THE REGULATION OF PYRUVATE DEHYDROGENASE IN SKELETAL MUSCLE *IN VIVO* AND PERFUSED HEART

By

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ABSTRACT

The goal was to determine the physiological significance of pyruvate dehydrogenase (PDH) activation in muscle. The PDH activity was measured in small samples of muscle obtained by needle biopsy from normal volunteers. In heart PDH was measured using an isolated perfused rat heart preparation. The PDH activity was measured by determining $^{14}Co_2$ production from pyruvate-1-^{14}C using whole tissue homogenates.

The total PDH (PDH_t) activity in human skeletal muscle was 302 ± 10 nmol/g/min. with about 40 ± 3% in the active form (PDH_a). With aerobic exercise (60% $\dot{V}O_2max$) more than 88% of PDH was converted to PDH_a within 5 min. Aerobic training did not increase the resting muscle PDH_t activity (untrained 304 ± 4, trained 309 ± 8 nmol/g/min.) but it increased the PDH_a from 124 ± 9 nmol/g/min. to 215 ± 15 nmol/g/min.

Muscle immobilization by arm cast for 6 weeks decreased the PDH_a from 112 ± 4 to 28 ± 4 nmol/g/min. but it had no effect on the PDH_t activity. Exercise following immobilization decreased the rate and the extent of PDH activation while exercise after weight training increased the extent of PDH activation.

Starvation for 24 and 48 hr. in rat heart decreased PDH_a from 2768 ± 73 to 1286 ± 109 and 920 ± 149 nmol/g/min. respectively.

In the perfused heart PDH was fully activated by epinephrine (1.3μg/ml), insulin (1 mU/ml) and high (>0.2 mM) pyruvate concentration. PDH activation was not mediated by cyclic AMP since insulin activated PDH but did not change the heart cyclic AMP concentration. Propranolol prevented epinephrine from activating PDH. Octanoic acid decreased the proportion of PDH in the active form.

In the heart a detailed study of the relationship between the rate of heart pyruvate oxidation and PDH activation was undertaken. In all experimental conditions where the rate of pyruvate oxidation was changed, parallel changes in the PDH_a activity was seen. The correlation between PDH activation and rate of heart pyruvate oxidation was greater than 0.92. These findings suggest that interconversion of PDH_a and PDH_i probably control the rate of heart pyruvate oxidation. A similar correlation was not seen in skeletal muscle during exercise. The calculated rate of muscle pyruvate oxidation was several fold greater than the extent of PDH activation. Although the activation of PDH regulates pyruvate oxidation in both heart and skeletal muscle, in heart this is achieved through the interconversion of active and inactive PDH whereas in skeletal muscle allosteric conversion of the active form of PDH plays a greater part.

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ABBREVIATIONS

Biochemical and Chemical Compounds

ADP	-	Adenosine-5'-diphosphate
ALD	-	Alanine dehydrogenase
AMP	-	Adenosine-5'-monophosphate
cAMP	-	Cyclic adenosine-3',5'-monophosphate
ATP	-	Adenosine-5'-triphosphate
CK	-	Creatine kinase
CoA	-	Coenzyme A
CP	-	Creatine phosphate
CPK	-	Creatine phosphate kinase
Cr	-	Creatine
DNA	-	Deoxyribonucleic acid
F-1-P	-	Fructose-1-phosphate
F-1.6-P	-	Fructose-1,6-phosphate
F-6-P	-	Fructose-6-phosphate
F-6-Pase	-	Fructose-6-phosphatase
FFA	-	Free Fatty Acids
FA-CoA	-	Fatty Acid Coenzyme A
G-1-P	-	Glucose-1-phosphate
G-6-P	-	Glucose-6-phosphate
G-6-PD	-	Glucose-6-phosphate dehydrogenase
HK	-	Hexokinase

LDH	-	Lactate dehydrogenase
NAD^+	-	Nicotinamide-adenine dinucleotide, oxidized
NADH	-	Nicotinamide-adenine dinucleotide, reduced
$NADP^+$	-	Nicotinamide-adenine dinucleotide phosphate, oxidized
PDH	-	Pyruvate dehydrogenase
PDH_i	-	Pyruvate dehydrogenase, inactive (phosphorylated)
PDH_a	-	Pyruvate dehydrogenase, active (dephosphorylated)
PDH_t	-	Pyruvate dehydrogenase, total: sum of (PDH_a + PDH_i)
PEP	-	Phosphoenol pyruvate
PFK	-	Phosphofructokinase
PK	-	Pyruvate kinase
TPP	-	Thiamine pyrophosphate

DTT	-	Dithiothreitol
EDTA	-	Ethylenediaminetetraacetate
KRB	-	Krebs-Ringer bicarbonate
PCA	-	Perchloric acid
Tris	-	Tris(hydroxymethyl)aminomethane

SYMBOLS AND UNITS

dpm	-	Disintegrations per minute
r	-	Correlation coefficient
γ	-	Femtomoles
g	-	Grams
ht	-	Heart
i. units	-	International Units
Kpm	-	Kilopond meter
$\dot{V}O_2$ max	-	Maximum oxygen consumption
MVIC	-	Maximum voluntary isometric contraction
$\bar{x}$	-	Mean value
K_m	-	Michaelis constant
μl	-	Microliter
μg	-	Microgram
mmHg	-	Millimeter mercury
min.	-	Minute
ng	-	Nanogram
nmol	-	Nanomole
n	-	Number of observations
PCo_2	-	Partial pressure carbon dioxide
Po_2	-	Partial pressure oxygen
RQ	-	Respiratory exchange ratio (quotient)
sec.	-	Second
SEM	-	Standard error of the mean

VO_2 - Total oxygen consumption

Wet m. - Wet muscle weight

INTRODUCTION

The metabolism of glucose or glycogen to pyruvate and its subsequent oxidation to Co_2 is essential for normal muscle function. Its partial oxidation to pyruvate or lactate generates a limited but essential amount of energy (ATP) required for rapid muscle contraction. With sustained exercise it is necessary to increase and maintain ATP generation as the fundamental driving force for continued work. When glucose or glycogen serve as the major muscle energy source the regulation of pyruvate oxidation to Co_2 is perhaps the most important regulating step in muscle metabolism. Such metabolic regulation could be exerted at a number of different sites in the TCA cycle. On the basis of numerous studies[1-7] it has become generally acknowledged that the oxidation of pyruvate to acetyl CoA is a major, if not the primary regulatory step in the whole process of pyruvate oxidation to Co_2.

Before pyruvate can enter the tricarboxylic acid cycle (TCA), pyruvate must be oxidatively decarboxylated to acetyl CoA. The reaction responsible for this action is made up of several different enzymes known as the Pyruvate Dehydrogenase complex (PDH). In all tissues studied to date, PDH has been found to be a three enzyme complex which is

directly involved in oxidation of pyruvate in the inner mitochondria.[1-7]

The three enzymes are as follows:

1. Pyruvate decarboxylase (EC 1.2.4.1)-Enz_1.
   This enzyme is also known trivially as pyruvate dehydrogenase.*
2. Dihydrolipoate transacetylase (EC 2.3.1.12) -Enz_2.
3. Dihydrolipoate dehydrogenase (EC 1.6.4.3)-Enz_3.

The enzymes catalyze the following overall reaction in a sequential order.[1,7-9]

$$\text{Pyruvate} + \text{CoA} + NAD^+ \longrightarrow \text{Acetyl Co-A} + Co_2 + NADH + H^+$$

The various steps occur in the manner set out below:

(R1) Pyruvate + Thiamine pyrophosphate (TPP)-Enz_1 $\longrightarrow$

Co_2 + hydroxyethyl-thiamine pyrophosphate - Enz_1

(R2) Hydroxyethyl-TPP-Enz_1 + Lipoyl - Enz_2 $\rightleftharpoons$

S-Acetyldihydrolipoyl - Enz_2 + TPP - Enz_1

(R3) S-Acetyldihydrolipoyl - Enz_2 + CoA $\rightleftharpoons$ Acetyl CoA

+ Dihydrolipoyl - Enz_2

---

* The use of pyruvate dehydrogenase here refers to the first reaction of the PDH complex, elsewhere in the thesis PDH is used to indicate the PDH complex.

(R4) Dihydrolipoyl - Enz_2 + FAD - Enz_3 $\rightleftharpoons$ Lipoyl - Enz_2 + FADH - Enz_3

(R5) FADH - Enz_3 + NAD^+ $\rightleftharpoons$ NADH + H^+ + FAD - Enz_3

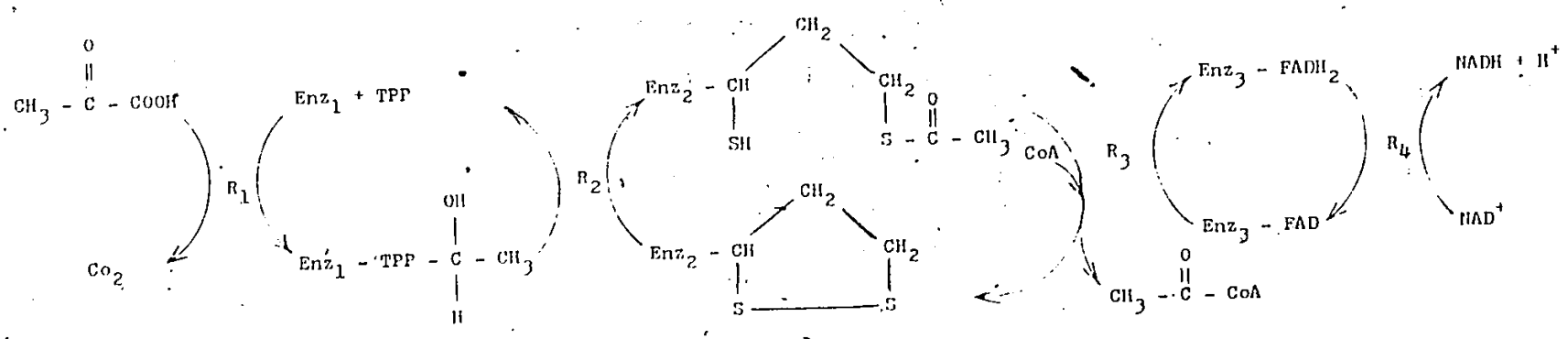
The multienzyme complex forms a structural, functional and regulatory single unit of three enzymes and two other enzymes in higher organisms.[1,8] The first reaction in the sequence is nonreversible while all the remaining reactions are reversible. (Figure 1)

The PDH multienzyme has been isolated from numerous organisms. The enzyme from pig heart muscle was the first to be isolated from the mammalian system.[5,23] Since the 1950's the enzyme has been shown to exist in Escerichia coli,[10,11] Azotobacter,[12] Neurospora crassa,[14,15] Streptococcus,[13a,13b] yeast,[16-18] potatoes,[19] pigeon breast muscle,[3,4,20,21] skin fibroblasts[22] and many mammalian tissues.[5,6,23-25]

1. MAMMALIAN PDH COMPLEX

The original work on the PDH complex (E. coli) postulated that there were 24 polypeptide chains of each enzyme in the even ratio of 1:1:1, for the three enzymes. However, upon closer analysis the ratio was found to be 1:1:0.5,[42] where there were two polypeptide chains of Enz_1 and Enz_2 for each polypeptide chain of the flavoprotein

Figure 1



The reactions and components that constitute the pyruvate dehydrogenase complex. (for explanation refer to text). Enz_1-(pyruvate dehydrogenase) catalyes reaction 1 (R_1), Enz_2-(dihydrolipoate transacetylase) catalyzes reaction 2 (R_2), and Enz_3-(dihydrolipoate dehydrogenase) catalyzes reactions 3 (R_3) and 4 (R_4).

Enz_3. Since then, the exact ratio has not been resolved nor has the molecular weight or polypeptide chain for each enzyme been definitely established.

Like the E. coli complex, the mammalian complex appears to vary between tissue types but the general concensus is that the molecular weight is about 10×10^6 and the complex may contain a nucleus of approximately 60 identical transacetylase units, (enzyme 2, Figure 1), each containing one moiety covalently bound to lipoic acid. When isolated, the pyruvate dehydrogenase (Enz_1, Figure 1), has a molecular weight (MW) of about 154,000 and consists of two subunits, one of 41,000 (α-subunit) which binds TPP and Mg^{++} and decarboxylates pyruvate to give hydroxethyl-TPP. The other subunit of 36,000 (β-subunit), catalyzes the reduced acetylation of the oxidized lipoic acid on the transacetylase.[43] It is postulated, therefore, that the α-subunit of pyruvate dehydrogenase may catalyze reaction 1, while the β-subunit may be responsible for the catalyzing of reaction 2.[34,42] (Figure 1)

Dihydrolipoate dehydrogenase (Enz_3)(Figure 1) has a MW of 110,000 and contains two similar or identical polypeptide chains, each containing one FAD molecule. The purified enzyme has 10 - 12 polypeptide chains of dihydrolipoate dehydrogenase.[25] The transacetylase (Enz_2) functions both catalytically and in a structure forming way. It

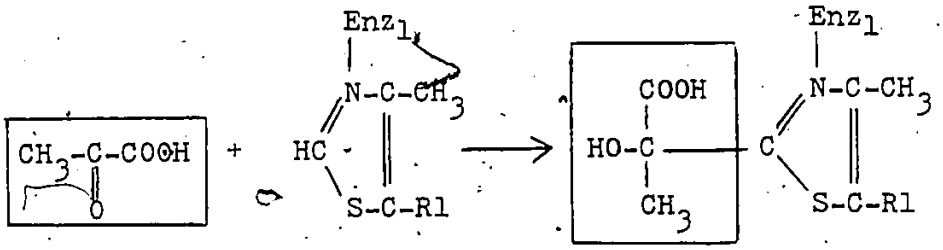
forms the nucleus of the complex with the other two enzymes covalently bound on its surface (surrounded by Enz_1 and Enz_3 subunits).[25,48]

In addition to the three enzymes above, the mammalian enzyme complex contains a phosphatase and a kinase[1,7] which catalyzes the dephosphorylation (reactivation) and the phosphorylation (inactivation) of pyruvate dehydrogenase. This will be discussed in more detail later in the introduction.

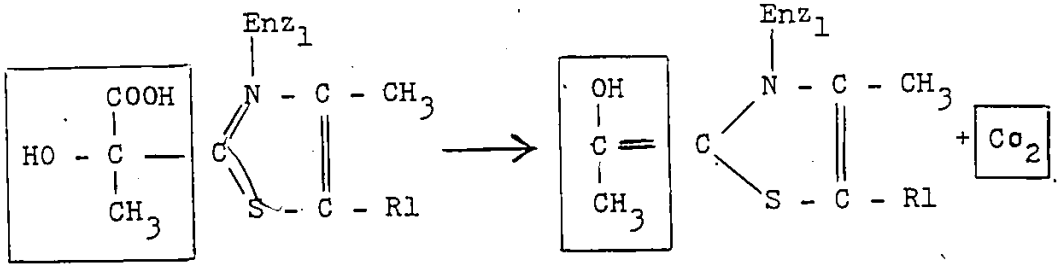
2. ENZYME MECHANISMS

A. Pyruvate Dehydrogenase (Enz_1)

The decarboxylation of pyruvate is initiated by its binding to the 2-position of the thiazole ring of TPP (reaction 1).[23,27-31]



After the binding of pyruvate, Co_2 is released from the enzyme complex of Enz_1 to form Co_2 and hydroxyethyl thiamine pyrophosphate[31-35] (reaction 2).

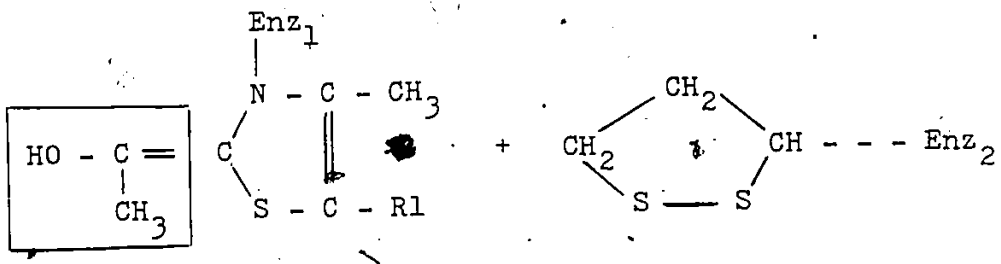
(2) 

Enz_1 - TPP - pyruvate

Enz_1 - TPP - hydroxyethyl

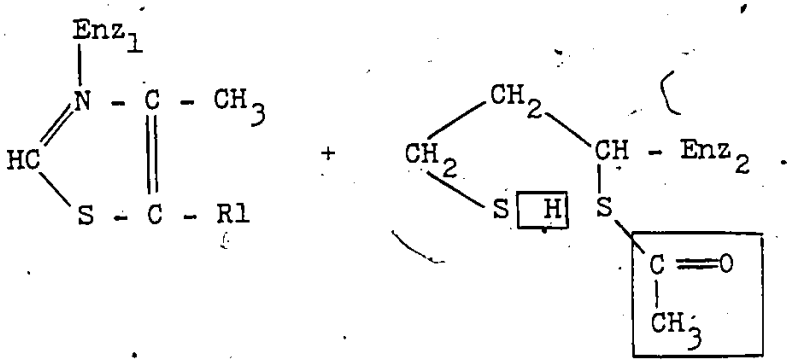
B. Dihydrolipoate Transacetylase (Enz_2)

In mammals this enzyme catalyzes the transfer of the hydroxyethyl group from pyruvate dehydrogenase (Enz_1) to dihydrolipoate transacetylase (Enz_2)(reaction 3).[11]

(3) 

Enz_1 - TPP - hydroxyethyl

Enz_2 (oxidized)

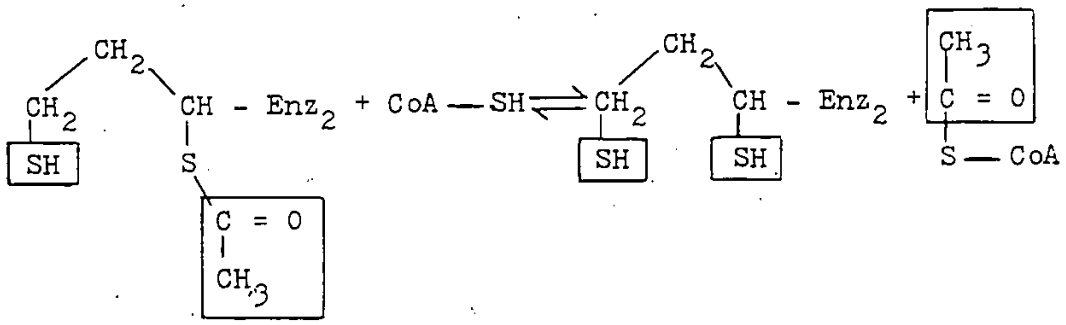


Enz_1 - TPP

Acetyl - Enz_2

Lipoic acid contains 2-sulphur groups which are reduced with the oxidation of the hydroxyethyl group (reaction 3).[13a,13b] The next stage of the reaction involves the formation of acetyl CoA (reaction 4).

(4)

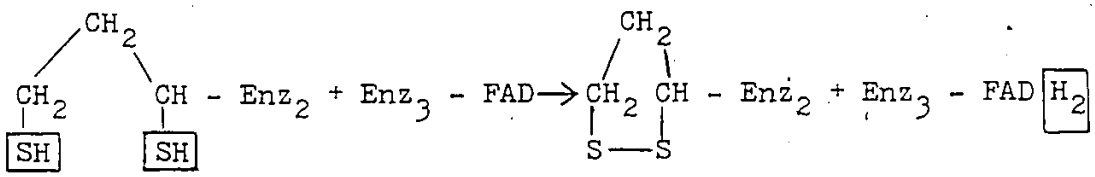


acetyl - Enz_2 Coenzyme A Enz_2 (reduced) Acetyl CoA

Unlike any part of the PDH complex this reaction is freely reversible.[10,11] In fact the reversibility of this reaction is used as a test of purity in the separation of the various enzymes in this sequence. Acetyl CoA is a potent product inhibitor of Enz_2; but its regulatory role remains to be established.[8,36,80,84]

C. Dihydrolipoate Dehydrogenase (Enz_3)

The transfer of electrons from the reduced sulphur groups to NAD^+ involves a complex series of steps. FAD is covalently linked to Enz_3 and is involved in the eventual transfer of electrons to NAD^+.[37,38] The initial electron transfer is thought to occur as follows:

(5) 

Enz_2 - reduced Enz_3-oxidized Enz_2-oxidized Enz_3-reduced

The reduced Enz_3 reacts with free NAD^+ to form NADH (reaction 6).

(6) 

The transfer of electrons from Enz_3 to NAD^+ involves sulfhydryl groups in addition to the FAD.[8,13a,13b,39-41] The detailed sequence of electron transfer is poorly understood. While dihydrolipoate dehydrogenase accepts electrons from lipoate bound to acetyl transferase, the lipoate dehydrogenase does not appear to contain much, if any, lipoate itself.[2,8,37,38]

3. INTERCONVERSION AND REGULATION OF THE ENZYME

Recent work has shown the PDH complex to exist in two enzymatically interconvertible forms, one which is catalytically active and the other which is inactive.[1,7,51,52] The two additional enzymes are a kinase which inactivates the PDH complex in the presence of ATP and low Mg^{++} concentrations by phosphorylation, and a phosphatase which activates or dephosphorylates the complex in the presence of high Mg^{++} and Ca^{++} concentrations.[66]

The PDH kinase is very tightly bound to dihydrolipoate transacetylase (Enz_2).[42] The PDH kinase has been separated from the transacetylase but has very low activity unless reconstituted with the transacetylase.[25] The PDH kinase catalyzes the transfer of the terminal phosphate of ATP to at least two or three serine-hydroxyl groups per polypeptide chain of Enz_1.[1,45] There are only about five PDH kinase molecules in the isolated complex and each of these molecules phosphorylates several Enz_1 chains.[43,44] From the work of Davis et al.[45] and others[43,46,47] there appears to be good evidence that PDH is phosphorylated at different sites on the enzyme complex from different tissues. For example, the pig and bovine kidney and heart and pigeon breast muscle are phosphorylated on the same peptide subunit.[25,43,46-48]

The PDH phosphatase, consisting of five polypeptide chains, is not as tightly bound as the PDH kinase and can be separated from the complex by ultra centrifugation.[1,7,48] The site and mechanism of the binding of the PDH phosphatase is not yet clearly known. The PDH kinase and PDH phosphatase regulate the PDH complex activity by altering the activity of the first step in the complex (i.e. pyruvate decarboxylation). This step may be rate limiting for the whole PDH complex but it has never been confirmed experimentally.

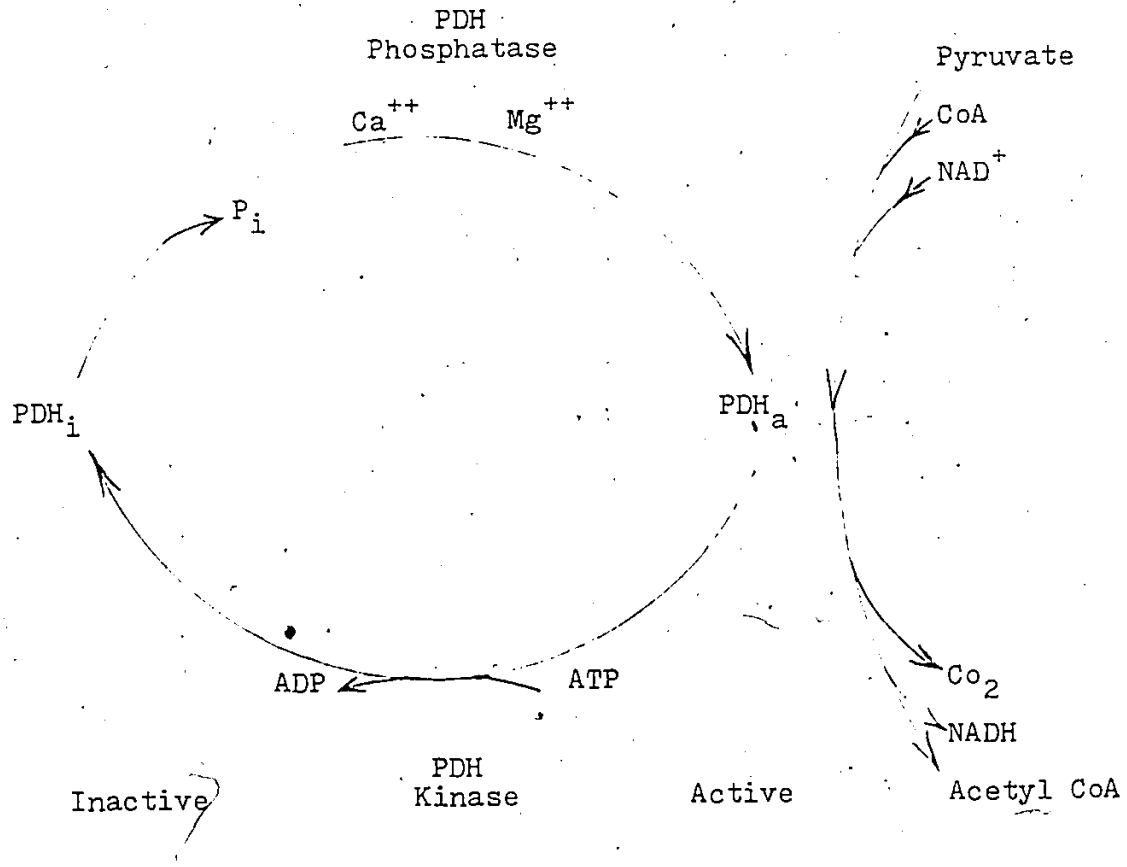
The inactivation and reactivation by PDH kinase and phosphatase are under metabolic control and are modulated

by a number of effectors. Pyruvate, pyrophosphate and ADP inhibit the PDH kinase and hence activate the pyruvate dehydrogenase (Enz_1, Figure 1).[49-51] Other effectors like Mg^{++} and Ca^{++} inhibit the PDH kinase reaction and activate the PDH phosphatase reaction.[34,52-55,61] (Figure 2)

Pyruvate, the substrate for the enzyme, has been shown to protect PDH from phosphorylation and inactivation by the PDH kinase.[1,7,51,56] The protection afforded the enzyme is eleven times more powerful for the heart than for kidney.[8,51] These studies show that the protection by pyruvate is available to the heart, so that the oxidation by pyruvate cannot be blocked by cellular ATP generation. Kinetic studies have shown that there is a regulatory binding site for pyruvate that probably does not occupy the same position with the substrate binding site of Enz_1 or with the binding site of the ATP of the PDH kinase.[51,57]

The protective effect of pyruvate has been shown by Krebs[58,59] to be important physiologically because the reducing equivalents necessary for gluconeogenesis have to be generated by the oxidation of pyruvate in the mitochondria when lactate is not the initial substrate. During these conditions, gluconeogenesis by way of oxaloacetate is only possible if some of the pyruvate is oxidized and, thus provides the NADH needed for that option. Pyruvate, therefore, prevents PDH inactivation by protecting the enzyme complex

Figure 2.



Legend: The above scheme for PDH complex interconversion in mammalian tissue. A kinase phosphorylates and inactivates the enzyme while a phosphatase dephosphorylates and reactivates the enzyme. The properties that influence the activity are also summarized.

against the inactivation by the PDH kinase. Hucho[51] and Burgett[60] have shown that pyruvate inhibits phosphorylation of casein by the PDH kinase when separated from the enzyme complex, and feel that the PDH kinase may, therefore, bind and act at separate substrate and regulatory sites. Under certain conditions, especially when TPP is available, pyruvate concentrations lower than 100 μM, activate the PDH kinase while higher concentrations inhibit it.[55,56] In the presence of TPP, pyruvate stimulates its own decarboxylation. This suggests that pyruvate or a decarboxylation product may be responsible for the inhibition of PDH kinase. Acetaldehyde and acetoin are some of the products available, but acetaldehyde has no effect, while acetoin activates the PDH kinase in low concentrations.[9] The process does not occur if TPP is not available or if pyrophosphate is substituted.[9] The reasons for the activation of the PDH kinase by acetoin formed from pyruvate with TPP, is not yet resolved.

Some carboxylic acids can also inhibit the PDH kinase while proprionic acid appears to have a similar potency as pyruvate.[9,51] Halogenated carboxylic acid such as dichloroacetate also inhibit the PDH kinase either in the presence or absence of TPP, but when compared to pyruvate and proprionic acid, dichloroacetate is less effective. Therefore, the halogenated carboxylic acids can bind to PDH, do not get metabolized, and thus can activate the PDH complex.

It is suggested that the halogenated carboxylic acids activate PDH by stimulating the active or dephosphorylated form.[9] It appears that the halogenated carboxylic acids activate PDH by inhibiting the PDH kinase reaction in some way, allowing the PDH phosphatase to dephosphorylate and, therefore, causing the conversion to the active PDH form.[9]

Work from Reed's and Wieland's laboratories has shown that Mg^{++} is required for PDH phosphatase activity.[1,6,48] In the presence of EDTA, however, the enzyme was completely inactivated. These studies prompted Denton et al.[61] and Pettit et al.[50] to explore the effects of calcium ions on PDH activity in various tissues. The effect was to lower the K_m of the phosphatase for its substrate PDH phosphate from about 30 μM in the absence of Ca^{++} to about 1.6 μM with a calcium concentration of 10 μM.[62] There also seems to be a reduction of the K_m for Mg^{++} if Ca^{++} is available.[50,61,64] Hayakawa[5] and Hucho et al.[51] have observed that the divalent metal Mn^{++} may replace Mg^{++}, and Denton et al.[9] further showed that some other divalent metals such as Sr^{++} and La^{++} could also mimic the effect of Ca^{++} while Ba^{++}, Cu^{++} and Fe^{++} had no effects. Two other metals, Ni^{++} and Zn^{++}, inhibited the effect of Ca^{++} also. Siess and Wieland[63] with their work, carried out at the same time, showed that fluoride could also inhibit the enzyme.

The adenine nucleotides may play a regulating role by the activation and inactivation of PDH by affecting the PDH kinase activity. ATP is an essential substrate for the PDH kinase, ADP is a weak PDH kinase inhibitor, while AMP, cyclic AMP and other nucleotides appear to have little influence on the regulation using purified, and partially purified mitochondria.[1,15,49,52,65,69-74,168] As Hucho[51] has pointed out the K_m of ATP for PDH kinase is low, so that the cell ATP concentration always saturates the PDH kinase. ADP and AMP, because of their weak inhibitory effects, are probably ineffective as regulators for the PDH kinase. The PDH phosphatase is not influenced by the nucleotides. Consequently, it has been difficult to determine how the interconversion of PDH changes according to the demands of the cell. It has also been difficult to show how the PDH enzyme complex activity is regulated by the nucleotides.[9,67] It seems that the studies on the PDH kinase at least show that the regulation of the enzyme is very complex. There is some evidence, in heart, that the ATP:ADP ratio may exert an effect on the PDH activity.[68-74] This will be discussed later.

Regulation by glycolytic intermediates in mammalian tissue PDH complex is subject to product inhibition by numerous metabolites.[68-77] However, most of the studies with the intermediates have been conducted on the stimulation

of PDH in E. coli rather than in mammalian tissue. Such intermediates as fructose-diphosphate, fructose-6-phosphate, glyceraldehyde-3-phosphate, phosphoenol pyruvate, dihydroxyacetone phosphate and glucose-6-phosphate stimulate the first step of the PDH complex (Enz_1). Very recently Ngo and Bardeau[90] reported that they were unable to observe any effects of brain PDH by the glycolytic intermediates. In addition to the activation of glycolytic products, product inhibition by NADH on Enz_3 in E. coli, and other tissues of the mammalian organs has been described.[9,51,70,73-75,90] Acetyl CoA also inhibits PDH activity in all tissues tested to date. In contrast to E. coli a change in the free $NADH:NAD^+$ ratio in mammalian tissue is more important in cellular metabolic regulation than the acetyl CoA:CoA ratio.[74,75,81-83,85,86] The TCA cycle intermediates inhibit pyruvate oxidation in mammalian tissues with citrate influencing, as an example, the interconversion of the inactive to the active PDH.[78,79] (see p. 23 for further discussion).

