level background of PAH from natural sources, but it seems clear that the major source of PAH in the present surface sediment at this location is anthropogenic combustion. A more detailed investigation of the depth distribution of PAH in sediments from a variety of depositional environments, near and remote from urban areas, is needed to confirm this conclusion. For example, studies of PAH in anoxic, rapidly depositing, nearshore, marine or lake sediments would be ideal because these locations would not be subjected to the uncertainties in reconstructing historical records which are introduced into sediments with bioturbated surface layers (*22*).

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5 April 1977; revised 13 June 1977

Pulmonary Metabolism During Diving: Conditioning Blood for the Brain

Abstract. *During experimental diving by the awake Weddell seal, blood glucose concentration falls consistently. A large fraction of the glucose consumed from the central circulating blood appears as lactate. During diving, the lung utilizes blood lactate in preference to blood glucose as a source of both carbon and energy, and it is able to release glucose into pulmonary venous blood to supplement the supply available for brain metabolism.*

Although the role of the lung in gas exchange was defined more than 100 years ago by Magnus (*1*), many of its metabolic functions in the living organism remain obscure in part because arteriovenous (AV) metabolic gradients across the lung are diluted by the large pulmonary blood flow (*2*). However, we repeatedly measured AV metabolic differences across the lungs of a diving mammal. Although the lung ceases to be a significant gas-exchange organ during diving, it remains perfused by the entire cardiac output, while the 70 to 90 percent fall in cardiac output during diving (*3*) should increase the measurable pulmonary AV metabolite concentration differences. In our studies of experimental diving by the awake Antarctic Weddell seal (*Leptonychotes weddelli*), a species that can dive for more than an hour (*4*), we observed a decrease in glucose concentrations in systemic and pulmonary artery blood and an increase in lactate concentrations; at the same time, pulmonary AV differences of blood lactate and glucose were reciprocal. As all the enzymes necessary for lactate use and for glucose formation were present in fresh seal lung tissue, our study suggests that lactate is used by the lungs as a preferred substrate and that the lungs may supplement the circulating blood supply of glucose for the brain.

Table 1. Relationship between the decrease in glucose concentration in systemic arterial blood and the rise in lactate concentration. Whenever two dives were studied, we allowed the seal to recover completely from the first before beginning the second. In all cases, from half to all of the glucose utilized appeared as blood lactate. As the seals were awake and varied in condition and degree of stress, no close correlation between diving time and glucose utilization was expected or evident.

Seal	Diving length (min)	Change in glucose concentration (μmole/ml)	Change in lactate concentration (μmole/ml)
1	5	−0.6	+0.5
2			
Dive 1	10	−1.5	+1.3
Dive 2	22	−1.0	+1.7
3			
Dive 1	20	−1.0	+1.1
Dive 2	25	−1.5	+1.5
4			
Dive 1	26	−0.9	+0.8
Dive 2	46	−1.0	+2.5

Weddell seals were captured at Turtle Rock on the Ross Island fast ice. Adult seals weighing 450 to 500 kg were herded into a large wooden box and hauled on a sled to the McMurdo Eklund Biological Laboratory. Here, the seal was tranquilized (intramuscular ketamine hydrochloride, 2 mg per kilogram of body weight); the box was positioned with a forklift, and the animal was transferred to a mobile operating table. During surgery (*5*), anesthesia was maintained by the spontaneous breathing of halothane in oxygen.

A large drop in glucose concentration was consistently seen during short- and long-term dives (Table 1) in samples of whole blood taken either from the pulmonary artery (mixed venous) or the aorta (*6*, *7*). We believe that a notable fraction of the glucose used was being fermented for two reasons. (i) If all the glucose used were being fully oxidized metabolic rates would be far higher than expected (*8*) and P_{O_2} would drop further than observed (Fig. 1). (ii) Even in dives

of relatively short duration, a large fraction (50 percent or more) of the glucose used was represented by a concomitant lactate accumulation; in more extreme situations, the fall in blood glucose concentration was approximately equal to the rise in that of lactate (Table 1).

