Effect of insulin on membrane potential and potassium content of rat muscle

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ZIERLER, KENNETH L. Effect of insulin on membrane potential and potassium content of rat muscle. Am. J. Physiol. 197(3): 515-523. 1959.—Insulin increased resting membrane potential of excised rat muscle, extensor digitorum longus, by about 5 mv in less than 1 hour. In 1 hour insulin caused no increase in the ratio of intra- to extracellular potassium, but in 2–3 hours intracellular K increased by about 10%. It is concluded that the increase in intracellular K is probably too small and too late to account for the hyperpolarization on the basis of conventional theory and it is suggested that the hyperpolarization produced by insulin is the cause of the potassium shift.

It has been known since 1923 (1) that insulin, administered to the intact animal, causes a decrease in the concentration of potassium in serum. Despite several alternative hypotheses offered in the past, it is now clear that at least part of the explanation for insulin-induced hypokalemia lies in increased net uptake of potassium from extracellular fluid by skeletal muscle (2–4). If, as is generally agreed, a major portion of the resting potential across the muscle membrane is described by the potassium equilibrium potential in accordance with the Nernst equation, \( E_K = \frac{RT}{F \ln \frac{(K^+)_i}{(K^+)_o}} \) (where \( E_K \) is the potassium equilibrium potential, \( R \), \( T \) and \( F \) have their usual meaning, and \( (K^+)_i \) and \( (K^+)_o \) are the activities of potassium inside and outside the cell, respectively), then an increase in intracellular potassium concentration, a decrease in extracellular potassium concentration, or a combination of both should lead to an increase in the resting membrane potential. That is, if insulin increases the ratio \( \frac{(K^+)_i}{(K^+)_o} \), then the muscle membrane should be hyperpolarized, as, in fact, it is (5).

However, the cause and effect relation placed speculatively between the ratio \( \frac{(K^+)_i}{(K^+)_o} \) and membrane potential can be reversed. If by some independent means the membrane is hyperpolarized and held at the new higher potential difference, then the equilibrium ratio \( \frac{(K^+)_i}{(K^+)_o} \) must be increased. Indeed, there are objections to earlier explanations of the net movement of potassium into muscle induced by insulin and it remains possible that the correct explanation of the action of insulin leading to increased \( \frac{(K^+)_i}{(K^+)_o} \) lies in a more primary effect of insulin on the cell membrane, causing hyperpolarization.

The question can be investigated by comparing the time-course of the increasing resting membrane potential following exhibition of insulin to the time-course of the increasing ratio \( \frac{(K^+)_i}{(K^+)_o} \). If hyperpolarization precedes the increased ratio \( \frac{(K^+)_i}{(K^+)_o} \), providing analytical sensitivity is adequate, it may be concluded that insulin hyperpolarizes the membrane in some way and that the intracellular migration of potassium is a result of hyperpolarization. The present report elaborates on an earlier one (5) and extends the observations to include more detailed studies of the time-course of potassium movement. With reservations to be discussed, it is concluded that hyperpolarization precedes attainment of potassium equilibrium.

Methods

Excised mammalian muscle survives poorly, compared, for example, to frog muscle. However, although frog muscle responds to mammalian insulin (6), its response is slow, quantitatively less, and perhaps even qualitatively different. For these reasons it seemed best to explore the effect of insulin on mammalian muscle and to this end it was necessary to develop a technique which would provide a stable preparation for at least several hours. Because such a preparation may be generally useful it is described below.

Johnson and Fisher (7) introduced the use of the excised rat extensor digitorum longus muscle as a tool for study of muscle metabolism. The suggestion was adopted by Gourley (8), Gourley and Jonas (9), McLennan (10) and Manery, Gourley and Fisher (3). In the last instance it was reported that the mammalian muscle was abandoned largely because it yielded inconsistent results. The muscles used by these investigators may have been larger than those which we use, although the weights are not reported. McLennan (10) used muscles...
Muscles used for measurement of potential weighed approximately 20 mg and were approximately 20 mm long. A cylinder of equivalent length and volume would have a radius of about 0.5 mm, but owing to the fusiform shape of the muscle the largest radius exceeded this value. Under a dissecting microscope with calibrated eyepiece the diameter of surface fibers was estimated at 80-100 μ. From these measurements it can be estimated that there were probably about 300 fibers in each muscle.