4. Differences Between Organs in the Regulation of Mammalian Pyruvate Dehydrogenase

A. Brain: The brain PDH complex has been shown to be interconvertible by the phosphorylation and dephosphorylation process.[63] From data available so far it appears

that brain PDH activity can be regulated by different factors when compared with other organs. For example, when compared with kidney, heart, liver and adipose tissue, the brain PDH_a activity decreases far less during times of starvation. The heart, kidney and adipose tissue PDH_a are drastically reduced to between 15-20% of the PDH_t during starvation, whereas in the brain the PDH_a is never reduced by more than 50% of the PDH_t.[1,7,87,88] This suggests that the rat brain has the ability to maintain a constant metabolic environment which is reflected as a minimal change in the PDH_a:PDH_t ratio.

There is some controversy whether acetyl CoA inhibits the PDH activity in the brain. Siess et al.[86] have shown in rat brain that acetyl CoA was inhibitory, but Blass and Lewis[89] reported that in ox brain it was not. Following up on the controversy, Wieland et al.[88] and very recently Ngo and Bardeau[90] have shown that under their experimental conditions, acetyl CoA inhibits PDH activity which is consistent with the results reported in most of the other tissues tested to date.[74,75,86,91,94]

Jope and Blass[92,93] have recently reported that in isolated rat brain mitochondria Mg^{++} and Ca^{++} when added to the "medium" do not activate the enzyme complex. In contrast, using liver mitochondria they showed an activation, under the same conditions, to be more than threefold. However,

Booth and Clark[95] found that Mg^{++} and Ca^{++} were needed for maximal brain PDH activation. What is agreed upon is that the PDH_a activity in brain is quite high. It appears to be about 70% of the PDH_t in mature fed rats which is somewhat higher than in most other tissues measured to date.[9,87] Wilbur and Protel[96] have shown that in immature rats, PDH_a in brain is low but increases quickly during the first month of life. Other research has described that the PDH_a varies with many different manipulations such as ischemia, insulin, starvation and the change in the energy charge.[93,97] It seems in the above cases reported, that the proportion of the PDH_a in brain changes rather than the PDH_t. However, when mice have been exposed to ether, morphine, ethanol or diazepam the PDH_a does not change, but those treatments do change pyruvate oxidation in the brain in vivo. Therefore, it can be concluded that treatments which alter pyruvate oxidation in brain tissue do not necessarily alter the PDH_a:PDH_t ratio.[93]

B. Adipose Tissue: This tissue has been comprehensively studied in many laboratories.[98-115] The activation of PDH in fat pads exposed to insulin has shown increases of approximately 2-3 fold when incubated with glucose or fructose. The response is very variable and may be due to high Ca^{++} concentrations found in the mitochondria of fat cells.[116] As with brain PDH, the fat-pad PDH_t activity is

not changed under most circumstances, however, the PDH_a portion can be. This has been confirmed when Mg^{++} and Ca^{++} are incubated with PDH phosphatase and the activation and insulin effect are lost.[103,106,107,116] The mechanism by which insulin activates PDH has not been elucidated. The known effects of insulin, like accelerated glucose uptake and inhibition of lipolysis are perhaps secondary to other effects because accelerated glucose uptake has been observed in tissue incubated with fructose.[117,118] The glucose uptake is not altered by insulin.[105,108] Denton[9] and others[26,87,98,101,103,108,111,112] have found that many lipolytic agents such as epinephrine and dibutyryl cAMP increase PDH activity. However, when epinephrine and dibutyryl cAMP are added with insulin these agents stop the effect of insulin on PDH activity. Denton[102,104,110,122] found no simple correlation between changes in rates of lipolysis and fatty acid synthesis, and changes in PDH activity. In the presence of nicotinic acid PDH activation was unaffected. The conclusions of Denton et al.[104,120] and Stansbie et al.[114,115] is that there could be a link between the rate of lipolysis and PDH activity under certain conditions, but the insulin effects are probably not dependable solely on changes in the rate of lipolysis. Short-term effects of insulin in decreased cAMP concentration are observed only in the presence of epinephrine or other agents which increase cAMP concentrations. An example of

this is the decrease of glycogen phosphorylase$_a$ activity produced by insulin in the presence of epinephrine.[8,9] Changes such as this, which are associated with a decrease of the activity of protein kinase and a lowering of cAMP concentration, seem to show that the lowering of cAMP may play an integral part in insulin action.[122] Other effects are also observed and include increases in glucose transport, glycogen synthetase and PDH activation. Under these experimental conditions, the protein kinase, adenylase cyclase activation and cAMP concentration were not changed, signifying that the insulin effects do not appear to be cAMP dependent.[122] With PDH, insulin appears only to act by activating the enzyme by increasing the rate of dephosphorylation in this tissue. However, whether insulin only activates the PDH phosphatase has not been resolved. Denton et al.,[119,122] Severson et al.,[62,116] Kerbey et al.,[73,169] and Hughes and Denton[121] have been actively investigating effects that insulin has upon the mitochondrial concentration of the effectors of the PDH phosphatase and PDH kinase (see p. 11 and p, 12). It is well known that the PDH enzyme complex is altered by a number of effectors. High pyruvate and ADP, both inhibitors of the PDH kinase and Ca^{++} which activates the PDH phosphatase and inhibits the PDH kinase, have all been found to increase the proportion of the PDH complex in the active form. However, no convincing evidence has been obtained that the insulin action is mediated by changes in

whole tissue ATP:ADP ratio, NADH, NAD^+, acetyl CoA, CoA or potassium concentration.[110,111,120,123] Up to this point in time, no effects have been shown with mitochondrial concentrations of adenine nucleotides or pyruvate within intact cells because techniques are not available to measure them. However, the effect of insulin is evident during the preparation of mitochondria from fat-cells or fat pads[122] when pyruvate is absent. During this time it is presumed that pyruvate is not present in the mitochondria and as Denton et al.[119,121] state, the ATP concentration in mitochondria treated with and without insulin is the same. This, then appears to suggest that changes in adenine nucleotides and pyruvate are not important as mediators of the effect of insulin.

A fairly recent approach to measuring the rate of the PDH kinase reaction within intact mitochondria has been to follow the incorporation rate of ^{32}P from $[^{32}P]$ P_i into PDH phosphate.[34,51,121] From such experiments it has been found that the specific radioactivity of ATP in fat-pad mitochondria approaches that of the "medium" phosphate within seconds. The rate of the incorporation of ^{32}P into PDH phosphate is taken as a measure of the rate of the PDH kinase reaction within intact mitochondria. The rate is greater in mitochondria from insulin treated fat-pads suggesting that the increase in the proportion of the active

form of the complex in the mitochondria was not caused solely by the inhibition of the PDH kinase.[122]

Another possible mechanism by which insulin activates PDH has recently been suggested by Paetzke-Brunner et al.[131] They suggested that the increase in PDH_a in adipose tissue is a result of insulin reducing the acetyl CoA:CoA ratio in the mitochondria if acetyl CoA carboxylase is activated.

Fluoroacetate and insulin have similar effects on fat cell metabolism which has been interpreted to indicate a similar mechanism of action.[9,128,129] Schiller et al.[130] and Brownsey et al.[128] have shown that both agents accelerate glucose transport, increase lipogenesis, and activate PDH but both decrease lipolysis and lower the cAMP concentration. The major difference between insulin and fluoroacetate seems to be that fluoroacetate induces the inhibition of PDH kinase while insulin does not.

Denton and Halperin,[120] Denton,[119] Denton et al.,[122] Coore et al.[100,101] and Halestrap and Denton[125] have shown parallel changes in PDH_a and acetyl CoA carboxylase proportions when the fat pad is treated with insulin and epinephrine which may suggest that the regulatory mechanism could be clearly linked.[119] Both enzymes are involved in lipogenesis and as with PDH, acetyl CoA carboxylase exists in an inactive and active form. As an example, changes in cytoplasmic concentrations of fatty acid coenzyme.A (FA-CoA)

might be responsible for activity changes of both PDH and acetyl CoA carboxylase if FA-CoA concentrations do, in fact, regulate the concentration of an effector of the PDH complex. Various workers[9,124-126] have suggested that a decreased cytoplasmic FA-CoA concentration may be responsible for a decrease in the mitochondrial ATP:ADP ratio and hence the activation of PDH; but a conclusive role of FA-CoA has not been resolved.

The changes in citrate concentration has also been suggested as a possible regulator of the respective PDH and acetyl CoA carboxylase enzymes. Taylor and Halperin[79] and Schiller et al.[130] have shown that citrate appears to decrease PDH activity by inhibition of PDH phosphatase possibly by chelating Mg^{++} and Ca^{++}. Both Mg^{++} and Ca^{++} have been described as activators of the phosphatase.[1,7] Volpe and Vagelos[127] have found that citrate activates acetyl CoA carboxylase and is inhibited by FA-CoA, but the importance of the effector is difficult to explain because there is little correlation between rates of fatty acid synthesis and whole tissue concentrations of citrate.

In summary, it is well established that the interconversion of the PDH_a and the PDH_i forms of the PDH complex in adipose tissue is influenced by hormones. Insulin results in an increase of PDH_a. However, there seems to be confusion as to the actions of agents such as epinephrine. Certain laboratories have reported that PDH_a activity in

fat-pads is increased after treatment by lipolytic agents in the absence of insulin,[99,108,111,113,117,118] while another laboratory has shown that PDH_a is not influenced by epinephrine used by itself.[101] The same authors have also found that some lipolytic agents oppose the reported increases of PDH_a when insulin is available. When isolated fat-cells were used with epinephrine alone large PDH_a decreases have been shown.[100-103,119,125,126,225] Possibly one reason for the differences is that the buffer used is often very different between laboratories.[100-102,119,125,126,225] An example is that wide variations in pyruvate concentrations are used. Others use glucose or fructose which allows some re-esterification of FFA derived from TG.[132,144,148]

C. Liver: A large number of studies have produced direct evidence that phosphorylation and dephosphorylation observed originally by Linn et al.[147] in Reed's laboratory occurs in the liver of various different animals such as rats,[67,86,92,113-137,145,146] mice,[138] guinea pigs,[139,140] pigs[141] and developing immature rats.[142] Crude extracts from freshly prepared rat liver mitochondria and freeze-clamped samples of whole rat liver yields different activity concentrations but almost identical ratios of PDH_a:PDH_t.[135] This is similar to other tissues such as skeletal muscle where the proportions are almost identical but the absolute activities may be different. (see p. 112)

In livers from normal fed rats, the PDH_a form accounts for only about 20% of the PDH_t activity.[144,145] This is different from other tissues in the fed state such as heart muscle, kidney, brain and adipose tissue where the PDH_a is at least 50% of the PDH_t activity.[86,87]

During starvation there is a marked decrease in PDH_a activity in the kidney and other tissues to approximately one sixth of the PDH_t.[87,107,132,144] In liver, starvation decreases the PDH_a to about 10% of the PDH_t activity.[145] This indicates that under normal fed conditions oxidative decarboxylation of pyruvate in vivo occurs at a much lower rate in liver than in many other tissues. The feeding with glucose appears to cause similar PDH reactivation in the liver, heart and kidney.[145]

In general, metabolic states associated with decreased plasma FFA concentrations result in an increase of PDH_a activity whereas a rise in plasma fatty acids is associated with a lowering of PDH_a activity. This, in turn, leads to an increase or decrease of mitochondrial ATP:ADP ratios which suggests that the mitochondrial energy state may be an important control of PDH_a. However, other studies with adenine nucleotides appear to show that the ATP:ADP ratio remains essentially unchanged and possibly, therefore, does not effect the active or inactive PDH concentrations.[79,147]

It has been observed also that the addition of octanoate causes a large 4-fold decrease in PDH_a activity, without a change in the ATP:ADP ratio, indicating that the inactivation of PDH with this fatty acid is not due to changes of the ATP:ADP ratio.[70,72,140,147,149-151] If octanoate is added to mitochondria oxidizing pyruvate, it does not change the ATP:ADP ratio.[9,67,152] Pyruvate abolishes the effect of octanoate and shifts the PDH complex into a more active form.[67,152] Wieland[88] and others[51,135] have shown earlier that incubation with 2.0 mM pyruvate converts PDH to over 80% of its total activity. This activation appears to be similar to heart tissue. (see p. 28)

Pyruvate stimulation of PDH_a kinase probably is not a major regulatory feature for the enzyme regulation in vivo. However, it does appear that, after decarboxylation of pyruvate, the acetylation of the lipoyl moiety reacts more slowly than the subsequent steps of the PDH reaction.[154] The fraction of the lipoyl moieties in the acetylated form tend to reflect the acetyl CoA:CoA ratio and $NADH:NAD^+$ ratio. Nevertheless, stimulation by pyruvate affords an important approach for detecting the stimulation of PDH_a kinase activity. From the recent work of Cate and Roche[154] pyruvate is shown to either stimulate or inhibit PDH_a kinase activity. Both acetyl CoA and low pyruvate concentrations stimulate PDH_a kinase to approximately the same level, while

the combination of pyruvate and acetyl CoA does not cause further stimulation.

NADH also has been shown to induce inactivation of PDH_a in liver.[72,140,152-154] This PDH kinase activity increase appears to be modulated by the reduction of the lipoyl moiety bound to the dihydrolipoyl transacetylase component and interaction of the dihydrolipoate at a site that activates PDH_a kinase activity. Significantly higher levels of PDH_a kinase activity are achieved with acetyl CoA or pyruvate than with NADH or dihydrolipoamide. The results of these studies suggest that both acetyl CoA and pyruvate stimulate PDH_a kinase activity by different mechanisms. Cate and Roche[154] suggest that a common mechanism, such as stimulation of PDH_a kinase activity resulting from the release of inhibition by the oxidized form of the lipoyl moiety,[73] seems unlikely. According to Cate and Roche, lipoic acid mediates the stimulation of PDH_a kinase activity, suggesting both the acetylated form of the lipoyl moiety is more effective than the reduced form in stimulating PDH_a kinase activity.[141,154,155] Further experimentation seems to be required before the mechanisms are confirmed.

Very low $NADH:NAD^+$ and acetyl CoA:CoA ratios reduce the activity in liver, as well as kidney and heart of the PDH_a activity by enhancing PDH_a kinase activity, while higher ratios are required for product inhibition of the overall PDH reaction.[1,53,67,140,141,150,154-156]

Insulin loading in liver produces marked activation of PDH_a but when FFA was infused the increase in PDH_a was abolished.[87,148] In more recent work by Topping et al.[157] Wieland's earlier work was confirmed, but showed in addition, that insulin modified in some way the effects of FFA on PDH_a. The effects appear to be mediated by changes in adenine nucleotides and their ratios. The ability of insulin to increase PDH activity may be due to an anti-lipolytic effect of the insulin.[148] This may decrease the intracellular FFA available and the amount of FFA required for the reaction.

Calcium ions also effect PDH interconversion and resemble the observed effects that occur in various other tissues. Calcium and magnesium have both been shown to be essential cofactors in liver and without their addition to the reaction medium very little activity occurs.[67,88,145,154]

In summary, the interconversion of PDH in liver is regulated by adenine nucleotides, pyruvate, divalent cations, free fatty acids (FFA) and by modulation by the oxidation-reduction state of the mitochondrial ratios of $NADH:NAD^+$ and by acetyl CoA:CoA ratios. The liver PDH_a activity in normal fed animals is much lower than in any other tissues.[79,146,149]

D. Heart: The activity of the heart PDH complex, originally isolated from mitochondria by Linn et al.[1,7] is regulated by phosphorylation and dephosphorylation. The site of the regulation is the pyruvate dehydrogenase

component of the multienzyme complex. Phosphorylation and concomitant inactivation of the three site (PDH) reaction is catalyzed by an ATP-specific kinase, first isolated in beef kidney mitochondria (a PDH kinase).[1,7] The dephosphorylation and concomitant reactivation is catalyzed by a phosphatase (a PDH phosphatase). Beside the pyruvate dehydrogenase component, the PDH phosphatase has been shown to have been isolated from the dihydrolipoate transacetylase and dihydrolipoate dehydrogenase enzymes of the PDH complex and the three are functionally interchangeable. The PDH kinase has also been isolated from the three site sequence reaction and is active at low levels of Mg^{++}. The PDH phosphatase requires Mg^{++} at a concentration of approximately 10 mM and up to 10 mM of Ca^{++} for maximal activity.[7,62] However, the Ca^{++} seems not to be as essential in the heart as it is in other tissues.[79] There is recent evidence that the PDH phosphatase and PDH kinase components of the reaction are cyclic in their regulations. The PDH kinase has also been shown to be bound to the transacetylase component of the enzyme, while the two other enzymes of the PDH complex are also bound to the transacetylase.[6,7] It has not as yet been conclusively shown that the PDH phosphatase is bound to the transacetylase in the heart although it has been shown that the transacetylase facilitates dephosphorylation of phosphorylated pyruvate dehydrogenase by the PDH phosphatase.[7]

The PDH kinase in the heart is inhibited by pyruvate and ADP, especially if K^+ or NH_4^+ ions are present.[51,52]

Pyruvate protects the heart PDH complex against inactivation by ATP.[51] Pyruvate has been shown by Hucho et al.[51] and others[9,53] to decrease the inactivation of PDH kinase at low concentrations. Heart PDH in mitochondria appears to be activated by larger concentrations of pyruvate. Pyruvate increases the PDH_a proportion of the enzyme by inhibiting the PDH kinase activity.[9,53,87] The inhibitory effect of pyruvate is more pronounced in the heart than in the kidney and other tissues such as brain and liver. According to Denton et al.[9] fatty acids, ketone bodies and diabetes may interfere with the inhibitory effect of pyruvate on the PDH kinase resulting in phosphorylation and PDH inactivation. Pyruvate evidently produces less activation of PDH in mitochondria from alloxan-treated diabetic rats.[9,162] It does seem likely that up to 75% of the inhibition of pyruvate oxidation by fatty acids and ketone bodies is due to phosphorylation and the rest to end product inhibition. This probably occurs by the increased ratios of $NADH:NAD^+$ and acetyl CoA:CoA facilitating phosphorylation and thus inactivation. No direct evidence supporting this is available.[9,158,159,179]

Numerous effectors have been implicated in the regulation of the PDH kinase and PDH phosphatase, including changes in ATP:ADP, acetyl CoA:CoA and $NADH:NAD^+$ ratios.

When fatty acids (octanoate, butyrate, palmitate, oleate and linoleate) are infused into the heart it has been shown that PDH is inhibited and thus inactivated,[87,88 107,144,161,163,179] while the oxidation of pyruvate is also substantially inhibited.[151,160,161,165-167,171] These conditions lead to an increase of CoA concentration and a large decrease in the acetyl CoA concentration.[74,75,94,168] Recently, the PDH kinase has been shown to be activated by decreases in ratios of $NADH:NAD^+$ and acetyl CoA:CoA.[70,71,73, 181-184] The implications of the interconversion of PDH have been explained by the changes in the ratios of mitochondrial $NADH:NAD^+$ and acetyl CoA:CoA ratios in heart mitochondria of various different animals.[72,163,169] The effect of the palmitoylcarnitine oxidation on PDH_a activity has also been implicated in similar ratio changes.[151,169] From all the above studies it seems quite apparent that the effect of fatty acids and ketone bodies on pyruvate metabolism by the heart tissue is mediated by changes in $NADH:NAD^+$ and acetyl CoA:CoA ratios and the decrease of PDH_a activity. Thus, the increased acetyl CoA:CoA ratio observed with FFA, could explain why FFA inhibited PDH_a activity. In addition, there probably is also direct feedback inhibition of the PDH_a by end products such as NADH and acetyl CoA. There is some evidence that small increases in the ATP:ADP ratio do not suffice to explain the changes in PDH_a activity in the

presence of palmitoylcarnitine and rotenone. It is fairly obvious that there must be other mechanisms leading to the PDH kinase activation. Portenhauser and Wieland[164,181] appear to show that large NADH:NAD^+ ratio increases in rotenone inhibited mitochondria with palmitoylcarnitine, and small increases of the ATP:ADP ratio, may modify the PDH complex sufficiently to cause some change in the PDH activity. When the heart mitochondria are incubated without substrates, the ATP:ADP ratio decreases up to a third and consequently the PDH_a increases considerably.[180-184]

Dichloroacetate was shown by Lorini and Ciman[174] to elevate the R.Q. and lower the blood glucose concentration in alloxan-diabetic rats. Later studies in the early 1970's demonstrated that sodium dichloroacetate also stimulated pyruvate and glucose oxidation in rat heart and diaphragm, especially when the carbohydrate oxidation was inhibited by diabetes or by fatty acids or ketone bodies.[175-178] The studies also concluded that acetate, ketone bodies and palmitate oxidation were inhibited by dichloroacetate. Later, McAllister et al.[178] and others[55,158,179] demonstrated that the PDH_a portion of the PDH complex increased considerably in heart, diaphragm muscle, psoas muscle, fat and kidney after treatment with dichloroacetate. This agent has also been involved with the lowering of tissue citrate concentrations especially when the heart was perfused

with acetate, ketone bodies or fatty acids.[178] Dichloroacetate also increases tissue concentrations of acetyl CoA, acetyl carnitine, glutamate and lowers malate, aspartate and pyruvate concentrations if perfused with glucose plus insulin.[176,178]

It has been suggested that citrate fails to accumulate when perfused with acetate or ketone bodies because of a decrease in pyruvate and perhaps oxaloacetate. The reason for the suggestion came from the studies which showed dichloroacetate not to decrease tissue concentration when perfused with pyruvate.[178] Dichloroacetate lowered pyruvate and lactate concentrations in plasma and increased glucose, pyruvate and lactate concentrations in the heart. The studies of McAllister et al.[178] and Whitehouse et al.[179] suggest that dichloroacetate activates PDH_a by increasing the acetyl CoA concentration which in turn may inhibit the PDH kinase.

The concensus of opinion at this time seems to show that the effects of fatty acid oxidation on PDH_a activity are not seen either in the presence of very low ATP:ADP ratios or in the presence of Ca^{++} at about 10^{-6} M. The ATP:ADP ratio physiologically probably changes minimally in the heart[170,171] but fluctuations of Ca^{++} concentrations may influence the PDH activity considerably more.[172]

Calcium activates the PDH phosphatase reaction and can also inhibit the PDH kinase enzyme. Studies with EGTA

(ethylene-glycol-bis(aminoethyl)-tetra-acetate) and Ca-EGTA buffers in the heart mitochondria of pigs suggest that the PDH phosphatase is activated by μM Ca^{++} concentrations in the presence of Mg^{++}.[50,61,172] In the heart it was found that Sr^{++} can be substituted for Ca^{++} to activate PDH. Randle et al.[172] have also described that in the presence of EGTA the K_m of the PDH phosphatase was lowered for its substrate PDH phosphate from 30μM to 1.6μM. It is interesting that the concentration dependence of the effect of Ca^{++} is similar to skeletal muscle phosphorylase b kinase.[173] In comparison, Ca^{++} variations below 10^{-6} M cause little or no change in the oxygen uptake of mitochondria in rat heart so that an indirect effect of Ca^{++} on PDH interconversion involving an energy demand of Ca^{++} uptake and increased NADH:NAD^+ and ATP:ADP ratios become very unlikely.[72,106] Hansford and Cohen[162] feel, however, that the possibility of such an indirect mechanism could be shown if Ca^{++} concentrations above 10^{-6} M were used.

The site of calcium binding in the heart, is unknown. Transacetylase has been suggested as the binding site because Ca^{++} may induce conformational changes that inhibit the PDH kinase while facilitating the binding and activity of the PDH phosphatase.

Calcium in the heart appears to activate PDH phosphatase in a similar way to the Ca^{++} in kidney and

adipose tissue, except lower (μM) amounts of Ca^{++} are needed in the heart.[9,62] Calcium also appears to reduce the K_m for Mg^{++} in all tissues tested. In addition, the PDH kinase is inhibited by Ca^{++} in the heart but it is not known to what extent this applies to adipose tissue PDH or kidney PDH.

5. General Background to Thesis

These experiments were prompted by the physiological role, PDH activation might play in the regulation of skeletal muscle oxidation of pyruvate, derived from muscle glycogen and blood glucose during exercise.

There was considerable evidence that muscle ATP generated aerobically during heavy exercise was largely [redacted]med from muscle glycogen breakdown, with only a small proportion coming from other sources such as amino acids[214] or FFA.[211,212] Thus, the major metabolic end point for glycogenolysis in muscle is pyruvate, which may be metabolized to lactate, or decarboxylated to acetyl CoA catalyzed by PDH. As the muscle O_2 consumption and Co_2 production increases at least 10 fold during heavy aerobic exercise,[205,206] it follows that the major sources of aerobic metabolism is pyruvate oxidation derived from glycogen/glucose.

Since the PDH reaction is the committed step in pyruvate oxidation and aerobic generation of ATP, it seemed likely that control of glycogen/glucose oxidation was

probably largely at PDH.

When this work was started there were few published reports on muscle PDH and these were in diaphragm muscle[49,74] and pigeon breast muscle[3,4,20,21] and only in mitochondria, so it was decided to pursue studies in human and animal skeletal muscle PDH during rest and exercise without isolating mitochondria.

The purpose of the study was to determine whether PDH activation or changes in other factors known to inhibit or stimulate PDH activity, changed in a way which would implicate PDH as the major control point in regulating the TCA cycle activity and oxidative phosphorylation in skeletal muscle with heavy aerobic exercise.

Beside the various studies carried out in the mammalian skeletal muscle under different conditions, other studies were pursued in the rat heart. As PDH is the primary reaction that determines the metabolic fate of pyruvate in the heart and as the interconversion of the active and inactive PDH is regulated by a large number of factors,[1,7,42,43,50,51] it was decided to explore which events in the cell between the cellular signal and final molecular interconversion were possibly accountable for the interconversion of the inactive and active PDH.

There is a basic assumption that if a main regulatory enzyme exists in an inactive and active state, the interconversion of the two states of the enzyme is the

primary factor in controlling the flow of carbon through that enzyme reaction. As that had not been established for PDH in the heart, studies in the perfused rat heart were carried out to determine to what extent of the PDH activation was the primary factor controlling the heart pyruvate oxidation.

Since this study was begun, several publications have appeared using mammalian skeletal muscle to explore certain aspects of the regulation of the enzyme PDH.[79,237-240] These authors have used mitochondria to measure the PDH activity and none of these reports have described PDH activities in human muscle biopsies during exercise and training.

Accounts by Ward et al.[241-244] and Toews et al.[245] of some of these experiments have appeared.

MATERIALS AND METHODS

1. Materials

A. Animals

Male Sprague-Dawley rats (Bio-Breeding Corporation, Ottawa, Ontario, Canada) 200-300 g were used in all the animal experiments. They had free access to food (standard Purina laboratory chow) and water until the day before the experiments. The specific dietary regime for a day prior to the experiments is outlined in the text.

B. Chemicals

All the enzymes, cofactors and metabolic intermediates used in the fluorometric analysis of tissue and perfusion fluids were obtained from C.F. Boehringer-Mannheim Corporation, Montreal, Quebec, Canada or from Sigma Chemical Company, St. Louis, Missouri, U.S.A. All enzymes were checked for contaminating activity and were used only when their purity was established. The other chemicals were of the highest quality available commercially. Insulin [—Toronto (zinc insulin crystals)] was obtained from Connaught Laboratories Limited, Toronto, Ontario, Canada; Propranolol (Inderal) from Ayerst Laboratories Incorporated, New York, N.Y., U.S.A. and bovine serum albumin (Cohn fraction V) was obtained from Sigma Chemical Corporation. All ^{14}C-compounds

were obtained from New England Nuclear Corporation, Boston, Massachusetts, U.S.A. or Amersham-Searle Limited, Oakville, Ontario, Canada. Sodium Heparin (Hepalein) was obtained from Harris Laboratories, Brantford, Ontario, Canada; epinephrine hydrochloride from Parke-Davis and Company Limited, Toronto, Ontario, Canada and sodium pentobarbital (Somnotol) from MTC Pharmaceuticals, Hamilton, Ontario, Canada.

2. Methods

A. MUSCLE MODELS

I. Heart Perfusion

All the rats were fasted for 18 hours before the experiments. They were anaesthetized with ether and the abdomen was opened. Two hundred i. units of sodium heparin was injected into the inferior vena cava to prevent coagulation in the myocardial vessels before any manipulation of the heart. The chest was opened and the heart was removed and placed on ice within 10 seconds of opening the chest. The aorta was then cannulated and flushed with ice-cold perfusion medium before the heart was placed on the perfusion apparatus.

In all the experiments the arrested hearts (weighing between 1 and 1.5 g fresh wt.) were restarted by perfusion with Krebs-Ringer bicarbonate buffer (KRB)[186,187] containing

0.5 g bovine serum albumin (Cohn fraction V)/100 ml buffer. The albumin was dialyzed as previously described.[188] In most of the experiments 10 mM D-glucose and 200 μU/ml insulin was added to the buffer. In many of the experiments, substrates other than glucose and the hormone insulin were used, and these details are described in the appropriate legends.

After the aorta was cannulated the heart was placed in the perfusion chamber at 38°C and perfused at an aortic pressure of 80 mmHg at an average flow rate of 10 to 15 ml/g ht/min. The heart was pre-perfused for 10 min. before 0 time. The perfusion was done in a non-recirculating manner with identical conditions, both before and after 0 time. At 0 time the heart was switched to a recirculating perfusion system with a reservoir volume of 200 ml. The heart was not electrically stimulated (except as outlined in the legends) and the heart did not contract against a ventricular pressure (non-working model); the heart maintained a ventricular rate of 120-160 beats/min. If this beating rate was not achieved the heart was discarded.

After 0 time the outflow samples were collected every 5 or 10 min. up to 40 min. (unless otherwise stated in the appropriate legends). At 40 min. the heart was freeze-clamped with aluminun clamps pre-cooled in liquid nitrogen. The heart was stored in liquid N_2 until analyzed.

II Rat Skeletal Muscle

Fasted male rats were anesthetized with an intraperitoneal injection of 4 mg/100 g body weight sodium pentobarbital.

During anesthesia, the skin covering both hind legs was peeled back, leaving the gastrocnemius-plantaris muscle group or the quadriceps-femoris muscle tissue exposed. Depending upon the experiment, the gastrocnemius or quadriceps muscle groups in each leg were carefully dissected free from other muscle groups ensuring that the blood vessels and nerves were left intact. The sciatic nerves or femoral nerves (whichever was used for the experiments as shown in the appropriate legends) were transected bilaterally with as little manipulation as possible. The exposed areas were bathed with normal saline, recovered with the skin, and the animal was placed in a holding area at a temperature of 30-35°C for approximately 15 min. before any stimulation was begun. The sciatic or femoral nerve was cut in order to provide a direct point of nerve stimulation to the muscle and to prevent any neural influence from the brain. Blood lactate, arterial pH, Po_2 and Pco_2 were monitored to ensure that the experiments were as physiological as possible. Nerve transection resulted in minimal muscle contraction. To ensure a steady state during the experiments the rats were placed in the holding area for 15 min. prior to the

start of the experiment. The side to be stimulated was selected randomly; the contralateral leg always served as the control. The details of electrical nerve stimulation are outlined in the appropriate legends. At the end of the experimental period the particular muscle group used was freeze-clamped *in situ* by the use of aluminum tongs precooled in liquid nitrogen.[189] The frozen muscle (approximately 300 mg) was rapidly dissected free from the leg and stored in liquid nitrogen until analyzed. The maximal time between muscle sampling and freezing was 2-3 sec. As shown previously by Edington, Ward and Saville, 1973,[190] the deep muscle freezing time, as monitored by a thermistor, was 0.5 sec. to 0°C and 4.0 sec. to -100°C. Only one muscle sample per side was obtained because the sampling technique did not allow for multiple muscle biopsies.

