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chapter 8

THE METABOLIC SYSTEMS: ANAEROBIC METABOLISM (GLYCOLYTIC AND PHOSPHAGEN)

George A. Brooks and L. Bruce Gladden

IN this chapter, we address the history of the study of anaerobic metabolism during exercise. Our emphasis is on the early origins and progression of studies on phosphagen and glycolytic metabolism. Further, although our topic is "anaerobic" metabolism, we consider the interrelationship between oxygen and lactic acid production to be of primary importance. Other areas of this broad topic receive less attention; space limitations required us to focus on what we believe to be the more important events and their consequences. For information on studies and scientists before approximately 1900, we relied heavily on Fletcher and Hopkins (63), Keilin (99), Leicester (105), von Muralt (153), Rothschuh (141), Williams (164), and Zuntz (168).

THE PRELACTIC ACID ERA

Fermentation and the Pasteur Effect

Despite the fact that von Muralt (153) called the study of muscle metabolism before 1907 the "prelactic acid era," numerous important observations relating to glycolysis and lactic acid were made during this period. The notion that lactic acid is formed as the result of oxygen lack can be traced to alcohol fermentation technology of the

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eighteenth century. David Keilin (99, pp. 65–72) and Henry M. Leicester (105, pp. 177–179) describe scintillating controversies in the 1850s and 1860s among Louis Pasteur (1822–1895), Justus von Liebig (1803–1873), Claude Bernard (1813–1878), and Marcellin Berthelot (1827–1907) about the nature of fermentation. The key observations of Pasteur were that some microorganisms can live and proliferate in the absence of air and cannot use oxygen. In fact, O_2 poisons those organisms. He also found that some facultative cells are capable of living in both the presence and absence of oxygen. Moreover, Pasteur found that these facultative cells respire normally in the presence of oxygen and cause very little fermentation, but in anoxia they show very active fermentation (99, p. 68). Not only did Pasteur recognize the existence of aerobic and anaerobic organisms (130), but he also recognized that different types of the organisms which he called yeast formed very different products (129). For instance, one kind of organism fermented sugar to lactic acid whereas another organism produced alcohol.

Lactic Acid

In 1808, Jöns Jacob Berzelius (1779–1848), "almost the final authority on chemical matters of his day" (105), found an elevated concentration of lactate in "the muscles of hunted stags" (124). In 1845, Hermann von Helmholtz (1821–1894) reported findings that were consistent with lactic acid formation at the expense of glycogen (153). Shortly thereafter (1859), Emil H. Du Bois-Reymond (1818–1896) (63, 153) noted that activity caused muscles to become acidic and actually related this finding to the increased lactic acid reported by Berzelius. Soon after (1864), Rudolph P.H. Heidenhain (1834–1897) (153) reported that the amount of lactic acid increased with the amount of work done. In the next year, Ranke (1865) (63) indicated that resting muscle is alkaline but becomes acid as it survives. As emphasized by R. James Barnard (1937–) and John O. Holloszy (1933–) in Chapter 6, Nathan Zuntz (1847–1920) (168) in 1911 reviewed other significant papers of the 1800s. For example, Zuntz noted that Minot's paper in 1876 reported that the hindlimbs of dogs perfused with anoxic serum produced lactate during electrical stimulation.

Zuntz was a disciple of Eduard Friedrich Wilhelm Pflüger (1829–1910). Through the invention of the portable Zuntz-Geppert breathing machine, "Zuntz became involved in those physiological events which were associated with sports, hiking and high altitude ... He was a popular lecturer who could present in simple terms a series of rather complex biological phenomena, a gift which made him contribute to the dissemination of physiological knowledge to the persons interested in sports activities" (141).

Despite all of the evidence that seems obvious from our modern-day perspective, other studies including those of Astaschewsky (1880), Warren (1881), Blome (1891), Heffter (1893), and von Furth (1903) (63) found little or no increase in lactic acid over time in surviving muscle. The results of Astaschewsky and of Warren were probably explainable by the presence of an intact circulation that removed the lactic acid (63). Nevertheless, confusion persisted until the classic studies of Walter Mor-

ley Fletcher (1873–1910) and Frederick Gowland Hopkins (1861–1947) in 1907 (63). Fletcher was mentor to A.V. Hill and in 1929 Hopkins was awarded the Nobel Prize for "his discovery of growth-stimulating vitamins" (152).

These early studies of lactic acid should be viewed in the context of the theory of muscle contraction in the late 1800s. Ludimar Hermann (1838–1914) (63, 105) postulated that a hypothetical *inogen* molecule was an unstable precursor of both lactic acid and carbon dioxide. Presumably, oxygen was already combined into the inogen molecule and was ready to combine with carbon and hydrogen in an explosive breakdown of inogen. In recovery, fresh carbon bodies perhaps along with lactic acid would be combined again into a reoxygenated unstable inogen molecule. Pflüger supported this theory, referring to a giant molecule (inogen) which was unstable due to the fact that it contained what he called "intramolecular oxygen" (63).

Major advances were made by Fletcher and Hopkins in their classic studies of 1907 (63). Much of the uncertainty surrounding lactic acid formation in muscle at the beginning of the twentieth century was due to methodological problems mainly associated with muscle glycogenolysis prior to study. Fletcher and Hopkins (63) developed a method which prevented significant lactic acid formation in resting muscles before the extraction and analysis of the lactate. Accordingly, they were able to show that: (1) freshly excised resting muscle contains only a small amount of lactic acid, (2) lactic acid concentration increases in excised, resting, anaerobic muscles, (3) lactic acid accumulates to high levels during stimulation of muscles to fatigue, and (4) when fatigued muscles are placed in oxygen-rich environments, lactic acid disappears. Interestingly, Fletcher and Hopkins (63) were not certain that glycogen was the precursor of lactate at this time and they further assumed that muscles could form more lactate than could be provided by their glycogen stores. In 1910, Archibald Vivian (A.V.) Hill (1886–1977) (81) published data indicating that the immediate processes of muscle contraction did not require or involve the consumption of oxygen, the heat of contraction being the same in the presence or absence of O_2. However, extra heat was liberated in recovery, but only if O_2 was present.

Glycolysis

In 1912 Gustav Georg Embden (1874–1933) and associates (55) showed that yeast and working muscle produced the same intermediate which was believed to be a glyceraldehyde. Subsequently, in 1914 Embden and Laquer (56) found a phosphorus compound in muscle that caused production of lactic acid. They called this substance "lacticidogenen" and afterward identified it as a hexose monophosphate (57). As reviewed by Leicester (105, pp. 203–204) this "Embden Ester" turned out to be a mixture of hexose monophosphates that were subsequently identified: fructose-6-P (Neuberg Ester, 1918), glucose-6-P (Robison Ester, 1922), and eventually glucose 1-P (Cori Ester, 1936). In 1920, using frog muscle preparations (Fig. 8.1) Otto Fritz Meyerhof (1884–1951) (118–120) definitively identified glycogen as the precursor of lactic acid and also provided evidence strongly linking contraction to lactate formation and oxidative recovery to glycogen restoration.

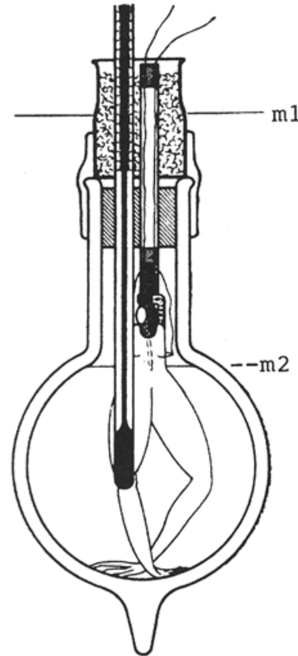

Abb. 2. Calorimeter mit Anordnung zur indirekten elektrischen Reizung eines Schenkelpaares. Der horizontale Strich entspricht dem Wasserstand des Thermostaten; der Strich im Gefäß dem Spiegel der Ringerlösung.

Fig. 8.1. Meyerhof's frog muscle calorimeter, 1920. Translated: "Figure 2. Calorimeter with electrode for indirect electrical stimulation of a hemicorpus. The first horizontal mark (m1, inserted) shows the level of the thermostatically controlled water bath. The mark in the inside of the vessel (--m2, added) shows the level of the Ringers solution." See (120).

Elaboration of the full Embden-Meyerhof (glycolytic) pathway took another two decades (121). However, in the early twentieth century, the essence of the phenomena described by Pasteur was confirmed on lactic-acid-producing cells and tissues from vertebrates; when yeast cells were incubated or muscles were made to contract without oxygen, lactic acid accumulated. And, even though 1931 Nobel laureate Otto Heinrich Warburg (1883–1970) (152) reported extensively on glycolysis leading to lactate accumulation in some types of well-oxygenated cells (e.g., cancer cells) (155), the phenomenon describing glucose–oxygen–lactic acid interactions came to be known as the "Pasteur effect" in textbooks of biochemistry (e.g., 104, p. 408). Retrospectively, because lactate removal is primarily by oxidation we now understand that in the absence of oxygen, cultured facultative cells and isolated frog muscles could only produce, but not remove, lactate and other glycolytic or fermentation products. *In this context the notion of a causative relationship between hypoxia and lactate accumulation evolved.*

Current Knowledge

Today we know that some types of facultative cells can be cultured with lactate as a preferred fuel because their mitochondria possess means to consume and oxidize lactate directly without conversion to pyruvate in the cytosol. For instance mitochondria of yeast (*Saccharomyces cerevisiae*) contain flavocytochrome b_2, a lactate–cytochrome c oxidoreductase (45) that couples lactate dehydrogenation to reduction of cytochrome c (46). In fact, the association between cytochrome b_2 and LDH in yeast can be traced to the 1940s (99, p. 274). A similar phenomenon occurs in mammalian muscle (31, 151). Contemporary studies have reported a balance of lactic acid production and oxidation in exercising men at sea level and 4,300 m altitude (136); such data were simply not obtainable with the technology of the 1920s. Similarly, the nuclear magnetic resonance (NMR) technology that allows us to know that contractions, independently of O_2 availability, stimulate glycolysis in muscle (41), and that lactate is oxidized in working skeletal (20) and cardiac (38) muscle, is a recent development.

THE O_2 DEBT HYPOTHESIS

Following the 1907 work of Fletcher and Hopkins (63), in 1913 Hill (78) provided data indicating that the heat of recovery following muscle contraction was greater when O_2 was present. Hill concluded that the "processes of muscle contraction are due to the liberation of lactic acid from some precursor" and that "the lactic acid precursor is rebuilt after the contraction is over in the presence of, and by use of oxygen with the evolution of heat" (78, p. 79).

As already noted, using frog muscle preparations in 1920, Meyerhof (120) definitively identified glycogen as the precursor of lactic acid. Further, Meyerhof observed that after contractile activity, when lactate disappeared, glycogen reappeared in a corresponding amount, less a quantity which, calculated from heat production and O_2 consumption, approximated the enthalpy of a fraction (one-fourth to one-third) of the lactate that disappeared. Independently of Meyerhof, in 1914 Hill (79) found that the recovery heats of isolated frog muscles could account for combustion of about one-sixth to one-fifth of the lactate removed in recovery. The fraction of frog muscle lactate oxidized in recovery to restore the remainder to glycogen came to be known as the "combustion coefficient." While the results of Meyerhof (Fig. 8.2), and Hill (Fig. 8.3) contained quantitative differences, their results and conclusions were broadly the same: a minor fraction (roughly one-fifth) of lactate in muscle after contractions is oxidized to provide energy for the reconversion of the majority (about four-fifths) to its precursor, glycogen.

In 1922, Meyerhof and Hill shared the Nobel Prize in physiology and medicine. Meyerhof was recognized "for his discovery of the fixed relationship between the consumption of oxygen and the metabolism of lactic acid in the muscle," whereas Hill was recognized "for his discovery relating to the production of heat in the muscle" (152). The awards were richly deserved, but the studies were on ex vivo prepa-

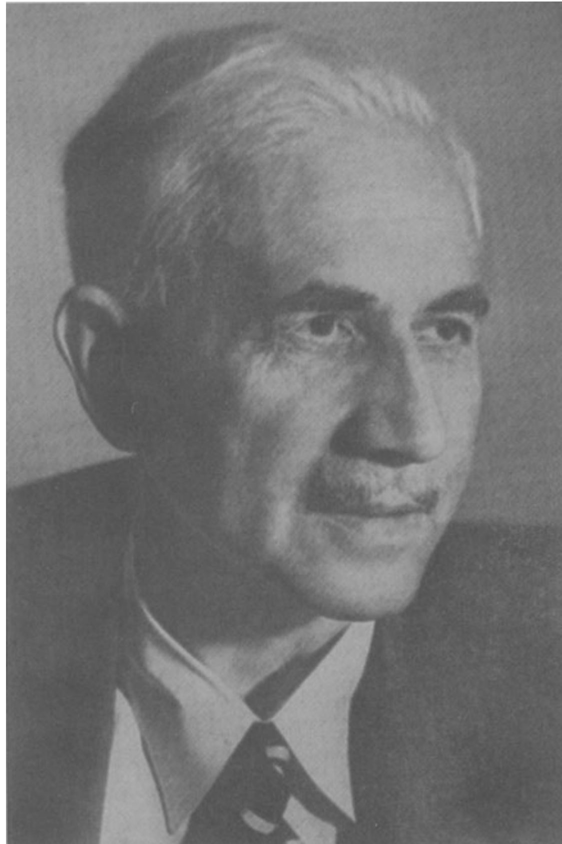

Fig. 8.2. Portrait of Otto Meyerhof, 1922. Source, Nobel Prize Archives.

rations. Linking the experimental results to human physiology was a challenge that Hill took up in the early 1920s although, as noted later, he and others were either unaware of, or ignored, the efforts of others in this area.

