ENZYME ACTIVITIES OF THE EPITHELIAL OUTGROWTH OF THE HAIR FOLLICLES IN TISSUE CULTURE

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INTRODUCTION

The viability of cells in tissue culture can be expressed by several biochemical activities such as glycolysis, respiration, protein and nucleic acid syntheses, enzyme activity, etc. Since all of the standard analytical procedures for these activities

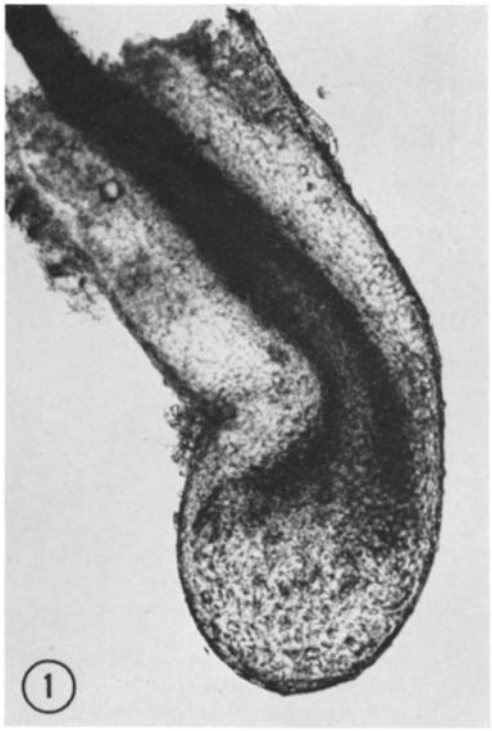

FIGURE 1 The dissected growing hair follicle, bulb portion. × 250

require a relatively large number of cells, they are not suitable for testing a small group of cells in culture.

Lowry developed fluorometric microenzyme assay methods that are sufficiently sensitive to measure glycolytic enzyme activities in a single cell (8). We found that this fluorometric technique is applicable in testing the viability of the epithelial outgrowth of hair follicles where only a limited number of cells are available. In this study, the activities of five enzymes participating in the carbohydrate metabolism of cells in the outgrowth of hair follicle culture were assayed.

MATERIALS AND METHODS

Single hair follicles were dissected from fresh skin biopsies obtained from the occipital region of adult stump-tailed macaques (Fig. 1). The individual follicles were explanted on the bottom of Falcon plastic T-30 flasks in a clot of equal parts of chick embryo extract and chicken plasma (Baltimore Biological Laboratory, Baltimore, Md.). After the clot was firmly set, 3 ml of supernatant medium consisting of medium 199 enriched with 30% fetal calf serum (Grand Island Biologicals Co., Grand Island, N.Y.) were added to the flasks. The cultures were incubated at 37°C in a chamber with humidity control and an atmosphere of 5% CO_2 in air.

Epithelial cells began to grow out from the explants in a few days, and 20–30 days after the beginning of incubation the cells were in sheet formation (Fig. 2). At this time the cultures were ready for enzyme studies.

After removal of the supernatant fluid, the cultures were thoroughly washed twice with physiological saline and quickly frozen with dry ice. The frozen cells were dried under vacuum at − 15°C overnight.

After the frozen-dried samples had been equilibrated to room temperature, a portion of the epithelial outgrowth was scraped from the wall of the culture vessel with a razor blade under a stereomicroscope. The samples were weighed with a microbalance (7) and transferred to microtest tubes for assay of the following enzymes: hexokinase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, isocitrate dehydrogenase, and fumarase. The assay conditions are summarized in Table I. Tissue DNA concentration was measured according to the method of Kissane and Robins (6).

RESULTS AND DISCUSSION

The results are summarized in Table II. The enzyme activities in the bulb portions of growing hair follicles (9) are also listed in this table for comparison. Generally, the activities of glycolytic enzymes in the outgrowth were twice as high as those in the original hair follicle cells, and the activities of tricarboxylic acid cycle enzymes were approximately the same in the outgrowth as in the original tissue. If the enzyme activities are compared on a per cell basis instead of on a per dry weight basis, they are two to three times higher in the outgrowth than in the original hair follicle cells since DNA concentration in the outgrowth was 0.92 g/100 g dry weight tissue (± 0.12 SE, $n = 8$) and that in the follicle was 1.8 g/100 g dry weight (± 0.10 SE, $n = 8$). However, the profile of enzyme pattern was similar for both in vitro (outgrowth) and in vivo (original follicle) samples. For example, the activities of glucose-6-phosphate and lactate dehydrogenases, both in vitro and in vivo, were approximately 2 and 20 times greater, respectively, than those of hexokinase.

