Intracellular pH Determination by Means of pH Glass Microelectrodes*

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A number of years ago I decided to attempt to measure the intracellular pH of skeletal muscle by a more direct method than those in current use, that is, by an electrode. I excluded all forms of pH electrodes except the glass electrode and then set up a certain number of characteristics or requirements that this electrode should have.

First, the electrode should be insulated in such a manner that only the pH-sensitive portion is in the cell cytoplasm. Second, the reference side of the electrode group should also be within the cell, because the cell has a transmembrane potential; and the only way to circumvent this transmembrane potential in actual electrical measurements is to have the reference electrode within the cell. Finally, the tip diameter of the combined pH and reference electrode should be less than 1 μ in order to reduce damage of the single cell by penetration or impalement.

Insulation of Electrodes

I soon discovered that one of the major problems in constructing an electrode was the method of insulating. My associates and I tried a number of methods for obtaining the required amount of insulation. One method we developed at about the same time as Kostyuk and Sorokina (1961) was the so-called concentric capillary method in which the pH capillary is run down a lead glass shield. This method has certain deficiencies. First, the insulated portion of the lead glass shield is too thick to enter the cell, so that one can never be sure that some pH sensitive glass is not on the outside of the cell membrane. Second, the numerous organic materials we tried as sealants between the pH glass and the lead glass imbibed solution and ultimately lost their high specific resistance. In addition, although listed as industrial adhesives, many of these materials do not adhere well to very small glass capillaries. These organic materials, then, although usually very satisfactory for electrical insulation, are not very satisfactory for the electrodes used in the concentric capillary method. For these reasons, none of the organic coverings were found useful as insulating materials.

Our first successful insulating technique, which was first reported in 1961, was the use of a glass stain (Carter). Of the several methods of staining glass, we chose the silver glass stain, which is a good method for insulation unless one wants to make a large number of electrodes. It has been known for a long time that, if one takes a silver salt and lays it on glass and then brings the glass to a temperature close to its annealing point, some of the alkaline cations within the glass will exchange with the silver ions. Furthermore, the silver ions that enter the glass will nucleate or form a colloidal suspension. Thus, in essence, what this glass stain does is reduce the sensitivity of the glass to hydrogen ion. Theoretically it is a good method, but it is not a very practical one.

The final method we tried is the one we are currently using and have been using for a number of years. This consists of covering the pH glass with a glaze or fired-on enamel. The principal drawback with this method is that one has to find a glaze or enamel that very closely matches the thermal characteristics of the pH glass. Furthermore, this glaze, which is really glass after being fused, has to have high chemical resistance so that it does not dissolve away from the pH glass. We have developed a ceramic glaze which we believe has the two properties just mentioned, and it is now commercially available. It is a high titanium, low melting point, lead glass. If one takes a small capillary of pH glass and coats it with this glaze, fires the glaze on to the capillary and then pulls the capillary out to a tapered tip, one finds that, depending upon several factors mentioned below, it is possible to pull out a small length of pH-sensitive glass that has no glaze. The length of this pH-sensitive tip is determined by: 1) the initial thickness of the glaze on the capillary; 2) the thickness of the wall of the capillary or the diameter of the capillary; 3) the ultimate length of the overall taper. These factors can be worked out empirically. For example, it is possible not only to select a capillary size that will allow the tip to be pulled closed, but to select a capillary size which, when

* Presented at the *Symposium on Intracellular pH, Pco_2 and PO_2*, September 29, 1967, Medical College of Virginia, Richmond, Virginia.

coated with a certain thickness of glaze, will result in a tip of pH-sensitive glass that varies between 15 and 20 μ in length. It is also possible to add a small capillary of lead glass parallel to the glazed pH capillary and pull these capillaries simultaneously so that a single tip made up of two components will result and the reference side of the electrode will remain open. If the glaze is applied correctly, the pH side can be what we call "insulated" down to within a few microns of the tip. This is the ideal electrode.

Testing of Electrodes

The electrode is placed in a Teflon holder which contains a silver-silver chloride electrode and the mechanical attachments for mounting it in a micromanipulator. With this type of construction a number of tests must be made before any given electrode can be used to make a biologic measurement. Figure 1 shows the results obtained from an electrode which was checked in several buffers against its millivolt reading. The electrode had a fairly linear relationship over the range from pH 4.0 to pH 10.0 and was quite sensitive in the pH range in which we were most interested. The tips of these electrodes are extremely delicate, of course. Not all types of pH glasses are very durable by present day standards. It is not good practice to leave the electrodes in either pH 4.0 or pH 10.0 for any length of time, because they will deteriorate. Contrary to what had been previously published about Corning 0150 pH glass, we found it maintained its properties best, although not very well, in distilled water. Therefore, once one obtains a workable electrode, it is mandatory to perform all necessary tests and then use it without delay. The electrode depicted in Figure 1 had, at 25°, a slope of 58.7 mv for a tenfold change in H^+ activity. Most of the electrodes have somewhat smaller than theoretical slope factor. We have selected electrodes that have slopes above 40 mv per pH unit.

