A microelectrometric titration method for measurement of total intracellular Cl^- concentration

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BOMSZTYK, KAROL, MIHAIL B. CALALB, LAURA SMITH, AND THOMAS H. STANTON. *A microelectrometric titration method for measurement of total intracellular Cl^- concentration.* Am. J. Physiol. 254 (Cell Physiol. 23): C200–C205, 1988.—A microelectrometric titration method is described to measure picomole amounts of Cl^- present in solutions at micromolar concentrations. This method was used to measure total intracellular chloride concentration ($[Cl^-]_i$) in leukocytes. Through the use of submicroliter samples, $[Cl^-]$ can be measured in the range of 5–500 μM. There is no measurable interference from other ions normally present in the cell and no intracellular ion is falsely measured as Cl^-. $[Cl^-]_i$ determined by the conventional coulometric titration and the new microelectrometric titration method was the same. Among the commonly used substituting anions, thiocyanate was the only one falsely measured as Cl^- and should be avoided in experiments using this method. Because only picomole amounts of Cl^- are required, measurements of intracellular pH and total concentrations of other intracellular ions can be done in the same lysate of as few as 5×10^5 cells. This feature should make it possible to study the time course of changes in $[Cl^-]_i$ together with measurements of other intracellular ions following various physiological or experimental maneuvers.

cell chloride; sodium; calcium; magnesium; pH

VARIOUS METHODS have been introduced in the past to measure total intracellular chloride concentration ($[Cl^-]_i$). The most commonly used methods employed coulometric titration of cell lysates or isotopic dilution using ^{36}Cl (2, 6, 10–12, 14). These methods work well but require large numbers of cells (10^6–10^7) and cannot be easily used to measure total intracellular concentrations of other ions in the same sample of cell lysate.

We describe here a new method to measure total $[Cl^-]_i$. The method required fewer number of cells (5×10^5), was very reproducible, was not affected by other ions normally present in cells, and was used to measure $[Cl^-]_i$ in leukocytes together with the measurement of intracellular pH and the total concentration of sodium, calcium, and magnesium.

METHODS

Cells. The murine pre-B lymphocyte cell line, 70Z/3, was derived from a nitrosourea-induced tumor and was grown as described previously (19). Human tonsilar B lymphocytes were prepared and kindly provided by Drs. Mary Valentine and Edward Clark, University of Washington, Department of Microbiology and Immunology. This preparation contains at least 95% lymphocytes (7). Human neutrophils were isolated from fresh blood treated with 0.02% potassium EDTA by Dextran sedimentation followed by Ficoll-Hypaque gradient centrifugation. Contaminating red cells were then removed by NaCl hypotonic lysis (8). Thymocytes from C3Heb/FeJ mice were obtained by mincing the thymus with sterile scissors in RPMI media and passing it over a screen (12). Rat resident peritoneal macrophages were harvested by peritoneal lavage with Hanks' balanced salt solution without divalent cations and containing 100 mg/l EDTA. Cells were sedimented by centrifugation (100 *g*), layered onto preformed continuous Percoll gradient, and centrifuged at 2,200 *g* (4°C). Macrophages were harvested with a pipette and washed free of Percoll with excess buffer (1).

Reagents and materials. ^3H_2O and [^{14}C]inulin were obtained from New England Nuclear. Silver cleaner from J. A. Wright (Keene, NH) was used to clean the silver plate (electrode). Dibutyl phthalate and bisphthalate oils were obtained from Kodak (Rochester, NY) and RPMI 1640 media from GIBCO (Santa Clara, CA). Gelatin and agar were obtained from DIFCO (Detroit, MI). Capillary glass tubing was from Glass Company of America (Bargaintown, NJ).

Experimental procedures. Cell water volumes were determined by the double isotope method (19). Aliquots of $0.5–1.0 \times 10^6$ cells suspended in RPMI 1640 media at 2×10^6 cell/ml were added to 0.7 ml choline chloride overlayed on 0.3 ml dibutyl phthalate:bisphthalate oil (1:1) in 1.5-ml Eppendorf tubes kept on ice. The samples were spun (12×10^3 *g*) at 4°C for 30 s. Aliquots of the overlay were used for scintillation counting and ion measurements. Care was taken to remove all overlay and oil. Double distilled water (0.2 ml) was added to the pellet and the tubes were vortexed, boiled for 2 min, vortexed again, and centrifuged at 12×10^3 *g*. Aliquots (100 μl) of the cell lysate and the overlay were used for volume determination in a liquid scintillation counter (model LS 7000, Beckman). [^{14}C]inulin was used as a marker for the trapped extracellular volume and ^3H_2O as a marker for the intracellular plus the trapped extracellular volume. The difference between the two is the intracellular water volume.

