22. B. S. McEwen and D. W. Pfaff, *Trends Neurosci.* **8**, 105 (1985).
23. For all groups, 128 cultures were examined before treatment and 75 cultures after treatment (59% survival rate). Cultures failed to survive due to cell death or bacterial contamination. The survival of cultures was independent of treatment. The survival rate of cultures without inhibitors (control, 1× 5-HT, and 5× 5-HT) was 58% (22 of 38). Cultures treated with the protein synthesis inhibitors (±5-HT) had a survival rate of 58% (28 of 48), and those treated with the transcriptional inhibitors (±5-HT) had a survival rate of 60% (25 of 42).
24. R. T. Ambron *et al.*, *J. Neurosci.* **5**, 2857 (1985).
25. We thank R. Woolley and K. Hilten for their technical assistance, N. Dale, G. Clark, and R. Hawkins for critical review of the manuscript, and H. Ayers and A. Krawetz for typing the manuscript. Supported by fellowships from the European Molecular Biology Organization (P.G.M.), Helen Hay Whitney Foundation (P.G.), and McKnight Foundation (S.S.); NIH grant NS 19595 (V.F.C.); and Howard Hughes Medical Institute (E.R.K. and J.M.).

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Lactate Transporter Defect: A New Disease of Muscle

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New methods were used to identify the abnormality in a patient who showed evidence of neuromuscular dysfunction on extensive clinical examination. The methods revealed that the lactate content of the patient's skeletal muscle does not decline normally after exercise and that his red cells are defective in lactate transport. These results suggest that skeletal muscle and erythrocyte membranes share the same genetic lactate transporter (or a common subunit), which is deficient in this patient. This defect may be a common cause of elevated serum creatine kinase levels, as seen in the patient described here and of unexplained episodes of rhabdomyolysis and myoglobinuria.

STRIATED MUSCLE HAS A GREATER DYnamic range than any other tissue. Upon strenuous exercise its oxidative demands may increase a hundredfold, and its glycolytic rate, a thousandfold (*1–3*). Moreover, it is the inherent paradox of the contractile machinery that attainment of maximal force by the recruitment of all fiber groups, with the greatest demand for oxidative energy provision, simultaneously produces the greatest blockade of capillary blood flow and hence the most anoxic state. It is particularly for this tissue that the distinction between conditioning and disease becomes blurred, since poor conditioning may produce more functional compromise in muscle than many diseases produce in other target organs. Muscle is thus a prime candidate for functional or metabolic diseases, that is, enzyme or carrier defects that limit maximal performance, but may be unaccompanied by anatomic pathology. Examples of such defects have thus far been limited to enzyme deficiencies, such as those involved in glycolysis and glycogenolysis (*4–6*) and myoadenylate deaminase (*7*). I have previously referred to some of these enzymes as "perquisitory" catalysts, because, although not essential for muscular activity, they provide important perquisites for maximal performance (*8*). One may anticipate, therefore, that defects in such catalysts may produce "diseases of healthy people."

Since most patients whose major complaint is of muscle pain or weakness are never identified with a specific disease or pathologic diagnosis (*9*), it occurred to me that a logical candidate for the pathogenesis of some of these cases would be the lactate transporter of the cell membrane. This transporter has been known for about a dozen years. It was first demonstrated in bacterial cells, then later studied in detail in mammalian tumor cells (*10*) and human red blood cells (*11*). It is now clear that it is present in most animal cells and tissues that produce lactate above their utilization level, which include erythrocytes and skeletal muscle.

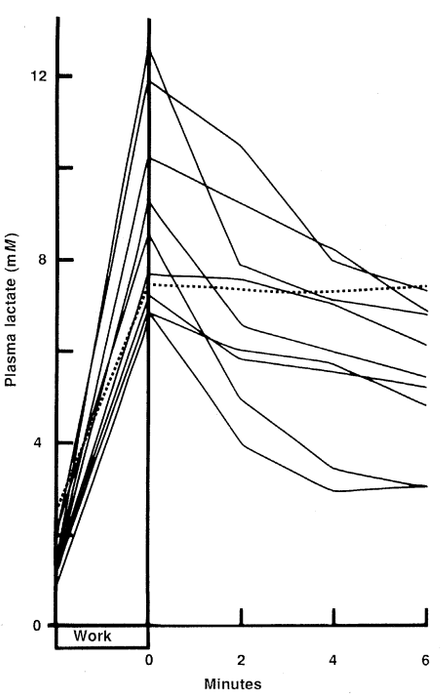

Fig. 1. Rise and decline of plasma lactate in nine control subjects and the affected patient (dotted line) after 2 minutes of ischemic forearm exercise. All cases show a peak lactate immediately after exercise, and all, except the patient, show a considerable decline during 6 minutes of reperfusion.

