Effect of CO_2 concentration on phospholipid metabolism in the isolated perfused rat lung

William J. Longmore, Carolyn M. Niethe, Deborah J. Sprinkle, and Rodolfo I. Godinez

Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104

Abstract Studies have been carried out on the incorporation of [U-^{14}C]glucose, [2-^{14}C]pyruvate, [2-^{14}C]acetate, and [1-^{14}C]-palmitate into the phospholipids of the isolated perfused rat lung in the presence of either 6 or 45 mM total CO_2 concentration in the perfusion medium. Incorporation of [U-^{14}C]glucose into total phospholipid and into the phosphatidylcholine fraction was increased 19–53% over the 2-hr perfusion period in lungs perfused with medium containing 45 as compared with 6 mM CO_2. The incorporation of [2-^{14}C]acetate, [2-^{14}C]-pyruvate, and [1-^{14}C]palmitate was not affected by the change in medium CO_2 concentration. Increased incorporation of [U-^{14}C]glucose combined with a shift toward greater incorporation into the fatty acids of the phosphatidylcholine fraction produced a maximum increase of 90% in [U-^{14}C]glucose incorporation into the fatty acids of phosphatidylcholine after 2 hr of perfusion in the presence of medium containing 45 mM CO_2 as compared with 6 mM CO_2. The increase in medium CO_2 concentration produced as much as a 150% increase in [U-^{14}C]glucose incorporation into palmitate derived from the phosphatidylcholine fraction.

The results provide evidence that glucose functions as an important precursor of palmitate in the phosphatidylcholine fraction of lung phospholipids and that the CO_2 concentration of the perfusion medium affects the incorporation of glucose into palmitate.

Supplementary key words [U-^{14}C]glucose · [2-^{14}C]pyruvate · [1-^{14}C]palmitate · [1-^{14}C]acetate · phosphatidylcholine

Although lipid metabolism in the lung, and in particular that of dipalmitoyl glycerophosphorylcholine as a major component of the surface-active material (1–3), is directly associated with lung function, relatively little is known regarding the regulation of lipid metabolism in the lung. It has been established that glucose, acetate, and palmitate are incorporated into lung phospholipids in rabbit lung slices (4–6) and in the isolated perfused rat lung (7). The rate of incorporation of exogenous palmitate into lung phospholipids has been found to be dependent upon the palmitate concentration of the medium (6, 7), and its incorporation is decreased by the presence of oleate (7).

In studies of lipid metabolism in rat liver, it has been determined that increasing the concentration of total CO_2 within physiological limits at pH 7.4 increases markedly the incorporation of acetate and pyruvate into fatty acids of both triglycerides and phospholipids (8–10). Observed increases in citrate concentration with increased CO_2 concentration in rat kidney (11), diaphragm (12), and liver (13), and the inhibition of bovine liver aconitase (14) by CO_2, have suggested that the altered citrate concentration may then regulate fatty acid synthesis through its effect as an activator of acetyl CoA carboxylase activity (15) and as a source of acetyl CoA for extramitochondrial fatty acid synthesis.

In order to establish whether phospholipid metabolism in the lung is affected by changes in CO_2 concentration, as it is in the liver, studies were carried out on the effect of CO_2 concentration in the range of 6–45 mM on the incorporation of glucose, palmitate, pyruvate, and acetate into the phospholipids of the isolated perfused rat lung.

EXPERIMENTAL PROCEDURE

Male rats of Wistar origin weighing between 200 and 275 g were used. They were fed commercial laboratory chow ad lib. Preparation of the lungs for perfusion, perfusate preparation, and procedures of the perfusion have been previously reported (7). The perfusion medium was adjusted to contain approximately 6 or 45 mM total CO_2 by the addition of the appropriate ratio of $NaHCO_3$:NaCl to the medium so that equilibration with CO_2–O_2 gas mixtures in the range of 5–15% CO_2 (and 95–85% O_2) gave initial pH values of 7.35–7.40.