The microelectrometric titration method for measurement of $[Cl^-]_i$ is based on the method developed by Ramsay, Brown, and Croghan (17). Total amount of Cl^- in a sample is determined by titration with Ag^+ ions that

are added to the solution by passing a constant current through a AgCl-Ag electrode. The following is a schematic representation of the electrode-sample system

Ag/1.0 M $AgNO_3$: 1.0 M $NaNO_3$: sample : AgCl/Ag
electrode holder electrode solution silver plate

When a DC current is applied with AgCl-Ag as the anode, Ag^+ is liberated to remove Cl^- ions from the sample. The emf (voltage) of the system is the sum of all junction potential differences. The voltage across AgCl-Ag junction is determined by the Cl^- ion concentration, and as Cl^- ions are precipitated out voltage falls. Figure 1 is a tracing of the potential in such a system as constant current is passed. The titration for each sample is automatically stopped at the same half point (250 mV) of the rapid fall of the voltage. This provides a sharp cut off point at which practically all Cl^- ions are removed from the solution and the total amount of Cl^- removed is then equal to the charge that was passed (current × time). This method has been used to reproducibly measure <10 pmol of Cl^- (3, 17). We decided to use electrometric titration to measure total [Cl^-] but were initially not successful because the cell lysate Cl^- concentration was in the micromolar range, too low to allow titration. To avoid this problem we developed two similar methods to shrink the sample droplet to bring the [Cl^-] to levels sufficiently high to allow current titration.

Method 1. The system is illustrated in Fig. 2. Aliquots (0.2–0.6 μl) of the undiluted lysate of the cell pellet were deposited on the silver plate using a glass micropipette containing mineral oil (tip diam 20–30 μm). To ensure accurate volume delivery each sample was aspirated into the micropipette up to the same mark (~5 mm from the tip). Because the same mark was used for the standards and the cell lysates, [Cl^-] in the lysates was determined from the standards without the need to measure the volume of the deposited samples. The micropipettes were mounted in micromanipulators (model 3301, WPI) and were used with a stereo microscope at ×10 magnification (Olympus). Enough oil was expelled with the sample from the pipette so that while shrinking no portion of the sample adhered to the silver plate. Ten to twenty samples were measured in a run. By the time (10 min) the last sample was deposited, enough water evaporated from the first sample to increase [Cl^-] by 50–100 times (a decrease in the droplet diameter by 4–5 times). After a constant volume of a solution of 0.05% gelatin in 1.0 M H_2SO_4 was added (~0.01 μl) to the shrunken droplet, a double-barreled micropipette (tip diam 6–12 μm) was inserted into the sample droplet and titration was begun. Addition of gelatin decreases AgCl solubility and improves sharpness of the titration curve (9). A solution of 1% gelatin dissolved in water was prepared once per week and stored at 4°C.

Method 2. Through the use of a 30-gauge needle and 1-ml glass syringe, a row of 10–20 bisphthalate oil droplets (0.5–1.0 μl) was deposited first on a silver plate (electrode). Under ×10 magnification (Olympus stereomicroscope) 0.5–1.0 μl aqueous sample containing 1:200 dilution of 0.05% gelatin in 1.0 M H_2SO_4 was then deposited onto one of the oil droplets using an Eppendorf ultramicropipettor (Brinkman, Westbury, NY). To speed up the shrinkage process the silver plate was heated to 60–70°C with a thermofoil heater (Minco, Minneapolis, MN). Through the use of a micromanipulator (model 330, WPI, New Haven, CT), the double-barreled microelectrode was placed in the shrinking aqueous droplet and the titration was initiated within 15–20 s. During titration of the first sample, a second sample was deposited on another oil droplet. The second sample, in turn,

