Role of Pyruvate Transporter in the Regulation of the Pyruvate Dehydrogenase Multienzyme Complex in Perfused Rat Liver†

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ABSTRACT: Metabolic substrates such as octanoate, β-hydroxybutyrate, and α-ketoisocaproate which produce acetoacetate stimulate the rate of pyruvate decarboxylation in perfused livers from fed rats at perfusate pyruvate concentrations in the physiological range (below 0.2 mM). A quantitative relationship between pyruvate oxidation ($^{14}CO_2$ production from [1-^{14}C]pyruvate) and ketogenesis (production of acetoacetate or total ketone bodies) was observed with all ketogenic substrates when studied over a wide range of concentrations. The ratio of extra pyruvate decarboxylated to extra acetoacetate produced was >1 with octanoate and α-ketoisocaproate, but it was <1 with β-hydroxybutyrate. The stimulatory effect of β-hydroxybutyrate on pyruvate decarboxylation was abolished completely in the presence of 0.1 mM α-cyanocinnamate, an inhibitor of the pyruvate transporting system in the mitochondrial membrane. The data suggest that the mechanism by which the flux through the pyruvate dehydrogenase reaction is stimulated in liver under ketogenic conditions involves an acceleration of the net rate of pyruvate transport into the mitochondria compartment due to an exchange with acetoacetate and/or acetoacetate plus β-hydroxybutyrate.

Initiation of fatty acid oxidation in various tissue preparations causes an inhibition of pyruvate oxidation (Garland & Randle, 1964; Bremer, 1969) and an inactivation of the pyruvate dehydrogenase complex (Portenhauser & Wieland, 1972; Taylor et al., 1973, 1975; Wieland & Portenhauser, 1974; Batenburg & Olson, 1976; Kerbey et al., 1976; Hansford, 1977; Olson et al., 1978). Mechanisms proposed for these phenomena have accentuated, first, the direct inhibitory effects of NADH, acetyl-CoA, and long chain length acyl-CoA on the active, dephospho, form of the enzyme complex (Garland & Randle, 1964; Bremer, 1969; Wieland et al., 1969; Tasi et al., 1973) and, second, the effects of various regulatory species on pyruvate dehydrogenase kinase and/or phosphatase (Linn et al., 1969a,b; Portenhauser & Wieland, 1972). Elevation of each of the mitochondrial NADH/NAD$^+$, acetyl-CoA/CoA-SH, and ATP/ADP ratios has been implicated as a primary effector of the inactivation of pyruvate dehydrogenase upon addition of fatty acids (Portenhauser & Wieland, 1972; Martin et al., 1972; Wieland & Portenhauser, 1974; Watajtys-Rode et al., 1974; Taylor et al., 1975; Pettit et al., 1975; Cooper et al., 1975; Batenburg & Olson, 1976; Kerbey et al., 1976; Hansford, 1976).

From the perspective of economy in a metabolic system, the inhibition of pyruvate oxidation by fatty acids is an attractive regulatory event, since the availability of fatty acids as substrates for energy generation would allow the "sparing" of pyruvate derived from lactate or amino acids for other metabolic fates, such as gluconeogenesis. In the perfused rat liver, however, this often reported phenomenon can be demonstrated only with high pyruvate concentrations. At concentrations in the physiological range, i.e., below 0.2 mM pyruvate, pyruvate decarboxylation is stimulated by medium and long chain length fatty acids as well as by β-hydroxybutyrate (Scholz et al., 1978; Dennis et al., 1978; Patel et al., 1982a,b). Since the addition of these substrates results in the production of acetoacetate at high rates and since acetoacetate is reported to be a counterion for the exchange of pyruvate across the mitochondrial membrane (Papa & Paradies, 1974; Halestrap, 1975, 1976; Halestrap & Denton, 1975; Pande & Parvin, 1978), we formulated the following hypothesis (Scholz et al., 1978). At low pyruvate concentrations and in the absence of an appropriate counterion for the transport of pyruvate, the intramitochondrial concentration of pyruvate is set at a level such that the flux through the pyruvate dehydrogenase reaction is maintained at a relatively low rate. Under conditions in which ketogenesis is stimulated, the efflux of acetoacetate from the mitochondrial compartment enhances the inward transport of pyruvate, results in an increase in the mitochondrial pyruvate concentration, and leads to an increased rate of pyruvate metabolism. Thus, we suggested that a site of interaction between fatty acid and pyruvate oxidation is at the level of the pyruvate transporting system.

In order to test this hypothesis for the fatty acid mediated enhancement of pyruvate oxidation at pyruvate concentrations in the physiological range (Scholz et al., 1978), we extended our initial studies to include α-ketoisocaproate, another ketogenic compound. Moreover, we varied the concentrations of octanoate, β-hydroxybutyrate, and α-ketoisocaproate over a wide range, in order to answer the question of whether or nor there is a direct correlation between rates of ketogenesis and pyruvate oxidation. The data support our conclusion that pyruvate oxidation can be stimulated under ketogenic conditions, by means of an accelerated $pyruvate_{cyt}/acetoacetate_{mit}$ exchange across the mitochondrial membrane.

Materials and Methods

[1-^{14}C]Pyruvate was purchased from Amersham Buchler Co., Braunschweig. All chemicals were of reagent grade and were purchased from Merck Co., Damstadt, and Carl Roth Co., Karlsruhe. All enzymes and coenzymes used in the metabolite determinations were products of Boehringer-Mannheim. α-Ketoisocaproate and α-ketoisovalerate were obtained from Sigma, Munich. α-Cyanocinnamate (2-cyano-3-phenylacrylic acid) was prepared from cyanoacetate and benzaldehyde (Lapworth & Baker, 1941).

