

To study the mechanism of periodic paralysis, we investigated the properties of intact muscle fibers biopsied from a patient who had adynamia episodica hereditaria with electromyographic signs of myotonia. When the potassium concentration in the extracellular medium, $[K]_e$, was 3.5 mmol/l, force of contraction, membrane resting potential, and intracellular sodium activity were normal, but depolarizing voltage clamp steps revealed the existence of an abnormal inward current. This current was activated at membrane potentials less negative than -80 mV, reached a maximum within 50 msec, and was not inactivated with time. The inward current was completely and reversibly blocked by tetrodotoxin, which indicates that it was carried by sodium ions. In a solution containing 9 mmol/l potassium, normal muscle would depolarize to -63 mV and yet be capable of developing full tetanic force upon stimulation. The muscle from the patient depolarized to -57 mV and became inexcitable, i.e., it was paralyzed. A contracture did not develop. Lowering of the extracellular pH did not influence the resting potential, but it effectively antagonized or prevented the paralytic effect of high $[K]_e$ by changing the inactivation characteristics of the sodium channels. Hydrochlorothiazide, which had a therapeutic effect on the patient, did not prevent paralysis in vitro. An abnormal rise of the intracellular sodium activity was recorded when the extracellular potassium concentration was raised to 10 mmol/l.

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ADYNAMIA EPISODICA HEREDITARIA WITH MYOTONIA: A NON-INACTIVATING SODIUM CURRENT AND THE EFFECT OF EXTRACELLULAR pH

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Adynamia episodica hereditaria, first described in 1955,¹⁵ is a rare muscle disease with autosomal dominant inheritance. Clinically it is similar to the much more common hypokalemic periodic paralysis; however, during episodes of muscle weakness the serum potassium rises rather than falls. The

disease, therefore, is also often called hyperkalemic periodic paralysis. We prefer the original name given to it by Gamstorp,¹² because the attacks occur episodically with no recognizable periodicity and are usually characterized by muscle weakness—adynamia—rather than by complete paralysis. Moreover, the name adynamia episodica is better suited to point out the genetic and pathophysiological distinction from hypokalemic periodic paralysis.³³

Three variants of adynamia episodica have been described: (1) in combination with clinical or electromyographical myotonia,^{6,8,18,19,24-26,28,31} (2) without any signs of myotonia,^{3,4,16,24,27} and (3) in combination with paramyotonia.^{2,10,11,29,30} The latter variant has also been called paralysis periodica paramyotonica.^{2,30} The affected members of a given family always show the same variant.

In a previous study some of us have reported the membrane and contraction parameters of a patient with variant (1) and a patient with variant (2).²⁴ The membrane parameters of a patient with variant (3) were reported in a study on paramy-

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otonia congenita²³ at a time when we were not aware of this patient's episodic attacks of work- or potassium-induced weakness (case "PWMD"). (In the meantime, an extensive report has been published describing the clinical differentiation of adynamia and paramyotonia in this patient and the selective treatment of the two symptoms by the use of different drugs.²⁹) As a major result of these first studies,^{23,24} each of the three clinical variants was characterized by different abnormalities in the electrophysiological parameters. But since these differentiations were based on one patient for each variant only, more investigations seemed necessary. We now describe further *in vitro* experiments with intact muscle fibers biopsied from another adynamia patient with variant (1). The new results confirm the earlier finding²⁴ that adynamia episodica is related to an abnormal sodium conductance of the muscle fiber membranes and characterize the voltage and time dependence of this conductance. Additional experiments were performed to study *in vitro* the effect of low extracellular pH on muscle paralysis.

A clinical study of this patient has appeared.³⁰ Part of the results have been presented to the German Physiological Society.¹³

MATERIAL AND METHODS

The study was approved by the Ethics Commission of the Technical University of Munich and was carried out in accordance with the Helsinki convention. The patient, a 22-year-old woman of athletic constitution (case 1 of Ricker et al.³⁰) has suffered from attacks of adynamia from her late teens on, as has her younger brother. During rest after work on a bicycle ergometer, her serum potassium would rise to > 7 mmol/l, causing severe muscle weakness. She has never experienced muscle stiffness in connection with adynamia, but lid lag and myotonic runs in the EMG were clearly present between attacks. The patient gave informed consent for an external intercostal muscle biopsy to be taken. The specimen was obtained under general anesthesia without the use of muscle relaxants. Small muscle fiber bundles were dissected for the measurements of contraction force, membrane parameters, and the intracellular Na activity. Control values of normal human intercostal muscle that were not available from references^{20,21} were measured in a muscle specimen from a patient without muscle disease who had to undergo thoracic surgery.

For the measurement of contraction force, a bundle containing about 500 fibers was suspended

in a plexiglass chamber with one end fixed and the other end fastened to a quasi-isometric semiconductor strain gauge. The length of the bundle was so adjusted that the force during tetanic stimulation was maximal. A silver wire ran along either side of the preparation for direct ("massive field") stimulation. Supramaximal square current pulses of 0.2 msec duration were applied as 300-msec trains of 50 Hz frequency at intervals of at least 5 minutes.

For the measurement of membrane parameters and current-voltage relationships, the voltage clamp set-up and the experimental protocol were used as already described.²³ Data analysis was carried out off-line with the use of a PDP 11/23 computer (Digital Equipment Corporation, Maynard, MA).³²

For the determination of the availability of the sodium current, the fibers were impaled with two microelectrodes at a distance of ~100 μm . DC currents were applied through one electrode so that the membrane potential, measured through the other electrode, assumed holding values between -120 and -50 mV (in 10 mV steps). When the holding potential had been maintained for >1000 msec, a brief (1-3 msec) depolarizing pulse was applied through the current electrode so that an action potential was elicited. The rate of rise of this action potential was obtained by electronic differentiation. The maximum rate of rise, plotted against the holding potential, gives a measure of the availability of the sodium channels (" h_{∞} curve").

The determination of the intracellular sodium activity (a_{Na_i}) of the muscle fibers was carried out with a set-up described elsewhere.¹⁴ Na-sensitive microelectrodes were made from double-barrelled glass pulled to a tip diameter of 0.5 μm . One barrel was filled with a "Na cocktail" (71176, Fluka, Neu-Ulm, West Germany), the other one with 1 mol/l magnesium acetate.