(a) Rat Training Stimulus

The training stimulus was by running on a motor driven treadmill (Quinton Instruments, Seattle, Washington, U.S.A.) adapted for simultaneous use by 12 animals by the addition of wooden boxes. The training was carried out once daily for 5 days a week over a peribd of 10 weeks. The aerobic training program was initially begun at a speed of 19 meters/min. at 0% grade for 15 min. Each week the workrate was increased by 1.0% grade. In the final 2 weeks of the training program, the rats were running at 19 meters/min. at 5% grade for 45 min. daily.

(b) Surgical Procedure

Rats were anaesthetized with ether and the dorsal area of the lower leg exposed. The gastrocnemius-plantaris muscle group was thus divided and the soleus muscle and gastrocnemius muscles were dissected out from the leg. Both muscles were frozen *in situ* by liquid N_2 cooled aluminum tongs. The separate muscle samples were stored in liquid nitrogen until analyzed.

III Human Skeletal Muscle

(a) Short Term Exercise - Maximum Voluntary Isometric Contraction (MVIC)

Normal healthy male subjects (age 20-36) were used for all experiments. Two days before each experiment, the subjects were tested on a Cybex II dynamometer (Lumex Isokinetic Systems Inc., N.Y., U.S.A.) in a sitting position as described by Edwards et al.[191,192] to determine their maximal voluntary isometric contraction (MVIC) of their lateral quadriceps femoris muscle group.

The experimental isometric contraction was at 50% MVIC until exhaustion. The time to exhaustion at a constant power output was 65-73 sec. Fifty percent MVIC was selected as the work load because an increase above this level decreased the time to exhaustion dramatically.

The muscle biopsies were obtained from the lateral side of the thigh about 12-16 cm above the upper part of the patella. Two percent xylocaine epinephrine free (Astra Pharmaceuticals Company, Toronto, Ontario, Canada) was injected into the skin subcutaneous area and the fascia lata. A 4 mm incision was made in the skin and fascia lata. The needle biopsy was obtained using a 4 mm diameter needle as described by Bergstrom.[193] After sampling, the muscle sample (40-80 mg) was frozen with the needle in liquid nitrogen. The time from sampling to freezing was 2-3 sec. The samples were stored in liquid nitrogen until analyzed.

In all human studies, each subject was informed about the nature of the procedures and the risks involved. Written consent was obtained and each subject was informed that they were free to leave the studies at any time. Each study was approved by the McMaster University Ethics Committee.

(b) Long Term Exercise - Progressive Work

Normal volunteers starved for 12-16 hr, exercised on a cycle ergometer (Monark-Crescent, A-B, Varberg, Sweden) starting at 100 Kpm/min. and increasing the work rate by 200 Kpm every 2 min. Thus, the maximum work rate was 1700 ± 200 Kpm/min. and the total duration of the exercise was 20 min. The subjects exercised until exhaustion.

Prior to the start of the exercise regime, an arterialized venous polyethylene catheter (#18 Angiocath) was inserted into a superficial vein of the hand for intermittent blood collections. The hand was placed in a temperature controlled air chamber and the temperature was maintained at 40-45°C. The venous Po_2 under these conditions was 75-84 mM Hg. The O_2 consumption, Co_2 production and ventilation was continually monitored.[194] Exercise was not permitted 24 hr prior to the experiments. Muscle biopsies (40-80 mg) were obtained at rest and at exhaustion and handled as outlined earlier (p. 44).

(c) Short Term High Intensity Exercise - Intermittent Work

Six normal subjects, fasted 12-16 hr were exercised on a cycle ergometer for 1 min., followed by rest for 3 min. This cycle of exercise was repeated until exhaustion. The calculated work load was 140% of their maximal O_2 consumption ($\dot{V}O_2$ max).[194] The average time between the start of exercise and exhaustion was 10.1 $\pm$ 1.6 min. An arterialized venous catheter was inserted for blood sampling prior to the beginning of the experiment and kept patent by an infusion of heparin-free isotonic saline (Abbotts Laboratories, Illinois, U.S.A.) and muscle biopsies were taken at rest and at exhaustion and frozen as described previously (outlined on p. 44).

(d) Short Term Maximal Exercise - Anaerobic Type Work

Normal subjects were exercised at 100% $\dot{V}O_2$ max until exhaustion on a cycle ergometer. The $\dot{V}O_2$ max for each subject was determined on a cycle ergometer[194] a week prior to the experiments.

Muscle biopsies were taken at rest and at exhaustion. The exercise time to exhaustion varied from 63 to 68 sec. The muscle biopsies were obtained as outlined earlier (p. 44).

(e) Training Induced Adaptation of Human Skeletal Muscle

(i) STUDY 1

Heavy Resistance Weight Training and Immobilization With the Arm

Nine healthy male subjects completed this study (age 19-22 years, wt 61-75 kg). The variation in body weight during the study was less than 2% in all subjects. None had performed any regular resistance or weight training during their life time. The scheme for the study is outlined in Figure 3. Upper arm girth was measured by a spring-loaded constant tension tape. Measurements were made before, during and after each section of the programme. Maximal voluntary contractile strength for elbow extention was measured concentrically on a Cybex II dynamometer at 30° per sec.

The muscle biopsy technique for the triceps brachii was similar to the technique used for the vastus lateralis

muscle as described earlier on p. 44. The long head of the triceps brachii was anaesthetized locally with 2% xylocaine (epinephrine free) and a 4 mm diameter needle was inserted through a 4-5 mm incision to a depth of about 4 cm below the skin. A 20-40 mg muscle sample from the non-dominant arm was obtained and frozen within 2-3 sec. and stored in liquid N_2 until analyzed.

(a) Training Programme: The subjects were trained in the following manner. The elbow extensors were trained by performing a bench press on the Universal Gym (Universal Sales Company, Fresno, California, U.S.A.), elbow extension and pullovers on Nautilus apparatus (Nautical Sports/Medical Industries, De Land, Florida, U.S.A.), and vertical dips between parallel bars with a suspended weight. For each work period, resistances were chosen to make it impossible for the subject to continue beyond 10 repetitions, and a maximum of 5 sets was performed with 2 min. rest periods between.

(b) Immobilization: Each subject had their non-dominant arm immobilized in a fiberglass elbow cast (Lightcast II, Merck and Company, West Point, Pennsylvania, U.S.A.) from the shoulder to the hand including the thumb, at 120°. The subjects' movement was not restricted except for the non-dominant limb. The subjects were assigned at random into 2 groups.

Figure 3.

Programme for Training Induced Adaptation of Human Skeletal Muscle To Heavy Resistance Weight Training Immobilization and Exercise

Study 1			
Group 1 (4)	Control	Weight Training (4 days/week : 5 months)	Immobilization (5 weeks)
Group 2 (5)	Control	Immobilization (5 weeks)	Weight Training (4 days/week : 5 months)
Study 2			
Group 3 (3)	Control + Exercise (15 min)	Weight Training (4 days/week : 5 months) + Exercise (15 min)	Immobilization (6 weeks) + Exercise (15 min)
Group 4 (3)	Control + Exercise (15 min)	Immobilization (6 weeks) + Exercise (15 min)	Weight Training (4 days/week : 5 months) + Exercise (15 min)

Legend:

Group 1 - muscle biopsies were taken pre-weight training (control), after weight training, and after immobilization.

Group 2 - muscle biopsies were taken pre-immobilization (control), after immobilization, and after weight training.

Group 3 - muscle biopsies were taken pre-training and after exercise, post training and after exercise, post immobilization and after exercise.

Group 4 - muscle biopsies were taken pre-immobilization and after exercise, post-immobilization and after exercise, and post training and after exercise.

Group 1.: Members of this group resistance weight trained 4 days weekly for 1 hr daily on alternate days for 5 months, then had their non-dominant arm immobilized for 5 weeks.

Group 2.: In contrast, group 2 members had their non-dominant arm immobilized for 5 weeks first, before beginning their 5 months of resistance weight training as above.

All girth and strength measurements were recorded on each subject in both groups before and after training periods, before the arms were placed in casts and within 12 hr of removing the casts after immobilization.

The muscle biopsies were taken before the study began and within 12 hr after the casts were removed, but 4-6 days were allowed to elapse after the final training session before the post training biopsies were taken. This procedure was followed to avoid any acute effects that may have occurred from the training programme. The biopsies were stored in liquid N_2 until analyzed.

(ii) Study 2

Heavy Resistance Weight Training, Immobilization And Exercise With the Arm

Six male subjects completed the study (age 19-23 years, wt 68-73 kg). None had performed regular resistance or weight training before the study.

The scheme of the study is outlined in Figure 3. The protocol was almost identical to the one outlined on p. 48 (Figure 3) except that the muscle was also biopsied before and after 15 min. of arm ergometry, and the arm cast period was extended to 6 weeks.

(a) Exercise: The exercise was carried out on a Monark ergometer adapted for the use of arm ergometry for 15 min. The exercise was done at 500 ± 50 Kpm producing a heart rate of between 145-155 beats/min. which was estimated to be about 50% of their $\dot{V}O_2$ max.[195] The arm ergometry exercise took place at the beginning of the study (control), after heavy resistance weight training for 5 months and after 6 weeks of immobilization. The subjects were randomly assigned to two, 3 member groups (groups 3 and 4).

Group 3.: Members of this group resistance weight trained 4 days weekly for 1 hr daily on alternate days for 5 months and immediately thereafter exercised by arm ergometry for 15 min. Their non-dominant arm was then immobilized in a cast for 6 weeks, the cast removed and exercised again by arm ergometry for 15 min.

Group 4.: In contrast to group 3, the reverse procedure was followed for group 4. The non-dominant arm was immobilized for 6 weeks, the cast removed and the arm exercised by arm ergometry. Immediately after the exercise,

the 5 month resistance weight training programme was begun and when finished, exercise by arm ergometry was carried out.

All girth and strength measurements were recorded as described previously on p. 46.

Muscle biopsies were obtained in the following way:

(a) Before the experimental portion of the study began and after 15 min. of arm ergometry exercise. (These served as controls).

(b) Within 12 hr after the 6 week old immobilizing casts were removed, and after 15 min. of arm ergometry exercise.

(c) After weight training for 5 months, and after the 15 min. of arm ergometry exercise.

B. ANALYTICAL AND PREPARATION TECHNIQUES

I. Handling and Storage of Tissues, Blood, And Perfusate Samples

All the tissue biopsies were immediately frozen and stored in liquid nitrogen until analyzed. Prior to the extraction, the human skeletal biopsies were dissected free of blood, tendon and fat at -20°C in a cold room or on a block of solid carbon dioxide. The rat heart samples were dissected free of all non-ventricular material. The rat skeletal muscle was treated in a similar manner. The heart

perfusate samples and plasma samples were stored at -20°C until analyzed.

When the muscle samples were analyzed for PDH_a and PDH_t, the muscle (human skeletal muscle, 20-40 mg; rat heart, 200-400 mg; rat skeletal muscle, 200-400 mg) was chipped into 20-40 mg pieces on solid Co_2 and homogenized in 30 (human skeletal muscle) or 4 volumes (rat skeletal muscle and heart) of an ice-cold buffer containing 10 mM potassium phosphate, 1.0 mM diethiothreitol (DTT), 1 mM Na-EDTA and 1 g/l of crystalline bovine serum albumin (BSA), pH 7.4 for 40 sec. using a Polytron RT10 homogenizer.

The homogenate obtained was used directly for the assay of PDH_a and PDH_t as outlined on p. 63. The PDH assays were completed within 24 hr of muscle sampling.

The muscle samples used to assay the concentration of metabolic intermediates were extracted with 30 (human skeletal muscle) and 4 volumes (rat skeletal muscle, heart, perfusate and plasma samples) of ice-cold 6% perchloric acid (PCA). Immediately after adding PCA the mixture was homogenized for 4 x 5 sec. bursts with a Polytron RT10 homogenizer. After centrifugation at 0°C, the supernatant was neutralized with 1N KOH containing 50 mM triethanolamine to a final pH of 6.0-7.0. The metabolites in question were assayed within 4 hr of neutralization.

The muscle samples used for glycogen analyses, however, were usually treated differently after they were frozen. As the muscle tissue to be used was often very small (usually less than 20 mg) the sample was homogenized with a Polytron directly in a volume of 1 ml 0.1 M acetate buffer pH 4.75 (0.05 M acetic acid : 0.05 M sodium acetate) and used directly for the assay of glycogen as outlined on p. 60. If larger muscle samples were available they were homogenized with a Polytron in 1N HCl at 0°C, boiled for 5 min. and analyzed immediately or stored at 5°C. All assay methods are outlined on p. 60.

II Fluorometric Analysis of Metabolites

(a) Tissue Metabolites

The methods used to determine the metabolite concentrations were based upon those of Bergmeyer 1963[196] and modified in this laboratory. Measurements were usually carried out on an Aminco Fluoro-Microphotometer (American Instrument Company, Silverspring, Maryland, U.S.A.) or an Eppendorf-Geratebau 1030 Photometer with an Eppendorf Recording Adapter 2134 (Hamburg, West Germany), but when the concentrations of the intermediate would be low, the measurements were made on a Farrand Ratio Fluorometer-2 (Farrand Optical Company Inc., New York, N.Y., U.S.A.).

The Excitation wavelength was 340 nm and the Emission wavelength was 460 nm.

(i) Adenosine Triphosphate (ATP) and Creatine Phosphate (CP)

The principle of these assays involved the reaction outlined below:

(1) glucose + ATP $\xrightarrow{\text{hexokinase}}$ G-6-P + ADP

(2) G-6-P + $NADP^+$ $\xrightarrow[\text{dehydrogenase}]{\text{G-6-P}}$ 6-phosphogluconolactone + NADPH + H^+

(3) CP + ADP $\xrightarrow[\text{phosphokinase}]{\text{creatine}}$ creatine + ATP

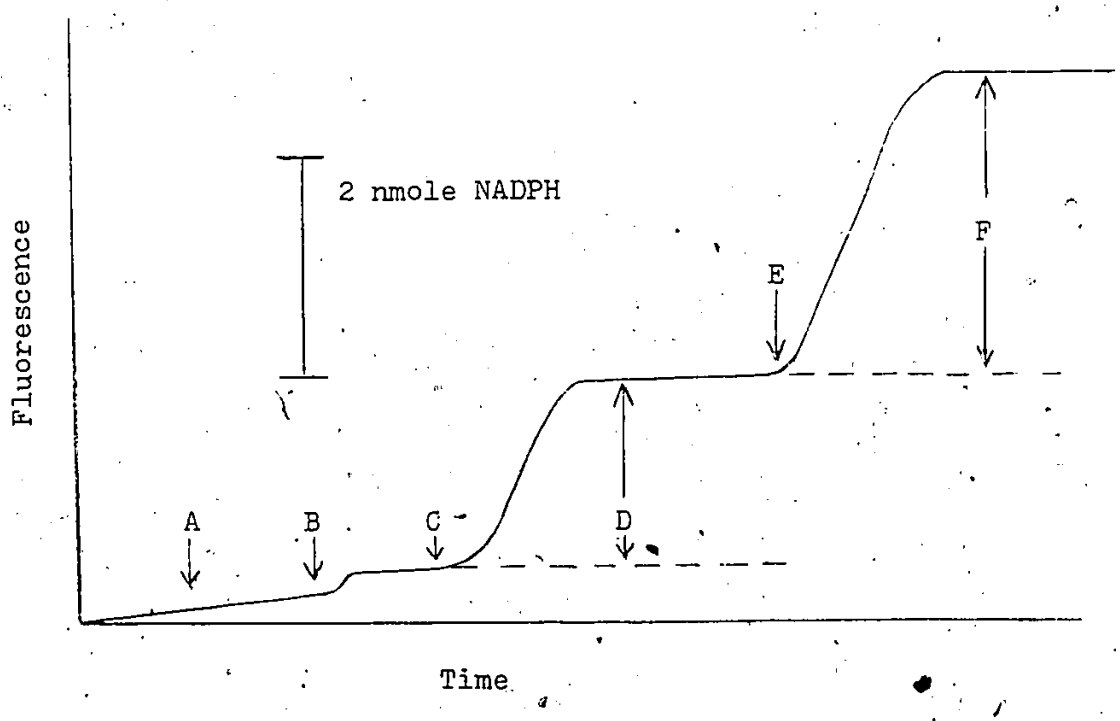
The 2 ml reaction mixture contained:

25 mM Tris
0.5 M Hydrazine hydrate
5 mM D-Glucose
100 μ M $NADP^+$
5 μ M Mercaptoethanol
100 μ M Na-EDTA
5 mM $MgCl_2$ with a final pH 8.5

The ATP concentration was determined as follows: Two ml of reaction mix containing an acidified-neutralized extract of muscle (equivalent of 2-10 mg of wet muscle), 10 μg of glucose-6-P dehydrogenase, and the change in baseline fluorescence with time was established. When the baseline was stable, 10 μg of hexokinase was added. This resulted in a rapid increase in fluorescence due to conversion of the ATP to ADP by hexokinase, which in turn,

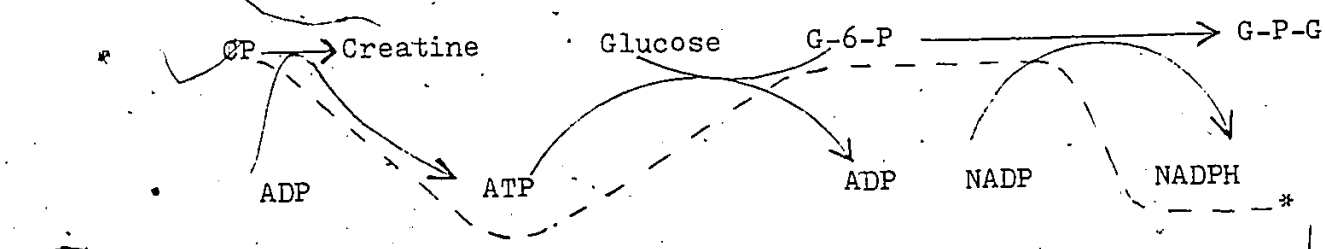
generates equimolar quantities of glucose-6-P and NADPH via glucose-6-P dehydrogenase. The assay was considered accurate when standard quantities of ATP and NADPH produced the same increase in fluorescence.

A representative assay for ATP and CP is shown below:



At point A 2 ml of reaction mix with muscle extract was placed in the fluorometer (Eppendorf). At point B 10 μg of glucose-6-P dehydrogenase (G-6-PdeH) was added. The small but rapid increase was due to muscle glucose-6-

phosphate content. At point C 10 μg of hexokinase (HK) was added. The rapid increase in fluorescence was due to conversion of ATP to ADP catalyzed by HK. The end point measurement was formation of fluorescent NADPH by G-6-P dehydrogenase. The fluorescence change shown as Point D represents the quantity of ATP present in the sample. At point E 10 μg of creatine phosphokinase was added. The rapid increase in fluorescence was due to the following sequence of reactions: * ---cyclic pathway of reaction



(ii) Pyruvate, Adenosine Diphosphate (ADP) and Creatine (C)

The principle of these assays is shown in the reaction below:

(1) pyruvate + NADH + H^+ $\xrightarrow{LDH}$ lactate + NAD^+

(2) PEP + ADP $\xrightarrow{PK}$ pyruvate + ATP

(3) creatine + ATP $\xrightarrow{CK}$ CP + ADP

The reaction mixture contained in tube (2 ml) the following:

25 mM Triethanolamine
5.0 mM KCl
5 mM $MgCl_2$
0.25 mM Phosphoenol pyruvate
20 μM NADH, final pH 7.4

Since reaction (1) and (2) are coupled by pyruvate that is formed in reaction (2), it was imperative to react all pyruvate already present in the reaction solution.

Two ml of reaction mix containing an acidified-neutralized extract of muscle (equivalent of 2-10 mg of wet muscle), and 10 μg of lactate dehydrogenase (LDH) was added to drive the reaction. The resulting decreased fluorescence was measured, this was the pyruvate formed (1) from NAD^+. The assay was considered completed after a further 10 μg LDH was added to check if there was no further fluorescence. The second stage of the reaction was begun by the addition of 10 μg pyruvate kinase (PK) and after 30 min. the reaction was complete. The decrease of fluorescence was due to the conversion of ATP to ADP (2) by the PK. The fluorescence was checked by the addition of a further 4 μg PK.

A new baseline was then established when excess ATP (100 μM) was added to the assay mix. After baseline, 20 μg creatine kinase (CK) was added to produce reaction (3) which is the creatine. A further 20 μg CK was added to check fluorescence. Appropriate known standards were used for each section of the assay reaction. The decrease in

fluorescence was quantitated by comparison with the decrease in the fluorescence obtained with the known amounts of standard.

(iii) Lactate

The reaction is shown below:

$$\text{lactate} + NAD^+ \xrightarrow{LDH} \text{pyruvate} + NADH + H^+$$

The lactate was measured enzymatically utilizing a $NADH:NAD^+$ coupled reaction which recorded the increase in fluorescence due to NADH formation.

The reaction ran to completion because pyruvate was removed by hydrazine. The reaction mixture (2 ml) contained:

25 mM Tris
0.5 M Hydrazine hydrate
200 μM NAD^+
5 μM Mercaptoethanol
100 μM Na-EDTA with a final pH of 8.5

Lactate dehydrogenase (10 μg) was added to 2 ml of incubation medium in the cuvette.

Varying amounts of muscle extracts were used to determine the lactate present. The assay system was standardized using known amounts of lactate and NADH. The assay was considered satisfactory if the lactate and NADH standards changed the fluorescence to the same extent. The execution of the assay was as outlined as on p. 55.

(iv) Glucose

Glucose was measured enzymatically by fluorometric techniques in the reaction set out below:

$$(1)\quad \text{glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\text{hexokinase}} \text{G-6-P} + \text{ADP}$$

$$(2)\quad \text{G-6-P} + NADP^{+} \xrightarrow[\text{dehydrogenase}]{\text{G-6-P}} \text{6-phosphogluconoluctone} + NADPH + H^{+}$$

The reaction mixture contained the following:

25 mM Tris
0.5 M Hydrazine hydrate
1.0 mM ATP
5 μM Mercaptoethanol
100 μM Na EDTA
1.0 mM $NADP^{+}$ with a final pH 8.5

The glucose concentration was determined by the following manner. Two ml of reaction mix containing 5-500 μl acidified-neutralized extract of muscle (equivalent of 2-10 mg of wet muscle), 10 μg of glucose-6-phosphate dehydrogenase (G-6-PD), and the change in baseline fluorescence with time was established. When baseline was established and the first reaction stable, 10 μg HK was added and the glucose formed, measured.

(v) Glycogen

The reaction is as follows:

(1) $(\text{glucose})_n + (H_2O)_{n-1} \xrightarrow{\text{amyloglucosidase}} n\text{ - glucose}$

(2) $\text{glucose} + \text{ATP} \xrightarrow[Mg^{++}]{\text{hexokinase}} \text{G-6-P} + \text{ATP}$

(3) $\text{G-6-P} + \text{NADP}^+ \xrightarrow[\text{dehydrogenase}]{\text{G-6-P}} \text{6-phosphogluconate} + \text{NADPH} + H^+$

Glycogen analysis was performed by enzymic hydrolysis with the enzyme amylo-α-1,4-α-1,6-glucosidase followed by enzymic assay of glucose as described by Passoneau and Lauderdale,[197] Nahorski and Rogers[198] and modified in this laboratory.

The reaction mixture contained:

50 mM Triethanolamine
1.0 mM ATP
1.0 mM $NADP^+$
5 mM $MgCl_2$ with a final pH 7.8

The incubation mixture contained:

100 mM Acetate (50 mM acetic acid : 50 mM Na acetate) with a final pH 4.75

The skeletal muscle (50-100 mg wet muscle) was homogenized in the acetate buffer and to an aliquot of the

buffer which contained (2-10 mg wet muscle equivalent) 50 ng of amylo-glucosidase in a volume of 5 μl was added to begin the reaction and the mixture incubated at room temperature for 1 hour. After incubation 200 mM Tris was added to each incubated aliquot.

A 25 μl samples of the incubation mixture was then added to the reaction mixture. Glucose-6-phosphate dehydrogenase (2 μg) is added directly to the reaction mixture or added to each aliquot separately. The resulting fluorescence was measured and 2 μg HK was added to drive the reaction and the glucose formed from the enzymatic degradation of glycogen measured. The NADPH formed was also measured after 10 min. at room temperature. The glucose and G-6-phosphate from the NADPH was also measured and substracted from the sample containing amylo-glucosidase.

The amylo-α-1,4-α-1,6-glucosidase liberates the glucose from glycogen by hydrolyzing α-(1-6)-linkages as well as α-(1-4)-linkages (1). Glucose (2) was converted to 6-phosphogluconate in one step by glucose-6-phosphate dehydrogenase and hexokinase. Appropriate standards of glycogen and glucose were used at each step.

(vi) Cyclic AMP

Muscle cyclic AMP was determined by a radioimmunoassay of Steiner et al., 1972.[246]

The assays were kindly performed by Dr. M. Rathbone of the Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada.

(b) Blood Gases and Blood pH

Arterial blood Pco_2, Po_2 and pH were determined on a Radiometer (Copenhagen) PH M72-MK2-Digital Acid-Base Analyzer and a BMS3-MK2-Blood Micro system.

(c) Plasma Glucose

Plasma glucose concentration was measured by enzymic fluorometric techniques in PCA and nuetralized with potassium bicarbonate as described by Lowry and Passoneau, 1972[199] and on p. 59 of the text.

(d) Plasma Lactate

Plasma lactate concentration was measured using enzymatic fluorometric methods in PCA extracts by the method of Toews et al., 1970.[200]

III Preparation of Mitochondria

For preparation of mitochondria, fresh rat heart and skeletal muscle was removed under anaesthesia (p. 39) and the procedure was a modification described by Chappell and Hansford, 1972[201] and Hansford, 1974.[202] The tissue was

rinsed in ice-cold sucrose medium (0.25 M sucrose, 5 mM Tris-HCl and 2 mM ethylene-glycol-bis-(aminoethyl)-tetra-acetate (EGTA), pH 7.4). The tissue was then cut up with scissors in 5 ml sucrose medium and homogenized with a Polytron PT10 homogenizer for 3 x 10 sec. at one third the maximum speed at 0-2°C. The cell debris was precipitated by slow-speed centrifugation (600-650 g for 10 min.) The supernatant was then centrifuged at 10,000 g for 1 hr. The pellet was resuspended in sucrose medium and diluted to 20 mg of protein/ml. The yield was approximately 5-10 mg of mitochondrial protein/g muscle. All operations were carried out at 0-4°C.

Pyruvate dehydrogenase activity in unfractionated rat skeletal muscle was approximately 300 ± 16 nmol/g/min. In unfractionated rat heart muscle it was approximately 1240 ± 30 nmol/g/min. Mitochondrial PDH activity was 5-7% of the total muscle activity.

Mitochondrial protein was determined by the method of Lowry et al., 1951.[203]

IV Pyruvate Dehydrogenase (PDH) Assay

The muscle samples used for the PDH assays were stored and extracted as outlined on p. 51.

Pyruvate dehydrogenase was assayed by a modification of the method described by Taylor et al.[105] The assay mix

for PDH_a contained 25 mM potassium phosphate, 2.5 mM Na-EDTA, 2.5 mM $MgCl_2$, 0.5 mM CoA, 2.5 mM dithiothreitol (DTT), 30 mM NAD^+, 1.5 mM thiamine pyrophosphate (TPP), 0.8 mM Na pyruvate, Na-pyruvate-1-^{14}C (1.7 μ C/ml) and 2 g/l crystalline bovine serum albumin, (pH 7.4). One quarter ml of the assay mix was added to a 10 ml Erlenmeyer flask cooled on ice in a cold room, with a disposable plastic center well (Kontes Glass, Vineland, N.J., U.S.A.) containing 0.2 ml of 10 N Hyamine hydroxide. The flasks were sealed with a rubber stopper containing the center well. The assay mixture was incubated at 37°C, and 0.2 ml of ice cold muscle homogenate was added 1 min. after incubation.

The Na-pyruvate-1-^{14}C was added just prior to addition of muscle homogenate to minimize nonspecific pyruvate-1-^{14}C decarboxylation. The control incubation was identical except no muscle extract was present. The assay mixture was incubated for 2 min. after enzyme added and then stopped by adding 0.8 ml of a solution containing 0.08 M citric acid and 0.04 M Na_2HPO_4, pH 3.0 under closed conditions. All the $^{14}Co_2$ was trapped in the Hyamine hydroxide center well after 60 min. The $^{14}Co_2$ was determined in the center well using standard scintillation counting techniques. The counting efficiency was determined using the channels ratio method and/or by use of an external standard.

The PDH_t activity was determined as described above except that the muscle extract was incubated for 6 min. in the buffer also containing 10 mM $MgCl_2$ and 10 mM $CaCl_2$. The Mg^{++} and Ca^{++} dephosphorylated the PDH_i forming PDH_a within the 6 min. incubation.

The lowest background counts were obtained when the Na-pyruvate-1-^{14}C was used just prior to assay.

V The Method for Determining the Specific Activity of Pyruvate

This method was developed to measure the specific activity of pyruvate in muscle extracts.

The principle of the method is as follows:

Pyruvate behaves as an anion and alanine as a cation on a cation exchanger at pH 7.4. Pyruvate can be quantitatively converted to alanine by the following reaction:

(1) $$\text{pyruvate} + \text{NADH} + NH_4^+ + H^+ \xrightarrow[\text{dehydrogenase}]{\text{alanine}} \text{alanine} + NAD^+$$

In any muscle extract any added labelled pyruvate will be partially present as alanine because of the rapid interconversion of these two intermediates. In the initial acidified-neutralized extract all the cations, including alanine, were removed by passing the solution through a cation exchange resin (Dowex (50 x 12 - 100) -50 W, Sigma Chemical Company, Missouri, U.S.A.). The effluent from the

column contained all the anions, including pyruvate. The pyruvate was then converted to alanine with alanine dehydrogenase and excess NADH, and repassed through a cation exchange resin. Since alanine dehydrogenase is specific for pyruvate, any ^{14}C retained by the column will be derived from pyruvate-1-^{14}C. The alanine is extracted from the cation exchange resin using 5N KOH and counted as ^{14}C as outlined on p. 68. The validity of the ion exchange chromatography was checked using alanine-1-^{14}C and Na-pyruvate.

Preparation of Sample

Frozen heart muscle was pulverized at -20°C into small pieces and homogenized with a Polytron as described on p. 51. After centrifugation at 4°C the supernatant was decanted, neutralized to pH 7.4 with 5N KOH and the sample recentrifuged. The supernatant was divided into 2 aliquots: one 2 ml aliquot was immediately freeze-dried and stored as powder at -20°C for future analysis of metabolites. The remaining was treated as outlined below:

Assay

(1) A 2 ml aliquot of muscle supernatant pH 7.4 was added directly to cuvette and placed in an Eppendorf 1030 photometer or a Farrand ratio-2-recording fluorometer.

(2) After a stabilization period for baseline fluorescence, 15 μ M NADH was added to cuvette and converted

to NAD^+, baseline readjusted, and 5 mM $(NH_4)_2SO_4$ was added and the baseline adjusted again.

(3) To the mix was added 5 μg alanine dehydrogenase to begin the reaction.

