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Lactate Consumption by Hepatocytes in Monolayer Culture (41083)

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Abstract. Hepatic lactate metabolism was studied in confluent primary hepatocyte monolayer cultures from fasted rats. The cultures were maintained in Krebs–Henseleit buffer containing 1% gelatin and 50 m*M* of both Tricine and Hepes. Under standard conditions of pH 7.4, P_{O_2} > 280 mm Hg, and P_{CO_2} of 22–27 mm Hg, little glucose or lactate was produced unless the media was made 10 m*M* with either fructose, dehydroxyacetone, or pyruvate. While the addition of glucose did not lead to lactate production, added lactate was converted to glucose. Lactate utilization was unaffected by lowering the P_{O_2} to 100 mm Hg, but between 100 to 20 mm Hg lactate consumption was increasingly inhibited. At 20 mm Hg no lactate was consumed. At low pH, below 7.0, lactate utilization was inhibited. The effects of low pH and anoxia, separately and together, were readily reversed by returning the cultures to standard conditions. Varying the P_{CO_2} from 4.4 to 101 mm Hg had no effect on lactate consumption.

Lactic acidosis is a serious clinical condition that can become irreversible and lead to death. While the cellular metabolic pathways utilizing lactate are well defined, little is known as to how the body regulates lactate homeostasis. As has been discussed in several reviews (1–3), a contributing factor in lactic acidosis appears to be an inability of the liver to consume lactate. This study was undertaken to understand better hepatic lactate utilization relative to P_{O_2}, P_{CO_2}, and pH.

Several studies have been done on hepatic lactate uptake with perfused liver (4–7), but the method has several technical drawbacks. The report presented here involves initially developing a primary hepatocyte monolayer culture system that yields viable and easily manipulated cells. These cultures were found to metabolize various glycolytic and gluconeogenic substrates, and to be hormonally responsive in a manner consistent with hepatic functions. The consumption of lactate was inhibited by low pH and anoxia, the inhibition being reversed by returning the cultures to normal pH and atmosphere. Varying the P_{CO_2} from 4.5 to 103.1 mm Hg in heavily buffered medium at pH 7.5 had no effect on lactate consumption except at excessive P_{CO_2} levels (103.1 mm Hg) which were associated with a lowered pH.

Materials and Methods. Experiments were performed with 150- to 210-g male Wistar rats, fasted for 24 hr prior to use. Animals were anesthetized with sodium pentobarbital ip (30 mg/kg).

Hepatocytes were prepared by the collagenase perfusion method of Katz *et al.* (9). The final cell pellet was brought up to 1 $\times 10^6$ cell/ml in Eagle's basic medium supplemented with glutamine (4 m*M*), Hepes (20 m*M*), and made 10% with fetal bovine serum. The suspension was kept at 4°. Viability was tested by the exclusion of 0.2% trypan blue in saline.

Monolayer cultures were established in 35-mm tissue culture dishes (Falcon) by adding 2.0 ml of cell suspension and incubating at 37° in an atmosphere of 95% O_2–5% CO_2 for 45–60 min. The media and unattached cells were aspirated off the monolayers, and an additional 2.0 ml of cell stock was added. At the end of the second 45- to 60-min period, the cultures were again aspirated and washed gently with warm saline.

At this point, experiments were initiated by adding to each dish 1.0 ml of incubation medium: Krebs–Henseleit buffer (10)

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supplemented with 1.5% gelatin, 50 m*M* tricine, and 50 m*M* Hepes to which the various substrates were previously added. Except where noted, the cultures were kept under what are here defined as standard conditions of 37°, pH 7.4, in an atmosphere of 95% O_2–5% CO_2.

The production or consumption of glucose and lactate was determined as the difference between samples collected at zero time and at later times indicated in the experimental results. At the second collection, the attached monolayer cells were harvested for protein determinations. The net change for glucose or lactate concentration was corrected to a per milligram of protein basis.

The concentration of lactate in the medium was determined with the Calbiochem rapid lactate kit and glucose by the Sigma UV glucose kit. The protein determinations were by the method of Lowry *et al.* (11) using bovine serum albumin as a standard. Glutamic-oxalacetic (GOT) and glutamic-pyruvic transaminase (GPT) were measured with the Sigma 155-UV kit using either 25 or 50 μl of sample to 1.0 ml of reagent at room temperature. A recording Gilford 2400-S spectrophotometer was used to measure the decrease in optical density.