Solutions. The standard solution⁵ used for transportation, dissection, and basic experiments contained (in mmol/l): NaCl, 107.7; KCl, 3.48; CaCl₂, 1.53; MgSO₄, 0.69; NaHCO₃, 26.2; NaH₂PO₄, 1.67; sodium gluconate, 9.64; saccharose, 7.6; glucose, 5.5. The pH was set at 7.4 by gassing this solution with carbogen (a mixture of 5% CO₂ and 95% O₂). Solutions with higher than normal potassium concentrations were obtained by the addition of the appropriate amounts of KCl to the standard solution.

For the blockade of the sodium channels, tetrodotoxin (TTX, Roth, Karlsruhe, West Germany or Sigma, München, West Germany) was used. In some experiments the pH of the solutions was low-

ered to about 6.5, either by gassing the solution with a mixture of 40% CO₂ and 60% O₂ or by the addition of the appropriate amount of HCl. Hydrochlorothiazide (Esidrix, CIBA-Geigy, Wehr, West Germany), when present, was at 0.125 mmol/l, and ouabain (Sigma) was at 0.3 mmol/l.

All experiments were conducted at 37°C. Results are given as mean values ± SD.

RESULTS

Contraction Measurements and the Effect of Low pH.

In the standard solution, the twitches and tetani of the bundles were of normal force amplitude. When the extracellular potassium concentration, [K]_e, was raised to 7 mmol/l, the contraction force decreased similar to the way it does in vivo during hyperkalemia. When at that stage the bathing solution was gassed with 40% CO₂, the extracellular pH quickly decreased to 6.5, and the force recovered (Fig. 1)

although [K]_e was still at 7 mmol/l. When the solution was gassed again with normal carbogen, the force amplitude decreased. This decrease was very much accelerated when [K]_e was raised slightly more, to 9 mmol/l. When the 9-mmol/l-[K]_e solution was gassed with 40% CO₂, the force amplitude recovered quickly, and when finally [K]_e was lowered to the normal 3.5 mmol/l, the force amplitude returned to 100%.

To investigate whether this protection from paralysis was caused by extracellular acidity, we also lowered the pH by the addition of HCl to the bathing solution, keeping the CO₂ concentration constant. Figure 2 shows an experiment in which the pH was lowered to 6.5 by HCl before [K]_e was raised to 9 mmol/l. Under these conditions the contraction force did not decrease. When a bathing solution with 9 mmol/l [K]_e and pH 7.4 was flushed into the muscle bath, complete paralysis set in very

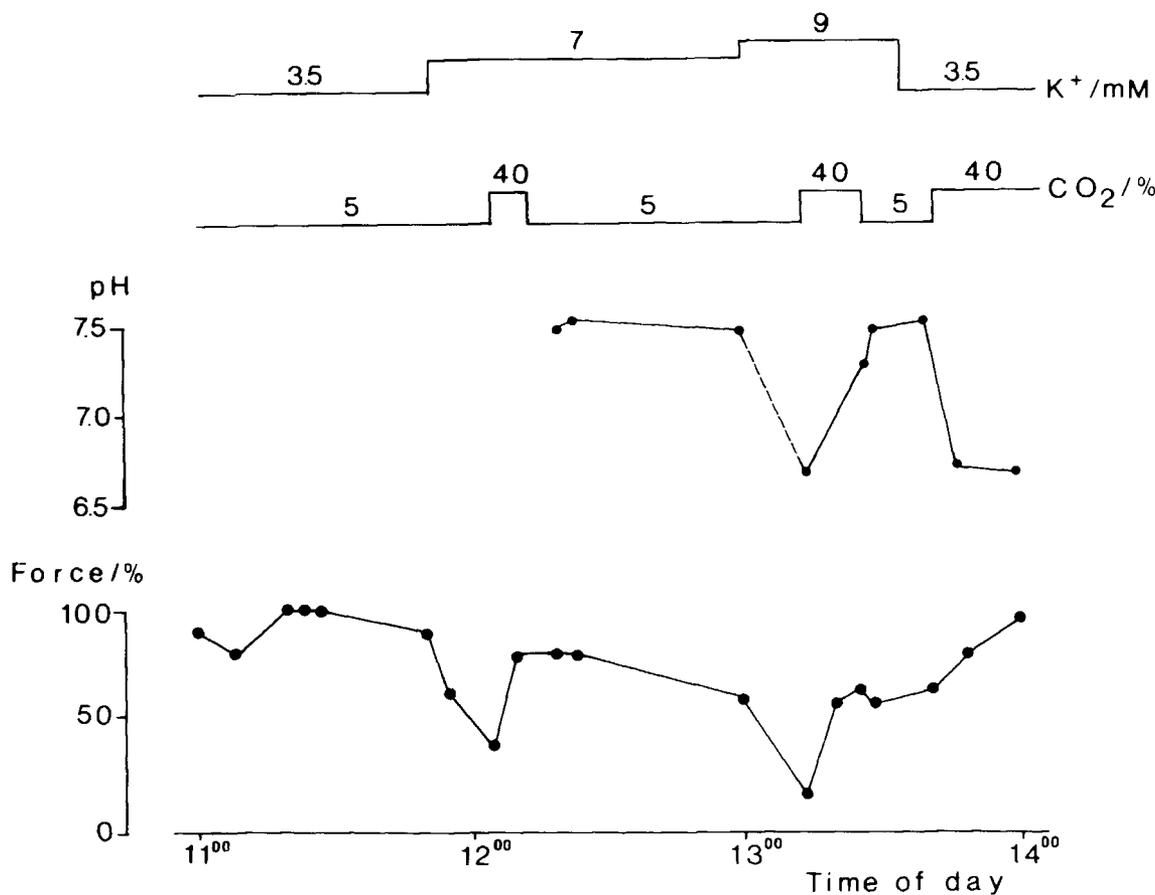


FIGURE 1. Maximal isometric force of tetanic contractions of a muscle bundle from the adynamia patient. The force decreases when the extracellular potassium concentration is raised and increases again when the high-potassium solution is gassed with a mixture of 40% CO₂ and 60% O₂. The pH in the muscle bath was measured with a glass electrode. Dashed Line: the change in pH occurred after the increase in CO₂.

