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Evidence for Aerobic Glycolysis in λ-Carrageenan-Wounded Skeletal Muscle[1]

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Classically, increased lactate production in wounded tissue is ascribed to anaerobic glycolysis although its oxygen consumption has been found to be similar to normal tissue. This apparent inconsistency was studied in a standardized isolated perfused wound model. Male Sprague–Dawley rats were wounded (group W) with intramuscular injections of λ-carrageenan and fed *ad lib.*; not wounded and pair fed to the decreased food intake of the wounded animals (group PFC); or not wounded and fed *ad lib.* (group ALC). After 5 days, the hindlimbs of animals from each group were either perfused using a standard perfusate with added [U-^{14}C]glucose or [1-^{14}C]pyruvate or assayed for the tissue content of lactate and pyruvate. In addition, the effect of a 30% hemorrhage on the tissue lactate and pyruvate concentration was examined. Wounding increased glucose uptake and lactate production by 100 and 96%, respectively, above that seen in ALC animals. Oxygen consumption was unchanged by wounding (5.74, 5.14, and 5.83 μmole/min/100 g in W, PFC, and ALC, respectively). Glucose and pyruvate oxidation were also unaltered among the groups. Hemorrhage resulted in a comparable increase in lactate and pyruvate in tissue from wounded and pair-fed control animals (above those concentrations found in tissue harvested without preexisting hemorrhage). As a consequence, the same relationship in L/P ratio was maintained after hemorrhage. Taken together, these results confirm the presence of aerobic glycolysis in wounded tissue (unchanged oxygen consumption, glucose, and pyruvate oxidation). In addition, pyruvate dehydrogenase activity in the wound was apparently the same as that found in muscle from pair-fed control animals.

INTRODUCTION

Glucose metabolism in wounded tissue is characterized by increased glucose uptake and lactate production [9, 19, 22]. Because the measured oxygen tension of wounded tissue was low [8], these findings have been classically ascribed to anaerobic glycolysis [23]. Anaerobic glycolysis is characterized by a lack of oxygen utilization with presumably a Pasteur effect leading to increased glucose uptake and an anoxia-induced change in the cytosolic redox state resulting in increased lactate production. In contrast aerobic glycolysis maintains pyruvate oxidation via the tricarboxylic acid (TCA) cycle and the increased lactate production is presumably due to decreased activity of NADH shuttles [12]. Therefore, aerobic glycolysis differs from anaerobic glycolysis in that pyruvate oxidation proceeds normally even in the face of increased lactate production.

Chang and Goldberg have evaluated the relationship between oxygen consumption and acetyl group oxidation and have shown a near constant relationship regardless of the substrate [4]. Previous studies have suggested that oxygen consumption in wounded tissue does not differ from that of normal tissue [23]. This finding, taken in the context of Chang and Goldberg's work, implies equal substrate oxidation in wounded and nonwounded tissue. It is, therefore, difficult to use the concept of anaerobic glycolysis to explain the altered metabolism noted in wounded tissue. This study

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was designed to determine whether glucose metabolism in wounded skeletal muscle was aerobic or anaerobic in nature and, therefore, give insight into the cause for increased lactate production.

METHODS

Animals

Male Sprague–Dawley rats (mean wt 236 ± 15 g) were divided into three groups: wounded (W) with intramuscular injections of λ-carrageenan and fed *ad lib.*; not wounded and pair fed to the decreased food intake of W animals (PFC); or not wounded and fed *ad lib.* (ALC). The animals were housed in metabolic cages under constant environmental conditions with 12-hr light cycles and maintained on R-M-H 3000 rat chow (Agway Inc., Syracuse, N. Y.) with free access to water. Treatment, feeding, and sacrifice of the pair-fed controls were staggered by 24 hr in order to match the conditions of their wounded cohort.

Experimental Groups

Experiment 1—in vivo determination. Wounded (n = 7), pair-fed control (n = 7), and *ad lib.* control (n = 5) animals were fasted overnight prior to sacrifice, which occurred 5 days following initiation of their individual treatments. At this time, each animal was anesthetized with pentobarbital sodium as described below. The skin and subcutaneous fat were dissected from the hindlimbs. The hindlimb muscles were immediately frozen with Wollenberger tongs cooled in liquid N_2 [26].

Experiment 2—acute hemorrhage. Wounded (n = 12), pair-fed control (n = 12), and *ad lib.* control (n = 8) animals were treated as in Experiment 1 with the exception that a 30% hemorrhage over a 2-min period via cardiac puncture was performed prior to collection of the hindlimb muscle by freeze clamp technique.

