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STUDIES ON THE CONTROL OF PYRUVATE OXIDATION IN ISOLATED FETAL RAT LIVER CELLS

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SUMMARY

The control of pyruvate oxidation in isolated fetal rat liver cells has been studied by incubating these cells with glucose, in the absence and in the presence of uncouplers of oxidative phosphorylation.

1. It was found that cells suspended in Krebs–Ringer–phosphate medium accumulated lactate and pyruvate under aerobic conditions. The lactate/pyruvate ratio was found to be 7.5 in the presence of glucose, increasing to 70, 193 and 48 in the presence of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), valinomycin and nigericin, respectively. Measurements of glycolytic intermediates and adenine nucleotides showed that the changes in the lactate/pyruvate ratio correlated with changes in the phosphate potential and the 3-phosphoglyceric acid/glyceraldehyde 3-phosphate ratio, according to the equation:

$$\frac{[\text{pyruvate}]}{[\text{lactate}]} = \frac{K_{LDH}}{K_{GAPDH} \cdot K_{PGK}} \cdot \frac{[ATP]}{[ADP] \cdot [P_i]} \cdot \frac{[\text{3-phosphoglyceric acid}]}{[\text{glyceraldehyde 3-phosphate}]}$$

where K_{LDH}, K_{GAPDH} and K_{PGK} are the equilibrium constants of lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase.

2. The flux through pyruvate dehydrogenase has been measured with [3,4-$^{14}C_2$]glucose. In the absence of uncouplers, this flux was found to be 0.4 nmole/min per mg protein, increasing to 0.6 and 0.9 nmole/min per mg protein in the presence of FCCP and valinomycin, respectively. Measurement of the specific activity of lactate permitted the calculation that 90% of the pyruvate formed from glucose in the absence of uncouplers is reduced to lactate and only 10% is oxidized by the mitochondria.

Intraperitoneal injection of pregnant rats with thiamine increased the percentage of pyruvate oxidized by the mitochondria of the fetal liver cells to 30. It is concluded that in fetal liver cells pyruvate oxidation is controlled at the level of pyruvate dehydrogenase.

Abbreviation: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone.

INTRODUCTION

Much work has been done on the regulation of glycolysis and the Krebs cycle in adult rat tissues. These studies have shown that the reduction state of nicotinamide adenine dinucleotides and the phosphorylation state of adenine nucleotides are important regulatory factors for the overall activity of the two processes (for review see refs 1 and 2). Phosphofructokinase (ATP : D-fructose-6-phosphate phosphotransferase, EC 2.7.1.11), which appears to be rate limiting for glycolysis, is inhibited by ATP and activated by ADP and AMP [3]. The adenine nucleotides are also important regulators of the tricarboxylic acid cycle [4]. Furthermore, Krebs cycle activity is probably regulated by the availability of oxaloacetate which in turn is controlled by the reduction state of mitochondrial NAD [5]. Recently Linn et al. [6, 7] described the regulation of the pyruvate dehydrogenase complex, (Pyruvate : lipoate oxidoreductase, acceptor-acetylating, EC 1.2.4.1) by phosphorylation and dephosphorylation, suggesting that Krebs cycle activity might also be controlled at the level of pyruvate dehydrogenase.

Fetal rat liver is characterized by an enzyme pattern that in many respects differs from that of adult liver. The activity of several glycolytic enzymes is rather low [8] as are some of the Krebs cycle enzymes [9], including pyruvate dehydrogenase [10]. In view of these differences one might speculate that in fetal rat liver, factors different from those operative in adult liver control the flux through glycolysis and the Krebs cycle. The observation of Hommes et al. [11] that glycolysis in isolated fetal rat liver cells produces more pyruvate than the mitochondria can oxidize, as measured by net lactate accumulation, has been further investigated to gain more insight into the factors regulating the oxidation of pyruvate in these cells. For this purpose isolated fetal rat liver cells were incubated in the presence of uncoupling agents in order to study the effect of a low cytosolic or mitochondrial phosphate potential on the flux through pyruvate dehydrogenase.