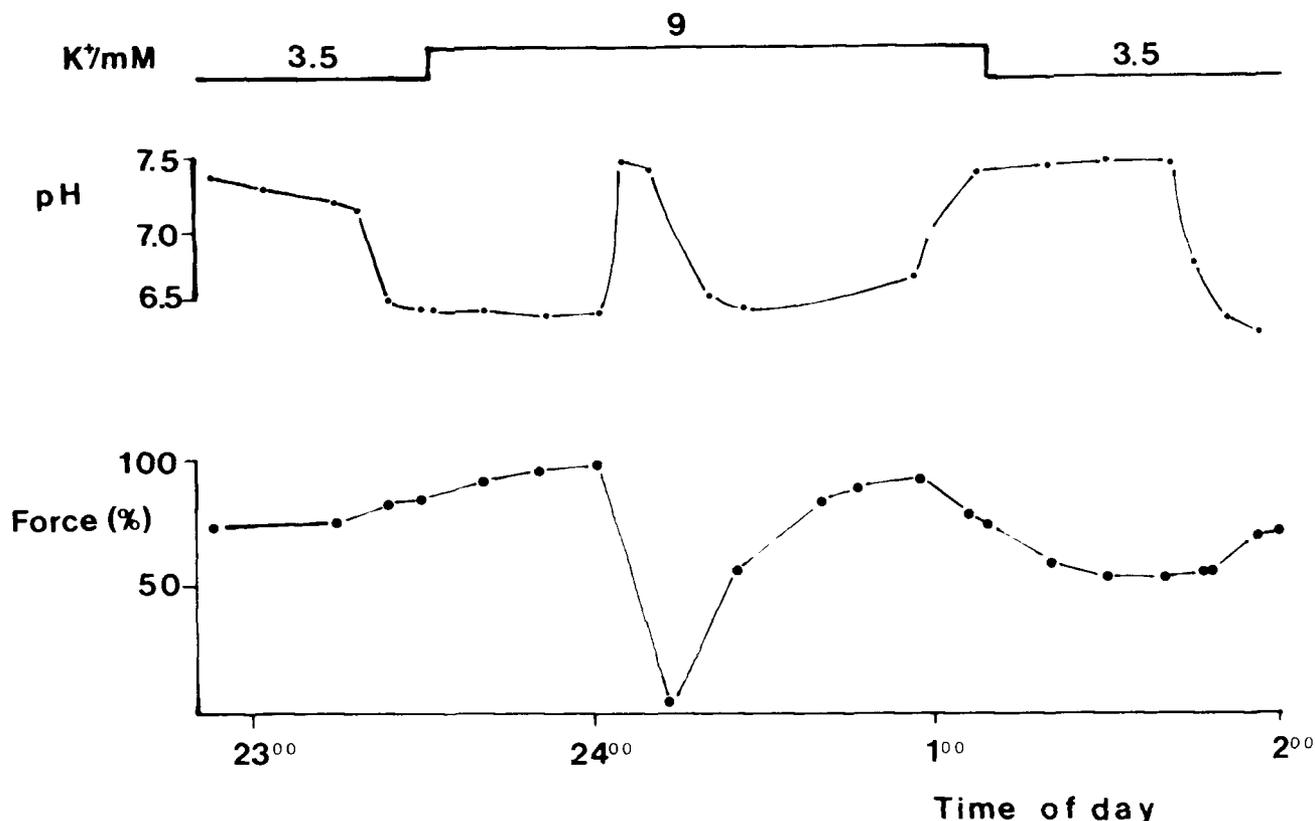


FIGURE 2. Maximal isometric force of tetanic contractions as in Fig. 1 but with another bundle from the patient. The extracellular pH is lowered by the addition of HCl to the bathing fluid. This prevents the decrease of force on increase of [K]_e. When a high-[K]_e solution with normal pH is flushed into the bath, the muscle becomes paralyzed. When the pH is lowered to 6.5, the force recovers.

quickly, demonstrating the protective effect of a low pH. Again, this effect was completely reversible.

Hydrochlorothiazide (HCT) prevents paralytic

attacks in adynamia patients.²⁶ To test whether the drug has a direct effect on the muscle fibers or acts perhaps via its well-known systemic effect of lowering the serum potassium, we did a similar ex-

Table 1. Resting potentials and membrane conductances of external intercostal muscle fibers from the patient with adynamia episodica hereditaria and from a healthy control person. Measurements were made in solutions containing 3.5 or 10 mmol/l potassium, with or without 0.3 mg/l TTX, and that were gassed with 5% or 40% CO₂ (means ± SD; number of fibers investigated in parentheses).

Solution	Resting potential (mV)		Membrane conductance (μS/cm ²) at resting potential	
	Patient	Control	Patient	Control
3.5 mM K 5% CO ₂	-83.5 ± 5.4 (14)	-80.2 ± 2.4 (9)	110† ± 12 (3)	165 ± 25 (9)
3.5 mM + TTX 5% CO ₂	-85.8 ± 3.2 (7)	-81.4 ± 3.2 (7)	183 ± 23 (7)	158 ± 28 (6)
9.0 mM K 5% CO ₂	-57.3* ± 5.4 (30)	-62.8 ± 1.9 (21)	108† ± 41 (8)	337 ± 41 (6)
9.0 mM K + TTX 5% CO ₂	-67.5* ± 3.7 (13)	-64.8 ± 4.8 (14)	376 ± 69 (6)	321 ± 46 (6)
9.0 mM K, 40% CO ₂	-58.5 ± 3.1 (29)	-62.0 ± 2.7 (7)	137† ± 42 (5)	341 ± 38 (7)

*The difference of the resting potential values (in 9 mM K with and without TTX) was statistically significant ($P < 0.001$) when tested with Student's *t*-test.

†Slope of current-voltage relationship. Because of the abnormal sodium conductance not representative for membrane conductance (see text).

periment in which instead of lowering of the pH we added 0.125 mmol/l HCT to the bath before raising $[K]_e$ to 9 mmol/l. HCT had no direct protecting effect.

Membrane Parameters in Solutions with Normal Extracellular Potassium Concentration. In the standard solution, the fibers had normal resting potentials of -83.5 ± 5.4 mV ($n = 14$) and -85 ± 3.2 mV ($n = 7$) in the presence and absence of 0.3 mg/l TTX, respectively (see Table 1). Current-voltage relationships determined with the three-microelectrode voltage clamp were significantly different from control. When in normal fibers the membrane potential is clamped to a value between -80 mV (resting potential) and -65 mV (threshold of the fast sodium current), an outward current flows that assumes a steady value about 10 msec after the onset of the clamp pulse. In fibers from the patient, the membrane current flowed in the outward direction for about 20 msec only and then reversed its direction to assume a steady negative value (Fig. 3).