In 1920, Danes, Shack August Steenberg Krogh (1874–1949) and Jens Peter Johannes Lindhard (1870–1947), were the first to report the exponential decline in O_2 consumption in men after exercise (102). August Krogh was a 1920 Nobel Prize winner "for his discovery of the capillary motor regulating mechanism" (152). Following the report of Krogh and Lindhard, Hill and associates turned their attention to studies of humans in an attempt to unite the new knowledge of muscle biochemistry and human metabolism. In 1923 Hill and Lupton (86) articulated the O_2 debt hypothesis, and the following year Hill, Long, and Lupton (83–85) published a series of noteworthy reports. The "oxygen debt" was defined (86, p. 142) as the "total amount of oxygen used, after cessation of exercise in recovery therefrom." Recognizing that during exercise onset and maximal exercise conditions there was a "deficit" in oxygen consumption, Hill and associates sought to measure the "excess post-exercise O_2 consumption" (O_2 debt) (64) to obtain an energy equivalent of the anaerobic lactate-

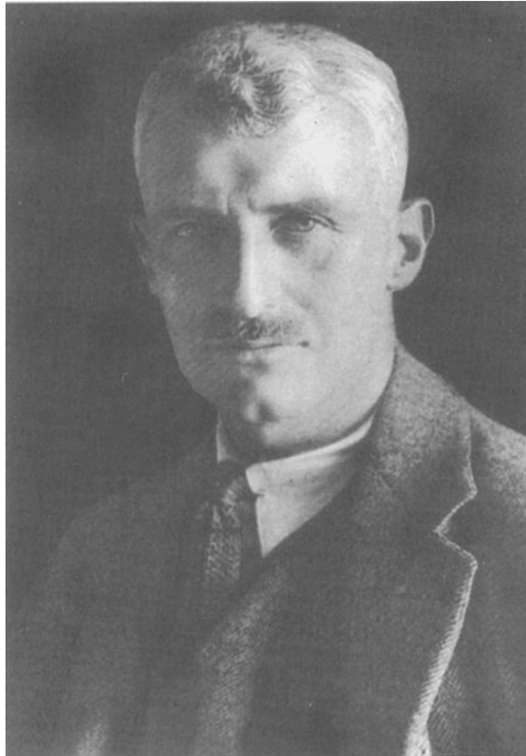

Fig. 8.3. Portrait of Sir Archibald Vivian (A.V.) Hill, 1922. Source, Nobel Prize Archives.

producing work done during exercise. Hill and associates attributed the first (fast) phase of decline in postexercise $\dot{V}O_2$ to oxidative removal of lactate in the muscles of formation and the second (slow) phase of decline in postexercise $\dot{V}O_2$ to oxidation of lactate that had escaped from muscles by diffusion.

In formulating their hypothesis, Hill and associates assumed that the energetics and metabolism in muscles of healthy humans were the same as in isolated frog muscles. With regard to the assumption of similar energetics, the supposition was appropriate as the laws of thermodynamics apply regardless of species differences. With regard to the phenomena of extra heat released from isolated muscles when they recovered in the presence, versus the absence, of O_2, and the extra O_2 consumed by persons recovering from exercise, it was logical for Hill and associates to link the two sets of phenomena together. That metabolic pathways, activities, and strategies differ significantly between mammals and lower vertebrates, particularly with regard to pathways of lactate disposal, is more recent knowledge (163).

As noted by Peter Harris in 1969 (73), given their experimental results and assumptions, it was natural for Hill and associates to conclude that in the human body, just as in isolated frog muscle, the "recovery volume of oxygen" (i.e., the O_2 debt) was caused by the oxidation of lactate. Further, because a "combustion coefficient" of

approximately one-fifth could be assumed, a stoichiometry between the extra O_2 consumed in recovery and the lactate produced, but not removed, during exercise could be established. Thus, by their definitions, measurement of the O_2 debt provided a means to quantitate the energy provided by anaerobic mechanisms during human exercise. Though simple in concept, assessment of anaerobic metabolism during exercise from postexercise O_2 consumption measurements proved difficult because of problems associated with establishing the postexercise baseline O_2 uptake (see ref. 64 for explanation). With the benefit of hindsight, investigators should have been suspicious of extrapolating results obtained on nonperfused, highly glycolytic, frog white muscles that are poorly circulated and seldom recruited in nature, to mammalian systems containing extensive capillary beds, elaborate mitochondrial reticula, and high metabolic rates, cardiac outputs, and muscle and liver perfusion rates. Nonetheless, until recently, O_2 debt theory dominated scientific thinking; the theory still dominates some clinical fields.

Partitioning the O_2 Debt

As described in more detail in the section The Phosphagen Buffer System, in 1930 Einar Lundsgaard (112) discovered "phosphagen" (later shown to be ATP + PCr) and demonstrated the role of that energy system in sustaining muscle contraction. Additionally, Lundsgaard established the terms "lactacid" and "alactacid" in describing nonoxidative energy systems in muscle. Working in the Harvard Fatigue Laboratory in 1933, Rodolfo Margaria (1901–1983), Harold T. Edwards (1897–1937), and David Bruce (D.B.) Dill (1891–1986) (114) adopted Lundsgaard's terminology and used it to reinterpret the biphasic curve describing whole-body $\dot{V}O_2$ during recovery from exercise. Using human subjects and running protocols of 3–10 minutes duration, Margaria et al. (114) observed that immediately after hard exercise, blood [lactate] remained elevated while $\dot{V}O_2$ fell rapidly during the first, fast O_2 debt period. Subsequently, blood [lactate] declined during the second, slow O_2 debt period. Therefore, Margaria et al. (114) concluded that the rapid O_2 debt phase was a result of the restoration of phosphagen in recovering muscle. This rapid O_2 debt phase was termed "alactacid"—that is, not having to do with lactic acid removal. The investigators also termed the second, slow O_2 debt phase that coincided with the decline in blood [lactate] the "lactacid" O_2 debt. Though consistent with Lundsgaard's' terminology, Margaria et al. had no data on muscle lactate or PCr concentrations or flux rates. Yet, from their analyses of the changes in O_2 consumption and blood lactate in recovery they knew that the classic Hill–Meyerhof O_2 debt theory had to be revised.

There are several reasons why the 1933 paper of Margaria et al. became one of the most influential papers in the history of exercise physiology. Over the course of decades, this seminal work on oxygen debt withstood numerous challenges and is still fundamental to ideas expressed by proponents of the "anaerobic threshold." Of the several reasons responsible for longevity of the O_2 debt concept in a scientific enterprise in which hypotheses are discarded daily, the distinction of the investigators

Fig. 8.4. Portrait of Rodolfo Margaria, 1960, age 59. Courtesy P. Cerretelli.

must be recognized as primary. August Krogh, who first described recovery O_2 consumption in humans, was awarded a Nobel Prize in 1920, and as already mentioned, A.V. Hill and Otto Meyerhof shared a Nobel Prize in 1922. In the United States, D.B. Dill (of Margaria et al.) replaced Lawrence Joseph Henderson (1878–1942) as director of the Harvard Fatigue Laboratory; eventually Dill served as president of the American Physiological Society. When the Fatigue Laboratory closed after World War II, those trained there took positions of eminence in physiology departments and laboratories around the United States. Similarly, as professor of physiology in Milan, Rodolfo Margaria (Fig. 8.4) ascended to a position of preeminence in Europe. World War II intervened; work in the field halted in favor of more applied efforts; and the O_2 debt model was ensconced in textbooks of physiology and biochemistry.

It is appropriate to note here that despite the many contributions of the "British School" physiologists led by A.V. Hill, in some ways this group also hindered understanding of muscle metabolism during exercise. While their focus on reconciliation of their studies of isolated amphibian muscle with their studies of humans during exercise was laudable, it may also have led them astray. As Barnard and Holloszy emphasized in Chapter 7, Zuntz in 1911 was convinced that glycogen is the precursor of lactic acid and further that both fat and carbohydrate are substrates for energy during exercise in humans. Following the experiments of Fletcher and Hopkins and

of A.V. Hill, as well as Hill's hypothesis that lactate mediates muscle contraction (thereby indicating that carbohydrate is the sole energy source for muscular contraction), in 1920 Krogh and Lindhard (103) reinvestigated Zuntz's findings. Their (103) results were largely confirmatory of Zuntz's view that fat could also serve as a fuel for exercise, findings that are now established (14, 15). Hill must have been aware of these studies since he cited other work of Krogh and Lindhard. Nevertheless, he and Lupton stated that "It would seem probable that carbohydrate alone provides the energy for the excess metabolism of exercise: this certainly appears to be the case in isolated muscle" (86). In 1939, Erik Hohwü Christensen (1904–1996) and Ove Hansen (1907–1990) (40) resurrected the idea that both fat and carbohydrate can be used as energy substrates for muscular exercise.

CHALLENGES TO O_2 DEBT THEORY

Ole Bang (1901–1988) was the first to question the fundamental assumptions and conclusions of O_2 debt theory (3, 4). His work benefited greatly from development of a micro-method for sampling and analysis of lactate content in arterialized capillary blood. This technical advance permitted repeated blood sampling during as well as after exercise. Through the study of exercises of varied intensities and durations, Bang showed that the results of Hill and Margaria and their colleagues were fortuitous consequences of the duration of their experiments. Using exercise bouts lasting only a few minutes, Bang found that the concentration of lactic acid in the blood reached a maximum after exercise had ended, and depending on the intensity of exercise, remained elevated long after the oxygen consumption had returned to preexercise levels. With prolonged exercise, Bang showed that the blood lactate level reaches a maximum after about 10 minutes of exercise and then declines whether exercise ceases or not. In some cases, basal lactate levels can be achieved during exercise itself. After exercise, however, there was always an "O_2 debt," with predictable kinetics to be "paid." The results could not be reconciled with the idea that lactic acid determines oxygen consumption after exercise (4).

In two letters to George A. Brooks (1944–) (September 10, 1972, and December 18, 1972) Bang offered opinions on why his work appeared to have been overlooked. Firstly, A.V. Hill did not acknowledge the work. Secondly, Bang's clinical commitments and the events of World War II served to deflect his efforts from exercise physiology. Likely important also was the fact that the German language journal selected for publication of results (*Skand. Arch. Physiol.*) ceased publication.

In addition to Bang's experiments on exercising humans, three notable studies on anesthetized dogs also cast doubt on the traditional O_2 debt concept. As early as 1927 Abramson et al. (1) tested the lactic acid/O_2 debt hypothesis by infusing lactate into anesthetized dogs. The change in $\dot{V}O_2$ was not different from that observed in their $NaHCO_3$ control. Those results were corroborated decades later (1954) by Norman R. Alpert (1922–) and Walter S. Root (2), who found no correlation between $\dot{V}O_2$ and the lactate dose given to anesthetized dogs. Alpert and Root in-

duced O_2 deficits in dogs by means of cardiac tamponade and having them breathe O_2-deficient gases. Magnitude of the subsequent O_2 debt was poorly correlated with the preceding O_2 deficit. As the result of those experiments and subsequent data obtained in 1964 on lactate removal in anesthetized dogs after electrically stimulated exercise, Herbert L. Kayne and Alpert (98, p. 1004) stated that

> the basic assumptions of the 'oxygen debt' hypothesis are that lactate, unknown anaerobic metabolites, or 'excess lactate' act as security for the debt and force repayment of the debt during recovery from the stress. The experiments described above indicate that oxygen missed [i.e., O_2 deficit] during stress does not relate to the recovery oxygen and the removal of lactate or 'excess lactate' which are [*sic*] infused at rest or produced during exercise are [*sic*] not causally related to recovery oxygen consumption [i.e., O_2 debt].

While space prevents a complete discussion, it should be noted that Meyerhof's results from amphibian muscle in vitro were later shown to differ from the results obtained on lower vertebrates in vivo (13, 69). Further, we now know that lower vertebrates differ from mammals in the mechanisms of clearance of an exercise-induced lactate load (163). The important insight is that the isolated frog muscle systems studied by Hill and Meyerhof did not directly apply to humans, mammals in general, or even lower vertebrates in vivo.

O_2 Availability, Lactate Production, and O_2 Debt

In 1923, David Barr and Harold Himwich (10) reported comparisons of arterial and venous blood drawn simultaneously from the arm 3 minutes following vigorous leg exercise. They (10) also took blood samples after vigorous exercise with the forearm. The conclusion was that less active tissues actually removed lactic acid from the blood. Very little was made of this remarkable finding for over 30 years. In 1955, Drury, Wick, and Morita (51) reported that a significant fraction of the respiratory CO_2 is derived from lactate in eviscerated and nephrectomized rabbits. Presumably, most of the metabolism in this preparation was due to skeletal muscle. In the very next year, Omachi and Lifson (126) studied $^{13}CO_2$ derivation from ^{13}C-labeled lactate in isolated, perfused dog gastrocnemius preparations at rest and during contractions. They found that lactate oxidation was greater when the muscles were contracting. While both of these studies in the 1950s suggested consumption of lactate by skeletal muscle, these results went largely unnoticed, perhaps because both preparations were far removed from normal physiological conditions.

It was left to Wendell N. Stainsby (1928–) (Fig. 8.5) to challenge the foundations of traditional theory regarding the relationship between oxygen and lactate production. Stainsby did his dissertation work on the effect of passive stretch on resting $\dot{V}O_2$ of the surgically isolated canine gastrocnemius (GP) in situ. Subsequently, over a period of 40 years (1957–1997), Stainsby and associates would use the canine GP preparation to study phenomena such as the "critical oxygen tension" in

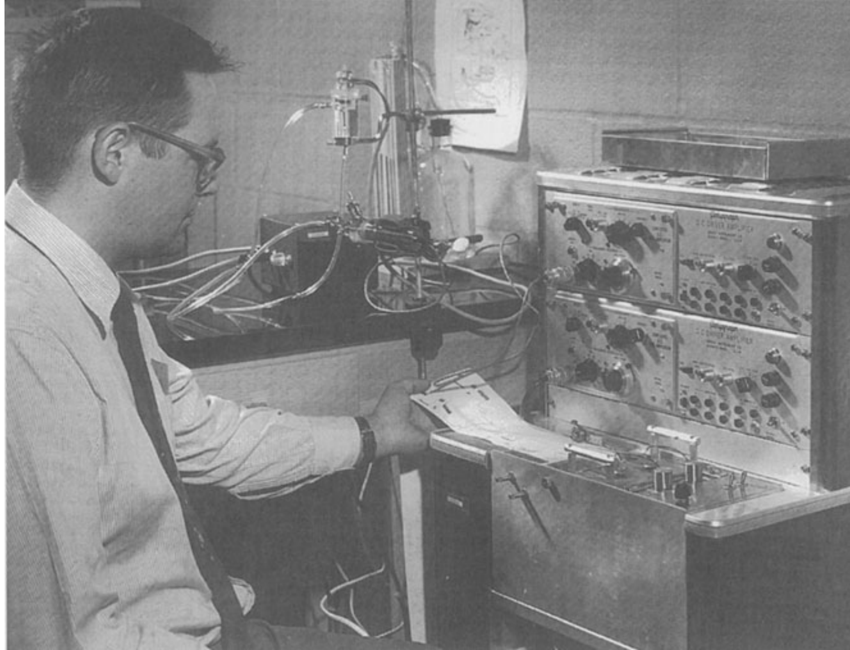

Fig. 8.5. Wendell Stainsby, University of Florida, Gainesville Laboratory, 1958. Courtesy of Wendell Stainsby.

muscle and the relationships among muscle O_2 delivery, lactate production and release, and O_2 debt.