The muscle was placed in a chamber (fig. 1) which was part of a recirculating bathing system (fig. 2). An air-driven stirrer motor (which required no electrical shielding) drove stainless-steel paddles of a Lucite-cased pump which lifted bathing solution to a reservoir. Gas (95% O₂, 5% CO₂) was admitted to the reservoir at a point just below the tube through which solution entered the reservoir and gas and solution travelled together to the far end of the container. On the roof of the far end was an open turret permitting escape of any undissolved gas. At the base of the far end was an outlet from which the gassed solution flowed through a connecting tube to the muscle chamber. The connecting tube was made of a number of short lengths of gently-curving glass tubing linked by Tygon tubing. This arrangement gave a flexible connection and yet minimized the area of Tygon through the walls of which gas diffuses. The advantage of gassing the reservoir rather than the muscle chamber is that gas bubbles are eliminated from the chamber; otherwise the bubbles obscure the field and cause motion artifacts. The gassed solution entered one end of the muscle chamber through a small orifice drilled in line with the longitudinal axis of the muscle but at a lower level so that the solution, jetting into the chamber with high linear velocity, was driven beneath the muscle surface, permitting freshly gassed solution to flow upwards through the muscle. The chamber outlet orifice was connected by Tygon tubing to supply the inflow to the pump. In preliminary experiments the solution in the muscle chamber was explored with an oxygen electrode (11) and it was easy to establish pump and gassing rates which provided an oxygen tension to the chamber as high as could be attained by gassing the chamber directly; that is, there was essentially 100% oxygen. The volume of fluid in the chamber was only about 5 ml but the whole system circulated about 150 ml of solution.

The bathing solution (referred to in the text as saline-HCO₃-glucose solution) had the following composition (mM/l.): Na, 145; K, 4.7; Ca, 2.5; Mg, 1.2; Cl, 123; HCO₃, 28; Pi, 1.2; glucose, 11; pH was 7.4. When insulin was used, it was added directly to the reservoir to yield a final concentration of approximately 0.1 U/ml of bathing solution.

To reduce the muscle’s metabolism the experiments were done at room temperature, approximately 26°C, rather than at mammalian body temperature.

Microelectrodes were glass capillaries drawn on a mechanical puller made after the plans of Nastuk (19). The technique for drawing microelectrodes was devised by Mr. William J. Sullivan. The capillaries were threaded into a short length of polyethylene tubing, both
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glass and polyethylene were filled with a solution of 2 M KCl and the polyethylene tubing was led into a well filled with the same solution. Electrodes were selected by their electrical impedance, usually 15-30 MΩ. The glass capillary pipette was connected through an agar salt bridge and Ag-AgCl electrode to the grid of a 5879 tube. The circuit was completed symmetrically through a grounded Ag-AgCl electrode connected to the bathing solution through an agar salt bridge. Grid current on the 5879 cathode follower was checked with each experiment and was always 10⁻¹³ amp. or less, usually 10⁻¹⁴. The signal was amplified and displayed on a cathode ray oscilloscope at a gain of 25 mv/in. A short circuit between the metal conductors of the probe and the ground electrodes was operated manually through a relay which permitted grounding of the grid of the cathode follower. The signal through the short circuit was accepted arbitrarily as zero. If the potential difference with electrodes in the bathing solution was greater than 5 mv beyond arbitrary zero, the microelectrode was rejected. The difference, largely due to microelectrode tip potential, was usually 2 mv or less. In practice a surface fiber was impaled and the electrode then driven through a number of fibers deep in the muscle, the membrane potential of each fiber recorded as the electrode penetrated. When the electrode had penetrated as far as possible it was withdrawn and the system short-circuited to check the tip potential. When the tip potential changed by more than 1 mv between impalements, measurements were rejected.

Criteria for accepting a display on the oscilloscope as valid representation of the resting membrane potential were simply that the potential rose rapidly (either from zero base line or from some slightly higher value to which it fell between fibers) and was maintained sufficiently long to be read. No values were rejected simply because they were low. This latter fact may explain in part the lower mean values and, perhaps, greater variance in this report than in those reported for other muscles.