When TTX was added to the bath, the flow of inward current was abolished, i.e., the time course of the current flow was indistinguishable from that in normal fibers. This finding suggests that in the fibers from the adynamia patient, depolarization slowly activates a sodium inward current that cancels—and even supersedes—the potassium outward current. A plot of the clamp currents flowing 55 msec after the onset of the voltage clamp pulses as a function of the clamp potential is shown in Fig. 4A. It illustrates the relatively large increase of the slow inward current with increasing depolarization.

When a normal muscle fiber is voltage-clamped to potentials less negative than -64 mV, a fast and transient inward current is triggered, the excitatory sodium current responsible for the upstroke of the action potential. The three-microelectrode voltage clamp does not control the membrane potential along the whole fiber. During such pulses, therefore, a twitch is often elicited whereby the recording is terminated. Sometimes, in clamp pulses that exceed the threshold by not too much, the elec-

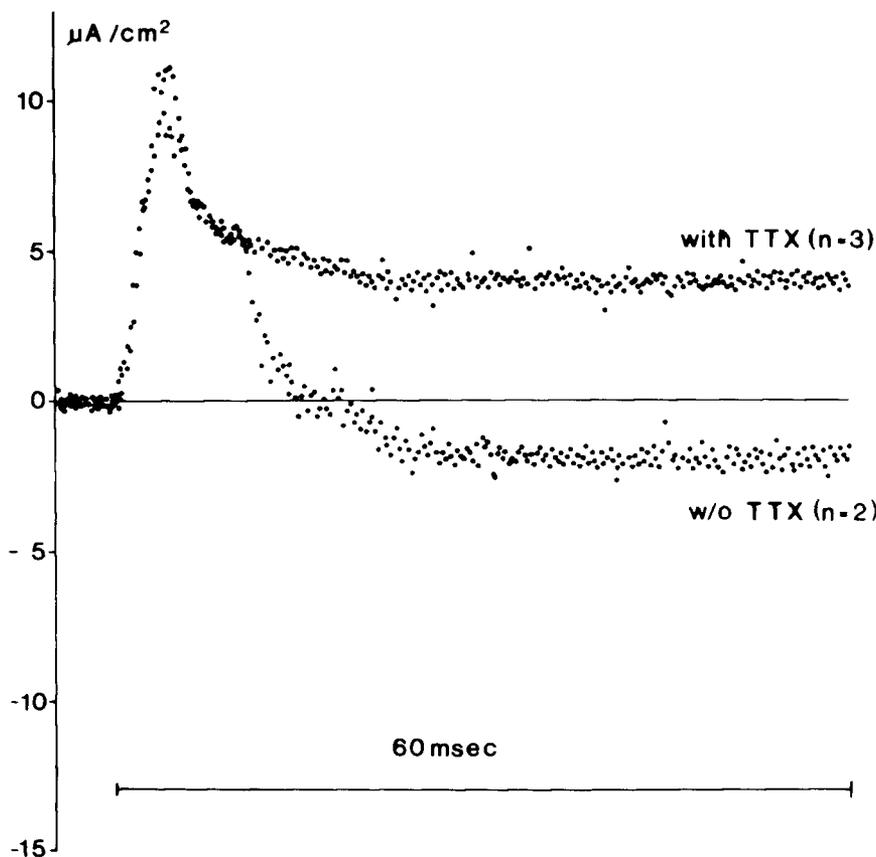


FIGURE 3. Time course of membrane current density in fibers from the adynamia patient during square voltage clamp pulses going from -80 mV to -68 mV for 60 msec. The traces are the digitized and averaged data from 3 fibers in the presence of 0.3 mg/l TTX (upper) and from 2 fibers in the absence of TTX (lower). The difference between the traces represents the slow sodium current. Current densities recorded from normal fibers would be identical to the upper trace, both in the presence and absence of TTX.

trode positions remain stable, and the slow currents following the fast inward current can be recorded. Whenever this was the case with fibers from the patient, a fast and a slow inward current were observed. Both currents were abolished by 0.3 mg/l TTX. When voltage clamp pulses into this potential range were performed with normal fibers, the steady-state currents always flowed in the outward direction.²¹

It is well known that the membrane conductance, g_m , of a muscle fiber is dependent on the membrane potential. To obtain values comparable among patients with various muscle diseases we adopted the standard procedure of determining g_m from the slope of the current-voltage relationship at -80 mV. This convention leads to spurious results in the case of episodic adynamia. The slope of the curve in Fig. 4A is almost zero at -80 mV, suggesting that the membrane conductance is extremely low. A better insight into the situation is obtained when the sodium conductance is abolished by the application of TTX so that only the

potassium and chloride conductances play a role. The current-voltage relationships recorded from six fibers in the presence of TTX (Fig. 4A, asterisks) were indistinguishable from control curves obtained from normal human muscle in the presence or absence of TTX.²¹ In particular, the slope of the curve at -80 mV was normal. The simplest explanation of all these findings is that the passive potassium and chloride currents are normal in these fibers and that in the absence of TTX an active sodium current is triggered by depolarization in the potential range only slightly below the resting potential. This active current reduces the net current flowing at -80 mV to almost zero, and therefore the slope conductance is low, although the membrane conducts large currents.

Membrane Parameters in Solutions with Increased Potassium Concentration. Figure 5A illustrates an experiment in which we tested the effect of increased extracellular potassium on the resting potential. In the beginning, the bundle was bathed in

