Metabolic activities of heart, lung, and brain during diving and recovery in the Weddell seal

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MURPHY, B., W. M. ZAPOL, AND P. W. HOCHACHKA. *Metabolic activities of heart, lung, and brain during diving and recovery in the Weddell seal.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48(4): 596–605, 1980.—The metabolic potentials and activities of the heart, lung, and brain were studied in the Weddell seal, a species displaying outstanding diving abilities. The activities of representative enzymes in oxidative and fermentative metabolism were similar to those in homologous bovine organs, but the brain and heart contained elevated levels of lactate dehydrogenase. Isozyme analyses indicated the potential in all three organs for either lactate production or lactate utilization, both conditions being found during diving-recovery cycles. During awake simulated diving, overall energy needs were supplied by glucose in a mixed aerobic and anaerobic metabolism; the consequent fall in blood glucose levels and rise in lactate levels were due predominantly to peripheral hypoperfused tissues, but the central organs influenced these metabolite pools as well. The brain utilized blood glucose at a rate of 0.3 $\mu mol \cdot g^{-1} \cdot min^{-1}$, releasing 20–25% as lactate; this proportion did not change through diving-recovery cycles. The lung utilized blood lactate preferentially at a rate of about 0.5 mmol/min and thus diminished its accumulation in the blood during diving. The main fate of lactate taken up by the lung was oxidation inasmuch as $^{14}CO_2$ was the only measurable derivative found in aortic blood following [^{14}C]lactate infusion into the right ventricle. During recovery, when blood lactate levels rose above 6 μmol/ml, the brain switched from lactate release to lactate uptake at a rate high enough to readily support the normal metabolic rate of this organ (about 8 $\mu mol\ ATP \cdot g^{-1} \cdot min^{-1}$. Enzyme and metabolite measurements suggested that the lung and heart also contributed to lactate clearance and reestablishment of metabolic homeostasis following diving.

diving metabolism; brain metabolism; lung metabolism; seal metabolism

BY COMPARISON WITH MAN, marine mammals display startling capacities for breath-hold diving. Perhaps the most capable of pinniped divers, the Weddell seal, can dive to depths of 500 m for over 1 h (13). During such prolonged diving, the organism by definition becomes a self-contained life-support system. All energy and oxygen requirements must be supplied from internal stores. Utilization of potentially limiting substrates (including oxygen) must be optimized so that internal stores are not fully depleted during the diving period. All anaerobic end products must be deposited or further metabolized elsewhere in the body. To satisfy such needs of a closed isolated system, both physiological and biochemical mechanisms must be harnessed.

For over four decades (8, 10, 18, 19) the central physiological features of diving in marine mammals have been known to involve bradycardia and markedly reduced perfusion of hypoxia-tolerant tissues, but only recently have quantitative measurements on the selective redistribution of blood flow particularly to the heart, lung, and brain become available (28). In our companion studies on the Weddell seal, we learned that tissues of the central nervous system retained a constant blood flow during 8–12 min of simulated diving. All other tissues and organs, except for the adrenals where perfusion decreased somewhat, sustained drastically reduced blood flow. Even coronary and pulmonary arterial blood flows decreased to less than one-sixth the resting values (28).

The physiological purpose of circulatory alterations during diving is to conserve oxygen for the most oxygen-dependent organs, usually assumed to be the brain, heart, and lung (8, 10, 18, 19). The metabolic functions of circulatory adjustments, however, are less clear. Whereas the metabolic consequences of diving are well known in hypoperfused tissues and organs such as muscle and kidney (8), little information is available on the partitioning by the brain, heart, and lung of substrates between anaerobic and oxidative metabolism, or on metabolic interactions between these key central organs. For example, the arterial P_{O_2} in the Weddell seal (7) during simulated diving may decrease to 25 Torr, a level that is distinctly hypoxic to the brain of terrestrial mammals (22), but how the seal brain deals with this metabolic problem is unknown. To be sure, some indirect data are available. Brain and heart lactate dehydrogenases in seals and whales (2, 21) display relatively high proportions of M-type subunits. Together with unusually abundant stores of glycogen in seal heart and brain (11), these data imply an improved anaerobic capacity (9). But there is no direct information on substrate preferences of the brain, heart, and lung during diving and recovery cycles, or on the contribution of these organs to changes in blood metabolite pools.

To obtain a more comprehensive picture of diving metabolism in the Antarctic Weddell seal, we examined metabolic potentials and activities of the brain, heart, and lung during diving and recovery cycles. We learned that during diving the brain, heart, and lung display different substrate preferences and thus minimize competition for potentially limiting supplies of any one energy source. The brain utilizes glucose in a mixed (anaerobic and oxidative) metabolism that releases some lactate. By utilizing lactate formed here and in other hypo-

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perfused tissues, the lung and probably the heart diminish its accumulation in arterial blood during diving while simultaneously sparing glucose for the brain. During recovery from diving, when a large pulse of lactate is washed out of many tissues, the brain, heart, and lung all appear capable of preferentially utilizing lactate as a carbon and energy source. This organization contributes to the maintenance of metabolic homeostasis at widely varying perfusion rates (at rest and during diving) and facilitates metabolic transitions in diving and recovery cycles.

MATERIALS AND METHODS

Metabolite Studies

Adult seals (*Leptonychotes weddelli*), weighing 350–500 kg, were captured near Turtle Rock on the Ross Island Antarctic fast ice and transported for study to the Eklund Biological Laboratory at McMurdo Station. Unless otherwise noted, all in vivo manipulations were as previously described (28).

