

## Resting transmembrane potential difference of skeletal muscle in normal subjects and severely ill patients

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### Research Article

The resting membrane potential difference ( $E_m$ ) of skeletal muscle was measured in 26 normal human subjects, 7 patients with mild illness, and 21 patients with severe, debilitating medical disorders. A closed transcutaneous approach to the muscle was made by needle puncture and the  $E_m$  was measured utilizing standard Ling electrodes. Measurements revealed an  $E_m$  of  $-88 \pm 3.8$  mv in healthy subjects and  $-89 \pm 2.1$  mv in patients hospitalized for minor medical problems. The mean  $E_m$  in 21 in-hospital patients, judged to be severely ill clinically from a variety of causes, was  $-66.3 \pm 9.0$  mv. Open deltoid muscle biopsies were performed in 7 of the healthy subjects and in 13 of the severely ill group. Estimation of the intra-extracellular water partition was made by calculating the chloride space from the previously measured  $E_m$ . Analysis of the muscle samples revealed no significant difference in the intra-extracellular potassium ratios of the two groups biopsied. Intracellular  $\text{Na}^+$  concentrations were uniformly increased in the muscle samples of the severely ill subjects and averaged 42.3% higher than those of the normal subjects. The mechanisms which might account for the elevation of intracellular  $\text{Na}^+$  and a depression of  $E_m$  independent of changes in intra-extracellular  $\text{K}^+$  ratios are discussed and it is suggested that this defect may be a generalized cellular abnormality which is a common quality of [...]

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# Resting Transmembrane Potential Difference of Skeletal Muscle in Normal Subjects and Severely Ill Patients

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**ABSTRACT** The resting membrane potential difference (Em) of skeletal muscle was measured in 26 normal human subjects, 7 patients with mild illness, and 21 patients with severe, debilitating medical disorders. A closed transcutaneous approach to the muscle was made by needle puncture and the Em was measured utilizing standard Ling electrodes. Measurements revealed an Em of  $-88 \pm 3.8$  mv in healthy subjects and  $-89 \pm 2.1$  mv in patients hospitalized for minor medical problems. The mean Em in 21 in-hospital patients, judged to be severely ill clinically from a variety of causes, was  $-66.3 \pm 9.0$  mv. Open deltoid muscle biopsies were performed in 7 of the healthy subjects and in 13 of the severely ill group. Estimation of the intra-extracellular water partition was made by calculating the chloride space from the previously measured Em. Analysis of the muscle samples revealed no significant difference in the intra-extracellular potassium ratios of the two groups biopsied. Intracellular  $\text{Na}^+$  concentrations were uniformly increased in the muscle samples of the severely ill subjects and averaged 42.3% higher than those of the normal subjects. The mechanisms which might account for the elevation of intracellular  $\text{Na}^+$  and a depression of Em independent of changes in intra-extracellular  $\text{K}^+$  ratios are discussed and it is suggested that this defect may be a generalized cellular abnormality which is a common quality of serious illnesses.

## INTRODUCTION

In the past several years, considerable interest has developed about the study of the resting transmembrane potential difference (Em) of skeletal muscle in human subjects (1-11). Published results of data obtained in human subjects vary greatly and appear to be a function of at least two variables; the difference in technique used

to measure membrane potential in human subjects and the clinical state of the human subjects examined. This present study was performed on skeletal muscle in human volunteers with the following four objectives: first, to establish a technique for consistent and accurate recording of Em in human skeletal muscle; second, to define the mean value and ranges of muscle Em in the normal population; third, to explore Em changes in altered clinical states; and finally, to determine the relation of electrolyte composition of muscle cells to the changes in membrane potential occurring in disease states.

## METHODS

### Selection of human subjects

Three separate groups of human subjects were selected as follows.

**Normal subjects.** 26 healthy volunteers were selected at random from the general population. Care was taken to insure that no one in this group had a history of past illness of a systemic nature and that no medications were being received at the time of the study. The mean age was 28.9 yr with an approximate equal distribution of males and females, Caucasians and Negroes.

**Mildly ill subjects.** Seven in-hospital patients with a mean age of 41.9 yr were selected. This mildly ill group was characterized as having illnesses which did not incapacitate them and allowed ambulation at the time of study. Four of the patients were hospitalized for evaluation and treatment of medical illnesses which responded well to conventional management. Two patients were followed after uncomplicated surgical procedures (E. M. and R. H.). An additional patient (J. M.), who had sporadic periodic paralysis, was studied at a time during which his serum potassium concentration was normal and there had been no recent paralytic episodes. All of these patients were discharged in good clinical condition after relatively uncomplicated hospital courses.

**Severely ill subjects.** 21 in-hospital patients, mean age 43.8 yr, judged to be severely ill on a clinical basis were studied. All of these patients had systemic illnesses of a severe nature with complications resulting from their basic

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disease. In contrast to the group of mildly ill subjects, this entire group of severely ill patients had as their common denominator: hospital courses characterized by the need for intensive medical management, prolonged hospital stays, generalized weakness and exercise intolerance which for the most part necessitated confinement to bed, and an extremely poor clinical prognosis during hospitalization and at the time of final discharge. None of the patients were hypotensive or clinically anoxic at the time of study, although a few had reduced  $PO_2$  (Table III). Some of the patients studied in both the mildly ill and severely ill groups (Tables II and III) were receiving drugs at the time of the study. As can be seen from the tables, the most commonly administered drug was digitalis. A significant number of patients were receiving antihypertensive medication which included guanethidine, trichloromethazine, and methyldopa. However, 4 of 7 patients in the mildly ill group and 11 of 21 patients in the severely ill group had received no drugs for a reasonable period of time (at least several days) prior to study.