The procedures concerning lung and medium sample preparation, pH measurement, analyses of glucose, phosphorus, and protein, lipid extraction, fractionation and designation of phospholipid fractions, and the preparation, separation, quantitation, and trapping of fatty acid methyl esters by gas–liquid chromatography have been reported (7). Medium CO_2 was measured by the manometric method of Van Slyke and Neill (16), and pyruvate concentration was determined by enzymatic analysis (17). Mild hydrolysis of the phospholipid fractions and isolation of the resulting fatty acids and water-soluble phosphate esters (glycerophosphorylcholine) was carried out by the method of Dittmer and Wells (18). Because of the consistent quantitative recovery of the water-soluble phosphate esters and the variable quantitative recovery of the fatty acids from the hydrolysate, data obtained to determine the percentage of incorporation of radioactive precursors into these moieties were based upon the percentage incorporation into the water-soluble phosphate esters. The percentage incorporation into the fatty acid moiety of the phospholipids represents the difference between 100% and that found in the water-soluble phosphate esters.

The amounts of glucose, pyruvate, acetate, or palmitate incorporated into the phospholipid fraction were calculated from the specific activity of each in the medium and the pool size and specific activity of each phospholipid fraction at the time of sampling. All ^{14}C-labeled compounds were purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Glucose was present in the medium perfusing the isolated lungs at a normal concentration of approximately 5.6 mM initially in all experiments. The glucose concentration of the medium after 2 hr of perfusion did not decrease below an average of 5.0 mM. Medium total CO_2 concentrations were varied from 6 to 45 mM, approximating the extreme extracellular physiological range. No addition of acid or base to the medium was necessary during the perfusions to maintain a pH in the approximate range of 7.3–7.4, normal for the extracellular compartment of the lung.

Reported in these studies are results obtained for the incorporation of labeled substrates into the total lung phospholipid fraction and the phosphatidylcholine fraction. However, the fraction designated phosphatidylethanolamine (7) was followed throughout these studies, and incorporation into that phospholipid was approximately 10% or less of the incorporation into phosphatidylcholine. It was therefore not possible to obtain data on the phosphatidylethanolamine fraction that could be considered reliable.

Effect of CO_2 concentration on incorporation of [U-^{14}C]glucose into lung phospholipids

A series of experiments was performed to determine if the CO_2 concentration of the perfusion medium would alter the incorporation of [U-^{14}C]glucose into the phospholipids of the lung. The results, presented in Table 1, indicate that the incorporation of [U-^{14}C]glucose into both the total phospholipid fraction and the phosphatidylcholine fraction was increased approximately 20% at 40 min and 50% at 120 min in lungs perfused with medium containing 45 mM CO_2 compared with those perfused with medium containing 6 mM CO_2. A similar series of experiments was performed in which palmitate (0.14 mM) or acetate (4 mM) was added to the perfusion medium in order to determine whether the presence of either of these compounds would decrease glucose incorporation into the phospholipids or decrease the CO_2 effect on glucose incorporation. The data, also presented in Table 1, show that the presence of palmitate or acetate had essentially no effect in either case. Both the rate of incorporation and the increase in incorporation of [U-^{14}C]-glucose with increased CO_2 concentration of the medium were approximately the same as when only glucose was present in the medium.

Effect of CO_2 concentration on incorporation of [1-^{14}C]palmitate, [2-^{14}C]pyruvate, and [2-^{14}C]acetate into lung phospholipids

The incorporation of [1-^{14}C]palmitate (0.11 mM), [2-^{14}C]pyruvate (0.29 mM), and [2-^{14}C]acetate (4 mM) into the total phospholipid fraction and the phosphatidylcholine fraction of lungs perfused with medium containing approximately 5.6 mM glucose and either 6 or 45 mM total CO_2 is shown in Table 2. In no case did the incorporation of the radioactive substrate increase significantly in response to the increase in medium CO_2 concentration. This is in contrast to the results reported in similar studies in liver (8–10) in which incorporation of both pyruvate and acetate into phospholipid fatty acids was markedly increased by a similar increase in medium CO_2 concentration. Palmitate incorporation into phospholipid fatty acids of rat liver, however, was reported to be unaffected (19).