For monitoring metabolites, 2-ml samples were drawn from a PE-190 catheter positioned in the thoracic aorta. Samples were taken at various times before, during, and after diving. Metabolites in acid-extracted blood samples were assayed enzymatically as described elsewhere (4). To assess the capacity of the lung to metabolize lactate, a 150-cm-long, 8-French-diameter, balloon-flotation Swan-Ganz thermodilution catheter was surgically introduced into an exposed internal jugular vein at the thoracic inlet and advanced into the pulmonary artery with pressure monitoring. The proximal port of the Swan-Ganz catheter in the right ventricle was used as an injection site, and the distal port was used for collecting blood samples from the pulmonary artery. The lactate injectate or bolus contained [U-^{14}C]lactate (4 μCi/l blood), 2-g carrier lactate to bring concentrations up to about 3–4 μmol/ml, and 200 mg Evan's blue dye; the injectate was made up in a 15-ml total volume of normal saline (9 g/l) at pH 7.4. At 20-s intervals following rapid (5-s) manual injection of the lactate bolus into the right ventricle, 5-ml blood samples were drawn simultaneously from the sampling port in the pulmonary circulation and from the thoracic aorta. In a preliminary study, two additional sampling sites were used (the left and right ventricles), but these samplings added to the complexity and difficulty of the experiment without adding significantly more information; thus they were subsequently omitted. The blood samples taken from the pulmonary artery and aorta were added directly to equal volumes of 1.4 M perchloric acid (PCA) in stoppered tubes containing Hyamine (methyl benzethonium) hydroxide CO_2 traps. Thus, $^{14}CO_2$ formed from [^{14}C]lactate during a single circulation through the lung could be readily detected. On removal of the CO_2 traps, these blood samples were treated as before and assayed for dye content, lactate concentrations, and [^{14}C]lactate radioactivity. The marker dye concentration was estimated in arbitrary optical density units read at 600 nm. Lactate was measured enzymatically, following NAD^+ reduction at 340 nm. [^{14}C]lactate radioactivity in aliquots dissolved in Aquasol (New England Nuclear) was determined with a Nuclear Chicago Unilux 2A liquid scintillation counter. $^{14}CO_2$ trapped in Hyamine hydroxide was taken up in 10 ml of Aquasol for counting. The methods of Wolfe et al. (27) produced no evidence of blood [^{14}C]glucose formation from [^{14}C]lactate in these short-term experiments. Thin-layer chromatography indicated that no metabolic derivatives of [^{14}C]lactate other than $^{14}CO_2$ were released into the blood during the 7-min time course of these experiments.

For monitoring cerebral venous metabolites, a PE-5(catheter was introduced via a 13-gauge steel Tuohy needle into an epidural vein in the cervical region and advanced in an anterior direction to within a few centimeters of the occiput. In pinnipeds, cerebral venous blood is principally drained by these veins without admixture by venous blood from other tissues (12).

In all in vivo studies, routine physiological parameters were monitored as described elsewhere (28). After recovery from anesthesia (8–12 h), blood samples were drawn to establish metabolite levels in the control or resting state. This was usually followed by a relatively short simulated dive (10–20 min) and a recovery period. Another simulated dive was performed when the seal had fully recovered (usually 2–3 h) as judged by the return to resting levels of arterial pH, P_{O_2}, and P_{CO_2} and heart rate. When the desired in vivo experiments were completed, the animal was killed with an overdose of anesthetic, and the position of all catheters was verified at autopsy.

Enzyme Extraction and Assay

Tissues for enzyme extraction were excised as quickly as possible after the animal was killed; because of the massive size of the Weddell seal, this process took 20–30 min. A transmural left ventricular myocardial sample was obtained from each seal. Each sample contained both endocardium and epicardium. The same regions of the brain (anterior cerebral cortex), containing both gray and white matter, and of lung parenchyma (left lower lobe periphery without major vessels or airways) were sampled in each seal.

Glucose-6-phosphatase was assayed by following the release of inorganic phosphate, which was measured according to Nordlie (16). Assay conditions were 5 mM glucose-6-phosphate, 7.5 mM Mg^{2+}, 50 mM K^+, imidazole buffer, pH 7.4, 37°C. All other enzymes were assayed as before (4).

Lung Slice Studies

Samples of lung tissue were excised, washed in chilled Ringer solution, blotted on filter paper, and weighed to the nearest milligram. Slices weighing 25–50 mg were placed in Ringer solution of the following composition (in mM): NaCl 122, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.3, KH_2PO_4 0.4, and $NaHCO_3$ 25, pH 7.8. The O_2 uptake rate by lung slices was determined at 37°C using a Gilson Oxygraph. The oxidation of [U-^{14}C]lactate and [6-^{14}C]glucose by lung slices was determined using Warburg-type flasks containing a small Hyamine hydroxide CO_2 trap. Slices were incubated for 20–30 min in the presence of labeled and carrier substrates (protocol given below), holding

specific activity of glucose and lactate constant but varying total concentration. The lung slice experiments were terminated with PCA to stop the reaction and release CO_2. $^{14}CO_2$ collected in the Hyamine hydroxide traps was counted as described above.

Reagents and Coupling Enzymes

All enzyme substrates and coupling enzymes were purchased from Sigma Chemical (St. Louis, MO). Isotopically labeled glucose and lactate were purchased from New England Nuclear (Cambridge, MA). All reagents were of analytical grade.

RESULTS

Enzyme Levels in Seal Heart, Lung, and Brain

Because enzyme levels clearly correlate with outstanding hypoxia tolerance in many invertebrate animals (5), it was considered essential at the outset to explore enzymatic potentials of the heart, lung, and brain in the Weddell seal. These three organs differ greatly in metabolic organization (15, 22, 24), so it is not surprising that their enzyme activity profiles differ greatly (Table 1). However, organ-by-organ comparisons indicate that the activities of oxidative (mitochondrial marker) enzymes in seal heart, lung, and brain are similar to, or somewhat lower than, those measured under identical conditions in homologous tissues of the ox (Table 1) and of other species (20).

With respect to glycolytic potential, a few differences were noted that may be significant. First, hexokinase and lactate dehydrogenase in the seal brain occur at 4- and 2-fold higher levels than in the ox brain. And second, the activities of seal heart pyruvate kinase and lactate dehydrogenases are 1.5- to 2-fold higher than in the ox heart, the latter enzyme occurring at activities of about 1,000 μmol product$\cdot$min$^{-1}\cdot$g^{-1} at 37°C. With the exception of the lungfish heart (6), this is the highest activity of lactate dehydrogenase in any vertebrate heart thus far studied and undoubtedly the highest thus far measured in mammals regardless of size (20).

Electrophoretic studies show that in all three organs both heart- and muscle-type subunits of lactate dehydrogenase occur, thus generating multiple isozymes and indicating the potential either for lactate production, catalyzed most effectively by muscle-type lactate dehydrogenases, or for lactate utilization, catalyzed most effectively by heart-type lactate dehydrogenases (9). Empirically this can be expressed by the ratio of pyruvate reductase activity to lactate oxidase activity, which is strikingly higher for the skeletal muscle lactate dehydrogenases (Fig. 1).

Glucose-6-phosphatase catalyzes the terminal step in the formation of glucose either from triose precursors or glycogen (20). Interestingly, the enzyme occurs in all three central organs of the Weddell seal (Table 1). The ratio of glucose-6-phosphatase to hexokinase activities, which may supply an indication of the potential for glucose release vs. glucose phosphorylation, is highest for the lung (0.4) and lowest (about 0.1) for the brain, as observed also for two species of Arctic whales (25). The occurrence of this enzyme in the brain is in agreement with recent studies by Anchors et al. (1).