### Preparation of electrodes

Electrodes were made from dichromate acid-washed borosilicate capillary glass of 0.9 mm i.d. They were pulled on an automatic horizontal pipette puller to a tip diameter of approximately  $0.5 \mu$ . The electrodes were filled under vacuum with a solution of 2.0 M KCl/0.5 M  $KNO_3$ . The tip resistance and tip potential of the electrodes were tested

before use as follows. The electrode was mounted in a plastic holder the chamber of which was filled with the same KCl/ $KNO_3$  solution as the electrode and which contained a Ag-AgCl electrode at the opposite end of the chamber. The electrode was connected to the input of a unity gain amplifier (Medistor Instrument Co., Seattle, Wash.), the output of which was displayed on a Tektronic oscilloscope. The low impedance side of the circuit consisted of a Beckman calomel half-cell reference electrode dipped in a solution of normal saline and connected in series to the reference side of the Medistor. When both the microelectrode tip and the calomel reference electrode tip were immersed in the isotonic sodium chloride solution, the potentiometer built into the Medistor was adjusted so that zero potential existed between the two electrodes. The tip resistance was measured according to the method of Frank and Fuortes (12). The electrodes used had tip resistances of 5–7 megohms. The magnitude and variability of microelectrode tip potential was checked by measuring the potential difference between the reference calomel electrode and the microelectrode, first in a solution of isotonic Ringer's lactate simulating extracellular fluid and next in a phosphate buffer solution simulating the internal environment of the cell (150 mEq potassium/liter, 300 mOsm/kg). The change in potential difference between these two solutions was taken as the maximum effect of tip potential; only electrodes with tip potential changes of less than 5 mv were selected for use.

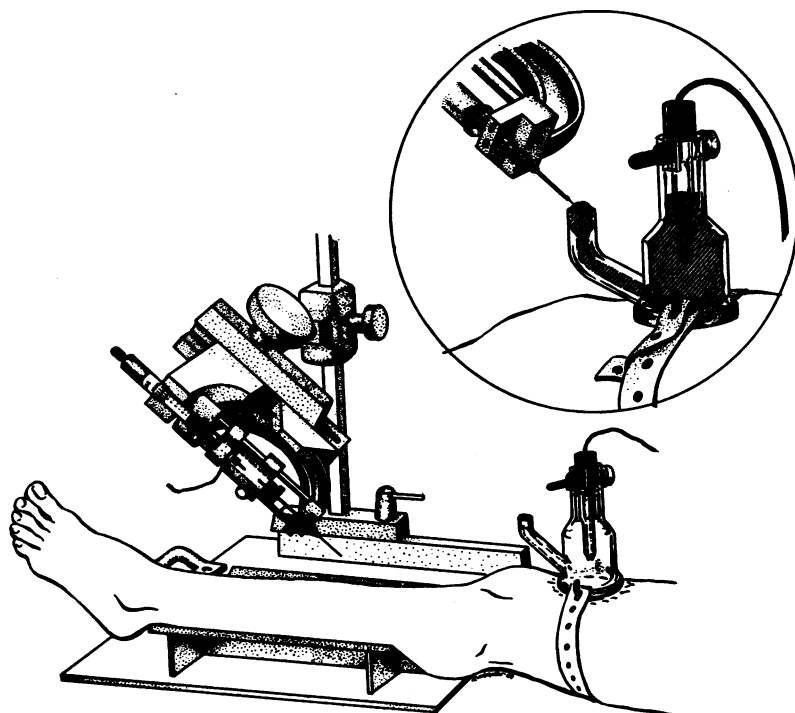


FIGURE 1 Electrode manipulator in position to make puncture into the anterior tibial compartment of the stabilized leg. Note that the V-shaped trough and the pedestal holding the electrode manipulator are mounted on a heavy steel plate to afford stability to the entire unit and leg. Insert shows microelectrode in reference solution in side arm of the skin-liquid junction-calomel reference unit (see text).

## Measurement of membrane potential

Transmembrane potential of human skeletal muscle was measured by transcutaneous puncture of the anterior tibial compartment of the leg utilizing the method described by Beranek (1, 2). After extensive experimentation with a variety of locations, this particular muscle group was found to yield the most constant and reproducible results. The subjects were placed in a supine position on a standard hospital bed around which was constructed a portable Faraday cage. The subject's leg was immobilized in a V-shaped trough (Fig. 1). The microelectrode holder was mounted on a movable chassis above the leg. A glass microelectrode was inserted, blunt end first, through an 18 gauge thin-bore hypodermic needle into a plastic chamber filled with KCl/KNO<sub>3</sub>. This plastic chamber was attached to a micrometer advance mechanism. Both the 18 gauge needle and micrometer advance mechanism were firmly attached to a vertical rack and pinion control (Fig. 2). The solution in the plastic chamber was connected through a Ag-AgCl electrode to the high impedance side of the recording equipment. The low impedance side of the circuit consisted of a Beckman calomel half-cell reference electrode whose tip was in electrical contact with the skin of the patient's leg via a potassium phosphate buffer solution ( $K^+ = 150$  mEq/liter). This was made possible by a plastic cylinder which could be strapped to the anterior surface of the suprapatellar region of the leg (Fig. 1, insert) and filled with the buffer solution. The underlying skin was first cleaned with acetone. Prior to puncture the microelectrode was placed in the buffer solution in the plastic cylinder; tip resistance was checked and the electrode was zeroed against the reference electrode. This use of liquid buffer for establishing contact between skin and reference electrode avoided the erratic surface potentials occurring with the use of solid skin electrodes.