In those cases where the P_{O_2} and P_{CO_2} were varied, cultures were placed into chambers connected to a gas inlet line. The relative flow of oxygen, carbon dioxide, and nitrogen was regulated through separate flow meters. The chambers were gassed for 3 min, disconnected from the gas line, sealed, and kept at 37° for 115 min. At the end of the incubation the media were immediately analyzed with a Radiometer-Copenhagen blood–gas analyzer for pH, P_{O_2}, and P_{CO_2}. Samples were collected for lactate assay and the attached cells were harvested for protein determinations.

Results. The initial cell suspension resulting from dispersion of the collagenase digested livers had a viability, between preparations, of 55–95%. In order to obtain consistently high viabilities and have the added advantage of allowing for media changes with a minimum of trauma to the cells, we took advantage of the fact that in the presence of serum, viable cells preferentially attach to plastic tissue culture dishes (12). Under the conditions employed here, viable cell attachment was maximal between 45 and 60 min, while longer times allowed attachment of nonviable cells. Two attachment periods were necessary in order to obtain confluent monolayers. Efforts to increase attachment by adding more cells at one time (increased volume or increased cells/ml) only resulted in the cells attaching to each other instead of the culture dish. The net result of the procedure used here was a 35-mm culture dish containing 1.5–2.0 mg protein, with a cell viability of 90–100%.

Cell viability based on trypan blue exclusion remained unchanged during 2 hr of incubation in Krebs–Henseleit buffer. In addition, there was no detectable leakage of either GOT or GPT into the media. If the cells and media were collected together and frozen to lyse the cells, 25 μl of the 1000*g* supernatant contained a decreasing absorbance at 340 μm of 0.062/min for GOT and 0.046/min for GPT.

The performance of these cultures with respect to the production of glucose and lactate from various substrates and the effects of glucagon (1 μM) on those parameters are summarized in Table I. In the absence of a medium substrate, there was a slight production of glucose and lactate. This has been reported in similar systems (8, 13, 14) and is attributed to the breakdown of residual cellular glycogen that survived the 24-hr fasting period.

When glucose was supplied as a substrate, there was little production of lactate; yet lactate was found to be readily metabolized to glucose. Added fructose, alanine, and pyruvate were metabolized to lactate; fructose was the best source. Fructose was also the best substrate for glucose formation. While pyruvate and lactate were equally good as gluconeogenic substrates, added pyruvate resulted in lactate and glucose production in a 2:1 *M* ratio.

The effects of glucagon were found to be consistent with the glucogenic function of the hormone. The trend was to increase glucose production and to inhibit lactate

TABLE 1. GLUCOSE AND LACTATE PRODUCTION FROM VARIOUS SUBSTRATES IN THE ABSENCE AND PRESENCE OF 10 μM GLUCAGON[a]

Added substrate (10 μM)	Glucose production (mmole/mg protein/60 min) × 10^{-3}		Lactate production (mmole/mg protein/60 min) × 10^{-3}	
	Glucagon		Glucagon	
	−	+	−	+
None	0.047 ± 0.004	0.063 ± 0.006	0.031 ± 0.003	0.007
Glucose	0.071 ± 0.006	0.051 ± 0.002	0.053 ± 0.003	0.003
Fructose	0.160 ± 0.010	0.241 ± 0.021	0.429 ± 0.036	0.273 ± 0.021
Alanine	0.044 ± 0.003	0.065 ± 0.007	0.172 ± 0.013	0.051 ± 0.005
Pyruvate	0.064 ± 0.006	0.128 ± 0.011	0.122 ± 0.008	0.117 ± 0.011
Lactate	0.075 ± 0.006	0.161 ± 0.010	−0.314 ± 0.048	−0.403 ± 0.030

[a] All values are the means of duplicate determinations on duplicate cultures ± SD.

production. While alanine conversion to glucose was not stimulated, the amino acid's metabolism to lactate was inhibited. Glucagon was found to be effective in stimulating glucose production from fructose.

The rate at which the hepatocyte monolayer cultures consumed lactate was found to be linear with time to 120 min (Fig. 1) and is quantitatively similar to that reported by others (12, 14). The fact that there was no latent period and consumption continued for 120 min indicates the cultures were biocheimcally viable and readily responded to exogenous lactate. The rate at which lactate was consumed was found to be proportional to the initial concentration of lactate between 1 to 8 mM (Fig. 2). Above 8 mM consumption was maximal and unchanged. All experiments on the effects of the various parameters on lactate consumption were done at an initial lactate concentration between 8 and 10 mM.