Effect of CO_2 on the incorporation of [U-^{14}C]glucose into the glycerophosphorylcholine and fatty acid moieties of the phosphatidylcholine fraction of lung phospholipids

The distribution of the incorporation of [U-^{14}C]-glucose into the glycerophosphorylcholine and fatty acid moieties of the phosphatidylcholine fraction of lung phospholipid was determined. The results obtained from lungs perfused with medium containing approximately 5.6 mM glucose in the presence or absence of

TABLE 1. Effect of CO_2 concentration on incorporation of [U-^{14}C]glucose into phospholipids of isolated perfused rat lung in the presence or absence of palmitate or acetate in the perfusion medium

Substrate	Perfusion Medium: pH, Time Elapsed (min) 0	pH, Time Elapsed (min) 120	Total CO_2, Time Elapsed (min) 0	Total CO_2, Time Elapsed (min) 120	Incorporation of [U-^{14}C]Glucose into: Total Phospholipids, Time Elapsed (min) 40	Total Phospholipids, Time Elapsed (min) 80	Total Phospholipids, Time Elapsed (min) 120	Phosphatidylcholine Fraction[a], Time Elapsed (min) 40	Phosphatidylcholine Fraction[a], Time Elapsed (min) 80	Phosphatidylcholine Fraction[a], Time Elapsed (min) 120
			mM			*μmoles/g protein*[b]			*μmoles/g protein*[b]	
Glucose (5.7 mM)	(A) 7.34	7.30	6.1	5.9	1.91 ± 0.15 (5)	4.13 ± 0.23 (5)	6.29 ± 0.32 (5)	1.81 ± 0.19 (5)	3.51 ± 0.36 (5)	5.03 ± 0.33 (5)
	(B) 7.39	7.36	45.4	45.9	2.28 ± 0.17 (5)	5.12 ± 0.39 (5)	8.82 ± 1.04 (5) ($P < 0.05$)[c]	2.22 ± 0.19 (5)	4.75 ± 0.30 (5)	7.72 ± 0.63 (5) ($P < 0.01$)
Glucose (5.4 mM) + palmitate (0.14 mM)	(A) 7.39	7.36	6.2	6.1	1.74 ± 0.38 (2)	3.29 ± 0.12 (2)	4.86 ± 0.17 (2)	1.57 ± 0.41 (2)	3.02 ± 0.20 (2)	4.70 ± 0.63 (2)
	(B) 7.40	7.36	45.9	46.7	1.77 ± 0.46 (2)	4.60 ± 0.31 (2)	7.28 ± 0.12 (2) ($P < 0.01$)	1.63 ± 0.40 (2)	4.19 ± 0.47 (2)	6.46 ± 0.42 (2)
Glucose (5.8 mM) + acetate (4 mM)	(A) 7.36	7.35	5.9	6.3	2.04 ± 0.34 (2)	4.16 ± 0.39 (2)		1.92 ± 0.21 (2)	3.78 ± 0.37 (2)	
	(B) 7.40	7.40	45.7	46.3	2.60 ± 0.27 (3)	5.53 ± 0.52 (3)		2.53 ± 0.25 (3)	5.21 ± 0.41 (3)	

[a] See Experimental Procedure for designation of fractions.
[b] Values are averages ± SE of number of experiments in parentheses.
[c] Probability that value in line A is not significantly different from that in line B.

TABLE 2. Effect of CO_2 concentration on incorporation of [1-^{14}C]palmitate, [2-^{14}C]pyruvate, and [2-^{14}C]acetate into phospholipids of isolated perfused rat lung