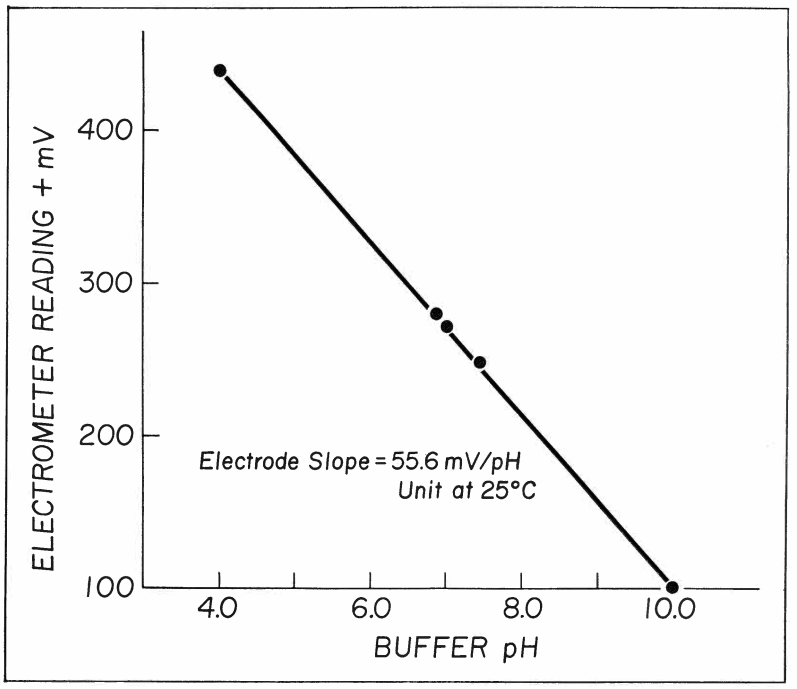

Fig. 1—Voltage response of a double-barrelled micro pH electrode to various buffer solutions. (Reprinted with permission from *Federation Proc.*, 1967a.)

Electrodes are tested in several ways. One is to check the electrode's ability to correctly read pH of whole blood measured with a Beckman blood pH electrode. Another way is to test the electrode in a muscle homogenate by homogenizing muscle in as small an amount of water as possible, measuring the pH with a microelectrode and comparing the results with those of the pH electrode. What we are looking for, principally, is not a real fault of the glass membrane, i.e., its "protein error." As a matter of fact, we believe it is possible to reduce the so-called protein error to nearly zero by lightly siliconizing the surface of the microtips. What we are interested in is what the various micromolecules, and particularly the various anions, do to the tip potential of the reference side of the electrode. An electrode which otherwise seems to be a very good pH electrode may, because of its reference electrode, be worthless in a biologic solution. For example, the Beckman electrode may read pH 7.4, whereas the reference side, for reasons unknown, may give an apparent pH of 9.0. When you put the electrode into the muscle homogenate, it may pick up a new tip potential of perhaps 40 or 50 mv, which it never loses. The electrode, then, has to be discarded.

The tip potential of the reference side is generally quite low, and, as you will see later, we bucked the tip potential to where we wanted it to be and kept checking to make certain that it had not shifted. There are electrodes that go through all the checks and seem to be good electrodes until one starts making muscle punctures. Then the slope of the pH side of the electrode or the tip potential of the reference side changes tremendously. When this happens, the electrode usually is not recoverable. There is no consistent direction in which bad electrodes change. Providing there is

no mechanical injury to the electrode, the operational life of a truly good electrode varies somewhere between several hours to three weeks.

As I indicated earlier, insulation is one of the most important aspects of the entire operation of building and using microelectrodes, and for a long time we did not have an in vitro method of testing insulation. Several years ago we developed a method of testing insulation which, because it entailed micropuncture and perfusion of the rat kidney tubule, would never have gained acceptance. (Rector, Carter and Seldin, 1965) If one is fairly adept at perfusion in single tubules and puncturing the tubule, this is a satisfactory way of checking the insulation of electrodes. Finally, an in vitro method was developed. We found it possible to make a membrane out of the latex used in comparative anatomy laboratories for injecting cats. The latex was poured on a piece of paraffin® film that had a hole cut in it. We obtained a membrane about 1 μ thick that sealed around the microtip well enough to prevent the buffer running down between the membrane and into a buffered agar below the membrane. We used agar in the lower buffer to give mechanical strength to the membrane. It was possible by direct microscopic observation to measure how far the microtip had penetrated the membrane into the agar. It was therefore possible to determine how much of the electrode tip was pH sensitive.