In 1966, Stainsby and his doctoral student, Hugh G. Welch (1937–) reported the surprising result that net lactate output by the contracting GP was always transient. The net output peaked and declined within 15–20 minutes, even becoming net lactate uptake as the contractions continued at a constant stimulation rate (147). Subsequently, Welch's dissertation work (162) further established the transient nature of net lactate output by the contracting dog GP and additionally demonstrated that recovery O_2 uptake (O_2 debt) in the anesthetized dog GP was not related to lactate metabolism.

If oxygen deficiency was the cause of lactate production in contracting muscle, then why would net lactate output decline to near zero or even revert to net uptake with continued contractions? To answer this question, Stainsby collaborated with Frans Jöbsis (1929–) who with Britton Chance (1913–) had invented the surface fluorescence method for NAD/NADH detection. In 1968, Jöbsis and Stainsby (94) reported that NAD/NADH was highly reduced at rest and became highly oxidized quickly after the onset of contractions in the canine GP; NAD/NADH was becoming oxidized at a time when lactate production was known to be prevalent. Lactate production appeared *not* to be the result of muscle hypoxia. Although this study (94) has been criticized over the years, it became one of the key pieces of evidence against classic ideas of lactate production associated with O_2 insufficiency.

For years it has been known that the critical mitochondrial oxygen tension is very low, on the order of 1 mm Hg (e.g., ref. 142); see Gladden (67) for review. The critical oxygen tension is the partial pressure below which there is insufficient oxygen for mitochondria to achieve maximal rates of respiration. While the critical mitochondrial O_2 tension for isolated mitochondria has been known to be low, a major technical limitation has been the inability to measure the oxygen tension in working skeletal muscle as well as to understand the relationship between working muscle Po_2 and lactate formation. In 1984, Richard Connett (1943–), Thomas Gayeski (1946–), and Carl Honig (1925–1993) (42) observed that the intramuscular Po_2 remained above the critical O_2 tension in contracting canine muscles in situ. Their results suggested that lactate was produced in exercising muscle under fully aerobic conditions.

More recently (1998), Russell Richardson (1965–), Peter Wagner (1944–), and associates (137, 138) (Fig. 8.6) utilized progressive exercise protocols and the dual technologies of (1) NMR spectroscopy to measure myoglobin saturation and (2) classical (a−v) concentration measures to evaluate lactate balance in resting and exercising human quadriceps muscle. Their results for resting muscle indicated that the intracellular Po_2 is probably very close to that in the venous effluent from the muscle, approximately 40 torr, a value that is actually too high for accurate assessment by the NMR method. Nevertheless, these resting, well-perfused muscles released

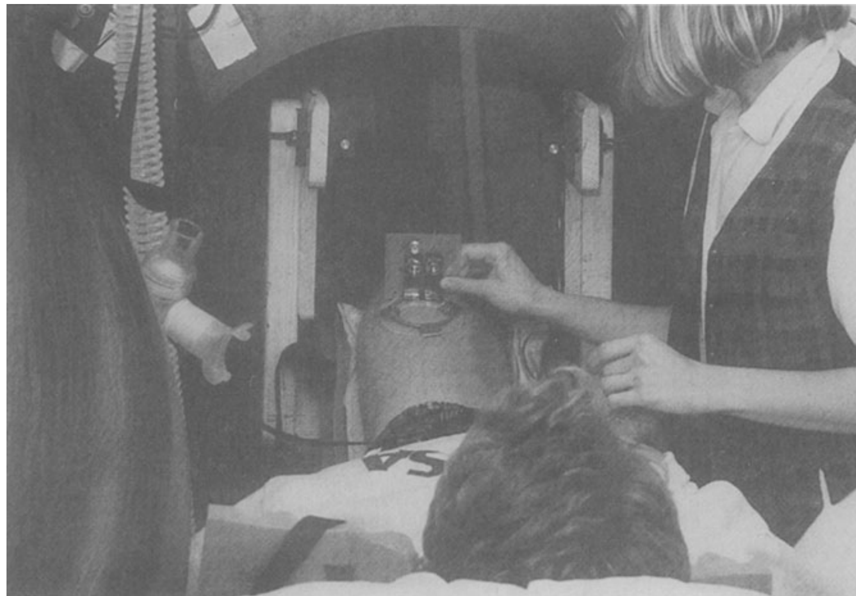

Fig. 8.6. Application of a surface coil to Russ Richardson (University of California, San Diego) by Liz Noyszewski for NMR studies of muscle oxygenation at the University of Pennsylvania. Note equipment for pulmonary gas-exchange measurements (left), ergometer support flanking coil, and magnet in background. Courtesy of R. Richardson and P.D. Wagner (see ref. 137).

lactate on a net basis (138). However, at exercise onset, Richardson et al. observed a dramatic decline in muscle PO_2 to approximately 4 torr, a value well within the range of detection but well above the critical mitochondrial PO_2 as determined in cellular and mitochondrial suspensions. Moreover, the intracellular PO_2 was maintained well above the critical mitochondrial PO_2 as power output increased to maximum.

In contrast to intramuscular PO_2 that fell rapidly at exercise onset and then remained constant as power output was progressively increased, Richardson et al. (138) reproduced the result that muscle lactate release changed little at low exercise intensities. However, at a power output corresponding to approximately 65% of $\dot{V}O_2$max, coinciding with a rise in arterial epinephrine, muscle lactate release began a steep rise, again an observation made previously. When studies were performed on subjects breathing hypoxic gas mixtures (11% O_2), intramuscular PO_2 changed little, but lactate net release accelerated. Net lactate release was accentuated when epinephrine began to rise under both normoxic and hypoxic conditions. Like results obtained previously on men using isotope tracers and working limb lactate balance (36), results obtained using NMR spectroscopy are consistent with the interpretation that lactate production is the consequence of glycolysis, but not necessarily O_2 lack.

In 1969 and 1970, R. James Barnard, Kenneth M. Baldwin (1942–), and Merle Foss (1936–), working in the University of Iowa laboratory of Charles M. Tipton (1927–), approached the O_2 debt hypothesis with different types of experiments. Barnard and coworkers (7–9) studied recovery oxygen kinetics in intact dogs after treadmill running. Postexercise $\dot{V}O_2$ was measured via a tracheotomy tube surgically implanted prior to studies. Further, they used pharmacological agents to block processes such as gluconeogenesis and the β-adrenergic functions. At the University of Michigan John A. Faulkner (1923–) and his graduate student George Brooks were impressed by several aspects of work in the Tipton laboratory, including the size of the postexercise O_2 consumption volumes. Brooks postulated that the tracheotomy procedure used in the dog studies of Barnard et al. affected the ability of the dogs to cool during exercise. Further, Brooks hypothesized that an elevated body temperature due to exercise might loosen the coupling of oxidative phosphorylation in mitochondria, thus increasing O_2 consumption during recovery and giving rise to at least part of the O_2 debt. In 1971 Brooks and associates (33) reported experimental results that largely confirmed his hypothesis; at high incubation temperatures similar to those found in working muscle the rate of mitochondrial O_2 consumption was raised and the efficiency of coupling was loosened. Further, in rats during recovery from exhausting exercise, the first, fast phase of whole-body O_2 consumption tracked muscle temperatures while the second, slow recovery O_2 phase tracked rectal temperature in recovery (34).

In combination, the work of several groups: (1) Stainsby and coworkers' dissociation of O_2 availability from muscle lactate production (94, 147, 162), (2) data on the effects of temperature on mitochondrial respiration (33), (3) the correlation between tissue temperatures and rate of O_2 consumption during recovery from exercise (34), and (4) the effects of β-blockade on recovery O_2 kinetics (8) led to the conclusion that exercise causes several perturbations in homeostasis (e.g., tissue tem-

peratures, hormones, and ions) that individually and collectively affect postexercise $\dot{V}O_2$ recovery volume (64). Hence, data acquired in the 1960s and 1970s led to loss of confidence that the postexercise recovery O_2 uptake could be used as a surrogate marker of the anaerobic metabolism which occurred during exercise. Measurement of anaerobic ATP provision by any means, invasive or noninvasive, remains a major challenge to muscle and exercise physiologists.

After the work of Meyerhof it was long assumed that reconversion to glycogen was the primary fate of lactate during recovery from physical exercise. Since it was believed that mammalian muscles lacked the necessary enzymes to reverse glycolysis, it was presumed that most of the reconversion of lactate to glycogen or glucose would occur in the liver as a part of the Cori cycle (101). In 1973, Brooks et al. (28) at the University of Wisconsin, ran rats to exhaustion, injected them with [U-^{14}C]lactate, placed them in a metabolism chamber, and collected $^{14}CO_2$ in recovery. The results were clear; most of the lactate was oxidized in recovery and injected lactate tracer yielded $^{14}CO_2$ almost like an injection of tracer bicarbonate. Further, parallel experiments indicated that lactate was cleared with little muscle glycogen restoration in recovery (28). Subsequently at Berkeley in 1978, Brooks and Ph.D. student Glenn A. Gaesser (1950–) combined the techniques of whole-animal calorimetry, two-dimensional paper chromatography, autoradiography, and ^{14}C isotope infusion to trace the pathways of lactate removal during recovery from exhausting exercise (32). Again, lactate disposal in recovering rats was largely by means of oxidation; glycogen restoration depended on carbohydrate feeding.

In 1977, Lars Hermansen (1933–1984) and Odd Vaage (1939–) (76) revived the idea that lactate could be resynthesized into muscle glycogen during recovery from intense exercise in humans. Their estimate that ≈70% of the lactate remaining in muscle after short-term, intense exercise was converted back to glycogen was most likely inflated by errors in their assessment of blood flow. In 1991, also studying human subjects, Jens Bangsbo (1957–) and colleagues (6) attempted to quantify the fate of lactate during recovery from short-term, high-intensity exercise performed by a single muscle group, the quadriceps muscles. While they calculated that 13%–27% of the muscle lactate present at the end of the exercise was reconverted to glycogen, this still left a majority of the lactate accounted for by oxidation. Although the percentage of lactate converted to glycogen might be higher in whole-body exercise with higher blood lactate concentrations (128), the results of these human studies are in general agreement with the radioactive tracer studies on the rat; the primary fate of lactate in recovery from intense exercise is oxidation.

Oxidation of lactate is enhanced by moderate exercise during recovery from intense exercise. As early as 1928, Otto Jervell (1893–1973) (93) observed that blood lactate concentration declined more rapidly in recovery when moderate exercise was performed instead of a passive resting recovery. This observation was made in two experiments on one subject. In 1937, Dill's group at the Harvard Fatigue Laboratory (125) extended Jervell's work, finding that the rate of blood lactate decline during recovery increased with the intensity of recovery exercise up to a critical level of activity. They (125) speculated that the increased rate of lactate decline during an ex-

ercising recovery might be due to (1) an increased blood flow with more rapid transport of lactate to removal centers and (2) an increased utilization of lactate as a fuel for the exercise.

THE PHOSPHAGEN BUFFER SYSTEM

In the late 1920s, Embden's group (58, 59) discovered muscle adenylic acid, but its significance was not immediately obvious (123, p. 14). In 1928, Karl Lohmann (1898–1978) found a substance in trichloroacetic acid extracts of muscle that could be hydrolyzed into inorganic phosphate (107, 108). Lohmann thought the substance might be inorganic pyrophosphate, but his experiments did not definitively support that point of view (123, p. 14). Lohmann (109, 110) and independently, Cyrus H. Fiske and Yellapragada SubbaRow (1895–1948) (62) solved the puzzle in 1929 when both groups deduced the structure of ATP in muscle. Meanwhile, parallel experiments were uncovering important information about another muscle phosphate compound. In 1927, Philip Eggleton (1903–1954) and Grace Palmer Eggleton (1901–1970) (54) observed this second labile phosphate compound in trichloroacetic acid extracts of muscle and called it phosphagen. Shortly afterward, it was again Fiske and SubbaRow (61, 62) who observed the same behavior, and subsequently identified the compound as phosphocreatine (PCr).

In the 1930s Einar Lundsgaard (1899–1968) (111,113) demonstrated that muscles were able to contract even though lactic acid production was prevented by iodoacetate poisoning. Instead, there was a decrease in a phosphorylated compound (later shown to be PCr) and an increase in inorganic phosphate. Schwartz and Oschmann (145) had made similar observations in 1924, but their results were apparently overlooked. On the basis of Lundsgaard's work, it was hypothesized by 1932 that the energy for muscle contraction was derived from the breakdown of phosphocreatine and that this phosphocreatine was resynthesized in recovery via a mixture of anaerobic (lactic acid formation) and aerobic metabolism. These new ideas concerning muscle energetics in 1932 were so profoundly different from the state of knowledge in 1926 that Hill (82) referred to the experiments over that period of time as "the revolution in muscle physiology."

The discoveries of ATP and PCr had weakened the prevailing theories concerning the mechanism and energetics of muscle contraction (22, 123). There were two phosphagens in muscle, ATP and PCr, with PCr being the more quantitatively important (22, 123: p. 17). However, this information alone provided no insight into which of the compounds might be the direct energy donor for muscle contraction (123, p. 17). Once again, Lohmann came to the forefront. He found that PCr was only dephosphorylated in muscle extracts when AMP or ATP was present (109). In the late 1930s and early 1940s, investigations by Englehardt and colleagues (60) which were extended by reports from Banga and Szent-Gyorgi (5) and Straub (150), illustrated that actomyosin contracts only with the addition of ATP. This research established the idea that direct energy donation for muscle contraction is

from ATP hydrolysis and that PCr serves as a buffer for ATP resynthesis (22; 123: p. 18).