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Table I: Effects of Octanoate Concentration on Metabolic Rates in Perfused Rat Liver[a]

time of perfusion (min)	octanoate concn (mM)	metabolic rates [μmol·h^{-1}·(g of liver wet wt)$^{-1}$] oxygen consumption	β-hydroxybutyrate production	acetoacetate production	$^{14}CO_2$ production ⟨pyruvate⟩	$^{14}CO_2$ production ⟨lactate⟩	rel specific radioactivity of venous lactate (arterial pyruvate = 1)
29–30	0	112 ± 7	1 ± 1	3 ± 1	4.1 ± 0.4	47 ± 5	0.092 ± 0.008
37–38	0.02	120 ± 6	3 ± 1	8 ± 1	5.4 ± 0.4	73 ± 7	0.078 ± 0.008
45–46	0.04	129 ± 7	6 ± 1	13 ± 2	5.6 ± 0.5	78 ± 9	0.077 ± 0.007
53–54	0.08	145 ± 7	13 ± 2	22 ± 2	5.8 ± 0.4	91 ± 8	0.067 ± 0.006
61–62	0.16	173 ± 8	33 ± 3	32 ± 3	6.3 ± 0.4	102 ± 5	0.063 ± 0.005
69–70	0.32	193 ± 9	57 ± 6	37 ± 3	5.9 ± 0.3	118 ± 7	0.052 ± 0.004
77–78	0.72	196 ± 9	52 ± 6	38 ± 3	5.1 ± 0.4	111 ± 6	0.047 ± 0.004
85–86	0	118 ± 6	3 ± 1	5 ± 1	3.7 ± 0.5	44 ± 4	0.082 ± 0.011

[a] Livers from fed rats were perfused with Krebs/Henseleit bicarbonate buffer containing 0.05 mM [1-^{14}C]pyruvate. Octanoate was infused with stepwise increasing concentrations (8-min intervals) from 30 to 78 min of perfusion. The perfusate was collected in 30-s fractions; samples were taken for metabolite analyses; the remainder was acidified, and CO_2 was trapped in phenylethylamine. The rates of $^{14}CO_2$ production from [1-^{14}C]pyruvate were calculated in that the radioactivity in CO_2 was divided by the specific radioactivity of either arterial pyruvate (⟨pyruvate⟩) or venous lactate (⟨lactate⟩). In the following tables and figures, the term "pyruvate decarboxylation" denotes $^{14}CO_2$ production from [1-^{14}C]pyruvate based on the specific radioactivity of lactate which was determined after fractionation of the effluent perfusate by anion-exchange chromatography. Triplicate analyses at the end of each 8-min interval, when a new steady state had been reached, were used for the calculation of the mean values (±SEM) from eitht experiments with identical protocol.

Male albino rats (Wistar strain; Thomae, Biberach; 180–220 g) received a standard laboratory chow diet (Altromin) and water ad libitum prior to the surgical removal of the liver under pentobarbital anesthesia. The isolated livers were perfused with Krebs/Henseleit bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, 37 °C, saturated with an oxygen/carbon dioxide mixture (95:5) in a nonrecirculating system (Scholz et al., 1973). Substrates were infused into the perfusion fluid prior to entry into the liver via the portal vein. The oxygen concentration in the effluent perfusate was monitored with a platinum electrode. Perfusate samples were collected at 1- or 2-min intervals and were analyzed for glucose, lactate, pyruvate, β-hydroxybutyrate, and acetoacetate by standard enzymatic procedures (Bergmeyer, 1974). In the presence of high α-ketoisocaproate concentrations (>1 mM), the enzymatic analyses of pyruvate and lactate were complicated due to the fact that this compound acts both as a substrate (Scholz et al., 1976) and as an inhibitor of lactate dehydrogenase (noncompetitive inhibition, K_i around 20 mM; Franz Zwiebel, unpublished observation). Metabolic rates were calculated from the arteriovenous differences of metabolite concentrations and the constant flow rate of the perfusate through the liver and were referenced to the wet weight of the liver.

The formation of $^{14}CO_2$ from [1-^{14}C]pyruvate was measured as described previously (Scholz et al., 1978). Lactate was isolated from the perfusate by anion-exchange chromatography in order to determine its specific radioactivity, which closely reflects the intracellular dilution of the infused pyruvate (Braun, 1976; Scholz et al., 1976). Recently it was shown by means of kinetic experiments that $^{14}CO_2$ produced from [1-^{14}C]pyruvate in the perfused liver is formed mainly due to pyruvate decarboxylation by pyruvate dehydrogenase (Braun, 1976; Scholz et al., 1976, 1978). The contribution of additional decarboxylation reactions was found to be less than 20%, and this percentage did not change in the presence of octanoate (Scholz et al., 1978). A quantitative evaluation of the flux rate through the pyruvate dehydrogenase reaction can be achieved by using the specific radioactivity of lactate released by the liver as a reference base. Thus, in the following experiments, the radioactivity in CO_2 was divided by the specific radioactivity of venous lactate and was used as a measure of the rate of pyruvate oxidation by pyruvate dehydrogenase. The rates were not corrected for the contribution of additional decarboxylation processes; the data, therefore, are approximate values, 10–20% higher than the real flux rates.

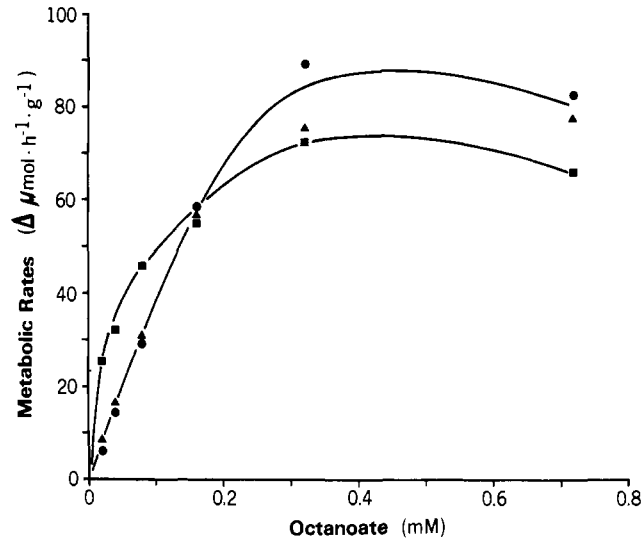

FIGURE 1: Increases in metabolic rates following infusion of octanoate. Mean values of the individual increases in each of the eight experiments which were summarized in Table I. The increases in the rates of β-hydroxybutyrate plus acetoacetate production (ketogenesis, ●), pyruvate decarboxylation (■), and oxygen consumption (▲) following infusion of octanoate were plotted vs. the concentration of octanoate.