Table 1 shows the use of both insulated and uninsulated electrodes in the latex membrane method. When tested in buffered solutions of pH 6.0 and pH 7.4, these electrodes both read pH reasonably well. One had a slope of 58 mv; the other, 55 mv. These measurements were made at room temperature. When the insulated electrode was placed so that its tip was 10 μ below the latex, and, thus, inserted into the pH 6.0 buffered agar, it gave approximately the same

TABLE 1
In vitro test of double-barrelled microelectrodes

	Insulated Electrode			Uninsulated Electrode		
	Depth of Penetration μ	Electrometer Reading mv	Apparent pH of Agar Buffer	Depth of Penetration μ	Electrometer Reading mv	Apparent pH of Agar Buffer
Buffer pH 6.0		+157	58 mv/pH unit		+155	55 mv/pH unit
Buffer pH 7.4		+ 76			+ 78	
Agar Buffer pH 6.0	10	+155	6.04	42	+157	5.96
Overlaid Buffer pH 7.4	10	+155	6.04	42	+ 86	7.24
Transmembrane Potential of 90 mv Imposed	10	+155	6.04	42	+101	6.97

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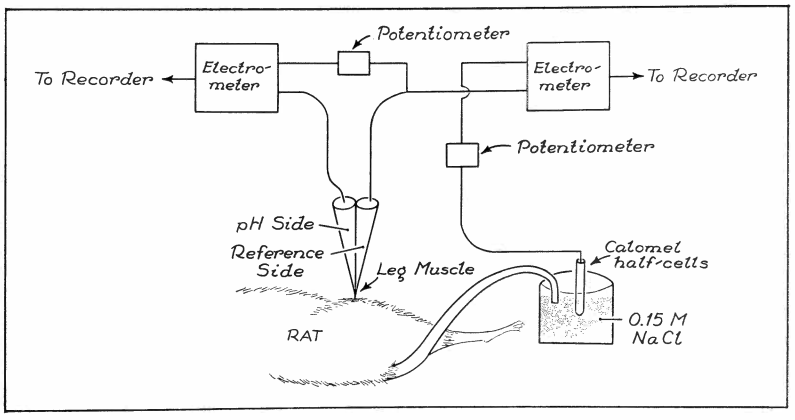

Fig. 2—Method for measuring intracellular pH and transmembrane potential simultaneously with a double-barrelled electrode. (Reprinted with permission from *J. Clin. Invest.*, 1967b.)

reading as it did when tested previously in a solution of pH 6.0 buffer. When we overlaid with pH 7.4 buffer on the upper surface of the latex membrane, there was no change in the electrometer reading; and the apparent pH still stood at approximately pH 6.0. Finally, when we imposed the 90 mv synthetic membrane potential, there was no effect whatsoever on the electrometer reading.

In the case of the uninsulated electrode, the tip penetrated 42 μ, an inordinate distance. Even with 42 μ below the surface of the latex membrane, however, we got an apparent pH change when we overlaid pH 7.4 buffer, which suggested that there was a significant amount of uninsulated pH glass above the membrane, or above the 42 μ point.

Finally, when we imposed the 90 mv transmembrane potential, we obtained a further change in the pH reading. Each electrode has to go through this test. Although this test is not technically difficult, it requires a large number of manipulations. One usually breaks the electrode at this point and has to make a new start.

Intracellular pH and Transmembrane Potential

With the use of these double-barrelled electrodes and two electrometers, one can simultaneously make readings of intracellular pH and record transmembrane potential. Figure 2 shows how this is done, namely, by connecting the reference side of the microelectrode to both an independent electrometer and the low impedance side of a pH electrometer.

During a series of measurements we removed the electrode and rechecked it by dipping the electrode in our test solutions to determine not only whether the electrode was still reading the same pH as previously in any given buffer, but whether the reference of the pH electrode has developed any significant change in tip potential. By utilizing the shunt circuit on the input of the electrometer, we were able to measure at will the resistance of both the reference and pH sides of the electrode even while they were inside of a muscle cell. It was possible by this means to ascertain whether we were dealing with an intact electrode or one that had been accidentally broken off by impalement. (Carter et al., 1967b)

In Table 2 we show data obtained in normal rats. Most scientists who study transmembrane potentials consider the highest transmembrane potential to be the correct one. Hence, we arbitrarily took a value of 89 mv plus or minus one standard deviation, which was 3 mv, a value that we obtained from almost 300 impalements in 24 rats, using Ling electrodes to measure normal transmembrane potential. We examined those pH measurements that fell within the emf range from 86 to 92 mv. In this range we had 38 measurements from seven rats. The blood pH of the rats was 7.41. The average potential of these 38 readings was 88.7 mv, which was close to the 88.9 mv potential previously found. The mean intracellular pH was 5.99. If this result means that hydrogen ions are in electrochemical equilibrium across the skeletal mus-