According to Bessman and Geiger (22), Parnas in 1934 demonstrated that PCr and ADP could be synthesized from ATP and creatine; this meant that the Lohmann reaction was reversible. In 1941, Fritz Albert Lipmann (1899–1986) (99), who had worked in Meyerhof's laboratory from 1927 to 1931, surveyed the available information on the interactions of ATP, PCr, and energy metabolism in general and concluded that ATP stands as the central link between energy-using and energy-yielding reactions in cells. Lipmann (106) also coined the phrase "energy rich phosphate compounds" and originated the notation of "~P" to indicate readily convertible energy from such phosphate compounds. In 1953, Lipmann won a Nobel Prize "for his discovery of co-enzyme A and its importance for intermediary metabolism" (152). However, despite the abundance of circumstantial evidence, the role of ATP relative to PCr in muscle contraction was not yet finalized; A.V. Hill was not completely satisfied. Hill's 1950 "A Challenge to Biochemists" (80) stated the following:

> In the lactic acid era the evidence that the formation of lactic acid was the cause and provided the energy for contraction seemed pretty good. In the phosphagen era a similar attribution to phosphagen appeared even better justified. Now, in the adenosine triphosphate era lactic acid and phosphagen have been relegated to recovery and ATP takes their place. Those of us who have lived through two revolutions are wondering whether and when the third is coming.
>
> It may very well be the case, and none will be happier than I to be quit of revolutions, that the breakdown of ATP really is responsible for contraction or relaxation: but in fact there is no direct evidence that it is. Indeed, no change in the ATP has ever been found in living muscle except in extreme exhaustion, verging on rigor.

Ultimately, Hill's challenge was answered with finality by Dennis Francis Cain (1930–) and Robert E. Davies (1919–1993) (37) in 1962 when they provided the proof for ATP as the immediate energy donor for muscle contraction. They (37) inhibited creatine kinase in muscles with the poison 1,fluoro-2,4-dintobenzene (DFNB) and then immediately froze the muscles after a series of contractions. Under these conditions in which ATP resynthesis from PCr was prevented, a decline in ATP concentration was observed. And so it was that PCr came to be viewed as an energy buffer for the replenishment of ATP (22). To be complete, it should be noted that in 1943 Herman M. Kalckar (1908–1991) (97) discovered the enzyme adenylate kinase (called myokinase in muscle), which can resynthesize ATP from the combination of two ADP molecules; AMP is also a product. The myokinase reaction is thought to play a role in intense exercise, but that role has not yet been firmly established.

After a long period of work from the 1950s to the 1970s by numerous investigators, Samuel P. Bessman (1921–) formally proposed the existence of a phosphocreatine shuttle in 1972 (21). In this role, the PCr system serves as a spatial as well as temporal buffer of ATP. The original proposal of the shuttle suggested that as ATP from oxidative phosphorylation was transported out of the mitochondria, a mitochondrial-bound isoenzyme of creatine kinase (CK) immediately used that ATP

to synthesize PCr. This PCr would diffuse to the myofibrils, where a second creatine kinase isoenzyme bound to the M band would replenish ATP from ADP + PCr, thus providing ATP to the contractile apparatus. The resulting creatine would diffuse back to the mitochondria to complete the shuttle. At present, two different models of the CK–PCr system are debated. One of these, an amplification of the original proposal, asserts that CK isoenzymes are highly compartmentalized at sites of energy production and utilization (154, 166). The alternative model of Martin Kushmerick (1937–) and colleagues proposes that specific localization of CK is not necessary for muscle performance; rather the CK–PCr system establishes facilitated diffusion that maintains CK-catalyzed fluxes near equilibrium in the cytosol (116, 117, 159, 166).

Changes in phosphagen concentration in human muscle during exercise were first studied by Eric Hultman (1925–) and colleagues (Fig. 8.7) (70, 90). Their results showed that PCr concentration declined linearly with increases in exercise intensity while changes in ATP concentration were comparatively minor. In 1968, Pietro di Prampero (1940–) and Margaria (49) and Piiper, di Prampero, and Paolo Cerretelli (1932–) (132) reported similar observations for the isolated dog gastrocnemius in situ. These findings have been borne out by numerous investigations to the present day (48, 146). Several studies have also shown that PCr degradation is instantaneous

Fig. 8.7. Eric Hultman (left) conducts one of the first muscle biopsy studies on colleague Aasmund Roch Norlund, 1967. Courtesy of E. Hultman.

at the onset of exercise (91, 95). The winner of the 2002 Olympic Prize in Sport Sciences, Bengt Saltin (1935–), and colleagues (143) found elevations in human muscle lactate concentration after only 10 seconds of intense cycling exercise; this led them to suggest that glycolysis is activated at the onset of exercise also. Subsequent studies have supported this hypothesis (146) so now we realize that essentially all of the metabolic pathways of energy metabolism are turned on at the initiation of exercise.

GLYCOGEN

Almost 150 years ago, it was established that glycogen is the body's carbohydrate storage form. Perhaps the first scientist to report this finding was Claude Bernard in 1855 (19). As noted above, the relationship between a decrease in glycogen content and an increase in lactate concentration was then clarified by Meyerhof in the early 1920s. Also, as briefly reviewed in the earlier section, The Prelactic Acid Era, the steps in the glycolytic pathway were determined in the late 1930s largely by Embden and Meyerhof but with important contributions by Warburg in Germany and Carl Ferdinand Cori (1896–1984) and Gerty Theresa Cori (1896–1957) in the United States (104, pp. 317–318). A question that was being investigated in parallel was to what extent glycogen and the glycolytic pathway were employed during exercise in humans.

As early as the 1860s, studies of substrate metabolism during exercise were underway. Max von Pettenkofer (1818–1901) and Carl Voit (1831–1909) (131) measured urinary nitrogen output and proposed that protein was not an important source of fuel during exercise. Before the turn of the nineteenth century, Jean Baptiste Auguste Chaveau (1827–1917) in France (39) and Zuntz and Loeb in Germany (167) were measuring the respiratory exchange ratio (RER) in subjects during work. Zuntz studied mild work and found RER not to be very different from rest, supporting the idea that both fats and carbohydrates were being used. Chaveau, however, studied heavier work, obtained RER values near 1.0, and argued that carbohydrates are the sole source of energy for muscular exercise (39). The next important studies were those of Francis Gano Benedict (1870–1957) and Edward Provan Cathcart (1877–1954) in 1913 (12) at the Carnegie Institution of Washington. They found that RER increased with exercise intensity. Subsequently, in 1920 Krogh and Lindhard (102) published the results of very careful experiments on the use of fats and carbohydrates during exercise; their findings supported the notion that both substrates were used during rest and exercise. Recall that in 1920, the ruling theory of muscle contraction from the work of Hill and his contemporaries was that lactic acid formation, likely from a carbohydrate source, was the immediate source of energy. Accordingly, Krogh and Lindhard (102) assumed that if fat was used during exercise, it must be transformed into carbohydrate first. The next landmark study in the area of substrate metabolism and exercise was the report by Christensen and Hansen (40) in 1939. From measurements of respiratory gas exchange, they confirmed that both carbohydrates and fats are metabolized during exercise. Further, they showed that carbohydrate utilization decreases with increasing exercise duration in moderate ex-

ercise while carbohydrate usage increases with increasing exercise intensity. As noted above, these advances ran counter to the hypotheses of the "British School" of physiologists.

In a series of landmark studies that are summarized in a 1967 supplement to the *Scandinavian Journal of Clinical & Laboratory Investigation* (89, 90), Hultman, Jonas Bergström (1929–2001) and their colleagues reported the effects of various types of exercise and diets on muscle glycogen concentration. Since glycogen breakdown measurements in muscle biopsies provide no direct information on the actual fate of the glucosyl units, it was important that Hultman and colleagues were careful to determine carbohydrate oxidation by indirect calorimetry in order to verify the linkage between glycogen degradation and glucosyl oxidation. Though limited by the inability to distinguish between oxidation of glycogen and other carbohydrates or to identify the pathways of glycogen disposal, the studies of Hultman and colleagues confirmed the importance of muscle glycogen availability for exercise performance and established the theoretical basis for glycogen loading.

The pioneering studies of Hultman, Bergström, and coworkers, as well as numerous studies since, demonstrated clearly that glycogen is not only the most important energy source in contracting muscles but also that glycogen can be almost completely depleted during cycle exercise to fatigue at 60%–80% of $\dot{V}O_2$max. Performance and fatigue appear to be closely linked to muscle glycogen depletion in this range of exercise intensities (44).

Tracers and Lactate Flux during Rest and Exercise

In science, there is typically an interaction between the evolution of new ideas and technology; new ideas spawn technological advances that in turn yield information permitting additional ideas to be formulated. Such is the case with the application of isotope tracers to the study of metabolism. As reiterated by Hetenyi and associates (77) from Schoenheimer (144):

> the concept of the dynamic steady state has been one of the most far-reaching ideas in biomedical science in this century. According to this concept, all constituents of the body are continuously formed and utilized.... At the cellular level substances are taken up or formed in the cell as well as metabolized or released into extracellular fluid. Substances released into the extracellular fluid reach the bloodstream and are carried to all organs. At the same time they are excreted or taken up by other cells; therefore substances undergo turnover in the circulation.

Isotope tracers have application for the study of metabolic processes because the turnover of isotopically labeled metabolites can be measured. Most studies using tracers have measured glucose flux rates because of the obvious importance of maintaining glucose homeostasis in health and disease. The same basic methodology has also proven useful in measuring the effects of exercise and training on free fatty acid, glycerol, amino acid, and lactate flux rates (14–18, 29).

In dynamic steady states such as rest or sustained exercise, metabolites like lactic acid and glucose are continuously formed and enter the circulation. Because lactate is formed continuously, at greatly variable rates in anatomically distributed tissues (26), measurements of tissue lactate content provide only meager information about rates of production and removal. For this reason, when the technology became available, radioactive isotope tracers were applied to the study of lactate flux rates in animals. More recently, stable (nonradioactive) tracers have been used to study lactate metabolism in humans. All the results on humans and other mammals are consistent; lactate turnover is prominent at rest and scales exponentially to metabolic rate during exercise. Lactate is actively exchanged both within as well as between cells, organs, and tissues (25–27).

Pioneer work in the field of lactate kinetics in exercising mammals was that of Florent Depocas (1923–) and colleagues. In 1969 using continuous infusion of [U-^{14}C]lactate into dogs during rest and continuous steady-state exercise Depocas and coworkers (47) made several key, fundamental findings regarding lactate metabolism. These findings included: (1) there is active lactate turnover during the resting postabsorptive condition; (2) a large fraction, approximately half, of lactate formed during rest is removed through oxidation; (3) the turnover rate of lactate increases during exercise as compared to rest even if there is only a minor change in blood lactate concentration; (4) the fraction of lactate disposal through oxidation increases to approximately three-fourths during exercise; and (5) a minor fraction (one-tenth to one-fourth) of lactate removed is converted to glucose via the Cori cycle during exercise. Though the fractions are subject to species and experimental variations, the essential results have been reproduced in rats (50) (Fig. 8.8), dogs (92), horses (161), and humans (18, 115, 148).

A most relevant aspect of the issue of blood lactate accumulation during exercise concerns the role of net release from active muscle. Surprisingly, while net lactate release from resting muscle is common, net release from working muscle is usually transient if power output or stimulation rate is held constant. As shown first by Stainsby and Welch in 1966–1967 (147, 162) using dog muscle preparations contracting in situ, this "Stainsby Effect" (35) of transient muscle net lactate release at exercise onset followed by a switch to net uptake from the blood by working muscle has been confirmed in exercising humans (35, 36). Thus, it is certain that working skeletal muscle is not the sole source of blood lactate in humans during whole-body exercise. Epinephrine is more likely to signal glycolysis and lactate production in noncontracting tissues than working muscle; in working muscle epinephrine augments glycolysis leading to increased lactate accumulation (160). Additionally, recent studies show that epinephrine reduces net glucose and lactate uptake in working humans (160) and canine gastrocnemius (72) muscles in situ, respectively. Lactate uptake by exercising human skeletal muscle has been well documented, both with (18) and without tracers (35). L. Bruce Gladden's (1951–) studies on dog muscles contracting in situ (68) clearly show that lactate uptake is concentration (substrate), and not O_2 dependent, a finding that also appears to be the case in human muscle (18) (Fig. 8.9).

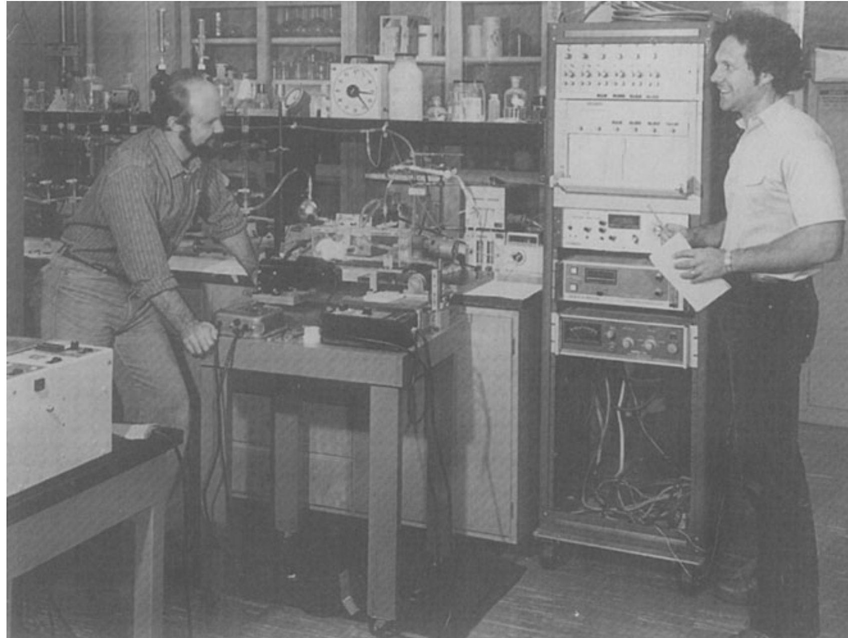

Fig. 8.8. Casey Donovan (1952–) (left) and George Brooks (right) and the Plexiglas metabolism chamber with mixing manifold suspended over hand-machined treadmill. At right are chart recorder and amplifiers for O_2 and CO_2 analyzers as well as vibrating reed electrometer in ionization chamber (for on-line detection of $^{14}CO_2$, left of metabolism chamber). Behind Donovan are the glass trapping columns to quantitate $^{14}CO_2$ excretion in expired air. The coiled wire above the equipment rack went to the Human Performance Laboratory where A-D interfaces to a digital computer served both animal and human experimentation (see refs. 30, 50).