The most important outcome of the above enzyme studies is not how different the enzyme profiles are in the seal heart, lung, and brain, but rather it is how similar they are to other mammals. Differences in enzyme levels are maximally two- to fourfold, and are often far less, as also shown by more extensive recent studies (M. Castillini, personal communication). Although unique regulatory properties may yet be uncovered for some of these enzymes, as found in diving turtles, for example (23), it can be tentatively concluded that the metabolic correlates of outstanding diving in the Weddell seal are not based on enzymatic potentials, but rather on how those

TABLE 1. *Enzyme activities in brain, heart, and lung of the Weddell seal*

Enzymes	Brain		Heart		Lung	
	Seal	Ox	Seal	Ox	Seal	Ox
Citrate synthase	17.8 ± 2.5 (15.6–20.5)	16.8 (15.4–18.1)	28.8 ± 6.9 (18.8–35.3)	61.7 (45.8–79.9)	1.52 ± 0.9 (0.8–1.7)	6.6 (6.4–6.9)
Glutamate dehydrogenase	7.5 ± 1.6 (5.7–8.6)	4.5	4.4 ± 1.3 (3.5–6.5)	2.8 (2.4–3.2)	0.90 ± 0.4 (0.6–1.7)	0.9
β-Hydroxybutyrate dehydrogenase	0.40 ± 0.10 (.28–0.5)	0.3 (1.9–3.6)	2.42 ± 0.7 (1.9–3.6)	2.8	1.7 ± 1.0 (0.5–2.5)	0.2
β-Hydroxybutyryl CoA dehydrogenase	3.4 ± 0.3 (3.2–3.7)	2.2	16.0 ± 3.5 (12.5–20.5)	21.6	1.2 ± 0.4 (0.5–2.5)	5.2
Hexokinase	5.2 ± 1.8 (3.0–5.7)	1.3 (1.3–1.4)	2.0 ± 0.9 (1.2–3.4)	2.9 (2.8–3.0)	1.7 ± 0.8 (1.1–2.9)	2.2 (2.2–2.1)
Glucose-6-phosphatase*	0.61		0.48		0.72	
Phosphofructokinase	8.6 ± 2.8 (5.3–12.3)	8.6 (8.2–9.0)	16.7 ± 5.9 (9.9–24.1)	14.0 (13.9–14.0)	3.7 ± 1.3 (2.3–5.4)	4.7 (4.2–5.2)
Pyruvate kinase	167.3 ± 11.1 (111–183)	196 (194–198)	217.5 ± 51.5 (183.3–294.0)	133.1 (128–137.9)	45.6 ± 17.7 (21.6–66.3)	98.0 (94.4–101.6)
Lactate dehydrogenase	228.8† (200–350)	128.2 (125.8–130.6)	1032.0 ± 45.7 (1013–1050)	556.0 (508–604)	69.6 ± 30.7 (50.6–107.8)	91.9 (79.8–104.0)

Values are means ± SE, with ranges in parentheses, expressed as μmol substrate converted$\cdot$min$^{-1}\cdot$g^{-1} wet tissue wt at 37°C, pH 7.4, and saturating levels of substrates, cofactors, or coenzymes. Detailed assay conditions given elsewhere (4, 16). Comparable values are included for the same organs in the ox. At least 6 seals were sampled. Sample number was usually 2 for the ox values, are included only for comparative purposes. For further mammalian values, see Refs. 16, 20. * n = 1; almost identical values obtained for two cetaceans (W. Vogl and B. Murphy, unpublished data). † n = 3.

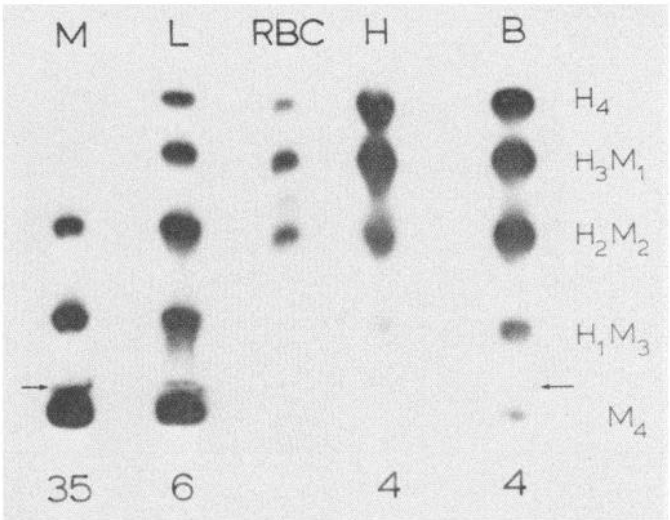

FIG. 1. Starch gel electrophoretic separation of lactate dehydrogenase isozymes in skeletal muscle (M), lung (L), red blood cells (RBC), heart (H), and brain (B) of the Weddell seal. Electrophoresis conditions: 25 mA; 200 V; 12 h at 4°C; anode at top; origin marked with an *arrow*. Subunit composition of each isozyme is shown on *right*. Numbers below refer to ratio of pyruvate reductase activity to lactate oxidase activity at pH 7.4 at saturating coenzyme and substrate concentrations, assayed at 37°C.

potentials are used in vivo. That is why the bulk of our emphasis in this study turned to assessing in vivo metabolic activities during simulated diving and recovery.

Blood Glucose, Pyruvate, and Lactate Profiles

As previously reported (7), a decrease in glucose concentration is consistently seen during short- and long-term dives in samples of whole blood taken either from the pulmonary artery or the aorta (Fig. 2). It is important to note that during the first 5–10 min of recovery, when cardiac output is high and the total 60-liter blood volume is well mixed (28), blood glucose levels continue to decrease; but within 5–10 min, they begin to return to normal and then often overshoot prediving concentrations. In contrast to glucose profiles during diving, blood lactate concentrations consistently rise in arterial blood usually from less than 1 μmol/ml in predive states to over 3 μmol/ml at the end of the diving period (Fig. 3). Pyruvate levels do not change greatly during diving (Fig. 4), but following simulated diving a large washout of both pyruvate and lactate is always observed. What was not expected was the large difference in the kinetics of pyruvate and lactate appearance in the blood, with the pyruvate washout peak always lagging behind the lactate washout peak (Fig. 5). As a result, during early stages of recovery, lactate concentrations may rise well before there is any measurable change in pyruvate levels. This means transient and very large changes in blood lactate-to-pyruvate ratios may occur during diving and recovery cycles.

The question arising is where the glucose and lactate concentration changes are generated and in particular how they are influenced by the central organs. In this study we obtained data on the brain and lung.