MATERIALS AND METHODS

Isolation of fetal rat liver cells and of mitochondria

Liver cells of fetal rats 0–2 days before birth were isolated according to the method of Hommes et al. [12] with some modifications. The perfusion medium consisted of 3.0 mM NaCl, 5.0 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 24.0 mM $NaHCO_3$, 2.0 mM glucose, 5.0 mM EDTA and 230 mM sucrose, the final pH was 7.0. After perfusion, the livers were removed from the fetuses and incubated with perfusion medium, containing 0.05% lysozyme, for 30 min at 37 °C under 95% O_2 and 5% CO_2. The livers were then dispersed mechanically by gentle pressing, filtered and centrifuged. The cells were washed 3–4 times with Krebs–Ringer–phosphate solution, final pH 7.0, containing 0.1–2 mM glucose. Mitochondria from fetal livers were isolated according to Hoogeboom and Schneider [13].

Incubations

Incubations were carried out in Krebs–Ringer–phosphate solution in 25-ml Erlenmeyer flasks in a Dubnoff shaker at 37 °C. Total volume was 2 ml. Further reaction conditions are indicated in the legends to the tables and to the figures. Reac-

tions were started by adding cells to the medium. Incubations with [3,4-$^{14}C_2$]glucose were carried out in stoppered Warburg vessels. $^{14}CO_2$ was trapped with hyamine (1 M in methanol).

Reactions were stopped by addition of $HClO_4$ to a final concentration of 2%. The mixture was centrifuged. An aliquot of the supernatant was neutralized with KOH to pH 6–7 and after standing in the cold for 1 h, $KClO_4$ was removed by centrifugation. The supernatant was used for further analysis. In some experiments, the cells were separated from the incubation medium by rapid centrifugation through silicone oil as described by Harris and Van Dam [14]. Their method was also used for corrections for extracellular phosphate by incubating the cells with ^{3}H-labelled water and [U-^{14}C]sucrose.

Measurements of metabolites

Lactate [15], glucose 6-phosphate, fructose 6-phosphate [16] and ATP [17], pyruvate [18], ADP and AMP [19], fructose 1,6-diphosphate and dihydroxyacetone phosphate [20], 3-phosphoglyceric acid, 2-phosphoglyceric acid, phosphoenolpyruvate [18] were determined enzymically by coupling to an NAD(P)-dependent system, using an Aminco-Chance dual-wavelength spectrophotometer. Inorganic phosphate was determined by the method of Fiske and SubbaRow [21]. $^{14}CO_2$ was measured by transferring the content of the inner well of the Warburg vessel to 10 ml of Bray's solution [22] and counted with a Nuclear Chicago Mark I liquid scintillation counter. [1-^{14}C]Lactate was separated from [3,4-$^{14}C_2$]glucose and organic acids by column chromatography (Dowex 1X8, column 10 mm × 140 mm) according to Van Korff [23]. The fractions (1 ml) were mixed with Bray's solution and counted.

Protein was estimated with the biuret method [24]. Thiamine was measured in $HClO_4$ extracts of mitochondria according to Cooper et al. [25], modified as described earlier [26].

Administration of thiamine

Rats, pregnant for 17 days, were injected intraperitoneally with thiamine dihydrochloride (25 mg/200 g body weight in 0.9% NaCl) for 3 consecutive days.

Reagents

All reagents were of analytical grade. [3,4-$^{14}C_2$]Glucose and $NaH^{14}CO_3$ were purchased from NEN Chemicals, GmbH, Germany. Valinomycin and nigericin were kindly donated by the Eli Lilly Company.

Carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was a gift from Dr P. G. Heytler. Antimycin was purchased from Sigma Chemical Co., St. Louis, U.S.A.

RESULTS

The time course of lactate and pyruvate accumulation during incubation of fetal rat liver cells in Krebs–Ringer–phosphate solution is shown in Fig. 1.

Approximately linear rates are obtained in the aerobic as well as in the antimycin-inhibited state. The rate of lactate accumulation under aerobic conditions is 2.5±0.4 nmoles/min per mg protein (n = 11, range 1.7–3.1), in the presence of anti-

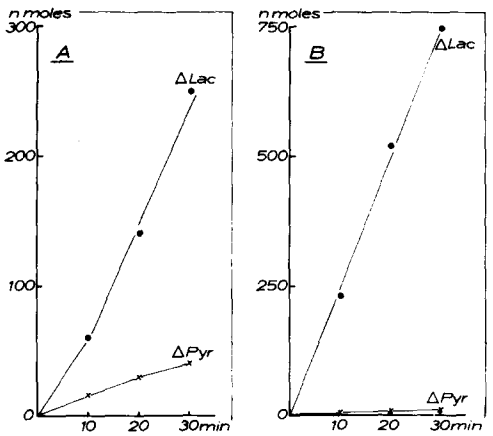

Fig. 1. Time course of lactate and pyruvate accumulation during glycolysis in isolated fetal rat liver cells. A: aerobic state. B: antimycin-inhibited state induced by adding antimycin (10 μg/ml) to the medium. Protein, 3.7 mg.

mycin 8.1$\pm$2.5 nmoles/min per mg protein ($n = 5$, range 6.7–10.9). The aerobic lactate/pyruvate ratio is 8.5$\pm$2.7 ($n = 15$, range 6–14); the aerobic ATP/ADP ratio is 4–7.