As a function of pH, under conditions of constant P_{O_2} and P_{CO_2}, the utilization of lactate was maximal above pH 7.0 (Fig. 3). In a separate experiment, not shown, lactate consumption was uneffected to a pH of 7.7. Below 7.0 (Fig. 3), the utilization of lactate was increasingly inhibited with decreasing pH. At a pH of 6.2, the low level of lactate consumption that did occur was found to be a linear function with time (data

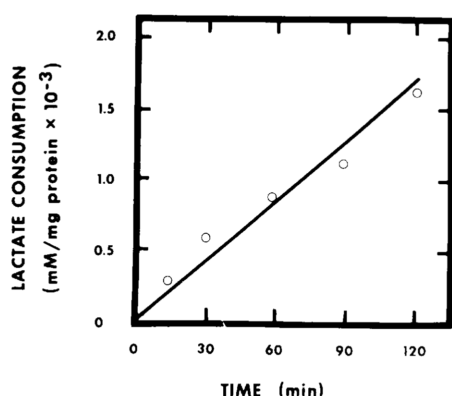

FIG. 1. The consumption of 10 mM lactate with time by hepatocyte monolayer cultures under standard conditions of 95% O_2, 5% CO_2, and pH 7.4. Each point is the mean of duplicate determinations on duplicate cultures.

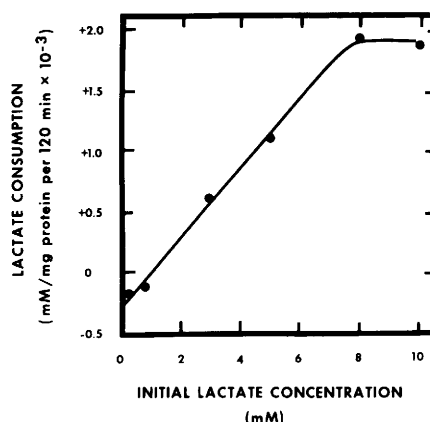

FIG. 2. The consumption of lactate versus the initial medium concentration of lactate by hepatocyte monolayer cultures under standard conditions of 95% O_2, 5% CO_2, and pH 7.4. Each point is the mean of duplicate determinations on duplicate cultures.

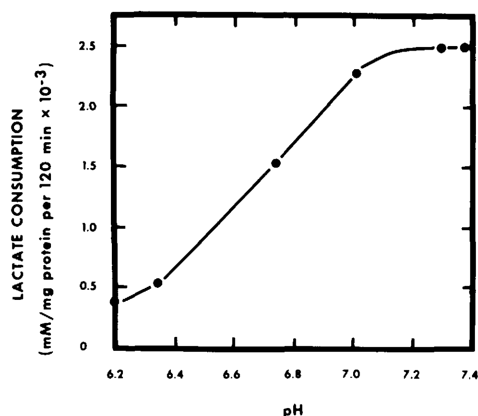

FIG. 3. The consumption of 10 m*M* lactate versus pH by hepatocyte monolayer cultures under standard conditions of 95% O_2, 5% CO_2, and pH 7.4. Each point is the mean of duplicate determinations on duplicate cultures.

not shown) and not due to a delayed impairment of the cells to use lactate. The pH of the medium remained unchanged during the course of the experiment.

At a pH of 7.4, and keeping the P_{CO_2} within a range of 22–35 mm Hg, lowering the P_{O_2} from >280 mm Hg (obtained in a 95% O_2–5% CO_2 atmosphere) to 120 mm Hg had little effect on lactate utilization (Fig. 4). Below 120 mm Hg, there was a progressive decrease in lactate consumption until no lactate was consumed.

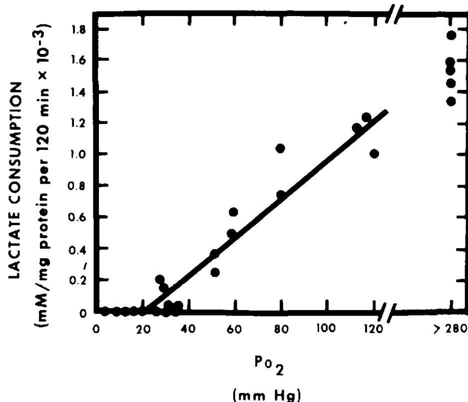

FIG. 4. The consumption of 10 m*M* lactate versus medium P_{O_2} by hepatocyte monolayer cultures at pH 7.4. The P_{CO_2} remained between 22–35 mm Hg. Each point is the mean of duplicate determinations on single cultures.

