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Metabolic Effects of Pent-4-enoate in Isolated Perfused Rat Heart

By J. KALERVO HILTUNEN
Department of Medical Biochemistry, University of Oulu, SF-90220 Oulu 22, Finland

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The metabolic effects of the hypoglycaemic agent pent-4-enoate were studied in isolated, beating or potassium-arrested rat hearts. The addition of 0.8mM-pent-4-enoate to the perfusion fluid increased O_2 consumption by 76% in the arrested heart and by 14% in the beating heart; the concentration ratio of phosphocreatine/creatine increased concomitantly by 47% and 27% respectively. Perfusion of the heart with pent-4-enoate resulted in a 30-fold increase in the concentration of the pool of tricarboxylic acid-cycle intermediates in the tissue, about 90% of this increase being due to malate. The sum of the concentrations of the myocardial free amino acids remained virtually unchanged during the accumulation of the tricarboxylic acid-cycle intermediates. It was concluded that pent-4-enoate can be effectively metabolized in the myocardium and that its metabolism probably proceeds via propionyl-CoA, since pent-4-enoate reproduces many of the metabolic characteristics of propionate in the cardiac muscle. The accumulation of the tricarboxylic acid-cycle intermediates is probably due to carboxylation of propionyl-CoA. The response pattern of the metabolite concentrations in the cardiac muscle is quite different from that in the liver, in which decrease of the concentrations of the tricarboxylic acid-cycle intermediates has been observed previously [Williamson, Rostand & Peterson (1970) *J. Biol. Chem.* **245**, 3242–3251].

The hypoglycaemic agent pent-4-enoic acid has been reported to inhibit the oxidation of long-chain fatty acids, pyruvate and 2-oxoglutarate and the synthesis of citrulline in isolated liver mitochondria (Senior & Sherratt, 1968*a*,*b*; Fukami & Williamson, 1971; Glasgow & Chase, 1976), and to inhibit gluconeogenesis in the isolated perfused liver (Williamson *et al.*, 1970). Inhibition of the citric acid cycle and cellular energy production has also been observed during the metabolism of pent-4-enoate in the liver (Williamson *et al.*, 1970).

Previous reports have shown that glucose oxidation in the isolated perfused rat heart is inhibited more than O_2 consumption during potassium-induced cardiac arrest (Hassinen & Hiltunen, 1975; Hiltunen & Hassinen, 1976). These results suggested that the carbohydrate-to-fat ratio of the fuel consumed by the heart is dependent on the cellular energy state. This prompted a study using inhibitors of fatty acid oxidation. The results obtained with pent-4-enoate, however, were unlike anything observed in other tissues.

The pool size of the citric acid-cycle intermediates in cardiac tissue can vary, depending on metabolic conditions such as the cellular energy state or the presence of the substrates than can form acetyl-CoA, i.e. pyruvate, glucose, acetate or fatty acids (Safer & Williamson, 1973; Randle *et al.*, 1970; Davis & Bremer, 1973; Garland & Randle, 1964). This must be the result of an 'anaplerotic' mechanism capable of replenishing the citric acid-cycle intermediates. Carbon skeletons of certain amino acids may be channelled to the citric acid cycle by coupled transaminations or the 'purine nucleotide cycle' (Safer & Williamson, 1973; Davis & Bremer, 1973; Lowenstein, 1972). The pyruvate carboxylase reaction in cardiac tissue is usually considered unimportant, because of the low activity of the enzyme (Scrutton & Utter, 1968). During the metabolism of propionate, however, the carboxylation of propionyl-CoA results in an accumulation of citric acid-cycle intermediates (Davis *et al.*, 1972; Smith *et al.*, 1965).

In the present study one of the main characteristics of the pent-4-enoate metabolism in the heart was large accumulation of intermediates of the citric acid cycle. The results suggest that, unlike other tissues, the heart is able to metabolize acrylyl-CoA rapidly after its initial reduction to propionyl-CoA, which is then metabolized further by mechanisms that are also used for the disposal of citric acid-cycle intermediates.