Following the final electrode check, the skin over the mid-third of the anterior tibial surface of the leg was surgically prepared and anesthetized over a 1.5 cm area with 1% procaine. Procaine was injected down to the outer fascial layers over the muscle and care was taken not to inject any of the anesthetic into the muscle belly itself. A small (2-3 mm) incision was made and the 18 gauge needle (containing the microelectrode tip retracted 1-1.5 cm) was advanced into the muscle mass of the leg with the vertical rack and pinion. Once the needle sheath was in a stable position inside the muscle belly, the microelectrode was advanced by means of the micromanipulator. In this manner the electrode tip could be advanced through numerous layers of muscle cells obtaining a membrane potential with impalement of each single cell.

To check the validity of the membrane potentials obtained with this technique, two different types of experiments were performed. In a small group of normal volunteers, membrane potentials measured with the technique described above were compared with those obtained through an open-skin incision through which the microelectrodes were advanced into the muscle fibers without the aid of a needle trocar. A similar group of comparative experiments were performed in rats measuring membrane potential both with the needle trocar method and with open-skin incisions.

## Analytical methods

Open deltoid muscle biopsies were performed at the time of membrane potential recording in some of the normal subjects and patients. Atraumatic excision of approximately

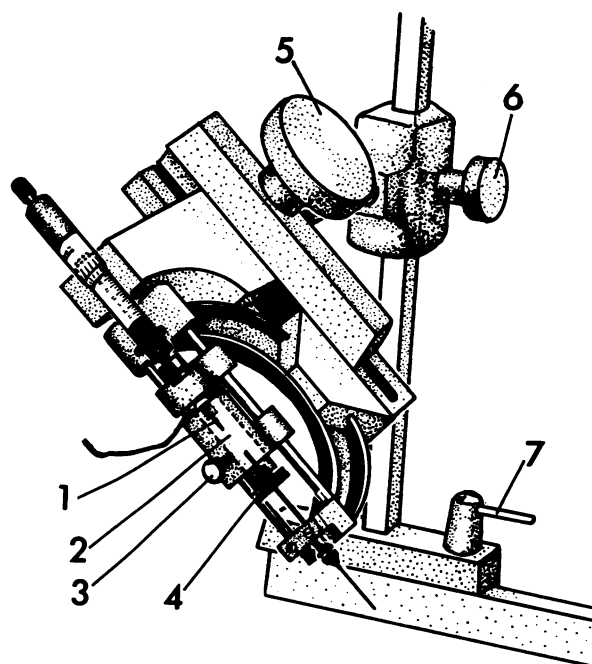


FIGURE 2 Detail of the microelectrode holder and manipulating unit. (1) Ag-AgCl electrode in plastic chamber filled with KCl-KNO<sub>3</sub> solution. (2) Plastic chamber held in place in micro-manipulator ways by thumb screw (3). (4) Glass microelectrode held in plastic chamber by plastic lock-nut seating against an O-ring of neoprene. (5) Macro rack and pinion advance for the micrometer-electrode chamber-electrode assembly. (6) Vertical ordinant control. (7) Horizontal ordinant control.

1 g of skeletal muscle was performed and the muscle samples were immediately transferred to a tared bottle and weighed. The samples were dried under vacuum at room temperature for 12 hr and then for 24 hr at 70°C to a constant dry weight. The dried samples were pulverized and fat removed by two 6-hr petroleum ether extractions. Fat-free dry solid weight was obtained after a final period of drying under vacuum at 70°C for 24 hr. Muscle electrolytes were leached out by adding 2.5 ml of 10% acetic acid to the fat-free residues and allowing the samples to shake for 12 hr. Sodium and potassium of serum and muscle extracts were determined by flame photometry, and chloride by a Cotlove chloridometer. Serum pH, Pco<sub>2</sub>, and Po<sub>2</sub> were determined using an Instrument Laboratory, Inc. pH meter.

## Microscopy

In association with each biopsy a deltoid muscle sample was also obtained for light and electron microscopy. Care was taken to preserve muscle fiber lengths by use of a special muscle clamp (13). Specimens for light microscopy were fixed in 10% formalin and specimens for electron microscopy were preserved in a glutaraldehyde phosphate buffer solution (pH 7.38) prior to postfixation in osmium tetroxide.

## Calculations

The partition of water and electrolytes between intra- and extracellular phases was calculated on the basis of the

chloride space as the estimate of extracellular fluid volume. Intra- and extracellular chloride distribution was considered to be a passive phenomenon related to the measured resting potential in a manner predicted by the Nernst equation (14). Chloride distribution between intra- and extracellular phases was calculated in the following manner:

$$E_m = -61.5 \log \frac{Cl_o^-}{Cl_i^-} \quad (1)$$

$$Cl_i^- = \frac{Cl_o^-}{\frac{\text{antilog } E_m}{-61.5}} \quad (1a)$$

where

$E_m$  = measured resting potential

$Cl_o^-$  = concentration of plasma water

$Cl_i^-$  = concentration of intracellular water chloride (calculated from the Nernst equation).