Often referred to as the organic anion or substituted monocarboxylate transporter because of its generic carrier properties (*12*), this transporter's main function is to export lactate, the major organic acid that accumulates intracellularly. Although, to my knowledge, there have been no definitive studies on its isolation and characterization, the transporter seems to be a membrane protein that cotransports a lactate anion and a proton externally (OH^- uptake has not been excluded), without a requirement for adenosine triphosphate (ATP), to reduce and prevent intracellular metabolic acidosis (*10–12*). The carrier must contain one or more essential sulfhydryl groups, since micromolar concentrations of organic mercurial compounds inhibit the transporter, and permit one to conclude that it is responsible for about 90% of lactate efflux; the remainder is carried by diffusion and by the chloride/carbonate exchanger (*13*). The transport rate is sensitive to the *p*H gradient across the cell membrane, and increases markedly as the intracellular *p*H falls with accumulation of lactic acid (*10–12*).

The human red blood cell, which has no aerobic energy-yielding pathway, is almost completely dependent upon glycolysis for its energy stores, and therefore upon rapid lactate export. Lactate efflux is even more critical for muscle, which produces more lactate than it uses under all conditions, even at rest. Although aerobic electron transport is a much more efficient energy producer, glycolysis is much faster, and muscle metabolism is designed to maintain an adequate supply of pyruvate (and hence lactate) for all oxidative capabilities. If the excess lactic acid is not exported rapidly, the developing acidosis will render inefficient both the contractile apparatus and the energy-yielding pathways.

Over the past year we have developed a clinical assay for the human erythrocyte lactate transporter and an ischemic exercise test suitable for evaluating muscle lactate transport. These have been applied to normal subjects and to patients with unexplained muscle dysfunction. I report here the first case of what may prove to be a common muscle disease.

The patient was a 26-year-old military drill instructor in superb physical condition. He had had three brief episodes of severe, diffuse anterior chest pain over the past 5

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years. On the last occasion he was hospitalized and examined for possible myocardial infarction, but results of a complete cardiac evaluation were normal except for elevated serum enzymes; in particular, the creatine kinase (CK) was 2100 IU (normal <200). He felt well and was discharged to regular activity, but monitoring of his CK level was scheduled twice weekly for the next month. His CK levels ranged from 1,272 to 13,700 IU, with 90 to 98% consisting of the skeletal muscle isozyme (MM). He was then transferred to a medical center for further evaluation of unexplained muscle disease. There, despite 2 weeks of inactivity, his CK remained between 700 and 900 IU, although results of complete cardiac and neuromuscular examinations, including electrocardiogram, electromyogram, and frozen and fixed muscle biopsies, were normal. The patient was adamant in his denial of any drug use, and he refused narcotic medication after his muscle biopsy.

He carried out an ischemic forearm exercise test in our laboratory, and his performance was impressive in terms of quantitative work and impulse (momentum), and he showed a normal increase in lactate and ammonia levels. However, his lactate level did not decline for 6 minutes after completion of work and restoration of blood flow. In our test, blood samples are equilibrated with muscle (*14*), and all subjects have shown maximum lactate levels immediately after exercise. Thus, the subsequent decline can be used to assess muscle lactate efflux. Figure 1 compares the patient's response to that of nine normal subjects. For each test, all lactate values can be normalized to the initial maximum lactate increase (above resting level), taken as 100%, to gauge the rate and pattern of decline. This has been done in Fig. 2 for 20 tests on 12 control subjects, and the patient's response is clearly outside the normal range.