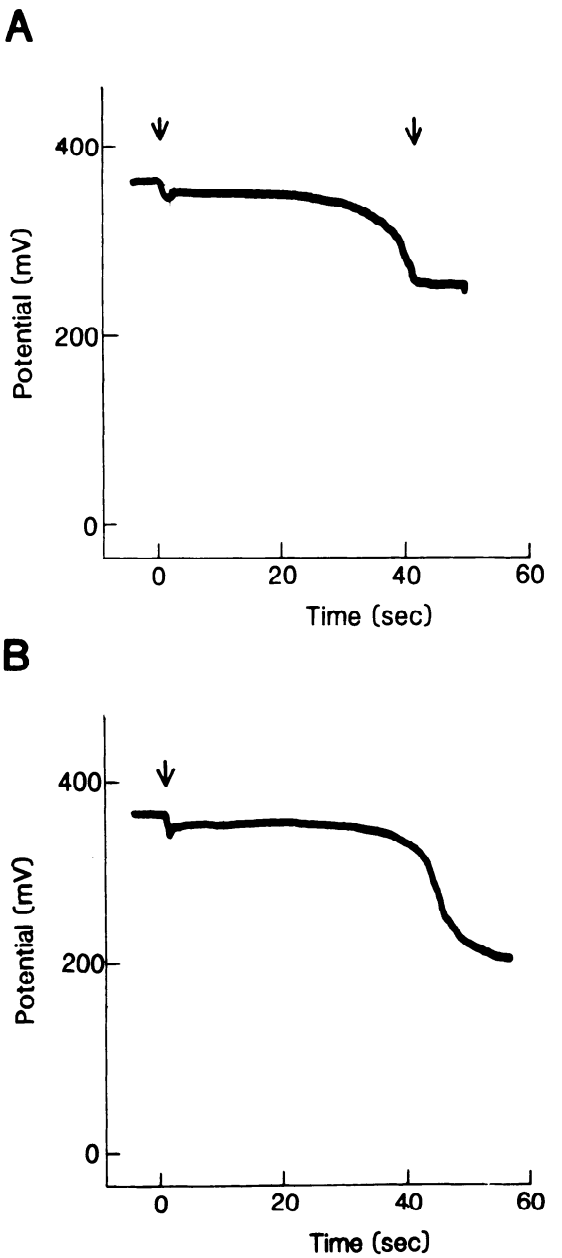

FIG. 1. Tracing of potential measured with a microelectrode in a sample droplet during DC current titration of Cl^- ions. Potential measured by microelectrode falls as Cl^- ions are precipitated out with Ag^+ ions liberated from Ag electrode (silver plate) by DC current. Titration is started (⬇ on left) and is automatically stopped at preset point corresponding to half point of sharp fall in voltage (*A* ⬇ on right). At this voltage, virtually all Cl^- has been precipitated out as AgCl (see text) and provides a sharp cut off point. *B*: voltage tracing when current titration is continued to full completion. After all Cl^- has been removed, voltage of system remains steady at 200 mV and represents sum of potentials of junctions other than AgCl/Cl.

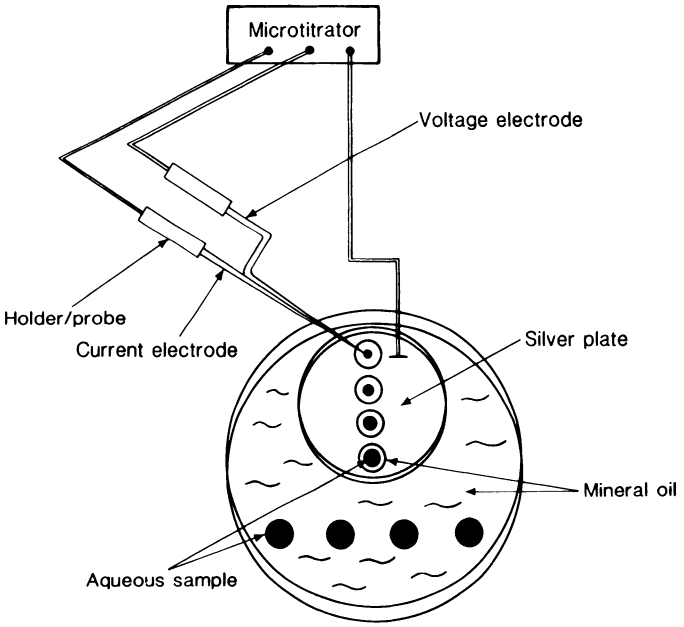

FIG. 2. Schematic representation of microelectrometric titration system. Aliquots (1–2 μl) of lysate of cell pellet and standards are deposited on a glass Petri dish covered with water preequilibrated mineral oil (●). Smaller aliquots (0.2–0.6 μl) of those samples are aspirated into a volume-calibrated sample micropipette containing mineral oil (not shown) and are deposited on a silver plate (anode) placed in a smaller Petri dish (●). Enough oil is expelled with sample so that while shrinking no portion of sample is lost due to adherence to plate. For purpose of illustration, size of droplets is shown magnified relative to size of Petri dish. Double-barreled (voltage and current) microelectrode is placed into shrunken sample droplet and Cl^- titration is begun by passing DC current and monitoring voltage (microtitrator).

