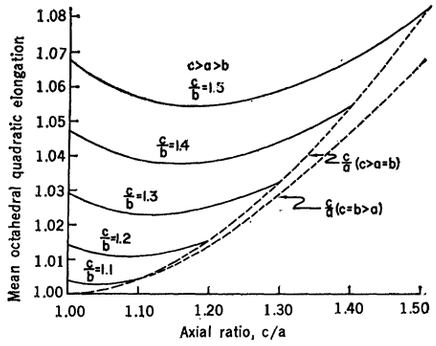

Fig. 3. Mean octahedral quadratic elongation as a function of axial ratios for octahedra with $a \neq b \neq c$; dashed curves are for tetragonally distorted octahedra.

larger M(2) octahedra are more distorted than the smaller M(1) octahedra. However, both the quadratic elongation and the angular variance parameters indicate that M(1) is slightly more distorted than M(2) for disordered olivines and for those containing only one type of octahedral cation.

Figure 2C shows that distortion of the SiO_4 tetrahedron in olivine is related to the effective size of the cations in the octahedra with which it shares edges. Likewise, distortion of the SiO_4 tetrahedron in garnet (Fig. 2D) is dependent on the effective size of the {X} cation in the edge-shared dodecahedra (*6*). In both structure types the tetrahedra become more regular as the cations in the edge-shared polyhedra become larger. For those distorted polyhedra whose bond angles are ideal, for example, an octahedron with D_{2h} or D_{4h} symmetry, the angular variance cannot be used as a measure of distortion. In this case, the quadratic elongation can be calculated with ease, since $l_0 = (a \cdot b \cdot c)^{1/3}$, where a, b, and c are the three center-to-vertex distances in the octahedron. Graphical solutions can be obtained with the use of Fig. 3 in the following manner. For octahedra with D_{2h} symmetry, one would (i) order the metal-anion distances $c>a>b$, (ii) find the value of the ratio c/a along the abscissa, and (iii) plot the ratio c/b among the family of solid curves. For tetragonally distorted octahedra, one would (i) compute the ratio c/a and (ii) use either the upper dashed curve if c is the unique axis ($c > a = b$) or the lower dashed curve if a is unique ($c = b > a$).

If the mean quadratic elongation is used as a quantitative measure of polyhedral distortion, it now should be possible to separate by regression analysis the roles played by distortion and by size in determining the distribution of cations among coexisting phases as well as the polyhedral site preferences within a single phase.

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Maintenance of Resting Potential in Anoxic Guinea Pig Ventricular Muscle: Electrogenic Sodium Pumping

Abstract. *Anoxic ventricular muscle maintained a normal resting potential despite a large loss of potassium. The resting potential was separated into two components: one that depended on the potassium distribution, and one that depended on the activity of an electrogenic sodium pump.*

The ionic hypothesis states that the resting potential of excitable cells results from the distribution of ions across a selectively permeable cell membrane (*1*). The potassium concentration gradient has been considered responsible for the resting potential of muscle cells. Slight measured deviations of the membrane from the behavior of a simple potassium electrode have been accounted for by permeability of the membrane to other ions such as sodium and chloride (*2*). If the resting potential is dependent on potassium distribution, and if both intracellular and extracellular potassium concentrations are known, then the value of the resting potential may be predicted by the Nernst equation

$$E_k = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}$$

where E_k is the potassium equilibrium potential, R is the gas constant, T is the absolute temperature, F is the Faraday constant, $[K]_o$ is the extracellular potassium concentration, and $[K]_i$ is the intracellular potassium concentration. On the other hand, if the sodium gradient also contributes to the resting potential (*3*), the resting potential observed will be lower than that predicted by E_k, particularly at external potassium concentrations in the physiological range (*4*).

Earlier studies (*5*) have shown that guinea pig papillary muscles incubated under anoxic conditions for up to 12 hours have a normal resting potential as judged from the amplitude of the action potential. On the other hand, anoxic muscles have been shown to lose much of their potassium (*6*). Analysis of ^{42}K efflux data indicated the possibility of cell compartmentalization of potassium (*6*), since a rapidly exchanging compartment contained five times more the amount of potassium than that attributable to the extracellular (inulin) space. It was tentatively pro-