Potassium content of muscle was measured by flame photometry following acid digestion of muscle. Calculations were based on quadruplicate determinations on both of duplicate dilutions of the muscle digest. Volume of distribution of inulin was measured essentially by the method described by Creese (13) which depends upon incubating the muscle in a solution containing inulin, transferring it to an inulin-free solution for further incubation and finally measuring the inulin that diffused from muscle interstitial space into the solution. Total water was measured by drying the muscle at 95°C to constant weight.

RESULTS

Resting membrane potential in excised rat muscle in glucose solutions. The resting membrane potential (E₉) in extensor digitorum longus from 12 rats (307 impalements) in saline-HCO₃-glucose (external potassium concentration, 4.7 mM/l.) was 74 ± 0.8 mv (S.E. of mean).

Figure 3 is a plot of frequency distribution of E₉ in six of the control muscles which were subsequently exposed to insulin. The distribution is skewed toward high values, raising the possibility that many if not all the values below 65 mv represent a different, and perhaps injured, population of fibers. Nevertheless, these low values are included because they met the criteria described earlier.

Falsely low values for Eₙ may be anticipated if excised muscle is damaged by trauma or by hypoxia. In the former case, surface fibers, more susceptible to trauma, may be expected to have lower E₉'s than deeper fibers. In the latter case, surface fibers may be expected to have higher E₉'s than the hypoxic core, as was shown by Creese, Scholes and Whelan (14) for excised and hypoxic rat diaphragm.

Of the 225 impalements plotted in figure 3, 133 impalements were of the outer three fibers and 76 were of the fourth to sixth fiber, inclusively. Of the nine fibers in which E₉ was 50 mv or less, eight were found among the group of outer three fibers. However, the cumula—

**Fig. 3.** Frequency distribution of E₉ in muscles of 6 rats before exposure to insulin. Distribution is weighted for difference in number of impalements per rat.

**Fig. 4.** Cumulative frequency distribution of E₉ in 6 rats before exposure to insulin, showing difference between E₉ measured in outer 3 fibers and in 4th to 6th fibers.

**Fig. 5.** Frequency distribution of E₉ in muscles of 6 rats after exposure to insulin. Compare with fig. 3.
tive frequency distribution of $E_r$ among the outer three fibers was indistinguishable from that of $E_r$ among the second layer of three fibers for values of $E_r$ greater than 55 mv (fig. 4). This suggests that the very low values of $E_r$ may have been owing to trauma to some of the outermost three fibers. With the exception of these lowest values of $E_r$ among 6% of the outer three fibers, there was no trend in $E_r$ as the probe electrode advanced from the surface to the center of extensor digitorum longus. Even if those fibers in which $E_r$ was 50 mv or less are omitted the mean value of $E_r$ in figure 3 remains approximately 75 mv.

If a sufficiently large number of fibers are damaged either by trauma or by hypoxia there would be expected a decrease in intracellular potassium concentration and an increase in potassium efflux. Details of measurements of potassium concentration will be presented later in this report. There was no decrease in potassium concentration with time. Potassium efflux from excised rat extensor digitorum longus was measured under slightly different conditions, but the differences were such as to make hypoxia more rather than less likely (higher temperature and less efficient oxygenation). Nevertheless, potassium efflux was at a rate constant of only about 0.2 hr. $^{-1}$, comparable to that reported for frog sartorius (15) and lower than that reported by others for the rat (10).

Thus, by several existing criteria, there is no substantial evidence that $E_r$ is low in excised extensor digitorum longus owing to trauma or hypoxia, although the possibilities remain that the criteria may be relatively insensitive and that fibers may deteriorate with repeated puncture by microelectrodes.

**Effect of insulin on $E_r$ in presence of glucose.** Insulin, nearly always at a concentration of about 0.1 u/ml, added to saline-HCO$_3$-glucose solution (external potassium concentration, 4.7 mm/l.) increased $E_r$. In seven rats (376 impalements), $E_r$ was 79 ± 1.6 mv. In six of these muscles was measured in experiments in which insulin was added after a series of control measurements of $E_r$ had been made. No measurements were recorded beyond 1 hour after initial exposure of the muscle to insulin. Data from these muscles and from a seventh rat in which the bathing solution, saline-HCO$_3$-glucose, contained 6.1 mm/l. of potassium, are shown in table 1. In each case insulin led to hyperpolarization; the mean increase in $E_r$ being 5.4 ± 0.8 mv.