Substrate	Perfusion Medium: pH, Time Elapsed (min) 0	pH, Time Elapsed (min) 120	Total CO_2, Time Elapsed (min) 0	Total CO_2, Time Elapsed (min) 120	Incorporation of Radioactive Precursor into: Total Phospholipids, Time Elapsed (min) 40	Total Phospholipids, Time Elapsed (min) 80	Total Phospholipids, Time Elapsed (min) 120	Phosphatidylcholine Fraction[a], Time Elapsed (min) 40	Phosphatidylcholine Fraction[a], Time Elapsed (min) 80	Phosphatidylcholine Fraction[a], Time Elapsed (min) 120
			mM			*μmoles/g protein*[b]			*μmoles/g protein*[b]	
[1-^{14}C]Palmitate (0.11 mM) + glucose (5.13 mM)	(A) 7.36	7.28	5.6	5.4	3.11 ± 0.16 (2)	5.23 ± 0.61 (2)	8.49 ± 0.79 (2)	2.98 ± 0.11 (2)	5.10 ± 0.65 (2)	8.27 ± 0.80 (2)
	(B) 7.39	7.36	45.2	45.4	2.72 ± 0.38 (2)	5.61 ± 0.95 (2)	6.92 ± 0.52 (2)	2.69 ± 0.20 (2)	5.26 ± 0.78 (2)	6.65 ± 0.96 (2)
[2-^{14}C]Pyruvate (0.29 mM) + glucose (5.42 mM)	(A) 7.35	7.35	6.2	5.9	1.61 ± 0.32 (2)	4.55 ± 0.68 (2)	6.48 ± 0.56 (2)	1.83 ± 0.14 (2)	4.37 ± 0.68 (2)	6.36 ± 0.50 (2)
	(B) 7.33	7.35	45.7	46.1	1.85 ± 0.44 (3)	3.60 ± 1.02 (3)	5.16 ± 0.82 (3)	1.79 ± 0.43 (3)	3.47 ± 0.98 (3)	4.96 ± 0.83 (3)
[2-^{14}C]Acetate (4 mM) + glucose (5.60 mM)	(A) 7.41	7.38	10.9	10.5	6.53 ± 0.64 (4)	11.22 ± 0.62 (4)	18.36 ± 1.25 (4)	5.51 ± 0.54 (4)	8.74 ± 1.19 (4)	12.84 ± 1.50 (4)
	(B) 7.42	7.40	42.8	43.4	5.93 ± 0.23 (4)	10.60 ± 0.15 (4)	18.51 ± 1.88 (4)	4.56 ± 0.65 (4)	8.15 ± 0.87 (4)	13.48 ± 1.33 (4)

[a] See Experimental Procedure for designation of fraction.
[b] Values are averages ± SE of number of experiments in parentheses.

TABLE 3. Incorporation of [U-^{14}C]glucose into glycerophosphorylcholine and fatty acids of the phosphatidylcholine fraction of isolated perfused rat lungs; effect of perfusate medium CO_2 concentration in the presence and absence of palmitate or acetate

Substrate	Perfusate Medium: Total CO_2 Time Elapsed (min) 0	120	Radioactivity Found in: Glycerophosphorylcholine Time Elapsed (min) 40	80	120	Fatty Acids[a] Time Elapsed (min) 40	80	120	Phosphatidylcholine Fraction Time Elapsed (min) 40	80	120	Incorporation of [U-^{14}C]Glucose into: Glycerophosphorylcholine Time Elapsed (min) 40	80	120	Fatty Acids Time Elapsed (min) 40	80	120
	mM		%			%			μmoles/g protein			μmoles/g protein			μmoles/g protein		
Glucose (5.7 mM)[b]	(A) 6.1	5.9	48.4[c] ± 2.5	43.9 ± 2.3	41.5 ± 3.4	51.6	56.1	58.5	1.64	3.27	4.81	0.79	1.45	2.03	0.85	1.81	2.78
	(B) 45.4	45.9	37.6 ± 1.6	32.6 ± 0.6	31.9 ± 1.2	62.4	67.4	68.1	2.21	4.66	7.72	0.83	1.51	2.46	1.38	3.15	5.27
% Increase, line A to line B												+5.1	+4.1	+21.2	+62.4	+74.0	+89.6
Glucose (5.4 mM) + palmitate (0.14 mM)[d]	(A) 6.2	6.1	48.4 ± 2.4	47.9 ± 0.4	49.2 ± 1.0	51.6	52.1	50.8	1.57	3.02	4.70	0.76	1.45	2.31	0.81	1.57	2.39
	(B) 45.9	46.7	35.1 ± 1.4	33.8 ± 0.3	33.7 ± 0.1	64.9	66.2	66.3	1.63	4.19	6.46	0.57	1.41	2.18	1.06	2.78	4.28
% Increase, line A to line B												−24.0	−2.8	−6.0	+30.9	+75.9	+79.1
Glucose (5.8 mM) + acetate (4 mM)[e]	(A) 6.1	6.4	36.3		35.5	63.7	64.5		1.62	3.25		0.59	1.15		1.03	2.10	
	(B) 43.5	43.7	28.0		25.9	72.0	74.1		2.01	4.87		0.56	1.26		1.45	3.61	
% Increase, line A to line B												−5.0	+9.6		+40.8	+71.9	

Average pH of perfusion medium was 7.37.
[a] % Fatty acids = total radioactivity − radioactivity found in glycerophosphorylcholine × 100/total radioactivity (see Experimental Procedure). [b] Averages of four experiments. [c] Average ± SE. [d] Averages of two experiments. [e] One experiment only.