Initially, it was unclear whether lactate in the central circulation was due to glucose fermentation in the lung, heart, or brain (*9*). In this connection, two observations made in preliminary studies of the lung were relevant. (i) Blood glucose concentration in the pulmonary artery during diving could be consistently slightly higher than, equal to, or slightly lower than the concentration in the aorta. That is, glucose could be taken up or released by the lung under different conditions. Glucose uptake by perfused lung and lung slices has been noted by others (*10, 11*), but to our knowledge, glucose release by the lung has not been previously reported. (ii) With respect to lactate, concentration changes across the lung again could be either positive or negative, but concentration decrements were observed only in animals with profound diving bradycardia. Although statistically significant (*12*), such results were unexpected since in most mammalian lungs, the Fick principle for studies of substrate use and release cannot be used effectively (*11*) because of two related problems: the large blood flow (cardiac output) and the relatively small lung mass (which implies a low absolute metabolic rate). By contrast, a 450-kg Weddell seal has about 5 kg of lung tissue. With its one unit of hexokinase per gram (Table 2), this lung could take up glucose at a rate of 5 mmole/min. Assuming a diving cardiac output of 5 liter/min (*13*), this enzyme activity could generate an easily measurable concentration difference of 1.0 μmole per milliter of blood perfusing the lung. The enzymic potential for glucose release is lower (Table 2), but it could still generate 0.1 to 0.2 μmole per milliliter of glucose concentration differences across the lung. And finally, the activity of the lactate dehydrogenase back reaction (lactate → pyruvate) is about 140 unit/g (Table 2), an amount that in theory could generate large changes in lactate concentration in the blood as it perfused the lung.

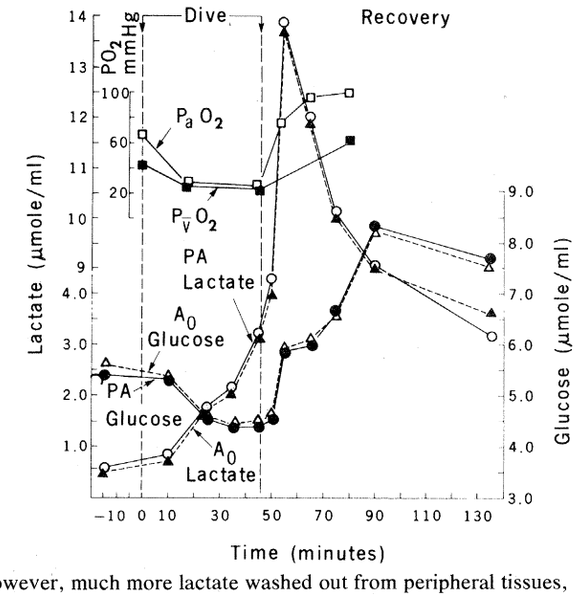

Fig. 1. Changes in glucose and lactate concentration in the blood of the central circulation during a 46-minute experimental dive of an awake, restrained 1100-lb (~ 500-kg) Weddell seal (seal 4, dive 2). Blood samples were taken from catheters in the aorta (A_0 samples) or pulmonary artery (*PA* samples). The total time period between the end of dive 1 and the beginning of dive 2 was 2 hours. In dive 1, about half the glucose used had appeared as lactate in blood; in the dive illustrated, all of the glucose used appears as lactate. During recovery from dive 1, only a modest amount of lactate had been observed to wash out of the peripheral tissues and circulation (peak concentrations were only about 3.5 μmole/ml); during recovery from dive 2, however, much more lactate washed out from peripheral tissues, which produced central blood concentrations nearly 30 times greater than that found in the predive blood samples. Oxygen tensions (PO_2) in the aorta (P_aP_2) and the pulmonary artery ($P_{\bar{v}}O_2$) are shown on the inset scale.

