did the controls, or that synthesis of DBH was reduced. In any case, a loss of fibers or a reduction in synthesis should not have affected our measure of transport, which depended on levels of DBH actually present in the nerves. Reduced accumulation points, therefore, to a defect in the system for transport of DBH in these abnormal nerves. Because DBH is ordinarily associated with catecholamine storage vesicles in adrenergic axons (*11*), reduced transport of this enzyme could reflect abnormalities in the vesicles or in the packaging of DBH into vesicles. On the other hand, reduced transport of DBH might well reflect an abnormality in the machinery for rapid axonal transport per se (*12*). These alternatives require further study. It does appear, however, that examination of the axonal transport of DBH and other marker substances may yield new insights into the mechanism underlying some peripheral neuropathies.

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References and Notes

1. P. Weiss and H. B. Hiscoe, *J. Exp. Zool.* **107**, 315 (1948).
2. A. Dahlström and J. Häggendal, *Acta Physiol. Scand.* **67**, 278 (1966); S. Barondes, *Neurosci. Res. Progr. Bull.* **5**, 307 (1967); R. J. Lasek, *Int. Rev. Neurobiol.* **13**, 289 (1970); B. Grafstein, *Advan. Biochem. Psychopharmacol.* **1**, 11 (1969).
3. For a review of the tissue and subcellular localization of DBH, see L. B. Geffen and B. G. Livett, *Physiol. Rev.* **51**, 98 (1971). For evidence that the sural and other cutaneous nerves contain sympathetic fibers, see G. A. G. Mitchell, *Anatomy of the Autonomic Nervous System* (Livingstone, Edinburgh, 1953), p. 207.
4. The methods, symptomatology, and usefulness of sural nerve biopsy have been discussed by P. J. Dyck and E. P. Lofgren [*Med. Clin. N. Amer.* **52**, 885 (1968)].
5. Composition of physiological saline solution: Na^+, 142 meq/liter; K^+, 5 meq/liter; Ca^{2+}, 3 meq/liter; Mg^{2+}, 2 meq/liter; Cl^-, 102 meq/liter; HCO_3^-, 24 meq/liter; PO_4^{3-}, 3 meq/liter; SO_4^{2-}, 2 meq/liter; and glucose, 5.6 m*M*.
6. P. B. Molinoff, R. Weinshilboum, J. Axelrod, *J. Pharmacol. Exp. Ther.* **178**, 425 (1971).
7. S. Brimijoin, *ibid.* **183**, 298 (1972).
8. ———, *J. Neurochem.* **19**, 2183 (1972); J. Coyle and F. Wooten, *Brain Res.* **44**, 701 (1972).
9. In the last case, DBH has presumably reached distal regions of the nerve by some process that is too slow to measure by the present techniques, for example, diffusion or slow transport.
10. J. L. Jammes, *Arch. Neurol.* **27**, 213 (1972).
11. H. Hörtnagl, H. Hörtnagl, H. Winkler, *J. Physiol. London* **205**, 103 (1969).
12. The present results should be compared with a previously reported defect in slow axoplasmic transport in cats with acrylamide-induced neuropathy [D. E. Pleasure, K. Mishler, W. D. Engel, *Science* **166**, 524 (1969)].
13. Research supported in part by NIMH grant MH-21413, NIH grants NS05811 and NS0741, and the Gallmeyer, Miller and Upton Funds.

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Heart Muscle Viability following Hypoxia: Protective Effect of Acidosis

Abstract. *The mechanical performance of hypoxic heart muscle is further depressed by an acid* p*H. In contrast to preparations at normal or alkaline* p*H, however, hypoxic preparations at acid* p*H do not develop contracture and exhibit full recovery of mechanical activity upon reoxygenation.*

It is generally agreed that an acid *p*H, particularly in the presence of myocardial hypoxia, is detrimental to cardiac contractile function and to the organism as a whole. An alkaline *p*H, on the other hand, improves the contractile function of hypoxic heart muscle.

Agents that enhance the performance of ischemic or hypoxic heart muscle, however, may accelerate deterioration (*1*), whereas myocardial depressants decrease cardiac energy requirements and preserve viability. In view of these observations and the results of the present experiment, it may be necessary to reevaluate our concepts of the significance of *p*H on hypoxic myocardium. Experiments were carried out to determine the effects of *p*H on heart muscle function during hypoxia and on recovery of mechanical activity after reoxygenation.