Training

Space constraints do not permit adequate treatment of the effects of training on lactate turnover and its parameters (concentration, production, removal, clearance). In brief, it is well established that trained individuals maintain lower lactate levels during a given exercise task than do untrained, but highly trained athletes can achieve and sustain exercise at higher lactate concentrations than can the untrained. First measurements of lactate kinetics using radioactive tracers in rats (50) showed that training lowered circulating lactate levels by increasing clearance (Fig. 8.8). More recently, using a longitudinal training design, and a combination of stable isotope tracers, arterial–venous difference measurements, and muscle biopsies on humans, training has been found to have a small, but significant effect on blood lactate appearance, but a major effect on improving lactate clearance, especially during hard exercise (Fig. 8.9) (18). Training improves the capacity of gluconeogenesis in rats (30) and humans (17), and that effect helps exercising individuals maintain glucose homeostasis during prolonged exercise. However, the major effect of training is to improve lac-

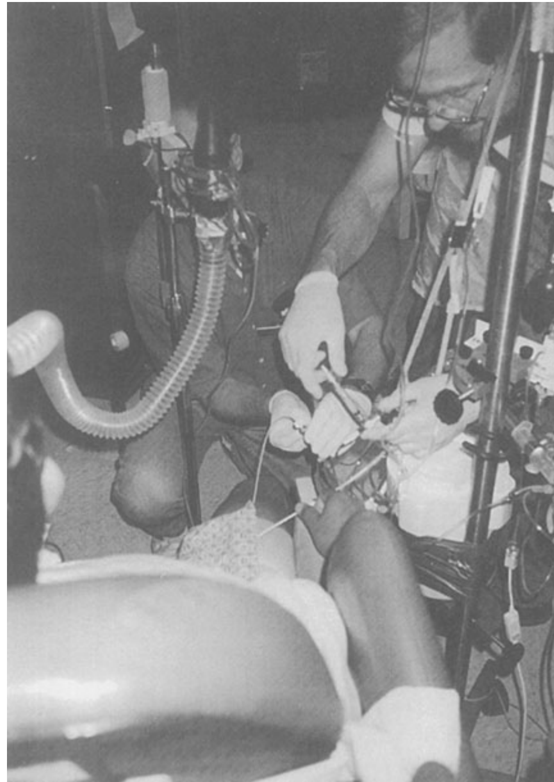

Fig. 8.9. Simultaneous femoral arterial and venous blood sampling during a resting phase of the 1996 lactate study in Gail Butterfield's Palo Alto laboratory. David Guido (left, obscured by the pneumotachometer) handles the venous side while Gene Wolfel takes arterial blood. Also visible on left are apparatus for thermal dilution measurement of limb blood flow and green dye measurement of cardiac output. A left arm vein was used for tracer glucose and lactate infusions into the seated subject (see refs. 15–18, 52).

tate oxidation within working muscles (17), an effect that may be partly attributable to the effects of training on increasing expression of lactate transporters in muscle sarcolemmal (52, 133) and mitochondrial (52) membranes. These adaptations facilitate operation of cell–cell and intracellular lactate shuttles (i.e., exchange of lactate between glycolytic and oxidative fibers, and the oxidative disposal of lactate within oxidative fibers, *vide infra*).

In summary, the use of isotopic tracers has either shown or confirmed that (1) lactate turnover is prominent at rest and scales exponentially to metabolic rate during exercise, (2) lactate is actively exchanged both within as well as between cells, organs and tissues, (3) lactate disposal through oxidation increases with exercise and is the dominant pathway, (4) only a minor fraction of lactate removed is converted to glucose via the Cori cycle during exercise, (5) net release from exercising muscle is usually transient at a constant power output, (6) contracting skeletal muscle is not

the sole source of blood lactate, and (7) training lowers tissue lactate levels during exercise mainly by increasing clearance.

The Lactate Shuttle and Lactate Transport

In 1985, George Brooks took a unique approach to explaining phenomena related to lactate responses to exercise when he articulated the "lactate shuttle hypothesis" (26). Key elements of the hypothesis were described as follows: "the shuttling of lactate through the interstitium and vasculature provides a significant carbon source for oxidation and gluconeogenesis during rest and exercise." As such, the lactate shuttle hypothesis represented a model of how the formation and distribution of lactate represents a central means by which the coordination of intermediary metabolism in diverse tissues and different cells within tissues can be accomplished. The initial hypothesis was developed from results of original isotope tracer studies conducted on laboratory rats in Brooks's own laboratory along with numerous other studies, many of which were cited in the previous section. Thus, the working hypothesis was developed that much of the glycolytic flux during exercise passed through lactate.

According to the cell–cell lactate shuttle hypothesis, lactate is a metabolic intermediate rather than an end product (25, 26). Lactate is continuously formed in and released from diverse tissues such as skeletal muscle, skin, and erythrocytes, but lactate also serves as an energy source in highly oxidative tissues such as the heart and is a gluconeogenic precursor for the liver. Lactate exchanges among these tissues appear to occur under various conditions ranging from postprandial to sustained exercise (25).

If lactate does serve as a key metabolic intermediate that shuttles into and out of tissues at high rates, particularly during exercise, then transmembrane movement is critical. For many years, lactate was assumed to move across membranes by simple diffusion. It is now known that cell–cell lactate exchanges among all tissues are facilitated by membrane-bound monocarboxylate transporters (MCTs) (66, 135, 139). Lactate and pyruvate (monocarboxylate, MCT) transport were first described by Andrew P. Halestrap (1949–) in red blood cells in 1974 (71). It was not until 1990 that the characteristics of membrane transport of lactate in skeletal muscle were first described by David A. Roth (1953–) and Brooks (139, 140). The study of cell membrane lactate transport proteins took a major leap in 1994 when, looking for the mevalonate (Mev) transporter gene in Chinese hamster ovary (CHO) cells, Christine Kim Garcia (1968–) and associates Michael S. Brown (1941–) and Joseph L. Goldstein (1940–) (66) cloned and sequenced a monocarboxylate transporter which they termed MCT1. In 1985, Goldstein and Brown shared the Nobel Prize "for their discoveries concerning the regulation of cholesterol metabolism" (152). Garcia et al. found that MCT1 was abundant in erythrocytes, heart, and basolateral intestinal epithelium. MCT1 was detectable only in oxidative muscle fiber types, and not in liver. With an interest to describe a role for MCT isoforms in the Cori cycle, Garcia

et al. (65) subsequently described isolation of a second isoform (MCT2) by screening of a Syrian hamster liver library; MCT2 was found in liver and testes.

Independently of Garcia et al. (65, 66), Halestrap and colleagues (71, 135) identified another MCT isoform (now known as MCT4) in 1998. In human skeletal muscle, two MCT isoforms (MCT1 and MCT4) with different kinetic properties have been described (135, 165). Recently (1999), the knowledge that lactate was formed and oxidized continuously within muscle and heart in vivo, led to extension of the cell–cell lactate shuttle to include an intracellular component, the "intracellular lactate shuttle," which is based on dual discoveries by Brooks and colleagues of the presence of lactate dehydrogenase (LDH) in liver, cardiac, and skeletal muscle mitochondria (31, 52), as well as a functional relationship between LDH and MCT1 in mitochondria (27), thus allowing mitochondria to directly oxidize lactate (31).

Understanding of the molecular biology and physiology of lactate transporters is in a state of rapid change, making it difficult to place the sequence of discovery in a clarifying perspective. However, this section would be incomplete without mentioning the contributions of Arend Bonen (1946–) and Carsten Jeul (1948–) to the field. Bonen is noted for his efforts to describe the effects of training (23) on expression of MCT isoforms. Additionally, Bonen and colleagues are pioneers in studying the role of transcription on expression of MCT isoforms (24). This work has relied heavily on use of laboratory animal models. Juel is distinguished for his development of the so called "giant sarcolemmal vesicles" technique, which permits lactate transport to be studied on small muscle samples, including human muscle biopsies (96). Further, Juel and associates have contributed to our understanding of the effects of training on MCT isoform expression in human skeletal muscle (134).

The Anaerobic Threshold

The concept of the "anaerobic threshold" is counter to the views of the authors of this chapter and to many of the views referenced here, but representative of an extensive body of scientific literature that has been adopted in clinical fields as diverse as cardiopulmonary assessment (156), treatment of septic shock (53), and field testing of athletes (88). Although the weight of evidence was provided by Karlman Wasserman (1927–) and associates (157, 158), very similar ideas were advanced independently by Wilfried Kindermann (1940–), Joseph Keul (1933–2000), and others (88, 100, 149) in Germany. Regardless of whether the anaerobic threshold concept is ultimately proven correct, partially correct, or incorrect, the originators of the concept have made notable contributions to the advancement of exercise physiology. Science is a self-correcting process that depends critically upon testable hypotheses. In this context, the anaerobic threshold has been one of the centerpieces of exercise physiology in the latter half of the twentieth century.

In the model of anaerobic threshold articulated by Wasserman and associates (156, 157), the assumption is that a series of metabolic and ventilatory events is precipitated by inadequate O_2 delivery. Wasserman and associates developed elaborate

technological and experimental protocols to identify the anaerobic threshold noninvasively; a key element was determination of the time at which respiratory compensation for metabolic acidosis (lactic acidosis) occurred. It was argued that the subsequent respiratory exchange alterations established the work rate and metabolic rate at which O_2 supply became inadequate; this was accomplished without the necessity of taking a blood sample. In parallel experiments, instead of relying on pulmonary gas-exchange determinations, Kindermann and associates took the more direct approach of using micromeasurements of capillary blood to assess the onset of lactic acidosis (100).

Proponents of anaerobic threshold theory are in far better position to argue their hypotheses than is possible here. Therefore, readers are referred to the text of Wasserman's Cannon Lecture given to the American Physiological Society in 1994 (156). Regardless of one's opinion concerning tissue O_2 lack as the precipitator of lactic acid production, Wasserman's review stands as an important descriptor of how metabolism and cardiopulmonary function are linked. One reason for the widespread acceptance of the anaerobic threshold concept may be the model developed by Wasserman and colleagues to illustrate the coupling of ventilation to the circulation and of both of those to muscle metabolism (letter from Wasserman to Brooks, 2/21/2000). The central tenet of anaerobic threshold theory had wide appeal to physiologists, especially against the backdrop of the well-known and established Pasteur effect. A PubMed search on "anaerobic threshold" in January 2002 retrieved 1911 documents published since 1973.

The basic notion begins with the observation that during a progressive incremental exercise test, there is a range of mild work rates at which there is only a minimal or no increase in blood (and presumably muscle) lactate concentration. With further increases in work rate, a "threshold" is reached beyond which there is an abrupt or rapid increase in blood lactate concentration as work increases further. This finding dates back to W. Harding Owles (127), who made the following conclusion, "*A critical metabolic level* {*italics* are ours} was found below which there was no increase in blood lactate as a result of the exercise, although above this level such an increase did occur." Interestingly, there were two subjects in Owles' study: one was Owles himself while the other was apparently his mentor, the famous physiologist Claude Gordon Douglas (1882–1963), inventor of the "Douglas bag," used to collect respiratory gases.

Within the anaerobic threshold concept, the abrupt rise in blood lactate concentration is attributed to the development of muscle hypoxia. In the words of Wasserman and colleagues (158), "if the number of muscle units which must contract to generate the required power exceeds the oxygen delivery and exhausts the O_2 stores, the oxygen level will drop to critical levels in each muscle unit and prevent the ATP, which is needed for muscle contraction, from being generated at an adequate rate by the respiratory enzymes in the mitochondria." In other words, as exercise intensity increases, O_2 delivery to muscle mitochondria is unable to keep pace, allowing P_{O_2} in the neighborhood of at least some mitochondria at least some of the time to decline below the critical level required for adequate oxidative phosphorylation. Further el-

evations in work rate increase the number and duration of hypoxic sites. With insufficient O_2 to accept electrons at the end of electron transport chains within mitochondria, there would be a "back-up" of reducing equivalents. Ultimately, the tricarboxylic acid (TCA) cycle would be inhibited and increased cytosolic concentrations of both pyruvate and NADH would result; pyruvate would become the preferred H^+ acceptor in this scenario and increased lactate production would occur.

Disregarding any polemic over the meaning of the ventilatory or blood lactate inflection points during graded exercise (87, 88, 100, 149, 156, 158), it is clear that valuable information has been obtained on the parameters which determine the kinetics of O_2 transport and delivery and the control of breathing. The predicament remains, however, that the fundamental assumption of O_2 lack in working muscle at the "anaerobic threshold" has never been shown conclusively (*vide supra*). Further, the assumption is in conflict with all existing data on the state of muscle oxygenation when net lactate release occurs (36, 41, 42, 122, 137). Nonetheless, arguments in favor of the anaerobic threshold have been sufficiently compelling to find widespread acceptance. For a possible reconciliation of the ideas of hypoxia versus adequate oxygenation regarding lactate production, see Gladden's chapter in the *Handbook of Physiology* (67).