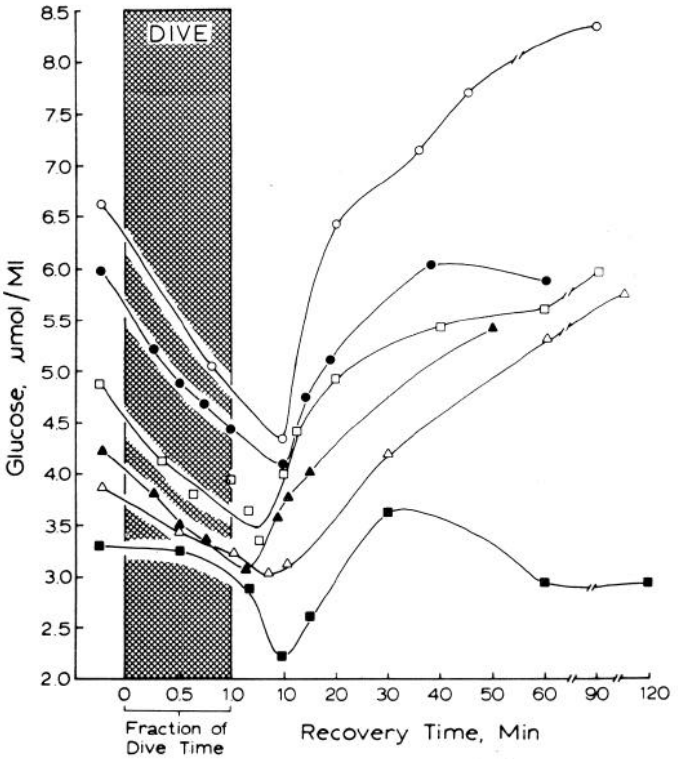

FIG. 2. Change in glucose concentration of whole arterial blood during diving and recovery in 6 representative seals whose initial blood glucose supplies varied by nearly 2-fold. Duration of dives, standardized to an arbitrary scale, varied between 10 and 20 min. Dive times: ○, 20 min; ●, 20 min; □, 15 min; ▲, 20 min; △, 20 min; ■, 10 min. Although blood may be poorly mixed during diving, it becomes well mixed in early recovery when cardiac output overshoots initial levels and is as high as 60 l/min. It is therefore instructive that glucose concentrations continue to fall during at least first 5–10 min of recovery from diving.

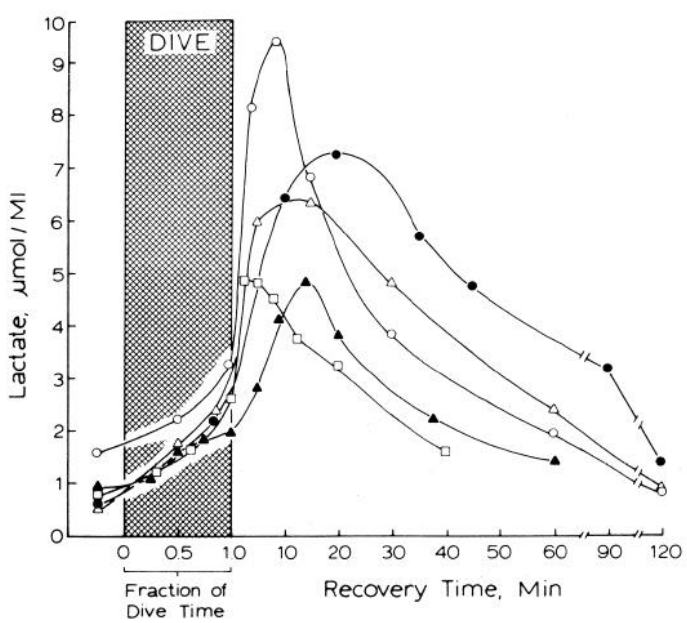

FIG. 3. Change in lactate concentration of whole arterial blood during diving and recovery in 5 representative seals. Dive duration varied between 10 and 20 min, but was standardized to facilitate comparison on an arbitrary scale. Dive times: ●, 20 min; △, 20 min; ○, 10 min; ▲, 20 min; □, 15 min.

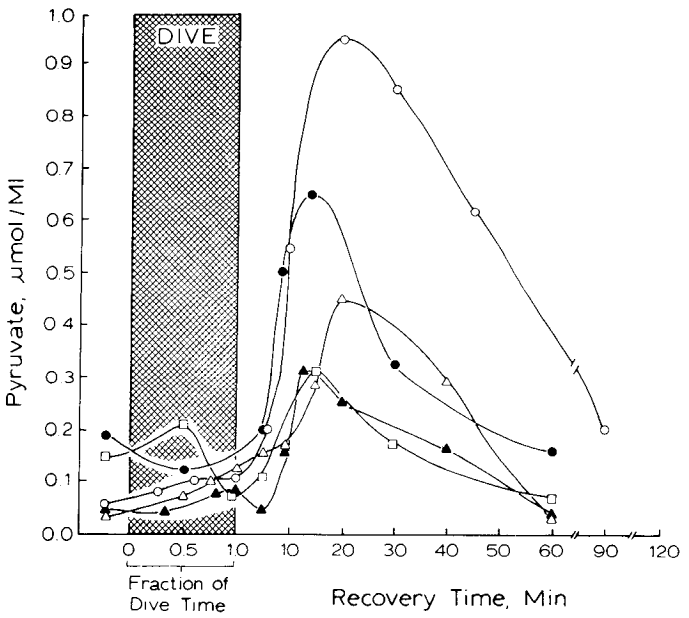

FIG. 4. Change in pyruvate concentration of whole arterial blood during diving and recovery in 5 representative seals. Dive duration varied between 10 and 47 min, but was standardized to facilitate comparison on an arbitrary scale. Dive times: ●, 10 min; □, 20 min; ○, 46 min; ▲, 15 min; △, 20 min.

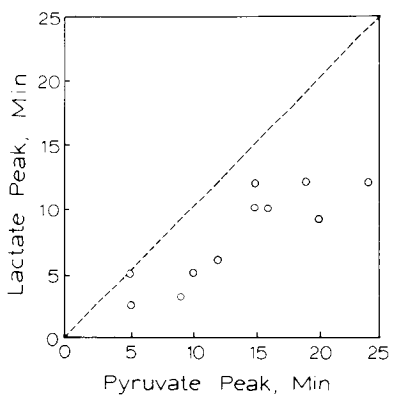

FIG. 5. Plot of recovery time required to reach peak washout concentrations of lactate and pyruvate following diving for 11 seals. Dive duration varied between 10 and 47 min. In essentially all cases, pyruvate washout trailed behind lactate washout by easily measurable times.