Total sample chloride

= extracellular chloride

+ intracellular chloride (2)

$$= (ECW) (Cl_o^-) + (ICW) (Cl_i^-) \quad (2a)$$

$$= (ECW) (Cl_o^-) + (\text{total sample water} - ECW) (Cl_i^-) \quad (2b)$$

where

ECW = extracellular water

ICW = intracellular water

Intracellular  $Na^+$

$$= \frac{\text{total muscle } Na^+ - (ECW) (Na_{ECW})}{ICW} \quad (3)$$

Intracellular ( $K^+$ )

$$= \frac{\text{total muscle } K^+ - (ECW) (K_{ECW})}{ICW} \quad (3a)$$

However, since the value for  $(ECW) (K_{ECW})$  is so small intracellular ( $K^+$ ) was calculated as follows:

$$\text{Intracellular } (K^+) = \frac{\text{Total muscle } K^+}{ICW} \quad (3b)$$

The concentrations of  $Na^+$ ,  $K^+$ , and  $Cl^-$  in ECW were calculated from their plasma concentrations correcting for plasma water of 0.94 and the following Donnan factors:  $Na^+$  0.99,  $Cl^-$  1.01 (15).

## RESULTS

**Pattern of membrane potential measurement in normal subjects.** As shown in Fig. 3 progressive advance of the microelectrode through the needle trocar into the muscle produced a stair-step increase in  $E_m$  beginning at approximately  $-40$  to  $-60$  mv and increasing to a plateau value of approximately  $-90$  mv. The plateau value is obtained after advancing the electrode 1–1.5 mm (Fig. 3). Low values on initial puncture with an increase to a plateau have also been observed by Bolte, Riecke, and Rohl (5), although they did not achieve a plateau value until the microelectrode tip was advanced approximately 1.3 cm into the muscle. An explanation

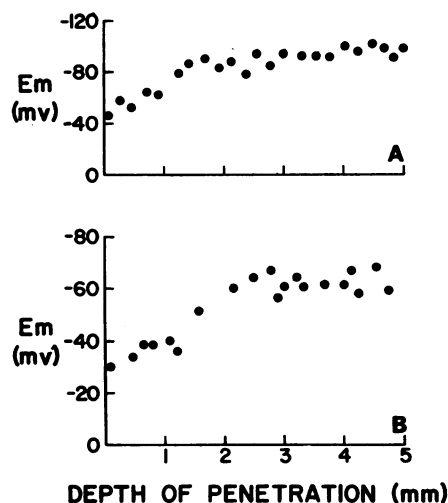


FIGURE 3 The increase of transmembrane potential with the depth of penetration of the microelectrode into the muscle tissue. Depth of penetration refers to the number of mm the tip of the microelectrode is beyond the metal trocar. (A) Control subject. (B) Severely ill patient.

for the early low membrane potentials is that the presence of the needle trocar injured the muscle fibers adjacent to the tip of the trocar. However, this same pattern of early low potentials was seen even when the measurements were made with Ling electrodes placed directly into previously exposed muscle without using the needle trocar. Perhaps this too is the result of injury; conceivably, exposure to air is sufficient to injure surface fibers, causing low transmembrane potentials.

After plateau values of membrane potential have been obtained, repeated impalements of fibers could be recorded as the electrode was advanced. As has been previously noted, the potential did not necessarily return to zero as the electrode passed from one fiber to the next (7). This is probably due to the fact that the speed with which the electrode passed through the very small inter-fiber space was greater than the response time of the high impedance electrode. Very similar results were obtained in rat hind limb muscle with this equipment both with and without the needle trocar. In five normal rats, the mean muscle  $E_m$  measured using the needle trocar technique was  $-88.7 \pm 5.0$  (mean  $\pm$  sd) as estimated from the plateau in each rat. This compares favorably with results obtained in this laboratory using more conventional techniques (16).

**Comparison of membrane potential in normal subjects and seriously ill patients.** For purposes of comparison among different groups of patients, the low values of membrane potential that occurred at shallow depths were ignored and only the high plateau values were used. Again, upon first entering the muscle tissue with the

microelectrode, low voltage readings were obtained in patients as in control subjects, but as in the controls, a plateau level was always obtained.

The control group of healthy volunteers had an average resting Em of  $-88.0 \pm 3.8$  mv (Table I). The group of mildly ill hospitalized patients had similar values of  $-89.8 \pm 2.1$  mv (Table II). In sharp contrast to these values the severely ill group consistently had depressed Em with an average value of  $-66.3 \pm 9.0$  mv (Table III). The Em for these patients ranged from  $-48$  to  $-79$  mv and did not overlap with the values for the control volunteers which ranged from  $-80$  mv to  $-98$  mv. Serum electrolytes, blood pH,  $P_{CO_2}$  and  $P_{O_2}$  were for the most part similar in all three groups. Note that the mildly ill group (Table II) had a transmembrane potential not different from the normal subjects. Moreover, the mean age of this group was similar to that of the patients in the severely ill group. This suggested that the low Em seen in the severely ill patient group are not the result of the increased age of this group compared with the normal subjects.

**Muscle analysis.** Open deltoid muscle biopsies were performed on 7 normal subjects (three normal controls and four mildly ill subjects) and on 13 of the severely ill group at the time of Em recording. The results of muscle analysis are shown in Table IV. There were no significant differences in extracellular sodium, potassium or chloride concentration in the two groups. The values for intracellular  $Na^+$ ,  $K^+$ , and  $Cl^-$  in the control subjects are in agreement with those previously reported (17). There were no significant differences in extracellular water content in the two groups.