Papillary and trabecular carneae muscles were dissected from the left ventricles of rats killed by decapitation and were mounted in a muscle chamber containing Krebs-Henseleit solution (*2*) gassed with 95 percent O_2 and 5 percent CO_2. Glucose (5 m*M*) was present in the bath solution, kept at a constant temperature of 28°C. Muscles were stimulated 12 times per minute by parallel platinum electrodes delivering 5-msec square-wave pulses at voltages 10 percent greater than the minimum required to produce a maximum mechanical response. Stimulation thresholds did not change significantly throughout the study. Experiments were carried out with preparations contracting isometrically at the apexes of their length tension curves, and unstable or poor preparations were discarded. Developed tension at *p*H 7.4 under oxygenated conditions was 5.72 ± 0.42 g/mm². Resting tension was less than 20 percent of developed tension and did not vary significantly prior to hypoxia.

The *p*H of the solution was changed by addition of 1*N* HCl or NaOH. With a change to *p*H 6.8 or 7.8 alone, tension declined to 93.7 or 90 percent of control values, respectively. After 15 minutes of stable contraction at the new *p*H, hypoxia was initiated by changing the gas mixture to 95 percent N_2 and 5 percent CO_2. After 60 minutes of hypoxia, reoxygenation with

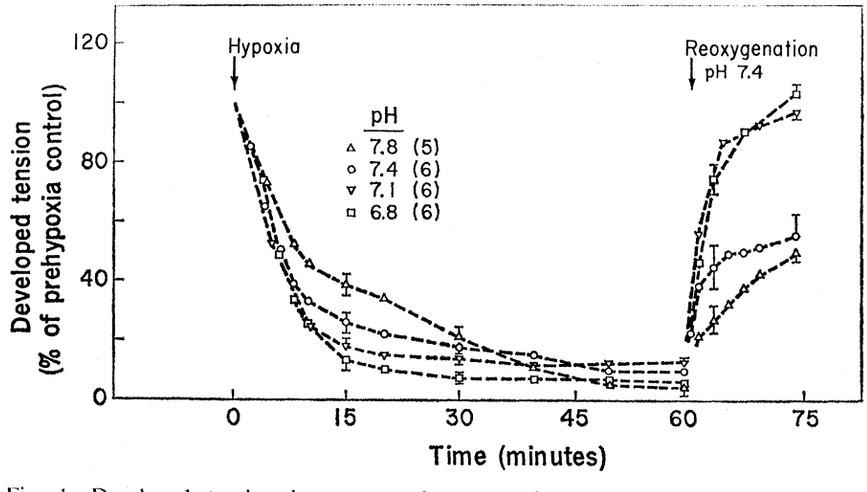

Fig. 1. Developed tension by rat muscle preparations during 60 minutes of hypoxia at varying *p*H values and 15 minutes of reoxygenation at *p*H 7.4. Glucose (5 m*M*) was in the medium. Prehypoxia developed tensions at each *p*H were as follows: *p*H 6.8, 5.25 ± 0.47 g/mm²; *p*H 7.1, 6.73 ± 0.63 g/mm²; *p*H 7.4, 5.70 ± 1.30 g/mm²; and *p*H 7.8, 4.96 ± 0.37 g/mm². The numbers in parentheses following the *p*H values indicate the number of muscle preparations in each group. Brackets represent ± 1 standard error of mean. Although tension is most depressed at an acid *p*H during hypoxia, full mechanical activity is restored upon reoxygenation.

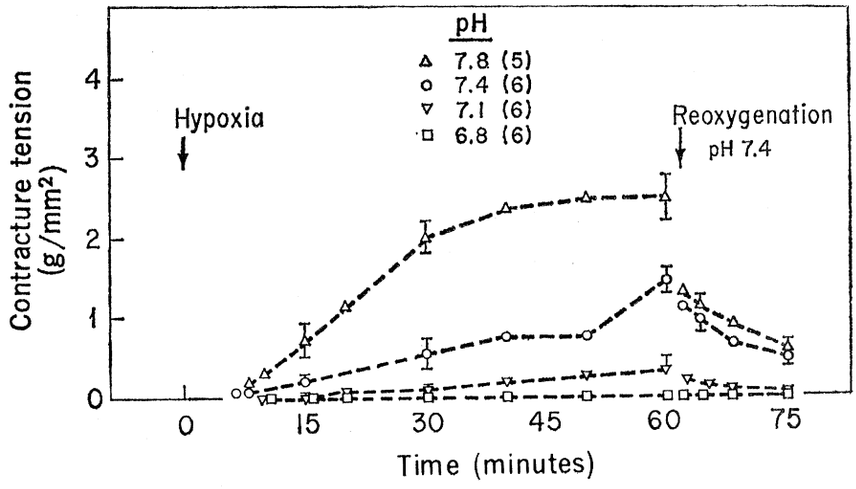

Fig. 2. Contracture tension, developed by rat heart muscle preparations during hypoxia and reoxygenation in the presence of 5 m*M* glucose. The numbers of muscle preparations are in parentheses; brackets show ± 1 standard error of mean. During hypoxia, the most severe contracture is seen at an alkaline *p*H. No contracture occurs during hypoxia at *p*H 6.8.