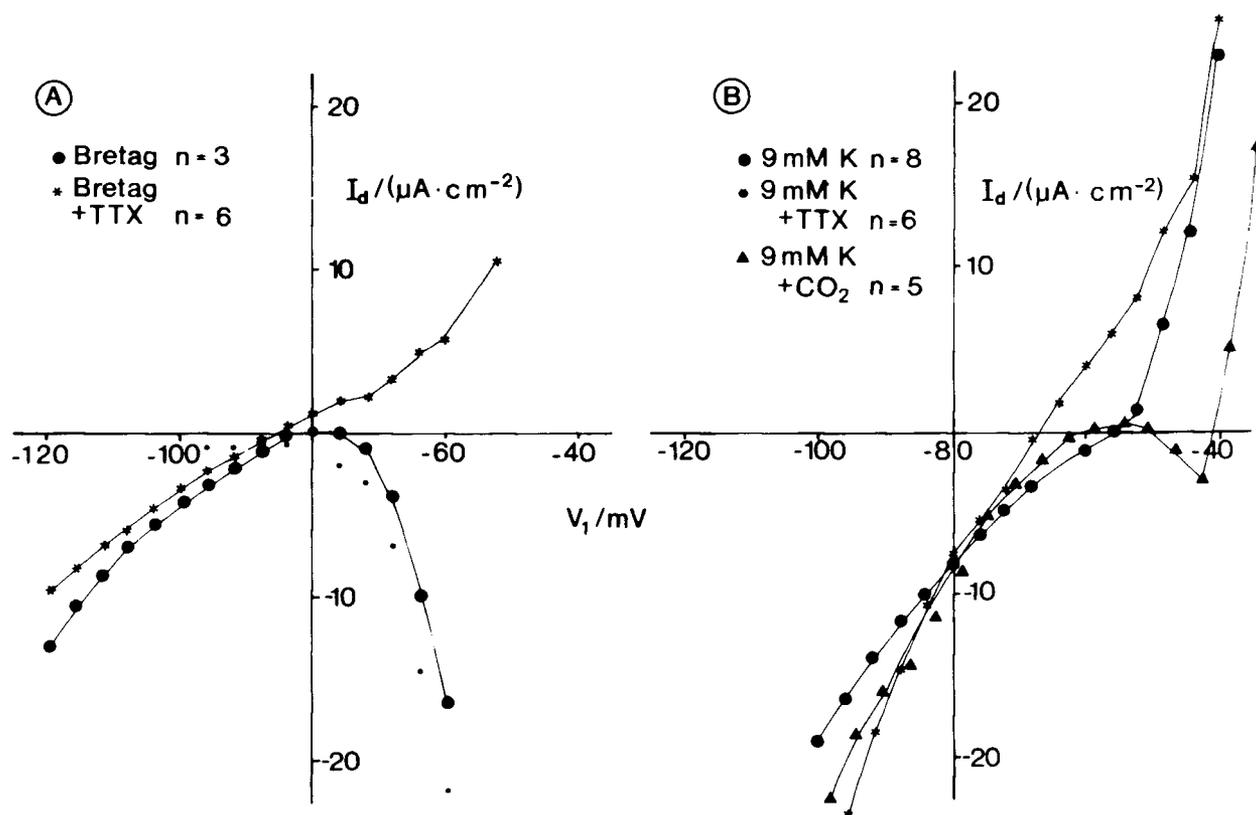


FIGURE 4. Current-voltage relationships of the muscle fiber membrane in adynamia episodica. The current amplitude at the end of square voltage clamp pulses of 60 msec duration is plotted against the clamp potential. In **A**, the fibers were bathed in Bretag's standard solution containing 3.5 mmol/l potassium without (●) or with (*) 0.3 mg/l TTX. The dots (-) represent the difference between the current amplitudes in the presence and absence of TTX, i.e., the voltage dependence of the abnormal sodium current. In **B**, the fibers were bathed in a solution containing 9 mmol/l potassium without (●) or with (*) 0.3 mg/l TTX. The curve marked by triangles (▲) was recorded in a solution that contained 9 mmol/l potassium and no TTX and was gassed with a mixture of 40% CO₂ and 60% O₂.

the standard solution with 0.3 mg/l TTX added to prevent the flow of the slow sodium current. The resting potential was negative to -80 mV. When $[K]_e$ was raised to 9 mmol/l, the resting potentials decreased to -67.5 ± 3.7 mV ($n = 13$), a value similar to that adopted by normal fibers in this solution (-64.8 ± 4.8 mV, $n = 14$, dashed lines in Fig. 5). When a TTX-free bathing solution was introduced, many fibers began to twitch. Five minutes later this spontaneous activity had subsided enough for the membrane potentials to be measured. Most fibers were now further depolarized so that the mean resting potential was -57.3 ± 5.4 mV ($n = 30$). The statistical distribution of the values was normal both in the presence and absence of TTX so that Student's t-test of significance could be applied. The difference of the membrane potential values in the presence and absence of TTX was highly significant ($P < 0.001$). Such additional depolarization and twitching on removal of TTX was never observed in normal muscles.²⁰

In a similar experiment carried out with another bundle, we investigated the effect of 40% CO₂ on the resting potential under the conditions in which the paralysis is prevented. In this experiment, illustrated in Fig. 5B, TTX was absent from the beginning so that all fibers depolarized to below -64.8 mV (dashed line) when $[K]_e$ was raised to 9 mmol/l. Gassing the bathing solution with 40% CO₂ (and thereby lowering the pH to about 6.5) did not lead to an increase in the mean resting potential.

As regards the slow sodium current, current-voltage relationships recorded in 9 mmol/l $[K]_e$ (Fig. 4B) allowed an interpretation similar to that given for normal $[K]_e$. In a TTX-containing solution, the curve (asterisks) had the same shape as the curve for normal fibers in high $[K]_e$.²¹ In particular, the slope at the resting potential was normal. The membrane conductance calculated from the slopes of 6 fibers was $376 \pm 69 \mu\text{S}/\text{cm}^2$. The corresponding figure evaluated in 6 fibers from a healthy control person was $321 \pm 46 \mu\text{S}/\text{cm}^2$. The curve re-