In figure 5 the frequency distribution of $E_r$ is displayed for the six rats in which external potassium concentration was 4.7 mm/l. This distribution is to be compared with that of figure 3 which shows control $E_r$ values from the same six rats. Besides showing the same skewed distribution as in figure 3, figure 5 is probably bimodal. There is a cluster of high frequency about 75 mv, reminiscent of that in figure 3, and a second cluster about 88 mv.

This bimodality is not the result of incomplete diffusion of insulin for if this were so $E_r$ would have been lower in deeper fibers, which was not the case. In fact $E_r$ in deeper fibers was higher than in the outer three fibers. This raises the possibility that there may have been somewhat more damage to outer fibers than had been estimated earlier. This damage may not have occurred in the initial preparation of the muscle but may have resulted from repeated puncture to which the outer fibers would be more vulnerable. The effect of insulin on $E_r$ in the layer of outer three fibers was only slight (fig. 6), but the effect of insulin on $E_r$ in the layer of fourth to sixth fibers was decidedly greater than the average effect cited above (fig. 7). This explains the bimodality shown in figure 5 and suggests that insulin is less effective on damaged fibers and that the quantitative effect of insulin on undamaged fibers is greater than estimated from table 1.

The time course of hyperpolarization induced by insulin could not be determined by observation of changes in a single fiber because stable resting potentials in a single fiber could not be recorded over a period of minutes, owing probably to mechanical oscillations in the system. Although there was a slight trend toward decrease of $E_r$ prior to addition of insulin, for any given muscle a plot of $E_r$ against time generally failed to show any trend after addition of insulin, perhaps because in any series of impalements at a single site on the muscle the range of $E_r$ was sufficiently large to obscure a trend and perhaps because with repeated impalements the preparation deteriorated. For these reasons the time course of insulin effect was appraised by plotting the mean increase in $E_r$ produced by insulin for each muscle against the mean time during which $E_r$ was measured after addition of insulin (fig. 8). Hyperpolarization increased with time.

**Water content and insulin space of extensor digitorum longus.** In seven muscles, water content was 76.6 ± 0.6% of the wet weight of the extensor digitorum longus. The mean

<table>
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<th>Insulin</th>
<th>Difference</th>
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<td>No. of fibers</td>
<td>$E_r ± S.E.M., \text{mv}$</td>
<td>No. of fibers</td>
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<td>41</td>
<td>61.5 ± 1.04</td>
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<tr>
<td>27</td>
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<td>53</td>
<td>81.5 ± 1.00</td>
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Mean difference 5.4 ± 0.8

* Solution was the saline-HCO$_3$-glucose solution described in the text. Potassium concentration was 4.7 meq/l, except in the first pair of observations in which it was 6.1 meq/l. Time refers to the elapsed time in minutes after addition of insulin to the bathing solution. The first number is elapsed time at which first measurement of $E_r$ was made; the second number is time at which last measurement was made.
wet weight of this group of muscles was 25.5 mg; the content of these muscles was 118.3 mEq/kg (S.D. ± 8.2 mEq/kg) immediately after excision from anesthetized rats. Potassium content in glucose solution. Forty muscles were frozen immediately after excision and before incubation. The two weights agreed to within 0.5 mg only on 10 occasions. They differed by 1.0 or 1.5 mg in eight instances and never differed by more than 2.5 mg. There were 18 instances of weight loss and only five of weight gain but the mean change in weight was not significantly different from zero (−0.46 ± 0.27 mg) and was not influenced by the duration of incubation. Nevertheless, the fact that there was a weight change in an individual muscle raised the question of whether the ‘before’ or ‘after’ incubation weight was the correct reference for measured potassium content.

If the weight changes were due to gain or loss of extracellular fluid, the former to failure to remove adherent excess perfusion fluid, the latter due to impure clotting or evaporation associated with delays in weighing, then the before weight would be more appropriate. However, if there were true changes in volume of fluid, then fiber water is reduced by 4%, that is, it is returned to the 1st hour of incubation. Our techniques for measuring total muscle water and inulin space are too crude to discriminate among these possibilities, perhaps both occurred.