palmitate (0.14 mM) or acetate (4 mM) and either 6 or 44 mM CO_2 are presented in Table 3. It is observed that the incorporation of glucose into the fatty acid moiety of phosphatidylcholine is more than 50% of the total incorporation into phosphatidylcholine under all conditions studied. The percentage of [U-^{14}C]glucose incorporation into the fatty acid moiety of the phospholipids in each case was greater when the lungs were perfused with medium containing 44 mM CO_2 as compared with 6 mM CO_2. Thus, increasing the CO_2 concentration of the medium not only increased the incorporation of [U-^{14}C]-glucose into lung phospholipids (Table 1) but also increased the percentage of [U-^{14}C]glucose incorporated into the fatty acid moiety. The combined effect of the increase in medium CO_2 concentration on the incorporation of [U-^{14}C]glucose may be seen in Table 3 when the actual incorporation of [U-^{14}C]glucose into the glycerophosphorylcholine and fatty acid moieties is calculated by multiplication of the percentage distribution by the incorporation of [U-^{14}C]glucose into the phosphatidylcholine fraction. When glucose alone was present in the medium, [U-^{14}C]glucose incorporated into glycerophosphorylcholine was increased only 5 and 4% after perfusion for 40 and 80 minutes, respectively, and 21% after perfusion for 120 minutes. However, incorporation into the fatty acid moiety was increased progressively over the 2-hr perfusion from 62 to 90%.

The presence of either palmitate (0.14 mM) or acetate (4 mM) in the medium did not alter markedly the effect of CO_2 concentration on the incorporation of [U-^{14}C]-glucose into the glycerophosphorylcholine or fatty acid moieties. The incorporation of [U-^{14}C]glucose into glycerophosphorylcholine was either decreased or not markedly affected and incorporation into the fatty acid moiety increased progressively with time from 31 to 79% by the presence of increased medium CO_2 concentration (6 to 44 mM) when either palmitate or acetate was present.

Similar procedures carried out on phosphatidylcholine isolated from lungs perfused with medium containing [1-^{14}C]palmitate, [2-^{14}C]pyruvate, or [2-^{14}C]-acetate indicated that essentially 100% of the radioactivity incorporated from each of the precursors into the phosphatidylcholine fraction appeared in the fatty acid moiety.

Effect of CO_2 concentration on the incorporation of [U-^{14}C]glucose, [1-^{14}C]palmitate, [2-^{14}C]pyruvate, and [2-^{14}C]acetate into palmitic acid isolated from the phosphatidylcholine fraction of lung phospholipids

Gas–liquid chromatography was performed on fatty acid methyl esters prepared after the mild alkaline hydrolysis of the phosphatidylcholine fraction of lungs perfused

TABLE 4. Effect of CO_2 concentration on incorporation of [U-^{14}C]glucose, [1-^{14}C]palmitate, [2-^{14}C]pyruvate, and [2-^{14}C]acetate into palmitic acid of the phosphatidylcholine fraction of isolated perfused rat lung

Perfusion Medium			Specific Activity of Methyl Palmitate		
	Total CO_2				
	Time Elapsed (min)		Time Elapsed (min)		
Substrate	0	120	40	80	120
	mM			*dpm/nmole*	
[U-^{14}C]Glucose (5.5 mM, 1.75 × 10^5	(A) 6.0	6.0	1.80	2.64	4.89
dpm/μmole)[a]	(B) 45.9	45.9	2.70	6.59	10.14
% Change, line A to line B			+49.9	+149.4	+107.5
[U-^{14}C]Glucose (5.4 mM, 1.7 × 10^5	(A) 6.2	6.1	1.11	2.06	3.21
dpm/μmole) + palmitate (0.14 mM)[a]	(B) 45.9	46.7	2.80	5.22	7.63
% Change, line A to line B			+153.1	+153.5	+137.8
[U-^{14}C]Glucose (5.8 mM, 1.9 × 10^5	(A) 5.9	6.3	1.86	2.89	
dpm/μmole) + acetate (4 mM)[b]	(B) 45.7	46.3	2.39	6.19	
% Change, line A to line B			+28.4	+114.2	
[1-^{14}C]Palmitate (0.11 mM, 6.8 × 10^5	(A) 5.6	5.4	22.63	48.45	74.05
dpm/μmole)[b]	(B) 45.2	45.4	26.49	49.89	61.86
% Change, line A to line B			+17.1	+3.0	−16.5
[2-^{14}C]Pyruvate (0.29 mM, 5.3 × 10^5	(A) 6.2	5.9	12.19	25.04	33.50
dpm/μmole)[b]	(B) 45.7	46.1	14.32	33.23	44.04
% Change, line A to line B			+17.4	+32.7	+31.5
[2-^{14}C]Acetate (4 mM, 1.3 × 10^5	(A) 15.3	14.5	9.00	17.31	27.28
dpm/μmole)[b]	(B) 38.7	40.7	10.43	18.68	28.45
% Change, line A to line B			+15.9	+7.9	+4.3