Table I shows the effect of FCCP, valinomycin and nigericin on lactate accumulation, lactate/pyruvate ratio and ATP/ADP ratio. In the presence of FCCP or valinomycin, lactate accumulation is stimulated, the lactate/pyruvate ratio is raised and a drop in the total amount of ATP is observed resulting in a lowering of the ATP/ADP ratio. Similar effects are observed, although less pronounced, when nigericin is present in the incubation medium. Addition of uncoupling agents results in a stimulation of the glycolytic flux and a shift to a more reduced state of the nicotinamide adenine dinucleotides of the cytoplasm as indicated by the rise in the lactate/pyruvate ratio. As the volume fraction of the mitochondria in the fetal rat liver cells is low [27, 28] the contribution of mitochondrial adenine nucleotides to total cell adenine nucleotides is relatively small and therefore the ATP and ADP values presented here represent predominantly the phosphorylation state of the adenine nucleotides of the cytoplasm.

TABLE I

EFFECT OF FCCP, VALINOMYCIN AND NIGERICIN ON LACTATE, PYRUVATE, ATP AND ADP DURING GLYCOLYSIS IN FETAL RAT LIVER CELLS

Fetal rat liver cells (4.8 mg protein) were incubated in Krebs–Ringer–phosphate solution and glucose (0.1 mM) for 30 min. Temperature 37 °C. Metabolite concentrations are given in nmoles/mg protein. L/P means the lactate/pyruvate ratio.

Expt	Additions	Δ Lactate	Δ Pyruvate	L/P	ATP	ADP	ATP/ADP
1	None	115	15.4	7.5	5.17	0.75	6.9
2	FCCP (2.5 μM)	406	4.2	96.7	2.98	2.13	1.4
3	Valinomycin (1 μM)	375	2.1	178	1.96	1.75	1.1
4	Nigericin (1 μM)	219	9.6	22.8	4.16	1.17	3.6

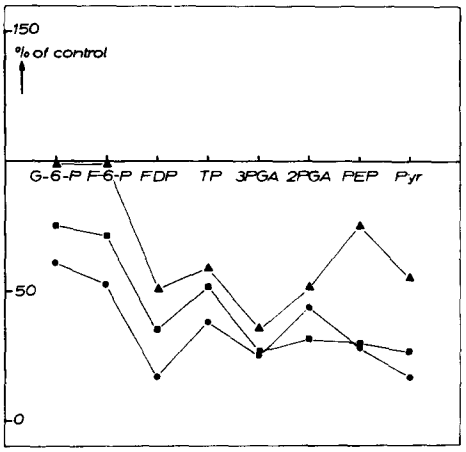

Fig. 2. Effect of 1 μM FCCP (▲–▲), 1 μM valinomycin (●–●), and 1 μM nigericin (■–■), on glycolytic intermediates in fetal rat liver cells. Values are expressed as percentage of control. Fetal liver cells (2–5 mg protein) were incubated as described under Materials and Methods. G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; TP, triosephosphate; 3-PGA, 3-phosphoglyceric acid; 2-PGA, 2-phosphoglyceric acid; PEP, phosphoenolpyruvate; Pyr, pyruvate.

The effect of FCCP, valinomycin and nigericin on glycolysis in fetal rat liver cells was investigated in more detail by analysis of glycolytic intermediates, resulting in the cross-over plots as shown in Fig. 2. It can be seen that the concentration of all glycolytic intermediates is lowered in the presence of these agents. This is consistent with the observed stimulation of the glycolytic flux. It should be noted that, although the ATP/ADP ratio is lower under these conditions no cross-over at phosphofructokinase is observed.