Results

In order to document our contention that a direct relationship exists between rates of ketogenesis and pyruvate decarboxylation at low pyruvate concentrations (Scholz et al., 1978), experiments were designed to correlate these and other parameters in the isolated perfused liver. The experiments summarized in Table I and Figure 1 refine our previous observation on the effects of octanoate and appropriately depict the relationship between the rate of $^{14}CO_2$ production from [1-^{14}C]pyruvate at 0.05 mM pyruvate and the rates of ketogenesis and oxygen consumption. As the concentration of octanoate was increased sequentially from 0 to 0.7 mM, the rates of ketogenesis, oxygen consumption, and $^{14}CO_2$ production increased concomitantly. In Figure 2, the increase in either acetoacetate production or total ketone body production was plotted against the increase in pyruvate decarboxylation. The data suggest a quantitative relationship between these processes.

The metabolism of octanoate is complex, and interactions with the overall process of pyruvate metabolism could occur at various levels other than solely at the pyruvate$_{cyt}$/aceto-

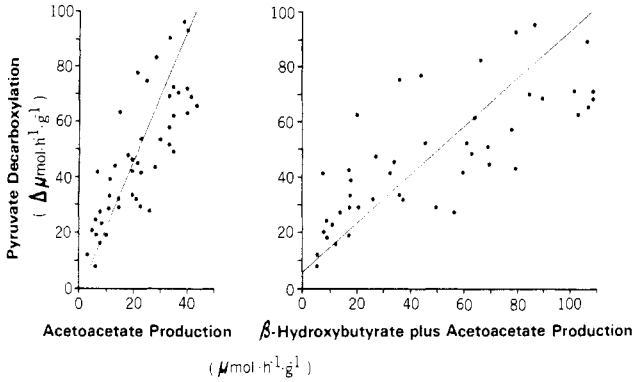

FIGURE 2: Relationship between increases in the rates of pyruvate decarboxylation and ketogenesis following octanoate addition. Pairs of individual data from the experiments summarized in Table I. The increases in the rates of pyruvate decarboxylation following infusion of octanoate (0.02–0.7 mM) were plotted vs. the increases in the rates of acetoacetate production (left) or total ketogenesis (right). The regression lines each calculated from 48 pairs of data follow the equations $y = 2.3x - 1$ ($r = 0.78$) and $y = 0.9x + 7$ ($r = 0.75$) for the left and right plots, respectively.

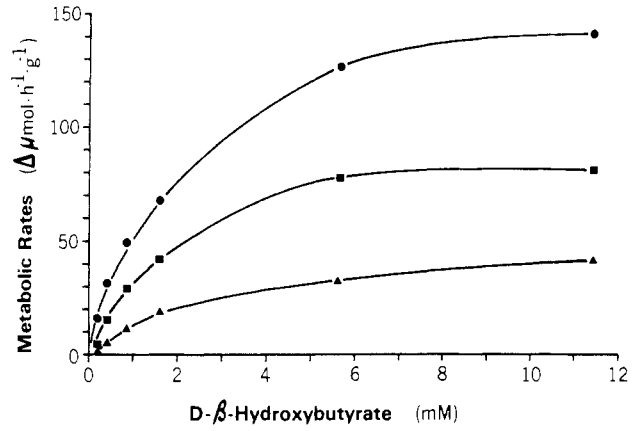

FIGURE 3: Increases in metabolic rates following infusion of β-hydroxybutyrate. The liver from a fed rat was perfused with 0.05 mM [1-^{14}C]pyruvate and with stepwise increasing concentrations of DL-β-hydroxybutyrate (0.2–22 mM) in the perfusate similar to the experiments described in the legend of Table I. The increases in the rates of acetoacetate production (●), pyruvate decarboxylation (■), and oxygen consumption (▲) at the end of each 8-min infusion interval (mean of triplicate analyses) were plotted vs. the concentration of D-β-hydroxybutyrate measured in the arterial perfusate.

acetate$_{mit}$ exchange. On the other hand, production of acetoacetate from β-hydroxybutyrate seemed a more direct approach to test our hypothesis that at low pyruvate concentrations, the rate of pyruvate decarboxylation is limited by pyruvate transport across the mitochondrial membrane. Perfusion experiments were performed in which DL-β-hydroxybutyrate was infused, stepwise increasing the concentration from 0 to 24 mM in the presence of either 0.05 or 5 mM [1-^{14}C]pyruvate. The infusion of β-hydroxybutyrate resulted in an increase in the rates of oxygen consumption and acetoacetate production under all conditions studied, whereas the rate of pyruvate decarboxylation was increased only at low (0.05 mM) but not at high (5 mM) pyruvate concentrations. In Figure 3, the increases in the metabolic rates at 0.05 mM pyruvate were plotted as a function of the perfusate concentration of D-β-hydroxybutyrate. In this experiment, an apparently linear relationship between pyruvate decarboxylation and acetoacetate production was observed at the low pyruvate concentration (Figure 4). The stoichiometry of extra CO_2 formed from pyruvate to extra acetoacetate produced was approximately 1:2 on a molar basis. It should be mentioned, however, that linearity was not observed in all experiments and

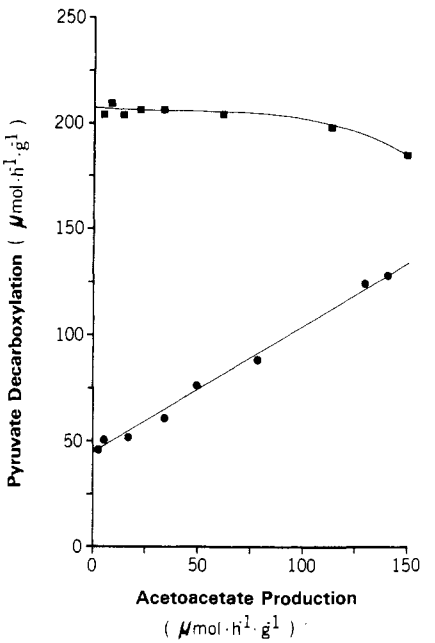

FIGURE 4: Relationship between rates of pyruvate decarboxylation and acetoacetate production following infusion of β-hydroxybutyrate. In a liver perfusion experiment, DL-β-hydroxybutyrate was infused with stepwise increasing concentrations (0.1–10 mM, 6-min intervals) twice: first (30–78 min), in the presence of 0.05 mM pyruvate (●), and second (100–148 min of perfusion), in the presence of 5 mM pyruvate (■). The rates of pyruvate decarboxylation were plotted vs. the corresponding rates of acetoacetate production. With 0.05 mM pyruvate, the regression line calculated from eight pairs of data follows the equation $y = 0.6x + 45$ ($r = 0.91$).

that the stoichiometry gradually decreased to 1:5 with β-hydroxybutyrate concentrations higher than 10 mM (Table IV).