Average pH of perfusion medium was 7.37.
[a] Averages of two experiments.
[b] One experiment only.

with medium containing 6 or 45 mM total CO_2. An effluent stream splitter was used so that the radioactive fatty acid methyl esters could be trapped and the specific activities determined. Methyl palmitate was trapped separately whereas all fatty acid methyl esters with retention times greater than methyl palmitate were trapped together. This procedure was established after it was determined that more than 90% of the radioactivity in the fatty acids obtained from the phosphatidylcholine fraction was in palmitate. Palmitate represented approximately 59%, on a molar basis, of the fatty acids present. Insufficient radioactivity was present in the other fatty acids to provide accurate data on their specific activities. The specific activities of methyl palmitate calculated at the lower and higher medium CO_2 concentrations were corrected for any difference in the specific activity of the radioactive precursor in the media perfusing the lungs.

The results of this study appear in Table 4. Regardless of whether palmitate (0.14 mM) or acetate (4 mM) was present in the medium, the specific activity of labeled methyl palmitate in the phosphatidylcholine fraction derived from [U-^{14}C]glucose (5.6 mM) was increased 28 to 154% over the 2-hr perfusion. Although the percentage increase differed and was not progressive with time of perfusion (assumed to be largely due to the accumulation of errors involved with trapping and mass determination of methyl palmitate), it is seen that with increased medium CO_2 concentration there was no increase in the specific activity of methyl palmitate derived from [1-^{14}C]-palmitate (0.11 mM), [2-^{14}C]pyruvate (0.29 mM), or [2-^{14}C]acetate (4 mM) in the presence of glucose (5.6 mM).

DISCUSSION

It has been established that [1-^{14}C]palmitate is readily incorporated into both lung tissue and alveolar dipalmitoyl glycerophosphorylcholine (20, 21), and that lung tissue dipalmitoyl glycerophosphorylcholine is the source of the dipalmitoyl glycerophosphorylcholine found in the alveoli (21). The production of this phospholipid by the lung as a major component of the surface-active material necessary for pulmonary function has been established (1–3).

The relative importance of de novo synthesis of fatty acids within the lung as compared with the incorporation of exogenous fatty acids followed by elongation within the lung in providing fatty acids for lung phospholipid synthesis has not been established (5, 22). It has been reported that [1-^{14}C]palmitate served as a better precursor of phosphatidylcholine in lung than did [2-^{14}C]-acetate (7). It is observed from the present results that glucose may be a major source of carbon atoms for either de novo synthesis or elongation of fatty acids in the lung. The design of these studies does not permit direct judgment as to which means of synthesis is predominant. There are recent reports that both systems of synthesis are present in lung (23, 24) and that the importance of one

over the other is dependent upon the available precursors (24).

The mechanism by which the increase in CO_2 concentration produced the increase in [U-^{14}C]glucose incorporation into the fatty acids of phosphatidylcholine of the lung is not clear. Unlike the observed effects of CO_2 on liver phospholipid metabolism (8–10), increased CO_2 concentration did not markedly increase acetate or pyruvate incorporation into the fatty acids of lung phospholipids. However, as was found in the studies on the effect of CO_2 concentration on lipid metabolism in liver (19), CO_2 concentration did not alter the incorporation of [1-^{14}C]palmitate into phospholipids. A lack of competition between acetate and glucose for fatty acid synthesis, a positive effect of CO_2 concentration on glucose incorporation into fatty acid, and a lack of effect of CO_2 concentration on acetate and pyruvate incorporation into fatty acid have been observed in these studies. A possible explanation of these findings could be that in each case the precursor (glucose or pyruvate and acetate) is predominantly being incorporated by only one mechanism of fatty acid synthesis, de novo synthesis or elongation. This explanation would require that the increase in fatty acid synthesis due to a rise in CO_2 concentration would affect either de novo synthesis or elongation but not both. In addition, separate acetyl CoA pools, derived from glucose in one case and acetate and pyruvate in the other, would seem necessary. Postulation and support for the existence of two acetyl CoA pools within the mitochondrion has been presented (25, 26).