The studies of Williamson et al. [29] and of Veech et al. [30, 31] have clearly established that a direct relationship exists between the reduction state of the nicotinamide adenine dinucleotides and the phosphate potential (ATP/(ADP · P_i)) in the cytoplasm. This relationship is mediated by the system glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD oxidoreductase, EC 1.2.1.13), 3-phosphoglyceric acid kinase (ATP: 3-phospho-D-glycerate-1-phosphotransferase, EC 2.7.2.3), lactate dehydrogenase (L-lactate:NAD oxido-reductase, EC 1.1.1.17) which is in near equilibrium. They derived the following expression:

$$\frac{[\text{pyruvate}]}{[\text{lactate}]} = \frac{K_{LDH}}{K_{GAPDH} \cdot K_{PGK}} \cdot \frac{[ATP]}{[ADP] \cdot [P_i]} \cdot \frac{[\text{3-phosphoglyceric acid}]}{[\text{glyceraldehyde 3-phosphate}]} \quad (1)$$

where K_{LDH}, K_{GAPDH} and K_{PGK} are the equilibrium constants of the lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase.

Stubbs et al. [32] observed a 10-fold increase in lactate concentration in rat liver after intraperitoneal injection of FCCP, accompanied by a fall in the ATP/ADP ratio and an increase in the lactate/pyruvate ratio. The directly measured value of the phosphate potential was in agreement with that calculated from Eqn 1.

To determine whether Eqn 1 also holds for fetal rat liver cells the 3-phosphoglycerate/dihydroxyacetone phosphate ratio was measured. From this value the ratio

TABLE II

MEASURED AND CALCULATED PHOSPHATE POTENTIALS IN ISOLATED FETAL RAT LIVER CELLS IN THE PRESENCE OF FCCP, VALINOMYCIN AND NIGERICIN

Fetal rat liver cells (2–5 mg protein) were incubated in Krebs–Ringer–phosphate solution (+2.0 mM glucose) for 30 min. Reaction temperature 37 °C. Intracellular phosphate (P_i) concentration was estimated as described in Methods. The concentration of HPO_4^{2-} at pH 7.0 was taken to be 60 % of total P_i [30]. L/P, the lactate/pyruvate ratio. DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate. Metabolite concentrations are given in nmoles/mg protein. The values in parentheses are the number of observations.

Expt	Additions	L/P	3-PGA	DHAP	GAP calculated	3-PGA/GAP
1	None	8.5± 2.7 (15)	4.3	1.0	0.11	39.0
2	FCCP (10 μM)	70 ±30 (4)	1.1	0.5	0.05	22.0
3	Valinomycin (1 μM)	193 ±75 (6)	1.5	0.5	0.05	30.0
4	Nigericin (1 μM)	48 ±30 (5)	1.1	0.4	0.04	27.5

Expt	ATP	ADP	P_i (mM)	$\frac{[ATP]}{[ADP]\cdot[P_i]}$ (mM^{-1})	
				Measured	Calculated
1	4.7±0.7 (7)	0.8±0.3 (6)	9.5	1030	1600
2	2.8±0.2 (4)	1.9±0.5 (4)	6.7	370	340
3	1.9±0.1 (4)	1.6±0.4 (4)	10.4	190	92
4	3.7±0.8 (4)	1.3±0.4 (4)	6.1	770	400

of 3-phosphoglycerate/glyceraldehyde 3-phosphate was calculated, assuming that the concentration of glyceraldehyde 3-phosphate is 1/9.3 that of the concentration of dihydroxyacetone phosphate [33]. The results are presented in Table II. In the presence of FCCP or valinomycin the measured phosphate potential fell to a low value, whereas in the presence of nigericin only a small decrease of the phosphate potential was observed. The value of the phosphate potential calculated according to Eqn 1 was in satisfactory agreement with that obtained from direct measurements. The results indicate that the relationship between phosphate potential and redox state, given by Eqn 1, in adult rat liver, is also valid for fetal rat liver cells. (The effective concentration of FCCP, valinomycin and nigericin depends on the protein concentration, because these agents bind to protein. This could well explain the relatively large errors observed in the analysis presented in Table II).