As a result of the lower Em, the calculated intracellular chloride concentration was increased to 8.8 mEq/liter in the severely ill patients (compared to 4.1 mEq/liter in the normal subjects). The calculated intracellular concentration of sodium was also significantly higher in the severely ill patients (severely ill  $37.7 \pm 10.1$  mEq/liter vs. normals  $26.5 \pm 4.3$  mEq/liter). This average increase of approximately 10 mEq/liter in intracellular sodium in patients with severe illness represents a 42.3% increase, a change which is statistically significant to the 0.001 confidence level. It is particularly noteworthy that the increase in intracellular sodium concentration was not associated with any commensurate decrease in intracellular potassium (151.5 mEq/liter vs. 149.0 mEq/liter).

Light and electron microscopy of the muscle samples revealed no morphologic differences in the two biopsied groups.<sup>1</sup>

<sup>1</sup>We wish to acknowledge and thank Dr. Anthony N. D'Agostino of the Department of Pathology, University of Texas (Southwestern) Medical School at Dallas for preparing and examining both the light and electron microscopic sections of muscle.

TABLE I  
Normal Subjects

Subject	Race	Sex	Age	Em
			yr	mv
1. M. L.	C	F	21	-90.8
2. L. P.	C	F	29	-84.0
3. B. B.	C	M	23	-92.5
4. B. R.	C	F	30	-90.8
5. J. P.	C	M	24	-90.4
6. L. R.	C	F	25	-80.7
7. J. H.	C	F	25	-87.4
8. K. V.	C	F	26	-90.6
9. B. M.	C	M	23	-82.9
10. M. F.	C	M	25	-87
11. J. M.	C	F	31	-92
12. R. M.	N	F	36	-92.5
13. E. T.	N	F	41	-88
14. M. C.	C	F	24	-98
15. C. W.	N	M	40	-92.6
16. M. H.	C	F	30	-87.3
17. M. C.	C	F	25	-86.1
18. O. B.	N	F	38	-93.8
19. R. R.	N	F	39	-87
20. E. H.	N	F	33	-88
21. M. W.	N	F	33	-90
22. T. R.	C	M	28	-83
23. D. R.	C	F	22	-86.2
24. J. R.	C	M	29	-88
25. H. B.	C	M	29	-88.1
26. C. H.	C	F	25	-87.5
Mean			28.9	-88.8 $\pm 3.8$ (SD)

C = Caucasian; N = Negro.

## DISCUSSION

Previous attempts to measure resting membrane potential in human skeletal muscle have yielded values ranging from  $-65$  mv to nearly  $-90$  mv (3, 5-8, 10, 11) (Table V). There are at least two possible explanations for the wide range of potentials reported. (a) Some workers may never obtain very high potentials, presumably due to technical difficulties (e.g. electrode tips too large, electrode tips broken by puncture technique, etc.). We have observed that large tip microelectrodes with low tip resistance give consistently low transmembrane potentials in both man and rat. To avoid this difficulty electrodes should be selected with tip resistances greater than  $5 \times 10^6 \Omega$ . (b) In other cases, technically good measurements of Em may have been obtained, but the initially obtained low Em's were averaged with the higher plateau Em's to give a combined low value for the so-called normal value.

The low Em of superficial fibers is probably the consequence of injury; however, Goodgold and Eber-

TABLE II  
Patients with

Patient	Age	Sex	Race	Diagnosis	Reason for administration	Final disposition
<i>yr</i>						
1. M. M.	76	F	N	Steatorrhea 2° bowel resc.	Steatorrhea	Discharged Improved
2. E. C.*	27	F	C	Hypothyroidism Military TBC	Pulmonary TBC	Discharged Improved
3. R. C.‡	31	M	N	Hypertension Glomerulonephritis	Digitalis intoxic.	Discharged Improved
4. J. M.	26	M	C	Periodic paralysis	For studies	Unchanged
5. R. H.	28	M	N	Blunt trauma Abdomen and chest	Explor. Lap. Postoperative	Discharged Improved
6. T. M.	17	F	N	Graves disease Flu syndrome	Mild hyperthyroidism	Discharged Improved
7. E. M.	76	M	C	ASHD	Infected pacemaker	Discharged Improved
Mean	41.9					

\* Taking streptomycin, *p*-aminosalicylic acid, and isoniazid at time of study.

‡ Muscle biopsy (Table IV, normal subjects).

§ Taking digitalis, methyl dopa, and trichlormethiazide at time of study.

|| Taking digitalis at time of study.

stein (3) have suggested that, at least in rectus abdominus muscle of humans, there might be two families of muscle fiber each having a different Em. In support of this view there is good morphologic evidence for two distinct types of muscle fibers in human skeletal muscle (18). In our studies, however, low Em's were obtained only in the puncture of superficial fibers. After the plateau level was attained, only one population of Em was observed despite progressive impalement of multiple fibers over distances as great as 0.5–0.7 cm. In the anterior tibial compartment of the leg, therefore, there appears to be only one family of fibers, at least with respect to Em.