When L-leucine, a branched chain amino acid, is oxidized, acetoacetate is formed as an intermediate (Meister, 1965). In the liver, the capacity for the first step in leucine degradation, i.e., the transamination of leucine to α-ketoisocaproate, is considerably less than that for the subsequent processes (e.g., decarboxylation) (Wohlheuter & Harper, 1970; Shinnick & Harper, 1976). In the present studies, therefore, the α-keto acid analogue of leucine, α-ketoisocaproate, was used as a ketogenic substrate.

The experiment depicted in Figure 5 illustrates the effect of α-ketoisocaproate on the rates of oxygen consumption, $^{14}CO_2$ production from [1-^{14}C]pyruvate, and ketogenesis. α-Ketoisocaproate was infused at two perfusate concentrations of pyruvate, initially at 0.05 mM and thereafter at 5 mM pyruvate. The rate of ketogenesis increased rapidly from about 8 to 45 $\mu mol \cdot h^{-1} \cdot g^{-1}$ upon α-ketoisocaproate infusion at 0.05 mM pyruvate; the increase was smaller at the high pyruvate concentration. As expected from our previous experiments with octanoate and β-hydroxybutyrate (Scholz et al., 1978), α-ketoisocaproate infusion resulted in a substantial increase in the rate of $^{14}CO_2$ production at 0.05 mM pyruvate, while at 5 mM pyruvate the rate was decreased. Both the stimulatory and inhibitory effects of α-ketoisocaproate were reversed rapidly following termination of the infusion.

As in the experiments in which octanoate was infused, the question arises whether or not the increase in $^{14}CO_2$ production following α-ketoisocaproate infusion could be attributed to the decarboxylation of [1-^{14}C]pyruvate by pyruvate dehydrogenase. For this reason, tracer dilution experiments were performed as described recently (Scholz et al., 1978). The data from four kinetic experiments in the absence and presence of α-ketoisocaproate at low or high pyruvate concentrations are summarized in Table II. The subdivision of the total

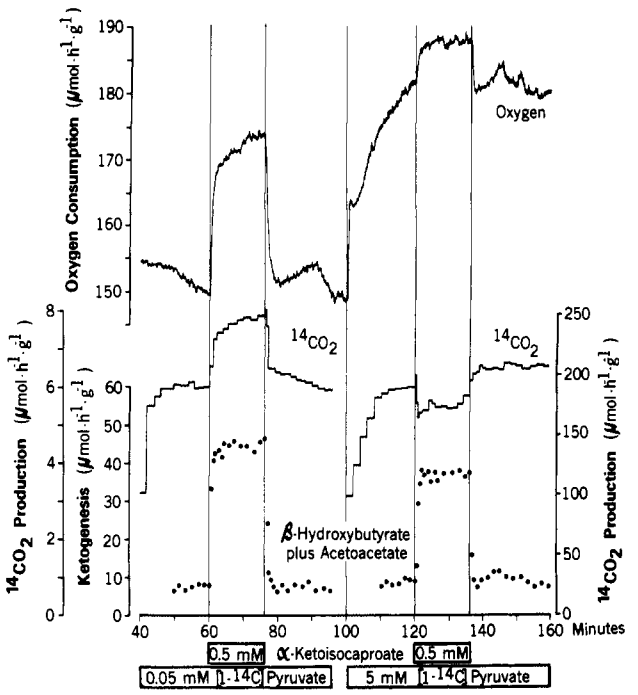

FIGURE 5: Effect of α-ketoisocaproate infusion on the rates of hepatic oxygen consumption, $^{14}CO_2$ production from [1-^{14}C]pyruvate, and ketogenesis in the presence of 0.05 or 5 mM pyruvate. Time course of an experiment in which the liver from a fed rat was perfused with Krebs/Henseleit bicarbonate buffer in a nonrecirculating system. Pyruvate and α-ketoisocaproate were infused as indicated by horizontal bars. Low and high concentrations of pyruvate differed in their specific radioactivities. The upper trace is the original recording of the venous oxygen concentration which was expressed as rate of oxygen uptake. Rates of $^{14}CO_2$ production are presented as a step curve; each step represents the rate during the interval indicated (15–60 s). Quantitation of the rates was based on the specific radioactivity of [1-^{14}C]pyruvate in the influent perfusate. Different scales for $^{14}CO_2$ production were used for the perfusion periods 40–96 min (0.05 mM pyruvate) and 100–160 min (5 mM pyruvate). Rates of β-hydroxybutyrate plus acetoacetate production (●) were calculated from arterial − venous concentration differences.

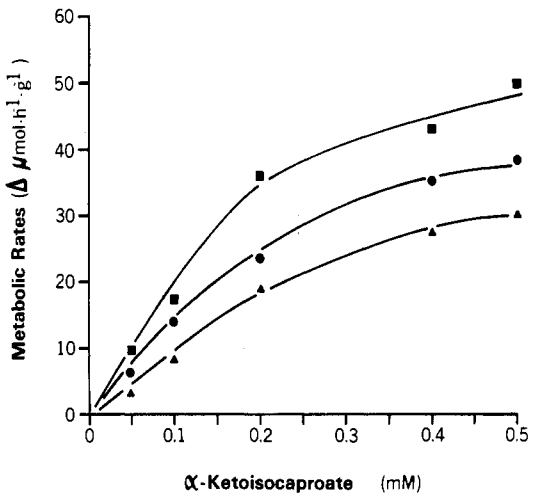

FIGURE 6: Increases in metabolic rates following infusion of α-ketoisocaproate. Data from five liver perfusion experiments in which α-ketoisocaproate was infused with stepwise increasing concentrations (0.05–0.5 mM) in the presence of 0.05 mM pyruvate in the perfusate. The mean values of the increases in the rates of ketogenesis (●), pyruvate decarboxylation (■), and oxygen consumption (▲) at the end of each 8-min infusion interval were plotted vs. the concentration of α-ketoisocaproate in the arterial perfusate.

