OBSERVED DIFFERENCES IN CO₂ REQUIREMENTS BETWEEN MAMMALIAN AND SALMONID FISH CELL LINES

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INTRODUCTION

During the course of metabolic studies with salmonid fish cell lines, an investigation of the in vitro CO_2 requirements of these cells was undertaken. Werkman (13) hypothesized that all forms of life assimilate CO_2 and that this essential physiological function is required for the synthesis of indispensable metabolic intermediates. The CO_2 requirement of mammalian cell cultures has been well established (4, 6, 12) but to our knowledge no such work has been reported with poikilothermic cells in vitro. Using cell lines derived from salmonid fish embryos (3) and maintained by serial passage in this laboratory, we have found that CO_2 is required for growth of salmonid fish cells in vitro. Differences in the CO_2 requirements between the fish cells and a mammalian cell line were observed.

MATERIALS AND METHODS

Cell lines CSE 119 and STE 137 were derived from embryonic tissue of coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*), respectively (3). Morphologically, CSE 119 cells are fibroblast-like and STE 137 are epithelial-like. HeLa cells used in this study were obtained from Microbiological Associates, Inc., Bethesda, Md. All three cell lines were maintained as monolayer cultures in Eagle's minimal essential medium (2) prepared with Hanks' salt solution and supplemented with 20% newborn agamma calf serum (Hyland Laboratories, Los Angeles, Calif.). For the CO_2 experiments, 5 mM Tris (Tris[hydroxymethyl]aminomethane) buffer was substituted for the bicarbonate, and dialyzed serum was used. All media contained 100 units of penicillin and 100 µg of streptomycin per ml. HeLa cells were grown at 35°C and the fish cells at 18°C. Cells were enumerated by Sanford and Earle's method of counting nuclei (11). Cultures of HeLa cells were initiated by using an inoculum of 100,000–200,000 cells per ml, and the fish cells were initiated at 200,000–500,000 cells per ml.

CO_2-free conditions were obtained in Conway microdiffusion dishes. 3 ml of cell suspension was inoculated into the center well of the dish and 5 ml of 10% KOH was added to the outer well to absorb any CO_2 produced by the cells. The culture dishes were sealed with silicone high-vacuum grease. CO_2 tensions of 3% (HeLa cells) and 2% (fish cells) were obtained in a plexiglass CO_2 incubator by reducing the pressure the desired amount with a vacuum pump and returning the pressure to one atmosphere by the addition of CO_2. Free gas exchange with the air was obtained by culturing the cells (8 ml) in 150-ml Pyrex milk dilution bottles closed with gauze-wrapped cotton plugs. Oxalacetate was prepared as the sodium salt and added to the culture medium to give a final level of 2 mM. The pH of all cultures was maintained between 7.0 and 7.9 by the addition of sterile 0.25% NaOH.

RESULTS

All three cell lines were tested for their ability to grow in the absence of CO_2 using the Conway microdiffusion dishes. Both salmonid cell lines demonstrated a growth requirement for CO_2 comparable to that shown for HeLa cells (Table I).

The salmonid fish cell lines were tested for their ability to grow in cultures open to the air (0.03% CO_2). Although growth of cell line CSE 119 seemed to be retarded at this low CO_2 level, both cell lines grew under these conditions (Fig. 1).

TABLE I

Growth of HeLa Cells and Two Salmonid Cell Lines in CO_2-Free and CO_2-Containing Atmospheres

Cell type	Culture condition		Cells × 10^5/ culture at:	
			Day 2	Day 8
HeLa*	CO_2 free‡	Exp. 1	3.1	3.6
	3% CO_2	Exp. 1	3.8	36.0
CSE 119§	CO_2 free	Exp. 1	5.6	6.1
		Exp. 2	11.3	10.2
	2% CO_2	Exp. 1	7.1	15.6
		Exp. 2	8.8	33.8
STE 137§	CO_2 free	Exp. 1	6.6	7.4
		Exp. 2	4.9	7.4
	2% CO_2	Exp. 1	7.2	21.0
		Exp. 2	5.4	25.8

* Cells grown at 35°C.
‡ Cells grown in Conway microdiffusion dishes with KOH trap.
§ Cells grown at 18°C.

HeLa cells under the same conditions showed almost no growth.