In order to measure directly the flux through pyruvate dehydrogenase the cells were incubated in sealed Warburg vessels in the presence of [3,4-$^{14}C_2$]glucose. As a result of glycolysis pyruvate and lactate will become labelled at the C-1 position. In the pyruvate dehydrogenase reaction the C-1 atom of pyruvate is removed and appears as $^{14}CO_2$ which can be trapped with hyamine. Depending on the age of the foetuses, label in glucose, pyruvate, lactate and CO_2 is diluted by the breakdown of glycogen which is present at 1–2 days before birth [34]. The dilution of the label is however equal for pyruvate, lactate and CO_2. Control experiments showed that, when cells are incubated with either [3,4-$^{14}C_2$]glucose or $NaH^{14}CO_3$, no label is

TABLE III

EFFECT OF FCCP AND VALINOMYCIN ON THE FLUX THROUGH PYRUVATE DEHYDROGENASE IN FETAL RAT LIVER CELLS

Fetal rat liver cells (4.9 mg protein in Expt 1, 3.2 mg protein in Expt 2) were incubated in Krebs–Ringer–phosphate solution containing 0.1 mM glucose in the presence of [3,4-$^{14}C_2$]glucose (1 μC_1). The incubations were carried out in stoppered Warburg vessels as described in Materials and Methods. Reaction time 30 min, temperature 37 °C.

Additions	Δ Lactate		Spec. act. (dpm/nmole)	ΔCO_2 (dpm)	Calculated		
	nmoles	dpm			ΔCO_2 (nmoles)	Flux (nmoles/min per mg protein)	
						Lactate dehydrogenase	Pyruvate dehydrogenase
Expt 1 a. None	410	10 200	2.50	1522	61	2.8	0.41
b. FCCP (2 μM)	1220	23 400	1.92	1740	91	8.3	0.61
Expt 2 a. None	215	16 160	75.0	2600	35	2.2	0.36
b. Valinomycin (1 μM)	816	82 000	100.0	8575	86	8.5	0.89

found in a chloroform–methanol (2 : 1, v/v) extract of the cells indicating that incorporation of $^{14}CO_2$ into fatty acids did not occur under these conditions. As the flux through the pentose phosphate cycle is about 2% of that through the glycolytic chain, rearrangement of the carbon atom skeleton is of minor importance [35].

In Table III the results are given of an experiment in which $^{14}CO_2$ production was measured in the presence of FCCP and valinomycin. Addition of FCCP or valinomycin increased the flux through pyruvate dehydrogenase as compared to controls. Addition of nigericin resulted in a decrease of this flux: the control yielded 532 dpm recovered in $^{14}CO_2$ while in the presence of 1 μM nigericin 365 dpm were recovered in $^{14}CO_2$. The difference in yield of $^{14}CO_2$ between experiments 1 and 2 is due to a difference in dilution by endogenous unlabeled glycogen as liver cell preparations of different ages were used (1/2 and 1 day before birth, respectively). Both [1-^{14}C]lactate and $^{14}CO_2$ are derived from [1-^{14}C]pyruvate and have therefore the same specific activity. By measuring the specific activity of lactate in each experiment it is possible to calculate the flux through pyruvate dehydrogenase and thus the distribution of pyruvate between reduction to lactate and oxidation by the mitochondria. From Table III it follows that in the control cells the flux through pyruvate dehydrogenase was 0.4 nmole/min per mg protein. In the presence of FCCP or valinomycin this value was increased to 0.6 and 0.9 nmole/min per mg protein, respectively. Furthermore it can be calculated that under aerobic conditions about 90% of the pyruvate formed by glycolysis, is reduced to lactate and only 10% is oxidized by the mitochondria, confirming the results reported earlier [11].

Roch and Reed observed that thiamine diphosphate inhibits the phosphorylation of pyruvate dehydrogenase thereby maintaining the enzyme complex in the active form [36]. To study the effect of thiamine in vivo, rats, pregnant for 17 days were injected intraperitoneally with thiamine (25 mg/200 g body weight in 0.9% NaCl), for 3 consecutive days. Mitochondria of the livers of the mother animal and of the fetuses were isolated on the 4th day and analysed for thiamine diphosphate content. The results are given in Table IV. Intraperitoneal injection of thiamine increases the thiamine diphosphate content of the fetal liver mitochondria by a factor 3.

TABLE IV

EFFECT OF INTRAPERITONEAL INJECTION OF THIAMINE INTO PREGNANT RATS ON THIAMINE DIPHOSPHATE CONTENT OF LIVER MITOCHONDRIA OF THE MOTHER AND OF THE FETUSES

Rats, pregnant for 17 days, were injected intraperitoneally with thiamine as described under Materials and Methods. Total thiamine diphosphate was measured in neutralized $HClO_4$ extracts of the mitochondria according to Cooper et al. [21]. Livers of fetuses from one litter were pooled. The number of animals or litters is given in parentheses.