In a letter of 2/21/2000 to G.A. Brooks, Wasserman (Fig. 8.10) described aspects of his career that motivated his investigations into noninvasive assessment techniques for pulmonary medicine. In 1959 Wasserman was a postdoctoral fellow under Julius H. Comroe (1911–1984) in the University of California, San Francisco (UCSF) Cardiology Research Institute. Recognizing the "epidemic" in cardiovascular disease, in November of 1960 Comroe challenged Wasserman to develop procedures for early detection of heart disease. Wasserman's response was that evaluation

> would be best done during exercise when the heart was being stressed.... The first sign of heart failure would be reflected in the failure of the circulation to deliver adequate O_2 to the metabolizing tissues (exercising muscles). Since the muscle O_2 requirement would be markedly increased by exercise, the failure of the heart to transport O_2 adequately would result in lactic acidosis (Pasteur Effect). (letter of 2/21/2000)

Aware of the earlier reports of Harrison and Pilcher (74, 75) who showed increased CO_2 production and reduced O_2 uptake in heart-failure patients, Wasserman thought "it possible to investigate how to detect the $\dot{V}O_2$ at which lactic acidosis developed during exercise, using non-invasive gas exchange techniques." His experience at UCSF led to an appointment at Stanford, where Wasserman co-authored a paper with Malcolm B. McIlroy (1921–) that appeared in 1964 in which "anaerobic threshold" was defined as the "$\dot{V}O_2$ above which lactate systematically increased in response to increase in work rate" (157). It is intriguing to note that Wasserman discussed his research with D.B. Dill prior to coining of the term "anaerobic threshold" in Wasserman and McIlroy's 1964 paper. Wasserman showed Dill his data and the method of gas exchange he was using to "detect the work rate at which O_2 transport was inadequate to prevent an exercise lactic acidosis." Wasserman states, "As close[ly] as I can remember, his [Dill's] comment was that I was detecting the *thresh-*

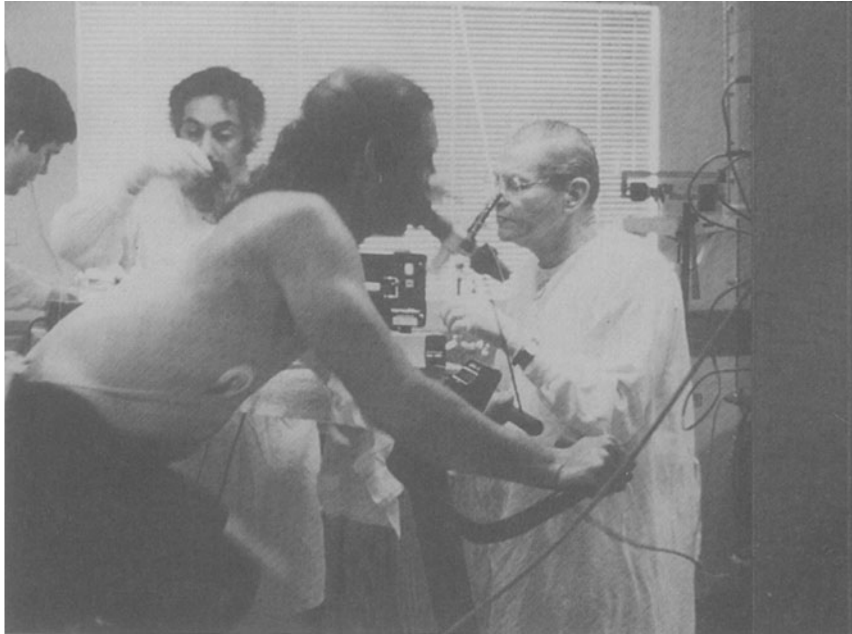

Fig. 8.10. Almost high noon during a study in Karl Wasserman's laboratory, Harbor General Hospital, 1992. Left to right: William Stringer, Richard Casaburi, subject, and Karl Wasserman. Investigators employed rapid arterial blood sampling to relate changes of pH, blood gases, and lactate to respiratory gas-exchange changes during exercise transitions. Courtesy K. Wasserman (see ref. 156)

old of anaerobic metabolism [*italics* are ours] during exercise by measuring exercise gas exchange. Thus, he put into words succinctly what I was trying to measure" (letter of 2/21/2000). In 1973, Wasserman, his colleague Brian J. Whipp (1937–), and other coworkers refined the concept in their classic paper, which generated tremendous interest in the topic (158).

For the subsequent span of over three decades Wasserman and colleagues vigorously pursued the development of technologies to identify the presence of lactic acidosis in graded clinical exercise protocols. For instance, early emphasis on using the RER to identify acidosis was abandoned in favor of using $\dot{V}E/\dot{V}O_2$ because the RQ of glycogen oxidation is unity and the increased reliance on glycogen during progressive exercise forced the RER to increase even without lactic acidosis (158). Subsequently, because some normal subjects and patients lacked the chemosensitivity necessary to respond to the CO_2 released from buffering of lactic acid, the $\dot{V}E/\dot{V}O_2$ method of threshold detection was reevaluated. Also, he found that obese patients and those with chronic obstructive pulmonary disease had limitations to pulmonary mechanics that limited their ability to develop ventilatory compensation to lactic acidosis. Therefore, Beaver, Wasserman, and Whipp developed the V-slope method to detect the "anaerobic threshold" (11). The V-slope method remains in common use today.

In a long and impressively productive career that included development of on-line computerized technologies for cardiopulmonary assessment and studies of ventilatory control, Wasserman remains best known for his work on using noninvasive means for detection of "anaerobic threshold." Clearly the work has led to methods for detection of lactic acidosis in pulmonary and cardiovascular medicine, but whether muscle hypoxia is identified remains an open question (43). As stated in his recent letter, Wasserman assumed that a Pasteur effect caused lactic acidosis during exercise both in patients with cardiovascular and cardiopulmonary limitations and in normal subjects. Again, justification for Wasserman's assumption of a Pasteur effect giving rise to lactic acidosis is found in his Cannon Lecture (156), a paper which is recommended reading for its description of physiological control in response to metabolic perturbations.

SUMMARY

By way of a summary of the history of anaerobic systems during exercise, we offer the following time line of key ideas, events, and eras:

1808–1907: *The prelactic acid era*. Lactic acid is discovered and related to physical activity and hypoxia. The prevailing theory of muscle contraction is that a giant molecule, inogen, contains bound oxygen, which combines with carbon and hydrogen in an explosive breakdown. In the late 1800s and early 1900s, Nathan Zuntz measures the respiratory exchange ratio in exercising humans and provides evidence that both fats and carbohydrates are substrates for energy during exercise.

1907–1926: *The lactic acid era*. This era was begun by the classical paper of Walter M. Fletcher and F. Gowland Hopkins detailing the formation of lactic acid in amphibian muscle. Archibald Vivian (A.V.) Hill and the "British School" of physiologists believed that the "processes of muscle contraction are due to the liberation of lactic acid from some precursor." Otto Meyerhof further detailed the relationship between glycogen and lactic acid formation. Hill developed the O_2 debt concept in human studies in the 1920s. August Krogh and Johannes Lindhard provided evidence supporting Zuntz's assertion that both fat and carbohydrate are substrates for energy during exercise in humans, but these studies were outside the prevailing theory of the "British School."

1926–1932: *The revolution in muscle physiology*. Hill refers to experiments on the "phosphagens" between 1926 and 1932 as "the revolution in muscle physiology." Gustav Embden's group discovers muscle adenylic acid. Independently, Karl Lohmann and Cyrus H. Fiske and Yellapragada SubbaRow deduce the structure of ATP. The Eggletons discover phosphagen, which is subsequently identified as PCr by Fiske and

SubbaRow. Einar Lundsgaard demonstrates muscle contraction with a decrease in PCr while glycolysis is inhibited.

1933: Rodolfo Margaria, Harold T. Edwards, and David Bruce Dill of the Harvard Fatigue Laboratory apply the new knowledge of the phosphagens to O_2 debt theory in humans.

1941: Fritz Lipmann postulates that ATP stands as the central link between energy-using and energy-yielding reactions in cells.

1962: D.F. Cain and R.E. Davies provide proof that ATP is the immediate energy donor for muscle contraction. Also in this year, Jonas Bergström reintroduces the needle biopsy technique. Over the next 5 years, Bergström and Eric Hultman and colleagues confirm the importance of muscle glycogen availability for human exercise performance and establish the theoretical basis for glycogen loading. Hultman and colleagues also use the needle biopsy technique to discern the behavior of muscle PCr and ATP during exercise.

1964: Karlman Wasserman and Malcolm B. McIlroy coin the term "anaerobic threshold."

1966–1968: Wendell Stainsby demonstrates the transient nature of muscle lactic acid output in canine muscle in situ and presents evidence that argues for the O_2 independence of lactic acid formation during muscle contractions. Also using canine muscle in situ, Margaria, Piiper, di Prampero, and Cerretelli confirm the relationship between muscle PCr concentration and contraction intensity.

1969: Florent Depocas and colleagues use radioactive tracers to study lactate turnover and oxidation in resting and exercising dogs. Fundamental discoveries of Depocas et al. showing active lactate turnover in resting and exercising individuals have been replicated in numerous species, including humans.

1972: Samuel Bessman proposes the phosphocreatine shuttle.

1973: Brooks and associates give ^{14}C-lactate to rats after exhausting exercise and find, contrary to classic O_2 debt theory, little incorporation into glycogen but major disposal as $^{14}CO_2$.

1974: Andrew Halestrap describes monocarboxylate transport in red blood cells.

1977: Lars Hermansen and Odd Vaage revive the idea of lactate resynthesis into glycogen during recovery from intense exercise in humans.

1980: Glenn Gaesser and George Brooks use ^{14}C-tracers, indirect calorimetry and two-dimensional chromatography to trace the paths of lactate and glucose disposal during recovery from exhausting exercise. Again, they find little incorporation of lactate-derived carbon into glycogen but major disposal as $^{14}CO_2$. In mammals, oxidation, not reconversion to glycogen, is the major fate of lactate after exercise.

1984: Richard Connett, Tom Gayeski and Carl Honig observe lactate production in canine muscle in situ when intramuscular PO_2 is apparently above the critical value for mitochondrial oxidative phosphorylation.

1985: George Brooks proposes the lactate shuttle.

1990: David Roth and George Brooks describe the characteristics of sarcolemmal lactic acid transport.

1991: Using human subjects, Jens Bangsbo and colleagues attempt to quantify the fate of lactate during recovery from short-term, high-intensity exercise performed by a single muscle group. Their results support radioactive tracer studies on rats, indicating that the primary fate of lactate in recovery from intense exercise is oxidation.

1994: Christine Kim Garcia, Michael S. Brown, Joseph L. Goldstein, and colleagues sequence and clone the gene encoding for a muscle cell membrane monocarboxylate transport protein (MCT). Subsequently, they identify a second isoform, MCT2, found mainly in liver.

1998: George Brooks proposes the intracellular lactate shuttle.

1998: Russ Richardson, Peter Wagner, and colleagues use magnetic resonance spectroscopy (MRS) to show lactate production and net release from fully aerobic, working human skeletal muscle.

1998: Andrew Halestrap and colleagues clone and sequence four new MCT isoforms and describe tissue variability in MCT isoform expression.

1999: Henriette Pilegaard (1962–), Andrew Halestrap, and Carsten Juel show MCT1 and MCT4 distribution in human skeletal muscle.

1999 & 2000: Brooks, Marcy Brown, Hervé Dubouchaud, and colleagues show LDH and MCT1 in muscle mitochondria of rats and humans.

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References

1. Abramson H.A., M.G. Eggleton, and P. Eggleton. The utilization of intravenous sodium r lactate. III. Glycogen synthesis by the liver. Blood sugar. Oxygen consumption. *J. Biol. Chem.* 75:763–778, 1927.
2. Alpert N.R. and W.S. Root. Relationship between excess respiratory metabolism and utilization of intravenously infused sodium racemic lactate and sodium L-(+)-lactate. *Am. J. Physiol.* 177:455–462, 1954.
3. Bang O. Blutmilchsaure und Sauerstoffaufnahme wahrend und nach Muskelarbeit beim Menschen. *Arbeitsphysiologie* 7:544–554, 1934.

4. Bang O. The lactate content of the blood during and after muscular exercise in man. *Skand. Arch. Physiol.* 74:46–82, 1936.
5. Banga I. and A. Szent-Gyorgyi. Preparation and properties of myosin A and B. *Stud. Inst. Med. Chem. Univ. Szeged* I:5–16, 1941–1942.
6. Bangsbo J., P.D. Gollnick, T.E. Graham, and B. Saltin. Substrates for muscle glycogen synthesis in recovery from intense exercise in man. *J. Physiol.* (*Lond.*) 434:423–440, 1991.
7. Barnard R.J. and K.M. Baldwin. The effect of training and various work loads on the lactacid-alactacid oxygen debt response of exercising dogs. *Int. Z. Angew. Physiol.* 28: 120–130, 1970.
8. Barnard R.J. and M.L. Foss. Oxygen debt: effect of beta-adrenergic blockade on the lactacid and alactacid components. *J. Appl. Physiol.* 27:813–816, 1969.
9. Barnard R.J., M.L. Foss, and C.M. Tipton. Oxygen debt: involvement of the Cori cycle. *Int. Z. Angew. Physiol.* 28:105–119, 1970.
10. Barr D.P. and H.E. Himwich. Studies in the physiology of muscular exercise. II. Comparison of arterial and venous blood following vigorous exercise. *J. Biol. Chem.* 55: 525–537, 1923.
11. Beaver W.L., K. Wasserman, and B.J. Whipp. A new method for detecting anaerobic threshold by gas exchange. *J. Appl. Physiol.* 60:2020–2027, 1986.
12. Benedict F.G. and E.P. Cathcart. *Muscular Work; A Metabolic Study with Special Reference to the Efficiency of the Human Body as a Machine*. Washington, DC: Carnegie Institution of Washington, 1913.
13. Bennett A.F. and P. Licht. Relative contributions of anaerobic and aerobic energy production during activity in amphibia. *J. Comp. Physiol.* 87:351–360, 1973.
14. Bergman B.C. and G.A. Brooks. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J. Appl. Physiol.* 86:479–487, 1999.
15. Bergman B.C., G.E. Butterfield, E.E. Wolfel, G.A. Casazza, G.D. Lopaschuk, and G.A. Brooks. Evaluation of exercise and training on muscle lipid metabolism. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39):E106–E117, 1999.
16. Bergman B.C., G.E. Butterfield, E.E. Wolfel, G.D. Lopaschuk, G.A. Casazza, M.A. Horning, and G.A. Brooks. Muscle net glucose uptake and glucose kinetics after endurance training in men. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40):E81–E92, 1999.
17. Bergman B.C., M.A. Horning, G.A. Casazza, E.E. Wolfel, G.E. Butterfield, and G.A. Brooks. Endurance training increases gluconeogenesis during rest and exercise in men. *Am. J. Physiol.* 278 (*Endocrinol. Metab.* 2):E244–E251, 2000.
18. Bergman B.C. E.E. Wolfel, G.E. Butterfield, G.D. Lopaschuk, G.A. Casazza, M.A. Horning, and G.A. Brooks. Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol.* 87:1684–1696, 1999.
19. Bernard C.M. Sur le mechanisme de la formation du sucre dans le foie. *C.R. Acad. Sci.* (*Paris*) 41:461–469, 1855.
20. Bertocci L.A. and B.F. Lujan. Incorporation and utilization of [3-^{13}C]lactate and [1,2-^{13}C]acetate by rat skeletal muscle. *J. Appl. Physiol.* 86:2077–2089, 1999.
21. Bessman S.P. Hexokinase acceptor theory of insulin action. New evidence. *Isr. J. Med. Sci.* 8:344–352, 1972.
22. Bessman S.P. and P.J. Geiger. Transport of energy in muscle: the phosphorylcreatine shuttle. *Science* 211:448–452, 1981.
23. Bonen A., K.J. McCullagh, C.T. Putman, E. Hultman, N.L. Jones, and G.J. Heigenhauser. Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37):E102–E107, 1998.
24. Bonen A., M. Tonouchi, D. Miskovic, C. Heddle, J.J. Heikkila, and A.P. Halestrap. Isoform-specific regulation of the lactate transporters MCT1 and MCT4 by contractile activity. *Am. J. Physiol.* 279 (*Endocrinol. Metab.* 5):E1131–E1138, 2000.