Experiment 3—perfusion studies. Wounded (n = 5), pair-fed control (n = 6), and *ad lib.* control (n = 5) animals were fasted overnight and then underwent hindlimb perfusion with standard perfusate containing [U-^{14}C]glucose. An additional group of wounded (n = 3) and pair-fed control (n = 3) animals were fasted overnight and underwent hindlimb perfusion with standard perfusate containing [1-^{14}C]-pyruvate.

Wounding

Rats were anesthetized by intraperitoneal injection of 5 mg/100 g body wt pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, Ill.) and then received bilateral hindlimb wounds by intramuscular infiltration of a total of 10 ml of 0.5% λ-carrageenan (Sigma Chemical Co., St. Louis, Mo.) as previously reported [16]. Twenty-four hours later, pair-fed control animals received only pentobarbital sodium anesthesia.

Perfusion. After adequate anesthesia was obtained (pentobarbital sodium), intact isolated rat hindlimbs were perfused as previously described [3]. Perfusate medium [14] was Krebs Ringer bicarbonate buffer containing 4% bovine serum albumin (Pentex fraction V, Miles Laboratories Research, Elkhart, Ind.), 10 m*M* glucose, and a physiological concentration of amino acids [3]. Washed, outdated, human erythrocytes were used to give a final hematocrit of 30%. The [U-^{14}C]glucose and [1-^{14}C]pyruvate (New England Nuclear, Boston, Mass.) were added to obtain a final concentration 0.05 μCi/ml.

Following a 20-min preperfusion, hindlimbs were perfused at a constant flow rate of 10 ml/min by a recirculating system for a total of 80 min. Arterial and venous perfusate samples were taken at 20-min intervals and analyzed for pCO_2 and pO_2, and pH using a blood gas analyzer (IL model 213 blood gas analyzer, Instrumentation Laboratories, Lexington, Mass.). Oxygen content was determined in arterial and venous samples using a Lex-O_2-Con-TL (Lexington Instruments, Wartham, Mass.). At the end of perfusion, approximately 1 g of muscle was rapidly frozen using Wollenberger tongs and stored at −80°C.

Analytical Methods

Perfusate glucose was determined by glucose oxidase assay utilizing a YSI-23A glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). The $^{14}CO_2$ production was measured by injecting arterial and venous perfusate samples (1 ml), obtained simultaneously, into counting vials with center wells (Kontes) containing 0.3 ml of Oxifluor-CO_2 (New England Nuclear, Boston, Mass.) and filter paper wicks. After injection of 1 ml of 0.7 *M* perchloric acid (PCA) into the main compartment, the vials were incubated in a shaking water bath at 37°C for 1 hr. The center wells are then counted in 5 ml of Oxifluor-CO_2. Specific activities of [U-^{14}C]glucose and [1-^{14}C]pyruvate were determined using HPLC methodology.

Frozen tissue was pulverized in a percussion mortar at liquid nitrogen temperature and homogenized in frozen 0.7 *N* PCA using a Polytron homogenizer (Brinkman Laboratories, Westbury, N. Y.). Pyruvate [2] and lactate [13] were determined by fluorometric enzymatic analyses. Chemicals and enzymes were reagent grade and were purchased from Sigma Chemical Company, St. Louis, Mo. Statistical analysis was performed by using a Newman–Keul evaluation of analysis of variance [24].

RESULTS

As previously demonstrated, λ-carrageenan injury to skeletal muscle is associated with decreased food intake. The use of pair-fed control animals demonstrated that the diminished food intake contributed largely to the decreased nitrogen balance and weight loss associated with this tissue injury [20]. Therefore, to adequately separate the contribution of semistarvation to the altered metabolism of tissue injury, pair-fed controls were added to *ad lib.* controls in this study.

Wounding resulted in an 100% increase in glucose clearance above that seen in ALC animals. Serum lactate concentrations were increased above *ad lib.* controls in the semistarved (35%) and wounded (48%) animals. Evaluation of lactate production in the perfused hindlimb preparation correlated well with the increased serum lactate in wounded animals. The lactate production from wounded tissue was 96% above that seen in *ad lib.* control animals (Table 1).

Examination of glucose oxidation using [U-^{14}C]glucose and [1-^{14}C]pyruvate showed no difference between wounded and nonwounded tissue (Table 2).

When oxygen consumption was assessed in the perfused hindlimb preparation, there was no difference among the groups (Table 1).