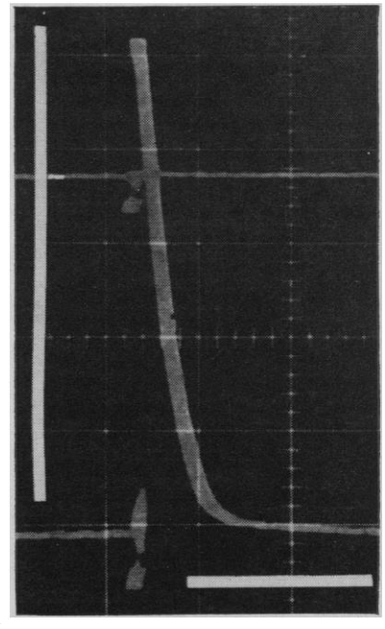

Fig. 1. Action potential of a guinea pig papillary muscle after 8 hours of anoxic incubation in 5 m*M* glucose medium. Voltage calibration is 100 mv and time calibration is 100 msec. Horizontal line across voltage indicates zero potential.

posed that within this restricted compartment the potassium concentration was maintained at a level sufficient to explain the observed resting potentials. Intracellular compartmentalization of potassium in cardiac muscle has been suggested (*7*).

An alternative explanation proposed for the maintenance of resting potential in the face of low intracellular potassium was the existence of an electrogenic sodium pump. This mechanism implies that during a cycle of the pump more sodium is pumped out than potassium pumped in, resulting in a current source in the membrane. An electrogenic pump has been demonstrated in guinea pig atria (*8*), cat atria (*9*), and cat papillary muscle (*10*) after hypothermia. We provide evidence that electrogenic sodium pumping contributes to the maintenance of the resting potential in anoxic cardiac muscle.

Muscles were incubated in a modified Krebs-Ringer medium of the following composition (meq/liter): Na, 138.5 K, 4.6; Ca, 4.9; Mg, 2.3; HCO_3, 21.91; PO_4, 3.48; and Cl, 125.91. The medium was equilibrated with either 95 percent O_2 and 5 percent CO_2 (O_2) or with 95 percent N_2 and 5 percent CO_2 (N_2); the glucose concentration was either 50 m*M* (G_{50}) or 5 m*M* (G_5). Rapid temperature changes were accomplished through the use of parallel water circulators. Electrical activity was recorded as described (*11*), and sodium and potassium were determined by flame photometry after digestion of the muscle in nitric acid. Water content was taken as the difference between wet and dry weight, and the extracellular space was measured with the use of [^{14}C]inulin. No significant changes were observed in the water content or the inulin space during these experiments. The calculation of intracellular sodium and potassium was based on an extracellular space of 267 ml per kilogram of wet tissue, and water content in papillary muscle of 816 ml per kilogram of wet tissue and in ventricular strips of 766 ml per kilogram of wet tissue.

Papillary muscles were incubated for 1 hour in $G_{50}O_2$ medium and then for 8 hours in G_5N_2 medium. Throughout the incubation period the transmembrane electrical activity of the muscle was monitored.

At completion of incubation, the potassium content based on six muscles was 16.7 ± 2.0 mmole/kg, wet weight; the E_k was −47.4 mv; and based on 24 measurements, the E_m was −77.1 ± 0.6 mv. The values are the mean ± S.E.M. Although the action potential duration was only about 15 percent of that seen in fresh muscle (Fig. 1), the resting potential had declined by less than 5 mv. It is evident that potassium distribution in the accepted sense cannot account for the observed resting potential. It is of some interest that despite the severe lack of adenosine triphosphate (ATP) in such muscles (*11*) and the resultant loss of potassium, contractile activity although severely depressed can still be detected.

The operation of an electrogenic sodium pump may be assumed to involve the utilization of an energy source. Marmor and Gorman (*12*) have proposed that the resting potential of *Anisodoris* giant neuron can be separated into an ionic dependent component and a metabolic dependent component. If a similar situation exists in potassium-poor cardiac muscle, it should be possible to examine the ionic component by an interruption of metabolism. Experiments were therefore carried out in which the resting potential of guinea pig papillary muscle and the sodium and potassium contents of right ventricular strips were measured during short exposures to cold at the beginning and at the end of 8 hours of anoxic incubation.

The effect of incubation at 8°C on the resting potential of papillary muscle before and after anoxic incubation was quite different (Fig. 2). An explanation for this difference may be found in the results presented in Table 1. At the end of 1 hour of incubation in $G_{50}O_2$ medium, E_k and the measured resting potential (E_m) were the same. Following 15 minutes of incubation at 8°C both E_k and E_m were reduced 13 mv. The