TABLE 2
Intracellular pH of Normal Rat Skeletal Muscle

	Number of Measurements	Range	Mean ± S.D.
Blood pH	7	7.38–7.44	7.41 ± 0.06
Transmembrane Potential	38	85–92 mv	88.7 ± 2.2 mv
Intracellular pH	38	5.77–6.29	5.99 ± 0.14

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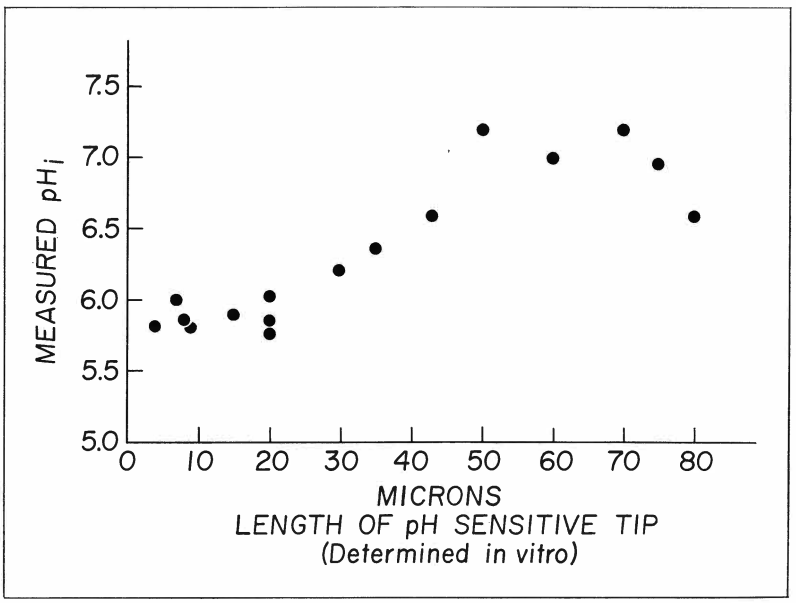

Fig. 3—Measured intracellular pH of rat skeletal muscle (with a simultaneous Em measurement of —85mv or higher) with electrodes having various lengths of pH-sensitive tips. (Reprinted with permission from *J. Clin. Invest.*, 1967b.)

cle membrane, then we should be able to determine mathematically the accuracy of our method.

If there is electrochemical equilibrium between blood pH and intracellular pH, then these results should fit the Nernst equation:

$Em = 61.5 (pH\ blood - pH_i)$, where Em is the transmembrane potential in mv and pH_i intracellular pH. If the observed Em (−88.7 mv) and blood pH (7.41) are used, the calculated pH_i is 5.97, which is very close to the observed value of 5.99, strongly suggesting electrochemical equilibrium for H^+ activity.

In Figure 3 one sees the results of a group of experiments which show the importance of insulation and its relationship to intracellular pH. Originally this work was deliberately undertaken with poorly insulated electrodes because of the findings of Dr. Peter Caldwell from Bristol, England, and Kostyuk and Sorokina in Russia. We now feel it was not poor insulation that affected the data obtained by Dr. Caldwell (1958). The only reason we believe the Russian workers' electrodes were poorly insulated is that they used as insulators organic materials which we have never found satisfactory. Both Caldwell in his work with the crab muscle, and Kostyuk and Sorokina with the frog muscle have shown by their electrode methods that there is no electrochemical equilibrium between extracellular and intracellular compartments for H^+. This figure shows that, if insulation is less than adequate, one will obtain a reading for intracellular pH that increases steadily until the length of the exposed tip is about 50 μ. From that point on the values will mostly fall around a pH of 7.0.

Single-Barrelled pH Electrodes

Despite the fact that we are getting very small tip diameters with our double-barrelled electrodes, it was thought necessary to use a single-barrelled pH electrode which can be pulled to a tip diameter well under .5 μ, thus reducing the possibility of cell damage by impalement. If one had a pH electrode outside the cell in the extracellular fluid, read its potential against a reference electrode in this fluid, and set this potential to zero, there would be no potential change when the cell membrane was impaled if H^+ were in electrochemical equilibrium. The reason for this is that the potential across the membrane would exactly balance the reading obtained from the pH electrode because of the change in H^+ activity inside. This only holds for electrodes that have a theoretic slope of 61.5 mv at 37C. If the slope is less than theoretic, one reads a potential greater than zero, but this potential can be calculated.