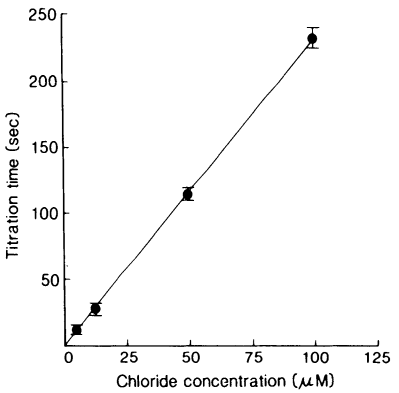

FIG. 3. A typical calibration curve of the microelectrometric titration *method 1*. Samples titrated with 25 nA. Points represent mean ± SD.

could be measured immediately after the preceding titration was completed, thus speeding up the entire procedure.

As is shown in Figs. 3 and 4 both methods were very reproducible. *Method 1* takes longer and was more difficult to learn but it allowed measurement of 5 μM $[Cl^-]$ in submicroliter samples. *Method 2* was simple, required no practice, was quicker and very reproducible in samples containing >50 μM $[Cl^-]$. Thus *method 2* was the preferred way to measure $[Cl^-]$ in cell lysates.

The double-barreled sample and H_2SO_4-containing pipettes were made from Pyrex glass tubing as previously described (3–5). Each barrel of the double-barreled pipette was backfilled with 1 M $NaNO_3$ in 2% agar and

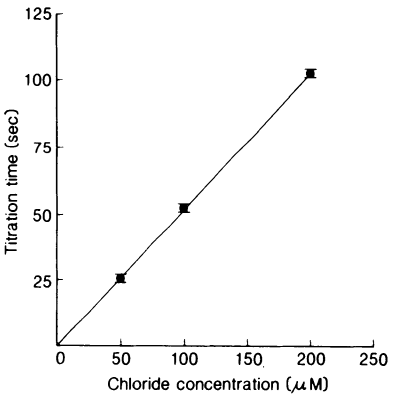

FIG. 4. A typical calibration curve of microelectrometric titration *method 2*. Samples titrated with 300 nA. Points represent mean ± SD.

mounted in microelectrode holders (WPI) filled with 1.0 M $AgNO_3$ solution. Between uses the double-barreled electrodes were stored in 1.0 M $NaNO_3$ solution. The microelectrode was connected to a microtitrator (Microtiter, model ET-1 WPI); one side of the double-barreled electrode served to measure voltage in the sample-AgCl-Ag system and the other barrel to pass DC current to liberate Ag^+ to titrate Cl^-. The microtitrator has a built-in precision stop clock with 0.1-s resolution to display the titration time. The clock starts timing when the current is applied and is automatically halted when the voltage reading reaches the preset endpoint (250 mV) (Fig. 1).

Because the calibration curves go through the origin (Figs. 3 and 4) in practice $[Cl^-]$ in the unknown was determined by the following equation

$$Cl^- = S \times T$$

where S is the slope of the calibration line obtained as a mean of the ratios of $[Cl^-]$ in the standards to the titration time, and T is the titration time of the unknown. Three calibration solutions were used and were prepared from NaCl. For a given sample volume the DC current was adjusted (25–300 nA) to keep the titration times in the 20–60 s range. Total $[Cl^-]_i$ was calculated as the difference between Cl^- content in the pellet minus the amount of Cl^- trapped in the extracellular space divided by the volume of cell water. $[Cl^-]$ in the trapped extracellular space was assumed to be equal to the $[Cl^-]$ that was measured in the overlaying medium. This way we corrected for the dilution of the choline chloride overlay (Cl^-; 150 mM) with the RPMI-incubating media (Cl^-; 113 mM).