If all potentials recorded are included, then the average value for membrane potential will be lower than if some rational method of selection is used. From our studies, as well as others (5), it would appear that the first few fibers transversed by the electrode are injured and necessarily have variably low potentials. In the trocar technique, the needle no doubt causes this injury. In the open method, exposure to air and/or sudden drop in temperature may be two factors that result in low potentials for the surface fibers. In any event, since deeper fibers tend to give a more consistent and higher Em value (Fig. 3), it would seem most reasonable to evaluate the high plateau potentials only, and not include the Em recorded from the more superficial fibers.

For purposes of comparison between normal subjects and patients with various clinical disorders, we have used only the average value for the high plateau Em. This plateau averaged  $-88.8 \pm 3.8$  mv in control subjects. The value for an individual tends to remain the same when skeletal muscle fibers are impaled more than one time. This value is similar in both males and females, does not vary with age in the range of 20–50 yr, and is not affected by minor illness requiring hospitalization.

On the other hand, serious debilitating illnesses of a variety of different causes markedly depressed the high plateau value of skeletal muscle Em. It is interesting to note that severe depression of muscle Em was sufficiently great in some cases that there should have been depolarization block, yet by clinical evaluation there was no paralysis or neuromuscular defect other than subjective weakness. This depression did not appear to be the consequence of any medications. Nine of the seriously ill patients were taking digitalis and/or antihypertensive medication. However, the results in these patients were similar to the 11 seriously ill patients who were taking no medication. Moreover, two of the mildly ill patients were taking digitalis and one was receiving antihypertensive drugs, yet membrane potential was normal in these patients. The seriously ill patients had a wide variety in blood chemistries, but no specific

# Mild Illness

BUN	Serum creatinine	[Na <sup>+</sup> ]	[K <sup>+</sup> ]	CO <sub>2</sub> content	Arterial pH	Arterial PCO <sub>2</sub>	Em
mg/100 ml	mg/100 ml	mEq/liter	mEq/liter	mmoles/liter		mm Hg	mv
13	—	133	4.0	31	7.42	49	—89
11	0.6	131	3.9	27	7.45	40	—87‡
101	8.1	123	3.6	27	7.34	46	—87‡
14	0.8	138	3.8	24	—	—	—89
10	—	129	4.6	27	7.40	44	—88‡
12	—	136	4.3	26	—	—	—92.6
25	—	142	4.1	26	—	—	—91.9‡
							—89.8
							±2.1 mv (SD)

abnormality could be correlated with the depressed Em: BUN and serum creatinine were elevated in some but normal in others; serum sodium was low in some patients but normal in most; serum potassium was normal in most patients but ranged from a low of 2.5 to a high of 10.4 mEq/liter; blood pH was depressed in some, elevated in some; arterial Po<sub>2</sub> was low in one patient but relatively normal in the remainder in which it was measured. The decrease in Em in the seriously ill patients, therefore, cannot be attributed either to specific drugs or abnormalities in blood chemistries and suggests the possibility of a generalized but rather nonspecific defect in cell membrane function associated with serious illness.

This is in accord with the view of Welt and coworkers (19, 20) who have observed that red cell sodium is frequently elevated in terminally ill patients dying from a variety of causes. He has found that this increase in red cell sodium is not a consequence of an increase in red cell membrane permeability but rather a defect in active outward transport of sodium. Welt has suggested the possibility that this defect in sodium transport in association with serious illness is a reflection of a widespread disorder affecting a variety of cell systems in the body (20).

In order to evaluate the possible causes of the low Em in the seriously ill subjects in our study, it is necessary

to consider the origin of the transmembrane potential. Two theories as to the origin of the membrane potential may be considered. The first theory, originally suggested by Conway (21), attributes the membrane potential to the active outward extrusion of sodium from muscle fibers by an electrogenic redox system. A second theory, which has been more widely accepted in recent years, attributes the membrane potential to the passive outward diffusion of potassium down a chemical concentration gradient. According to this view, the active outward movement of sodium is coupled to an inward movement of potassium through an electrically neutral ion-exchange pump. Such a mechanism, though electrically neutral, generates concentration gradients of both sodium and potassium across the cell membrane. The diffusion of these ions down their respective chemical concentration gradients can generate a cell membrane potential depending on the relative permeabilities of the membrane to the two ions. In this instance, the observed membrane potential can be explained on the basis of the Hodgins-Katz-Goldman equation in which the P<sub>Na</sub> (relative sodium permeability) is 0.01 (22, 23). Because of the low P<sub>Na</sub> the Em is essentially a potassium diffusion potential.

Considering these two theoretical mechanisms as possibilities, serious illness might lower membrane potential by (a) inhibiting the ion-exchange pump and thereby



decreasing the concentration gradient of potassium across the cell membrane, (b) by increasing the relative permeability of the cell membrane to sodium, or (c) by inhibiting an electrogenic redox pump.

To test these various possibilities, membrane potential and muscle electrolyte concentrations were measured in both normal subjects and seriously ill patients (Table IV). In the normal subjects, intracellular potassium was 149.0 mEq/liter and sodium was 26.5 mEq/liter of intracellular water. When these values were substituted into the Goldman equation using a  $P_{Na}$  of 0.01, the calculated or predicted value for the membrane potential in these subjects was  $-88.4 \pm 1.0$  mv and was in excellent agree-

ment with the actual measured value of  $-88.8 \pm 2.8$  mv. In contrast the seriously ill patients had markedly depressed membrane potentials. Intracellular potassium concentration was, however, normal (151.5 mEq/liter) although intracellular sodium concentration was substantially increased (37.7 mEq/liter).