Experimental

Reagents

Enzymes were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and Boehringer G.m.b.H., Mannheim, Germany. The standard reagents were obtained from E. Merck A.G., Darmstadt, Germany, the nucleotides and coenzymes from Boehringer,

and dithiothreitol was from Sigma. Pentanoic acid, pent-4-enoic acid and hexanoic acid were obtained from Fluka A.G., Buchs, Switzerland.

Animals and the method of perfusion

Female Long–Evans rats from this Department's own stocks were used. No period of food deprivation preceded the experiments. The rats were anaesthetized by intraperitoneal injection of Nembutal (80–100mg/kg body wt.) and injected intravenously with 500i.u. of heparin 1min before excision of the heart. The hearts were perfused with Krebs–Ringer bicarbonate solution, pH7.4 (Krebs & Henseleit, 1932), containing 2.5mM-$CaCl_2$ and 10mM-glucose in equilibrium with O_2/CO_2 (19:1), as described by Langendorff (1895), but without the recirculation at a hydrostatic pressure of 7.84kPa (80cm of water). The O_2 concentration in the venous perfusate was monitored with a Radiometer E5046 oxygen electrode.

Heart extracts

Samples were obtained from the heart by using aluminium clamps cooled with liquid N_2 (Wollenberger *et al.*, 1960). Initial acid extraction from the frozen, pulverized sample was performed by using 8% (v/v) $HClO_4$ in 40% (v/v) ethanol, precooled to −20°C to ensure quenching in the frozen state (Williamson & Corkey, 1969). Extraction was repeated with 6% (v/v) $HClO_4$, and the filtrate neutralized to pH6 with 3.75M-K_2CO_3 containing 0.5M-triethanolamine hydrochloride.

Metabolites

The concentrations of metabolites in the heart extracts were assayed by enzymic methods; the formation or disappearance of NAD(P)H was calculated from $A_{340}-A_{385}$ measured in an Aminco DW-2 dual-wavelength spectrophotometer by using $\varepsilon_{340-385} = 5330$ litre·mol^{-1}·cm^{-1}. Citrate was measured with citrate lyase (EC 4.1.3.6) (Gruber & Möllering, 1966), 2-oxoglutarate by the method of Narins & Passonneau (1970), pyruvate by the method of Bücher *et al.* (1963), lactate as described by Hohorst (1963), and malate and oxaloacetate essentially by the method of Williamson & Corkey (1969).

CoASH and acetyl-CoA were measured with 2-oxoglutarate dehydrogenase (EC 1.2.4.2) and phosphotransacetylase (EC 2.3.1.8) (Tubbs & Garland, 1969). 2-Oxoglutarate dehydrogenase was prepared from pig heart by the method of Sanadi (1969). Creatine was determined by the method of Bernt *et al.* (1970), ATP was determined by using hexokinase (EC 2.7.1.1) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49) (Lamprecht & Trautschold, 1970), and phosphocreatine in the same assay by the subsequent addition of ADP and creatine kinase (EC 2.7.3.2).

Free amino acids

A sample of the neutralized $HClO_4$ extract of the tissue was freeze-dried and dissolved in 0.1M-citric acid solution, and the pH adjusted to 2.2 by additions of 1M-NaOH. The amino acids were measured by ion-exchange chromatography by using a JEOL JLC-5AH automatic amino acid analyser.

Results

The metabolic effects and metabolism of pent-4-enoate were largely dependent on the energy state of the myocardial cells. Two energy states were studied: (1) the beating, retrogressively perfused heart, and (2) the resting, arrested heart. The latter condition was induced by increasing the perfusate potassium concentration to 15mM.

Oxygen consumption and tissue concentrations of ATP, phosphocreatine and creatine

After KCl-induced arrest, the cardiac O_2 consumption was rapidly inhibited by 64% (Table 1). Hexanoate increased the O_2 consumption by 30% in the presence of 10mM-glucose, and under the same

Table 1. *Effects of hexanoate, pentanoate and pent-4-enoate on O_2 consumption and perfusate lactate/pyruvate ratio in isolated perfused rat heart*

The hearts were pre-perfused for 15min with buffer containing glucose (10mM) before switching to a perfusate containing either glucose alone, or glucose plus the fatty acid to be tested, and the perfusion was then continued. Values are means±S.E. of measurements at 15min after the commencement of perfusion with the medium indicated. The numbers of independent biological observations are given in parentheses. *P* (versus beating heart) † <0.0005. *P* (versus control) * <0.05, ** <0.0025, *** <0.0005.