(4) When the reaction was completed another 5 μg alanine dehydrogenase was added to check whether the reaction was indeed completed.

(5) As a further check for the completion of reaction, a small amount of Na-pyruvate was added directly to cuvette. If the reaction was complete a further reaction would then take place with the pen travelling off scale.

(6) After the pyruvate to alanine reaction was completed, the complete assay mix was saved.

(7) The saved 2 ml mix was carefully passed through another cation exchange resin column and eluted with 2 ml 5N KOH and washed with 2 ml water. The alanine produced during the assay was trapped on the column and the pyruvate passed from the column. The effluent was neutralized to pH 7.4 with PCA , and the precipitate was centrifuged at 10,000 rpm to remove the potassium perchlorate, and the supernatant decanted directly into a scintillation vial. The resulting pellet was mixed and rinsed once with 0.5 ml water and centrifuged. The supernatant was combined with the first spin supernatant in the scintillation vial and immediately freeze-dried.

(8) Once freeze-dried, the vials were either stored, or 10 ml of scintillation mix was added directly to the vial. Before counting, it was desirable to add fumed silica 3-4 g/100 ml (Cab-O-Sil, grade 5 - Cabot Corporation, Boston, Mass., U.S.A.). A uniform mix and a slightly better counting efficiency resulted. The vials were counted by standard scintillation counting techniques as described on p. 69 .

VI ^{14}C Determinations

The scintillation fluid for counting ^{14}C determinations was toluene:ethanol (9:1, v/v) containing POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene] 50 mg/l, PPO (2,5-diphenylo-xazole) 5 g/l napthalene 130 g/l and dioxane.

The radioactivity was determined at 4°C in a Beckman LS-230 liquid scintillation counter. Efficiency was determined by the channels ratio method.

VII $^{14}Co_2$ Production

$^{14}Co_2$ production was determined by collecting the effluent from the hearts in flasks under a layer of mineral oil in preweighed beakers. The effluent volume was determined gravitationally. A 4 ml aliquot was transferred to a 10 ml Erlenmeyer flask sealed with rubber stopper and disposable plastic center well containing 0.4 ml 10 N Hyamine hydroxide.

The aliquot was then acidified with 4 ml 0.5 M citric acid and incubated for 60 min. at 20°C. The liberated $^{14}Co_2$ absorbed into the Hyamine hydroxide was placed directly into a scintillation vial and counted in toluene-based scintillator as described above. The recovery of $^{14}Co_2$ in the center well from $^{14}C\text{-}HCO_3^-$ in the flask was 97 ± 3%.

VIII Calculations and Statistical Analysis

Statistical analysis was evaluated by using the student's 't' test.

RESULTS

1. Development of PDH Assay Method

In the initial experiments, skeletal muscle mitochondria were isolated and prepared as described earlier on p. 62. The isolation technique resulted in only 5-10% recovery of the mitochondria. The effect of PDH_a and PDH_t activation versus a function of incubation time is shown in Figure 4. It is apparent that the PDH_a and PDH_t activity was linear with enzyme concentration to a mitochondrial concentration of 0.5 mg mitochondria protein/0.5 ml. In other experiments, (Figure 5), it was demonstrated that the rate of pyruvate oxidation by PDH was linear with time.

Because the recovery of mitochondria from muscle is unpredictable and low[201,202] and because the human muscle biopsy samples are small, it was necessary to develop a PDH assay that could be reliably used in muscle homogenates obtained from a needle muscle biopsy. A variety of different techniques were used and the one described on p. 63 was found to be the most reproducible.

Using the muscle homogenate preparation of PDH, the activity was linear with muscle content and the PDH activity was linear with time (Figure 6).

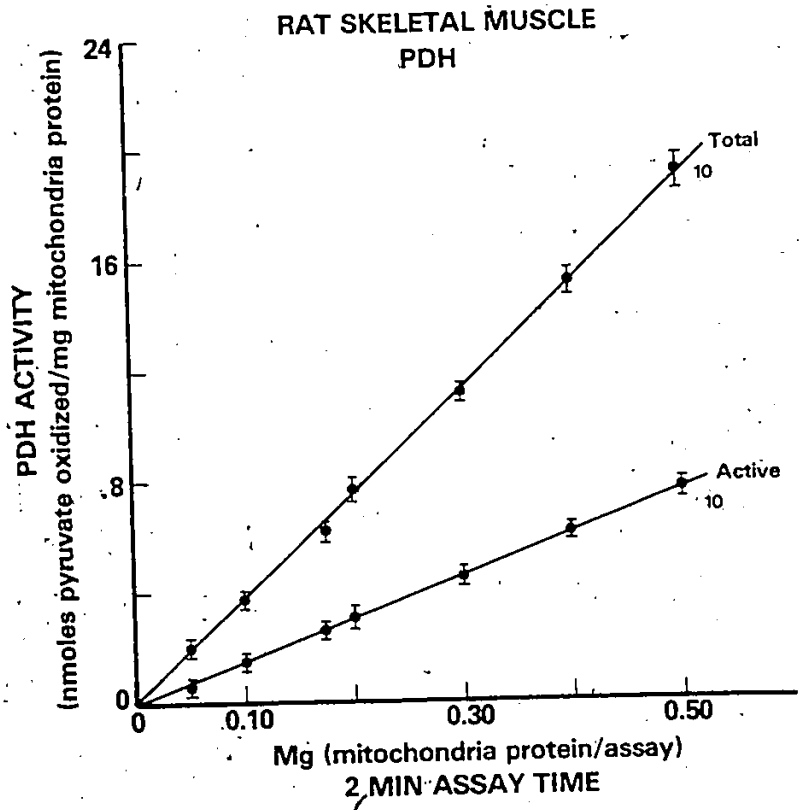


Figure 4.

The effect of mitochondrial protein on PDH activity. Mitochondria from rat skeletal muscle was used for determining PDH. Enzyme activity was calculated before and after preincubation at 37° with 10 mM Ca^{++} and 10 mM Mg^{++}. The PDH activity was expressed in nanomoles pyruvate oxidized per mg of mitochondrial protein. Results are means ± S.E.M. and the number of observations for each point is as indicated at the end of the linear line.

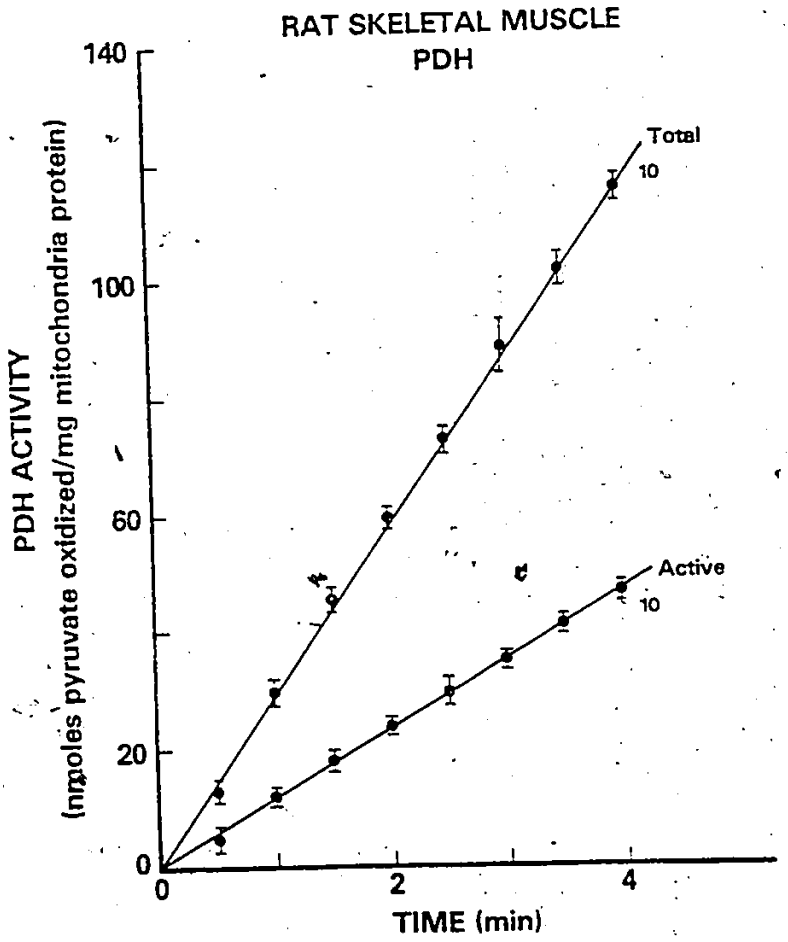


Figure 5.

Relationship between initial velocity versus time and enzyme concentration of PDH. The activity of PDH was determined as in "Materials and Methods". PDH_a and PDH_t activity was measured over varying time periods. Mitochondria were prepared from rat skeletal muscle and were used for PDH assays. Samples for PDH_t were incubated at 37°C. The $^{14}Co_2$ produced was calculated and the pyruvate oxidized, expressed as nmoles per mg of mitochondrial protein. There were 10 observations for each time point and the vertical bars represent ± S.E.M.

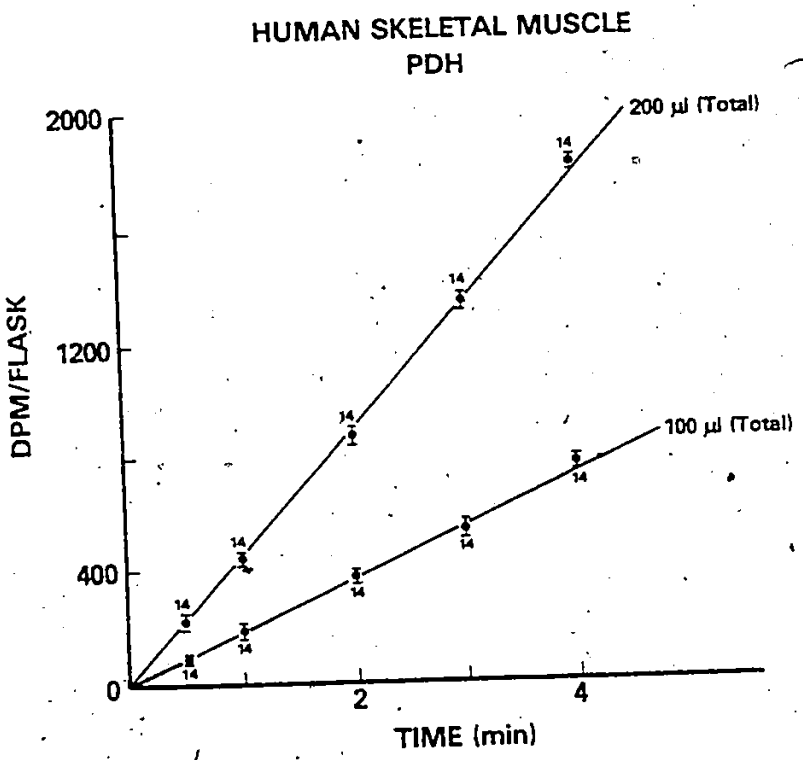


Figure 6.

Counts of total enzyme activity versus assay time. The muscle homogenates were prepared and enzyme assays with human skeletal muscle were performed as outlined in "Materials and Methods". The vertical bars represent ± S.E.M. and there were 14 observations for each point and the results are expressed as DPM/flask.

In other experiments, the same observations were made in heart (not shown). In the experiments it was demonstrated that by increasing the enzyme concentration to 300 μl (9.75 mg wet muscle) and extending the assay time to 4 or 5 min., it did not shift the line from linearity. As the assays were all run for only 2 min. and the maximal muscle added never exceeded 6.5 mg of muscle equivalent, the assay was linear with respect to time and concentration during these experiments. In fact, the enzyme activity was linear for at least 10 min. at 37°C (results not shown). The cofactor requirements for the PDH assay in muscle were characterized in more detail (Figures 7 and 8). There was less than 2% maximal activity without the presence of Mg^{++} in skeletal muscle. Full activity was achieved by addition of 10 mM Mg^{++}, 10 mM Ca^{++}, 0.5 mM CoA, 30 mM NAD^{+}, and 1.5 mM TPP. Omitting CoA, NAD^{+}, or TPP decreased the activity by 79% 7% and 82% respectively (Figure 7). The heart muscle responded to the lack of cofactors in a similar manner (Figure 8).

2. Physiological Regulators of PDH Activation

A. Nerve Stimulation in the Rat

The purpose of these experiments was to determine whether PDH activation could be mediated by nerve stimulation rather than a general hormonal effect.

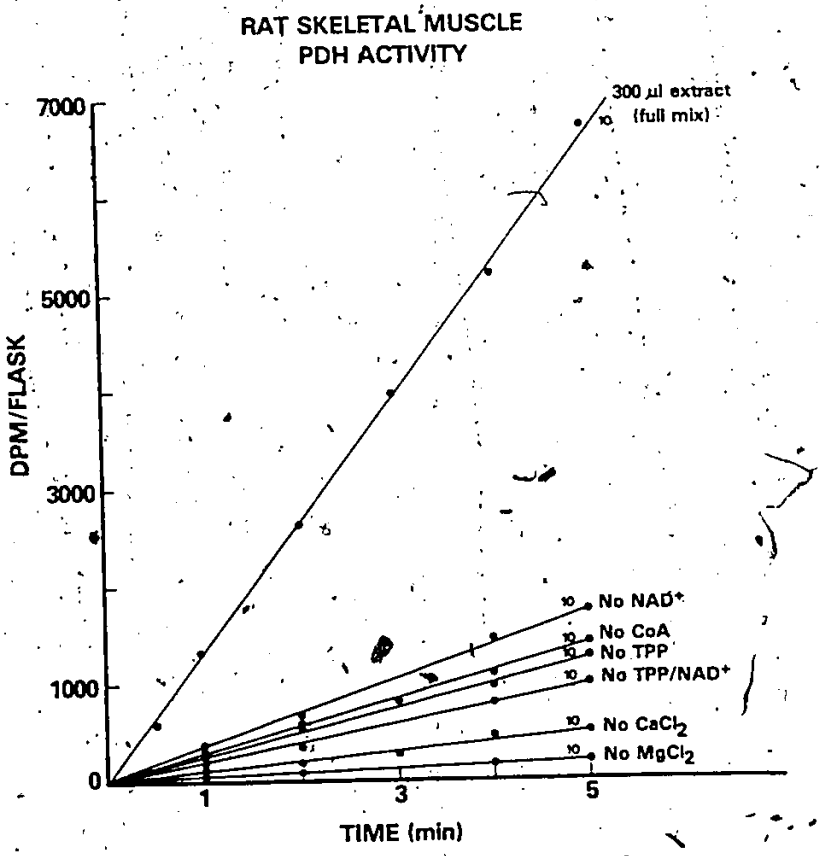


Figure 7.

The effect of various cofactors on the activity of rat skeletal muscle PDH. The components and conditions were as described in "Materials and Methods". The PDH activity was measured over various time periods with and without certain cofactors as indicated. There were 10 samples for each time point.

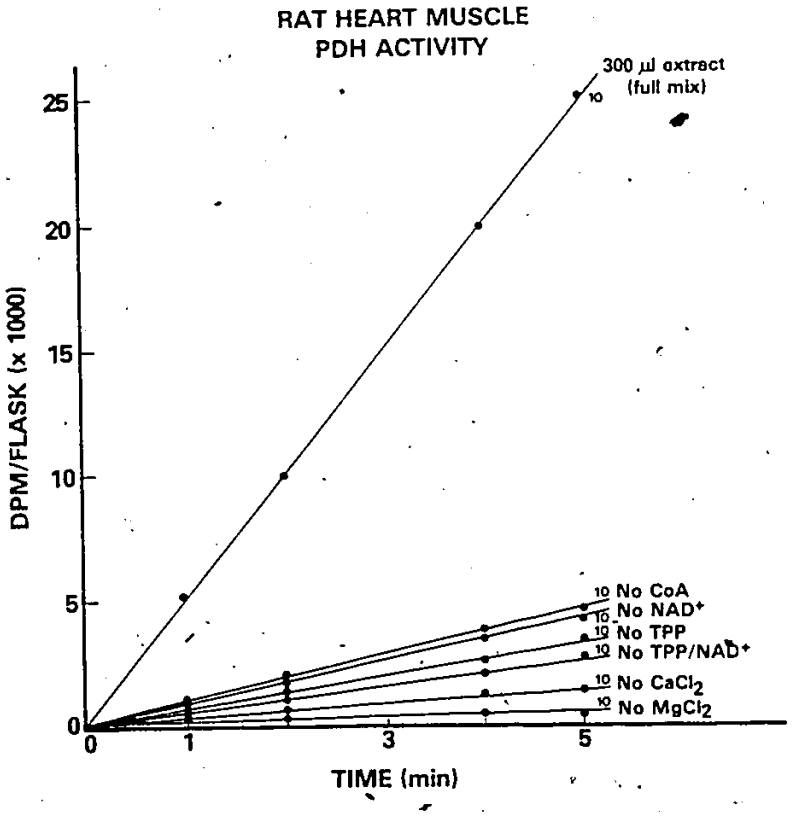


Figure 8.

The effect of various cofactors on the activity of rat heart muscle PDH. The PDH activity was measured with and without cofactors over varying time periods. All conditions were as described in "Materials and Methods". There were 10 samples for each time point.

I The Effect of Sciatic Nerve Stimulation on Gastrocnemius Skeletal Muscle With a Constant Pulse Frequency and Voltage

The effect of electrioal stimulation on PDH activity in rat gastrocnemius skeletal muscle is shown in Table 1. The PDH_a at rest was 296 ± 15 nmol/g/min. and increased to 591 ± 29 nmol/g/min. after 1 min. electrical stimulation. The maximal PDH_a activity was achieved after 10 min. of nerve stimulation. At this point more than 95% of the PDH_t was in the active form.

Table 2 shows the effect of electrical stimulation on PDH activity in rat gastrocnemius skeletal muscle but during these experiments the muscle biopsies were obtained at more frequent intervals to detect the rapidity of the activation of PDH_a conversion. The PDH_a at rest was 276 ± 15 nmol/g/min. ,and electrical stimulation rapidity, increased the PDH_a activity. The PDH became activated linearly with time during the first 2 min. of nerve stimulation. Less rapid activation of PDH was shown after that (Table 2).

The PDH_t did not change significantly during any of the experiments in Tables 1 and 2 with the average PDH_t concentration for both being 840 ± 39 nmol/g/min. indicating that there was no significant muscle sampling errors.

The effect of electrical nerve stimulation on some key muscle metabolite concentrations is shown in Table 3.

Table 1

The Effect of Sciatic Nerve Stimulation On

Pyruvate Dehydrogenase Activation in Rat Gastrocnemius Skeletal Muscle

Time (Min.)	0	1	2	4	5	10
PDH_a:PDH_t (%)	36	67	77	91	92	97
PDH_a (nmol/g/min.)	296±15*	591±29	667±47	773±23	789±45	804±22
PDH_t (nmol/g/min.)	831±28	886±41	859±45	851±31	856±43	821±27
Observations	20	12	12	6	6	6

Legend: * mean ± S.E.M.

There were varied observations at each time period. The sciatic nerve was stimulated at a frequency of 10 pulses/sec. at 5 volts. PDH_a and PDH_t (PDH_a + PDH_i) was assayed as outlined in "Methods" section. Rat gastrocnemius skeletal muscle was used for each experiment. Controls were taken from the contralateral leg during each stimulation period. The results are expressed as nmol/g/min.

Table 2

Effect of Sciatic Nerve Stimulation On The Rapidity

Of Pyruvate Dehydrogenase Activation In Rat Gastrocnemius Skeletal Muscle

Time (Sec.)	0	5	15	30	60
PDH_a:PDH_t (%)	35	47	51	60	66
PDH_a (nmol/g/min.)	276±15*	397±11	433±46	539±38	555±30
PDH_t (nmol/g/min.)	792±28	849±36	836±65	889±48	836±35
Observations	20	6	6	12	12

Legend: * mean ± S.E.M.

There were varied observations at each time period. The sciatic nerve was stimulated at a frequency of 10 pulses/sec. at 5 volts. PDH_a and PDH_t (PDH_a + PDH_i) was assayed as outlined in "Methods" section. Gastrocnemius skeletal muscle was used for all the above experiments. Controls were taken from the contralateral leg during each stimulated period. The results are expressed as nmol/g/min.

Table 3

The Activity of Selected Metabolites in Extracts of

Rat Gastrocnemius Skeletal Muscle Before and After Electrical Stimulation

Time (Sec.)	0	5	15	30	60	120	240	300	600
ATP	5.02±0.30*	4.63±0.15	4.37±0.21	3.98±0.28	3.76±0.27	3.59±0.23	3.44±0.26	2.99±0.22	3.20±0.29
ADP	0.63±0.04	0.66±0.04	0.70±0.03	0.70±0.03	0.80±0.03	0.87±0.05	- -	0.96±0.05	1.15±0.06
CP	17.94±1.03	14.32±1.20	13.17±0.95	11.73±1.36	10.24±1.17	8.81±0.72	8.68±0.43	7.40±0.68	4.86±0.88
Lactate	1.32±0.11	4.92±0.57	8.32±0.54	11.29±0.98	13.24±0.42	15.19±0.83	14.31±0.83	14.02±0.77	13.73±0.86
Pyruvate	0.12±0.01	0.10±0.01	- -	0.21±0.01	0.26±0.03	0.32±0.03	- -	0.37±0.04	0.39±0.04
Glycogen	37.18±3.14	- -	- -	- -	- -	31.04±4.74	- -	- -	11.50±3.18
ATP:ADP	8	7	6	6	5	4	- -	3	3
Lactate:Pyruvate	11	49	- -	54	51	48	- -	38	35

Legend: * mean ± S.E.M. Concentrations are tabled for each metabolite and reported as μmol/g wet m. There were 6 observations at each time point. The nerve stimulation was of 10 pulses/sec. at 5 volts. The samples were obtained from the muscle biopsies as shown in Tables 1 and 2.

The ATP concentration and the ATP:ADP ratio decreased significantly within 15 sec. after the start of nerve stimulation (Table 3). The CP concentration decreased to 10 from 17.9 μ mol/g at 60 sec. and continued to decrease to 4.9 μ mol/g after 10 min. of stimulation. The lactate concentration at rest was 1.3 μ mol/g and increased at every time period, but after 2 min. it reached a peak concentration of 15.2 μ mol/g. Pyruvate also increased as nerve stimulation progressed. At rest, the pyruvate concentration was 0.12 μ mol/g and after 10 min. of the nerve stimulation the concentration increased to 0.39 μ mol/g. The L:P ratio increased from 11 at rest to 35 after 10 min., however, the highest ratio was observed at 30 sec. The resting glycogen concentration was 37.2 μ mol/g. Nerve stimulation was associated with a decrease in glycogen concentration during the entire stimulation period. The lowest concentration of 11.5 μ mol/g was recorded after 10 min.

II The Effect of Sciatic Nerve Stimulation on Gastrocnemius Skeletal Muscle by Varying Pulse Frequency and Voltage

The following experiments extend the previous studies and show the effect of the stimulation rate on the activation of PDH in rat gastrocnemius skeletal muscle. The studies involved a varying pulse frequency of 5, 10 and 40

pulses/sec. at a constant voltage of 5 volts (Figure 9) and a constant 40 pulse frequency/sec. with a varying voltage of 5, 10 and 40 volts (Figure 10).

The resting PDH was essentially the same for all groups (Figures 9 and 10). Increasing the frequency of nerve stimulation increased the rate of PDH_a formation. For example, at 30 sec. of nerve stimulation at 5 pulses/sec. resulted in 52% PDH_a activation, while at 30 sec. of nerve stimulation at 40 pulses/sec. PDH was 85% activated.

The effect of changing the stimulation voltage at a constant pulse frequency is shown in Figure 10. The rate of PDH activation was slowest at 5 volts and the most rapid at 40 volts. At 120 sec. the three voltage groups had PDH_a activated to over 90%.

As shown in Tables 4 and 5, tissue metabolites were determined in the freeze-clamped skeletal muscle at rest and at the end of each time period in only the rats which were treated by a nerve stimulation of 5 pulses/sec. at 5 volts, and 40 pulses/sec. at 40 volts respectively. The metabolite concentration changes were similar to those obtained earlier (Table 3), except that the metabolites which increased or decreased their concentration did so more slowly at a pulse frequency of 5/sec. than at 10/sec. The ATP, CP and glycogen concentrations decreased considerably in the 5 pulses/sec. at 5 volts stimulation while ADP,

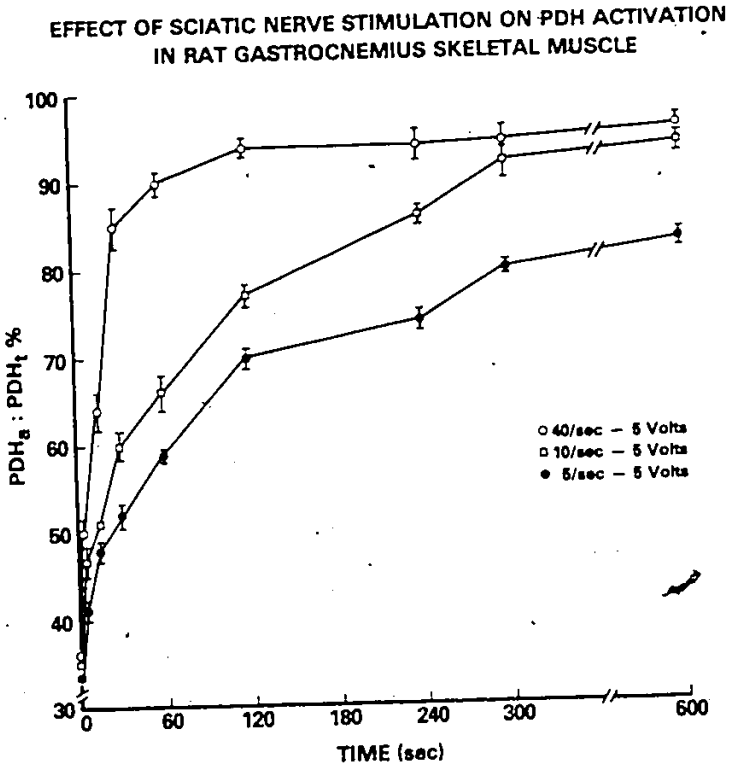


Figure 9.

At 0 time the sciatic nerve of the rat was stimulated in a square wave fashion with 5 volts at a frequency of ○40 pulses, □10 pulses and ●5 pulses/second and pulse duration of 5 milliseconds. Gastrocnemius muscle biopsies were obtained at 0, 5, 15, 30, 60, 120, 240, 300, 600 seconds from the contralateral side and the stimulated leg. There were 6 stimulated observations for all time points. Each stimulated muscle sample was accompanied by a control sample. The control sample is the mean of 54 observations. The PDH_a activity for 40 pulses/second and 5 volts was 306, 397, 439, 568, 697, 821, 831, 819, and 862 nmol/g/min from 0 to 600 seconds. For 10 pulses/second at 5 volts the PDH_a activity was 296, 397, 433, 539, 591, 639, 773, 789 and 764 nmol/g/min. For 5 pulses/second at 5 volts the PDH_a activity was 280, 330, 399, 460, 524, 634, 660, 667 and 697 nmol/g/min from 0-600 seconds. The PDH_t averaged about 845 nmol/g/min. The vertical bars are ± S.E.M.

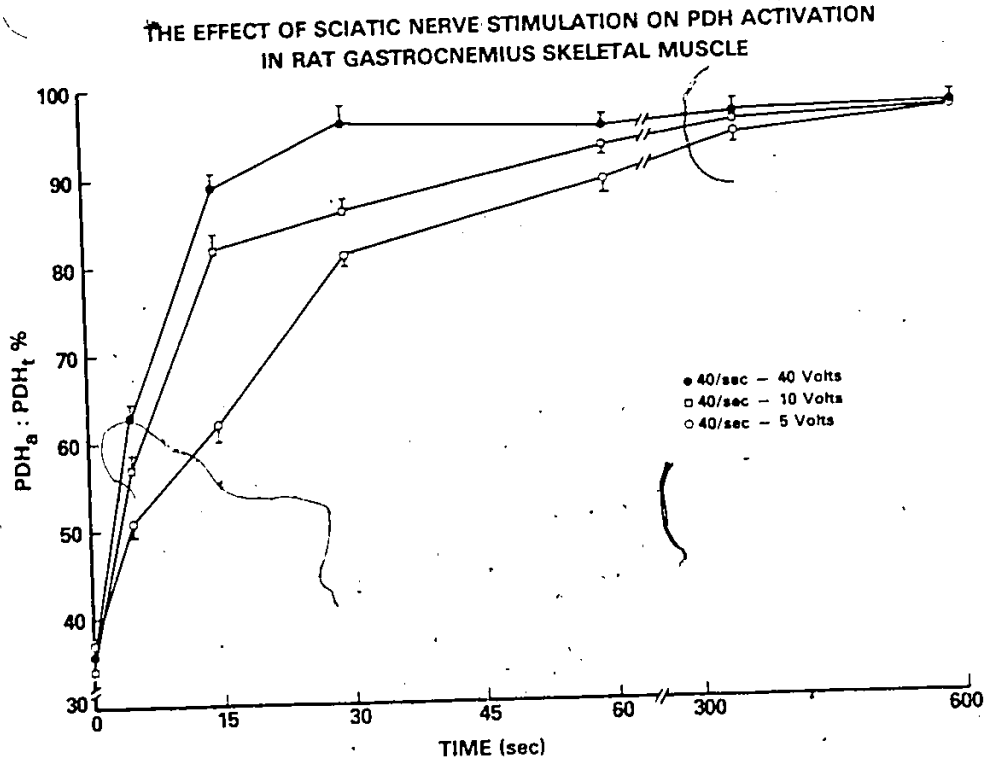


Figure 10.

For particulars of this experiment refer to Figure 9. In this case, however, the nerve was stimulated at a constant pulse but with a varied voltage. The pulses were 40/second but the voltage range was ●40 volts, □10 volts and ○5 volts respectively. Muscle biopsies were obtained at the same time periods but as the 120-240 second points were very similar to the 300 second period they were not included. There were 6 observations for the stimulated muscle at each point and a control was sampled with each. The PDH_a activity for 40 pulses/second at 40 volts was 312, 506, 759, 842, 826, 859 and 841 from 0-600 seconds respectivly. For 40 pulses/second at 10 volts PDH_a was 287, 482, 676, 732, 804, 820, 847 from 0-600 seconds while for 40 pulses/second at 5 volts PDH_a was 313, 422, 531, 688, 760, 821, 840 from 0-600 seconds respectively. The PDH_t averaged about 865 nmol/g/min. The vertical bars are ± S.E.M.

Table 4

The Concentration of Metabolites Before and After Sciatic Nerve Stimulation in Rat Gastrocnemius Skeletal Muscle

Time (Sec.)	0	5	15	30	60	120	240	300	600
ATP	5.17±0.32*	4.66±0.18	4.46±0.22	4.57±0.20	4.61±0.20	4.42±0.19	4.26±0.22	3.94±0.24	3.88±0.33
ADP	0.67±0.05	0.80±0.03	0.76±0.02	0.74±0.04	- -	0.77±0.03	- -	0.86±0.04	1.08±0.04
CP	17.67±0.82	14.87±0.74	13.71±1.08	13.26±1.05	11.86±0.89	11.00±0.83	9.92±0.84	8.40±1.14	6.36±0.92
Lactate	1.38±0.19	3.40±0.43	4.47±0.37	6.82±1.00	9.62±0.83	12.82±0.76	13.45±1.05	14.05±0.94	12.83±0.81
Pyruvate	0.14±0.01	0.25±0.03	- -	0.23±0.01	- -	0.30±0.02	0.29±0.03	0.29±0.03	0.36±0.02
Glycogen	37.70±2.06	- -	- -	- -	- -	34.32±5.48	- -	- -	27.06±6.96
ATP:ADP	8	6	6	6	- -	6	- -	5	4
Lactate:Pyruvate	10	14	- -	30	- -	43	47	49	36
Observations	6	6	6	6	6	6	6	6	6

Legend: * mean ± S.E.M. There were 6 observations for each time period shown. The sciatic nerve was stimulated in vivo at a frequency of 5 pulses/sec. at 5 volts. All assay procedures were as outlined in "Methods" section. Gastrocnemius skeletal muscle of the rat was used in all the above experiments. Values for the resting muscle samples are from the unstimulated contralateral leg. The results are expressed in µmol/g wet m.