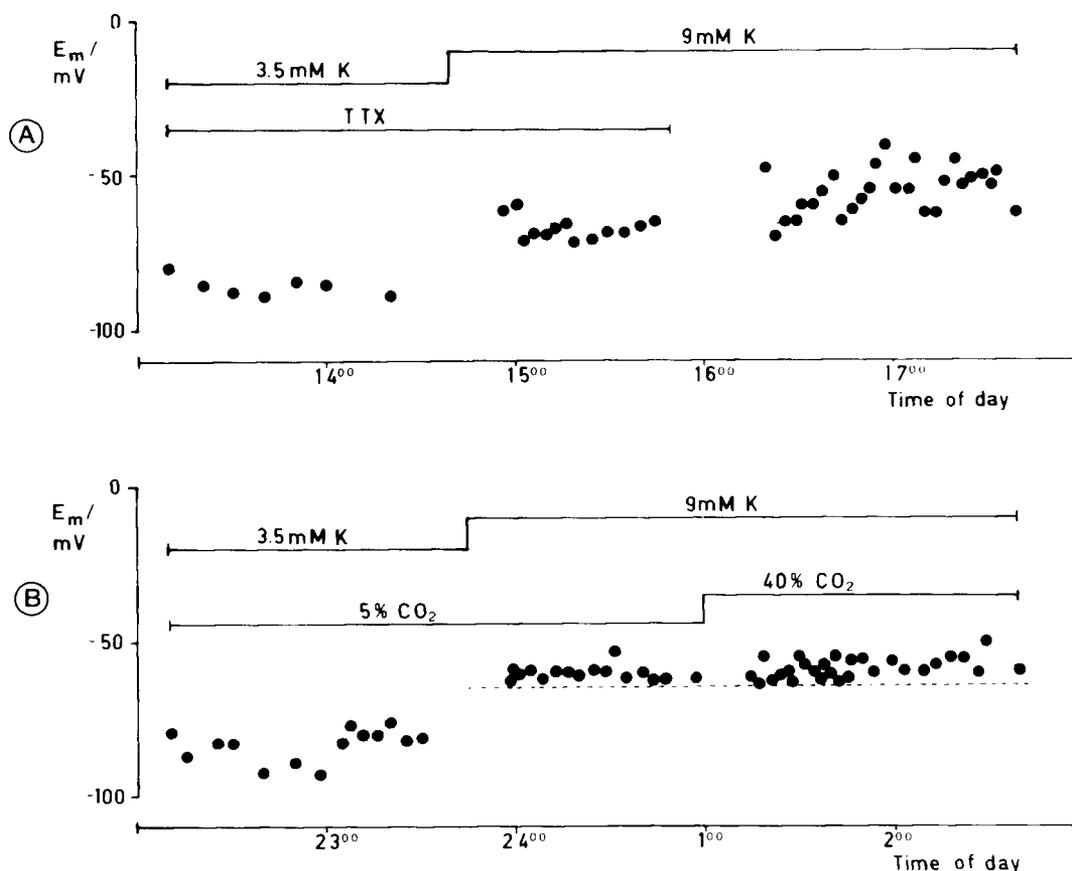


FIGURE 5. Dependence of the resting potential, E_m , recorded in many fibers from the adynamia patient on various extracellular conditions. In **A**, the bundle is in the standard solution containing 0.3 mg/l TTX. When $[K]_e$ is increased to 9 mmol/l, E_m assumes a value similar to the mean value assumed by normal human muscle fibers (dashed lines). When a 9 mmol/l $[K]_e$ solution without TTX is flushed into the bath, E_m falls to a mean value of -57.2 mV. In **B**, another bundle is investigated, and the protocol differs from **A** in that no TTX is present from the beginning. At the end, the bathing fluid is gassed with a mixture of 40% CO₂ and 60% O₂; this has no effect on E_m .

corded in TTX-free solution (filled circles) deviated from the curve in TTX-containing solution in a direction that can be explained by the additional flow of a sodium current. This deviation was largest in the potential range between -80 and -50 mV. Contrary to the curve in 3.5 mmol/l $[K]_e$, the curve in 9 mmol/l $[K]_e$ did not have a region of negative slope. This might be due to the fact that an increase in $[K]_e$ increases the membrane conductance for potassium to a degree that the potassium outward current effectively cancels the slow sodium inward current.

We also recorded current-voltage relationships in the absence of TTX when the pH was lowered by gassing the bathing fluid with 40% CO_2 (Fig. 4B, triangles). The most obvious change was an increase in the slow sodium current between -50 and -40 mV, which resulted in a range of negative slope. The reduction of the pH by 0.9 units did not significantly affect the slope conductance at the resting potential (see Table 1). Also in normal muscle, lowering of the pH from 7.4 to 6.5 did not change the slope conductance at the resting potential. (The absolute slope conductance values are lower than control for the muscle from the patient because of the abnormal sodium conductance, as explained above.) These results indicate that the chloride component conductance is not affected by this pH change.

The fast sodium current flowing during large clamp pulses was also increased in 40% CO_2 . Since this was unexpected, we investigated the availability of the sodium current measuring the rate of rise of action potentials as described in Material and Methods. The maximal rate of rise of the action potentials starting from a holding potential of -90 mV was 25% higher (~ 500 V/sec) at pH 7.5 (5% CO_2) than at pH 6.5 (40% CO_2), in agreement with the literature.¹⁷ But with a holding potential of -60 mV, the rate of rise was higher at pH 6.5 (53 V/sec) than at pH 7.4 (29 V/sec). Thus, a lowering of the pH decreased the sodium current of fibers having a normal resting potential but increased the sodium current of depolarized fibers.

Intracellular Sodium Activity, aNa_i . This parameter was investigated in muscle fibers from the patient and from three normal subjects. During the first 5 – 35 minutes after the impalement of a cell, usually a small drift of Na_i towards lower values was observed that was most likely due to some injury of the cell membrane caused by the impalement. The following data were recorded after aNa_i had reached

a steady level. When a fiber bundle from the patient was in the standard solution, aNa_i was 6.9 ± 1.0 mmol/l ($n = 10$) and the resting potential, E_m was -84.6 ± 2.8 mV. Control values from a normal subject were very similar ($aNa_i = 6.6 \pm 0.9$ mmol/l; $E_m = -84.2 \pm 2.7$ mV, $n = 8$). An elevation of $[K]_e$ to 7 mmol/l produced the same amount of membrane depolarization in the fibers from the patient and the normal subject and was without any effect on aNa_i (Fig. 6). In retrospect it appears that the application of 7 mmol/l $[K]_e$ to the bundle from the patient was too short-lasting, because the depolarization had not yet reached a steady level (Fig. 6B). Thus, with longer exposure, even at 7 mmol/l $[K]_e$ the fibers from the patient might have shown the abnormal behavior that was clearly visible when $[K]_e$ was raised to 10 mmol/l: (1) the depolarization was much greater than in the control fibers (Fig. 6A), indicating that high $[K]_e$, in addition to shifting the potassium equilibrium potential, had another depolarizing effect, i.e., activation of an inward current; (2) aNa_i increased, indicating that the inward current was carried by sodium; and (3) the membrane did not repolarize when the standard solution was reapplied, suggesting that the inward current was not inactivated. Only when 3 μ mol/l TTX were added to the standard solution did E_m and aNa_i return to normal. The rise of aNa_i on application of 10 mmol/l $[K]_e$ was observed in all fibers from the patient and was never seen in control fibers. Figure 7 shows another experiment carried out with two other preparations. In a normal muscle fiber, the application of 10 mmol/l $[K]_e$ had similar effects in the absence and in the presence of TTX (Fig. 7A): the membrane depolarized to about -65 mV, and aNa_i was unaffected. Application of 0 mmol/l $[K]_e$ produced the expected upward deflection of the aNa_i trace, indicating that the microelectrode was functioning well. Figure 7B illustrates that in a fiber from the patient the application of 10 mmol/l $[K]_e$ in the absence of TTX produced an abnormally large depolarization and an increase in aNa_i , whereas, in the presence of TTX, the depolarization had the amount expected from the Goldman equation, and aNa_i returned to normal.