	O_2 consumption (μmol/min per g dry wt.)		Lactate/pyruvate (molar ratio)	
	Beating	Potassium-arrested	Beating	Potassium-arrested
Control	27.2±1.6 (9)	9.7±1.3 (8)†	2.01±0.31 (6)	2.40±0.37 (7)
0.8mM-Hexanoate	28.8±1.3 (7)	12.8±0.7 (7)*	11.3±1.7 (7)***	10.1±1.5 (6)***
0.8mM-Pent-4-enoate	31.0±1.5 (9)	17.2±1.7 (7)**	2.76±0.39 (5)	7.89±0.97 (6)***
0.8mM-Pentanoate	31.0±1.3 (6)	16.9±0.7 (6)***	1.50±0.17 (6)	8.41±1.32 (6)

Table 2. *Tissue content of ATP, phosphocreatine and creatine in isolated perfused rat heart*
Hearts from rats fed *ad libitum* were perfused by the Langendorff (1895) procedure with Krebs–Ringer bicarbonate medium containing 10mM-glucose. After pre-perfusion for 15min the hearts were arrested with potassium or allowed to beat and the perfusions were continued for another 15min with added pent-4-enoate, pentanoate or hexanoate. At the end of the perfusion period they were freeze-clamped and analysed for metabolites. Values are means±S.E. for the number of independent biological observations in parentheses. *P* (versus beating heart) † <0.05. *P* (versus control) * <0.05, ** <0.0025.

	[ATP] (μmol/g dry wt.)		Phosphocreatine/creatine (molar ratio)		Phosphocreatine+creatine (μmol/g dry wt.)	
	Beating	Arrested	Beating	Arrested	Beating	Arrested
Control	23.5 (5) ±1.5	22.4 (6) ±1.0	1.71 (5) ±0.22	2.59 (5)† ±0.36	61.9 (5) ±2.0	59.7 (5) ±2.42
0.8mM-Hexanoate	21.9 (7) ±1.0	21.9 (7) ±1.0	2.85 (5) ±0.44	2.51 (5) ±0.42	63.1 (5) ±4.2	62.9 (5) ±3.2
0.8mM-Pent-4-enoate	23.0 (5) ±1.3	23.1 (6) ±1.5	2.17 (6) ±0.23	3.81 (6)* ±0.45	62.3 (6) ±3.9	61.1 (6) ±2.8
0.8mM-Pentanoate	20.2 (5)* ±1.0	20.5 (5) ±0.5	2.78 (5) ±0.14	4.49 (5)** ±0.15	57.1 (5)* ±1.3	60.9 (5) ±2.5

Table 3. *Effects of pent-4-enoate, pentanoate and hexanoate on the tissue content of CoASH and acetyl-CoA in rat heart*
The conditions are as given in Table 2. *P* (versus control heart) * <0.05, ** <0.0025, *** <0.0005.

	[Acetyl-CoA] (nmol/g dry wt.)		[CoA] (nmol/g dry wt.)	
	Beating	Arrested	Beating	Arrested
Control	46± 5 (7)	54± 4 (5)	370±26 (7)	337±20 (5)
Pent-4-enoate				
0.05mM	25± 9 (3)*	22± 7 (3)**	167±34 (3)**	255±38 (3)
0.2mM	32± 6 (4)	31± 4 (4)**	54± 6 (4)***	66±15 (4)***
0.8mM	36± 7 (4)	33±15 (6)	39± 7 (4)***	26± 7 (6)***
3.2mM	—	11± 6 (3)***	—	13± 7 (3)***
Pentanoate				
0.8mM	126±13 (6)***	131±12 (6)***	39± 3 (6)***	72± 6 (6)***
Hexanoate				
0.8mM	332±28 (7)***	320±17 (7)***	32± 5 (7)***	32± 5 (7)***

conditions pent-4-enoate and pentanoate both increased the O_2 consumption by 75% (the absolute value of the increase being about 7μmol of O_2/min per g dry wt.).