Table 2. Enzyme activities in lung tissue of the Weddell seal. Units of activity are in micromoles of substrate converted to product per minute per gram at 37°C. Averages of three determinations are given. Assay procedures have been described (*6*). Lactate dehydrogenase (LDH) and pyruvate kinase were assayed at *p*H 7.4 in 100 m*M* tris (hydroxymethyl) aminomethane (tris); all other enzymes were assayed at *p*H 7.0 in 100 m*M* imidazole. The hexokinase preparations were in 250 m*M* sucrose in *p*H 7.0 imidazole containing 5 m*M* MgATP complex. Mitochondrial pellets were isolated as described (*7*). Abbreviations: G6P, glucose 6-phosphate; $NADP^+$, nicotinamide adenine dinucleotide phosphate; G6PDH, glucose 6-phosphate dehydrogenase; FDP, fructose 1,6-diphosphate; GPI, glucosephosphate isomerase; F6P, fructose 6-phosphate; NAD^+, nicotinamide adenine dinucleotide; NADH, the reduced form of NAD^+; α-GPDH, α-glycerolphosphate dehydrogenase (E.C. 1.1.1.8); ADP, adenosine diphosphate; and DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid).

Enzyme	Assay conditions	Units of activity
Glucose-6-phosphatase	10 m*M* G6P, 1m*M* Mg^{2+}, *p*H 7.0	0.08
Hexokinase (56 percent mitochondrial bound)	1 m*M* glucose, 5 m*M* MgATP, 1 m*M* $NADP^+$, excess G6PDH	1.1
Fructose 1,6-diphosphatase	1 m*M* FDP, 1 m*M* $MgCl_6$, excess GPI and G6PDH, 1 m*M* $NADP^+$	0.1
Phosphofructokinase	1 m*M* F6P, 1 m*M* ATP, 1 m*M* $MgCl_2$, 0.1 m*M* NADH, excess aldolase, triose-P isomerase, and α-GPDH	1.8
Pyruvate kinase	2 to 4 m*M* phosphoenolpyruvate, 1 m*M* ADP, 1 m*M* $MgCl_2$, 0.1 m*M* NADH, excess LDH	32.4
Lactate dehydrogenase	Back reaction: 50 m*M* lactate, 1 m*M* NAD^+, *p*H 7.4 Forward reaction: 1 m*M*, pyruvate, 0.1 m*M* NADH, *p*H 7.4	143.0 557.0
α-Glycerolphosphate dehydrogenase	0.5 m*M* dihydroxy acetone phosphate, 0.1 m*M* NADH, *p*H 7	0.16
Citrate synthase	0.5 m*M* acetyl CoA, 0.5 m*M* oxaloacetate, 0.1 m*M* DTNB, *p*H 7.5 imidazole	1.05

These considerations encouraged us to examine changes in the AV concentration across the lung during diving. A typical result for a 46-minute dive (Fig. 1) shows lactate gradually accumulating in peripheral tissues during diving and being washed out of them into the blood with their reperfusion (*3*). During diving, pyruvate concentrations rose slightly (from 0.06 to 0.12 μmole/ml) while alanine levels remained unchanged. Arterial pressure fell by about 5 mm-Hg during the second half of the dive; at this time, the lung was no longer serving as a gas-exchange organ, the change in arterial and venous pressure being identical (Fig. 1, inset).

At all sampling times except the last (taken 75 minutes after completion of the simulated dive, at which time the lung appeared to be using glucose and releasing lactate), the lactate concentration was measurably lower in aortic blood than in pulmonary blood (mean of 0.105 μmole/ml for four samples taken during the dive). The probability that these values would all differ in the same direction is $P < .01$.

This type of experiment seems to demonstrate that the lung of the Weddell seal uses lactate as a substrate for metabolism, and, therefore that it cannot be the source of the rising blood lactate observed in the central circulation during diving. If half the lactate consumed were fully oxidized by the lung, a yield of 0.8 μmole of adenosine triphosphate (ATP) per gram of lung tissue per minute could be obtained, with a measured diving cardiac output of 5 liter/min; this metabolic rate is similar to that measured (1.2 μmole of ATP per gram per minute) in respiring lung slices (*10*). One fate of any remaining lactate may be reconversion to glucose. If half of the consumed lactate were converted to glucose, it would generate a transpulmonary glucose gradient of about 0.025 μmole/ml, a value close to that observed, the average AV difference for the dive in Fig. 1 being 0.035 μmole/ml. These AV differences in glucose concentrations across the lung were consistently in the opposite direction to those for lactate. If such differences were due to manipulative artifacts, lactate and glucose concentration profiles should be identical and not mirror images of one another. Our results therefore indicate that lactate is used in preference to glucose as substrate for the lung and that one fate of the consumed lactate may be reconversion to glucose for subsequent metabolism by other tissues of the central circulation.