The following example illustrates the effect of the difference between before and after weights on the final estimate of potassium concentration in fiber water. Assume that the potassium content of a digest of an entire muscle was 3.125 μEq. Assume also that the before weight was 25 mg and the after weight was 24 mg, a loss of 4%. If this loss is assumed to be the result of net efflux of fiber water in response to hyperosmotic perfusion fluid, then fiber water is reduced by 4%, that is, it is reduced from 52 to 50% of the wet weight of muscle. Given an external potassium concentration of 4.7 mEq/l, and an inulin space of 25% of muscle wet weight, the calculation of potassium in fiber water is...
where $K^+_i$ is potassium content per unit fresh weight of muscle, $W_e$ and $W_i$ are extra- and intracellular water, respectively, as gram per gram fresh weight.

On the basis of before weight, $[K^+]_i = \frac{(3125 - 1.2)}{24} / 0.52 = 238$, and on the basis of after weight, $[K^+]_i = \frac{(3125 - 1.2)}{24} / 0.5 = 258$. Thus, in this example, if the before weight is correct, use of the after weight leads to an overestimate of intracellular potassium concentration by about 8%.

Owing to uncertainty as to which weight is correct, the potassium content of muscle, per unit wet muscle weight, is presented in table 2 on the basis of both before and after weight. Concentration of potassium in milliequivalents per kilogram of fiber water can be estimated by subtracting 1.2 mEq from each value given in table 2 and dividing the difference by 0.52, or by 0.5, if the reader prefers. After 2 or 3 hours of incubation there were approximately 240 mEq of potassium per kilogram of muscle fiber water.

**Effect of insulin on potassium content of extensor digitorum longus in glucose solutions.** In an earlier report (5), potassium content of muscle after exposure to insulin was compared to the potassium content of the paired muscle in the opposite leg which had been excised and frozen immediately for subsequent chemical analysis. Such comparison is irrelevant because, as was shown in the previous section, the potassium content of the muscle increased when it was incubated in saline-HCO₃-glucose solution in the absence of insulin. To examine the effect of insulin on muscle potassium content the following experiments were conducted.

Both right and left extensor digitorum longus from a rat of the weight group used in the previous experiments were placed side by side in the perfusion chamber immediately after excision and incubated in saline-HCO₃-glucose solution. The first muscle was removed either after 1 or after 2 hours. The second muscle remained in the chamber for an additional 1 or 2 hours, but never for more than 3 hours. Within this time framework three types of experiments were conducted: 1) insulin was never added to the solution (control vs. control), 2) insulin was added to the solution (0.1 U/ml) after the first muscle was removed (control vs. insulin), 3) insulin was present in the solution from the beginning (insulin vs. insulin). Owing to conflicting reports of the persistence of insulin effect on excised mammalian tissues (17, 18), no attempt was made to incubate both muscles in the presence of insulin, and then, after removal of the first muscle, to incubate the second muscle for an additional time in an insulin-free solution.

In 5 of 10 control vs. control experiments potassium content of muscle increased with further incubation, but in 12 of 15 paired experiments in which insulin was present, either control vs. insulin or insulin vs. insulin, potassium content increased with time. These results in paired experiments, tested by chi-square method, show that the potassium content of muscle increased with time in the presence of insulin but not in its absence.

If the effect of pairing is ignored and experiments are combined in which muscles were incubated for similar times (table 3 and fig 9), although the mean concentration of potassium in muscle became higher than in the appropriate control muscle whenever insulin was added, owing to the small numbers and large variance within each group the differences between means are not significant.

When groups are pooled (table 4), the effect of insulin can be described with greater confidence. At the end of the 1st hour's exposure to insulin the potassium concentration of muscle could not be distinguished from that of muscles not exposed to insulin. When muscle was incubated with insulin for 2 or 3 hours there was clearly an accumulation of potassium, but the effect was not demonstrably greater after three hours than after two.

If we are to compare $E_1$, with changes in muscle potassium we are interested in the quantitative aspects of the increase in muscle potassium. Since tables 3 and 4 are based on after weights, the question arises as to whether insulin affected the difference between before and after weights observed in the absence of insulin. There was no evidence that it did. The same variation
in before and after weights occurred in the presence of insulin as in its absence, independent of duration of exposure to insulin. Among 22 muscles exposed to insulin, in which both before and after weights were obtained, the mean decrease in wet weight was 0.68 ± 0.36 mg.