25. Brooks G.A. Current concepts in lactate exchange. *Med. Sci. Sports. Exerc.* 23:895–906, 1991.
26. Brooks G.A. Lactate glycolytic end product and oxidative substrate during sustained exercise in mammals—the lactate shuttle. In: *Proceedings in Life Sciences. Circulation, Respiration, and Metabolism: Current Comparative Approaches; First International Congress of Comparative Physiology and Biochemistry, Liege, Belgium, Aug. 1984,* edited by R. Gilles. Secaucus, New York: Springer-Verlag, pp. 208–218.
27. Brooks G.A. Mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 120:89–107, 1998.
28. Brooks G.A., K.E. Brauner, and R.G. Cassens. Glycogen synthesis and metabolism of lactic acid after exercise. *Am. J. Physiol.* 224:1162–1166, 1973.
29. Brooks G.A., G.E. Butterfield, R.R. Wolfe, B.M. Groves, R.S. Mazzeo, J.R. Sutton, E.E. Wolfel, and J.T. Reeves. Increased dependence on blood glucose after acclimatization to 4,300 m. *J. Appl. Physiol.* 70:919–927, 1991.
30. Brooks G.A. and C.M. Donovan. Effect of endurance training on glucose kinetics during exercise. *Am. J. Physiol.* 244 (*Endocrinol. Metab.* 7):E505–E512, 1983.
31. Brooks G.A., H. Dubouchaud, M. Brown, J.P. Sicurello, and C.E. Butz. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 96:1129–1134, 1999.
32. Brooks G.A. and G.A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. *J. Appl. Physiol.* 49:1057–1069, 1980.
33. Brooks G.A., K.J. Hittelman, J.A. Faulkner, and R.E. Beyer. Temperature, skeletal muscle mitochondrial functions, and oxygen debt. *Am. J. Physiol.* 220:1053–1059, 1971.
34. Brooks G.A., K.J. Hittelman, J.A. Faulkner, and R.E. Beyer. Tissue temperatures and whole-animal oxygen consumption after exercise. *Am. J. Physiol.* 221:427–431, 1971.
35. Brooks G.A., E.E. Wolfel, G.E. Butterfield, A. Cymerman, A.C. Roberts, R.S. Mazzeo, and J.T. Reeves. Poor relationship between arterial [lactate] and leg net release during exercise at 4,300 m altitude. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 4):R1192–R1201, 1998.
36. Brooks G.A., E.E. Wolfel, B.M. Groves, P.R. Bender, G.E. Butterfield, A. Cymerman, R.S. Mazzeo, J.R. Sutton, R.R. Wolfe, and J.T. Reeves. Muscle accounts for glucose disposal but not blood lactate appearance during exercise after acclimatizatioin to 4,300 m. *J. Appl. Physiol.* 72:2435–2445, 1992.
37. Cain D.F. and R.E. Davies. Breakdown of adenosine triphosphate during a single contraction of working muscle. *Biochem. Biophys. Res. Commun.* 8:361–366, 1962.
38. Chatham J.C., C. Des Rosiers, and J.R. Forder. Evidence of separate pathways for lactate uptake and release by the perfused rat heart. *Am. J. Physiol.* (*Endocrinol. Metab.*) 281: E794–E802, 2001.
39. Chauveau A. Source et nature du potentiel directement utilise dans le travail musculaire, d'apres les echanges respiratoires, chez l'homme en etat d'abstinence.. *C.R. Acad. Sci.* (*Paris*) 122:1163–1169, 1896.
40. Christensen E.H. and O. Hansen. I. Zur Mehodik der respiratorischen Quotient-Bestimmungen in Ruhe und Arbeit. II. Untersuchungen über die Verbrennungs-Vorgange bei langdauernder, schwere Muskelarbeit. III. Arbeitsfahigkeit und Ernahrung. *Skand. Arch. Physiol.* 81:137–171, 1939.
41. Conley K.E., M.J. Kushmerick, and S.A. Jubrias. Glycolysis is independent of oxygenation state in stimulated human skeletal muscle in vivo. *J. Physiol.* (*Lond.*) 511:935–945, 1998.
42. Connett R.J., T.E. Gayeski, and C.R. Honig. Lactate accumulation in fully aerobic, working, dog gracilis muscle. *Am. J. Physiol.* 246 (*Heart Circ. Physiol.* 15):H120–H128, 1984.

43. Connett R.J., C.R. Honig, T.E. Gayeski, and G.A. Brooks. Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J. Appl. Physiol.* 68:833–842, 1990.
44. Connett R.J. and K. Sahlin. Control of glycolysis and glycogen metabolism. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*, edited by Loring B. Rowell and John T. Shepherd, American Physiological Society. New York: Oxford University Press, 1996, pp. 870–911.
45. Daff S., W.J. Ingledew, G.A. Reid, and S.K. Chapman. New insights into the catalytic cycle of flavocytochrome b2. *Biochemistry* 35:6351–6357, 1996.
46. Daum G., P.C. Bohni, and G. Schatz. Import protein into mitochodria. Cytochrome b2 and cytochrome c peroxidase are located in the intermembrane space of yeast mitochondria. *J. Biol. Chem.* 252:13028–13033, 1982.
47. Depocas F., Y. Minaire, and J. Chatonnet. Rates of formation and oxidation of lactic acid in dogs at rest and during moderate exercise. *Can. J. Physiol. Pharmacol.* 47:603–610, 1969.
48. di Prampero P.E. Energetics of muscular exercise. *Rev. Physiol. Biochem. Pharmacol.* 89: 143–222, 1981.
49. di Prampero P.E. and R. Margaria. Relationship between O_2 consumption, high energy phosphates and the kinetics of the O_2 debt in exercise. *Pflugers Arch* 304:11–19, 1968.
50. Donovan C.M. and G.A. Brooks. Endurance training affects lactate clearance, not lactate production. *Am. J. Physiol.* 244 (*Endocrinol. Metab.* 7):E83–E92, 1983.
51. Drury D.R., A.N. Wick, and T.N. Morita. Metabolism of lactic acid in extrahepatic tissues. *Am. J. Physiol.* 180:345–349, 1955.
52. Dubouchaud H., G.E. Butterfield, E.E. Wolfel, B.C. Bergman, and G.A. Brooks. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 278:E571–E579, 2000.
53. Duke T. Dysoxia and lactate. *Arch. Dis. Child.* 81:343–350, 1999.
54. Eggleton P. and G.P. Eggleton. XXV. The inorganic phosphate and a labile form of organic phosphate in the gastrocnemius of the frog. *Biochem. J.* 21:190–195, 1927.
55. Embden G., K. Baldes, and E. Schmitz. Über den Chemismus der Milchsaurebildung aus Traubenzucker im Teirkorper. *Biochem. Zeit.* 45:108–133, 1912.
56. Embden G. and F. Laquer. Über die Chemi des Lactocidogens. I. Isolierungsversuche. *Hoppe-Seyler's Z. Physiol.* 93:94–123, 1914.
57. Embden G. and F. Laquer. Über die Chemie des Lactacidogens. II. *Hoppe-Seyler's Z. Physiol.* 98:181, 1917.
58. Embden G. and G. Schmidt. Über Muskeladenylsaure und Hefeadenylsaure. *Hoppe-Seyler's Z. Physiol.* 181:130–139, 1929.
59. Embden G. and M. Zimmermann. Über die Bedeutung der Adenylsaure fur Muskelfunktion. I. Mitteilung: Das Vorkommen von Adenylsaure in der Skelettmuskulatur. *Hoppe-Seyler's Z. Physiol.* 167:137, 1927.
60. Englehardt W.A. and M.N. Ljubimowa. Myosine and adenosinetriphosphatase. *Nature* 144:668–669, 1939.
61. Fiske C.H. and Y. SubbaRow. The nature of the "inorganic phosphate" in voluntary muscle. *Science* 65:401–403, 1927.
62. Fiske C.H. and Y. SubbaRow. Phosphorus compounds of muscle and liver. I. Muscle. *Science* 70:381–382, 1929.
63. Fletcher W.M. and F.G. Hopkins. Lactic acid in amphibian muscle. *J. Physiol.* (*Lond.*) 35:247–309, 1907.
64. Gaesser G.A. and G.A. Brooks. Metabolic bases of excess post-exercise oxygen consumption: a review. *Med. Sci. Sports Exerc.* 16:29–43, 1984.

65. Garcia C.K., M.S. Brown, R.K. Pathak, and J.L. Goldstein. cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. *J. Biol. Chem.* 270:1843–1849, 1995.
66. Garcia C.K., J.L. Goldstein, R.K. Pathak, R.G. Anderson, and M.S. Brown. Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 76:865–873, 1994.
67. Gladden L.B. Lactate transport and exchange. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*, edited by L.B. Rowell and J.T. Shepherd, American Physiological Society. New York: Oxford University Press, 1996, pp. 614–648.
68. Gladden L.B., R.E. Crawford, and M.J. Webster. Effect of lactate concentration and metabolic rate on net lactate uptake by canine skeletal muscle. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35):R1095–R1101, 1994.
69. Gleeson T.T. Metabolic recovery from exhaustive activity by a large lizard. *J. Appl. Physiol.* 48:689–694, 1980.
70. Gollnick P.D., and L. Hermansen. Biochemical adaptations to exercise: anaerobic metabolism. *Exerc. Sport. Sci. Rev.* 1:1–43, 1973.
71. Halestrap A.P. and R.M. Denton. Specific inhibition of pyruvate transport in rat liver mitochondria and human erythrocytes by alpha-cyano-4-hydroxycinnamate. *Biochem. J.* 138:313–316, 1974.
72. Hamann J.J., K.M. Kelley, and L.B. Gladden. Effect of epinephrine on net lactate uptake by contracting skeletal muscle. *J. Appl. Physiol.* 91:2635–2641, 2001.
73. Harris, P. Lactic acid and the phlogiston debt. *Card. Res.* 3:381–390, 1969.
74. Harrison T.R. and C. Pilcher. Studies in congestive heart failure. I. The effect of edema on oxygen utilization. *J. Clin. Invest.* 8:259–290, 1929.
75. Harrison T.R. and C. Pilcher. Studies in congestive heart failure. II. The respiratory exchange during and after exercise. *J. Clin. Invest.* 8:291–315, 1929.
76. Hermansen L. and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am. J. Physiol.* 233 (*Endocrinol. Metab.* 2):E422–E429, 1977.
77. Hetenyi G. Jr., G. Perez, and M. Vranic. Turnover and precursor-product relationships of nonlipid metabolites. *Physiol. Rev.* 63:606–667, 1983.
78. Hill A. The energy degraded in recovery processes of stimulated muscles. *J. Physiol. (Lond.)* 46:28–80, 1913.
79. Hill A. The oxidative removal of lactic acid. *J. Physiol. (Lond.)* 48:x–xi, 1914.
80. Hill A.V. A challenge to biochemists. *Biochim. Biophys. Acta* 4:4–11, 1950.
81. Hill A.V. The heat produced in contracture and muscle tone. *J. Physiol. (Lond.)* 40: 389–403, 1910.
82. Hill A.V. The revolution in muscle physiology. *Physiol. Rev.* 12:56–67, 1932.
83. Hill A.V., C.N.H. Long, and H. Lupton. Muscular exercise, lactic acid and the supply and utilisation of oxygen. Pt I–III. *Proc. R. Soc. B* 96:438–475, 1924.
84. Hill A.V., C.N.H. Long, and H. Lupton. Muscular exercise, lactic acid and the supply and utilisation of oxygen. Pt IV–VI. *Proc. R. Soc. B* 97:84–138, 1924.
85. Hill A.V., C.N.H. Long, and H. Lupton. Muscular exercise, lactic acid and the supply and utilisation of oxygen. Pt VII–VIII. *Proc. R. Soc. B* 97:155–176, 1924.
86. Hill A.V. and H. Lupton. Muscular exercise, lactic acid and the supply and utilization of oxygen. *Q. J. Med.* 16:135–171, 1923.
87. Hofmann P., V. Bunc, H. Leitner, R. Pokan, and G. Gaisl. Heart rate threshold related to lactate turn point and steady-state exercise on a cycle ergometer. *Eur. J. Appl. Physiol.* 69:132–139, 1994.
88. Hollmann W. Historical remarks on the development of the aerobic-anaerobic threshold up to 1966. *Int. J. Sports Med.* 6:109–116, 1985.