The same three fatty acids only slightly increased the O_2 consumption of a beating heart, the increase being larger with the C_5 acids than with the C_6 acid. The concentration ratio of phosphocreatine/creatine was higher in the arrested heart, but there was little difference in the ATP concentration (Table 2). It is noteworthy that the fatty acids studied increased the concentration ratio of phosphocreatine/creatine, and that the highest ratios were in arrested hearts perfused with pentanoate (0.8mM), though pent-4-enoate also led to an increase.

Tissue concentrations of CoASH and acetyl-CoA

The mechanical work done by the heart had only a small effect on the concentrations of CoASH and acetyl-CoA. The three fatty acids tested exercised quite different effects on the concentrations of free CoASH and acetyl-CoA. At a concentration of 0.8mM they decreased the free CoASH concentration by the same amount (Table 3). Hexanoate increased the acetyl-CoA concentration by about seven times and pentanoate by about three times, but pent-4-enoate decreased it by 12%. In the hexanoate perfusion the free CoASH was converted into acetyl-CoA, whereas in the pentanoate perfusion the loss of CoASH was greater than the formation of acetyl-CoA. As far as CoASH and acetyl-CoA are concerned, the effects of pent-4-enoate in the heart are qualitatively similar to its effects on the liver, i.e. sequestering of CoA in acid-soluble derivatives other than acetyl-CoA (Fukami & Williamson, 1971; Williamson *et al.*, 1969).

Tricarboxylic acid-cycle intermediates

As work in this laboratory has shown previously (Hiltunen & Hassinen, 1977), cardiac arrest increased the amount of the combined citric acid cycle-intermediates by about 100%. However, perfusion of an arrested heart with a saturated or unsaturated odd-number-carbon short-chain fatty acid resulted in a 30-fold increase in the tissue concentration of the tricarboxylic acid-cycle intermediates compared with that in a beating control heart. About 80% of this increase was due to malate (Tables 4 and 5).

Lactate and pyruvate

Perfusion of the arrested heart by hexanoate, pentanoate and pent-4-enoate results in a decrease in the concentration of pyruvate (Table 5). In the beating

Table 4. *Effect of pent-4-enoate, pentanoate and hexanoate on the tissue content of citrate, 2-oxoglutarate and malate in beating and potassium-arrested rat hearts*

The conditions were as given in Table 2. Values are means±S.E. for the number of independent biological experiments given in parentheses. *P* (versus beating control) † <0.01, †† <0.0005. *P* (versus control) * <0.05, ** <0.01, *** <0.001.

	[Citrate] (μmol/g dry wt.)		[2-Oxoglutarate] (μmol/g dry wt.)		[Malate] (μmol/g dry wt.)	
	Beating	Arrested	Beating	Arrested	Beating	Arrested
Control	0.480 (5) ±0.049	1.46 (5)†† ±0.16	0.249 (7) ±0.021	0.364 (5)† ±0.035	0.266 (7) ±0.030	0.237 (5) ±0.045
Pent-4-enoate						
0.05mM	0.601 (3) ±0.099	1.88 (3) ±0.23	0.168 (3)* ±0.054	0.394 (3) ±0.039	0.966 (3)** ±0.140	2.83 (3)*** ±0.22
0.2mM	0.835 (4)* ±0.062	2.31 (4)* ±0.32	0.196 (4)* ±0.009	0.633 (4)* ±0.042	4.57 (4)*** ±0.28	13.7 (4)*** ±2.2
0.8mM	0.633 (5) ±0.118	2.55 (6)* ±0.44	0.229 (4) ±0.026	0.773 (6)*** ±0.067	4.18 (6)*** ±0.83	30.2 (6)*** ±2.7
3.2mM	—	2.08 (3)* ±0.10	—	0.732 (3)*** ±0.032	—	26.6 (3)*** ±2.0
Pentanoate						
0.8mM	0.719 (5)** ±0.051	4.93 (6)*** ±0.29	0.317 (6) ±0.033	0.704 (6)*** ±0.017	13.7 (6)*** ±0.6	21.8 (6)*** ±2.2
Hexanoate						
0.8mM	2.16 (7)*** ±0.11	2.39 (7)*** ±0.06	0.167 (7)** ±0.022	0.196 (7)** ±0.019	0.389 (7)* ±0.039	0.388 (7)* ±0045