It is assumed, then, that insulin had no effect either on total muscle water or on the distribution of water between fibers and interstitial space. If this be correct, then, at the range of concentrations in muscle and in extracellular fluid in these experiments, the relative change in potassium content of fiber water is approximated closely by the relative change in potassium content of muscle, per unit wet weight.

From the data of table 4 it can be calculated that the ratio of mean potassium content of muscle after insulin to its content before insulin was: after 1 hour's exposure to insulin, 1.03 ± 0.024; after 2 hours' exposure, 1.11 ± 0.044; after 3 hours' exposure, 1.09 ± 0.043. When the results of 2 and 3 hours' exposure to insulin are combined the ratio of potassium in muscle after and before insulin is 1.10 ± 0.036.

**Discussion**

According to Nernst, during equilibrium the electrical potential difference between two segments of a solution produced by inequality of concentration of diffusible potassium is

\[ E = \frac{RT}{F} \ln \frac{[K^+]}{[K^+]_0} \quad (1) \]

where \( R \) is the gas constant, \( T \) the absolute temperature, \( F \) the faraday and \( [K^+]_0 \) and \( [K^+] \) are activities of the ion in the two segments, say in fiber water and in interstitial fluid, respectively.

It has become a common, though incorrect, practice to equate the ratio of concentrations to the ratio of activities (activity being the product of an activity coefficient and concentration) on the assumption that the activity coefficients for univalent ions inside and outside the fiber are identical. For frog sartorius Adrian (19) has shown that this assumption is approximately true, but it need not be true for extensor digitorum longus of young rats. If the chemical potential of water inside muscle is equal to that outside, as most observers agree, intracellular osmolality must be about 280 mOsm/l. Since the single cation, potassium, accounts for six-sevenths of this number on a concentration basis, there must be a large proportion of polyvalent anions, a condition which might reduce the estimate of the activity coefficient of potassium salts in fiber water.

If activity coefficients and potassium binding or sequestration are ignored, the potassium equilibrium potential, \( E_K \), for rat extensor digitorum longus at 26°C is, from equation 1, 101 mv.

The observed \( E_T \) in the absence of insulin was considerably less than the predicted potassium equilibrium potential. If it is assumed that \( E_T = E_K \), then the equation can be solved for the activity coefficient of intracellular potassium; it becomes a value so low as to be unlikely, for then it is difficult to account for the total osmolality of intracellular fluid.

Since flame photometry measures all the potassium in muscle while equation 1 considers only mobile potassium ions, the question arises as to whether or not the mobility of a relatively large fraction of intracellular potassium is restricted, say by association with proteins, or by anatomical sequestration within the cell, say in mitochondria. Actually, if activity coefficients of potassium on both sides of the muscle membrane are the same, at 26°C, and for \( [K^+]_0 = 4.7 \text{ mEq/l.} \), the observed \( E_T \) of 74 mv predicts \( [K^+]_0 = 83 \text{ mEq/l.} \). That is, if \( E_T \) and \( E_K \) were in fact equal then about two-thirds of intracellular potassium would be bound or sequestered. This is far too high a bound fraction to permit isosmolality of solutions inside and outside muscle fibers and is therefore unlikely. It remains possible, however, that some potassium is bound or sequestered and that \( E_K \) is less than calculated.

An alternative explanation for the discrepancy between \( E_T \) and \( E_K \) is that proposed by Hodgkin and Katz (20) for systems not in the steady state:

\[ E_T = \frac{RT}{F} \ln \frac{P_K(K^+)_0 + P_{Na}(Na^+)_0 + P_{Cl}(Cl^-)_0}{P_K(K^+)_0 + P_{Na}(Na^+)_0 + P_{Cl}(Cl^-)_0} \quad (2) \]