Although this response suggests a deficiency in muscle lactate efflux, it is not, of itself, sufficient evidence because it might reflect a high aerobic glycolysis during recovery. A metabolic defect in oxidative anabolism, for example, would render the regeneration of ATP during recovery (that is, upon reperfusion) entirely dependent on glycolysis, and hence on continued high lactate production. In such a case, the "oxygen debt" would be repaired by glycolysis rather than by oxidation, and the muscle lactate level might remain high (or even rise further) despite effective efflux.

In this patient, however, direct assay of the erythrocyte lactate transport revealed that this function was defective. Our transporter assay involves diluting the washed, lactate-loaded red blood cells into a glucose-free and lactate-free buffer (*p*H 7.5), and measuring the residual cell lactate at timed intervals (*15*). The lactate decline is fitted by least squares to a first-order exponential decay curve (Fig. 3). In the presence of 20 μM *p*-hydroxymercuribenzoate (PHMB), which specifically blocks the lactate transporter (*12, 13*), the same preparation shows minimal lactate decline.

From each assay a single, best-fit point was obtained consisting of the initial lactate level (from the intercept) and the initial efflux velocity (from the slope and intercept) for use in a substrate-velocity plot. For each subject, points were obtained from at least two assays at different lactate loads. Figure 4 shows a substrate-velocity plot of 90 such assays. The patient's data fall below all control values, and their best-fit rectangular hyperbola indicates a maximum velocity (V_m) 30% of the mean normal value. This patient thus shows both a defective red

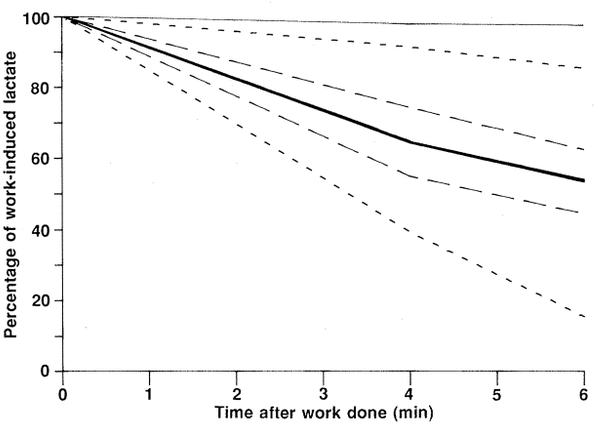

Fig. 2. Decline of the work-induced lactate increase (over resting levels) in 20 ischemic exercise tests on 12 control subjects. The increase immediately after exercise is taken as 100% in each test. Shown are the mean decline (heavy solid line), its 95% confidence limits (long dashed lines), and the extreme individual range (short dashed lines). The affected patient's decline (the topmost light line) is outside the extreme range for controls.

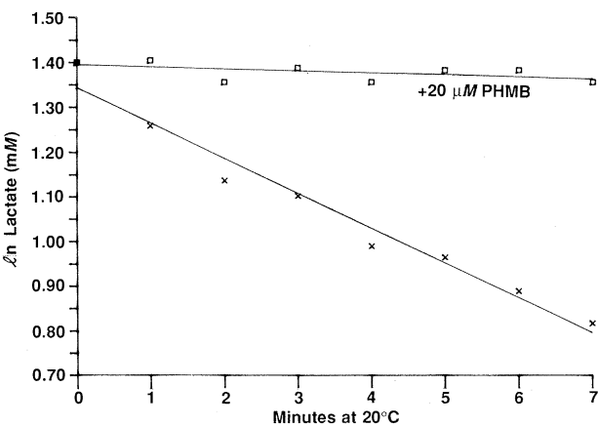

Fig. 3. Semilogarithmic plot of the decline of erythrocyte lactate levels in a representative efflux assay at 20°C. The least-squares fitted line is used to obtain the initial lactate level and initial velocity, providing one point for a substrate-velocity plot. If 20 μM PHMB, an inhibitor of lactate transport, is included in the assay medium, the same sample shows minimal lactate decline.