DISCUSSION

The present results are very similar to those obtained from another patient who had adynamia episodica with myotonia and differ from the results from the patient who had adynamia episodica without myotonia.²⁴

The results confirm the earlier finding that the resting potential of the muscle fibers is high when the potassium concentration in the bathing medium, $[K]_e$, is normal. This and the new finding of a normal resting sodium activity indicate that there is no defect in the Na/K pumps in this disease. The results further confirm that, in adynamia episodica with myotonia, elevation of $[K]_e$ produces an abnormally large and persistent depolarization of the muscle fiber membranes. The depolarization is large enough to cause inexcitability in most of the fibers

but not large enough to reach the threshold for contracture. The inexcitability explains the decrease of isometric force observed in vitro and, since serum potassium may easily rise to 7–9 mmol/l during paralytic episodes,³⁰ also the immediate mechanism of clinical adynamia.

Current-voltage relationships recorded at high $[K]_e$ in fibers from the earlier²⁴ adynamia patient with myotonia had shown that the reason for the abnormally large depolarization is the flow of an abnormal inward current carried by sodium. We

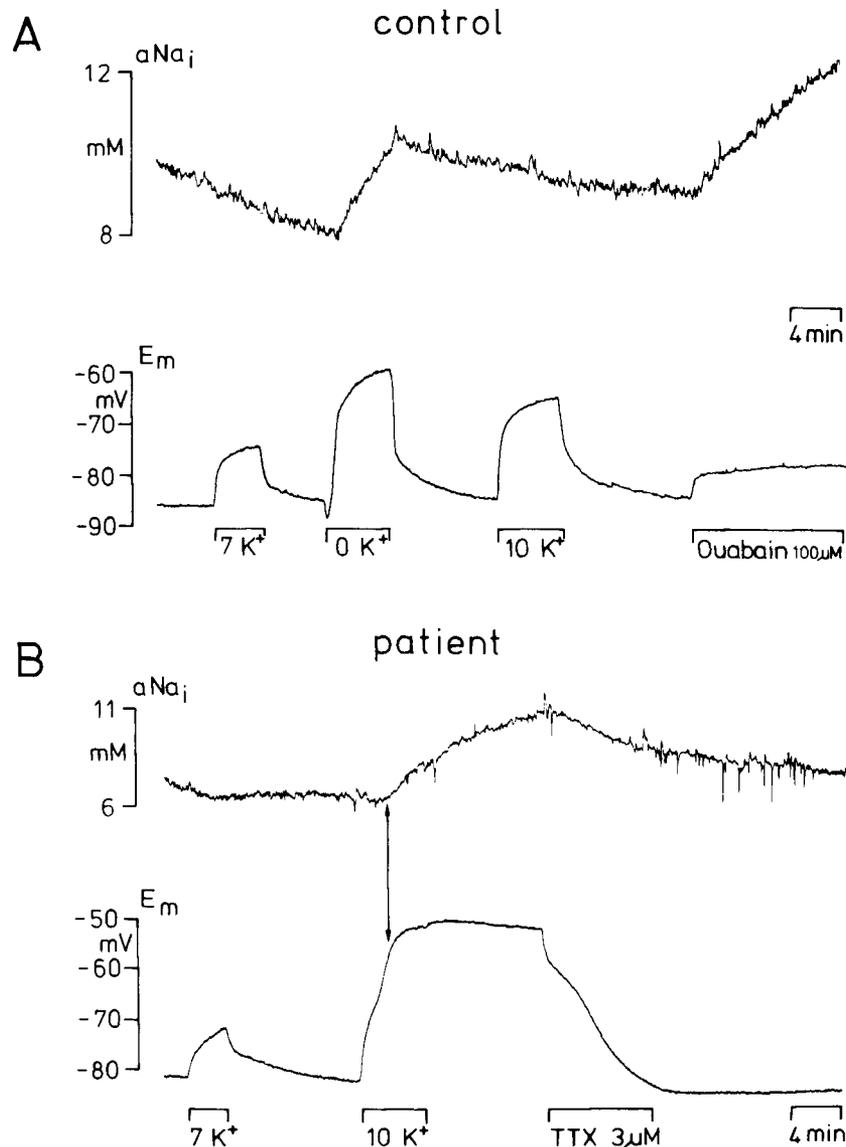


FIGURE 6. Recordings of the intracellular sodium activity, aNa_i , and the resting potential, E_m , in fibers from a healthy control person (**A**) and the adynamia patient (**B**). $[K]_e$ is 3.5 mmol/l unless otherwise indicated. In the presence of 10 mmol/l $[K]_e$, the fiber from the patient shows an abnormally large depolarization and, associated with it, an increase of aNa_i . The applications of solutions containing 0 mmol/l $[K]_e$ or 100 μ mol/l ouabain in **A** are standard tests for the Na sensitivity of the microelectrode based on the presumption that these changes block the Na/K pump. The large depolarization induced in the control fiber by the K-free solution is caused by the inhibition of the electrogenic Na/K pump and by changes of the Na and/or K permeabilities of the fiber membrane. For further details see text.