where \( P \) is an arbitrary coefficient defining the relative permeability of the membrane to the ions indicated by the subscript. The most important of the simplifying assumptions made in deriving equation 2 are that the
membrane is homogeneous and that the electrical field through it is constant. Assuming that $P_{Na}$ is small, as it is in the squid giant axon, then, if the ratio $(Cl^-)_o/(Cl^-)_i$ were less than the ratio $(K^+)_o/(K^+)_i$, $E_C$ would fall between $E_K$ and $E_{cl}$ if $E_{cl}$ is any non-negligible fraction of $P_K$. Unfortunately, we lack data on $(Cl^-)_i$. Nevertheless, if it is as large as $3 \text{mM/l}$, the ratio $(Cl^-)_o/(Cl^-)_i$ is less than $(K^+)_o/(K^+)_i$. There is, then, a real possibility that $E_C$ in rat extensor digitorum longus is more nearly related to the equilibrium chloride potential than to the equilibrium potassium potential.

Since the sign of the sodium equilibrium potential is the reverse of that of the potassium equilibrium potential, if $P_{Na}$ in equation 2 is greater than zero the potential defined by that equation must be less than $E_K$. Estimates of permeability coefficients have not been made for mammalian muscle. In the most recent exploration of this phenomenon in frog muscle no consistent values could be assigned permeability coefficients (19).

In any case, $E_K$ is almost certainly greater than the observed $E_C$. This fact implies that a fraction of the potassium moving into muscle, under equilibrium conditions, moves against a potential gradient; that is, is transported actively.

If potassium were impelled by insulin to accumulate in muscle fiber water and if this accumulation caused the observed hyperpolarization, how much would the intracellular concentration of potassium have to increase?

If the concentration of potassium in muscle fiber water is $(K^+)_i'$ before exposure to insulin and $(K^+)_i''$ after exposure to insulin, if the resting potential difference across the muscle membrane is $E_o$ before insulin and $E_o''$ after insulin and if all other elements of equation 2 remain constant during the period of observation, then from equation 2,

$$\frac{(K^+)_i''}{(K^+)_i'} = e^{E_o' - E_o''} = e^{\Delta E} = \frac{\alpha}{P_K(K^+)_o''} \left( 1 - \alpha \frac{P_{Na}(Na^+)_o'}{P_{cl}(Cl^-)_o'} \right), \quad (3)$$

where

$$\Delta E_r = E_o' - E_o'' \quad \text{and} \quad \alpha = P_{Na}(Na^+)_o' + P_{cl}(Cl^-)_o'.$$

If $\alpha = 0$, equation 3 reduces to

$$\frac{(K^+)_i''}{(K^+)_i'} = e^{\Delta E_r \frac{RT}{P_K(K^+_o)}.} \quad (4)$$

which is the solution one would expect if $E_r = E_K$.

At $26^\circ$, the temperature at which these experiments were conducted, and using the increase in $E_K$ reported in table 1, $\Delta E = 5.4 \text{mv}$, from equation 4, $(K^+)_i''/(K^+)_i' = -1.23$; that is, if the membrane potential was described accurately by the potassium equilibrium potential, and if hyperpolarization were caused only by an increase in intracellular potassium, $(K^+)_i'$ would have to increase by $23\%$ to account for a rise of $5.4 \text{mv}$ in $E_r$.

However, $E_r$, for rat extensor digitorum longus is less than $E_K$ and equation 3 is therefore more appropriate. At $26^\circ$ and for $\Delta E = 5.4 \text{mv}$, assuming that the only change in members of equation 2 is in $(K^+)_i$,

$$\frac{(K^+)_i''}{(K^+)_i'} = 1.23 + 0.23 \frac{\alpha}{P_K(K^+)_o'}. \quad (5)$$

Since the second term is positive, the increase in $(K^+)_i'$ predicted from equation 3 is greater than $23\%$ per cent and its magnitude grows with $\alpha$. If $\alpha = P_K(K^+)_i'$, and it is unlikely to be much higher, the predicted increase is $46\%$; if $\alpha = 0.5 P_K(K^+)_i'$, as is approximately true for squid giant axon, the predicted increase in $(K^+)_i'$ is $34\%$.