Table 5

The Concentration of Metabolites Before and After Sciatic Nerve Stimulation in Rat Gastrocnemius Skeletal Muscle

Time (Sec.)	0	5	15	30	60	120	240	300	600
ATP	5.29±0.26*	4.69±0.19	4.34±0.27	4.03±0.26	3.65±0.31	3.11±0.27	3.28±0.24	1.99±0.34	2.81±0.21
ADP	0.64±0.02	0.66±0.03	0.67±0.03	0.71±0.05	0.73±0.03	0.86±0.04	0,97±0.02	1.08±0.05	1.21±0.05
CP	20.09±1.03	16.30±1.11	14.66±0.97	11.89±0.88	8.38±0.95	5.31±1.00	3.27±1.11	1.38±0.92	1.61±0.80
Lactate	1.35±0.22	6.70±1.9	10.61±2.4	16.82±1.9	18.48±2.9	23.91±2.2	22.88±2.0	26.40±2.3	25.13±2.8
Pyruvate	0.11±0.01	0.15±0.02	0.19±0.02	0.37±0.06	0.41±0.03	0.55±0.04	0.60±0.09	0.64±0.08	0.74±0.12
Glycogen	34.11±3.20	32.85±2.33	30.11±1.82	26.32±3.41	20.82±2.04	20.42±3.23	17.56±4.40	14.37±5.12	10.83±3.13
ATP:ADP	8	7	7	6	5	4	3	2	2
Lactate:Pyruvate	12	45	56	46	45	44	38	41	34
Observations	6	6	6	6	6	6	6	6	6

Legend: * mean ± S.E.M. There were 6 observations for each time period. The sciatic nerve was stimulated in vivo at a frequency of 40 pulses/sec. at 40 volts. All assay procedures were as outlined in "Methods" section. Gastrocnemius skeletal muscle of the rat was used in all the above experiments. Values for the resting muscle samples are from the unstimulated contralateral leg. The results are expressed in μmol/g wet m.

pyruvate, lactate, and the L:P ratio increased (Table 4). When comparing the 40 pulses/sec. at 40 volts with the results above, Table 5 shows that all metabolite changes were similar, but the changes were more dramatic in response to the higher nerve stimulation. The ATP, CP, and glycogen concentrations and the ATP:ADP ratio showed a rather high degree of depletion while ADP, lactate, and pyruvate concentrations and the L:P ratio increased considerably.

III The Effect of Femoral Nerve Stimulation on Vastus Lateralis Skeletal Muscle By Varying Pulse Frequency and Voltage

These experiments using nerve stimulation on the rat were carried out in order to determine whether the nerve stimulation of another muscle group from a different nerve supply caused a variation in PDH activation. The vastus lateralis skeletal muscle from the quadriceps femoris group was stimulated via the femoral nerve. (Table 6)

The PDH_a in the vastus lateralis muscle increased in a manner which was similar to the gastrocnemius muscle response to nerve stimulation. (see Figure 9) The rate of increase of PDH_a activity was less with the vastus lateralis. This variation was not due to the pulse frequency or voltage applied to the nerve because the same conditions were used in both experiments. (see Figure 9) When the

pulse frequency was increased from 5 to 10 pulses/sec. at 5 volts, the rate of PDH_a formation was unaltered. (Tables 6 and 8)

In Tables 7 and 9 the metabolites measured displayed essentially the same concentrations at rest in the vastus lateralis as compared to the at rest gastrocnemius skeletal muscle, except for the glycogen concentration. The glycogen concentration in the vastus lateralis was 18 μmol/g compared to 38 μmol/g in the gastrocnemius muscle. (see Tables 4 and 5) The patterns of all the metabolites measured either increased or decreased in the vastus lateralis during nerve stimulation in a similar way to that observed in the gastrocnemius. (see Tables 4, 5, 7, and 9)

3. Aerobic Training in the Rat

Many studies in man and other mammalian species indicate that aerobic type training increases skeletal muscle capacity of O_2 consumption and Co_2 production.[204] The purpose of the following experiments was to determine whether PDH adaptation could be a major factor for these observations. The role of PDH in the adaptive process has not yet been studied.

The effect of 10 weeks of aerobic training in skeletal muscle PDH activation is shown in Table 10. The PDH_t activity was higher in the soleus than in the

Table 6

Effect of Femoral Nerve Stimulation on Pyruvate Dehydr ogenase Activation

In Rat Vastus Lateralis Skeletal Muscle Before and After Exercise

Time (Sec.)	0	7.5	15	30	60	120	240	300	600
PDH_a:PDH_t (%)	35	42	44	47	52	59	66	70	71
PDH_a (nmol/g/min.)	289±14*	341±13	355±18	386±16	424± 34	490±34	577±38	591±46	611±48
PDH_t (nmol/g/min.)	811±32	822±31	815±31	821±29	805± 48	825±32	865±39	829±36	861±57
Observations	6	6	6	6	6	6	6	6	6

Legend: * mean ± S.E.M. There were 6 observations for each time period. The femoral nerve was stimulated at a frequency of 5 pulses/sec. at 5 volts. All assays were as outlined in "Methods" section. Vastus lateralis skeletal muscle from the quadriceps group was used in each experiment. The results are expressed in nmol/g/min. The values for the resting muscle samples are from the unstimulated contralateral leg.

Table 7

Activity of Selected Metabolites in Extracts of Rat Vastus Lateralis Skeletal Muscle Before and After Electrical Stimulation

Time (Sec.)	0	7.5	15	30	60	120	240	300	600
ATP	5.06±0.17*	5.01±0.12	4.77±0.20	4.76±0.15	4.13±0.22	4.00±0.28	4.04±0.28	4.18±0.28	4.06±0.09
ADP	0.66±0.02	0.70±0.01	0.70±0.01	0.73±0.02	0.74±0.02	0.79±0.03	- -	0.89±0.03	1.02±0.04
CP	17.32±1.13	15.34±0.75	13.98±0.59	13.77±0.69	13.43±0.48	11.99±0.89	9.92±0.76	9.15±0.98	6.92±0.55
Lactate	1.52±0.16	3.47±0.41	3.73±0.42	5.11±0.52	6.48±0.58	7.53±0.38	- -	7.86±0.39	8.19±0.43
Pyruvate	0.13±0.01	- -	- -	0.20±0.01	0.21±0.02	0.23±0.02	- -	0.28±0.02	0.30±0.03
Glycogen	18.02±5.24	- -	- -	- -	- -	14.90±3.30	- -	- -	13.94±1.98
ATP:ADP	7.66	7.15	6.81	6.52	5.58	5.06	- -	4.69	3.98
Lactate:Pyruvate	11.69	- -	- -	25.55	30.85	32.73	- -	28.07	27.30

Legend: * mean ± S.E.M. Six (6) observations were used for each metabolite at each time period. The femoral nerve was stimulated at a frequency of 5 pulses/sec. at 5 volts. All assays were as outlined in "Methods" section. Vastus lateralis skeletal muscle was used for each experiment. All results are expressed in μmol/g wet m.

Table 8

Effect of Femoral Nerve Stimulation on Pyruvate Dehydrogenase Activation In Rat Vastus Lateralis Skeletal Muscle Before and After Exercise

Time (Sec.)	0	15	30	60	120
PDH_a:PDH_t (%)	34	43	45	48	63
PDH_a (nmol/g/min.)	278 ± 17*	351 ± 25	382 ± 45	422 ± 45	535 ± 45
PDH_t (nmol/g/min.)	819 ± 27	820 ± 33	859 ± 60	844 ± 53	845 ± 22
Observations	8	8	8	8	8

Legend: * mean ± S.E.M. There were 8 observations for each time period. The femoral nerve was stimulated at a frequency of 10 pulses/sec. at 5 volts. All assays were as outlined in "Methods" section. Vastus lateralis skeletal muscle from the quadriceps group was used in each experiments. The results are expressed in nmol/g/min. The values for the resting muscle samples are from the unstimulated contralateral leg.

Table 9

Selected Metabolite Concentrations From the Vastus Lateralis Rat Skeletal Muscle Before and After Femoral Nerve Stimulation

Time (Sec.)	0	15	30	60	120
ATP	4.72±0.18*	4.40±0.13	4.27±0.15	4.01±0.17	4.03±0.19
ADP	0.72±0.03	0.78±0.03	0.77±0.01	0.78±0.01	0.81±0.02
CP	18.74±0.36	14.65±0.55	12.27±0.73	0.75±0.94	7.83±0.80
Lactate	1.45±0.15	4.85±0.44	6.87±0.74	9.39±0.65	11.21±0.78
Pyruvate	0.13±0.02	0.19±0.01	0.19±0.01	0.28±0.02	0.30±0.03
Glycogen	17.50±2.04	- -	- -	- -	13.12±1.35
ATP:ADP	7	6	6	5	5
Lactate:Pyruvate	11	26	36	34	37
Observations	8	8	8	8	8

Legend: * mean ± S.E.M. There were 8 observations for each time period. Stimulation of the femoral nerve was carried out at 10 pulses/sec. at 5 volts. All assays were outlined in "Methods" section. Vastus lateralis skeletal muscle from the quadriceps of the rat was used in each experiment. All results are expressed as μmol/g wet m.

gastrocnemius. After 10 weeks of aerobic training the PDH_t activity was unchanged in either muscle group. However, the resting PDH_a in the soleus and gastrocnemius muscle was increased by about twofold as a result of the 10 week training programme.

4. Exercise in Human Skeletal Muscle In Vivo

The purpose of these experiments was to determine the forms of exercise in man that might be important in the regulation of skeletal muscle PDH activation.

A. Maximum Voluntary Isometric Contraction (MVIC)

Table 11 shows the results of skeletal muscle PDH activity in human subjects contracting isometrically at 50% MVIC. The PDH_a at rest was 111 ± 3 nmol/g/min. while the PDH_t was 302 ± 3 nmol/g/min. The resting PDH_a:PDH_t was 37%. After 10 sec. of contraction the PDH_a increased only slightly to 117 ± nmol/g/min. No significant changes in PDH_a were observed at 20, 30, or 60 sec. after contraction.

A number of key muscle intermediates were measured and are summarized in Table 12. The ATP concentration decreased progressively with time from 5.06 μ mol/g at rest to 2.90 μ mol/g at exhaustion. The ADP concentration increased from 0.57 μmol/g at rest to 0.78 μ mol/g after only 10 sec. but did not increase further thereafter. The ATP:ADP ratio

Table 10

Aerobically-Induced Training Changes of Pyruvate Dehydrogenase Activity In Rat Gastrocnemius and Soleus Skeletal Muscle With Running

	10 Week Sedentary Control		10 Week Aerobic Treadmill Running	
	Non-trained		Trained	
	Gastrocnemius	Soleus	Gastrocnemius	Soleus
PDH_a:PDH_t (%)	36	39	66	82
PDH_a (nmol/g/min.)	296 ± 15*	382 ± 15	553 ± 28	778 ± 33
PDH_t (nmol/g/min.)	811 ± 32	990 ± 36	827 ± 22	950 ± 37
Observations	10	10	10	10

Legend: * mean ± S.E.M. There were 10 observations for each condition. The rats ran aerobically on a motor driven treadmill once daily, 5 days per week for 10 weeks and the work rate was increased weekly. During the last two weeks the rats were running at 19 meters per min. at 5% grade for 45 min. The animals were not exercised for 18 hours before muscle samples were taken by freeze-clamping with liquid N_2 precooled tongs. For further details see "Methods" section. The results are expressed in nmol/g/min.

Table 11

The Changes of Pyruvate Dehydrogenase Activity By Exercise at 50% Maximum Voluntary Isometric Contraction For Various Time Periods in Human Quadriceps Femoris Skeletal Muscle In Vivo

Exercise Time (Sec.)	Pyruvate Dehydrogenase (nmol/g/min.) PDH_a	PDH_t	A:T %
0	111 ± 3* (23)	302 ± 3 (23)	37
10	117 ± 3 (4)	303 ± 2 (4)	39
20	129 ± 3 (5)	303 ± 4 (5)	42
30	137 ± 2 (5)	307 ± 2 (5)	45
69 ± 3 (Exhaustion)	127 ± 4 (8)	306 ± 2 (7)	44

Legend: * mean ± S.E.M. The subjects for each time period were exercised at 50% of their MIVC as outlined in "Methods". The number of observations are shown in brackets. The concentrations for PDH are expressed in nmol/g/min. For each exercise period a control sample was taken on the contralateral leg. The muscle biopsies were taken before exercise and immediately after the exercise ceased and frozen in liquid N_2.

Table 12

The Effect of 50% Maximum Voluntary Isometric Contraction for Various Time Periods On Human Quadriceps Femoris Skeletal Muscle Metabolites In Vivo

Metabolite	Exercise (Sec). 0	10	20	30	Exhaustion
ATP	5.06±0.19*(6)	3.92±0.15 (6)	3.99± 0.18 (6)	2.95±0.25 (6)	2.96± 0.13 (6)
ADP	0.57±0.04 (6)	0.78±0.03 (6)	0.68± 0.02 (6)	0.74±0.02 (6)	0.75± 0.03 (5)
CP	17.13±1.00 (6)	9.85±0.59 (6)	3.68± 0.39 (5)	5.82±0.78 (6)	2.85± 0.31 (6)
Lactate	1.83±0.39 (5)	9.25±1.60 (5)	11.82± 0.50 (5)	16.58±0.57 (5)	17.58± 0.99 (6)
Pyruvate	0.08±0.02 (5)	0.18±0.02 (5)	0.29± 0.03 (5)	0.46±0.02 (5)	0.54± 0.04 (6)
Creatine	9.17±0.42 (6)	11.90±0.18 (6)	18.39± 0.80 (6)	19.99±0.95 (6)	20.26± 0.81 (6)
Glycogen	71.85±8.88 (6)	58.00±6.46 (6)	47.82±10.26 (6)	39.87±9.42 (6)	44.26±13.55 (6)
ATP:ADP	9	3	6	4	4
Lactate:Pyruvate	23	51	41	42	40

Legend: * mean ± S.E.M. Each subject exercised for the time period as tabled above. Exhaustion time was 69 ± 3. The observations are in brackets for each metabolite. The concentration of all metabolites are reported in μ mol/g wet m. All procedures are as outlined in "Methods" section.

was lowest at exhaustion. The CP concentration decreased as contraction time increased, with 84% depletion occurring at exhaustion. As the CP concentration decreased the creatine concentration increased with the highest creatine concentration recorded at exhaustion. Muscle pyruvate accumulated at each exercise period and reached a peak concentration of 0.54 ± 0.04 μ mol/g at exhaustion.

The resting muscle lactate concentration was 1.8 μmol/g and increased with time. The concentration at exhaustion was 17.6 ± 0.99 μ mol/g. The L:P ratio at rest was 23 and increased to 40 at exhaustion, but the highest ratio of 51 was observed after only 10 sec. of contraction.

The glycogen concentration also decreased with time with 39% depletion of muscle glycogen stores occurring at exhaustion.

B. Progressive Exercise

The effect of progressively increasing aerobic exercise on the activation of human skeletal muscle PDH is shown on Table 13. At rest the PDH_a activity was 124 ± 9 nmol/g/min. which was 40% of the PDH_t. At exhaustion, after approximately 20 min. of exercise, and when the subject was working at about 90% $\dot{V}O_2$ max, the PDH_a activity increased more than twofold to 260 ± 7 nmol/g/min. Virtually all the PDH was in the active form at exhaustion.

Table 13

Effect of Various Types of Work On a Cycle Ergometer

In Vivo, On the Activation of Human Skeletal Muscle Pyruvate Dehydrogenase

	Progressive Exercise (20 ± 3 min.)			Intermittent Exercise (35 ± 6 min.)			Short Term Maximal Exercise (65 ± 3 sec.)		
	PDH_a	PDH_t	A:T %	PDH_a	PDH_t	A:T %	PDH_a	PDH_t	A:T %
Rest	124 ± 9*	301 ± 4	41	122 ± 10	316 ± 5	38	108 ± 4	299 ± 4	36
Exercise	260 ± 7	294 ± 2	88	193 ± 7	321 ± 4	60	118 ± 3	298 ± 5	39
Observations	5	5		6	6		3	3	

Legend: * mean ± S.E.M. The subjects in each type of exercise were exercised to exhaustion as outlined in the "Methods". The number of observations is shown on the bottom line. The values are in nmol/g/min. All the exercise was carried out on a cycle ergometer at the times as indicated.

C. Intermittent Exercise

The effect of intermittent short-term heavy exercise (140% $\dot{V}O_2$ max) until exhaustion is summarized in Table 13. The PDH_a increased from 122 to 193 nmol/g/min. at exhaustion. Despite the increased duration of the exercise period and the increased work accomplished, there was significantly less PDH activation than with the progressive exercise workload.* At exhaustion, about 30-40 min. after the start of the exercise, only 60% of the PDH_t was in the active state.

D. Short Term Maximal Exercise

The effect of the anaerobic-type exercise at the subjects' $\dot{V}O_2$ max on muscle PDH activation is shown in Table 13. The resting PDH_a activity was 108 $\pm$ 4 nmol/g/min. and increased to 118 $\pm$ 3 nmol/g/min. at exhaustion (exercise time; 65, 69, and 73 sec.). The change was not significantly different from the values obtained at rest. Essentially the same results were obtained with 50% maximum voluntary isometric contraction. (Table 11)

In all types of exercise situations the PDH_t did not change at rest or after exhaustive exercise suggesting that no significant errors in sampling of the muscle occurred.

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* Progressive Exercise = 1700$\pm$200 Kpm/min.: Intermittent Exercise = 2500 $\pm$ 180 Kpm/min.

The effect of the various exercise regimes on muscle metabolite concentrations are summarized in Table 14. The muscle pyruvate concentration increased threefold from 0.07 ± 0.01 to about 0.20 ± 0.03 μ mol/g in both progressive and intermittent exercise, while after short term exercise it increased slightly more, to 0.27 ± 0.02 μ mol/g/ The lactate concentration increased sevenfold in progressively increasing workload exercise, twelvefold in intermittent exercise, and with short term maximal exercise to exhaustion, it increased almost fifteenfold. The L:P ratio increased at the end of every work situation with the highest ratio increase of over fourfold being recorded with intermittent exercise.

There was a marked decrease in CP concentration with exercise which was associated with a parallel increase in the creatine concentration in each exercise group. The glycogen concentration with exercise in all three work regimens decreased, with the greatest decrease shown in the intermittent exercise.

E. Heavy Resistance Weight Training, Immobilization In Human Triceps Brachii Skeletal Muscle

The purpose of this study was to determine the effects of long-term heavy resistance weight training and 5 week long immobilization on skeletal muscle PDH activity

Table 14

Effect of Various Types of Work on Tissue Metabolite Concentrations

In Human Skeletal Muscle at Rest and After Exhaustive Exercise On a Cycle Ergometer

Time (Min.)	Progressive Exercise		Intermittent Exercise		Short Term Maximal Exercise	
	Rest	20 ± 3 Exercise	Rest	35 ± 6 Exercise	Rest	65 ± 3 sec. Exercise
Glycogen	84.09±12.39*	36.36±2.56	80.30±3.34	24.25±5.33	90.52±8.12	56.80±7.41
Lactate	1.29±0.06	9.41±0.30	1.18±0.16	13.11±0.48	1.24±0.37	18.22±0.76
Pyruvate	0.07±0.01	0.20±0.03	0.07±0.003	0.19±0.02	0.09±0.01	0.27±0.02
ATP	5.23±0.16	4.29±0.18	4.99±0.24	3.51±0.18	5.24±0.34	3.30±0.24
ADP	0.92±0.07	1.38±0.09	0.53±0.02	0.61±0.03	0.51±0.02	0.68±0.02
CP	15.62±1.30	3.30±0.88	16.05±0.79	5.05±1.01	17.41±1.74	3.32±0.45
Creatine	10.56±0.50	18.31±1.55	9.65±0.48	21.51±1.11	9.60±0.82	18.33±1.00
ATP:ADP	5.68±0.35	3.10±0.23	9.39±0.44	5.86±0.59	10.25±0.17	4.81±0.26
L:P	18.42±1.71	47.05±7.79	16.91±2.05	72.10±7.49	15.40±2.81	52.10±22
Observations	5	5	6	6	3	3

Legend: * mean ± S.E.M. The subjects in each type of work were exercised to exhaustion as outlined in "Methods" section. The values are in μmol/g wet m.

and determine whether this might explain the capacity of muscle to oxidize pyruvate with this type of training or be reversed by immobilization. The study was conducted as outlined in the "Methods" on p. 46.

I. Study 1 - (Group 1 and 2)

(a) Group 1: The muscle biopsies were taken from the arm in the following order: control, after 20 week training and following 5 week immobilization. (Figure 11) The control PDH_a activity was 107 ± 3 nmol/g/min. (PDH_a:PDH_t ratio 35%), while post training PDH_a had increased to 166 ± 10 nmol/g/min. (PDH_a:PDH_t ratio 54%). After 5 weeks of immobilization after the training period, the PDH_a decreased to 48 ± 2 nmol/g/min. The PDH_t activity was unaffected by the training or the immobilization.

(b) Group 2: The muscle biopsies were taken from the arm in the following order: control, after 5 weeks of immobilization and after 20 weeks of training.

The resting control PDH_a was 108 ± 4 nmol/g/min. similar to values obtained in Group 1. The PDH_a:PDH_t ratio was 36%. The PDH_a after 5 weeks of immobilization decreased to 38 ± 4 nmol/g/min. with a PDH_a:PDH_t ratio of 12%. The immobilization programme, where it preceeded the training period, resulted in substantially lower PDH_a activation (48 ± 2 vs 38 ± 4 nmol/g/min.).

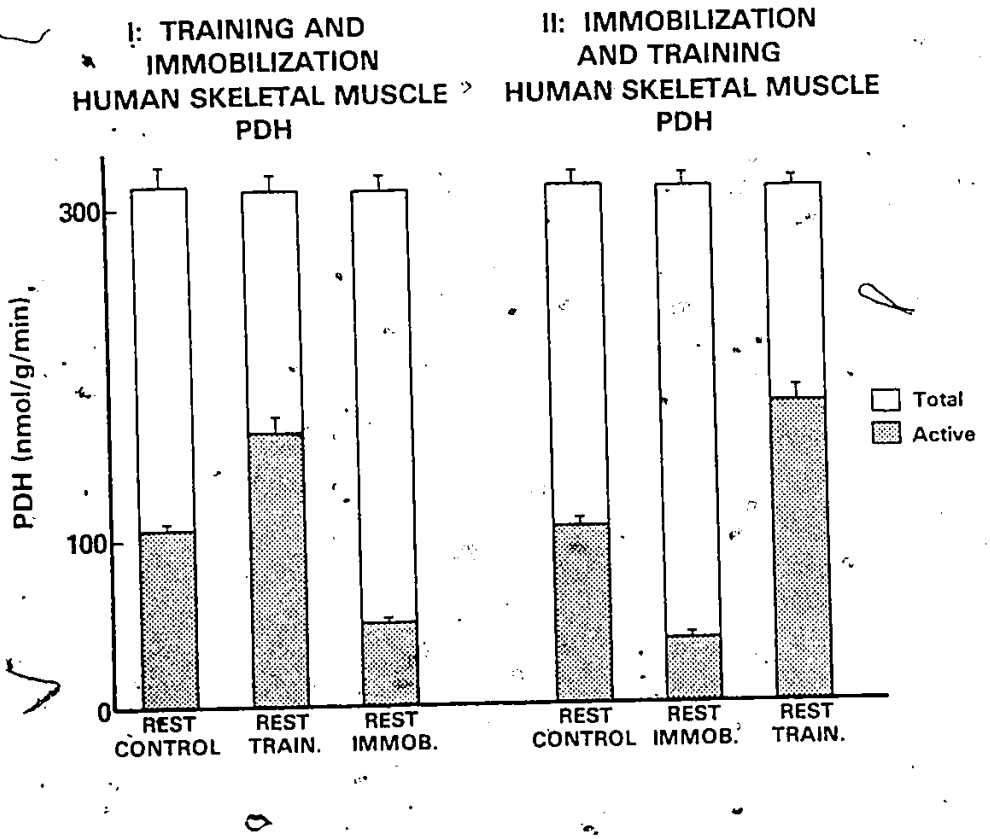

Figure 11.

I. The effect of training and immobilization upon PDH_a and PDH_t activity in human triceps brachii skeletal muscle. The training was with weights for 5 months and immobilization was by placing the arm in a cast for 5 weeks. Muscle biopsies were taken before and after training and immobilization. There were 4 observations for each variable in I.

II. The particulars are as for above (I), but were in reverse order. The vertical bars are $\pm$ S.E.M. and there were 5 observations for each variable in II.

After the 20 week weight training programme was completed, the PDH_a had increased to 179 ± 9 nmol/g/min. while the PDH_a:PDH_t ratio was 56%. (Figure 11)

(i) Metabolites: (Group 1 and 2)

The concentration of both CP and creatine were significantly higher ($p < 0.05$) after weight training in both groups (Table 15), while CP concentrations decreased significantly in both groups following immobilization. The decreases in concentration of creatine were only significant in group 1 where immobilization proceeded training even though both groups had decreases in creatine.

The ATP concentrations were significantly higher in both groups following training ($p < 0.05$) but were not significantly affected by immobilization.

When the training ATP and CP concentrations were combined and considered as total phosphagen, the phosphagen pool increased by 13% as a result of training. After immobilization, the phosphagen concentration decreased 18% from 44.7 to 36.1 μ mol/g ($p < 0.05$). The muscle glycogen concentration increased from 86 ± 7 to 114 ± 11 μ mol/g ($p < 0.01$) as a result of training (Groups 1 and 2). Immobilization decreased the muscle resting glycogen from 86 ± 7 to 57 ± 10 μ mol/g ($p < 0.01$). Immobilization, when it preceeded the training programme resulted in a larger

Table 15

Concentrations of Metabolites Before and After Training and Immobilization

	Group 1, N=4			Group 2, N=5		
	Control	Post Training	Post Immobilization	Control	Post Immobilization	Post Training
CP	16.68 ± 0.11	17.18 ± 0.25*	12.64 ± 0.95**	17.46 ± 0.23	13.33 ± 0.60**	18.70 ± 0.15**
Creatine	11.00 ± 0.26	15.61 ± 0.41**	11.48 ± 0.50*	10.47 ± 0.40	9.82 ± 0.80	13.42 ± 0.83*
ATP	5.52 ± 0.07	6.08 ± 0.22*	5.51 ± 0.24	4.62 ± 0.14	4.65 ± 0.36	5.85 ± 0.17*
ADP	0.93 ± 0.08	1.20 ± 0.03*	1.09 ± 0.02	0.99 ± 0.07	1.01 ± 0.10	1.12 ± 0.04
Lactate	1.20 ± 0.16	1.49 ± 0.21	0.71 ± 0.08	1.11 ± 0.19	0.82 ± 0.11	1.69 ± 0.15
Pyruvate	0.10 ± 0.01	0.16 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.05 ± 0.01	0.09 ± 0.01
Glycogen	84.32 ± 5.27	101.90 ± 6.54**	55.34 ± 10.72**	88.24 ± 7.91	59.46 ± 9.32**	125.90 ± 15.69**

Values are mean ± S.E.M., and are expressed in μ mol/g wet m. * $p < 0.05$; ** $p < 0.01$

decrease in the muscle glycogen concentration (Group 1: 102 ± 7 to 55 ± 11; Group 2: 88 ± 9 to 59 ± 9 μmol/g) ($p < 0.01$).

F. Heavy Resistance Weight Training, Immobilization and Exercise in Human Triceps Brachii Skeletal Muscle

The second study was similar to Groups 1 and 2 (p. 46) except muscle biopsies were also obtained after arm cranking exercise to exhaustion and the immobilization was extended from 5 to 6 weeks.

I Study 2: (Groups 3 and 4)

(a) Group 3: Resting and exercise muscle biopsies were obtained in the following sequence: control, after 20 weeks weight training and then 6 weeks after immobilization began.

The resting control PDH_a was 111 ± 4 nmol/g/min. and the PDH_a:PDH_t ratio was 37%. (Figure 12) After exercise the PDH_a increased to 288 ± 6 nmol/g/min. and the PDH_a:PDH_t ratio was 95%. As a result of the 20 weeks weight training programme, the PDH_a increased to 198 ± 7 nmol/g/min. at rest and the PDH_a:PDH_t ratio was 65%. Immediately after exercise the PDH_a was 295 ± 6 nmol/g/min. with 97% in the active form. After 6 weeks of immobilization the resting

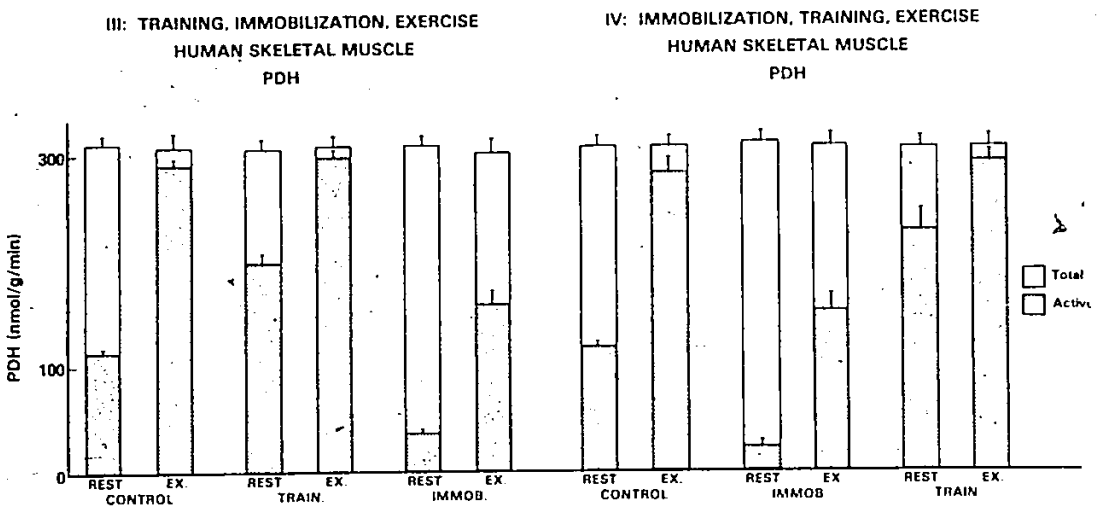

Figure 12.
III. Effect of training, immobilization and exercise upon PDH_a and PDH_t activity in human triceps brachii skeletal muscle. The biopsies were obtained at rest, after training and after immobilization and after exercise at each variable. There were 3 observations for each variable. The vertical bars are $\pm$ S.E.M. For furthur particulars see "Materials and Methods".
IV. Muscle biopsies were taken in a different order from Study III. At rest, after immobilization and after training and after exercise at each variable. There were 3 observations at each variable. The vertical bars are $\pm$ S.E.M. For further particulars see "Materials and Methods".

PDH_a decreased dramatically to 35 ± 3 nmol/g/min. or 11% of the PDH_t activity. Exercise following the immobilization period increased the PDH_a to 158 ± 9 nmol/g/min. with only 53% in the active form. The muscle PDH_t activity was not altered during any of these experimental conditions.