Impact of Diving on Brain Metabolism

Because the brain of most mammals is considered to be obligatorily dependent on blood glucose, it is not surprising that glucose is also a good substrate for the seal brain. Simultaneous sampling of arterial blood in the aorta and epidural venous blood close to the occiput (12) indicates that glucose uptake by the brain during simulated diving (0.3 $\mu mol \cdot g^{-1} \cdot min^{-1}$) is somewhat (140%) higher than during control resting states (Table 2). Only a small fraction (about 1/11) of the total brain hexokinase activity (Table 1) would be needed to sustain this rate of glucose metabolism. Interestingly, lactate release accounts for about 20–25% of the cerebral glucose uptake under both states (Table 2).

These data may be explained by changes in cerebral blood flow or by changes in uptake and release of the two metabolites. However, recent microsphere studies

TABLE 2. *Whole blood glucose and lactate concentration gradients across brain of the Weddell seal before and during diving*

Seal No.	Condition	Arteriovenous Differences		% Glucose Fermented	Rate of ATP Formation
		Lactate production	Glucose uptake		
9	Predive	0.13 ± 0.039 (5)	0.38 ± 0.042 (5)	17	6.9
17	Predive	0.18 ± 0.064 (6)	0.26 ± 0.031 (6)	34	3.9
	Dive [10]	0.24 ± 0.125 (2)	0.46 ± 0.030 (2)	23	10.8
18	Predive	0.13 ± 0.120 (3)	0.26 ± 0.141 (3)	25	4.4
	Dive [15]	0.13 ± 0.115 (3)	0.30 ± 0.109 (3)	22	6.3
18	Predive	0.13 ± 0.052 (4)	0.25 ± 0.106 (3)	26	4.2
	Dive [20]	0.31 ± 0.071 (4)	0.50 ± 0.038 (3)	31	9.5
20	Predive	0.14 ± 0.122 (4)	0.26 ± 0.092 (3)	27	3.6
	Dive [20]	0.11 ± 0.087 (4)	0.34 ± 0.119 (4)	16	7.8
Predive mean		0.14 ± 0.032 (20)	0.28 ± 0.033 (21)	25.0	4.7
Dive mean		0.16 ± 0.068 (12)	0.40 ± 0.051 (12)	20.0	8.3

Values are means ± SE, expressed as the difference in μmol/ml between arterial and venous blood samples drawn simultaneously. Maximum metabolic rate sustained by glucose catabolism is calculated in terms of $\mu mol\ ATP \cdot g^{-1} \cdot min^{-1}$ assuming a flow rate of $0.6\ ml \cdot g^{-1} \cdot min^{-1}$ in the control predive state, and $0.75\ ml \cdot g^{-1} \cdot min^{-1}$ during diving. Duration of dives, in min, shown in brackets; no. of samples given in parentheses.

(28) indicate that cerebral blood flow remains essentially unchanged during 8–12 min of diving, and this factor therefore cannot account for changes in glucose and lactate profiles. The blood flow and metabolite data, taken together, moreover imply that brain metabolism in diving is not O_2 limited. If it were, glucose uptake would need to increase by up to 18-fold due to the energetic inefficiency of anaerobic glycolysis; similarly, the fraction of glucose appearing as lactate would obviously have to rise. The estimated increase in glucose uptake rate of only 140%, with no change in the fraction fermented to lactate, indicates that the brain's dependence on anaerobic glycolysis does not rise much during simulated diving of up to 30 min duration, despite arterial P_{O_2} levels (down to 25 Torr) that may be hypoxic to nondiving mammals (22).

In addition to being capable of releasing lactate, the Weddell seal brain can also consume it. Routine lactate arteriovenous measurements upon recovery show a net brain uptake of this anaerobic end product whenever the mean arterial concentration reaches a critical level of about 7 μmol/ml; if this is surpassed either in recovery from diving (Fig. 6) or during lactate infusion (Fig. 7), the brain vigorously consumes lactate, generating an arteriovenous gradient of up to 1.25 μmol/ml (Figs. 6 and 7). The capacity for either lactate production or lactate uptake is consistent with the occurrence in seal brain of lactate dehydrogenases kinetically well suited for bidirectional function (Fig. 1, Table 1).

Impact of Diving on Lung Metabolism

In situ studies. Arteriovenous concentration gradients across the lung can be determined by simultaneous sampling in the pulmonary artery and left ventricle or aorta. In earlier studies, such data for the Weddell seal established that the lung takes up lactate from the blood during diving. Although glucose can be utilized by the

lung, it can also be released at a low rate (7), a result consistent with the relative activities of glucose-6-phosphatase and hexokinase in the lung (Table 1). We did not obtain evidence suggesting de novo synthesis of glucose from lactate. However, a major fate of lactate taken up by the seal lung appears to be oxidation. In part, this conclusion is based on in vivo experiments in which we sought metabolic derivatives of injected [^{14}C]lactate on a single circulatory pass through the lung. In these experiments (Fig. 8) a bolus of [^{14}C]lactate plus dye and carrier lactate was rapidly injected into the right ventricle after 10 min of simulated diving. Blood samples were taken every 20 s at two ports, the pulmonary artery and the aorta. Most of the [^{14}C]lactate (as well as the unlabeled carrier) traversed the pulmonary circulation simultaneously with the tracking dye. A small fraction of the lactate rapidly traversed the pulmonary circulation so that at the time of the first two samples (at 20 and 40 s), significant amounts of [^{14}C]lactate were already present in arterial blood. At the same time (20–40 s after injection), although the [^{14}C]lactate and absolute lactate concentrations were decreasing in the pulmonary circu-

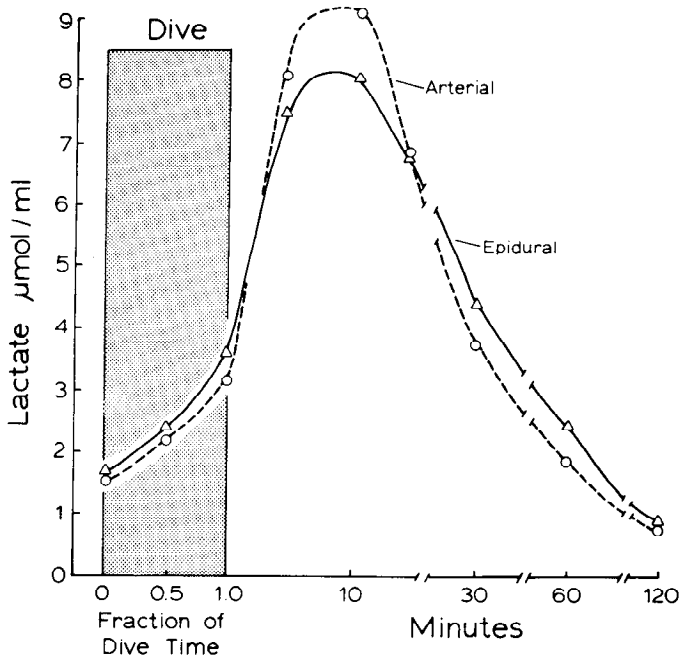

FIG. 6. Change in lactate concentration in arterial and epidural venous blood samples during diving and recovery in a seal showing an unusually large lactate washout. Dive time: 20 min.