Results obtained from studies on the effect of CO_2 concentration on the incorporation of [1-^{14}C]acetate into phospholipid fatty acids in rat liver suggest that the CO_2 effect is upon de novo synthesis.[1] In these studies, rat liver slices were incubated 90 min in medium containing either 10 or 40 mM bicarbonate at pH 7.40. [1-^{14}C]-Acetate incorporation into the major fatty acids of the phospholipids was then determined. The specific activity of the isolated palmitate was 11.9 times greater from the tissue incubated in the medium containing 40 mM bicarbonate than from tissue incubated in the medium containing 10 mM bicarbonate. The specific activities of palmitoleate, stearate, oleate, and arachidate isolated from the phospholipids were 4.2, 3.1, 2.0, and 1.7 times greater, respectively, from tissue incubated in the medium containing 40 mM as compared with the 10 mM bicarbonate-containing medium. Thus, the simplest explanation of those findings is that palmitate, derived mainly from de novo fatty acid synthesis, which is affected by CO_2 concentration, is then elongated and/or desaturated to form other fatty acids. Based upon this evidence, glucose would be utilized for the de novo synthesis of lung phospholipid fatty acids but not as a source of acetyl CoA for fatty acid elongation. This conclusion would imply the possible existence of two separate pools of acetyl CoA.

The mechanism by which CO_2 concentration may affect de novo fatty acid synthesis has been presented (9, 13). A preliminary report of the related effect of CO_2 on citrate metabolism in isolated rat liver mitochondria has appeared (27). Initial attempts to demonstrate an increase in the citrate concentration of rat lung mitochondria in response to an increase in medium CO_2 concentration, as was demonstrated in rat liver mitochondria, were unsuccessful (9).

The physiological importance of the effect of CO_2 in producing an increase in the incorporation of glucose into the fatty acids of phosphatidylcholine is unclear at this time. However, the significance of a marked change in lipid metabolism in the lung associated with a change in extracellular CO_2 concentration seems of importance considering the function of the lung as the regulator of CO_2 concentration for the animal. One report of CO_2-induced hyaline membrane disease has appeared (28).

This investigation was supported in part by grants HE-13405 and HE-05672 from the U.S. Public Health Service and grants from the Missouri Heart Association and the National Cystic Fibrosis Research Foundation.

Dr. Longmore is a Research Career Development Awardee, HE-11,113, of the U.S. Public Health Service.

Manuscript received 20 June 1972; accepted 30 October 1972.

REFERENCES

1. Pattle, R. E., and L. C. Thomas. 1961. Lipoprotein composition of the film lining the lung. *Nature*. **189:** 844.
2. Buckingham, S., and M. E. Avery. 1962. Time of appearance of lung surfactant in the foetal mouse. *Nature*. **193:** 688–689.
3. Frosolono, M. F., B. L. Charms, R. Pawlowski, and S. Slivka. 1970. Isolation, characterization, and surface chemistry of a surface-active fraction from dog lung. *J. Lipid Res.* **11:** 439–457.
4. Felts, J. M. 1965. Carbohydrate and lipid metabolism of lung tissue *in vitro*. *Med. Thorac.* **22:** 89–99.
5. Chida, N., and F. H. Adams. 1967. Incorporation of acetate into fatty acids and lecithin by lung slices from fetal and newborn lambs. *J. Lipid Res.* **8:** 335–341.
6. Wolfe, B. M. J., B. Anhalt, J. C. Beck, and D. Rubinstein. 1970. Lipid metabolism in rabbit lungs. *Can. J. Biochem.* **48:** 170–177.
7. Godinez, R. I., and W. J. Longmore. 1973. Use of the isolated perfused rat lung in studies on lung lipid metabolism. *J. Lipid. Res.* **14:** 138–144.
8. Longmore, W. J., A. B. Hastings, E. Harrison, and H. H. Liem. 1967. Effect of CO_2 and cations on fatty acid and cholesterol synthesis by liver *in vitro*. *Amer. J. Physiol.* **212:** 221–227.