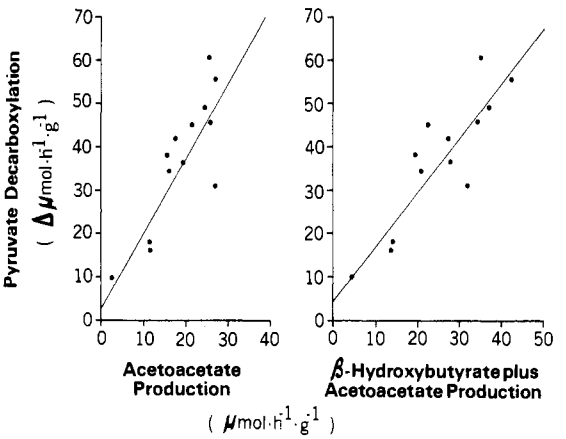

FIGURE 7: Relationship between increases in the rates of pyruvate decarboxylation and ketogenesis following infusion of α-ketoisocaproate. Pairs of individual data from the experiments summarized in Figure 6. The increases in the rates of pyruvate decarboxylation (0.05 mM pyruvate) following infusion of α-ketoisocaproate (0.05–0.5 mM) were plotted vs. the increases in the rates of acetoacetate production (left) or total ketogenesis (right). The regression lines each calculated from 13 pairs of data follow the equations $y = 1.8x + 3$ ($r = 0.88$) and $y = 13x + 4$ ($r = 0.93$) for the left and right plots, respectively.

$^{14}CO_2$ production into its components indicated that about 90% of $^{14}CO_2$ was attributable to direct pyruvate decarboxylation via pyruvate dehydrogenase at the low pyruvate concentration. This component was diminished to about 70% at 5 mM pyruvate. The distribution pattern appeared to be unaffected in the presence of α-ketoisocaproate, irrespective of changes in the total $^{14}CO_2$ production. Finally, the flux rate through the pyruvate dehydrogenase reaction was calculated from the relative specific radioactivity of venous lactate and from the component of $^{14}CO_2$ production which is attributable to direct pyruvate decarboxylation. Following α-ketoisocaproate infusion, the flux increased about 60% at 0.05 mM pyruvate but decreased slightly at 5 mM pyruvate.

The changes in the metabolic rates due to infusion of α-ketoisocaproate are plotted vs. the α-ketoisocaproate concentration in the arterial perfusate (Figure 6). Near maximal rates of pyruvate decarboxylation, ketogenesis, and oxygen consumption (about 50, 40, and 30 $\mu mol \cdot h^{-1} \cdot g^{-1}$, respectively) were reached at 0.5 mM α-ketoisocaproate. The relationship between the increase in the rate of pyruvate decarboxylation and the production of acetoacetate or total ketone bodies appeared to be linear (Figure 7). The ratio of pyruvate decarboxylation to ketogenesis was 1.8 in the case of acetoacetate and 1.3 in the case of total ketone bodies.

It has been reported that α-ketoisocaproate inhibits pyruvate oxidation in perfused rat livers (Williamson et al., 1979), isolated liver and brain mitochondria (McArthur & Bowden, 1972), and brain slices (Bowden et al., 1971). Those experiments, however, were performed with extremely high concentrations (>1 mM) of both pyruvate and α-ketoisocaproate. It should be emphasized that in our experiments the stimulatory effect of α-ketoisocaproate on pyruvate decarboxylation was observed only when low concentrations were employed. On the other hand, the stimulatory effect gradually decreased when the α-ketoisocaproate concentration was increased to values above 0.5 mM (Table III); with concentrations higher than 3 mM, pyruvate oxidation was inhibited strongly even at low pyruvate concentrations (data not shown).

In order to exclude a possible interaction between pyruvate decarboxylation and the overall metabolism of α-ketoisocaproate other than at the level of acetoacetate production, experiments with α-ketoisovalerate were performed. The structure and metabolism of α-ketoisovalerate are similar to that of α-ketoisocaproate except that the former compound

Table II: Kinetic Parameters from Tracer Dilution Experiments and Calculated Rates of Pyruvate Decarboxylation[a]

pyruvate concn (mM)	α-ketoisocaproate concn (mM)	half-times (s)			$^{14}CO_2$ production (μmol·h^{-1}·g^{-1})				rel specific radioactivity of lactate	flux through pyruvate dehydrogenase reaction (μmol·h^{-1}·g^{-1})
		a	b	c	total	A	B	C		
0.05	0	18	170	1000	5.2	4.8 (92)	0.40 (8)	0.02	0.054	89
0.05	0.5	19	110	800	7.8	6.9 (89)	0.84 (11)	0.03	0.049	141 (+58)
5	0	16	77	600	218	153 (70)	64 (30)	1	0.84	182
5	0.5	20	79	600	223	160 (72)	63 (28)	1	0.96	167 (−8)

[a] The data were derived from two perfusion experiments with livers isolated from fed rats and perfused with either 0.05 or 5 mM pyruvate in the perfusate. While the concentrations of unlabeled pyruvate were kept constant, trace amounts of [1-^{14}C]pyruvate were infused for 6 min in the absence or presence of α-ketoisocaproate (0.5 mM). The kinetics of $^{14}CO_2$ production following termination of the tracer infusion were studied. For illustration of these tracer dilution experiments, see Figure 2 in Scholz et al. (1978). The overall kinetics of $^{14}CO_2$ production exhibited a superposition of at least three exponential kinetic components, indicating the dilution of the radioactivity in different intracellular pools of substrates for decarboxylation processes. In our previous studies (Braun, 1976; Scholz et al., 1976, 1978), evidence was obtained that the rapid component (A with $t_{1/2}$ a) is due to the dilution of radioactivity in the mitochondrial pyruvate pool from which $^{14}CO_2$ is produced via pyruvate dehydrogenase. The slower component (B with $t_{1/2}$ b) probably reflects the malate oxaloacetate pool into which radioactivity was incorporated by a carboxylation of [1-^{14}C]pyruvate via pyruvate carboxylase. Component B, therefore, provides information about the contribution of other carboxylation processes, such as the tricarboxylic acid cycle, to the total $^{14}CO_2$ production, in addition to that directly derived via pyruvate dehydrogenase. The half-times of the three distinguishable components (a, b, and c) in the overall kinetics were calculated by computer evaluation (Braess, 1967). The rates of $^{14}CO_2$ production at the end of the 6-min period of tracer infusion, when an isotopic equilibrium had been reached, were calculated on the basis of the specific radioactivity of arterial pyruvate. A, B, and C are portions of total $^{14}CO_2$ production derived by extrapolation of the individual exponential curves to the time point where the tracer infusion was terminated. The percentage contribution of these components to the total $^{14}CO_2$ production is shown in parentheses. The relative specific radioactivity of venous lactate (arterial pyruvate = 1) was determined at the end of each [1-^{14}C]pyruvate infusion period. It was used as an indicator for the intracellular dilution of [1-^{14}C]pyruvate. In order to calculate rates of substrate flux through the pyruvate dehydrogenase reaction, the rates of component A were divided by the relative specific radioactivity of lactate. The percentage changes in the presence of α-ketoisocaproate are shown in parentheses.