Table 5. *Effect of pent-4-enoate, pentanoate and hexanoate on the pool size of the citric acid-cycle intermediates, oxaloacetate and pyruvate in isolated perfused rat heart*

The conditions were as given in Table 2. Since the sum of the concentrations of succinyl-CoA, succinate, fumarate, oxaloacetate and isocitrate is small compared with the total pool, the sum of the tissue concentrations of citrate, 2-oxoglutarate and malate is taken here to represent the pool size. Values are means±S.E. for the numbers of independent biological observations given in parentheses. *P* (versus control) ** <0.005, *** <0.0005.

	Sum of [citrate], [2-oxoglutarate] and [malate] (μmol/g dry wt.)		[Oxaloacetate] (nmol/g dry wt.)		[Pyruvate] (nmol/g dry wt.)	
	Beating	Arrested	Beating	Arrested	Beating	Arrested
Control	1.00	2.06	18±10 (7)	6.3±0.9 (5)***	172±15 (7)	163±34 (5)
Pent-4-enoate						
0.05mM	1.74	5.10	36± 8 (3)	23± 3 (3)***	—	—
0.2mM	5.60	16.6	73±12 (4)**	72±20 (4)**	—	—
0.8mM	5.04	33.5	78± 7 (6)***	87±12 (6)***	184±20 (5)	92±14 (6)**
3.2mM	—	29.4	—	96±20 (3)**	—	—
Pentanoate						
0.8mM	14.7	27.4	158±23 (6)***	35± 1 (6)***	254±19 (6)**	47± 4 (6)**
Hexanoate						
0.8mM	2.71	2.98	—	—	100± 8 (7)**	107±15 (6)

heart, only hexanoate affects the concentration of pyruvate.

The redox change in the cytosolic NAD^+ couple on the addition of the fatty acids to the medium used to perfuse the heart was largely dependent on the metabolic state of the heart. Pent-4-enoate had no effect, however, and pentanoate caused an oxidation of NADH in the beating heart (Table 1). In the arrested heart, all three fatty acids tested caused a reduction of NAD^+.

Free amino acids

Because of the great changes in the pool size of the citric acid-cycle intermediates, an attempt was made to identify the precursors of the cycle intermediates. Goldstein & Newsholme (1976) have pointed out that the citric acid cycle is capable of oxidizing only acetyl-CoA, and all substrates ultimately converted into the cycle intermediates must be disposed of by conversion into acetyl-CoA if they are oxidized to CO_2. These mechanisms are needed in most tissues. Some amino acids can be converted into the cycle intermediates, and among these amino acids the concentrations of glutamate and aspartate changed in the present experiments (Table 6). Cardiac arrest as such markedly decreased the concentration of aspartate, but the concentration of glutamate tended to increase simultaneously. Pent-4-enoate, however, increased the concentration of aspartate and decreased that of glutamate. The mass-action ratio of aspartate aminotransferase did not change during these changes in amino acid concentrations, indicating that this enzyme reaction remains in near-equilibrium. Cardiac arrest or the addition of pent-4-enoate decreases the concentration of alanine. The changes occurring in the equilibrium of the alanine aminotransferase reaction on the addition of pent-4-enoate are dependent on the metabolic state of the heart. In the beating heart, pyruvate and alanine concentrations change in opposite directions and the equilibrium shifts towards pyruvate and glutamate on the addition of pent-4-enoate.