TABLE II. EFFECT OF P_{CO_2} ON LACTATE CONSUMPTION

P_{CO_2} (mm Hg)	pH	Lactate consumption[a] (mmole/mg protein/120 min) × 10^{-3}
4.5	7.5	1.13 ± 0.08
17.2	7.5	1.11 ± 0.10
27.2	ND[b]	1.11 ± 0.09
52.9	ND	1.12 ± 0.09
67.5	ND	1.04 ± 0.10
72.4	7.2	1.02 ± 0.08
96.0	ND	1.00 ± 0.10
103.1	7.1	0.87 ± 0.08

[a] All values are the means of duplicate determinations on duplicate cultures ± SD.
[b] Not determined.

We wished to see if the inhibition of lactate consumption by acidosis and anoxia was reversible. In separate experiments (Table III), cultures were held for 30 min under standard conditions except for lowering either the pH, P_{O_2}, or both. Conditions were then restored to normal pH and P_{O_2} and lactate consumption measured. None of the conditions of pretreatment were found to significantly impair the ability of hepatocytes to consume lactate when the cultures were returned to normal pH and P_{O_2}.

Discussion. The establishment of the hepatocytes in monolayer culture resulted in a uniform, highly viable cell system that was easily manipulated. While in general primary cultures tend to lose their tissue-specific characteristics as a function of both the conditions and time in culture, the pattern of substrate utilization and hormonal response seen in Table I shows that these cultures maintain hepatic functions consistent with that reported with other systems (8, 12–14). This is particularly true in regard to their inability to produce lactate in the presence of glucose, yet to utilize lactate to produce glucose, all in a simple, well-defined medium.

The observation that low pH inhibits hepatic gluconeogenesis was initially made with perfused liver by Hems *et al.* (4), who reported a decrease in glucose production in the presence of lactate. Again using perfused livers, other (5–7) extended the study to show the decrease in glucose production

TABLE III. THE REVERSIBILITY OF ANOXIA AND LOW pH INHIBITION OF LACTATE CONSUMPTION

Pretreatment (30 min)		No. of	Lactate consumption[a] at pH 7.3, P_{O_2} > 280 mm Hg
pH	P_{O_2} mm Hg	cultures	(mmole/mg protein/90 min) × 10^{-3}
		Experiment 1	
7.4	>280	2	1.31 ± 0.09
6.2	>280	2	1.19 ± 0.11
		Experiment 2	
7.4	>280	3	1.60 ± 0.08
7.4	20	3	1.39 ± 0.11
		Experiment 3	
7.3	>280	4	1.38 ± 0.10
6.2	42	2	1.15 ± 0.09
6.2	25	2	1.01 ± 0.11
6.2	15	2	1.10 ± 0.10

[a] All values are the means of duplicate determinations on the indicated number of cultures ± SD.

was associated with a decrease in lactate uptake. Analysis of intermediate metabolites suggested the inhibition of lactate consumption was before phosphoenolpyruvate, and it was speculated that this site was at pyruvate carboxylase (6). These studies, however, using perfused liver, involved a complex aggregate of cell types and a system that could not be readily manipulated.

Using isolated hepatocytes in suspension culture, Rognstad (15) has recently reported that acidosis inhibited glucose production from lactate. Our data confirm in hepatocytes monolayer culture, that increased hydrogen ion concentrations inhibit lactate consumption. This effect is a steady-state response in that it was not found to be due to a latent period or an early inhibition of lactate utilization. In addition, it could not be attributed to death of the cells since the inhibition was reversible by adjusting the pH to 7.3 (Table III).

These results are consistent with the pH effect being solely due to the hydrogen ion concentration. Iles *et al.* (7), using perfused liver, have interpreted some of their data as indicating an involvement of CO_2 at 3 m*M* lactate under conditions simulating respiratory acidosis where the pH was allowed to decrease. As seen in Table II, we found no evidence for an effect of P_{CO_2} when pH was held constant.

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