95 percent O_2 and 5 percent CO_2 took place at *p*H 7.4. The in vitro length of each muscle was measured at the apex of its length tension curve. Following each experiment, the muscle was blotted and weighed, and cross-sectional area was calculated by assuming cylindrical uniformity and a specific gravity of 1.000. Developed and contracture tension were normalized for muscle cross-sectional area. Changes are expressed in absolute terms or as a percentage of prehypoxia control values.

The mechanical activity of isolated rat heart muscle during hypoxia at *p*H 7.4 has been described (*3*). At an acid *p*H, developed tension declined rapidly early during hypoxia (Fig. 1). At *p*H 7.8, on the other hand, higher levels of tension were present at this time. After 15 minutes of hypoxia, developed tensions at *p*H 6.8, 7.1, 7.4, and 7.8 were 13 ± 2.1, 18 ± 2.6, 26 ± 1.6, and 39 ± 3.4 percent of prehypoxia control values, respectively. Developed tension at *p*H 7.4 at this time was significantly different from that at *p*H 6.8 ($P < .001$) and *p*H 7.8 ($P < .01$). Thus, during early hypoxia, an alkaline *p*H enhanced the performance of hypoxic heart muscle while an acid *p*H depressed tension development. These observations are in agreement with those of others (*4*) and document the additional depressive effect of acid *p*H on the mechanical performance of hypoxic heart muscle. Despite the rapid decline in mechanical activity early during hypoxia at acid *p*H, developed tension stabilized, and after 60 minutes approximately 10 percent of prehypoxia tension was developed in all *p*H groups.

After 15 minutes of reoxygenation at *p*H 7.4, preparations previously hypoxic at *p*H 7.4 and 7.8 redeveloped approximately 50 percent of prehypoxia tension; in preparations previously hypoxic at *p*H 6.8 and 7.1, developed tension returned to almost 100 percent of prehypoxia values.

Contracture during hypoxia appeared earliest and to the greatest degree at *p*H 7.8 (Fig. 2). After 60 minutes, contracture tension was 44 percent of prehypoxia developed tension. Lesser degrees of contracture were seen at *p*H 7.4 and 7.1. At *p*H 6.8, no contracture was observed at any time during the 60-minute period of hypoxia. Upon reoxygenation, contracture gradually diminished as recovery took place. Most rapid and complete recovery was seen in those preparations previously functioning at acid *p*H.

During hypoxia, mammalian cardiac muscle must rely on limited stores of anaerobic substrate to maintain activity and preserve integrity. Recovery of function after hypoxia may depend on the extent to which energy stores are depleted during hypoxia. It is well recognized that *p*H influences the activity of several glycolytic enzymes. An acid *p*H, by inhibiting glycolysis, may conserve carbohydrate reserves and facilitate recovery after a period of hypoxia. An equally important mechanism by which a low *p*H may preserve energy stores of ischemic or hypoxic myocardium is by depression of contractile activity, a major energy-consuming reaction in the heart (*5*). It is also possible that *p*H changes during hypoxia may influence the transport of substrate into the cell.

If these observations on isolated heart muscle during hypoxia can be extended to myocardial ischemia in the intact animal, the present results may have clinical relevance. Acidosis is generally implicated in the development of irreversible deterioration and cell death following coronary occlusion. The present experiments have demonstrated a protective effect of acidosis during hypoxia on heart muscle function following hypoxia. It would seem possible, at least during early hypoxia, that acidosis may merely accompany and perhaps even retard other intracellular events that are responsible for cell deterioration.

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References and Notes

1. P. R. Maroko, J. K. Kjekshus, B. E. Sobel, T. Watanabe, J. W. Covell, J. R. Ross, Jr., E. Braunwald, *Circulation* **43**, 67 (1971); O. H. L. Bing, W. W. Brooks, J. V. Messer, *J. Mol. Cell. Cardiol.* **4**, 319 (1972).
2. H. A. Krebs and K. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33 (1932).
3. O. H. L. Bing, J. F. Keefe, M. J. Wolk, L. J. Finkelstein, H. J. Levine, *J. Clin. Invest* **50**, 660 (1971).
4. S. E. Downing, N. S. Talner, T. H. Gardner, *Amer. J. Physiol.* **210**, 1327 (1966); T. R. Gelet, R. A. Altschuld, A. M. Weissler *Circulation* **40** (Suppl. 4), 60 (1969).
5. A. M. Katz and H. H. Hecht, *Amer. J. Med.* **47**, 497 (1969).
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Phytoplankton Algae: Nutrient Concentrations and Growth

I believe that O'Brien misinterpreted the studies of Monod and others in his report "Limiting factors in phytoplankton algae: their meaning and measurement" (*1*). It is not true that ". . . changes in the concentration . . . of most factors that have been identified as limiting . . . cause changes in the growth rate, but not necessarily in the final yield."