(b) Group 4: In this series of experiments resting and exercise biopsies were obtained under control conditions, after 6 weeks of immobilization and after 20 weeks of weight training. The concentration of the control PDH_a was 113 ± 5 nmol/g/min. and increased to 279 ± 10 nmol/g/min. after exercise. (Figure 12) After the 6 weeks immobilization the PDH_a decreased to 20 ± 4 nmol/g/min or a PDH_a:PDH_t ratio of 6%. Following exercise the PDH_a activity increased to 151 ± 12 nmol/g/min. with 50% of the PDH_t activity in the active form. After the 20 week training programme the resting PDH_a was 224 ± 14 nmol/g/min. with the PDH_a:PDH_t ratio of 73%. After exercise the PDH_a activity increased to 299 ± 6 nmol/g/min. or 98% of the PDH_t activity. In summary, immobilization markedly decreased PDH_a activity while weight training increased it well above control levels. Arm exercise after immobilization resulted in minimal PDH activation while training resulted in rapid activation with exercise. No changes in PDH_t activity were observed.

(i) Metabolites: (Groups 3 and 4)

All the metabolite control resting values were similar to those obtained in Study 1 (see p. 104 and 105). Immobilization, whether it preceded or followed the training regime, significantly decreased the resting muscle CP concentration ($p < 0.05$). (Table 16) There was no consistent trend in the resting ATP concentration as a result of immobilization. Glycogen content was significantly altered by immobilization. The glycogen, ATP and CP concentration increased significantly as a result of the training programme. Immobilization followed the training, decreased the glycogen, ATP and CP concentration but only CP was below the values obtained in the control state.

Exercise, at the same workload and for the same time period (control and training) resulted in similar CP concentration decreases. Similarly, the decrease in ATP concentration as a result of exercise was essntially the same in control and after training. However, after immobilization with a reduced workload but over the same time period, the CP concentration was decreased most while ATP was essentially the same. The rise in muscle lactate with work was greatest after immobilization even though the workload was reduced. The absolute change in muscle glycogen (37 μ mol/g) was similar in all the groups.

Table 16

Concentrations of Metabolites for Before and After Training, Immobilization and Exercise

	Group 3: N=3						Group 4: N=3					
	Control	Ex	Post Training	Ex	Post Immobilization	Ex	Control	Ex	Post Immobilization	Ex	Post Training	Ex
CP	15.37 ± 0.30	9.56 ± 0.43	18.89 ± 0.42**	12.88 ± 1.02	12.43 ± 0.51**	5.10 ± 0.39	16.07 ± 0.32	9.94 ± 0.83	12.07 ± 0.93**	3.62 ± 0.66	18.46 ± 0.57**	12.00 ± 1.29
Creatine	9.12 ± 0.46	15.95 ± 0.35	10.78 ± 0.35	18.21 ± 0.21	9.38 ± 0.21	16.35 ± 0.49	10.07 ± 0.43	14.63 ± 0.68	9.18 ± 0.27	13.53 ± 0.43	12.34 ± 1.15*	17.84 ± 0.79
ATP	5.15 ± 0.16	4.09 ± 0.26	6.29 ± 0.22*	5.00 ± 0.27	5.61 ± 0.19	4.00 ± 0.13	5.31 ± 0.27	4.11 ± 0.24	4.40 ± 0.26	2.99 ± 0.11	6.98 ± 0.27*	4.34 ± 0.22
ADP	0.85 ± 0.04	1.04 ± 0.06	1.08 ± 0.03*	1.21 ± 0.05	0.83 ± 0.02*	1.02 ± 0.04	0.79 ± 0.10	0.94 ± 0.04	0.52 ± 0.08*	0.92 ± 0.23	1.10 ± 0.09**	1.28 ± 0.16
Lactate	1.04 ± 0.05	6.87 ± 0.65	1.60 ± 0.28	4.50 ± 0.76	0.83 ± 0.09*	6.39 ± 0.42	1.24 ± 0.20	7.81 ± 0.73	0.91 ± 0.10*	9.07 ± 0.20	1.79 ± 0.30	6.59 ± 0.72
Pyruvate	0.10 ± 0.02	0.25 ± 0.03	0.12 ± 0.02	0.26 ± 0.01	0.08 ± 0.01	0.28 ± 0.02	0.09 ± 0.005	0.21 ± 0.03	0.06 ± 0.01	0.24 ± 0.02	0.08 ± 0.002	0.29 ± 0.08
Glycogen	66.46 ± 2.19	33.32 ± 4.24	98.09 ± 5.04**	58.59 ± 1.85	68.00 ± 6.11**	26.12 ± 2.66	69.66 ± 6.55	30.60 ± 4.8	53.67 ±11.22*	22.59 ± 4.02	107.82 ±10.05**	70.26 ± 8.55

Legend: Values are mean ± S.E.M. and expressed as μmol/g wet m.

* $p < 0.05$; ** $p < 0.01$

5. Role of Hormonal Factors in Activating Skeletal Muscle PDH

A. A Comparison Between Unstimulated and Stimulated Pyruvate Dehydrogenase In Human and Rat Skeletal Muscle

This study was done to determine if PDH activation during exercise was hormonally induced or initiated by local muscle factors.

The human skeletal muscle non-exercised contralateral leg showed no PDH activation while in the exercising leg the PDH_a activity increased about twofold. (Figure 13)

In rat skeletal muscle the nerve stimulated leg showed a twofold activation of PDH within 1 min. while the non-stimulated contralateral leg at the same time showed no PDH_a activation. (Figure 13)

In both human and rat skeletal muscle the PDH_t did not change in either leg.

6. Effect of Various Factors on PDH Activation In the Perfused Rat Heart

The heart was used as a model for pyruvate oxidation and PDH activation because the oxidative rate in the heart varies by about threefold. The degree of PDH activation that might occur under these circumstances is roughly the

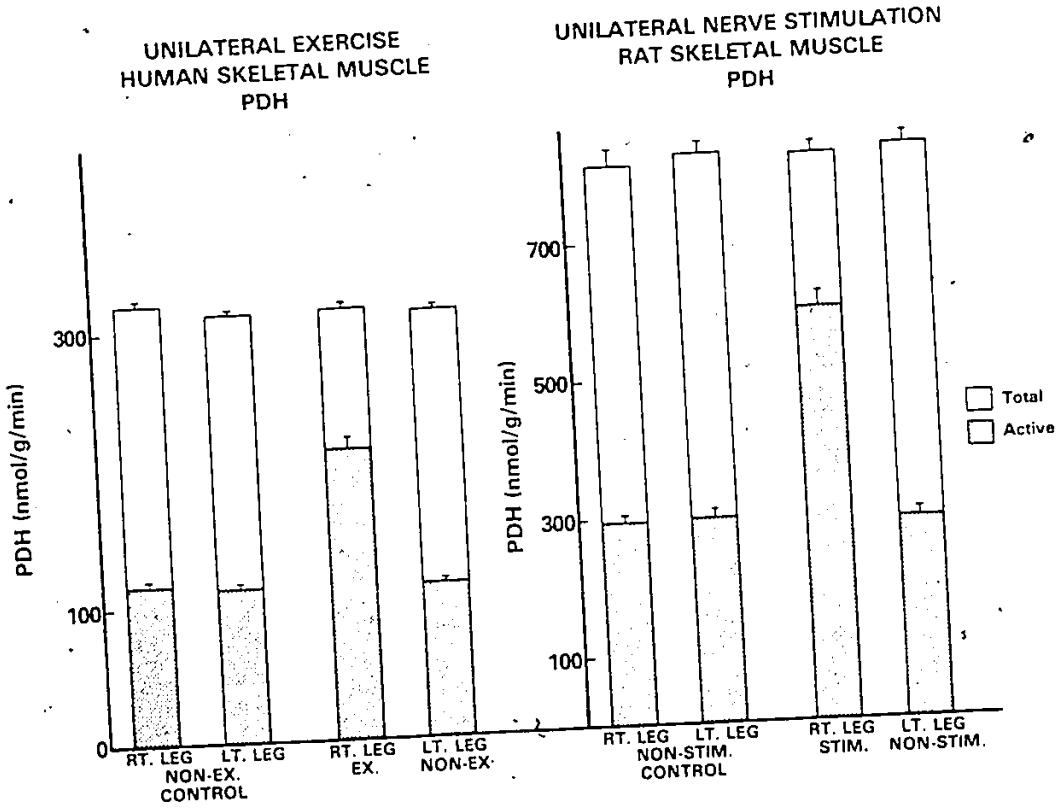

Figure 13.

Left: Three male subjects performed unilateral exercise on a cycle ergometer at 800 kpm for 2 minutes. The human skeletal muscle biopsies for PDH activity were taken at rest in both right and left legs and after exercise in the exercised and non-exercised leg. The vertical bars are ± S.E.M.
Right: Rat skeletal muscle was obtained from the vastus muscle group and the gastrocnemius muscle (not shown) after unilateral nerve stimulation. The PDH activity was measured at rest in both legs and after exercise in the exercised and non-exercised leg. There were 10 observations for each leg including the gastrocnemius muscle (not shown). The results were similar for both muscle groups. The nerve stimulation was performed in square wave fashion at 40 pulses/second at 5 volts and a duration of 5 milliseconds for 1 minute. The vertical bars are ± S.E.M.

same. The author is aware that factors other than PDH activation may regulate heart pyruvate oxidation but the model was used in order to determine whether the major changes in the rate of pyruvate oxidation in heart could be explained by PDH activation alone.

The effect of epinephrine (1.3 μg/ml) on the rate of heart pyruvate oxidation is outlined in Figure 14. The PDH_a increased from 1220 to a maximum of 4500 nmol/g/min. at 3-6 min. after addition of epinephrine. There was a similar increase in the rate of heart pyruvate oxidation from 1502 at 0 time to a maximum of 2960 nmol/g/min. at 6 min. after starting epinephrine infusion. The degree of PDH activation decreased between 6-9 min. after the start of epinephrine infusion with similar decreases in the rate of heart pyruvate oxidation. After starting the infusion of epinephrine the cyclic AMP increased from 330 fmol/μg DNA to a maximum of 944 at 9 min. (Figure 15) No significant difference of cAMP concentration was noted between 6-9 min. after the start of the epinephrine infusion. The PDH_a activity was increased by about fourfold by epinephrine while the rate of pyruvate oxidation increased about twofold.

When propranolol (4 μg/ml) and epinephrine (1.3 μg/ml) were infused together, the PDH activation was much less than with epinephrine alone. (Figure 16) There was a small increase in PDH_a activity suggesting that the propranolol

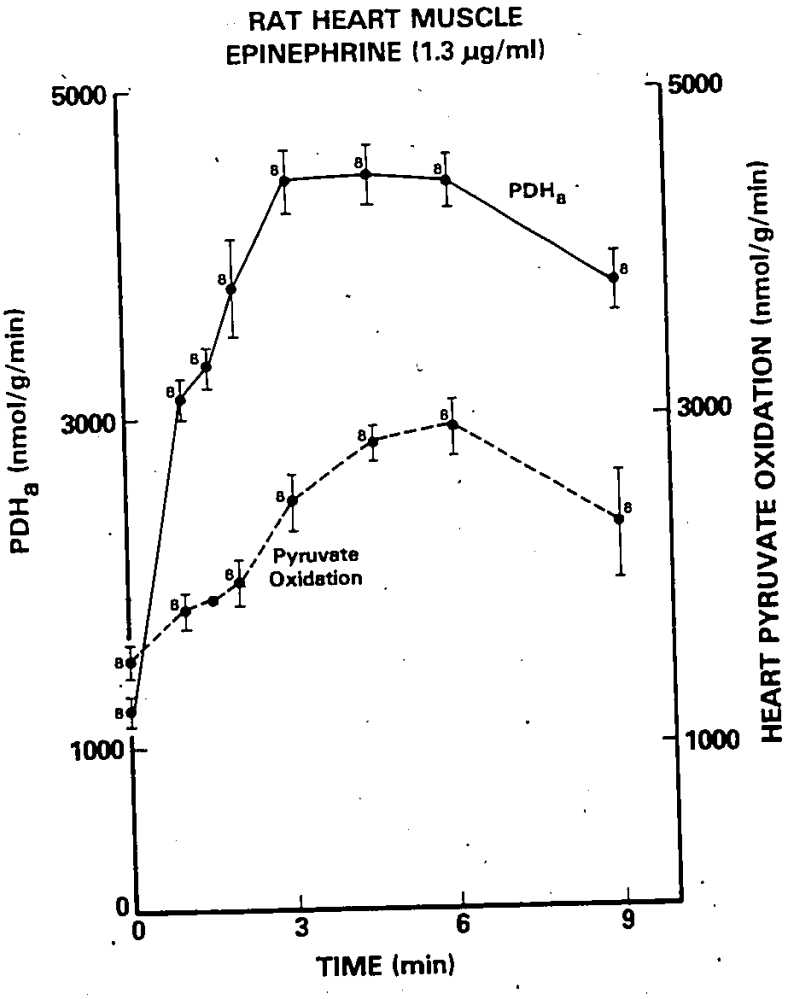

Figure 14.

Fasted rats (200-250 g) were anesthetized with ether and the hearts were removed after I.V. injection of heparin. The hearts were perfused through the aorta at a pressure of 80 mmHg for 10 min. with KRB. Epinephrine (1.3µg/ml) was then infused and the hearts perfused for 1, 3, 6, or 9 min., and at end of time period, the heart was freeze-clamped. The rat heart muscle homogenate of approximately 100 mg wet weight was assayed for active and total PDH and pyruvate oxidation was also determined. The PDH_t averaged 5048 nmol/g/min. All other procedures refer to "Materials and Methods". The means ± S.E.M. are shown on figure and the number of observations as indicated at each time point.

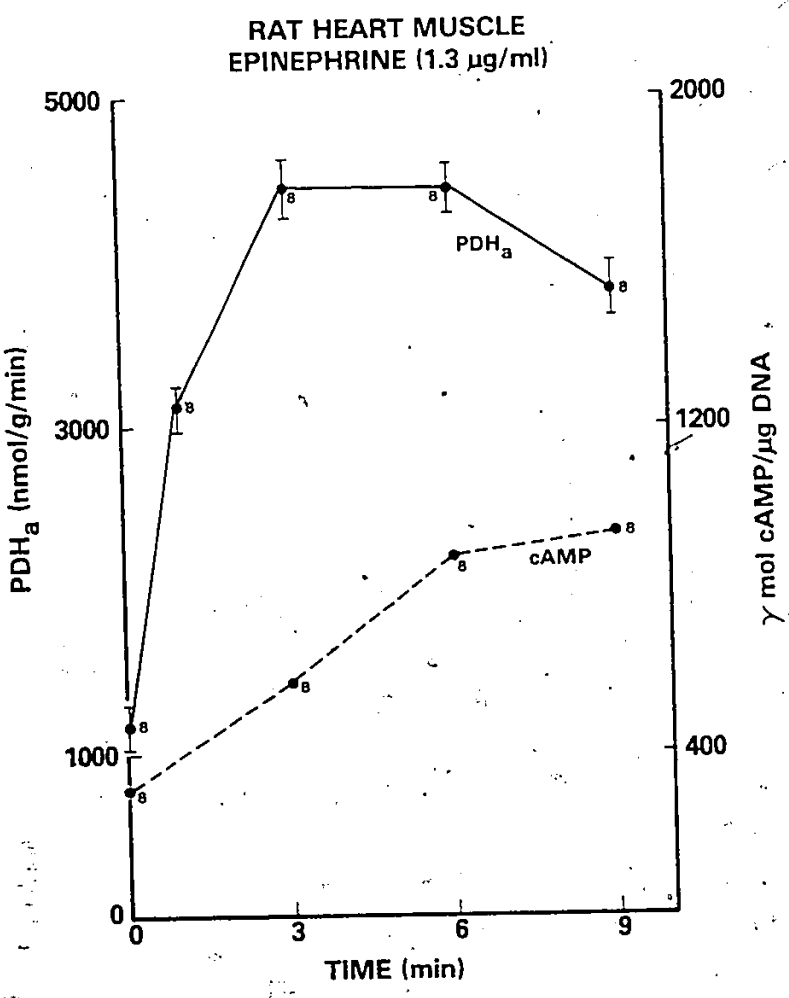

Figure 15.

Perfused rat heart muscle homogenates were assayed for PDH_a activity and cAMP concentrations after treatment with 1.3 µg/ml epinephrine as described in Figure 14. and "Materials and Methods". The number of observations are as indicated at each point and the vertical vars represent ± S.E.M. The total PDH averaged 5048 nmol/g/min.

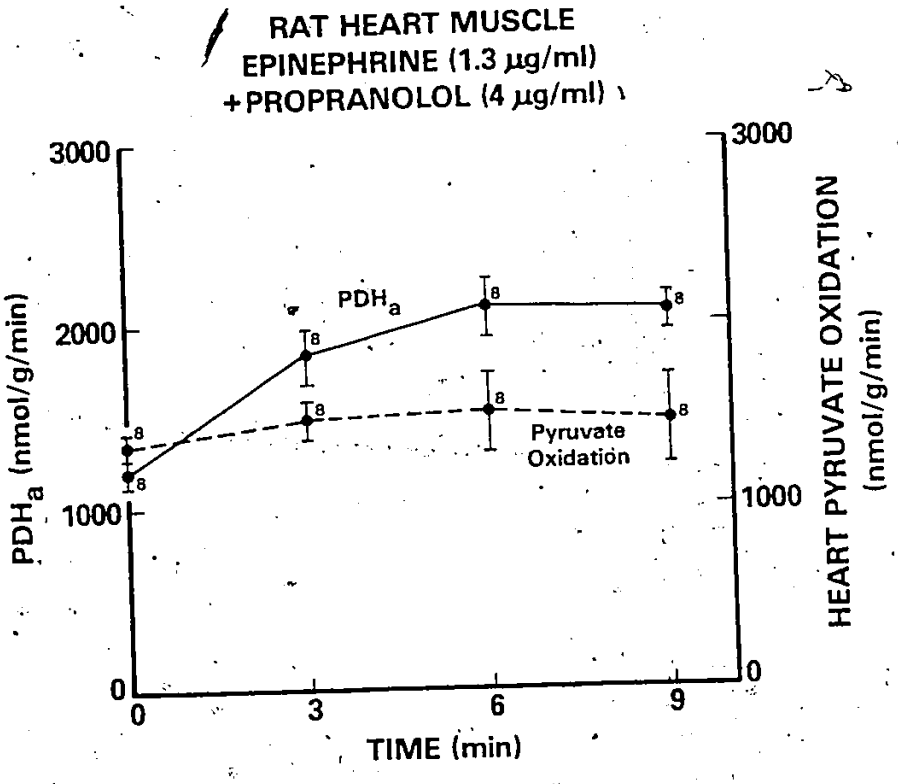

Figure 16.

Perfused rat heart muscle homogenates were assayed for changes in pyruvate oxidation and PDH activity over various time periods after treatment with 1.3 μg epinephrine and 4 μg/ml propranolol. The number of observations are as indicated and the vertical bars are ± S.E.M. All conditions of the experiments are as in "Materials and Methods" and Figure 14. The average total PDH was 4789 nmol/g/min.

was not present in a dose high enough to block the effect of epinephrine on PDH activation. The inhibition of PDH activation was followed in a parallel manner in that the rate of heart pyruvate oxidation was not changed. (Figure 16) The combination of epinephrine and propranolol almost completely prevented the activation of PDH, but the heart cAMP concentration increased substantially despite the ability of propranolol to block the activation of PDH. (Figure 17)

Propranolol alone had no effect on PDH activation, pyruvate oxidation (Figure 18), or on the heart cAMP concentration (Figure 19).

Insulin (1 mU/ml) resulted in almost complete activation of heart PDH (Figure 20) within 6 min. of insulin addition. The rate of pyruvate oxidation increased by about 100%. While insulin resulted in PDH activation, no changes in heart cAMP concentration were observed. (Figure 21)

The conditions of the heart perfusions described above did not involve pacing; spontaneous heart frequency varied between 120-160 beats/min. When the heart was electrically stimulated at 5 volts at a pulse frequency of 300 beats/min., there was rapid activation of PDH, with almost complete activation of the enzyme by 60 sec. of stimulation. (Figure 22) A similar increase in the rate of heart pyruvate oxidation was observed.

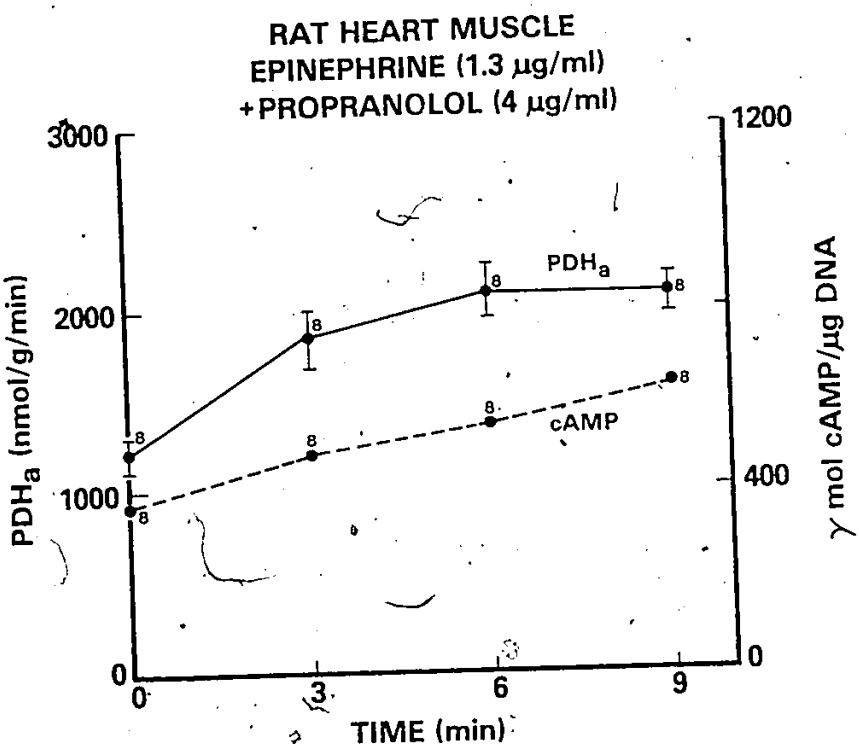

Figure 17.

Rat heart perfused with 1.3 µg/ml epinephrine and 4 µg/ml propranolol were homogenized and assayed for PDH_a activity and cAMP concentration. Hearts were freeze-clamped at various time periods and the number of observations were as indicated. The vertical bars are ± S.E.M. The total PDH averaged 4789 nmol/g/min.

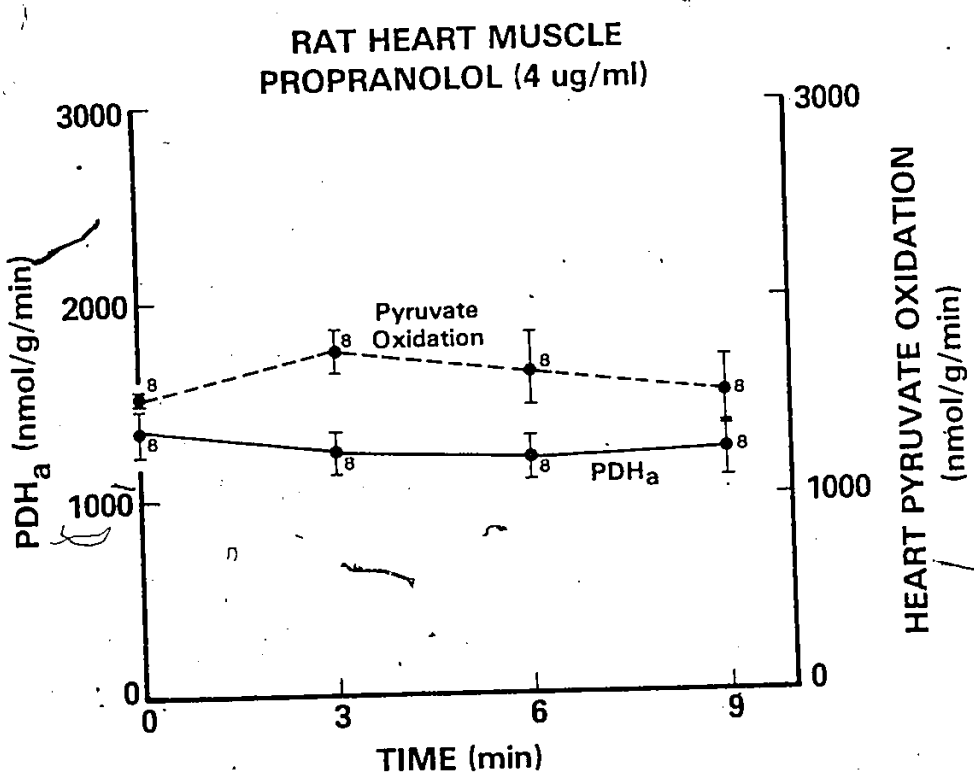

Figure 18.

Oxidation of pyruvate and PDH_a activity was measured as described in "Materials and Methods" and Figure 14, except the hearts were perfused with 4 μg/ml propranolol only. Each point represents a time period and 8 observations. The vertical bars are ± S.E.M. The total PDH activity averaged 4860 nmol/g/min.

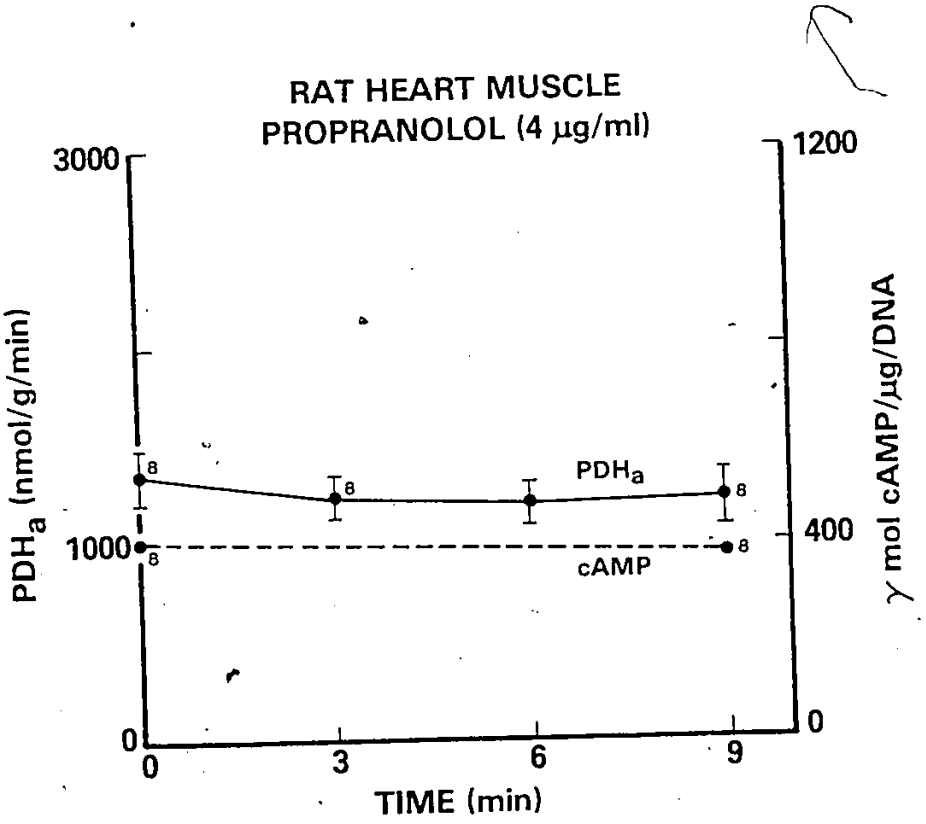

Figure 19.

PDH_a activity and cAMP concentration was measured from freeze-clamped perfused rat hearts treated with 4 µg/ml propranolol at various time periods. There were 8 observations at each point and the vertical bars are ± S.E.M. For other particulars refer to "Materials and Methods" and Figure 14. The total PDH activity averaged 4860 nmol/g/min.

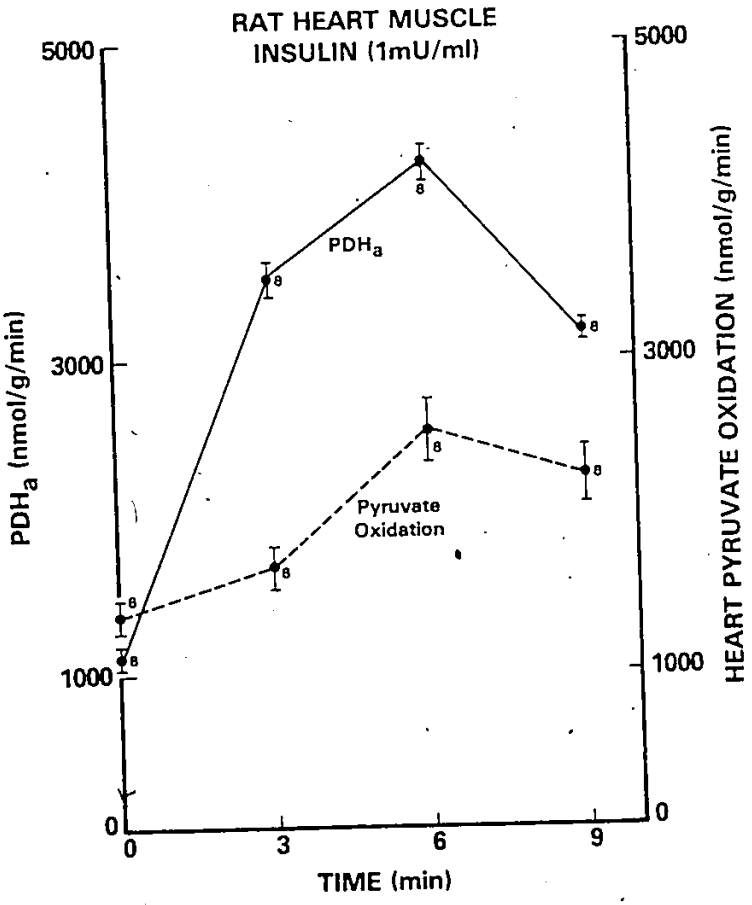

Figure 20.

Rat hearts were perfused with insulin (1 mU/ml) over various time periods and freeze-clamped. The homogenates were assayed for PDH_a activity. Pyruvate oxidation was also determined and the experimental conditions are as described in "Materials and Methods" and Figure 14. The PDH_t activity averaged 4942 nmol/g/min. There were 8 observations at each time period and the vertical bars are ± S.E.M.