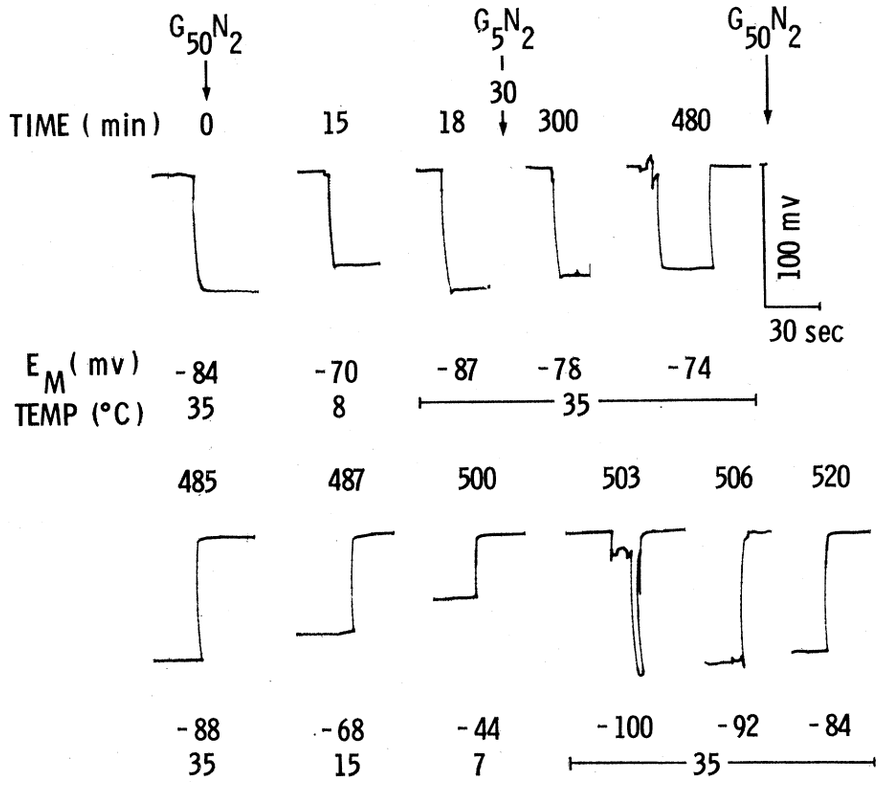

Fig. 2. Alterations in resting potential (E_m) of a guinea pig papillary muscle induced by temperature changes before and after 8 hours of anoxic incubation in 5 m*M* glucose medium. After 1 hour of incubation in 50 m*M* glucose medium in the presence of oxygen ($G_{50}O_2$) at 35°C the E_m was −84 mv. The incubation medium was changed to one without oxygen ($G_{50}N_2$) and the temperature lowered to 8°C for 15 minutes, then raised again to 35°C. The E_m fell to −70 mv during cooling and increased to slightly above control on rewarming. The muscle was then incubated for 450 minutes under anoxic conditions in medium containing 5 m*M* glucose (G_5N_2). The E_m declined during this incubation period, but 5 minutes after elevation of the glucose concentration ($G_{50}N_2$), the E_m increased to the same potential as after early rewarming. On cooling E_m fell to −68 mv in 2 minutes (15°C) and to −44 mv after 15 minutes (7°C). During the following 3 minutes in which the temperature was raised to 35°C the E_m increased to − 100 mv and then returned to control level over 20 minutes. Measurements of resting potential were made in different cells during the experiment and these cells were presumed to be superficial.

Table 1. The intracellular sodium $[Na]_i$ and potassium $[K]_i$ concentration (millimoles per kilogram of fiber water) of guinea pig right ventricular strips during anoxic incubation and two cooling-rewarming cycles. Calculations were based on extracellular space (inulin) of 267 ml per kilogram of wet tissue and water content of 766 ml per kilogram of wet tissue. Calculated potassium equilibrium potentials (E_k) are based on mean of $[K]_i$ and the resting potentials (E_m) measured in papillary muscle incubated under similar conditions. All values are mean ± S.E.M. Numbers in parentheses are either number of preparations analyzed for sodium and potassium or number of E_m measurements made.

Time (min)	Medium	T (°C)	$[Na]_i$ (mmole/kg)	$[K]_i$ (mmole/kg)	E_k (mv)	E_m (mv)
0	$G_{50}O_2$	35	36.7 ± 4.4 (14)	103.8 ± 2.4 (14)	−82.6	−81.1 ± 1.0 (12)
15	$G_{50}N_2$	8	64.8 ± 5.2 (14)	78.9 ± 4.8 (14)	−69.3	−67.9 ± 1.5 (12)
30	$G_{50}N_2$	35	60.2 ± 4.9 (12)	76.5 ± 3.8 (12)	−74.0	−81.8 ± 1.1 (12)
480	G_5N_2	35				−76.4 ± 1.7 (12)
485	$G_{50}N_2$	35	117.0 ± 5.0 (13)	36.7 ± 1.4 (13)	−55.3	−83.0 ± 1.5 (12)
500	$G_{50}N_2$	8	136.5 ± 3.8 (13)	30.5 ± 1.6 (13)	−46.3	−46.4 ± 2.5 (12)
515	$G_{50}N_2$	35	118.0 ± 3.0 (11)	29.4 ± 1.8 (11)	−48.8	−85.9 ± 2.2 (12)

intracellular sodium concentration ($[Na]_i$) had increased sharply and $[K]_i$ had decreased. On rewarming to 35°C for 15 minutes, E_m returned to control level some 8 mv greater than E_k. Neither potassium nor sodium changed significantly, although a slight loss of both may have occurred.