In Figure 4 we show the predicted potential difference that we

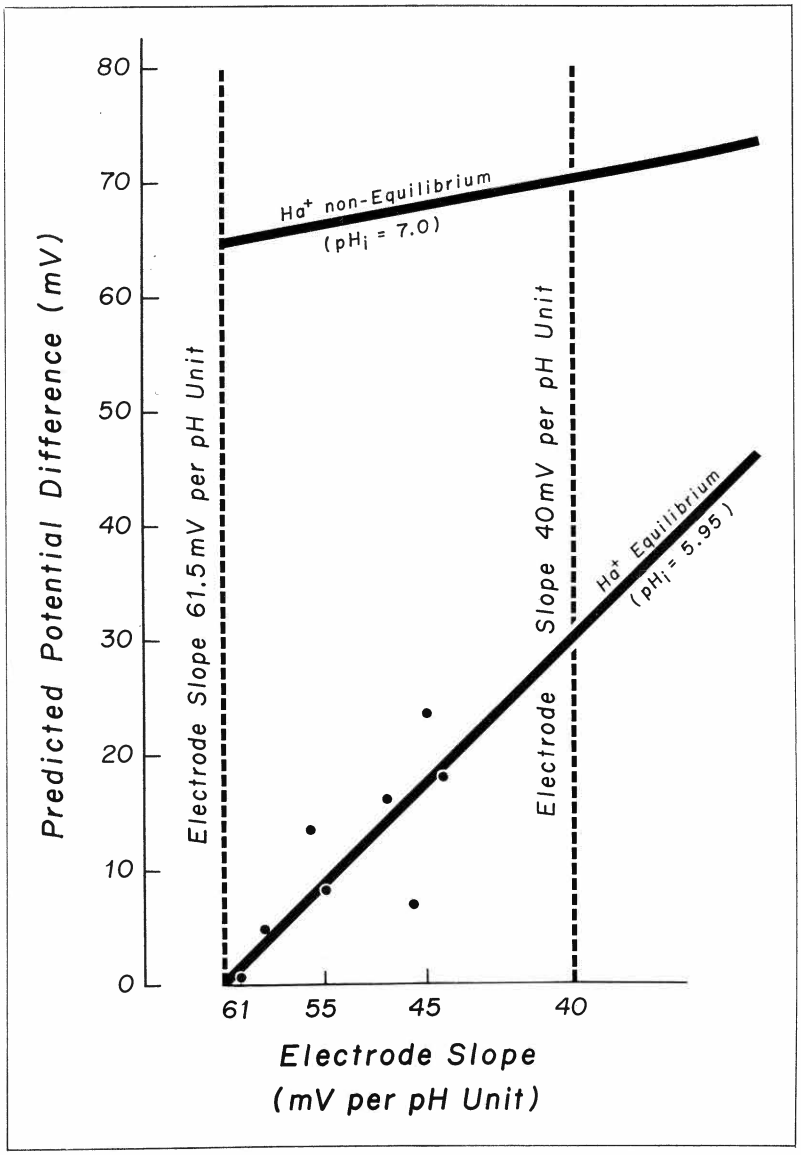

Fig. 4—Dots represent measured potential differences between intracellular and extracellular fluid using single-barrelled pH electrodes with different slopes. (Reprinted with permission from *Federation Proc.*, 1967a.)

would expect to get with the varying electrode slopes. We have drawn in the line that should be generated at various slopes with hydrogen ion equilibrium at an intracellular pH of about 5.95. The top line is what we would expect with non-equilibrium at an intracellular pH of 7.0 and different electrode slopes. We made the impalements and measured the potential. It is fairly clear that the points are clustering about the lower line. Certainly none of them are near the upper line. Hence, the data obtained with the single-barrelled electrode is in agreement with that obtained with the double-barrelled electrode; namely, H^+ appears to be in electrochemical equilibrium across the cell membrane.

Triple-Barrelled pH Electrodes

In Figure 5 we show the results of a single experiment made with a triple-barrelled electrode. In this electrode a third barrel was placed parallel to the other two, and the third barrel was used to impose a current across the muscle cell membrane. In other words, we applied electric current to alter the transmembrane potential and observed simultaneously changes in measured intracellular pH. We varied the transmembrane potential, at least in the area of the tip of the reference electrode, from a value of about +20 mv to −240 mv. The actual pH measurements we obtained at these various membrane potentials appear to fall more closely along what we call membrane slope, referring to the muscle cell, than along the electrode slope (the dotted line). We conducted five of these experiments with five different electrodes. Results were similar to those in Figure 5.

The results with these triple-barrelled electrode experiments further suggest that H^+ across the skeletal muscle cell membrane is in electrochemical equilibrium.