However, by the time $E_r$ had increased by some $5 \text{mv}$ under the influence of insulin, there was little or no increase in $(K^+)_i$. Only on prolonged exposure to insulin did $(K^+)_i$ rise and after 3 hours it had risen on the average by only $10\%$. The odds against the increase in potassium after 1 hour being as great as $23\%$ are greater than $10^{11}$ to 1. The odds against the increase in potassium after even 2 or 3 hours of insulin being as great as $23\%$ are about $3000$ to 1. That is, if the assumptions from which are derived equations 1 and 2 are valid for these experiments, the changes in potassium concentration produced by insulin are not adequate in themselves to account for the observed hyperpolarization. Indeed, the data so far at hand indicate that hyperpolarization precedes the change in potassium concentration and that in response to the new potential difference across the membrane, assuming no change in that fraction of potassium which is transported actively, potassium moves toward a new equilibrium ratio of concentrations on either side of the membrane. That is, the well-known effect of insulin as an hypokalemic agent can be attributed to hyperpolarization.

If the increased $E_r$ produced by insulin is not secondary to a change in concentration of potassium, to what is it due? Conceivably it may be due to an increase in the activity coefficient of potassium inside muscle, but this implies that insulin causes a decrease in intracellular ionic strength. If such occurs it is presumably due to a change in the intracellular anion population. It is possible that an appropriate change in intracellular organic anions might represent a metabolic effect of insulin.

### Table 4. Effect of Insulin on Potassium Content of Extensor Digitorum Longus

<table>
<thead>
<tr>
<th>No. of Control, 1, 2 and 3 hr.</th>
<th>Potassium Content, mEq/kg Wet Wt.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>125.7 ± 2.70</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>139.4 ± 10.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12</td>
<td>139.4 ± 1.45</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>9</td>
<td>139.4 ± 5.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>6</td>
<td>139.4 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>138.4 ± 3.90</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are pooled from those of table 3. $P$ is probability, estimated by $t$ test, that there occurred by chance the difference between mean potassium after indicated exposure to insulin and mean potassium content of control muscles. The indicated hours of exposure to insulin do not necessarily represent total hours of incubation, for which see table 3.
Against this possibility are the quantitative demands of the situation. Equations 3 and 4 may be rephrased by substituting the ratio of activity coefficients for the ratio of concentrations, if the concentrations are constant. If the activity coefficients were about 0.75 before insulin, an increase of 5.4 mv in $E_T$ would require that the activity coefficient of intracellular potassium rise by 23% to 0.92. It is improbable that intracellular ionic strength could decrease sufficiently to produce a change of this size. Furthermore, if an early change in activity coefficient accounted for the hyperpolarization there would be no simple explanation for the late increase in intracellular potassium concentration.

A second possibility is that insulin increases the permeability of the membrane to potassium or decreases its permeability to other ions whose equilibrium potentials are lower than $E_T$ in the absence of insulin. Preliminary measurements of $K^{\text{a}}$ flux from extensor digitorum longus failed to demonstrate that insulin increased permeability to potassium. If these observations are established as correct, there remains the possibility that insulin affects membrane permeability to other ions and, of these, owing to its quantitative importance, chloride would be most suspect, and sodium would be suspect because $E_{Na}$ is so far removed from $E_T$ that changes in permeability to sodium might exert a large effect on $E_T$.

A third possibility is that the assumptions on which equation 2 is based do not apply to the conditions of these experiments. In the absence of these simplifying assumptions it is difficult to make quantitative predictions of the relation between changing intracellular potassium concentration and changing $E_T$. If the membrane is not homogeneous and if it is spatially altered by insulin, conceivably hyperpolarization might occur without gross changes in potassium concentration.

Finally, the membrane itself may be a source of electrical potential with a potential difference between its two surfaces, possibly generated by metabolic processes within the membrane. If this is so, then it may be upon these processes that insulin acts. Against this possibility is the fact that although muscle membranes and their contiguous fluids do not behave precisely like a Gibbs-Donnan system, they do so nearly, as Boyle and Conway (21) showed for frog muscle, that if the membrane is not inert neither is it likely to be a large source of potential. Nevertheless, since we are examining a difference of only about 5 mv, or 10 mv, if one compares $E_T$ for deeper fibers, it is possible that this amount might be added by the membrane itself. This would be a happy solution, for it would not only account for the redistribution of potassium as a phenomenon secondary to hyperpolarization but it would also link metabolic effects of insulin to its effect on the cell membrane.

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REFERENCES