Additional evidence for a modified Cori cycle involving the lung comes from enzyme studies. We found that the seal lung displayed readily measurable amounts of fructose 1,6-diphosphatase (E.C. 3.1.3.11) (FDP) and glucose 6-phosphatase (E.C. 3.1.3.9) (G6P), the first capable of converting 0.1 to 0.2 μmole of substrate to product per gram of lung tissue per minute, the second occurring at about 0.08 such units of enzyme activity (Table 2). As these are gluconeogenic enzymes (*14*), we also looked for phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32) and pyruvate carboxylase (E.C. 6.4.1.1), two additional enzymes that are often (*14*), but not always (*18*), required for de novo synthesis of glucose from triose or amino acid precursors. Although they do not occur at levels greater than 0.05 unit/g, pyruvate kinase (E.C. 2.7.1.40) activity in the gluconeogenic direction can occur at as much as 5 percent of the thermodynamically "downhill" rate (*15*), and its activity (Table 2) could readily pace those of fructose-1,6-diphosphatase and glucose-6-phosphatase. Finally, as already emphasized, lactate dehydrogenase occurs at about 140 unit/g, assayed at *p*H 7.4 in the direction of lactate → pyruvate. Thus, all the enzymes for converting lactate to glucose occur in the seal lung, and under certain conditions, the lung may be able to supplement the supply of circulating glucose.

Although the quantitative role of this process remains to be assessed, there is little doubt that the lung of the Weddell seal uses lactate, not glucose, during prolonged awake diving. It therefore is unlikely to contribute to the drop in blood glucose and rise in blood lactate concentrations during diving. The heart and brain are the most likely alternative organs for this role (*3*), and the final question remains as to which makes the larger contribution. Although a definitive answer will require studies of the metabolite preferences of the two organs, a number of suggestive observations are already available. (i) Electron micrographs indicate extremely high quantities of glycogen (large rosette-shaped granules) in the heart but not in the brain (*16*). (ii) The heart, with 3.1 units of hexokinase per gram, contains only about one-half the brain's potential for competing effectively for glucose (*16*). (iii) During diving, coronary blood flow drops by about 75 percent, while blood flow to the brain rises by about 30 percent (*13*). From these data, we tentatively conclude that during diving, the heart removes less glucose from the central circulation than the brain does. From P_{O_2} and blood lactate measurements (Fig. 1), it would appear that some complete glucose oxidation and some glucose fermentation contribute to the uptake of glucose by the brain, a situation similar to that in hypoxia in other mammals (*17*).

Our studies indicate that during diving, the seal's lung conditions central circulating blood (i) by reducing lactate accumulation through the use of lactate in preference to glucose for energy metabolism and possibly (ii) by releasing glucose into the blood, a process that would supplement the circulating supply for the brain. It is possible that in the Weddell seal we have described a process uncommon in terrestrial mammals; if universal, however, similar metabolic functions of the lung may be important in conditions such as severe shock, when cardiac output is reduced and lactate acidemia prevails.