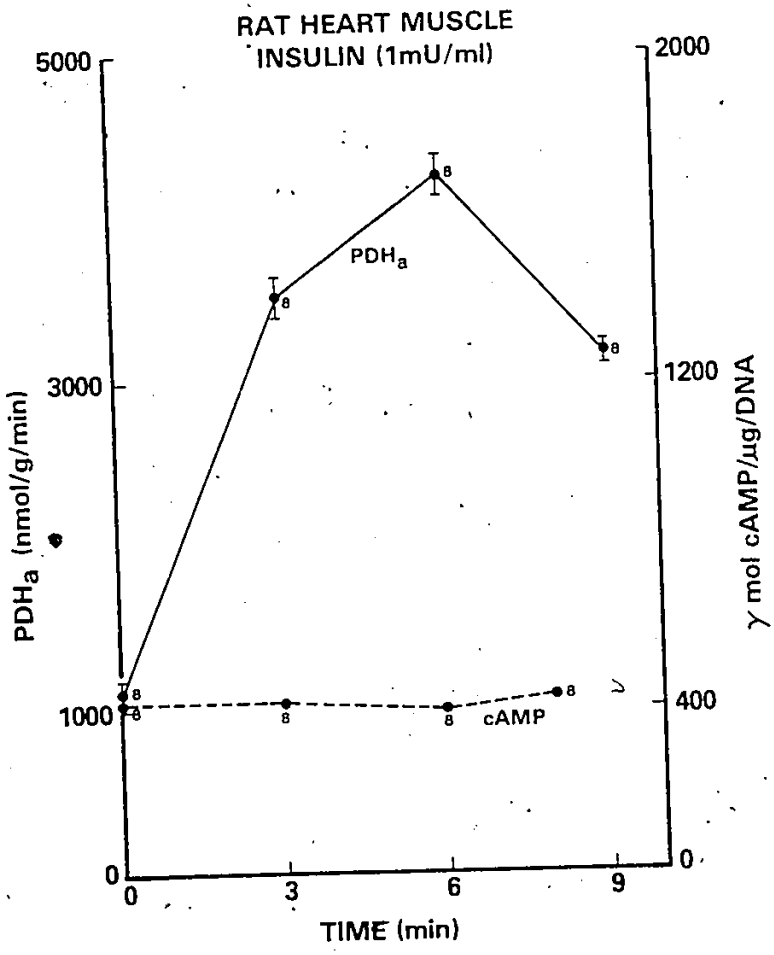

Figure 21.

Rat hearts were perfused with insulin (1 mU/ml) and the PDH_a activity and cAMP concentrations were measured. The PDH_t averaged 4942 nmol/g/min. and there were 8 observations at each time period. The conditions of the experiments are as described in the "Materials and Methods" and Figure 14. The vertical bars are ± S.E.M.

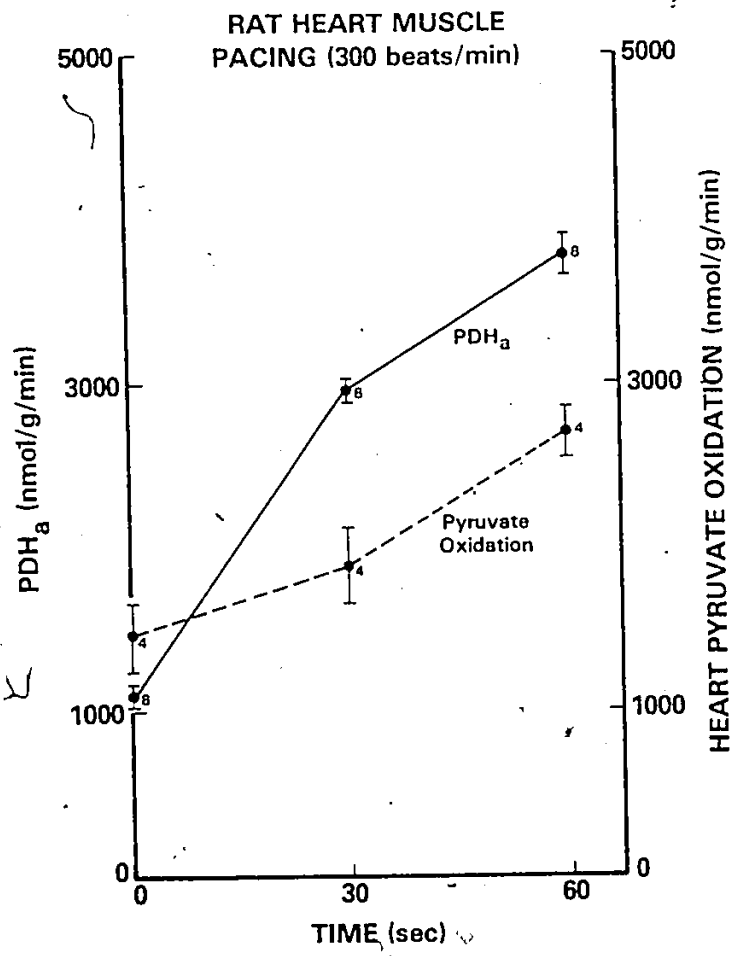

Figure 22.

Rat hearts were perfused, and paced by electrical stimulation which produced 300 beats/minute over 30 and 60 second time periods. The hearts were freeze-clamped, homogenized and assayed for PDH_a and pyruvate oxidation also measured as in "Materials and Methods". The vertical bars are $\pm$ S.E.M. and there were 8 observations at each time point for PDH and 4 observations for the measurement of pyruvate oxidation at each point. The total PDH activity averaged 4548 nmol/g/min.

FFA oxidation in heart inhibits cell glucose entry and oxidation.[9,158,159] In the experiments outlined in Figure 23, 1.0 mM octanoic acid resulted in marked decrease in the PDH_a activity. The rate of pyruvate oxidation was not decreased in a similar way. Octanoic acid resulted in a decrease in the heart cAMP concentration. (Figure 24)

The effect of pyruvate concentration (a substrate for pyruvate dehydrogenase) on PDH activation is shown in Figure 25. Almost complete activation of PDH was observed within 2-3 min. of 200 μ M pyruvate addition. The rate of pyruvate oxidation was increased but the extent of the increase was less than the degree of PDH activation. The effect of 200 μ M pyruvate on heart cAMP concentration is shown in Figure 26. It increased from 372 to 934 fmol/μ g DNA within 9 min. of pyruvate addition. (Figure 26) Increasing the pyruvate concentration to 1 mM resulted in the same rate and extent of PDH activation, as 200 μ M pyruvate. (Figure 27) The rate of pyruvate oxidation was increased by 1 mM pyruvate to a greater extent than 200 μ M pyruvate (Figure 25 and 27) The heart cAMP concentration was increased by about threefold by the 1 mM pyruvate. (Figure 28)

In order to determine whether PDH activation could account for the observed increase in pyruvate oxidation, the extent of PDH_a formation was correlated with the observed rates of pyruvate oxidation. (Figure 29) The

correlation of PDH activation and rate of pyruvate oxidation was greater than ($r = 0.92$), when all agents were compared and plotted.

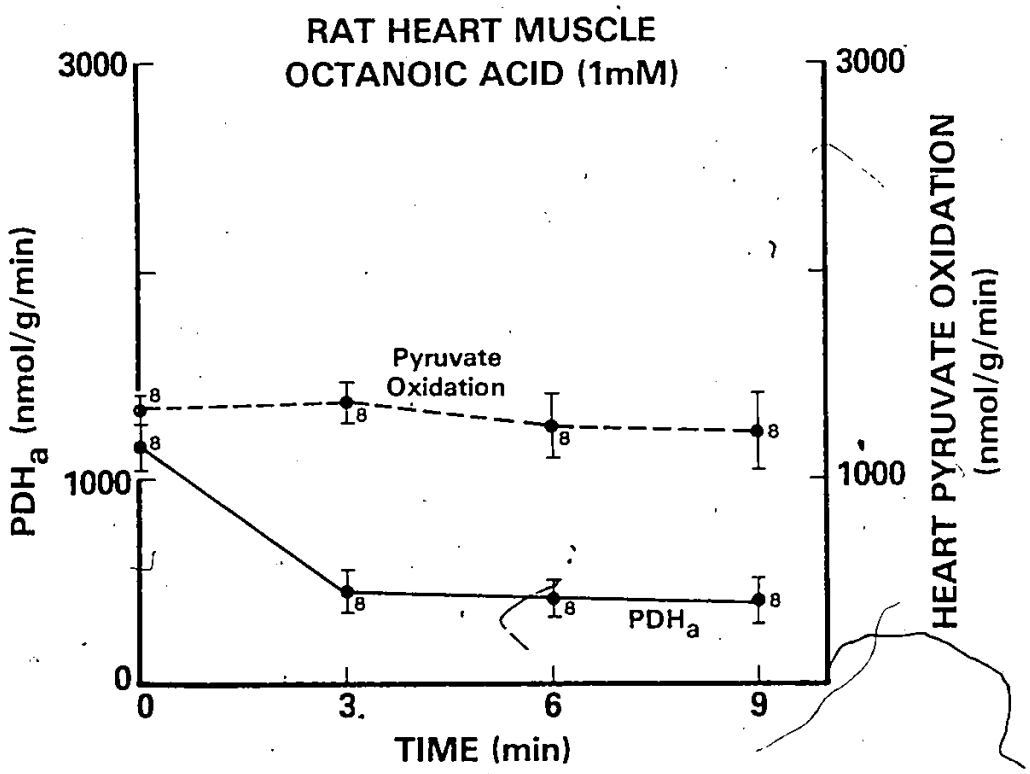

Figure 23.

Perfused rat hearts treated with octanoic acid (1 mM) were freeze-clamped at each time point and the activity of PDH_a and changes in pyruvate oxidation were measured. There were 8 observations at each time period and the vertical bars are ± S.E.M. The total PDH averaged 4901 nmol/g/min.

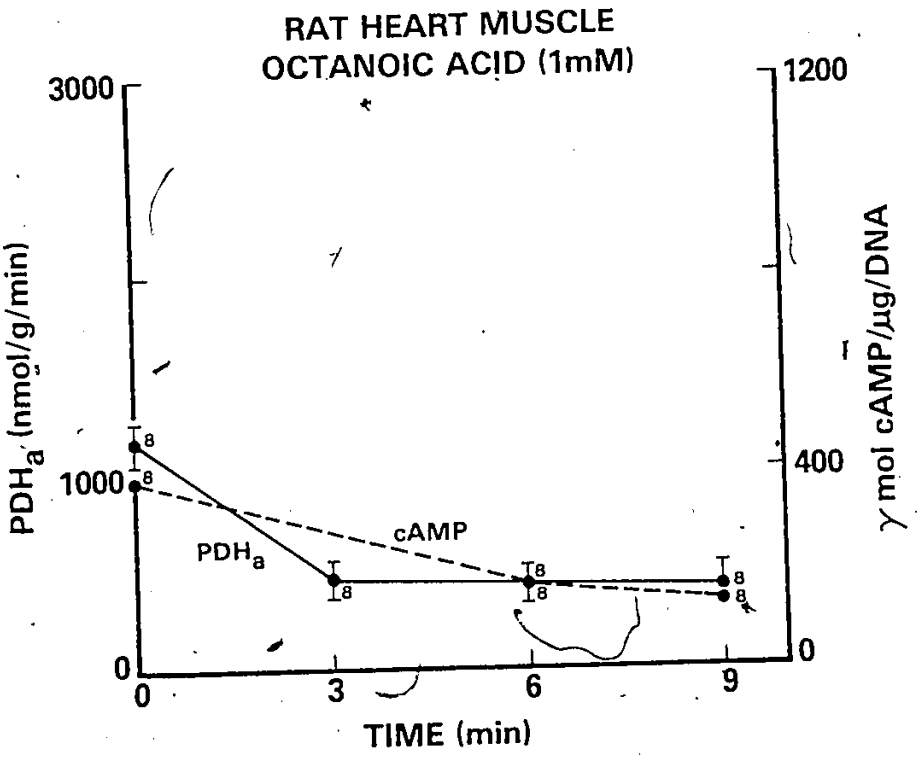

Figure 24.

Perfused rat hearts treated with octanoic acid (1 mM) were freeze-clamped and assayed for PDH_a activity and cAMP concentration. The total PDH activity averaged 4901 nmol/g/min. There were 8 observations at each time period and the vertical bars are ± S.E.M. All other conditions are as in "Materials and Methods".

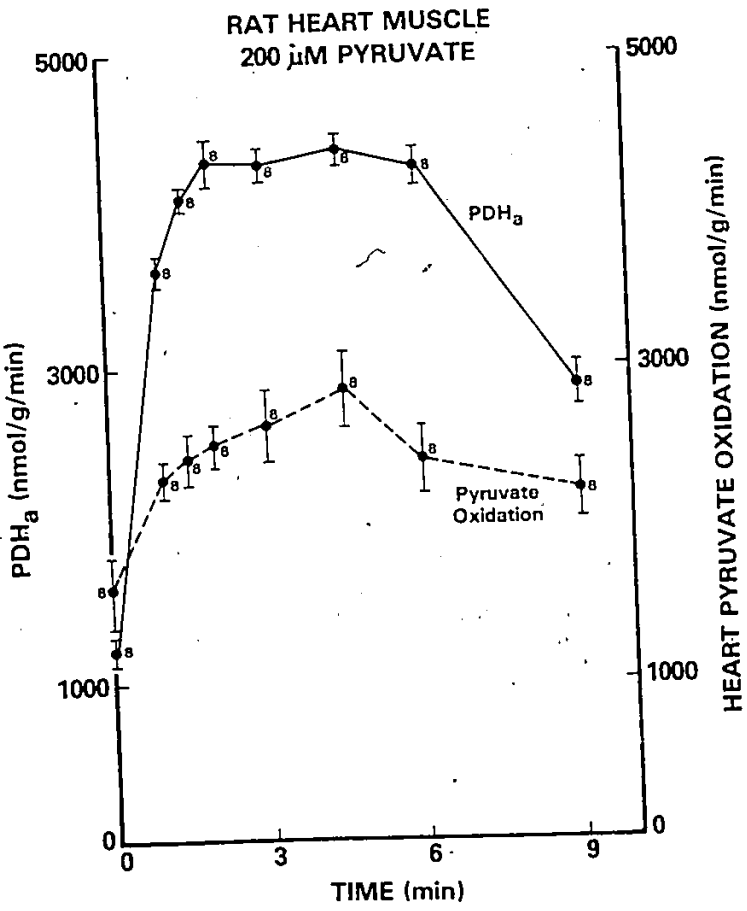

Figure 25.

The perfused hearts were treated with 200 µM pyruvate, freeze-clamped and the homogenates were assayed for PDH_a activity and pyruvate oxidation changes. The hearts were perfused for either 1, 1.5, 2, 3, 4.5, 6 or 9 minutes and there were 8 observations at each time point. The results are presented as ± S.E.M. The PDH_t activity averaged 4694 nmol/g/min.

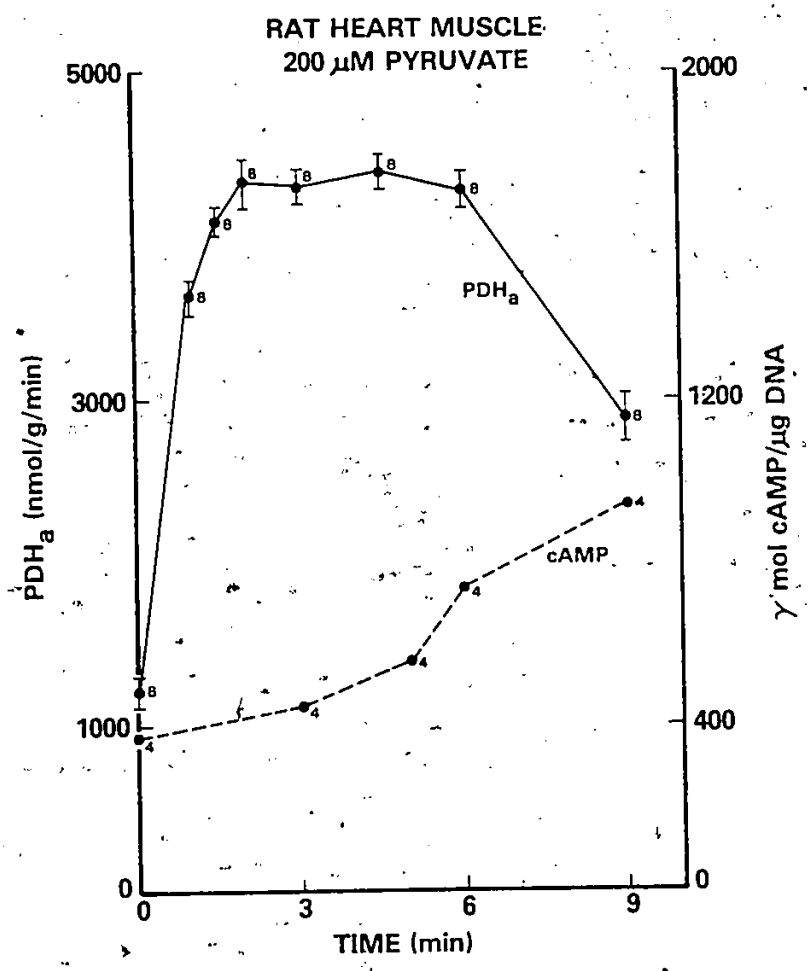

Figure 26.

The effect of 200 μM pyruvate infused into the rat heart is shown in this figure. The homogenate was assayed for PDH_a and cAMP concentration after a perfusion of 1, 1.5, 2, 3, 4.5, 5, 6, and 9 minutes. The PDH_t activity averaged 4694 nmol/g/min. in these experiments. There were 9 observations at each time point and the vertical bars are ± S.E.M.

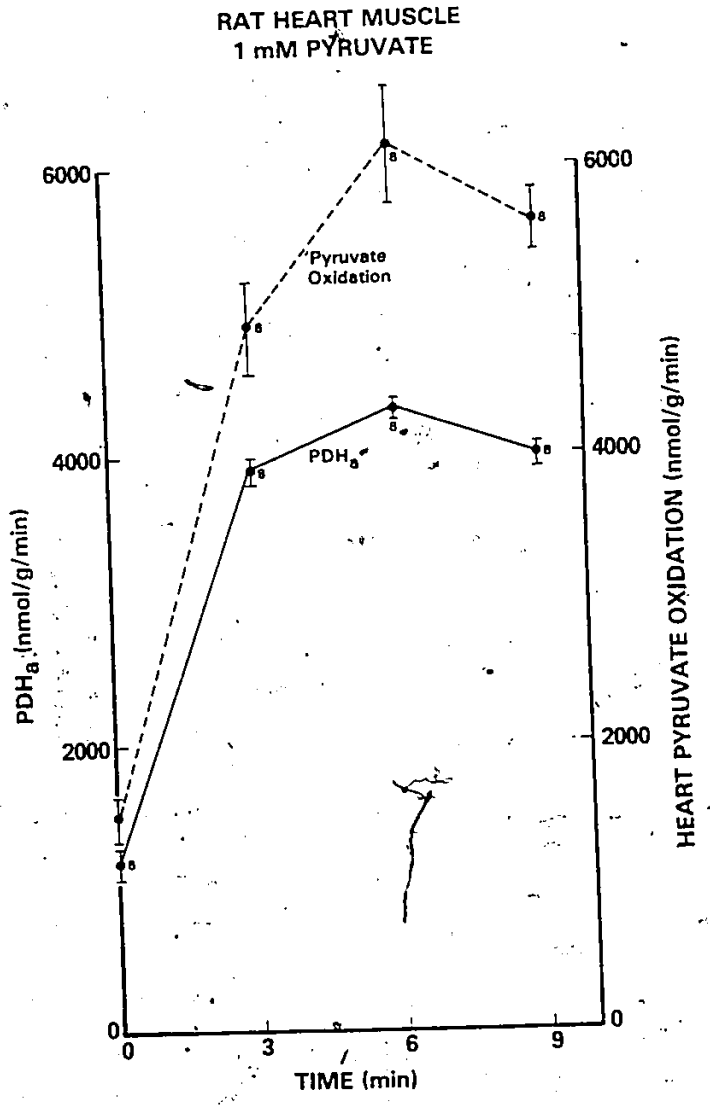

Figure 27.

Effects of 1 mM pyruvate on the activity of PDH_a in the perfused rat heart and the rate of pyruvate oxidation. Assays for PDH_a are as for Figure 14. and in "Materials and Methods". The total PDH activity averaged 4979 nmol/g/min. The perfusion times were 3, 6, and 9 minutes. The results are shown as mean ± S.E.M. and there were 8 observations at each time point.

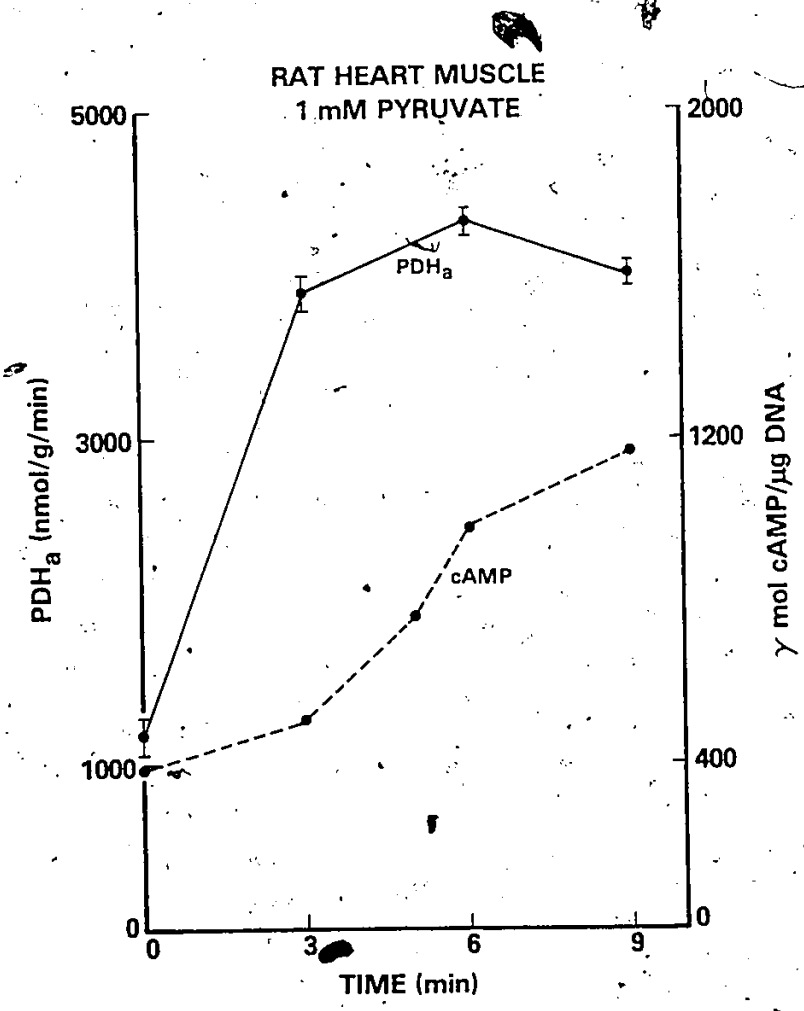

Figure 28.

Effects of 1 mM pyruvate on cAMP concentration and PDH_a activity in perfused rat heart homogenates. There were 8 observations for each time point and the perfusion was over 3, 5, 6, and 9 minutes for cAMP, and 3, 6, and 9 minutes for PDH. The PDH_t averaged 4979 nmol/g/min. The vertical bars are ± S.E.M. All other conditions are as in Figure 14. and in the "Materials and Methods".

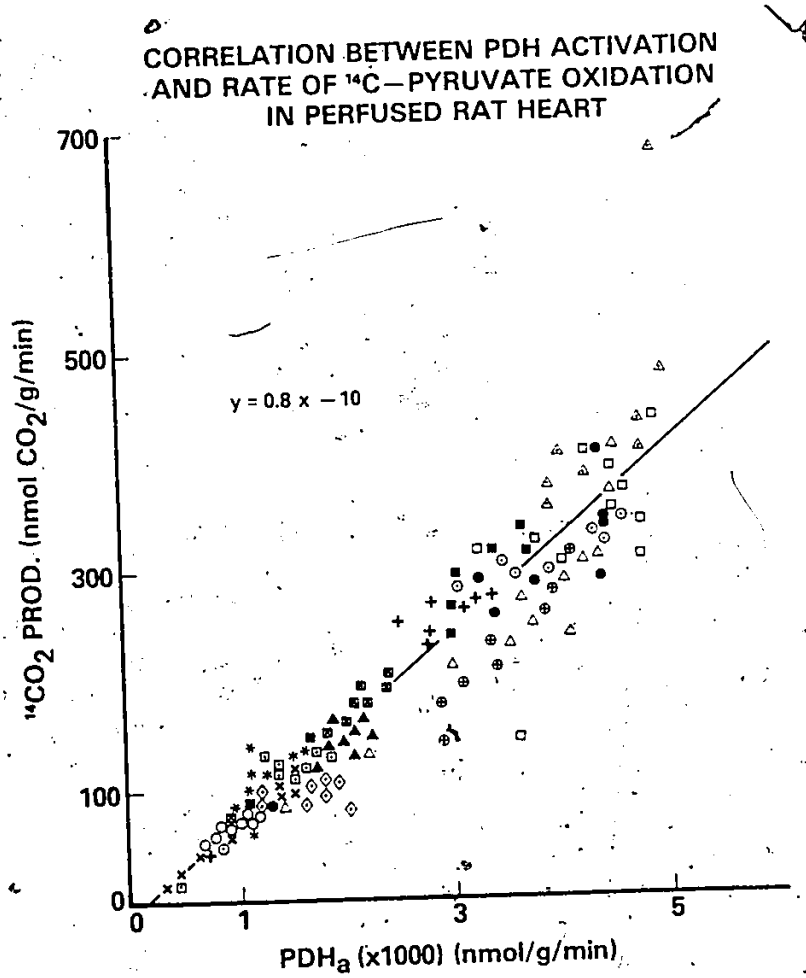

Figure 29.

The experimental results from all groups were compiled and plotted as PDH_a versus the rate of pyruvate oxidation. The rate of pyruvate oxidation was calculated from the rate of $^{14}Co_2$ production after correction for changes in heart specific activity. This was determined by converting pyruvate to alanine by alanine dehydrogenase and separating the alanine on a cation exchange resin column. Correlation resulted in an $r = 0.92$.

Symbols (Key) for Figure 29

⊠ 20 μ M Pyruvate (Control)

■ 50 μ M Pyruvate (Control)

□ 200 μ M Pyruvate (Control)

△ 1000 μ M Pyruvate (Control)

● 1.3 μ g/ml Epinephrine + 20 μ M Pyruvate

△ 1.3 μ g/ml Epinephrine + 50 μ M Pyruvate

\+ 1 mU/ml Insulin + 20 μ M Pyruvate

⊙ 1 mU/ml Insulin + 50 μ M Pyruvate

✳ 4 μ g/ml Propranolol + 20 μ M Pyruvate

◈ 4 μ g/ml Propranolol + 200 μ M Pyruvate

▲ 4 μ g/ml Propranolol + 1000 μ M Pyruvate

⊡ 1.3 μ g/ml Epinephrine + 4 μ g Propranolol + 20 μ M Pyruvate

× 1 mM Octanoic Acid + 20 μ M Pyruvate

⊕ Electrical Stimulation of Heart (Pacing) + 20 μ M Pyruvate

DISCUSSION

1. Methodology

Pyruvate dehydrogenase activity was assayed by the formation of $^{14}Co_2$ from pyruvate-1-^{14}C in muscle homogenates by a modification of the method described by Taylor et al., 1973.[105] By comparing the homogenate method with the mitochondrial preparation of Taylor et al.[105,150] and Taylor and Halperin, 1973,[79] it was found that the homogenate preparation provided essentially the same results for the PDH_a:PDH_t ratio. If a mitochondrial preparation was used, a considerable amount of tissue and time was required with less than 10% recovery of mitochondria.[201] Because of this low mitochondria yield, it was not possible to measure PDH activity in the isolated mitochondrial fraction from less than 50 mg of skeletal muscle biopsy.

The experiments comparing the homogenate and the mitochondrial preparations indicated that the rate of $^{14}Co_2$ production from pyruvate-1-^{14}C was linear with time and enzyme concentration (p. 70). Separate experiments indicated that PDH_a activity of an homogenate did not change during 60 min. of storage on ice. This suggests that there was no net enzyme phosphorylation or dephosphorylation. Since the time period between homogenization and enzyme

assay did not exceed 10 min., the observed PDH_a:PDH_t ratio probably reflects the state of the enzyme *in vivo*.

PDH activity requires a number of cofactors for its full activity (p. 74). The significance of the cofactors on the homogenate PDH activity is illustrated by the results on p. 75 . Mg^{++} and Ca^{++} were essential for the full activity of the enzyme. The maximum activity of PDH was achieved with 10 mM $MgCl_2$ and 10 mM $CaCl_2$. The addition of lower $CaCl_2$ concentrations (10-1.0 mM) did not have a major effect on the PDH activity. Other direct evidence that the assay does measure the PDH activity was provided by the observations that $^{14}Co_2$ liberated required the following cofactors - TPP, CoA and NAD^+ (p. 75). When NAD^+ or CoA were omitted separately, there still was considerable PDH activity. As NAD^+ and CoA are essential cofactors for PDH activity these observations suggest that some NAD^+ and CoA was bound to the enzyme complex.[51]

The spontaneous decarboxylation of pyruvate produced significant problems in determining the $^{14}Co_2$ produced from pyruvate-1-^{14}C. This was circumvented by minimizing the decarboxylation of pyruvate-1-^{14}C by freeze-drying the pyruvate-1-^{14}C and storing it dry in sealed vials at -20°C until required. Using these precautions, the basal $^{14}Co_2$ present prior to the assay was less than 5% of the DPM at the end of the assay. If these precautions were not

considered, the background $^{14}Co_2$ present at 0 time was not much lower than the $^{14}Co_2$ present at the end of the incubation. Such experimental conditions would not permit one to measure PDH activation with any accuracy.

2. Skeletal Muscle

PDH regulates and catalyzes the decarboxylation of pyruvate to form acetyl CoA.[1,8,9,13a,13b] In skeletal muscle the enzyme's activity is a major determinant affecting pyruvate oxidation from glycogen and blood glucose. Though the activity of the enzyme could be of major regulatory importance in regulating lipogenesis, in this regard, it is probably of little significance in skeletal muscle, since muscle capacity for lipogenesis is very small.[211-213] PDH activity is clearly of importance in regulating the oxidation of amino acids which have pyruvate as one of the intermediate steps. However, this is probably of little quantitative significance since amino acid oxidation forms a very small fraction of muscle pyruvate oxidation at rest or exercise.

Heavy aerobic exercise perhaps places the greatest demand on the muscle capacity to oxidize energy producing substrates. Work at close to the $\dot{V}O_2$ max results in 20-50 fold increases in the muscle O_2 consumption.[205-208,211,212] Most of the substrate for this is pyruvate derived from muscle glycogen and blood glucose.[207-209] Light work, on

the other hand, results in a relatively small increase in muscle O_2 consumption. In this case, some of the substrate oxidized is carbohydrate, but the majority of the Co_2 is derived from oxidation of fat, probably derived from increased lipolysis seen in these circumstances.[210-213] Because heavy work results primarily in muscle carbohydrate oxidation it would appear that regulation of PDH activity plays a major role in regulating pyruvate oxidation derived from glucose or glycogen. With heavy work, the flow through the enzyme step must be increased 20-50 fold, while only a small change in flow would be required with light work. It was a goal of this study to determine how muscle pyruvate oxidation was regulated during exercise.

The initial goal was to determine to what extent the increased muscle pyruvate oxidation with exercise was due to increased formation of PDH_a. The assay of PDH_a activity was not a major problem and is discussed elsewhere. The major difficulty was the quantitation of muscle Co_2 production derived from pyruvate. One method could involve determining muscle glycogen concentration changes and the muscle balance of key metabolic fuels by sampling femoral, arterial and venous blood samples if the leg blood flow is known. In this manner, if an exercising muscle mass was assumed, it would be possible to determine the balance of the key metabolites across the muscle vascular bed and

calculate the rate of pyruvate oxidation. While this technique is possible and has been used,[212,213] it was generally felt that such an approach could not be justified ethically. The other quantitative method would involve the use of a pyruvate-^{14}C tracer to measure the body pyruvate turnover. A major problem with this approach is that body pyruvate utilization does not necessarily equal muscle pyruvate oxidation since pyruvate can be metabolized in different ways in many different organs.[214,215] In addition, it would be difficult to measure quantitative changes in muscle pyruvate oxidation because the exchange between the muscle generated Co_2 and the bicarbonate pool would introduce a delay of at least 30 min.[216-218]

Because of these problems in quantitating the rate of muscle pyruvate oxidation, an advantage was taken of the physiological observation that heavy exercise is a situation where most of the muscle Co_2 is derived from muscle glycogen and blood glucose.[206,219] Thus, it was assumed that the increase in body Co_2 production during heavy exercise must have been metabolized through PDH. The relationship between calculated pyruvate oxidation and PDH_a activity is summarized in Figure 30. The rate of muscle pyruvate oxidation increased about 10-15 fold while the PDH_a varied from 94-126 nmol/g/min. at rest to 260 nmol/g/min. during exercise. It was unusual for PDH not to be almost fully activated during exercise.