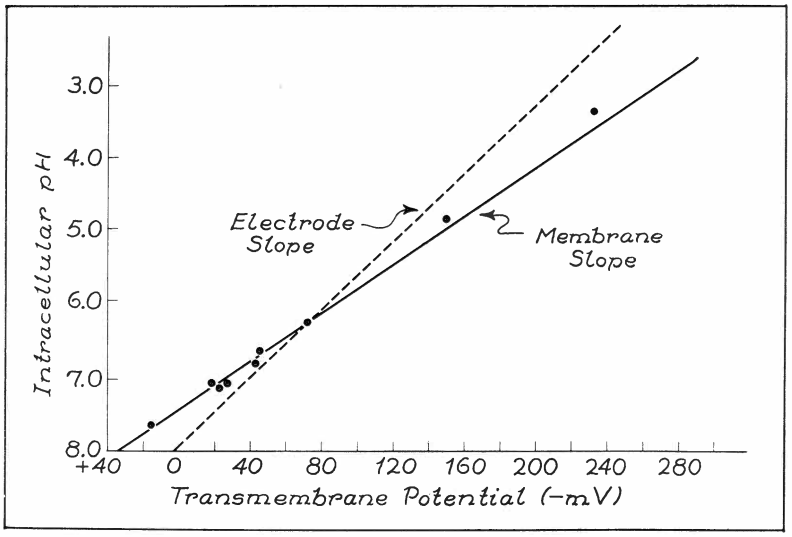

Fig. 5—Triple-barrelled pH electrode study wherein Em of cell could be changed by imposing a current through the third barrel of the electrode. When Em was so changed, measured pH_i was noted to change predictably in accordance with the "cell membrane slope," e.g., 1 pH unit for every 61.5 mv change in Em. (Reprinted with permission from *J. Clin. Invest.*, 1967b.)

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DISCUSSION

Dr. Patterson: Dr. Carter's paper is now open for discussion.

Dr. Robin: Some biologists claim that they have data which show that the hydrogen ions move very slowly into cells. It would seem to me that a simple way of testing your interpretation would be to administer hydrogen ions in the form of hydrogen chloride and show that hydrogen ions are in electrochemical equilibrium.

Dr. Carter: Yes. We've done this with hydrogen chloride, and also by producing respiratory acidosis and respiratory alkalosis. From intracellular pH measurements with the the pH electrode, it appears that hydrogen ions are in electrochemical equilibrium.

Dr. Jöbsis: I remember that Con-

way, defending himself against criticism raised by A. V. Hill, made some calculations on the speed of hydrogen ion movement into muscle. He came to the conclusion that these ions move only very slowly.

Dr. Carter: In his calculations, Conway made the rather arbitrary assumption that the mobility of hydrogen ion potassium in solution was the same. It turned out that it would take about 360 days to bring about one-half of a pH change in skeletal muscle. Nevertheless, many people hold to the view that hydrogen ions move very slowly, and they base their argument principally on the observation that Dr. Robin wanted us to look at, i.e., that metabolic acidosis does not seem to change the intracellular pH as measured by other than the pH electrode techniques.

Dr. Robin: Dr. Carter's work is obviously technically magnificent, and he has been put in the unfortunate position of being attacked from all sides because the methods that he uses are so advanced and technically so difficult. It is, therefore, hard to find experimenters who are capable of either confirming or denying his results. There are, however, other methods for measuring intracellular pH. I would like to mention three results obtained by independent methods which would be very difficult to reconcile with Dr. Carter's results, but are in good agreement with results obtained by indicator systems. I am not mentioning these findings to indicate that I think that they resolve the question. Firstly, if one assumes that the intracellular pH in the muscle is 5.95 to 6.00, then one can calculate the total amount of CO_2 which is present in the muscle; obviously, this would be very small in amount. If one uses the intracellular pH of 6.9 as obtained by indicator methods, then the amount of CO_2 which is in the body is considerably larger. One can measure CO_2 stores by independent methods, essentially dilution methods, that have nothing to do with the measurement of intracellular pH. Thus, one finds experimentally for the total body CO_2 a value which is much closer to the one predicted by calculation, assuming a value of 7, rather than 6, for intracellular pH. There is, however, the unresolved question whether a part of the CO_2 is present in the body as the "barium soluble" form. Secondly, in view of the difficulty just mentioned, another technique for measuring CO_2 stores has been used, which is as follows. The animal or human increases or decreases his CO_2 stores by hypo- or hyperventilation, and under these circumstances, by observing the amount of CO_2 which has been added to or subtracted from the body, one can estimate the CO_2 stores. If this calculated value for whole body CO_2 is compared with the value obtained by application of the DMO technique, or the H_2CO_3-bicarbonate indicator technique, the results are in good agreement. On the other hand, if one makes use of an intracellular pH value of 5.99 or 6.00, the results are quite disparate. This would mean either that the value of 5.9 for intracellular pH in muscle is low, or that all the CO_2 which has been added to the body was instantly converted into the barium soluble form. This is a rather unlikely possibility. Thirdly, if one administers mineral acid or base to an animal and then measures total CO_2 in muscle by gasometric procedures which are independent of any assumption about how that CO_2 is compartmentalized between dissolved CO_2, carbonic acid and bicarbonate, one can show that there is no evidence in terms of total CO_2 that the muscle is being titrated either by hydrogen ion or by bicarbonate. This leaves one in the rather uncomfortable position, accepting a pH value of 6.0, of saying that the gasometric techniques, which, as I emphasized, are independent of any assumptions about CO_2 compartmentalization, are fortuitously making precisely the same errors as are being made by measurements with pH indicators. That's not a question; that is a comment.