Tissue pyruvate concentration in pair-fed control animals was 35% higher than in *ad lib.* controls. Wounding increased tissue pyruvate 55% above that found in pair-fed control animals and 110% above those of *ad lib.* control animals. These relationships were maintained following hemorrhage. Wounding increased tissue lactate 40% above that seen in *ad lib.* controls. Under the stimulus of an assumed increased glycolysis associated with hemorrhage, tissue lactate increased markedly in the semistarved (107%) and in the wounded (231%) animals (Table 3). Hemorrhage resulted in a uniform increase in lactate/pyruvate ratios although there was no difference

TABLE 1

EFFECT OF WOUNDING AND SEMISTARVATION ON OXYGEN CONSUMPTION AND GLUCOSE METABOLISM

	Glucose clearance (ml/min/100 g)	O_2 consumption (μmole/min/100 g)	Lactate production (μmole/min/100 g)	Serum lactate (m*M*/liter)
ALC (11)	0.28 (0.01)	5.83 (0.44)	1.67 (0.26)	1.24 (0.12) (n = 8)
PFC (16)	0.41 (0.01)	5.14 (0.42)	2.32 (0.31)	1.67 (0.17) (n = 12)
W (11)	0.56 (0.07)*	5.74 (0.67)	3.27 (0.49)*	1.84 (0.12)* (n = 12)

* $P < 0.05$ vs ALC.

TABLE 2

EFFECT OF WOUNDING AND SEMISTARVATION ON GLUCOSE AND PYRUVATE OXIDATION

	$[^{14}C]O_2$ from [U-^{14}C]glucose (cpm/min/100 g)	$[^{14}C]O_2$ from [1-^{14}C]pyruvate (cpm/min/100 g)
ALC	154.2 (19)	
PFC	208.8 (30.1)	2549 (137)
W	271.5 (57.4)	2881 (81)

Note. Glucose and pyruvate specific activities were identical among the groups.

in these ratios between PFC and wounded animals (Table 3).

DISCUSSION

The λ-carrageenan wound model represents a significant tissue injury which is accompanied by a classical histological response and a concomitant increase in DNA concentration [16]. In addition, the increased glucose uptake, lactate production, and systemic metabolic alterations following this injury closely represent those changes seen with other models of tissue injury [20]. The λ-carrageenan model has the advantage of being suitable for *in situ* perfusion techniques because minimal neurovascular injury occurs [16].

Implied in the definition of aerobic and anaerobic metabolism is the different utilization of oxygen in each state. Systemic oxygen consumption has been noted to be unchanged by burn injury in guinea pigs [25] and in humans [23]. In addition, oxygen consumption measured in the human burned extremity has been noted to be unchanged from control unburned limbs [23]. The present study, using the perfused rat hindlimb wound model showed no difference in oxygen consumption in injured as compared with control animals. Chang and Goldberg have shown that it is possible to discern the amount of acetyl group oxidation by using a relationship of available acetyl groups to oxygen consumption. This relationship is relatively constant depending on the metabolic substrate used [4]. In our perfused model, glucose or pyruvate served as the oxidizable substrate and, therefore, equal oxygen consumption between wounded and nonwounded tissue would imply equal acetyl group oxidation in these circumstances. This implication was shown to be correct when [^{14}C]glucose and [^{14}C]pyruvate conversion to $[^{14}C]O_2$ was examined. Thus, the wounded hindlimb was capable of oxidizing glucose in a completely normal manner. This finding is in agreement with that of other investigators [15, 22]. However, wounds of skin and muscle appear to vary in the route of glucose carbon disposal. Im and Hoopes using an injured skin preparation found a major contribution of the pentose phosphate shunt to ultimate glucose oxidation [10]. The finding of identical pyruvate and glucose oxidation in wounded and nonwounded animals in the present study demonstrates the TCA cycle to be a major route of glucose oxidation in wounded muscle.

The most studied metabolic alteration associated with wounded tissue is that of glucose metabolism. Controversy has arisen regarding

TABLE 3

EFFECT OF HEMORRHAGE ON TISSUE LACTATE AND PYRUVATE CONTENT

	PYR (μmole/g)		LAC (μmole/g)		L/P ratio	
	In vivo	Hemorrhage	*In vivo*	Hemorrhage	*In vivo*	Hemorrhage
ALC	0.20 (0.02)	0.16 (0.008)	7.5 (0.4)	22.5 (1.8)	37.1 (4.2)	142.7 (11.6)
PFC	0.27 (0.02)*	0.25 (0.014)*	4.6 (0.4)*	46.5 (3.9)*	17.5 (0.9)*	188.8 (9.0)*
W	0.42 (0.02)*,†	0.36 (0.014)*,†	10.5 (0.6)*,†	74.6 (2.3)*,†	24.5 (0.9)*	208.9 (5.5)*