89. Hultman E. Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. *Scand. J. Clin. Lab. Invest. Suppl.* 94:1–63, 1967.
90. Hultman E., J. Bergström, and N.M. Anderson. Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with muscular work in man. *Scand. J. Clin. Lab. Invest.* 19:56–66, 1967.
91. Hultman E. and H. Sjoholm. Energy metabolism and contraction force of human skeletal muscle in situ during electrical stimulation. *J. Physiol. (Lond.)* 345:525–532, 1983.
92. Issekutz B. Jr. Effect of beta-adrenergic bockade on lactate turnover in exercising dogs. *J. Appl. Physiol.* 57:1754–1759, 1984.
93. Jervell O. Investigation of the concentration of lactic acid in blood and urine under physiologic and pathologic conditions. *Acta Med. Scand.* 24:1–135, 1928.
94. Jöbsis F.F. and W.N. Stainsby. Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir. Physiol.* 4:292–300, 1968.
95. Jones N.L., N. McCartney, T. Graham, L.L. Spriet, J.M. Kowalchuk, G.J. Heigenhauser, and J.R. Sutton. Muscle performance and metabolism in maximal isokinetic cycling at slow and fast speeds. *J. Appl. Physiol.* 59:132–136, 1985.
96. Juel C. and H. Pilegaard. Lactate/H+ transport kinetics in rat skeletal muscle related to fibre type and changes in transport capacity. *Pflugers Arch. Eur. J. Physiol.* 436:560–564, 1998.
97. Kalckar H. The role of myokinase in transphosphorylations. II. The enzymatic action of myokinase on adenine nucleotides. *J. Biol. Chem.* 148:127–137, 1943.
98. Kayne H.L. and N.R. Alpert. Oxygen consumption following exercise in the anesthetized dog. *Am. J. Physiol.* 206:51–56, 1964.
99. Keilin D. *The History of Cell Respiration and Cytochrome.* Cambridge: Cambridge U. Press, 1966.
100. Kindermann W., G. Simon, and J. Keul. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur. J. Appl. Physiol. Occup. Physiol.* 42:25–34, 1979.
101. Krebs H.A. and M. Woodford. Fructose 1,6-diphosphatase in striated muscle. *Biochem. J.* 94:436–445, 1965.
102. Krogh A. and J. Lindhard. The changes in respiration at the transition from work to rest. *J. Physiol. (Lond.)* 53:431–437, 1920.
103. Krogh A. and J. Lindhard. The relative value of fat and carbohydrate as sources of muscular energy. *Biochem. J.* 14:290–363, 1920.
104. Lehninger A.L. *Biochemistry; the Molecular Basis of Cell Structure and Function.* New York: Worth Publishers, 1970.
105. Leicester H.M. *Development of Biochemical Concepts from Ancient to Modern Times.* Cambridge: Harvard University Press, 1974, p. 286.
106. Lipmann F. Metabolic generation and utilization of phosphate bond energy. *Adv. Enzymol.* 1:99–162, 1941.
107. Lohmann K. Über das Vorkommen und den Umsatz von Pyrophosphat in Zellen. II. Mitteilung: Das Menge der leicht hydrolysierbaren P-Verbindung in tierischen und pflanzlichen Zellen. *Biochem. Zeit.* 203:164–171, 1928.
108. Lohmann K. Über das Vorkommen und den Umsatz von Pyrophosphat in Zellen. III. Mitteilung: Das physiologische Verhalten des Pyrophosphats. *Biochem. Zeit.* 203: 172–207, 1928.
109. Lohmann K. Über die enzymatische Augspaltung der Kreatinphosphorsaure: zugleich ein Beitrag zum Chemismus der Muskelkontraktion. *Biochem. Zeit.* 271:264–277, 1934.
110. Lohmann K. Über die Pyrophosphatfraktion im Muskel. Naturwissenschaften 17:624–625, 1929.

111. Lundsgaard E. Betydningen af faenomenet maelkesyrefrie muskelkontraktioner fer opfattelsen af muskelkontraktiones kemi. *Danske. Hosp.* 75:84–95, 1932.
112. Lundsgaard E. Untersuchungen über Muskel–Kontraktion ohne Milschsaurebildung. *Biochem. Zeit.* 217:162–177, 1930.
113. Lundsgaard E. Weitere Untersuchungen über Muskelkontraktionen ohne Milchsaurebildung. *Biochem. Zeit.* 227:51–83, 1930.
114. Margaria R., R.H.T. Edwards, and D.B. Dill. The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am. J. Physiol.* 106:689–715, 1933.
115. Mazzeo R.S., G.A. Brooks, D.A. Schoeller, and T.F. Budinger. Disposal of blood [1-^{13}C]-lactate in humans during rest and exercise. *J. Appl. Physiol.* 60:232–241, 1986.
116. McFarland E.W., M.J. Kushmerick, and T.S. Moerland. Activity of creatine kinase in a contracting mammalian muscle of uniform fiber type. *Biophys. J.* 67:1912–1924, 1994.
117. Meyer R.A., T.R. Brown, B.L. Krilowicz, and M.J. Kushmerick. Phosphagen and intracellular pH changes during contraction of creatine-depleted rat muscle. *Am. J. Physiol.* 250 (*Cell. Physiol.* 19):C264–C274, 1986.
118. Meyerhof O. Die Energieumwandlungen im Muskel I. Über die Beziehungen der Milchsaure zur Warmebildung and Arbeitsleistung des Muskels in der Anaerobiose. *Pflugers Arch. Physiol. Mensch. Tiere.* 182:232–283, 1920.
119. Meyerhof O. Die Energieumwandlungen im Muskel II. Das Schicksal der Milchsaure in der Erholungsperiode des Muskels. *Pflugers. Arch.. Physiol. Mensch. Tiere.* 182:284–317, 1920.
120. Meyerhof O. Die Energieumwandlungen im Muskel III. Kohlenhydrat und Milchsaureumsatz im Froschmuskel. *Pflugers Arch. Physiol. Mensch. Tiere.* 185:11–32, 1920.
121. Meyerhof O., University of Chicago, and University of Wisconsin. *A Symposium on Respiratory Enzymes.* Madison: The University of Wisconsin Press, 1942.
122. Molé P.A., Y. Chung, T.K. Tran, N. Sailasuta, R. Hurd, and T. Jue. Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46):R173–R180, 1999.
123. Mommaerts W.F.H.M. *Muscular Contraction; a Topic in Molecular Physiology.* New York: Interscience Publishers, 1950.
124. Needham J. and R. Hill. *The Chemistry of Life; Eight Lectures on the History of Biochemistry.* Cambridge, England: University Press, 1970.
125. Newman E.V., D.B. Dill, H.T. Edwards, and F.A. Webster. The rate of lactic acid removal in exercise. *Am. J. Physiol.* 118:457–462, 1937.
126. Omachi A. and N. Lifson. Metabolism of isotopic lactate by the isolated perfused dog gastrocnnemius. *Am. J. Physiol.* 185:35–40, 1956.
127. Owles W.H. Alterations in the lactic acid content of the blood as a result of light exercise, and associated changes in the CO_2-combining power of the blood and in the alveolar CO_2 pressure. *J. Physiol.* (*Lond.*) 69:214–237, 1930.
128. Pascoe D.D. and L.B. Gladden. Muscle glycogen resynthesis after short term, high intensity exercise and resistance exercise. *Sports Med.* 21:98–118, 1996.
129. Pasteur L. Mémorie sur la fermentation appelée lactique. *Ann. Chim. Phys.* 52:404–418, 1858.
130. Pasteur L. Recherches sur putrefaction. *C. R. Acad. Sci.* (*Paris*) 56:1189–1194, 1863.
131. Pettenkofer M.v. and C. Voit. Untersuchungen über den Stoffverbrauch des normalen Menschen. *Zeit. Biol.* 2:459–573, 1866.
132. Piiper J., P.E. di Prampero, and P. Cerretelli. Oxygen debt and high-energy phosphates in gastrocnemius muscle of the dog. *Am. J. Physiol.* 215:523–531, 1968.
133. Pilegaard H., J. Bangsbo, E.A. Richter, and C. Juel. Lactate transport studied in sarcolemmal giant vesicles from human muscle biopsies: relation to training status. *J. Appl. Physiol.* 77:1858–1862, 1994.

134. Pilegaard H., G. Terzis, A. Halestrap, and C. Jeul. Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am. J. Physiol.* 276 (*Endo-crinol. Metab.* 39):E843–E848, 1999.
135. Price N.T., V.N. Jackson, and A.P. Halestrap. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* 329:321–328, 1998.
136. Reeves J.T., E.E. Wolfel, H.J. Green, R.S. Mazzeo, A.J. Young, J.R. Sutton, and G.A. Brooks. Oxygen transport during exercise at altitude and the lactate paradox: lessons from Operation Everest II and Pikes Peak. *Exerc. Sport. Sci. Rev.* 20:275–296, 1992.
137. Richardson R.S., E.A. Noyszewski, K.F. Kendrick. J.S. Leigh, and P.D. Wagner. Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. *J. Clin. Invest.* 96:1916–1926, 1995.
138. Richardson R.S., E.A. Noyszewski, J.S. Leigh, and P.D. Wagner. Lactate efflux from exercising human skeletal muscle: role of intracellular PO_2. *J. Appl. Physiol.* 85:627–634, 1998.
139. Roth D.A. and G.A. Brooks. Lactate and pyruvate transport is dominated by a pH gradient- sensitive carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279:386–394, 1990.
140. Roth D.A. and G.A. Brooks. Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279:377–385, 1990.
141. Rothschuh K.E. *History of Physiology.* Huntington, NY: R.E. Krieger, 1973.
142. Rumsey W.L., C. Schlosser, E.M. Nuutinen, M. Robiolio, and D.F. Wilson. Cellular energetics and the oxygen dependence of respiration in cardiac myocytes isolated from adult rat. *J. Biol. Chem.* 265:15392–15402, 1990.
143. Saltin B., P.D. Gollnick, B.O. Eriksson, and K. Piehl. Metabolic and circulatory adjustments at onset of maximal work. In: *Onset of Exercise*, edited by A. Gilbert and P. Guille. Toulouse: University of Toulouse, 1971, pp. 63–76.
144. Schoenheimer R. *The Dynamic State of Body Constituents.* New York: Hafner, 1964.
145. Schwartz A. and A. Oschmann. Contribution au probleme du Mecanisme des contractures musculaires. Le taux de l'acide phosphorique musculair libre dans les contractures des animaux emposonnes par l'acide monobromecetique. *Social Biology* (*Paris*) 91:275–277, 1924.
146. Spriet L.L. Anaerobic metabolism during high-intensity exercise. In: *Exercise Metabolism*, edited by M. Hargreaves. Champaign, IL: Human Kinetics, 1995.
147. Stainsby W.N. and H.G. Welch. Lactate metabolism of contracting dog skeletal muscle in situ. *Am. J. Physiol.* 211:177–183, 1966.
148. Stanley W.C., E.W. Gertz, J.A. Wisneski, D.L. Morris, R.A. Neese, and G.A. Brooks. Systemic lactate kinetics during graded exercise in man. *Am. J. Physiol.* 249 (*Endocrinol. Metab.* 12):E595–E602, 1985.
149. Stegmann H., W. Kindermann, and A. Schnabel. Lactate kinetics and individual anaerobic threshold. *Int. J. Sports Med.* 2:160–165, 1981.
150. Straub F. B. Actin. *Studies from the Institute of Medical Chemistry, University Szeged.* II:3–16, 1942.
151. Szczesna-Kaczmarek A. L-lactate oxidation by skeletal muscle mitochondria. *Int. J. Biochem.* 22:617–620, 1990.
152. The Nobel Prize. The First 100 Years, edited by Agneta Wallin Levinovitz and Nils Ringertz. Singapore: World Scientific, London: Imperial College Press, 2001.
153. von Muralt A. The development of muscle chemistry, a lesson in neurophysiology. *Biochim. Biophys. Acta* 4:126–129, 1950.
154. Wallimann T., M. Wyss, D. Brdiczka, K. Nicolay, and H.M. Eppenberger. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues

with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostatis. *Biochem. J.* 281:21–40, 1992.

155. Warburg O. The origin of cancer cells. *Science* 124:269–270, 1956.
156. Wasserman K. Coupling of external to cellular respiration during exercise: the wisdom of the body revisited. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29):E519–E539, 1994.
157. Wasserman K. and M.B. McIlroy. Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am. J. Cardiol.* 14:844–852, 1964.
158. Wasserman K., B.J. Whipp, S.N. Koyal, and W.L. Beaver. Anaerobic threshold and respiratory gas exchange during exercise. *J. Appl. Physiol.* 35:236–243, 1973.
159. Watchko J.F., M.J. Daood, B. Wieringa, and A.P. Koretsky. Myofibrillar or mitochondrial creatine kinase deficiency alone does not impair mouse diaphragm isotonic function. *J. Appl. Physiol.* 88:973–980, 2000.
160. Watt M.J., K.F. Howlett, M.A. Febbraio, L.L. Spriet, and M. Hargreaves. Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. *J. Physiol.* (*Lond.*) 534:269–278, 2001.
161. Weber J.M., W.S. Parkhouse, G.P. Dobson, J.C. Harman, D.H. Snow, and P.W. Hochachka. Lactate kinetics in exercising Thoroughbred horses: regulation of turnover rate in plasma. *Am. J. Physiol.* 253 (*Endocrinol. Metab.* 22):R896–R903, 1987.
162. Welch H.G. and W.N. Stainsby. Oxygen debt in contracting dog skeletal muscle in situ. *Respir. Physiol.* 3:229–242, 1967.
163. Wickler S.J. and T.T. Gleeson. Lactate and glucose metabolism in mouse (Mus musculus) and reptile (Anolis carolinensis) skeletal muscle. *Am. J. Physiol.* 264 (*Regulatory Integrative Comp. Physiol.* 33):R487–R491, 1993.
164. Williams T.I. *A Biographical Dictionary of Scientists.* New York: Wiley, 1974.
165. Wilson M.C., V.N. Jackson, C. Heddle, N.T. Price, H. Pilegaard, C. Juel, A. Bonen, I. Montgomery, O.F. Hutter, and A.P. Halestrap. Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. *J. Biol. Chem.* 273: 15920–15926, 1998.
166. Wiseman R.W. and M.J. Kushmerick. Creatine kinase equilibration follows solution thermodynamics in skeletal muscle. ^{31}P NMR studies using creatine analogs. *J. Biol. Chem.* 270:12428–12438, 1995.
167. Zuntz N. and W. Loeb. Über die Bedeutung der verschiedene Nahrstoff als Energiequelle der Muskelkraft. *Arch. Anat. Physiol.* 18:541–543, 1894.
168. Zuntz V.N. Umsatz der nahrstoffe. XI. Betrachtungen über die beziehungen zwischen nahrstoffen und leistungen des korpers. In: *Handbuch der Biochemie des Menschen und der Tiere,* edited by K. Oppenheimer, G. Fischer, Jena, 1911, pp. 826–863.