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5. The thoracic aorta was catheterized via the radial artery, and a Swan-Ganz thermodilution catheter was inserted via a jugular vein into the pulmonary artery. Both catheters were connected to pressure transducers for continuously monitoring blood pressure; the electrocardiogram was recorded from subcutaneous needle electrodes. Ambient temperature in the laboratory was maintained at approximately 4°C, and the seal's flippers and dorsum were bathed with ice-cold seawater as needed to maintain body temperature below 38°C. To simulate diving, the animal's head was inserted through a round hole in a reinforced acrylic box 60 cm by 60 cm by 60 cm, a sleeve around the neck preventing water leaks when the box was filled with seawater and ice. The seal was restrained by a cargo net and allowed to recover from anesthesia for 4 to 6 hours. Then it was observed in simulated diving for a relatively short period (10 to 15 minutes); we monitored various physiological processes and collected blood samples for P_{O_2}, P_{CO_2}, and *p*H, and metabolite determinations before and during the dive and after complete recovery. Subsequently, a longer period of simulated diving was observed; physiological processes were again monitored to ensure full recovery. Cardiac output at various stages before, during, and after diving was determined through the use of a thermal dilution technique, and blood flow to various tissues was determined with labeled microspheres. [See G. F. Maruschak, E. A. Meathe, J. F. Schauble, A. Fronek, *J. Appl. Physiol.* **37**, 414 (1974); J. R. S. Hales, *Clin. Exp. Pharmacol. Physiol.* **1** (Suppl.), 31 (1974).]
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9. A possible "artifactual" source of lactate could be leakage from the peripheral circulation, normally closed during diving (*3*) by a powerful vasoconstricting reflex. Measurements of blood levels of lactate, pyruvate, and alanine makes such leakage unlikely. During recovery, when the peripheral circulation is known to open, the increase in blood pyruvate is greatest (up to 50-fold), that of alanine is 2- to 3-fold, and that of lactate about 10-fold (Fig. 1). In contrast, in the central circulation, during diving, the relative increase in lactate is about three times that of pyruvate, and alanine concentrations remain unchanged. Because of such differences in direction and the magnitude of change, we conclude that leakage from the peripheral circulation would not make a major contribution to the rising lactate concentrations in the central circulation during diving.
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12. Simultaneous pulmonary AV concentration differences were determined for glucose and lactate taken before diving from animals with normal heart rates and cardiac output. The values in micromoles per milliliter for glucose and lactate (in parentheses) were −0.24 (+0.15), −0.28 (+0.08), +0.03 (+0.10), −0.16 (+0.40), 0.0 (0.0), 0.0 (0.0), and −0.16 (−0.10) for seven samples. The average AV difference for glucose was −0.1083 ± 0.1357 (standard deviation), and for lactate it was +0.0900 ± 0.1592. These values are not significantly different from zero (*t*-test, $P > .05$). During bradycardia and 5 minutes of recovery, the values for glucose and lactate (in parentheses) were +0.11 (−0.6), +0.26 (−0.15), +0.37 (0.0), +0.14 (−0.03), +0.08 (−0.11), +0.02 (−0.13), +0.01 (−0.08), +0.05 (−0.10), +0.06 (−0.10), and +0.03 (−0.32) for ten samples. The average AV difference for glucose under these conditions was +0.1130 ± 0.1166, and the average AV difference for lactate was −0.1630 ± 0.1756 ($P < .02$). Under these conditions, therefore, the Weddell seal lung seems to use lactate and produce glucose.
13. Cardiac output in awake seals was about 35 to 40 liter/min; during intense diving bradycardia it dropped to 4 to 8 liter/min. In the dive shown in Fig. 1, cardiac output 25 minutes into the dive was 5 liter/min. In addition, our microsphere experiments established quantitative flow to various tissues and organs before, during, and after diving.
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22 February 1977; revised 16 May 1977

Mucopolysaccharidosis in a Cat with Arylsulfatase B Deficiency: A Model of Maroteaux-Lamy Syndrome

Abstract. *A Siamese cat that presented clinical signs similar to those seen in humans with mucopolysaccharidoses was studied. The animal excreted increased amounts of polymeric glycosaminoglycans in the urine, consisting almost entirely of dermatan sulfate. Electron microscopy of circulating polymorphonuclear leukocytes revealed the presence of many membrane-bound lamellar inclusion bodies. Sulfate incorporation studies with cultured skin fibroblasts indicated defective glycosaminoglycan degradation. These cells showed a deficiency in arylsulfatase B activity. The disorder appears similar or identical to the Maroteaux-Lamy syndrome described in humans.*

Increasing numbers of inborn errors of metabolism have been described in domestic animals in recent years, many of which have great potential as models of human diseases. Several lysosomal storage diseases, primarily lipid storage disorders, have been reported (*1*), and we have described the occurrence and clinical features of a mucopolysaccharidosis in a Siamese cat (*2*). We now report further evidence that this disease is a lysosomal storage disorder, that the mucopolysaccharide excreted in excess is dermatan sulfate, and that the defect is due to a deficiency in arylsulfatase B and represents an animal model of Maroteaux-Lamy syndrome.