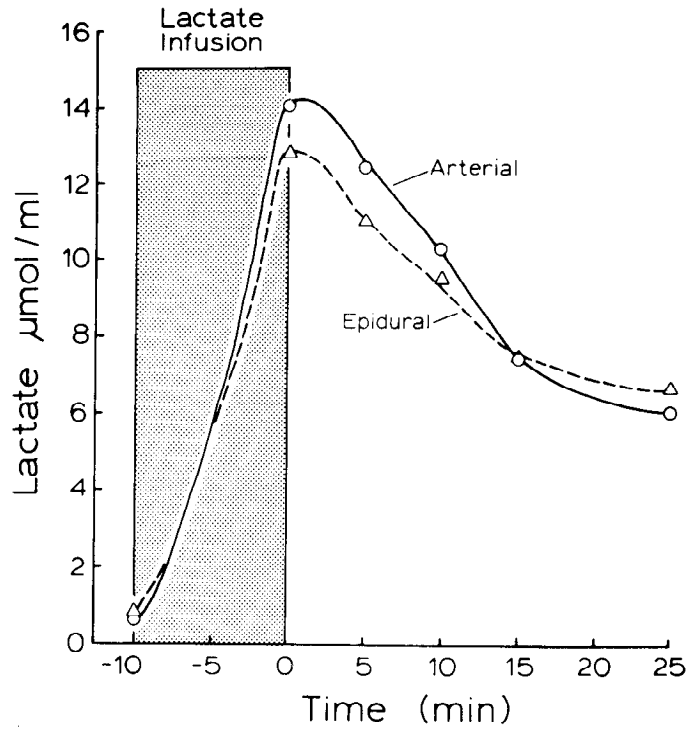

FIG. 7. Lactate concentration changes in arterial and epidural venous blood following infusion of 176 g of lactate. Lactate was infused in normal saline at pH 7.3 at a rate of about 100 ml/min.

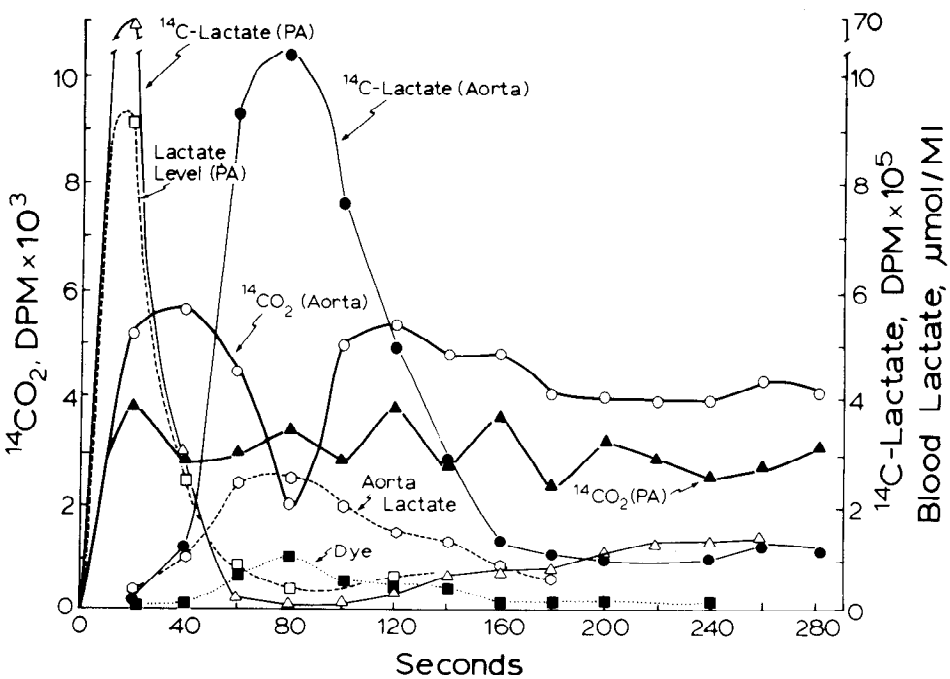

FIG. 8. [U-^{14}C]lactate oxidation by the Weddell seal lung in vivo. Multiple openings in right ventricular injection port of Swan-Ganz catheter assured well-mixed introduction of [^{14}C]lactate bolus. Rapid manual injection was performed after a stable bradycardia (heart rate of 15 beats/min) was established at 10 min into a 17-min simulated dive. At 20-s intervals simultaneous 5-ml blood samples were withdrawn from pulmonary artery (PA) and aorta and treated as described in MATERIALS AND METHODS. Cardiac output in this experimental seal (a 350-kg male) was 24 l/min before diving and decresaed to 4 l/min at 10 min into dive. Qualitatively similar results were obtained in a preliminary experiment with another seal during entry into a simulated dive.

lation, $^{14}CO_2$ had already appeared in aortic blood and could only have been generated by lung metabolism since at this time the lung was the only tissue (other than blood) to have received [^{14}C]lactate. The majority of the $^{14}CO_2$ pulse appeared on the left side of the heart, peaking at about 35 s after injection; the smaller initial peak of $^{14}CO_2$ in the pulmonary arterial blood probably arrived via the coronary circulation. That is probably why oscillations in blood $^{14}CO_2$ levels on the left and right sides of the heart initially are about 90° out of phase with each other. This situation holds for about the first 100 s. Nevertheless, for the duration of the experiment (7 min, of which only the first 280 s are shown in Fig. 8), it appears that lung oxidation of [^{14}C]lactate exceeded oxidation of injected [^{14}C]lactate by any other organs, a process that explains the consistently higher levels of $^{14}CO_2$ in the aorta than in the pulmonary arterial blood. The mean pulmonary-to-thoracic aorta circulation time (time for traversing from the right to the left side) is about 30–40 s under simulated diving conditions, and a complete circulation time is about 60–80 s (Fig. 8). Since the average cardiac output is 6 l/min (28), we can estimate a "central" blood volume of about 8 liters that exchanges slowly with the rest of the 60-liter blood volume presumably pooled in the venous system.