The muscles were then incubated for 450 minutes in G_5N_2 medium. This environment has been shown previously to induce large losses of muscle potassium and gains of sodium (*6*). At the end of this period of incubation, E_m had decreased slightly to −76.4 mv, but on elevation of the glucose concentration to 50 m*M* a slight increase (6.6 mv) in E_m was noted. The E_k at this time was −55.3 mv. An increase in the glucose concentration from 5 m*M* to 50 m*M* has been shown to reverse anoxia-induced shortening of the action potential duration in cat (*13*) and guinea pig (*5*) cardiac muscle. This effect was associated with an increased ATP production (*11*).

During rapid cooling to 8°C and incubation for 15 minutes, additional sodium was gained and potassium was lost (*14*), and E_m fell to a value that approximated E_k. On rewarming to 35°C, there was no change in potassium, a sharp loss of sodium, and a hyperpolarization of the membrane.

The observed responses during these cold-warm cycles indicate that the resting potential of anoxic cardiac muscle is dependent on the activity of an electrogenic pump rather than on a cellular compartmentalization of potassium. If the concentration of potassium in a particular compartment were the same in fresh muscle ($G_{50}O_2$) as in anoxic muscle, and if the potassium gradient between this compartment and the extracellular phase were responsible for the resting potential, E_m would be expected to decline to the same value during each period of cooling. Furthermore E_k, a function of total cell potassium, would not approximate E_m at 8°C. In experiments to be described elsewhere, $10^{-5}M$ ouabain decreased the membrane potential of anoxic muscle, and almost completely blocked the hyperpolarization and extrusion of sodium following hypothermia. Raising the external potassium concentration to 15 m*M* increased the hyperpolarization following hypothermia. This evidence, combined with the absence of chloride contribution to the hyperpolarization after hypothermia in cat papillary (*10*) and uterine muscle (*15*), does not support a role for chloride in the present case.

The results support the concept that the resting potential of anoxic guinea pig ventricular muscle can be separated into at least two components. One component is predicted by the potassium distribution and one is dependent on the activity of an electrogenic sodium pump. Energy from glycolysis has been implicated in the electrogenic pump of mammalian nonmyelinated nerve (*16*). Although glycolytic ATP provides sufficient energy to drive the pump described here, it is not sufficient to maintain the action potential duration and contractility, nor prevent large changes in ion content.

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14. There was a greater loss of $[K]_i$ during the 15-minute cold period in fresh muscle than during the 15-minute cold period after 8 hours anoxia. This finding has been confirmed in a further series of experiments. $[K]_i$ loss during the 0- to 15-minute period at 8°C was compared in fresh muscles and in muscles whose $[K]_i$ had been reduced by 33 or 66 percent after 3 or 8 hours of anoxia at 35°C. The $[K]_i$ loss was less in those muscles having a reduced $[K]_i$ than in fresh muscles, and the relationship between the rate of loss and $[K]_i$ was found to be linear.
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Lesch-Nyhan Syndrome: Rapid Detection of Heterozygotes by Use of Hair Follicles

Abstract. *A method is described which permits rapid phenotypic diagnosis of the Lesch-Nyhan heterozygote by direct assay of hypoxanthine guanine phosphoribosyltransferase activity in single hair follicles obtained from the scalp.*

The Lesch-Nyhan syndrome is a rare metabolic disorder of purine metabolism due to a deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT) (*1, 2*). The gene for HGPRT is X-linked and fibroblast skin cultures derived from female heterozygotes show two cell populations consistent with the Lyon hypothesis, one expressing the mutant *HGPRT*− allele and the other expressing the normal *HGPRT*+ allele (*3*). However, the cells of the hematopoietic system in heterozygotes appear to exhibit only one phenotype, that of the normal allele (*4, 5*). This has been attributed to a selective overgrowth by the normal (*HGPRT*+) cells in the bone marrow and may be related to some special requirement of the bone marrow for the salvage pathway of purine utilization (*6*). Consequently,