Table III: Effects of α-Ketoisocaproate and α-Ketoisovalerate Concentrations on the Rates of Acetoacetate Production and Pyruvate Decarboxylation[a]

α-ketoisocaproate concn (mM)	α-ketoisovalerate concn (mM)	metabolic rates [μmol·h^{-1}·(g of liver wet wt)$^{-1}$]	
		acetoacetate production	pyruvate decarboxylation
0		4	59
0.2		21	101
0.4		25	104
0.6		27	91
1.0		27	75
3.0		25	61
	0	4	52
	0.2	2	47
	0.4	2	46
	0.6	2	43
	1.0	2	38
	3.0	1	27

[a] Data from two liver perfusion experiments similar to those described in Table I. In the presence of 0.05 mM [1-^{14}C]pyruvate, branched chain α-keto acids were infused with stepwise increasing concentrations (0.2–3 mM) from 30 to 90 min of perfusion. The data are the means of three determinations at the end of each 12-min infusion period.

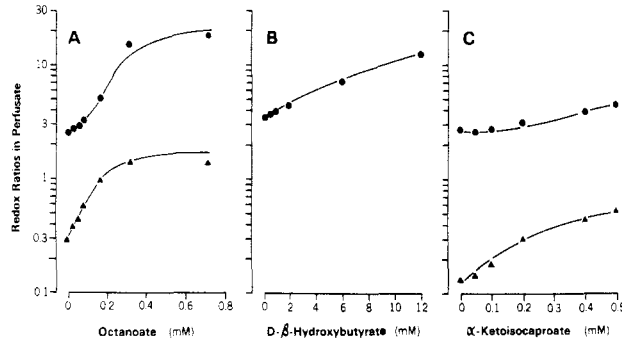

FIGURE 8: Redox ratios in the perfusate. Data from liver perfusion experiments in which (A) octanoate (0.02–0.7 mM), (B) β-hydroxybutyrate (0.1–11 mM), and (C) α-ketoisocaproate (0.05–0.5 mM) were infused. Lactate–pyruvate ratios (●); β-hydroxybutyrate/acetoacetate ratios (▲). Mean values from five to seven experiments in each group. The data were derived from the experiments summarized in Table I (A), Figure 3 (B), and Figure 6 (C). In all experiments, the perfusion medium contained 0.05 mM pyruvate.

is not ketogenic. As shown in Table III, the application of α-ketoisovalerate did not result in the production of acetoacetate, and the rate of pyruvate decarboxylation was decreased rather than increased.

The redox states of the cytosolic and mitochondrial NADH/NAD$^+$ systems as indicated by concentration ratios in the perfusate, i.e., lactate/pyruvate and β-hydroxybutyrate/acetoacetate, respectively, were increased when octanoate, β-hydroxybutyrate, or α-ketoisocaproate were added. These experiments were performed under the assumption that the perfusate lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios reflect the actual redox state of the cytosolic and mitochondrial compartments respectively, e.g., that the lactate and β-hydroxybutyrate dehydrogenases are in rapid equilibrium with the NADH/NAD$^+$ couple in their respective compartments and that the four substrates in question rapidly equilibrate with the perfusate. The data from experiments in which these compounds were infused at various concentrations are summarized in Figure 8. The redox changes were largest with octanoate (Figure 8A) and were less pronounced with α-ketoisocaproate (Figure 8C). A reduction of the mitochondrial NADH/NAD$^+$ system normally would be expected to inhibit/inactivate the pyruvate dehydrogenase complex due to a feedback inhibitory effect of NADH on the active form of pyruvate dehydrogenase (Bremer, 1969; Wieland et al., 1969; Tasi et al., 1973) or due to the effects of NADH and NAD$^+$ on the interconverting enzymes (Pettit et al., 1975; Cooper et al., 1975; Batenburg & Olson, 1976; Hansford, 1976) which would result in an inactivation of the multienzyme complex. Since the rate of pyruvate decarboxylation, however, was increased considerably under these experimental conditions, one may conclude that the redox state of the mitochondrial NADH/NAD$^+$ system plays a minor role in the