Lung slice experiments. Further evidence indicating that lactate is a good substrate for the lung comes from tissue slice experiments. By use of air-equilibrated Krebs-Henseleit-Ringer solution, the $\dot{Q}o_2$ for lung tissue was found to be similar to that reported for other mammals (26), at 30.19 ± 1.63(SE) μl $O_2 \cdot h^{-1} \cdot 100$ mg wet wt^{-1} (n = 14), and remained stable for over 1 h. Lung slices were found to oxidize [^{14}C]lactate at relatively high rates, but [^{14}C]6-glucose at substantially reduced rates. Increasing glucose concentration from 1 to 10 mM caused a threefold increase in the rate of oxidation, whereas increasing lactate concentration from 1 to 10 mM increased oxidation rate by nearly fivefold (Table 3*A*). Interacting effects of glucose and lactate were modest (Table 3*B*) in contrast to the mammalian heart, where high lactate levels strongly inhibit the rate of oxidation of glucose (14). Lactate oxidation exceeded glucose oxidation rates at all substrate concentrations, but this was accentuated at high lactate levels. Thus, it appears that lactate is utilized in preference to glucose as a substrate by the seal lung, a conclusion consistent with our previous studies (7) and with recent studies of the perfused rat lung (17, 27).

TABLE 3. *$^{14}CO_2$ production from [U-^{14}C]lactate and from [6-^{14}C]glucose by lung slices of the Weddell seal*

Conditions	$^{14}CO_2$ Production	
	[6-^{14}C]glucose	[U-^{14}C]lactate
A. Glucose and lactate concentrations varied independently		
10 mM glucose	247.8 ± 62.0 (6)	
5 mM glucose	122.2 ± 16.6 (5)	
1 mM glucose	63.3 ± 8.1 (6)	
10 mM lactate		531.5 ± 71.8 (6)
5 mM lactate		396.5 ± 35.3 (6)
1 mM lactate		201.3 ± 25.7 (6)
B. Glucose and lactate concentrations varied simultaneously		
10 mM glucose 1 mM lactate	204.0 ± 23.2 (11)	197.8 ± 15.2 (12)
1 mM glucose 10 mM lactate	50.1 ± 7.0 (12)	848.4 ± 74.7 (11)

Values are means ± SE; no. of experiments are given in parentheses. Conditions are given in MATERIALS AND METHODS. The rate of $^{14}CO_2$ release is expressed in nmol·h^{-1}·g^{-1} wet wt of tissue at 37°C. Specific activity of glucose and lactate was constant under all conditions.

DISCUSSION

Carbon and Energy Sources During Diving

Although the role of endogenous glycogen in different tissues during diving has not been quantified, it seems certain that blood glucose reserves are a critical carbon and energy source supporting the seal during diving. This is indicated not only by the extensive depletion of blood glucose during diving, but also by the observation of only minor simultaneous changes in the blood free amino acid pool (unpublished data). Because fatty acids are not likely to take on enhanced importance during the progressively hypoxic conditions developing in diving, it is tempting to suggest that blood glucose reserves are a major, if not the only, source of carbon for metabolism during diving.

Brain Metabolism in the Seal

The key metabolic feature of the Weddell seal brain, its vigorous uptake of blood glucose, was in fact expected. What was not expected was the relatively large fraction (20–25%) of the glucose taken up that was released as lactate. In the rat brain, only 5–15% of the glucose taken up is released as lactate owing to a limitation at the level of pyruvate dehydrogenase (3). The same mechanism may operate in the seal, but because of higher total lactate dehydrogenase activities and relatively more muscle-type isozyme a larger fraction of the pyruvate pool may be diverted to lactate. The routine and apparently "wasteful" release of lactate by the brain may therefore merely represent a minor "cost" of increasing its anaerobic potential somewhat.

Because the mean blood flow to the seal brain was measured, the glucose and lactate gradients allow estimation of metabolic rates in terms of μmol ATP·g^{-1}·min^{-1} that can be sustained with glucose as the carbon and energy source. The calculated metabolic rates (Table 2) sustainable by glucose catabolism are somewhat lower than for brain metabolism in man and other mammals (22). This may be expected from the scaling effects of body size inasmuch as brain metabolic rates of large mammals are reported to be lower than in small-sized species (22). Our estimates of cerebral metabolic rates do not take into consideration the flow of glucose carbon into glycogen, the pentose cycle, the free amino acid pool,

or other metabolic pathways, and this may explain the apparent differences between the diving and control states (Table 2). Under some conditions, glucose incorporation into the free amino acid pool accounts for a significant fraction of the glucose uptake by the brain in terrestrial mammals (22). Thus, the higher estimated values for cerebral metabolic rates during diving may merely reflect a pooling of glucose carbon in nonoxidative metabolic pathways. At least tentatively, therefore, no significance is attached to the differences noted.

Are these metabolic rates high enough to cause significant depletion of blood glucose reserves during diving? The answer is evident in a simple set of calculations. If we assume an average arteriovenous concentration gradient across the brain of 0.4 μmol/ml, and an average blood flow of 700 $ml \cdot kg^{-1} \cdot min^{-1}$, then a 500-g brain can take up glucose at a rate of 0.14 mmol/min or about 3 mmol/20 min dive. Assuming an 8-liter volume exchanging slowly with total blood volume during diving, this rate of glucose uptake would lead to an overall concentration change of 0.37 μmol/ml blood over a 20-min dive. This glucose utilization rate would decrease blood glucose levels by less than 0.05 μmol/ml when the total blood volume was well mixed. Complete mixing would be expected very early in the recovery process because cardiac output tends to overshoot prediving control levels, reaching values as high as 60 l/min during the 1st min of recovery (M. Snider, unpublished data). Because similar calculations indicate that brain anaerobic glycolysis can lead to only a modest increase in blood lactate levels, it is evident that during routine diving periods (of about 20 min duration) brain metabolism on its own does not markedly alter total blood pools of glucose or lactate. In longer dives, the brain's contribution to glucose depletion and lactate production in the central blood could become significant, so it is interesting that changes mediated by brain metabolism are to some extent counteracted by lung metabolism.