	Thiamine diphosphate of liver mitochondria (nmoles/mg protein)	Range
Control mother animal (9)	0.25±0.05	0.20–0.37
Control fetuses (9)	0.35±0.08	0.17–0.46
Thiamine-treated mother animal (6)	0.46±0.13	0.34–0.70
Thiamine-treated fetuses (4)	1.20±0.55	0.50–1.80

TABLE V

EFFECT OF THIAMINE TREATMENT ON THE ACCUMULATION OF [1-^{14}C]LACTATE AND $^{14}CO_2$ DURING GLYCOLYSIS OF [3,4-$^{14}C_2$]GLUCOSE IN FETAL RAT LIVER CELLS

Fetal rat liver cells isolated from fetuses of thiamine-treated mother animals were incubated in stoppered Warburg vessels in the presence of [3,4-$^{14}C_2$]glucose as described under Materials and Methods. Reaction time 30 min. Temperature 37 °C.

Expt	dpm recovered		% of pyruvate oxidized
	In lactate	In CO_2	
1	41 600	18 200	30
2	21 000	9 000	31

Liver cells, isolated from the fetuses of thiamine treated-mother rats were incubated in the presence of [3,4-$^{14}C_2$]glucose. It can be calculated from the data given in Table V, that in these cells 30% of the pyruvate is oxidized by the mitochondria in contrast to the untreated fetuses, where only 10% is oxidized. It can be concluded that the flux through pyruvate dehydrogenase is stimulated by the thiamine treatment.

DISCUSSION

When fetal rat liver cells are incubated in the presence of FCCP, valinomycin or nigericin a stimulation of lactate accumulation is observed concomittant with a fall in the ATP/ADP ratio and a rise in the lactate/pyruvate ratio. As can be seen from Fig. 2, cross-over plots do not show a control point at phosphofructokinase. As a control point should demonstrate itself as a cross-over point, whereas not every cross-over is necessarily a control point [37], it is unlikely that phosphofructokinase has a strong regulatory function in glycolysis of fetal rat liver cells under these conditions. This finding confirms the conclusion from earlier studies [11, 38] on the effect of pH on glycolysis in fetal rat liver cells. These studies suggested that phosphofructokinase contributed only to a minor degree to the control of glycolysis, but that hexokinase (ATP : D-hexose 6-phosphotransferase, EC 2.7.1.2) was the main controlling enzyme. The cross-over plot shown in Fig. 2 is consistent with control at hexokinase. However, it should be pointed out that glycogen breakdown contributed significantly to the glycolytic flux, in view of the low specific activity of the lactate recovered (Table III).

In the presence of FCCP or valinomycin production of $^{14}CO_2$ in the pyruvate dehydrogenase step is stimulated (Table III); in the presence of nigericin an inhibition is observed. These differences in results are probably related to differences in the mechanisms by which these agents act. FCCP and valinomycin are both uncouplers of mitochondrial oxidative phosphorylation [39]. Nigericin in low concentration is not an uncoupler but catalyzes an electroneutral K^+–H^+ exchange, resulting in K^+ depletion of the mitochondria. The action of these agents on cellular membranes is not clear. Poole et al. [40] observed that, when ascites tumor cells were incubated in the presence of 2,4-dinitrophenol, valinomycin or nigericin the cells lost K^+ and took up H^+ as measured by the decrease in intracellular pH. In the pres-

ence of nigericin the K^+ efflux was 3 times higher than in the presence of 2,4-dinitrophenol or valinomycin. The strong K^+-depletion of cells and mitochondria in the presence of nigericin may well be the cause of the inhibitory effect of nigericin on $^{14}CO_2$ production.

Regulation of pyruvate dehydrogenase by phosphorylation and dephosphorylation as described by Linn et al. [6, 7] could play a role in pyruvate oxidation. Such a regulatory mechanism could be operative in fetal liver cells as previous studies with isolated fetal rat liver mitochondria have yielded evidence for regulation of pyruvate dehydrogenase by phosphorylation and dephosphorylation [10].

According to Roche and Reed [36] thiamine phosphate at a concentration of 100 μM inhibits phosphorylation of the isolated pyruvate dehydrogenase complex.

Administration of high doses of thiamine could lead to a situation in which more pyruvate dehydrogenase is in the active form.

When fetal rat liver cells isolated from thiamine-treated animals were incubated with [3,4-$^{14}C_2$]glucose, it was observed that more pyruvate was oxidized (Table V). These results indicate that in fetal rat liver cells pyruvate oxidation is controlled at the level of pyruvate dehydrogenase, presumably by phosphorylation and dephosphorylation of the enzyme complex via the mechanism suggested by Roche and Reed [36].

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