Discussion

The research literature on pent-4-enoate is mainly concerned with its hypoglycin (α-amino-2-methylenecyclopropanepropanoic acid)-like and inhibitory effects in the liver and in the mitochondria. The present paper describes some characteristics of pent-4-enoate oxidation in cardiac muscle and demonstrates the potential of this compound in studies on the disposal and replenishment of citric acid-cycle intermediates in muscle tissue.

Cardiac arrest increases the total amount of the citric acid-cycle intermediates by about 1 μmol/g dry weight, but the anaplerotic mechanism responsible for this increase has not been positively identified. The simultaneous decrease in the amount of amino acids feeding into the cycle (mainly aspartate) is about 7 μmol/g dry weight. The concentration of alanine decreases, which may indicate that the increase in the cycle intermediates is not due to a coupled transamination involving aspartate transaminase and alanine transaminase (Safer & Williamson, 1973). The production of NH_3 decreases during cardiac arrest (T. Takala, unpublished work), which indicates that the purine-nucleotide cycle (Lowenstein, 1972) is not an important anaplerotic mechanism under these conditions.

Hexanoate increases the total amount of cycle intermediates 1.7-fold, and cardiac arrest then causes no further increase in the amount of these metabolites under these conditions. In the arrested heart, 0.8 mM-

Table 6. *Tissue content of free amino acids in isolated perfused rat heart*

The conditions were as given in Table 1. Values are means±S.E. from five independent biological observations. *P* (versus beating heart under same conditions) †† <0.0125; ††† <0.0005. *P* (versus beating or arrested control respectively) * <0.05, ** <0.0125, *** <0.0005.

	Content (μmol/g dry wt.)			
	Beating heart		Potassium-arrested heart	
Amino acid	Control	0.8 mM-Pent-4-enoate	Control	0.8 mM-Pent-4-enoate
Glutamate	25.4±2.1	10.4±1.1***	28.5±1.6	21.6±1.7†††**
Aspartate	13.2±0.6	28.0±1.9***	3.62±0.32†††	15.5±0.9***
Glutamine	18.2±0.8	16.8±0.7	16.2±1.2	15.2±0.7
Asparagine	2.1±0.3	1.99±0.14	1.43±0.17	1.92±0.30
Lysine	2.5±0.3	2.46±0.28	2.43±0.20	2.02±0.28
Sum	61.4	59.7	52.2	56.2
Alanine	3.24±0.29	2.32±0.12**	2.41±0.24††	1.73±0.07*
Glycine	1.73±0.21	1.63±0.149	1.36±0.13	1.59±0.12
Serine	1.32±0.20	1.40±0.16	1.01±0.11	0.98±0.23
Threonine	1.27±0.14	1.35±0.14	0.98±0.08	1.07±0.12

pent-4-enoate increases the total amount of the citric acid-cycle intermediates by 3000% in comparison with the beating heart perfused without pent-4-enoate. In the beating heart the total amount of citric acid-cycle intermediates constitutes one-sixtieth of the pool of the free amino acids, whereas in a heart perfused with pent-4-enoate it accounts for one-half of the free amino acid pool, the size of which remains almost unchanged. It is thus evident that the amino acids are not the precursors of the cycle intermediates under these conditions.

The results show (Fig. 1) that the rate of accumulation of the cycle intermediates is constant during the first 15min after the addition of pent-4-enoate. This also indicates that the capacity of the heart to dispose of the cycle intermediates is relatively limited. The results also show that this considerable metabolite accumulation can proceed without adverse effects on the cellular energy state.

Davis *et al.* (1972) found that the heart muscle is able to metabolize propionate at a high rate (2.7 μmol/min per g dry wt.). The metabolism of one molecule of propionate results in the synthesis of one molecule of citric acid-cycle intermediates and involves the fixation of CO_2 (Smith *et al.*, 1965). Propionate also causes an accumulation of citric acid-cycle intermediates (Davis *et al.*, 1972; Smith *et al.*, 1967). Therefore one could assume that propionate may be responsible for the metabolite pattern during the oxidation of pent-4-enoate.