The proposita was first seen at 21 months of age with an acute hindlimb lameness, small stature, a short broadened face with swollen drooping eyelids, corneal clouding, and a history of progressive difficulties with locomotion. Radiographic findings included fusion of cervical and lumbar vertebrae, flaring of the ribs, multiple exostoses, and epiphyseal dysplasia of the long bones. The cat excretes greatly increased amounts of cetylpyridinium chloride (CPC)-precipitable glycosaminoglycans (GAG) in her urine (*2*).

Measurement of the ratio of urinary polymeric GAG to oligosaccharides that contain uronic acid has been shown to be useful in detecting defects in mucopolysaccharide degradation (*3*). This ratio in normal human urine varies between 0.1 and 0.4 and is invariably less than 1. In pooled urine from eight clinically normal cats, the polymeric GAG: oligosaccharide ratio was 0.89 (1.16:1.30 mg of hexuronic acid per 25 ml of urine, respectively); and in the affected cat it was 5.89 (16.70 : 2.83), the absolute amount of polymeric GAG in the affected animal's urine being approximately 14 times greater than that in normal cat urine.

CPC-precipitable GAG's were isolated from the affected cat's urine and from pooled normal cat urine; Tamm-Horsfall glycoprotein was removed by salt precipitation, and the purified GAG was separated by electrophoresis on cellulose acetate (*4*). The primary GAG component from the affected cat migrated with dermatan sulfate (DS) with a small amount of chrondroitin sulfate (CS), and a trace of heparan sulfate (HS) also present, whereas normal cat urine contained primarily CS, with a small amount of DS and a trace of HS (Fig. 1a). Determination of the CS and DS content of similar urines by selective reaction with orcinol (*5*) confirmed the electrophoretic findings. Urine collected from eight clinical-

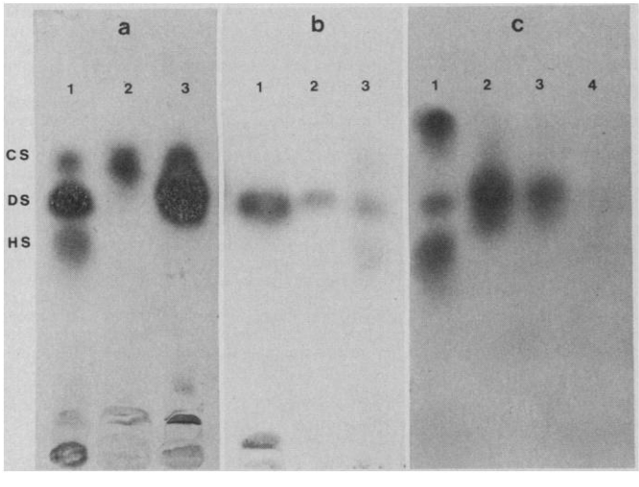

Fig. 1. Electrophoretic separation of urinary glycosaminoglycans (GAG). Separations were performed on strips (5 by 12.7 cm) of cellulose polyacetate (Sepraphore III, Gelman Instrument Co., Ann Arbor, Michigan) using 0.05*M* barium acetate, *p*H 8.0, at 100 volts for 180 minutes. The GAG were visualized with either toluidine blue or alcian blue GZ. (a) Total GAG from urine of a human patient with Hurler's syndrome (1), pooled cat urine (2), and urine from the affected cat (3). (b) The GAG fraction obtained by alkaline copper precipitation from the affected cat's urine (1), DS from pig skin (Sigma Chemical Co., St. Louis, Missouri) (2), total GAG from patient with Hurler's syndrome (3). (c) Mixture of commercially available GAG (1); from top to bottom chondroitin sulfates A and C (*CS*), dermatan sulfates (*DS*), and heparan sulfate (*HS*). Total GAG from the affected cat's urine (2), same, after treatment with chondroitinase AC (3), same, after treatment with chondroitinase ABC (4).