Dr. Carter: Unfortunately, one comment deserves another. We do not have time to go into our experiments with DMO, but suffice it to say that we think, as probably some of you have heard, that DMO is bound intracellularly. And although I have no data on the subject that is worth reporting here, it would appear that bicarbonate and DMO are located in the same space; perhaps one replaces the other. I'm not sure that this would explain all of the observations which you mentioned. But if allowance is made for some peculiar site, I think that the data could be reconciled on this basis. I have great reservations about the idea of the acid-labile CO_2, as put forth by Conway, for a variety of reasons. I don't think that new bicarbonate is formed either when the muscle is treated with potassium hydroxide for a short period of time, or when it is released with strong acid. I rather believe that some of the bicarbonate is not measured in the compartment that we call the bulk phase of the cell where we are getting the pH of 6. I don't think that negates the fact that there may be some other place where it is definitely measured and where, under a variety of physiologic alterations, it is purposefully utilized. I would agree with you that this is the meat of the whole issue. One might even go further and clarify it in very definite terms. At the current time there are almost 400 publications on intracellular pH utilizing either DMO or bicarbonate partition. With one exception, all of these agree in principle that the pH is near 7. It may vary from pH 6.7 to 7.2, but that is near pH 7. No one agrees with us that it is near pH 6, with one exception. We do have a problem indeed, but I can only show the data that we have. We are aware of the problem and anxious to reconcile the conflicting results.

Dr. Ramsey: Dr. Carter, is that one exception Fenn and Maurer?

Dr. Carter: Conway and Fearon *(J. Physiol. (London)* 103: 274 289, 1944).

Dr. Ramsey: I thought that around 1935 Fenn and Maurer, using the bicarbonate method, found that muscle had a pH around 6.

Dr. Carter: I know Fenn's various methods for measuring bicarbonate. I was not aware of the fact that he had a value of pH 6. He has several values of pH 7. Now he may well have one of 6, but he has several of 7. The only other pH measurement which agrees with our data is the one published by Netter, who used ammonia. I am inclined, however, to disregard his data, as I do Conway's result. Netter's data are quoted by Peter Caldwell in his review, and he gives a value of pH 7. However, if one looks at the original article, one finds out that the 7 value is arrived at by making certain chemical assumptions which may or may not be true. The absolute method of partition gives a value closer to 5.6, which is of some interest, particularly in regard to pK's of indicators. I think there are too many complications with ammonia, e.g., its binding, production, etc., applied to the whole cell; ammonia does not give a valid measurement.

Dr. Robin: There is hope with an ammonia technique different from the one that Netter used. One can load the animal with sufficient exogenous ammonia to swamp out endogenous ammonia. This would be critical, because if the indicator methods using negatively charged species give falsely high values because of heterogeneity within the cell, then positively charged species would be expected to give falsely low values. We have tested this in one system, the red cell. This is a bad one to use, because it is easy to measure pH in the red cell by any technique, and one can easily agree that it is in electrochemical equilibrium. By using the swamping-out technique, one can arrive at intracellular pH's measured in the red cell by the ammonia technique, by the DMO technique, by the CO_2 technique, if one corrects for carbamino CO_2, by chloride ratios and freeze-thaw, which are not significantly different.

Dr. Carter: We have given the swamping-out technique some thought. The trouble with its application to the whole animal is that we got into much difficulty with what we assume are effects of ammonium ion, particularly on the central nervous system. I am not sure of this, but, anyway, it is difficult to apply this technique. We probably came nowhere near a condition of swamping-out. Let me hasten to say that as far as the red cell is concerned, we would very much like to put the pH electrode into the red cell, in the absence of DMO, and see whether the pH is the same as the rather good pH values obtained with the other methods.

Dr. Robin: Let me caution you before you start, although you are much more skilled at puncturing red cells than we are. We have spent four years getting to the point where we can insert an electrode for measuring transmembrane potential. About 15 to 20 seconds after insertion of the electrode, the cells disrupt. Furthermore, baseline stability is reached very quickly. If you decide to try red cells, I suggest using the red cell of the bullfrog, because this cell is much larger and wider than the human. For two years we have also tried to use red cells of amphiuma or cryptobranchus. They are very big (90 μ, but they have a very thin membrane, and there is no way at all of preserving the transmembrane potential.

Dr. Carter: Yes. Could you measure the transmembrane potential?

Dr. Robin: We believe we have obtained some useful preliminary measurements which agree with chloride distribution.

Dr. Carter: It would be nice if we could insert the pH electrode into these cells. In a circumstantial sort of way, that would be at least one area for agreement, hopefully. On the other hand, if we obtained different measurements, we would really be in trouble.