[1] Longmore, W. J., A. B. Hastings, and E. Harrison. Unpublished results.

9. Longmore, W. J., A. M. Liang, and M. L. McDaniel. Symposium on CO_2 and metabolic regulation. Monaco, July 1971. Springer-Verlag, New York. In press.
10. Longmore, W. J. 1968. The effect of variation of CO_2 concentration on pyruvate metabolism *in vitro*. *Biochemistry*. **7:** 3227–3231.
11. Simpson, D. P. 1967. Regulation of renal citrate metabolism by bicarbonate ion and pH: observations in tissue slices and mitochondria. *J. Clin. Invest.* **46:** 225–238.
12. Adler, S. 1970. The role of pH, P_{CO_2}, and bicarbonate in regulating rat diaphragm citrate content. *J. Clin. Invest.* **49:** 1647–1655.
13. McDaniel, M. L., A. M. Liang, and W. J. Longmore. 1971. Effect of CO_2 concentration on intermediates of the Embden-Myerhof pathway and the citric acid cycle in isolated perfused rat liver. *J. Biol. Chem.* **246:** 4813–4817.
14. McDaniel, M. L., and W. J. Longmore. 1971. Activation and inhibition of the activities of bovine liver aconitase. *J. Biol. Chem.* **246:** 4818–4824.
15. Numa, S., W. M. Bortz, and F. Lynen. 1965. Regulation of fatty acid synthesis at the acetyl-CoA carboxylation step. *Advan. Enzyme Regul.* **3:** 407–423.
16. Van Slyke, D. D., and J. M. Neill. 1924. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J. Biol. Chem.* **61:** 523–573.
17. Czok, R., and L. Eckert. 1963. Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, New York. 224.
18. Dittmer, J. C., and M. A. Wells. 1969. Quantitative and qualitative analysis of lipids and lipid components. *Methods Enzymol.* **14:** 482–530.
19. Longmore, W. J. 1966. Effect of physiological variations in pH and CO_2 concentrations on metabolism in vitro. *Federation Proc.* **25:** 887–892.
20. Scarpelli, E. M. 1968. Lung surfactant: dynamic properties, metabolic pathways, and possible significance in the pathogenesis of the respiratory distress syndrome. *Bull. N.Y. Acad. Med.* **44:** 431–445.
21. Thomas, T., Jr., and R. A. Rhoades. 1970. Incorporation of palmitate-1-^{14}C into lung tissue and "alveolar" lecithin. *Amer. J. Physiol.* **219:** 1535–1538.
22. Tombropoulos, E. G. 1967. Synthesis of long-chain fatty acids by the mitochondrial fraction of lung tissue. *Progr. Biochem. Pharmacol.* **3:** 32–39.
23. Schiller, H., and K. Bensch. 1971. De novo fatty acid synthesis and elongation of fatty acids by subcellular fractions of lung. *J. Lipid Res.* **12:** 248–255.
24. Rhoades, R. A., L. N. Gasenheimer, R. W. Scholz, and E. R. Buskirk. 1972. Biosynthesis of phospholipid fatty acids by rat lung. *Federation Proc.* **31:** 335. (Abstr.)
25. Fritz, I. B. 1967. An hypothesis concerning the role of carnitine in the control of interrelations between fatty acid and carbohydrate metabolism. *Perspect. Biol. Med.* **10:** 643–677.
26. McGarry, J. D., and D. W. Foster. 1972. Regulation of ketogenesis and clinical aspects of the ketotic state. *Metab. Clin. Exp.* **21:** 471–489.
27. Liang, A. M., and W. J. Longmore. 1971. Effect of CO_2 on citrate metabolism in isolated rat liver mitochondria. *Federation Proc.* **30:** 1060. (Abstr.)
28. Schaefer, K. E., M. E. Avery, and K. Bensch. 1964. Time course of changes in surface tension and morphology of alveolar epithelial cells in CO_2-induced hyaline membrane disease. *J. Clin. Invest.* **43:** 2080–2093.