.

**Materials and Methods**

A mouse hybridoma line AB2-143.2 (Hornbeck and Levis, 1985) was cultivated in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum, MEM nonessential amino acids, and pyruvate. Buffering capacity was provided by 25 mM HEPES (N-2-Hydroxyethyl-piperazine-N'-2-ethanesulfonic acid) sodium salt (Shipman, Jr., 1969). pH adjustments were made twice daily (from day three onwards) with 0.5 N NaOH so as to keep the pH-value at 7.1-7.2, whereas the pH decreased from an initial value of 7.2 to pH 6.6-6.7 in the control experiment.

Viable and nonviable cells were estimated by hemacytometer counting (trypan blue staining). Glucose and lactate were determined by enzymatic assays; ammonia was measured with an ion-selective electrode. Fluorescent amino acid derivatives were separated by reversed phase HPLC and antibody concentrations quantitated by high pressure Protein A affinity chromatography.

Details on the hybridoma cell line, medium composition, and growth conditions as well as the assay procedures for metabolite and product concentrations are given elsewhere (Schmid et al., 1990).

**Results and Discussion**

The kinetics of growth, metabolism, and product formation of the murine hybridoma cell line AB2-143.2 were investigated in two experiments (Figures 1 and 2) performed in tissue culture flask with HEPES-buffered DME medium. In one case the initial pH of the medium (7.2) was maintained constant by twice daily additions of a 0.5 N NaOH solution; in the control experiment the pH was allowed to equilibrate freely with the gas flow (1% CO₂ in air) in the 37°C incubator.

When the pH value of the culture medium is maintained at 7.1-7.2 maximum viable cell concentrations (nᵥₘₐₓ) reach ca. 3 · 10⁸E6 cells/mL at day 5 (Figure 2a). At this time glucose and glutamine are both exhausted from the medium (Figures 2c and d) and the viable cell count drops off fast. If no addition of base is performed during the cultivation period the pH value falls to 6.6-6.7. Cells start to grow with the same max. growth rate of 𝜇ₘₐₓ 1.10±0.05 d⁻¹ but reach an nᵥₘₐₓ of 5 · 10⁸E6 cells/mL (Figure 1a). Glutamine becomes limiting at day 5 but there is then further growth at lower 𝜇 (resulting in an extended growth curve) using other amino acids, particularly valine, leucine and isoleucine, (Figure 1e-g) as substrates.