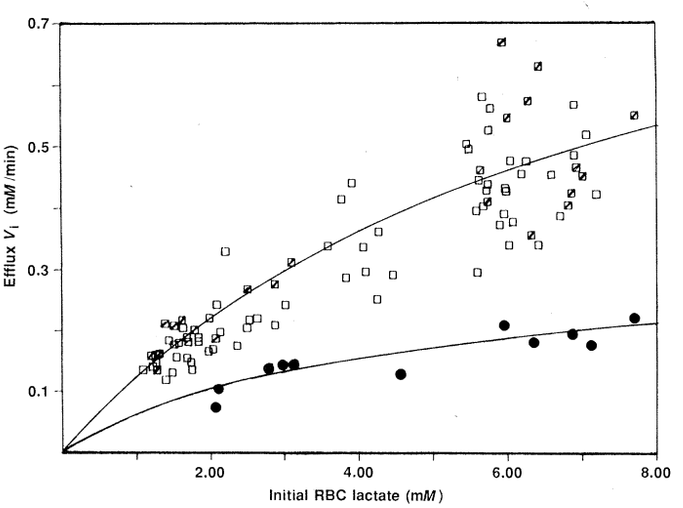

Fig. 4. Substrate-velocity (V_i) plot of the red blood cell (RBC) lactate efflux at 20°C. The results of 90 assays are shown, from 24 control subjects (14 males □, 10 females ⊠). For the affected patient, 11 assays are shown from three blood samples taken at weekly intervals. The upper curve shows the best-fit rectangular hyperbola for the control data, and yields a binding constant (K_m) of 7.2 ± 1.3 mM and maximum velocity (V_m) of 1.02 ± 0.11 mM/min (mean ± SEM). The lower curve shows the corresponding hyperbola for the patient, with K_m of 4.31 ± 0.31 mM and V_m of 0.31 ± 0.05 mM/min.

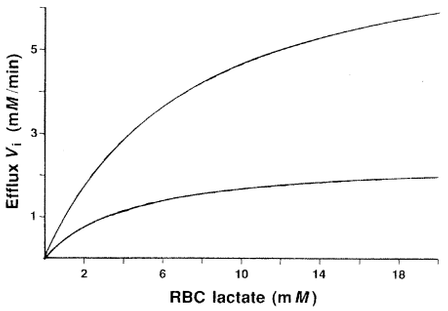

Fig. 5. Best-fit rectangular hyperbolas (Michaelis-Menten plots) for the mean normal values (upper curve) and for the patient's data (lower curve) in the red blood cell (RBC) efflux assay, extrapolated to 37°C and to high lactate levels. The K_m values are the same as in Fig. 4; the V_m values are 8.0 mM/min (SEM 11%) for the control subjects and 2.4 mM/min (SEM 16%) for the patient. At lactate levels above 8 mM (which normally occur only in muscle), the patient's cells cannot significantly increase lactate efflux to limit further elevations.

blood cell lactate transporter and a failure of muscle lactate to decline normally after exercise. The only parsimonious explanation is that human skeletal muscle and erythrocytes share the same genetic lactate transporter, or at least a common genetic subunit, which is defective in this patient.

Why call this a disease of muscle, when the defect is most convincingly demonstrated in the erythrocyte? The reason becomes clear from a comparison of the mean normal and the patient's hyperbolic plots at higher lactate levels (and at body temperature). This is easily done, since the binding constant changes little with temperature, and the activation energy is known (*11*). The discrepancy from normal only becomes marked above 8 mM lactate, where the patient's efflux can no longer increase significantly upon further lactate increase (Fig. 5). The only tissue in the body normally harboring such high levels is skeletal muscle, wherein the lactate may reach 25 to 30 mM upon extreme exercise. Indeed, an important role of the erythrocyte carrier may be to assist the muscle's lactate excretion. Since the red blood cells contain a third of the total water volume of blood, their rapid uptake of lactate egressing from muscle to plasma would retard the rise of plasma lactate concentration and facilitate continued efflux from muscle.

After extreme exercise, then, this patient may be unable to decrease his intramuscular acidosis with sufficient rapidity and might therefore be at risk of acute rhabdomyolysis and myoglobinuria. In the absence of extreme stress, however, he might encounter no difficulties; and this defect, like myoadenylate deaminase deficiency (*8*), may well be encountered in asymptomatic subjects, once the methods for detection are widely applied. Lactate transporter defect may ultimately be found to be a common cause of chronically elevated serum CK levels and of unexplained attacks of rhabdomyolysis and myoglobinuria in patients undertaking extreme muscular activity.