In this connexion it is noteworthy that the presence of pent-4-enoate in the liver causes a decrease in the citric acid-cycle metabolites (Williamson *et al.*, 1970). The metabolism of pent-4-enoate in the liver is usually thought to proceed from the initial formation of acrylyl-CoA and acetyl-CoA, but the fate of the acrylyl-CoA has remained obscure. It has been suggested that it is eliminated by the action of non-specific deacylases (Holland & Sherratt, 1973; Sherratt & Osmundsen, 1976), but this may not be true in cardiac muscle. It is more probable that acrylyl-CoA formed from pentenoate in the heart is metabolized via the propionate pathway (Smith *et al.*, 1967). For this to occur, a reduction to propionyl-CoA is necessary. The enzymes involved have not been identified, but one possibility could be the mitochondrial NADPH-dependent αβ-unsaturated acyl-CoA reductase (Seubert *et al.*, 1968). The substrate specificity of this enzyme spans at least C_4 to C_{16} fatty acyl derivatives (Seubert *et al.*, 1968), but C_3 fatty acyl derivatives have not been tested as substrates. The metabolic effects of the saturated C_5 fatty acid pentanoate in control experiments are very similar to those of pent-4-enoate. These results also suggest that cardiac muscle is able to reduce rapidly the double bond in pent-4-enoate.

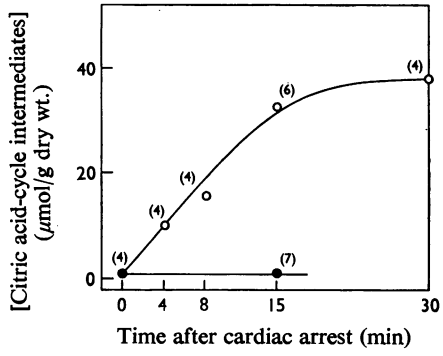

Fig. 1. *Accumulation of citrate, 2-oxoglutarate and malate in isolated potassium-arrested rat heart perfused with 0.8 mM-pent-4-enoate*

Each value is the mean for the number of observations given in parentheses. ○, Pent-4-enoate; ●, beating control heart.

That pent-4-enoate in heart muscle is metabolized via the propionyl-CoA and methylmalonyl-CoA pathway to succinyl (3-carboxypropionyl)-CoA is also indicated by the fact that pent-4-enoate increases the incorporation of [^{14}C]bicarbonate into the citric acid-cycle intermediates (M. Nuutinen, J. Peuhkurinen, K. Hiltunen & I. Hassinen, unpublished work). Complete oxidation of pent-4-enoate requires the conversion of C_4 metabolites into pyruvate, which could be effected by the NAD^+ and NADP-linked malate dehydrogenases (Lin & Davis, 1974; Davis *et al.*, 1972). In fact the concentration of pyruvate plus lactate increases during the metabolism of pent-4-enoate at the same time as the glycolytic flux decreases (results not shown). This may be due to the action of the 'malic' enzymes or phosphoenolpyruvate carboxykinase and pyruvate kinase.

The energy equivalence of the O_2 consumption is lower when pentanoate is oxidized to malate and CO_2 (0.205 mol of O_2/mol of ATP) than during the aerobic metabolism of glucose (0.158 mol of O_2/mol of ATP) or long-chain fatty acids (0.178 mol of O_2/mol of ATP). If the ATP consumption remains constant and the carbohydrate/fat ratio of the fuel oxidized by the arrested heart is 0.29 (Hiltunen & Hassinen, 1976), the O_2 consumption of the heart should rise by 18% on switching to pentanoate oxidation. This is considerably less than the 76% increase actually observed. The O_2 consumption of the heart allows for the oxidation of about 5 μmol of pent-4-enoate/min per g dry weight, which is in excess of the observed rate of propionate uptake in the heart (Davis *et al.*, 1972). It is evident that the oxidation of pent-4-enoate and pentanoate (and propionate) is limited by the mechanism for the disposal of the intermediates of the tricarboxylic acid cycle. The huge accumulation of metabolites under the conditions of the present study may indicate that the flux to and

from the citric acid cycle at steps other than citrate synthase is probably relatively small in cardiac muscle under physiological conditions, and that in this tissue the metabolic balance can be maintained by rather low activities of the anaplerotic mechanisms.

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