Dr. Huf: I want to take one step back. I am not sure, but I think the problem is still more complex. I am speaking here about the transmembrane potential. The question is: What is the source of the potential? Speaking about muscle and biological membranes in general, I am surprised that you haven't considered the possibility that there is a sodium pump effect. We begin to realize more and more that the membrane potential is generated by an electrogenic sodium pump. Other ions are distributed in accordance with the laws of thermodynamics. If one looks at the rate at which changes in the passive distribution of ions occurs, it seems to me that this would also depend on the rate at which the driving force, the sodium pump potential, changes. Alkalosis and acidosis are known to change the rate of active sodium transport. So maybe this is a determining factor, and my question is: Has this been considered?

Dr. Carter: It has, yes. I was not going to go into that in any great detail, because of time limitation. Certainly, as you state, currently there are many people who believe that the membrane potential arises from an electrogenic pump, pumping sodium out of the cell. It is certainly conceivable that changes in external pH would affect the pump. That should also affect the potential. Now I realize that there have been some people who claimed that not all of the electrogenic status is seen in the transmembrane potential, and I suppose that is a loophole. Stimulated by the ideas of Hodgkin and his workers on the origin of the transmembrane potential under steady-state conditions, we became interested in observing changes in transmembrane potential in experiments where we control potassium,

principally. This, certainly, is an oversimplification, but I also think that it is a point of departure. Electrogenic pumps are a convenient answer to the problem, but I also think that some of these so-called electrogenic pumps have arisen because investigators have found strange results on the ratios of various ions across the membrane. Finally, let me say that there may be yet a third mechanism for the production of the potential. It is conceivable that hydrogen ion sets the potential.

Dr. Huf: Then you should not be in agreement with the Nernst equation if this is the case.

Dr. Carter: Yes, why not? If you say potassium sets the potential, you are practically in agreement, and we are practically in agreement with hydrogen ion.

Dr. Huf: What I am driving at is this: Do you think that hydrogen ions are actively transported?

Dr. Carter: I know what you mean. No. I do not think this. I am saying this is another possibility, and you are absolutely right. If it is in absolute equilibrium, you do not have the potential, but looking at the case of potassium, hydrogen may behave exactly like the potassium.

Dr. Robin: There are various mechanisms for the development of transmembrane potential in biological systems. One of them is a Donnan distribution, which applies, for example, in the red cell. Here, with macromolecules present on the inside of the red cell, chloride distributes itself unequally, and under these circumstances, a potential difference arises. It has nothing to do with the sodium or potassium pump, either theoretically or actually. That is to say, one can stop the sodium or potassium pumping mechanisms, or change the sodium or potassium concentrations of the outside media, and this has no effect whatsoever on the distribution of chloride ratios or on the transmembrane potential.

Dr. Huf: All I am saying is that, if a pump is in operation, this pump, in effect, takes the place of a Donnan system.

Dr. Robin: Well, that depends on the relative mobility of the ions involved. Again, in the case of the red cell, where, presumably, chloride ions reach the thermodynamic equilibrium in microseconds—remember the half time for sodium is three weeks—one can modify the operation of the sodium pump to any extent, but it will not affect the transmembrane potential. Is it not true that, since potassium is in electrochemical equilibrium across the muscle, this suggested to Hodgkin that it wasn't a diffusion pump that was responsible for this distribution ratio?

Dr. Huf: Ion mobilities are important, but so is the driving force, the pump potential.

Dr. Kiesow: Dr. Carter, is it correct that, by altering the membrane potential, you can change the intracellular pH, assuming that there is an electrochemical equilibrium?

Dr. Carter: This is true if it is done by means of an electrode. Remember that, under those circumstances, the required anion or cation is supplied by the current-generating electrode. We were trying to look into this for more physiological conditions.

Dr. Kiesow: Since the pH changes are quite drastic ones, I would suggest using a physiological, intracellular pH indicator, which is already present and which can easily be observed—even in intact cells and tissues—by means of spectrophotometry. This is the $[DPN.H] \times [H^+]:[DPN]$ ratio and its response to such an intracellular pH change. Since it is a change in ion activity of at least an order of magnitude, it should affect this ratio considerably.

Dr. Carter: Yes, it is. That should follow, I would think. The only exception is that the part that you can measure might be principally located in the mitochondria, as I understand it. Of course, nothing that we have said necessarily has anything to do with mitochondrial pH.

Dr. Kiesow: You could very well also measure the cytoplasmic concentration of reduced pyridine nucleotides.

Dr. Carter: Is there enough to see, technically?

Dr. Jöbsis: Yes, fluorometrically you are better off. You can see the mitochondrial part, but in spectrophotometry you see both equally.

Dr. Carter: You can alter the membrane potential by a drastic amount, and this does indeed seem to alter the pH that we are measuring. Under those circumstances I would think this would be possible. These have to be quite rapid measurements.

Dr. Kiesow: Oh, this should not be a problem here.

Dr. Carter: This is not a very stable situation.

Dr. Kiesow: Even if it is only transient, these optical or fluorometric methods are very rapid.