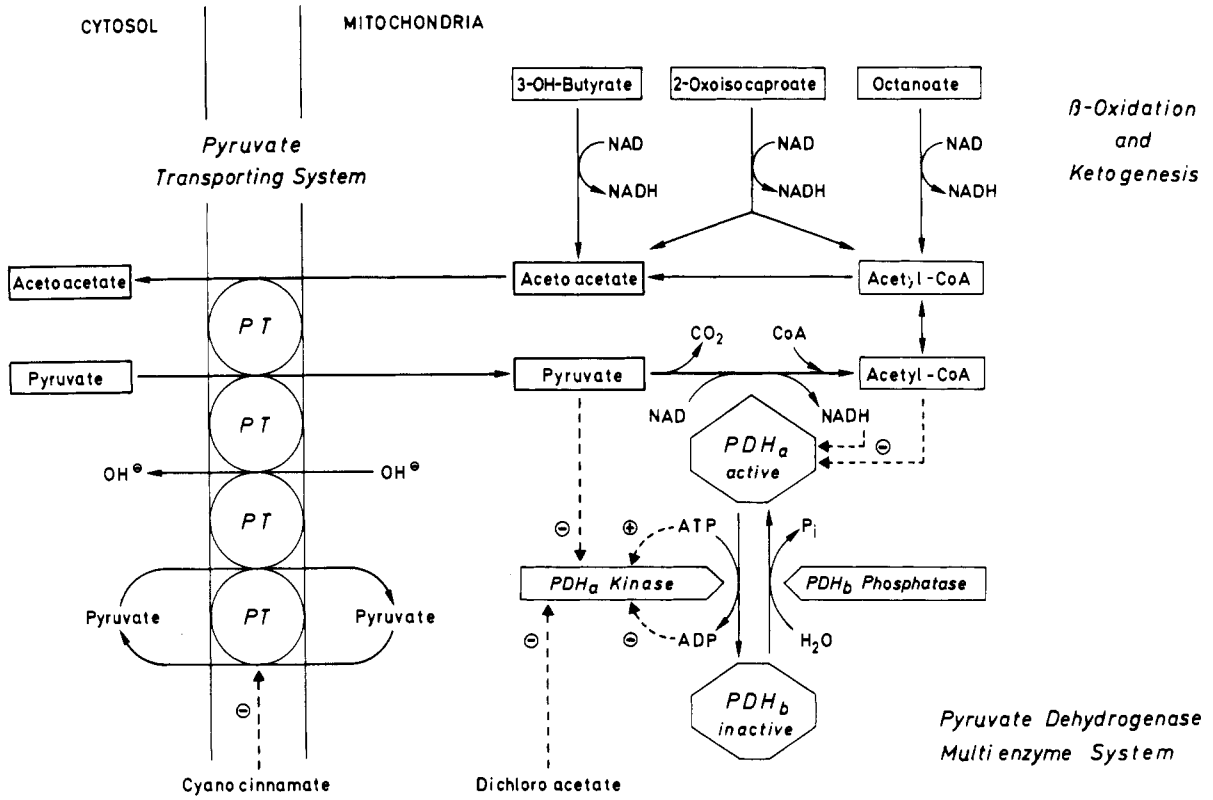

FIGURE 9: Metabolic scheme depicting the possible interaction between the pyruvate dehydrogenase multienzyme complex and ketogenesis via the pyruvate transporting system in the mitochondrial membrane. The experimental approaches by which the metabolic interaction was studied are illustrated (i.e., ketogenesis from various substrates with differences in their metabolism; application of inhibitors). Abbreviations: PT, pyruvate translocator; PDH_a and PDH_b, dephospho and phospho forms of pyruvate dehydrogenase, respectively.

regulation of pyruvate dehydrogenase, at least at pyruvate concentrations in the physiological range in the present experiments.

α-Cyanocinnamate is a known inhibitor of the exchange of monocarboxylates across biological membranes (Halestrap & Denton, 1974, 1975; Halestrap, 1975, 1976; Patel et al., 1980). The concentration required for half-maximal inhibition of pyruvate transport across the inner membrane of isolated mitochondria was found to be less than 1 μM (Halestrap, 1975). The monocarboxylate transport across the plasma membrane of liver cells appeared to be less sensitive to α-cyanocinnamate [i.e., half-maximal inhibition at 1 mM (Schwab et al., 1979)]. In our perfusion experiments, 0.1 mM α-cyanocinnamate was employed, a concentration which should affect the monocarboxylate exchange across the plasma membrane only slightly, but at which the rate of pyruvate decarboxylation was diminished to about 50%. The effects of α-cyanocinnamate were reversed totally following termination of its infusion.

When β-hydroxybutyrate was infused in the presence of α-cyanocinnamate, the increase in the rate of pyruvate decarboxylation was abolished almost totally (Table IV). It should be noted, however, that the rate of acetoacetate production also was affected, but not nearly to the same extent as that of pyruvate decarboxylation. This observation could indicate that acetoacetate is less dependent upon a carrier-mediated transport than pyruvate, i.e., a larger portion of acetoacetate diffuses passively across the mitochondrial membrane. The suppression of the stimulatory effect of β-hydroxybutyrate is evident when the rates of pyruvate decarboxylation are compared at similar rates of acetoacetate production in the absence and presence of α-cyanocinnamate. The data strongly support our hypothesis that the monocarboxylate translocator in the mitochondrial membrane is involved in the stimulatory effect of ketogenic compounds on the flux through the pyruvate dehydrogenase reaction.

Discussion

The theoretical background for our present experiments is depicted in Figure 9. Three ketogenic substrates, β-hydroxybutyrate, α-ketoisocaproate, and octanoate, were employed as metabolic substrates. In the process of acetoacetate generation—with or without acetyl-CoA as an intermediate—NADH is formed, and the mitochondrial oxidation–reduction state is affected (Figure 8). In contrast, acetoacetate can be generated from acetate when added to the liver in high concentrations, but without any effect on the redox state (Patel et al., 1981a,b). Oleate differs from octanoate in that its coenzyme derivative is formed outside the mitochondria. Despite differences in their metabolism, all of these substrates, including oleate (Scholz et al., 1978; Dennis et al., 1978) and acetate (Patel et al., 1982a,b), have in common the ability to enhance the rate of pyruvate oxidation when pyruvate is present in concentrations below 0.2 mM. The stimulatory effect of oleate was decreased when its β oxidation (and consequently ketogenesis) was suppressed by acyl derivatives of D(+)-carnitine (Scholz et al., 1978), indicating that it was not the compound itself but products of its metabolism which were responsible for the stimulation of pyruvate oxidation. Moreover, α-ketoisovalerate, a compound structurally similar to α-ketoisocaproate but a nonketogenic substrate, was not capable of enhancing pyruvate oxidation (Table III).

An apparently linear relationship between the increased rates of pyruvate decarboxylation and acetoacetate production was found with octanoate, β-hydroxybutyrate, and α-ketoisocaproate (Figures 2, 4, and 7). If the exchange of pyruvate for acetoacetate is tightly coupled, the expected stoichiometry of extra pyruvate decarboxylated to acetoacetate produced would be 1:1. Such a stoichiometry, however, was not observed. With β-hydroxybutyrate, the ratio was considerably

Table IV: Changes in the Rates of Acetoacetate Production and Pyruvate Decarboxylation following Addition of β-Hydroxybutyrate in the Absence and Presence of α-Cyanocinnamate[a]

DL-β-hydroxybutyrate concn (mM)	α-cyanocinnamate concn (mM)	changes in metabolic rates [Δμmol·h⁻¹·(g of liver wet wt)⁻¹] acetoacetate production	pyruvate decarboxylation
1		42	21
2		69	37
5		126	54
10		130	53
20		116	32
40		100	21
1	0.1	23	2
2	0.1	34	3
5	0.1	50	1
10	0.1	77	−2
20	0.1	85	−3
40	0.1	70	−4

[a] Data from a liver perfusion experiment similar to those described in Table I. In the presence of 0.05 mM [1-^{14}C]pyruvate, DL-β-hydroxybutyrate was infused with stepwise increasing concentrations (1–40 mM): first (30–66 min) in the absence of and second (100–136 min) in the presence of 0.1 mM α-cyanocinnamate. The infusion of α-cyanocinnamate was initiated at 80 min of perfusion. The values (mean of three determinations at the end of each 6-min infusion period) are the differences between the control values (before the β-hydroxybutyrate infusion was initiated) and those under the influence of β-hydroxybutyrate. The control rates of pyruvate decarboxylation were 70 and 26 μmol·h⁻¹·g⁻¹ in the absence and presence of α-cyanocinnamate, respectively. The control rates of acetoacetate production were below 3 μmol·h⁻¹·g⁻¹. All the changes were reversible when the β-hydroxybutyrate infusion was terminated.

below 1, whereas with octanoate and α-ketoisocaproate, the rate of pyruvate decarboxylation exceeded that of acetoacetate production by more than 50%. Thus, additional factors may be involved in the stimulatory effect.