Lung Metabolism in the Seal

As the lung was envisaged in earlier formulations of the diving response as a "favored" organ, it is interesting to note that the picture emerging from arteriovenous concentration gradients is a mirror image of metabolism in the brain. That is, during diving the lung does not release lactate, it utilizes it. At the same time, although glucose can be utilized by the lung, it can also be released at a low rate, a result consistent with the relative activities of glucose-6-phosphatase and hexokinase in the lung (Table 1). Neither the frequency of glucose release nor its significance have been clarified. Certainly, there is currently no evidence suggesting de novo synthesis of glucose from lactate. Rather, the main fate of lactate taken up by the seal lung seems to be oxidation, inasmuch as $^{14}CO_2$ is the only significant metabolic derivative of [^{14}C]lactate metabolized by the lung during simulated diving. Furthermore, in lung slices lactate is used in preference to glucose as a carbon and energy source, as subsequently shown in rat lung preparations (17, 26, 27) and in the harbor seal (B. Murphy, unpublished data). Whether this preference is shown under all metabolic conditions is not known, but at least during diving it is clear that, with a 0.1 μmol/ml arteriovenous concentration gradient (7), lactate uptake by 4-kg lung tissue will reduce blood lactate concentrations at a rate of about 0.5 mmol/min while sparing blood glucose for other organs, especially the brain. In these functions, the lung may be assisted by the heart; this may be particularly effective in reducing lactate accumulation in central blood during diving.

Potential Contributions of the Heart vs. Peripheral Organs

Interestingly, initially just the opposite situation was envisaged for the heart (i.e., lactate formation rather than utilization); this may in fact occur in emergency situations. There are several reasons for this view. In the first place, although the activities of hexokinase and other glycolytic enzymes (Table 1) are comparable to those in other species, heart lactate dehydrogenase activity and glycogen stores are the highest thus far measured in mammals. Moreover, glycogen is stored as large-diameter α-rosettes rather than the usual β-particles (W. C. Hulbert and P. W. Hochachka, unpublished data), a storage pattern typically observed only in organs storing unusual amounts of glycogen. As in other species, heart lactate dehydrogenase is potentially bifunctional, favoring either lactate formation or lactate utilization dependent on metabolic circumstances. Although all the above are consistent with a high potential for anaerobic glycogenolysis or glycolysis, it may rarely be utilized, for during diving, both cardiac output and coronary blood flow decrease by about 85%. Furthermore, at these low coronary flow rates, for the heart to produce all the blood lactate observed during diving the concentration gradient across the heart would have to be over 20 μmol/ml, representing a release rate 100 times greater than the brain. We consider this highly unlikely and the same considerations apply for glucose depletion. Finally, a dependence on anaerobic glycolysis would necessitate a large increase in coronary flow per watt of cardiac work. Measurements of blood flow, cardiac output, and arterial pressure (28) indicate that coronary flow per unit of cardiac work increases by only about 15% (from 75 to 87 $ml \cdot g^{-1} \cdot min^{-1} \cdot W^{-1}$). Thus, heart work during diving in the seal remains supported by oxidative metabolism. Whereas oxidative metabolism in the mammalian heart may be fired by a variety of substances (glucose, fatty acids, lactate), lactate is known to be preferentially utilized whenever concentrations rise above normal (14). That indeed is the situation developing through the diving period and it may explain why the Weddell seal heart retains exceptionally high levels of lactate dehydrogenase kinetically well suited for lactate oxidation.

From these considerations, we can conclude that only in emergency situations could the seal heart contribute to the lactate accumulating during diving; in routine diving of up to 30 min duration, it could not possibly account for all the lactate formed. The same considerations apply for glucose uptake. So by elimination, the glucose depletion and lactate accumulation observed must be largely due to metabolism of other hypoperfused organs and tissues. Although their blood flow is much

reduced during diving, it is not zero; skeletal muscles and skin, for example, receive a significant fraction of cardiac output (28). Because of the severely reduced blood supply, these organs must rely almost exclusively on anaerobic glycolysis (8); this explains the relatively close stoichiometry between glucose depletion and lactate accumulation in the central blood (Figs. 2 and 3) (7). This interpretation also explains why glucose utilization and lactate production are so high: first, because markedly hypoperfused organs and tissues constitute the bulk of the animal, and second, because anaerobic glycolysis is energetically inefficient and requires a large glucose consumption. This also explains why glucose concentrations in the blood continue to fall during early recovery even as complete mixing of the large blood volume is occurring. This is because glucose is depleted in the total blood volume, not only in the slowly exchanging volume of central blood. If only the latter occurred, glucose levels would return to near-normal levels within the 1st min of recovery as cardiac output rose and fully mixed the blood.

Role of the Brain, Lung, and Heart in Recovery

A first clue as to the roles played by the central organs in recovery comes from the kind and amount of lactate dehydrogenase isozymes present, because each has the potential for lactate utilization. Operationally, this can be expressed by the ratio of pyruvate reductase activity to lactate oxidase activity, which is low in all three organs (Fig. 1). It is not surprising, therefore, that during recovery, when lactate washout peaks are high, the seal brain switches from lactate release to lactate uptake. If all the lactate consumed were fully oxidized, it could support a metabolic rate of 9 μmol ATP$\cdot$g$^{-1}\cdot$min^{-1}, assuming that brain blood flow were normal. This value is equal to, or greater than, that sustainable by glucose metabolism and indicates that lactate metabolism under these conditions can readily supply all of the energy demands of the brain.

Because catheterization of the seal coronary venous sinus is formidable without radiography, similar data are not yet available for the heart. However, qualitative lactate dehydrogenase isozyme patterns for the brain and heart are similar although overall activities are fivefold higher in the heart. These are good reasons for suggesting that, during the lactate washout, blood lactate can serve as a carbon and energy source for the seal myocardium, as it does under conditions of high blood lactate levels in other mammalian species (14). A similar metabolic capacity for lactate conversion is displayed by the lung (7, 17, 27), particularly at the high blood lactate levels found during the recovery process.

In summary, the central organs display metabolic activities that are in a sense cooperative: the interactions between them facilitate transitions from high-perfusion resting states to low-perfusion diving states by minimizing perturbations in blood glucose and lactate pools and by accelerating the clearance of the postdiving lactate washout and sparing glucose for subsequent diving. These metabolic interactions, which undoubtedly contribute to extending diving duration, depend on how enzyme potentials are used and not on the development of any "new" or qualitatively different enzymic machinery. Indeed, only a few modest adjustments in enzyme levels seem to correlate with the observed metabolic organization. In this sense, the biochemical strategies associated with extending hypoxia tolerance appear to differ fundamentally from those utilized by many, more primitive, animal anaerobes (5).

As far as it goes, our interpretation (or metabolic model) of the brain, lung, and heart during diving seems capable of accounting for most currently available data. However, the model is based on simulated diving (of up to 30 min duration) when metabolism of the three central organs is not O_2 limited. If O_2 were to become seriously limiting in emergency situations, the use of lactate by the lung and heart would obviously become seriously curtailed, and the above key metabolic interactions would break down. What would the seal replace them with? That, for the moment, we do not know.

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