REFERENCES AND NOTES

1. P. Banks, W. Bartley, L. M. Birt, *The Biochemistry of the Tissues* (Wiley, New York, ed. 2, 1976), pp. 93–158.
2. L. D. Peachey and R. H. Adrian, Eds., *Handbook of Physiology*, section 10, *Skeletal Muscle* (American Physiological Society, Bethesda, MD, 1983).
3. P.-O. Astrand and K. Rodahl, *Textbook of Work Physiology* (McGraw-Hill, New York, ed. 2, 1977).
4. B. McCardle, *Clin. Sci.* **10**, 13 (1951).
5. S. Tarui *et al.*, *Biochem. Biophys. Res. Commun.* **19**, 517 (1965).
6. S. DiMauro, A. F. Miranda, S. Khan, K. Gitlin, R. Friedman, *Science* **212**, 1277 (1981).
7. W. N. Fishbein, V. W. Armbrustmacher, J. L. Griffin, *ibid.* **200**, 545 (1978).
8. W. N. Fishbein, in *Myology*, A. G. Engel and B. Q. Banker, Eds. (McGraw-Hill, New York, 1986), vol. 2, pp. 1745–1762.
9. K. R. Mills and R. H. T. Edwards, *J. Neurol. Sci.* **58**, 73 (1983).
10. T. L. Spencer and A. L. Lehninger, *Biochem. J.* **154**, 405 (1976).
11. W. P. Dubinsky and E. Racker, *J. Membr. Biol.* **44**, 25 (1978).
12. B. Deuticke, in *Membrane Transport in Erythrocytes*, U. V. Lassen, H. H. Ussing, J. O. Wieth, Eds. (Munksgaard, Copenhagen, 1980), pp. 539–555.
13. B. Deuticke, E. Beyer, B. Forst, *Biochim. Biophys. Acta* **684**, 96 (1982).

14. Venous trapping at half-diastolic pressure is used for 10 seconds before and during each specimen collection, for a total stasis time of 20 to 25 seconds. This is a reasonable time for equilibration with muscle by diffusion, as judged by our erythrocyte transport data (see text). Since lactate transport is not energy-coupled, the level of lactate in plasma cannot exceed that in the muscle and may well underestimate it. However, the main objective is to see how much the level falls during free blood flow between samplings.
15. Aliquots of the stirred sample are removed at 30- or 60-second intervals and diluted in ice-cold buffer (*p*H 5) to stop efflux. The cells are harvested and washed by centrifugation at 0°C, then lysis and assay follow (*11*). A detailed procedure will be published elsewhere.
16. I am indebted to S. M. Muldoon for referring this patient. Informed consent was obtained from all participants in the study, in accordance with institutional Human Use Committee guidelines. The control subjects were all healthy normal adult volunteers. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Energy Sources for Detritivorous Fishes in the Amazon

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Detritivorous fishes form an important part of the ichthyomass in the Amazon basin. Most of these fishes are contained in the orders Characiformes and Siluriformes (catfishes). The Characiformes constitute more than 30% of the total fish yield in the Amazon basin, whereas the catfishes are of minor importance. Stable isotope data indicate that Characiformes species receive most of their carbon through food chains originating with phytoplankton, while the Siluriformes receive a significant part of their energy from other plant sources.

THE PRIMARY PROTEIN SOURCE FOR the human population in the Amazon basin is fish (*1*). More than 30% of the fish consumed in the Amazon basin are detritivores (*2–4*), and evidence shows that this percentage has risen in recent years (*4, 5*). Most of these detritivorous fishes are contained in the order Characiformes (families Prochilodontidae and Curimatidae), whereas the catfishes (Siluriformes) form a second minor group.

Effective management of these populations will require an understanding of factors controlling their production. A critical first step will be to identify the plant carbon sources that fuel the detritivore food chain. There are four general groups of autotrophs in the Amazon region that could support detritivorous fishes, either directly or indirectly: trees, phytoplankton, periphyton, and aquatic macrophytes. Both aquatic macrophytes (*5, 6*) and trees (*7*) have been suggested as the principal carbon source for detritivorous fishes in the Amazon. There is also some evidence that algae can be important in the detritivore diet (*5, 7*). These hypotheses, however, are largely based on visual analyses of stomach contents, which can be misleading. These fishes are bottom feeders, and the stomach analyses generally reveal a complex mosaic of food items in their diet, including microinvertebrates, al-

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