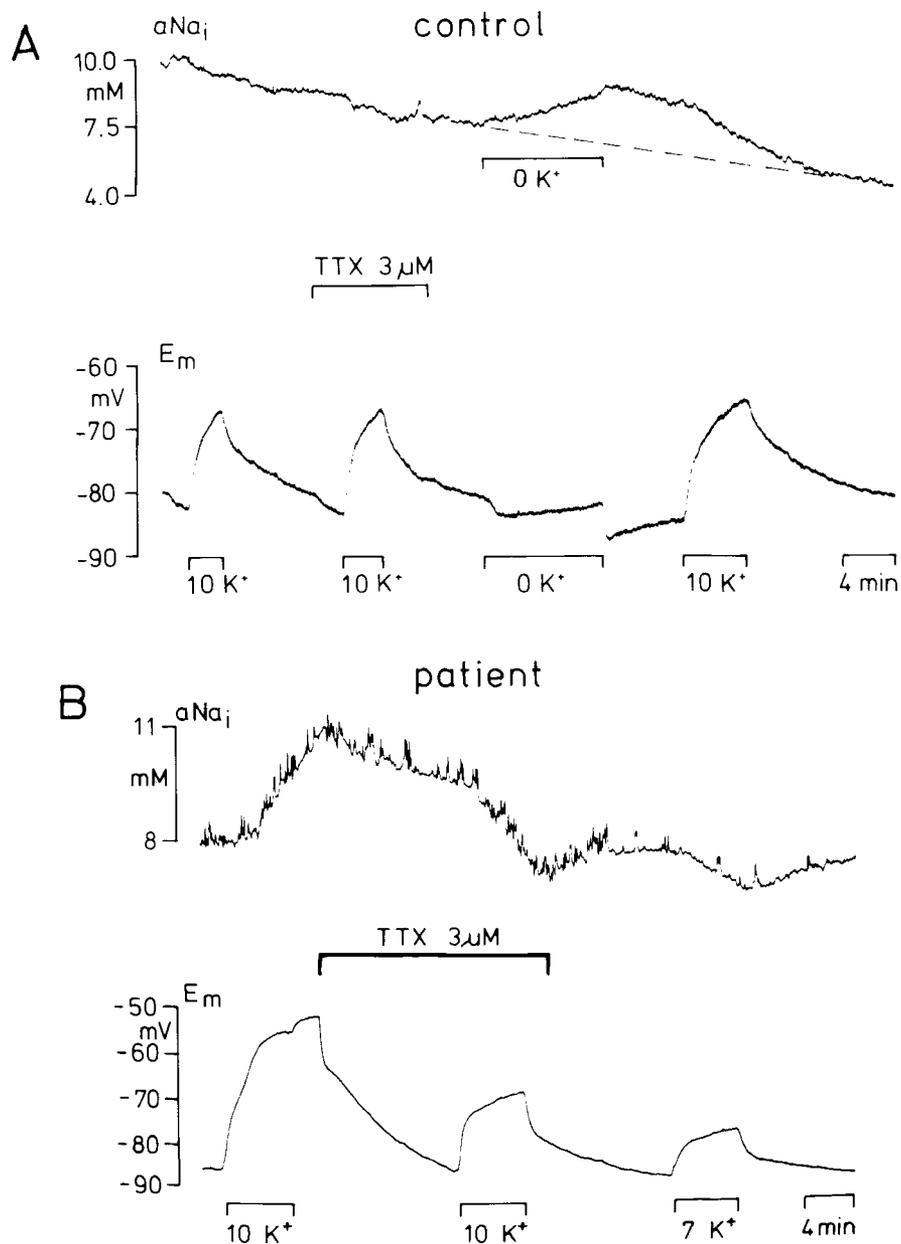


FIGURE 7. Recordings as in Fig. 6 from two other fibers. Again, the major result of the experiment is the abnormally large depolarization and the associated increase in aNa_i seen in the patient's fiber when $[K]_e$ is raised to 10 mmol/l. Abnormal depolarization and increase of aNa_i do not occur in the presence of TTX.

have now shown that this current is slowly activated by even slight membrane depolarization, and that in contrast to the fast sodium current, this current is not inactivated. By the additional measurement of the intracellular sodium activity, we have demonstrated more conclusively than with the mere application of TTX that this current is indeed carried by sodium. This makes other possible explanations of this current³⁴ very unlikely. We suppose that other depolarizing influences on the muscu-

lature of these patients, such as extreme cold or the use of muscle relaxants, may also trigger this abnormal sodium current and thus lead to adynamia, a supposition that is in agreement with the clinical experience.

The experiments show that the existence of non-inactivating sodium channels in muscle fiber membranes may lead to an abnormal function of the musculature. Such non-inactivating channels could either belong to an altered channel-type resulting

from a defect in the genetic code for sodium channels or to a physiological type recently detected in normal mammalian neurones.³⁵ In the latter case, these sodium channels would have to be abnormal in number. It is worth noting that lidocaine, which is known to block the fast sodium channels, did not block the non-inactivating sodium channels in Purkinje cells.³⁷ Tocainide and mexiletine, lidocaine derivatives that are very effective in the treatment of paramyotonic stiffness and weakness, do not prevent attacks of hyperkalemic weakness in paralysis periodica paramyotonica.^{29,30} Apparently, the effect of the local anesthetics is linked to the presence of a functioning inactivation gate. In contrast, TTX also blocked the non-inactivating channels in the preparations from the adynamia patients.

It is interesting that a lowering of the pH of the bathing fluid antagonized the force decrease caused by increased $[K]_e$, and that this effect was not based on an increase in the resting potential but on an appropriate change of the inactivation characteristic (h_∞ curve) of the sodium channels. The decrease of inactivation is an effect of increased extracellular H^+ concentration described in several tissues.^{7,17} Increase of the extracellular calcium concentration, known to alleviate paralytic attacks,

is probably effective by a similar mechanism, namely a binding of these ions to external sites of the membrane.¹⁷ Gassing the bathing fluid with 40% CO_2 is known to lower both the extra- and the intracellular pH of a submerged tissue,¹ whereas addition of HCl to the bathing fluid lowers the extracellular pH without an immediate effect on the intracellular pH.^{7,38} Since either method of lowering the pH had the same antiparalytic effect, the extracellular pH seems responsible for it.

It is well known that the serum potassium rises to high values as a consequence of heavy exercise, and yet adynamia patients do not experience paralytic attacks during the work. Our results suggest that the work-induced lowering of the pH may mediate this effect. The protective effect of slight exercise during an on-coming attack may not necessarily be explained the same way because the pH shift introduced in our experiments was much greater than that observed in ³¹P nuclear magnetic resonance spectroscopy of forearm muscles during light work.²² Other factors, like the hyperpolarizing effect of the catecholamines, may play a role.⁹ The observation that muscle rest per se may lead to weakness of single muscle groups³⁶ is as yet completely unexplained.

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