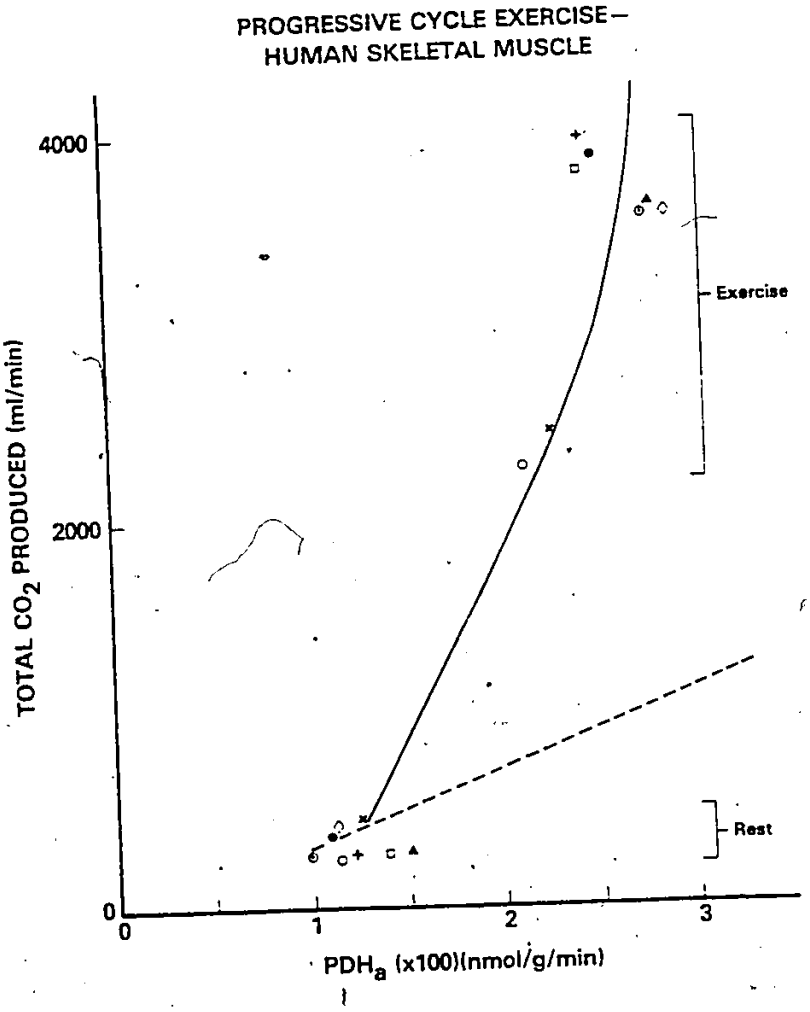

Figure 30.

The relationship between PDH activation and calculated muscle pyruvate oxidation. The points at the bottom of the graph represent the calculated rate of muscle pyruvate oxidation and the observed PDH_a activity at rest. The values at the top of the graph indicate the degree of PDH activation with exercise and the calculated increase in muscle pyruvate oxidation assuming that 80% of the increase in O_2 consumption is due to muscle pyruvate oxidation. The curved line represents the best fit with the data. The dotted line represents the increase in pyruvate oxidation anticipated if the increased oxidation was entirely due to PDH activation. The exercise was conducted in a progressive increase in the work load. The total $\dot{V}CO_2$ and $\dot{V}O_2$ was determined at 80% of the calculated $\dot{V}O_2$ max. Each point represents one subject at rest and during exercise.

It is important to note that close to 40% of the PDH_t was in the active form at rest. If this reflects the situation in the muscle cell, it follows that activation of PDH alone could only increase the rate of pyruvate oxidation by 2-3 fold. The calculated increase in the rate of muscle pyruvate oxidation was at least 10-15 fold. It is possible that the latter figure could be adjusted somewhat by altering the basic assumptions. However, it is clear that the manipulation of assumptions could not reduce the increase to 2-3 fold because this would not be consistent with published observations.[220,221] It follows that either PDH activation is not the primary control of pyruvate oxidation during exercise, or that there is a fundamental error in the assay of PDH_a. The latter possibly can be excluded for several reasons. Firstly, using the same assay it has been possible to demonstrate a dramatic decrease in PDH_a activity with immobilization. Thus, there is no fundamental flaw in the assay technique that prevents the measurement of low PDH_a activity using the same methods and same tissue sources. Secondly, the PDH_a activity tends to be higher in the fed state.[87] The subjects were all tested after a 12-14 hour fast which would tend to decrease, not increase, the resting PDH_a activity.

These results suggest that skeletal muscle pyruvate oxidation during exercise is controlled by factors

other than PDH_a formation. The formation of PDH_a during exercise could only explain an increase of pyruvate oxidation of 2-2.5 fold whereas the actual observed increase was at least 15 fold. The interpretation of these observations is that the rate of muscle pyruvate oxidation is largely regulated by factors which modulate the activity of PDH_a. For example, it is possible that the measured activity of PDH_a of 124 nmol/g/min. at rest (Table 13), represents the activity when all cellular inhibitory factors have been diluted out during the assay. Thus, a relatively high mitochondrial free $NADH/NAD^+$ ratio and other possible factors[163,164] could reduce the PDH_a activity in the cell to 10 from the measured activity of 124 nmol/g/min. During exercise a number of regulatory factors inhibiting PDH_a at rest would decrease and result in a marked stimulatory effect on PDH_a activity. In addition to these factors, there is formation of PDH_a from PDH_i by modulating the PDH kinase and PDH phosphatase activity. The combination of both mechanisms could explain the marked increase in the calculated rate of skeletal muscle pyruvate oxidation during exercise.

Some of the factors that might regulate PDH_a activity have been explored but interpretation of these data must be cautious because it cannot be assumed that the total tissue concentration of a metabolite necessarily reflects its concentration in the mitochondria at the location of PDH.

One of the factors controlling PDH_a activity is the pyruvate concentration. The resting muscle pyruvate concentration is about 50 nmol/g. With heavy exercise it increased up to 500 nmol/g. If the PDH_a K_m for pyruvate was well above 50 nmol/ml, a substantial increase in muscle pyruvate concentration could by itself increase the rate of muscle pyruvate oxidation. When this pyruvate concentration increase is combined with the increased formation of PDH_a, the calculated rise in pyruvate oxidation was about 5-7 fold. These calculations assume a relatively high (Figure 31) K_m of pyruvate which has not been observed with the purified enzyme.[51,73] Experiments which were subsequently obtained in the heart (Figure 32) indicate that the "observed" K_m is lower in intact muscle. If the observations in the heart apply to skeletal muscle in vivo, it is likely that the change in muscle pyruvate concentration with exercise would have a relatively minor effect on PDH_a activity.

It has been established that a decrease in ATP and an increase in AMP and ADP will stimulate PDH_a activity.[164,169] This effect has to be distinguished from the known effects of adenine nucleotides on PDH kinase and PDH phosphatase which control interconversion between PDH_a and PDH_i. In the experiments done for this purpose, it was observed that

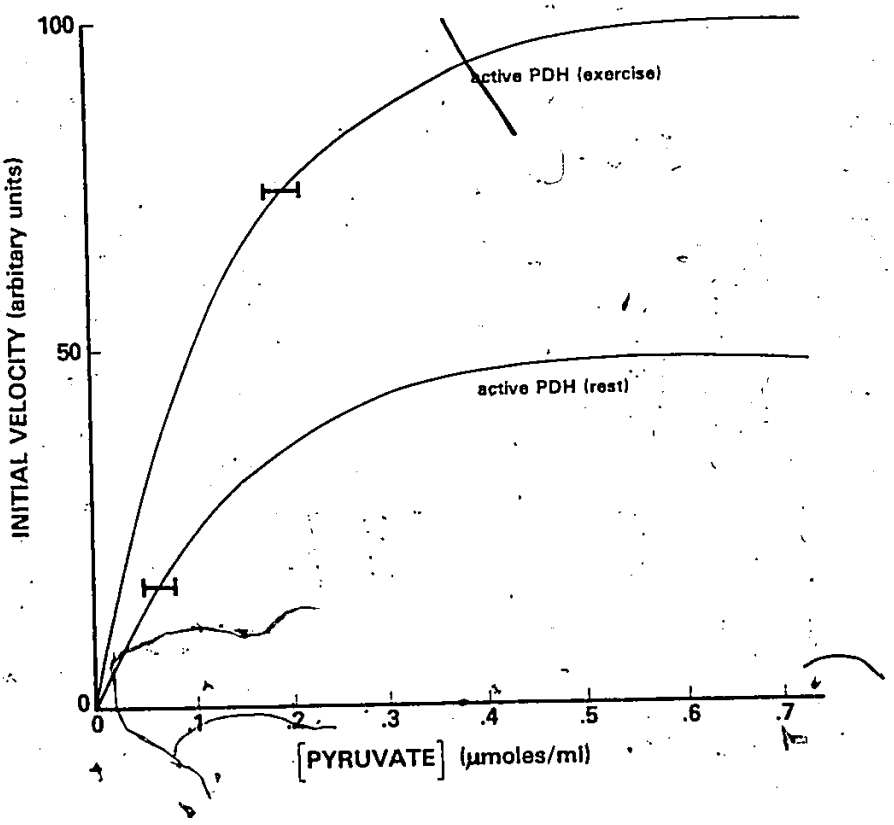

Figure 31.

The effect of pyruvate concentration on the active PDH activity in human skeletal muscle at rest and exercise. The graph is a schematic Michaelis-Menton plot combining the activation of PDH and pyruvate concentration. The plot assumes a K_m of 0.1 µM/ml. The pyruvate concentration increased from 0.07 to 0.2 µM/g wet wt with the progressive cycle exercise.

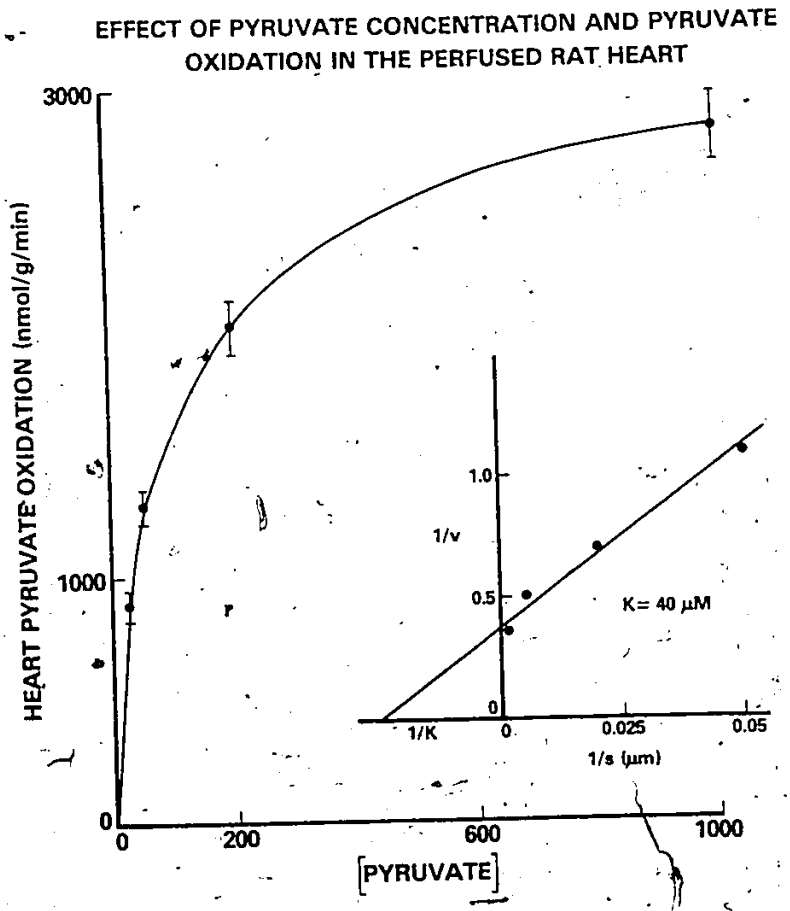

Figure 32.

Effect of pyruvate concentration and pyruvate oxidation in perfused rat heart: Lineweaver-Burk plot is inset. Changes in pyruvate oxidation rate were determined over a range of pyruvate concentrations. The K_m is indicated at 40 μM.

a significant decrease in muscle ATP with heavy exercise resulted (Table 14). These observations are consistent with the interpretation that relatively high ATP and low AMP and ADP concentrations at rest would inhibit PDH_a activity. During exercise, PDH_a would no longer be inhibited by the decrease in ATP and increase in ADP and possibly AMP. Similar experiments could have been done for other known modulators of PDH_a activity to see if the changes during exercise were consistent with this interpretation. These studies are now under way.

In summary, there is evidence that skeletal muscle pyruvate oxidation during exercise is regulated largely by factors regulating the active PDH activity, while a relatively minor part of the pyruvate oxidation changes can be explained by conversion of PDH_i to PDH_a. In the heart, however, the situation appears to be quite different. The capacity to increase pyruvate oxidation is less than in skeletal muscle and all the experimental manipulations used to alter heart pyruvate oxidation have the same effect on PDH_a formation (Figure 29). In fact, when the changes in the rate of pyruvate oxidation were correlated with PDH_a formation the correlation coefficient was greater than 0.92. This does not prove, however, that the heart pyruvate is not regulated by factors altering PDH_a activity, but it does indicate that no mechanisms other than PDH_a formation need be involved in order to explain the results.

It is possible that altered muscle PDH activity could be important in certain disease states because skeletal muscle is such a large "organ". To pursue this possibility further, some calculations to determine the capacity of the muscle PDH system were undertaken.

If a 70 kg athlete with a $\dot{V}O_2$ max of 70 ml of oxygen/kg/min. is exercising at a rate utilizing 50 ml of oxygen/kg/min. with 20 kg of muscle, one obtains a pyruvate oxidation rate of about 2,000 nmol/g/min., assuming that the only muscle substrate used was carbohydrate in nature. The average PDH_t activity in human skeletal muscle was about 310 nmol pyruvate/g/min. (most of the PDH assays were conducted at a pyruvate concentration of 20 μM based on the kinetic relationships outlined in Figure 29). The actual highest PDH activity and pyruvate concentration which was observed during exercise was about 1,100 nmol pyruvate/g/min. These calculations suggest that the capacity of the pyruvate dehydrogenase reaction may be close to the capacity of the maximal observed pyruvate oxidation in vivo. In this case, an inability to form PDH_a could have significant effects in a variety of disease states.

In one child where there was an opportunity to study pyruvate metabolism where the clinical presentation was severe lactic acidosis, it was observed that there was a large decrease in the muscle PDH_t and in the PDH_a activity

(Table 17). A number of other case reports of similar disease entities have been reported.[221-224] In most cases, the PDH activity is not absent, suggesting that it is not an inborn error of metabolism involving a failure to synthesize pyruvate dehydrogenase. It is possible that the decrease in PDH_a activity was secondary to some other problem. This is especially pertinent as it relates to other physiological situations we have studied and discussed later.' (Tables 18 and 19)

In a study involving normal young male athletes, it was demonstrated that an aerobic type training program did not change the muscle PDH_t activity but substantially increased the PDH_a activity. When the muscle groups were immobilized the PDH_t activity did not change but the PDH_a dropped substantially. In one subject where a fracture occurred requiring immobilization, the PDH_a activity dropped dramatically from a normal of 110 to 25 nmol/g/min. These observations indicate that immobilization or the training programme does not alter the total capacity for PDH activity, but substantially alters the amount of PDH in the active form. It is possible that under a variety of medical or surgical conditions that the associated immobilization results in a substantial reduction in PDH_a activity. This, in itself, may not present any significant problem, unless specific metabolic stress is applied. For example, how would

Table 17

Muscle PDH Activity

In a Child With Severe Lactic Acidosis

	PDH_a	PDH_t	A:T %
Controls (4)	110 ± 4	308 ± 2	37
Baby AM	12	44	27

Legend: The 8 week old infant presented with severe metabolic acidosis. The entire anion gap could be accounted for by lactate (20-31 mM). The controls were normal adult male subjects. All biopsies were obtained from the vastus lateralis muscle group.

such patients respond when large amounts of glucose were administered? It is possible that the glucose would enter the cell, be partially metabolized and subsequently be converted to lactate because of the inability to decarboxylate pyruvate. This could explain increased susceptibility to lactic acidosis under a variety of medical conditions in which immobilization is a major feature. It is recognized that circulatory problems by themselves would and could contribute to lactic acidosis purely on the basis of poor organ perfusion. However, the possibility that the lactic acidosis develops first, followed by dehydration and decreased cardiac function cannot be excluded.

Another general observation is that patients with severe medical or surgical problems have decreased glucose tolerance. The mechanisms for this may have never been clearly explained. It is possible that a significant or major contributing factor to this could be the inability of the muscle and perhaps other cellular systems to decarboxylate pyruvate derived from glucose.

It is difficult at the present time to know the significance of these generalizations. However, one cannot readily dismiss these possibilities since the capacity for pyruvate decarboxylation seems to have very little reserve based on the calculations in skeletal muscle. This does appear to be a unique position for enzyme systems, since

most regulatory enzymes are present in concentrations that would facilitate flux through the appropriate pathway higher than is normally observed.

Insulin and epinephrine have been shown to be important hormonal factors regulating the interconversion of PDH_i and PDH_a. In studies, outlined later in the heart (Figures 20 and 14), it was demonstrated that insulin and epinephrine resulted in activation of heart PDH. In other reported studies in the isolated fat cell, epinephrine decreased PDH_a activity dramatically.[100,101,225,226] Insulin in the fat cell results in complete activation of PDH.[98,101,113] The mechanisms by which the hormones exert their effect is not clear. On the basis of the studies in the heart, it would appear clearly that cyclic AMP is not an important factor regulating interconversion of PDH_i and PDH_a. Thus, epinephrine resulted in complete activation of PDH with a parallel increase in the cAMP concentration. Insulin resulted in PDH activation that followed a similar time course but the cAMP concentration under these conditions decreased. (Figures 15 and 21) Other investigators pursuing a similar approach have not been able to demonstrate the mechanism by which these hormones exert their effect on PDH activation and inactivation.[102,103,107,108,113,122]

Therefore, in the following studies, the possible role of hormones in regulating skeletal muscle PDH activation

was pursued in several ways. In the first series of studies the femoral nerve was stimulated unilaterally and the extent of PDH activation was determined bilaterally (Figure 13). The muscle PDH was activated in the stimulated leg while no activation was observed in the contralateral leg. If the primary mechanism for muscle PDH activation was hormonal, one would have expected bilateral activation occurring at the same rate. These studies were extended to a human situation where subjects exercised one leg while the other one was at rest (Figure 13). In the human study, as in the rat, muscle PDH was activated in the exercising leg while no changes were observed on the resting side. These observations strongly suggest that the physiological regulator of PDH activation is not hormonal but probably initiated by nervous impulses.

Additional studies were done in the rat to determine whether changes in voltage or frequency had any effects on PDH activation (Tables 1, 2, 6, and 8). Five volts is consistently used by investigators[192,237,238] to ellicit maximal nerve excitation. With regard to the effect of voltage on PDH activation, it appears from our data that voltage between 5 and 40 volts are sufficient to induce almost 100% activation of PDH. However, the time required to reach 100% activation appears to be affected by voltage and is directly proportional to the voltage applied. Thus, at 40 volts, 90% PDH_a activation was reached within 15-20 sec. whereas much longer times were required for similar

activation with voltages less than 5 volts. The data do not allow us to conclude about why or how voltages greater than 5 volts can affect the rapidity of PDH activation. It is perhaps somewhat difficult to extrapolate from these animal experiments to the in vivo situations since the results in human skeletal muscle were not what one would have predicted, based on the animal studies. For example, we observed that aerobic work at about 50% $\dot{V}O_2$max. resulted in fairly rapid PDH activation, whereas with more intense work, at about 140% $\dot{V}O_2$max, the rate of PDH activation was slower. At present, these different results obtained with nerve stimulation in the rate and exercise in the human cannot be readily explained.

A number of studies in the past few years have demonstrated that certain mitochondrial marker enzymes, such as succinate dehydrogenase and others, increase by up to 50% following appropriate aerobic training programmes.[204] In other similar studies, investigators have used morphometric techniques and have demonstrated that the number of mitochondria appear to have increased significantly with aerobic training programmes.[227-229] A similar study was performed to determine whether skeletal muscle PDH activity would change during various training programmes. The rationale was that the changes might be substantially greater for PDH because it is likely that the key enzyme regulating mitochondrial metabolism is from carbohydrate derived

substrates. In the aerobic running training programme for rats, it was observed that the PDH_a portion increased but no changes in the PDH_t activity was observed. Essentially the same results were obtained with weight training in humans. A very small number of competitive athletes were also tested for PDH concentrations and the results were essentially the same. (Table 18) While the $\dot{V}O_2$ max was much higher in the competitive athletes than in the matched controls, the muscle PDH_t activity was the same. The PDH_a activity in the athletes was significantly greater, however, than that in the control subjects. These experiments suggest that aerobic training, while it may increase the activity of certain mitochondrial marker enzymes, does not increase the activity or amount of PDH_t present in skeletal muscle. The PDH_a activity is invariably higher in the muscle of trained subjects. The physiological significance of this is not clear at the present time. The earlier calculations (p. 145) indicating that the aerobic capacity is very close to the measured PDH_t activity in muscle suggests that under both trained and untrained conditions the regulation of pyruvate oxidation in skeletal muscle is largely controlled by factors regulating the PDH_a activity. There clearly is activation of PDH during exercise but the contribution this makes to increasing the capacity of pyruvate oxidation in muscle appears to be relatively small.

Table 18

Muscle PDH Activity in Competitive Athletes

	$\dot{V}O_2$ max (ml $\dot{O}_2$/kg/min.)	PDH_a (-- nmol/g/min.--)	PDH_t	A:T %
Non-athlete controls (4)	47 ± 3	124 ± 9	304 ± 4	41
Competitive athletes (4)	71 ± 4	215 ± 15	309 ± 7	70

Legend: $\dot{V}O_2$ max was determined as outlined in the "Methods". Muscle biopsies at rest and PDH assays were conducted as outlined in the "Methods". The number in brackets refers to the number of subjects. The non-athlete controls were physical education students. The competitive athletes were two well-trained oarsmen and two trained distance runners.

3. Heart Muscle

The major goal of these studies was to determine whether changes in pyruvate oxidation in the heart was entirely regulated by endocrine conversion (interconversion) of PDH_i and PDH_a, unlike skeletal muscle. Heart is unique in that it cannot depend on anaerobic sources of energy for significant periods of time. It is different in other respects in that the range of oxygen consumption is substantially less than in skeletal muscle. Because at least 25% of the total pyruvate dehydrogenase is in the active form, it is difficult to see how pyruvate flow could increase by a factor of 10 or 20 on the basis of interconversion of PDH_a and PDH_i. The heart with the smaller range of oxidative metabolism could be regulated only by interconversion of the two enzyme forms because of the smaller range of oxidative capacity.

The perfused rat heart was used as a model because this permitted better control of all the variables. The general nature of the experiments involved the use of a variety of agents which have been demonstrated to affect the rate of either pyruvate or glucose oxidation. The anticipation was that these would be modulated by PDH activation and in this way information could be obtained about the significance of PDH activation versus the regulation of PDH_a by substrates, products, and allosteric factors.

In the individual experiments it was observed that activation of PDH occurred with epinephrine and insulin. (Figures 14 and 20) Octanoic acid and propranolol decreased PDH activity. (Figures 18 and 23) Additions of higher concentrations of pyruvate above 50 μ M, resulted in rapid activation of PDH. (Figures 25 and 27) In all these studies the change in pyruvate oxidation was paralleled by the amount of PDH_a formed. When the rate of heart pyruvate oxidation was correlated with the change in PDH_a activity, a very close correlation between the two was observed. The correlation coefficient was greater than 0.92, suggesting that any deviation from this correlation probably represented experimental error. Based on these correlations, one is tempted to conclude that the alteration in pyruvate oxidation was mediated by changes in PDH_a formation. While it is recognized that such correlations do not prove that PDH_a formation initiated the increase in pyruvate oxidation, it does indicate that one does not have to invoke any explanations other than PDH_a formation in the change of pyruvate oxidation in the perfused rat heart.

In order to pursue the mechanisms of heart PDH activation further, the rate of PDH_a formation, heart pyruvate oxidation, and changes in heart cAMP concentration were determined. Epinephrine had a marked stimulatory effect on PDH_a formation and a comparable increase in the

rate of pyruvate oxidation. The muscle cAMP concentration increased substantially and temporally with PDH_a formation. When propranolol was added with epinephrine, the rate of pyruvate oxidation was decreased to the same degree as the decrease in PDH_a activity. The cAMP concentration was still increased but was substantially less than with epinephrine alone. These results are consistent with cAMP playing a role as a second messenger in activating PDH. However, other experiments done with insulin, indicate that PDH was activated, the rate of pyruvate oxidation was increased, but the heart cAMP concentration was decreased. The results obtained with insulin in conjunction with those obtained with epinephrine indicate that hormonal activation of PDH can occur independent of changes in the cAMP concentration. These observations are consistent with studies by Reed, Hucho, Denton and Jungas,[8,9,51,99] who were unable to demonstrate any effect of cAMP on PDH kinase or PDH phosphatase activity. Some earlier experiments in the fat and liver cells suggested that cAMP may play a role in PDH activation,[48,111,112] however, subsequent experiments in the same cell systems have failed to establish an important role for cAMP in PDH activation and inactivation.[9,26,43,101,103,108]

In addition to the hormonal effects on PDH activation in the heart, it seems clear that high concentrations of

pyruvate result in a rapid PDH activation. To what extent these effects represent regulation of the physiological nature is not clear. It would appear that these may not be that important since the plasma pyruvate concentration rarely increases to concentrations higher than 100 μ M.

Earlier studies in the perfused rat heart attempted to separate the effects of epinephrine and other hormones in terms of its known effects on contractility and metabolic events.[161,230,231] The perfused rat heart model used in these experiments does not permit definitive conclusions to be made on this point since the heart was essentially a non-working model. However, an indirect assessment of the presumed role of contractility with this direct metabolic effect can be assessed by comparing the degree of PDH activation, pyruvate oxidation and oxygen consumption under the various experimental conditions. (Table 19) Epinephrine resulted in marked increases in pyruvate oxidation, PDH activation, as well as a significant increase in the rate of oxygen consumption. Insulin resulted in very similar increases in pyruvate oxidation and PDH activation, but had very little effect on oxygen consumption. High concentrations of pyruvate resulted in marked increases in the rate of pyruvate oxidation and active PDH formation. However, the oxygen consumption decreased. These observations suggest that the metabolic effects on PDH activation and pyruvate

Table 19

Effect of Different Manipulations

On Heart Pyruvate Oxidation, PDH Activation and O_2 Consumption

	Pyruvate (μM)	O_2 Consumption (natoms/ht/min.)	Pyruvate Oxidation (nmol/g/min.)	PDH_a (-nmol/g/min.-)	PDH_t
Control (48)	20	6.5 ± 0.1	1457 ± 131	1225 ± 48	4895 ± 176
Epinephrine (12)	20	9.6 ± 0.4	2333 ± 321	3844 ± 162	5017 ± 106
Insulin (8)	20	8.2 ± 0.4	2272 ± 198	3169 ± 66	4851 ± 74
Octanoic Acid (8)	20	- -	1220 ± 186	406 ± 95	4831 ± 212
Propranolol (8)	20	5.9 ± 0.6	1500 ± 171	1241 ± 133	4984 ± 170
Propranolol & Epinephrine (8)	20	5.8 ± 0.7	1466 ± 263	2100 ± 98	4881 ± 132
Control (8)	1000	4.0 ± 0.9	5604 ± 240	4001 ± 89	5051 ± 146

Legend: The pyruvate oxidation, PDH_a and PDH_t were all determined as outlined in the "Methods". The O_2 consumption was calculated from flow rate and the Po_2. The numbers in brackets refer to number of observations. The values represent ± S.E.M.

oxidation can occur independently of any changes in the rate of heart oxygen consumption. While this does not prove that PDH activation and pyruvate oxidation occur independently of contractility, it suggests that there may be separate effects of the various agents.

Insulin in the perfused heart like epinephrine, stimulated PDH activity and in a parallel way stimulated pyruvate oxidation. (Figures 14 and 20) The mechanism by which insulin activates PDH is unknown. Recently it has been demonstrated that insulin had no effect on PDH phosphatase in diabetic heart[184,232,233] suggesting that insulin may have an effect on the PDH kinase activity. Direct evidence for this is not available. The effect of insulin on PDH activation in the heart is essentially the same as observed in adipose tissue with or without added glucose. In fat cells, insulin increases lipogenesis from pyruvate[98,99,101,109] suggesting that one of the major effects of insulin is the stimulation of triglyceride formation from pyruvate. The heart has a relatively low rate of lipogenesis, suggesting that insulin in the heart must have a major effect on pyruvate oxidation rather than on lipogenesis.

It is possible insulin may act in some way to change the adenylate charge which in turn may activate PDH activation by decreasing the ATP-ADP ratio.[53,66,72,73,169,180]

It seems unlikely that this would be the major regulatory mechanism by the way insulin works, as the ATP:ADP ratio has not been shown to change with insulin administration in the heart.[232-234] On the contrary, insulin lack results in a decreased ATP:ADP ratio and decreased adenylate charge which should, if these arguments apply, result in PDH activation. Experimentally, insulin lack invariably results in PDH activation.

The PDH_t activity in the rat heart homogenate was not influenced by a 24 or 48 hr starvation period, however, the PDH_a was considerably decreased during the same starvation period. (Table 20) The inactivation suggests that the activity changes are hormonally or nutritionally regulated. Insulin lack, increased plasma FFA could be responsible. Most information has been primarily carried out on the regulation of increased fat oxidation and, therefore, the effects could possibly be mediated by this mechanism. The experiments <u>in vitro</u> suggest that the increase in the supply of FFA can lead to an increased phosphorylation of PDH in heart, liver, and kidney.[70,163,235] Perfusion of isolated rat heart with acetoacetate showed similar effects.[163] Insulin has been shown to act directly on adipose tissue[105,107] and on liver.[157] As phosphorylation is diminished in these tissues the PDH is greatly stimulated. As insulin has also exerted a stimulatory action in the heart, in this work,

Table 20

Rat Heart PDH Activity

In Various Stages of Starvation

	PDH_a	PDH_t	A:T
	(--nmol/g/min.--)		%
Feb ad libitum (12)	2768 ± 73	4863 ± 110	57
18-24 hr. starve (12)	1286 ± 109	4728 ± 168	27
48 hr. starve (12)	920 ± 149	4829 ± 91	19

Legend: The activity of PDH_a and PDH_t in rat heart of normal fed rats, 18-24 hr. starved rats and 48 hr. starved rats. The number in brackets refers to the number of rats. The values represent ± S.E.M.

insulin lack seems a likely candidate for producing the changes. The effects of insulin may be more widespread than those found in the results and the current literature. The degree of inactivation in the heart with starvation may be, therefore, directly due to the drop in insulin concentration in starvation. Of course, other factors may be responsible for the decrease in PDH_a activity.

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