We tentatively conclude that representative enzymes participating in the Embden-Meyerhof pathway and pentose cycle show increased activity when the cells adapt to the in vitro system. Obvi-

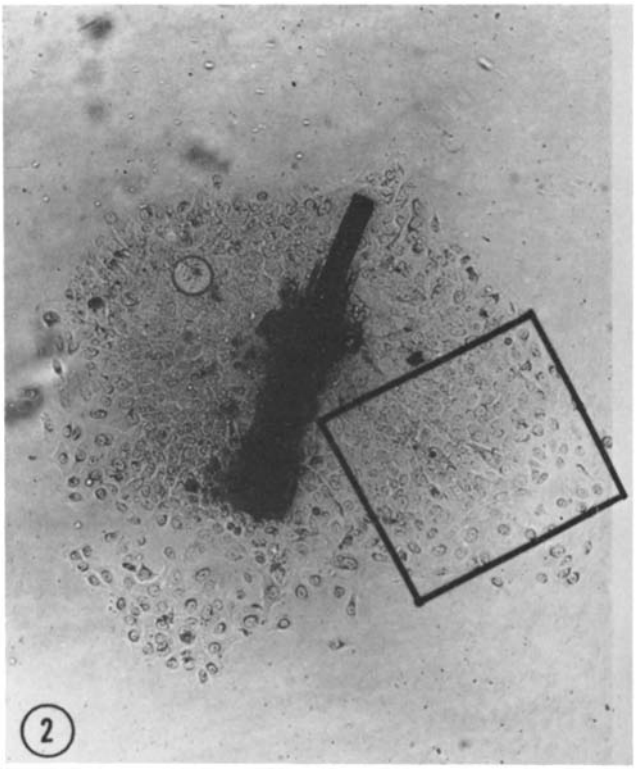

FIGURE 2 The epithelial outgrowth of the hair follicles. The cells within the rectangle show the typical size used for the enzyme assays. × 100

TABLE I

Substrate Reagent Mixtures for Enzyme Assays

Enzymes	Substrate	Cofactors and accelerators	Auxiliary enzyme system	Buffers	Reaction volume
					μl
Hexokinase (1)	glucose, 4 mM	ATP, 3 mM, Mg^{++}, 5 mM	G6PDH, 1 μg/ml, $NADP^+$, 0.3 mM	Tris-HCl, 0.1 M, pH 8.1	5–15
G6PDH (4)	glucose-6-phosphate, 2 mM	$NADP^+$, 0.3 mM, Mg^{++}, 2.5 mM, EDTA, 0.5 mM	None	AMP, 0.1 M, pH 8.8	10–15
LDH (5)	pyruvate, 1 mM	NADH, 2 mM	None	Tris-HCl, 0.1 M, pH 7.5	20–40
ICDH (2)	isocitrate, 5 mM	$NADP^+$, 1 mM, Mn^{++}, 0.25 mM	None	Tris-HCl, 0.1 M, pH 7.9	5–15
Fumarase (3)	fumarate, 20 mM	None	None	phosphate, 0.05 pH 7.1	5

The samples (0.2–2.0 μg) were incubated for 60 min at 37°C. Reaction products were measured fluorometrically.

The abbreviations used are as follows: G6PDH, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; ICDH, isocitrate dehydrogenase; ATP, adenosine triphosphate; $NADP^+$, nicotinamide adenine dinucleotide phosphate (TPN^+); NADH, nicotinamide adenine dinucleotide, reduced (DPNH); EDTA; ethylenediamine tetraacetic acid; and AMP = 2-amino-2-methyl-1,3-propanediol buffer.

Numbers in parentheses represent references.

TABLE II

Enzyme Activities in Outgrowing Cells in Tissue Culture

Enzyme sample	Outgrowing cells in tissue culture	qIn iBt1odon of hair follicle
	moles/kg dry wt/hr, ±SE	
Hexokinase	0.99 ± 0.03 (3)	0.65 ± 0.05
G6PDH	1.8 ± 0.17 (9)	1.09 ± 0.11
LDH	21.6 ± 2.2 (5)	13.9 ± 1.6
ICDH	1.3 ± 0.06 (4)	1.2 ± 0.07
Fumarase	5.1 ± 0.38 (7)	6.3 ± 0.42

Numbers in parentheses indicate number of determinations.

Data on the hair follicles represent the average of 20 determinations on the bulb portion of growing hair follicles in the scalp of the same animal. These samples were obtained by the microdissection of freeze-dried skin sections (20–40 μ in thickness) and were composed of epithelial tissues without keratin (see reference 9 for details).

ously, further experiments are required to determine whether general glucose metabolism is also increased in the cells of the outgrowth. Microenzyme assay methods to test cell viability in tissue culture systems may also be applicable in those systems containing groups of two or more different types of cells.

SUMMARY

By using highly sensitive fluorometric techniques, we assayed hexokinase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, isocitrate dehydrogenase, and fumarase in less than 1 μg quantities of epithelial outgrowth from hair-follicle cultures. All of the enzyme activities in the outgrowth either remained approximately the same as in the original hair-bulb cells or doubled. It is suggested that where limited groups of cells are available microenzyme assay methods may be useful in studying cell viability in tissue-culture systems.

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