Fig. 4 is a plot of the measured Em's vs. the ratio of intracellular to extracellular potassium concentration obtained in normal subjects and severely ill patients where muscle electrolyte data were obtained. The solid line is that generated by the Nernst equation, the broken line that of the Goldman equation. It can be seen that, although the values for the normal control subjects fall

TABLE III  
Studies on 21 Patients

Patient	Age	Sex	Race	Diagnosis	BUN mg/100 ml	Serum creatinine mg/100 ml
1. E. A.*	65	M	C	Chronic leukemia, bleeding ulcer	17	—
2. W. B.* <sup>1,2,4†</sup>	30	M	C	Uremia	75	12
3. E. B.* <sup>1,3,4</sup>	59	F	N	Uremia	53	8.2
4. G. B.* <sup>2,4</sup>	29	M	C	Uremia	150	21
5. G. B.*	39	F	N	Alcoholic, pancreatitis, bleeding ulcer	8	0.9
6. R. C.*	58	F	N	Alcoholic, malnutrition, myopathy	9	0.8
7. G. C.* <sup>1,5</sup>	48	F	C	Diabetes mellitus, congestive heart failure	31	1.4
8. F. C.	33	F	C	Polymyositis	11	0.6
9. O. E.*	45	F	N	Metastatic carcinoma	18	1.0
10. V. I.	48	F	N	Thyrotoxicosis, myopathy	8	0.5
11. O. L.*	45	M	C	Metastatic carcinoma	11	0.4
12. J. M.* <sup>1,3</sup>	46	M	C	Uremia	145	23
13. G. M.* <sup>1,2,4</sup>	59	M	N	Uremia	145	24
14. B. M.* <sup>3,4</sup>	29	M	C	Uremia	125	18
15. B. N.*	45	F	N	Scleroderma	9	0.5
16. V. P.* <sup>6</sup>	45	F	N	Diabetes mellitus, Ketoacidosis	50	3.3
17. V. S.* <sup>1,6</sup>	40	F	C	Myocardiopathy, congestive heart failure	11	0.6
18. C. S.	20	M	C	Thyrotoxic, myopathy	15	0.8
19. T. W.* <sup>2,3</sup>	67	M	N	Chronic renal disease	138	10.4
20. B. W.*	24	M	C	Polyneuropathy	5	1.7
21. G. R.*	41	M	C	Chronic renal disease, carcinoma of prostate	62	6.4
Mean	45.4					

\* Died within 6 months after study.

† Drugs taken during time of muscle studies: <sup>1</sup>, digitalis, <sup>2</sup>, methyl dopa; <sup>3</sup>, guanethidine; <sup>4</sup>, trichlormethiazide;

<sup>5</sup>, insulin; <sup>6</sup>, furosemide.

§ Muscle biopsy (Table IV, severely ill patients).

|| Lost to follow-up.

on the line for the Goldman equation, only one of the severely ill patients is even near the line and the remainder of these subjects are far below the line. The fact that the low Em is not associated with a decrease in the  $[K]_{iow}/[K]_{eow}$  ratio indicates that the basic defect is not inhibition of an ion-exchange mechanism.

The low Em, however, was associated with an increase in the intracellular concentration of sodium. It could be argued that the high intracellular sodium concentration noted in the group of severely ill patients is an artifact of the method of estimating extracellular volume from the partition of chloride between the intra- and extracellular spaces. As previously noted, this

was done by assuming electrochemical equilibrium for chloride and by calculating the intra- and extracellular chloride concentrations on the basis of measured Em. When this was done, the derived intracellular sodium concentration in the ill group was 42.3% greater than in the normal subjects. A second means of estimating intracellular sodium concentrations was to assume that the measured Em's in the seriously ill patients were incorrect and to calculate the chloride partition in both the normal subjects and the ill patients on the basis of an Em of -89 mv. When this was done, the intracellular sodium concentration in the ill patients was still 22.6% higher than in the normal subjects. Finally,

*with Severe Illness*

Na <sup>+</sup>	K <sup>+</sup>	CO <sub>2</sub> content	Arterial pH	Arterial Pco <sub>2</sub>	Arterial Po <sub>2</sub>	Em
<i>mEq/liter</i>	<i>mEq/liter</i>	<i>mmoles/liter</i>		<i>mm Hg</i>	<i>mm Hg</i>	<i>-mv</i>
128	4.2	34	7.48	34	88	-75
137	4.9	23	7.36	41	94	-62§
129	3.6	16	7.36	28	97	-53
136	4.5	15	7.33	33	123	-67§
130	4.0	21	7.38	36	—	-74
130	4.9	24	7.45	35	97	-65§
132	3.7	27	7.50	35	62	-62
136	3.3	22	7.41	37	—	-53§
129	4.0	23	7.44	34	—	-70§
141	2.5	29	—	—	—	-79
138	3.5	21	7.48	28	80	-72§
130	5.4	19	7.38	33	102	-59
136	3.6	14	7.23	34	90	-69§
137	5.5	21	7.34	45	111	-72§
135	4.1	23	7.45	33	98	-67§
128	4.8	24	7.47	33	—	-66
133	3.9	29	7.49	39	80	-48§
137	3.6	24	7.42	38	—	-75
133	10.4	23	7.39	38	—	-74§
140	3.8	26	7.45	46	—	-80§
136	4.9	45	7.60	48	—	-57§
						-66.3
						±9 mv
						(SD)

TABLE IV  
*Resting Muscle Potential and Intracellular Electrolyte Composition in Normal Subjects and Severely Ill Patients*

Group and number of individuals	Measured Em	Intracellular composition			Extracellular water	Extracellular composition*		
		Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>		Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>
	mv	mEq/liter			%†	mEq/liter		
Normal subjects§ (7)	-89 ±2.9	26.5 ±4.3	149.0 ±13.6	4.1 ±1.5	26.6 ±4.2	134.8 ±5.6	4.1 ±0.4	104.4 ±5.7
Severely ill patients (13)	-67 ±8.6	37.7 ±10.1	151.5 ±18.6	8.8 ±3.6	24.2 ±11.9	35.5 ±3.9	4.3 ±0.7	106.7 ±4.8

\* Plasma values corrected for Donnan effect and water content.