Another possible candidate as a counterion for enhancing the influx of pyruvate is β-hydroxybutyrate, although conflicting data from experiments with isolated mitochondria have been reported concerning its exchange for pyruvate (Papa & Paradies, 1974; Land & Clark, 1974). The ratio of pyruvate decarboxylated to total ketone bodies produced from either octanoate or α-ketoisocaproate approached a value of 1, suggesting that pyruvate may be exchanged with both acetoacetate and β-hydroxybutyrate. On the other hand, a possible exchange of pyruvate for β-hydroxybutyrate would imply that both metabolites should compete for the monocarboxylate transporting system during coinfusion. Indeed, such a competition could explain the relatively small stimulatory effect of β-hydroxybutyrate on pyruvate decarboxylation in comparison to the high rates of acetoacetate production and the gradual diminution of the effect with increasing β-hydroxybutyrate concentrations (Table IV).

Further support of our view that the site of interaction between fatty acid and pyruvate oxidation is at the level of the monocarboxylate transport system was provided by the experiments in which an inhibitor of the monocarboxylate translocator was infused. In the presence of 0.1 mM α-cyanocinnamate, the stimulatory effect of β-hydroxybutyrate was abolished (Table IV).

An important consideration in this discussion must be how an accelerated efflux of ketone bodies could affect the influx of pyruvate into the mitochondrial compartment, since the transport of pyruvate does not appear to be limiting in the overall process of pyruvate oxidation. [Note: The rate of pyruvate decarboxylation was increased following infusion of dichloroacetate even in the presence of low pyruvate concentrations; see Figure 7 in Scholz et al. (1978).] It is plausible that the rate of exchange of pyruvate across the mitochondrial membrane is much greater than the net rate of influx and that the exchange occurs mainly with pyruvate itself as the counterion [i.e., $pyruvate_{cyt}/pyruvate_{mit}$ exchange via the monocarboxylate translocator (Papa & Paradies, 1974)]. For each pyruvate metabolized inside the mitochondria, a counterion other than pyruvate, for instance, a hydroxyl ion, must be translocated out of the mitochondria (see scheme, Figure 9). A net inward transport of pyruvate in exchange for hydroxyl ions would require a proton gradient across the mitochondrial membrane [i.e., the mitochondrial matrix being more alkaline than the cytosol (Soboll et al., 1980)], which is maintained by the respiratory chain. The large increase in oxygen consumption following the addition of 5 mM pyruvate (Figure 5) could be explained in this way, i.e., an increased export of hydroxyl ions must be counteracted in order to avoid a dissipation of the proton gradient.

On the other hand, under conditions of enhanced ketogenesis, $pyruvate_{mit}$ could be replaced by $acetoacetate_{mit}$ as a counterion [i.e., $pyruvate_{cyt}/acetoacetate_{mit}$ exchange (Papa & Paradies, 1974)], thus increasing the net rate of pyruvate influx. It should be emphasized that the turnover of the monocarboxylate translocator need not be increased in order to accelerate the net influx of pyruvate.

In experiments with isolated mitochondria, it was found that the pyruvate concentration for half-maximal pyruvate/pyruvate exchange is considerably lower than the acetoacetate concentration for half-maximal pyruvate/acetoacetate exchange (Papa & Paradies, 1974). It follows from this observation that only an extremely high acetoacetate concentration in the cytosol could compete effectively for pyruvate influx. Conversely, relatively high mitochondrial concentrations of acetoacetate and low pyruvate concentrations are required in order to enhance the net influx of pyruvate. Our experimental data are consistent with the reported differences in the affinities of pyruvate and acetoacetae for the monocarboxylate translocator. Exceedingly high perfusate concentrations of acetoacetate (>10 mM) were required in order to suppress the stimulatory effect of octanoate or β-hydroxybutyrate on the rate of pyruvate decarboxylation (Scholz et al., 1978; Patel et al., 1982a,b). Moreover, in the presence of ketogenic precursors, the rate of pyruvate decarboxylation was stimulated only at low, but not at high, pyruvate concentrations [Figure 4 and Scholz et al. (1978)].

As a consequence of an increased net rate of pyruvate influx, the mitochondrial pyruvate concentration would be elevated. This would lead to an increased rate of pyruvate oxidation either due to an increased substrate supply (if the mitochondrial pyruvate concentration is below the K_m of pyruvate dehydrogenase) or due to an activation of the multienzyme complex [since pyruvate is a potent inhibitor of pyruvate dehydrogenase kinase, the inactivating enzyme (Hucho et al., 1972)]. In fact, an increase in the extractable activity of pyruvate dehydrogenase was found in perfused rat liver (Patel et al., 1982a,b) and in isolated rat liver mitochondria (Dennis et al., 1978) under conditions of octanoate-stimulated pyruvate oxidation. In contrast, no such effects were observed in the perfused rat heart (Olson et al., 1978), i.e., in an organ with little, if any, capability to generate acetoacetate from fatty acids. We admit, however, that the proposed mechanism underlying the phenomenon is based on the hypothetical in-

crease in the mitochondrial pyruvate concentration which, unfortunately, cannot be demonstrated experimentally at the present time. A definitive description of the mechanism, therefore, must await the development of a methodology which will allow the exact measurement of intramitochondrial pyruvate concentrations in intact tissues. Despite these uncertainties, however, the close relationship between the rate of pyruvate oxidation and ketogenesis and the suppression of the stimulatory effect by α-cyanocinnamate strongly supports our hypothesis (Scholz et al., 1978) that pyruvate transport across the mitochondrial membrane plays a role in the regulation of pyruvate metabolism.

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