* $P < 0.01$ vs ALC.
† $P < 0.01$ vs PFC.

tissue anoxia as a major contributing factor to the altered glucose metabolism in the wound. Several investigators have noted increased glucose uptake and lactate production in wounded tissue [9, 16, 19, 22, 25]. Since wounding has been associated with decreased local oxygen tension [8], and uptake of glucose and lactate production in muscle preparations are increased by anoxia [6, 17], it was thought that anoxia could be the driving force for the changes in glucose uptake and lactate production in wounded tissue [9, 23]. However, Turinsky has shown that incubation of wounded tissue in an anoxic atmosphere resulted in the same incremental increase in glucose uptake in injured and noninjured tissue [19]. In addition, an increase in wound fluid lactate concentration has been observed following an hypoxic challenge to dogs with implanted wound chambers [9]. These findings would be unexpected if anaerobic glycolysis were initially present in the wounded tissue.

In the present study lactate/pyruvate (L/P) ratios were the same in wounded and nonwounded tissues. In an attempt to stimulate glycolysis and possibly amplify any differences between the groups, the three groups of animals (*ad lib.* control, pair-fed control, and wounded) were subjected to a 30% hemorrhage over 2 min prior to hindlimb muscle harvest. The L/P ratios were maintained in the same relationship (despite an absolute increase) in the face of this hemorrhage. Similar L/P ratios under the above conditions suggest similar cytosolic redox potentials and, therefore, the increased lactate production should not necessarily be explained by a injury-specific decrease in the NAD/NADH ratio in wounded muscle.

Evaluation of tissue pyruvate concentrations in wounded and starved animals reveals markedly increased values over *ad lib.* control values. These differences were further accentuated following hemorrhage. Berger *et al.* during investigations of pyruvate dehydrogenase (PDH) activity in skeletal muscle following starvation, have shown a 75% decrease in glucose oxidation after 48 hr of starvation in the absence of alterations in glycolytic flux [1]. These observations were paralleled by changes in the activity of PDH [7]. In the present study examination of tissue pyruvate and lactate concentrations coupled with evidence of similar pyruvate oxidation in starving and wounded animals suggests comparable PDH activity.

Pyruvate dehydrogenase exists in two forms: an active dephosphorylated form (PDHa) and an inactive phosphorylated form (PDHb). Conversion of PDHa to PDHb (inactivation of PDH) is catalyzed by a protein kinase that requires Mg^{2+} and ATP and can be inhibited by pyruvate, ADP, and Ca^{2+}. Conversion of PDHb to PDHa (activation of PDH) is catalyzed by a phosphatase that requires Ca^{2+} and Mg^{2+} and can be inhibited by citrate and an increase in the acetyl CoA/CoA and NADH/NAD^+ ratios in the mitochondria [18]. Ruderman has suggested that acetyl CoA exercises product inhibition of PDH in starvation [18]. A comparable alteration in mitochondrial redox state to that found in the cytosol of wounded and pair-fed controls would help explain the similar PDH activity. However, explanation of the possible controlling factor or factors in inhibition of PDH activity in wounded tissue must await further study.

The cellular morphology of wounded tissue might help explain the metabolic changes seen. Wounding is associated with an increase in tissue DNA content which has been shown to reflect the increasing cellular infiltrate of polymononuclear leukocytes, monocytes, and macrophages [16]. In 1939, Kempner found the glycolytic rate of normal white blood cells to be high in comparison with the oxidative metabolism, and lactic acid to be formed aerobically [11]. Further evidence also suggested that aerobic glycolysis in these cells was so intense that it was often difficult to demonstrate a Pasteur effect [5]. Hunt *et al.* have attempted to quantitate pO_2 concentrations in wounds and have noted a decrease in local oxygen tensions [8]. This finding was further examined by Silver who postulated that the actively respiring cellular infiltrate may be a

major factor in the decreased pO_2 of wounded tissue [21]. Silver observed that if the cellular infiltrate was depleted by the administration of nitrogen mustards or anti-PMN serum, the tissue pO_2 more closely resembled that of normal tissue in the first hours after wounding. Studies that allow separation of the various components of healing wounds are necessary in order to better define the contribution of the cellular infiltrate to the altered metabolism following wounding.

In summary: (1) In wounded tissue glycolysis occurs *aerobically*. (2) Pyruvate dehydrogenase activity comparable to that found following starvation is seen in wounded tissue and contributes to the increased lactate production.

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