† Expressed as percentage of wet weight.

§ Includes three subjects from Table I and IV subjects from Table II.

|| Mean and sd.

the extracellular space was calculated by the classical method of Darrow which assumes that 1.0 mEq/100 g fat-free dry tissue represents the intracellular chloride (24). When this calculation was made, the intracellular sodium concentration was again found to be higher in the ill patients by 36.4%. Therefore, the intracellular concentration of sodium appears to be higher in the patients irrespective of the method of calculation.

One possible explanation for the combination of a low Em and a high intracellular sodium concentration in the ill patients is a selective increase in membrane permeability to sodium. Assuming that the transmembrane concentration gradients of Na<sup>+</sup> and K<sup>+</sup>, created by a nonelectrogenic ion-exchange pump, are responsible for generating the Em, it would be necessary to increase the value of P<sub>Na</sub> from 0.01 in control subjects to 0.07 in the ill patients to account for the observed values of Em.

An alternative possibility which cannot be definitely excluded or proved by the present study is that the membrane potential is the result of active outward transport of sodium via an electrogenic pump and that

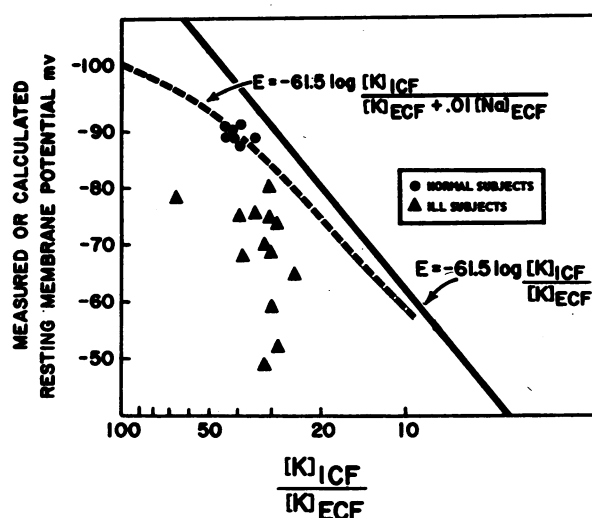


FIGURE 4 Plot of measured transmembrane potentials against the ratio of intracellular to extracellular potassium plotted logarithmically. The solid line is generated by the Nernst equation; the broken line by the Goldman equation. Solid dots represent mean Em values for control subjects; triangles represent mean Em values for severely ill patients.

TABLE V  
*Normal Human Muscle Resting Membrane Potential*

Method	No. of subjects	Membrane potential	Reference
		-mv	
Surgical exposure	4	77.8 ±15.5 (SD)	Johns (7)
Surgical exposure	—	70.0 ±6.0 (SD)	Norris (8)
Surgical exposure	1	87.4 ±8.9 (SD)	Creuzfeldt (6)
Surgical exposure	3	83.6 ±0.5 (SE)	McComas et al. (11)
In vitro	16	77.5 ±1.2 (SE)	Goodgold and Eberstein (3)
Needle cannula	19	87.2 ±5.2 (SD)	Bolte et al. (5)
Needle cannula	9	65 ±0.4 (SE)	Brooks and Hongdalarom (10)
Needle cannula	26	88.8 ±3.8 (SD)	Present study

TABLE VI

Alternate Derivations of Muscle Extracellular Water and Intracellular Sodium Concentration

Subjects	Method	Extracellular water	Intracellular Na <sup>+</sup> concentration
		%*	mEq/liter
Normal Subjects	A†	26.6 ±4.2	26.5 ±4.3
Ill Patients		24.2 ±11.9	37.7 ±10.1
Normal Subjects	B‡	26.6 ±4.2	26.5 ±4.3
Ill Patients		28.2 ±10.2	32.5 ±11.1
Normal Subjects	C	26.1 ±4.0	25.3 ±4.9
Ill Patients		29.7 ±12.9	34.5 ±12.1

\* Expressed as percentage of wet weight.

† A: data from Table IV. Calculation made by partitioning Cl<sup>-</sup> according to membrane potential (see text).

‡ B: data for normal subjects from Table IV. Cl<sup>-</sup> partition for ill patients based upon electrochemical equilibrium for Cl<sup>-</sup> but assuming a normal transmembrane potential (-89 mv) for each patient.

|| C: Cl<sup>-</sup> partition carried out by the Darrow method which assumes intracellular chloride is equal to 1 mEq/100 g FFDT (24).

this pumping process is significantly inhibited in the seriously ill patients. This possibility, rather than a selective increase in sodium permeability, is more consistent with Welt's observation that the sodium pump is inhibited in the red cell membrane's of terminally ill patients (20).

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