Oxalacetate has been reported to partially substitute for CO_2 in the growth of HeLa cells (10). Since the CO_2 requirements of the salmonid fish cell cultures seemed different from those of the HeLa cell cultures, the ability of oxalacetate to substitute for CO_2 in all three cell lines was studied. Oxalacetate partially substituted for CO_2 in the HeLa cell cultures, but little or no growth occurred in the salmonid fish cell cultures under the same conditions (Table II).

DISCUSSION

Although the salmonid cell cultures did demonstrate a growth requirement for CO_2, their ability to grow in bicarbonate-free Tris-buffered medium with free gas exchange with the air sets them apart from most mammalian cells. Swim and Parker (12) reported that strains of fibroblasts derived from human, mouse, and rabbit tissues failed to proliferate at a significant rate when cultured in a medium without bicarbonate in unstoppered flasks.

Oxalacetate alone or in combination with ribosides has been shown to replace the CO_2-bicarbonate system for L mouse fibroblasts and HeLa cells (5), for Yoshida ascites tumor cells (8), for three neoplastic lines of human cells (7), and for human

conjunctival cells (1). Runyan and Geyer (10) found only partial substitution by oxalacetate in L mouse fibroblasts and HeLa cells. The salmonid fish cells showed little or no growth when oxalacetate was substituted for CO_2.

Mechanisms involving the conversion of pyruvate (9) or phosphoenolpyruvate (14) in the presence of CO_2 to oxalacetate have been proposed for CO_2 fixation by mammalian cells in vitro. The results suggest that this form of CO_2 fixation may be
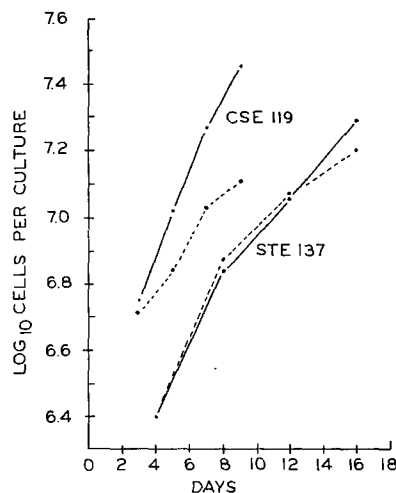
FIGURE 1 Growth of salmonid fish cell cultures under 2% CO_2 (solid line) and with free gas exchange with the air (broken line) in Tris-buffered medium without bicarbonate at 18°C.

TABLE II

Growth of HeLa Cells and Two Salmonid Cell Lines in CO_2-Free and CO_2-Containing Atmospheres in Medium Supplemented with Oxalacetate

Cell type	Cultural conditions			
	CO_2-free + 2 mM oxalacetate		CO_2* + 1 mM oxalacetate	
	Cells × 10^5/ culture at:		Cells × 10^5/ culture at:	
	Day 2	Day 8	Day 2	Day 8
HeLa‡	5.5	12.4	3.8	36.0
CSE 119§	9.2	7.4	8.8	33.8
STE 137§	6.0	8.9	5.4	25.8

* HeLa cells grown under 3% CO_2 and fish cells grown under 2% CO_2.
‡ Cells grown at 35°C.
§ Cells grown at 18°C.

utilized by HeLa cells since there was partial substitution of oxalacetate for CO_2 in these cells. This mechanism seems to be absent in in vitro CO_2 fixation by salmonid fish cells because of the lack of growth in oxalacetate-supplemented, CO_2-free fish cell cultures.

Since oxalacetate only partially substitutes for CO_2 in HeLa cells, it appears that an additional mechanism must be operating in CO_2 fixation by these cells. This mechanism may be related to the observation that when HeLa and conjunctival cells were grown in Tris-buffered medium containing labeled bicarbonate, most of the activity was found associated with the purines and pyrimidines of the cells (1). Additional studies to determine the site of CO_2 fixation in the salmonid fish cell cultures must be undertaken before the mechanism of CO_2 fixation in these cells can be determined.

A system for the growth of salmonid fish cells in cultures with free gas exchange with the air could be a valuable tool in the study of these cells and in the study of viruses found to replicate in these systems. For example, this would permit the preparation of monolayer cultures for virus plaque assays in Petri dishes without the need for a CO_2 incubator. The growth of the salmonid fish cell lines in Tris-buffered medium with free gas exchange with the air provides such a system.

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