To determine whether the methods of concentrating the cell lysate avoided the potential problem of loss of Cl^- due to adherence of part of the sample to the silver plate, we compared the measured and calculated titration time in a sample containing 100–200 μM chloride. Through the use of *method 1*, for a sample volume of 0.62 ± 0.02 μl (n = 6), there were 0.62×10^{-10} and 1.24×10^{-10} mol of Cl^- ions in 100 and 200 μM standards, respectively. With a titration current of 305 nA, the calculated titration times should be 19.4 and 38.9 s, which was close to the measured 19.5 ± 1.0 and 40.3 ± 0.1 s (n = 3). With *method 2*, using the Eppendorf ultramicropipettor, for a sample volume of 0.43 ± 0.02 μl (n = 3) and

titration current of 300 nA, the calculated time for 100 μM sample was 13.7 s compared with the measured 12.8 $\pm$ 0.8 s and 27.3 s compared with 28.3 $\pm$ 1.1 s ($n = 3$) for 200 μM sample. These results show that with both *method 1* and *2* there was no measurable loss of Cl^- ions and that at the half-point of the sharp voltage drop (Fig. 1) all Cl^- ions were titrated out. In the above measurements the volume of the standards deposited on the silver plate using the glass sampling micropipette (*method 1*) or the Eppendorf ultramicropipettor (*method 2*) was measured using [^{14}C]inulin. The results show that with either method the volume delivery is very reproducible.

To validate the microelectrometric titration method for measurements of cell [Cl^-], we added the same known amounts of chloride to a cell lysate and a standard. With the addition, the titration time increased by 33.1 $\pm$ 2.0 s in the lysate and by 33.4 $\pm$ 1.6 s ($n = 3$) in the standard. This indicates that the elements contained in the lysate of the cell pellet do not erroneously potentiate or inhibit chloride measurements. To determine whether any elements in the cell lysate other than chloride are falsely measured as chloride, we suspended 70Z/3 cells in chloride-free media (replaced with gluconate). After 30 min $[Cl^-]_i$ in these cells was 0.7 $\pm$ 0.2 ($n = 6$) mM compared with 39.2 $\pm$ 3.1 mM ($n = 3$) when suspended in chloride containing simplified media [(in mM) 145 NaCl, 5 KCl, 10 Na_2HPO_4, 0.5 $MgSO_4$, 5 glucose, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 1.5 $CaCl_2$; pH 7.24]. In this experiment, 150 mM $NaNO_3$ was used as the oil overlay rather than choline chloride. Nearly total depletion of Cl^- was also found when guinea pig vas deferens was bathed in Cl^--free media (2). The close to zero [Cl^-] in 70Z/3 suspended in Cl^--free media indicates that with the microelectrometric titration what was measured as Cl^- is in no part due to other molecules. To further test the method we examined the effects of other anions on chloride measurement. No interference was found with SO_4, NO_3, PO_4, HCO_3, HEPES, citrate, or gluconate. However, thiocyanate (SCN) was falsely read as Cl^- and thus should be avoided in experiments involving Cl^- measurements using this technique.

Because the microelectrometric titration method requires only picomoles of Cl^- per sample, we could use the same cell lysate to measure Cl^- and the total concentration of sodium, calcium, magnesium, and intracellular pH. Samples (1 μl) from the 1:8 diluted lysate were used to measure total intracellular Na^+ and Mg^{2+}. Samples (2 μl) from the undiluted lysate were used to measure total intracellular Ca^{2+}. These measurements were done by flameless atomic absorption spectrophotometry as previously described (3, 4, 19, 20). To validate the atomic absorption measurements we determined the total concentration of each ion in the cell lysate before and after adding the same amount of a given ion to the lysate and each of the standards. Measured Na^+ concentration in a cell lysate was 13.8 $\pm$ 0.1 before and 14.5 $\pm$ 1.0 μM ($n = 3$) after adding 100 μl of 40 μM NaCl to 100 μl of lysate and to 100 μl of each of NaCl-containing standards. Measured Ca^{2+} was 3.07 $\pm$ 0.32 and 3.13 $\pm$ 0.06 μM ($n = 3$) before and after adding 100 μl of 10 μM of $CaCl_2$ to 100 μl of the cell lysate and the $CaCl_2$ standards. Measured Mg^{2+} was 2.40 $\pm$ 0.10 and 2.45 $\pm$ 0.15 μM ($n = 3$) before and after adding 100 μl of 20 μM $MgCl_2$ to 100 μl of cell lysate and each of the $MgCl_2$ standards. Because the relative measured values of Na^+, Ca^{2+}, and Mg^{2+} were the same before and after the additions, other elements present in the cell lysate did not potentiate or inhibit the flameless atomic absorption measurements.

Intracellular pH was determined by the distribution method of the ^{14}C-labeled weak acid 5,5-dimethyl-2,4-oxazolidinedione([^{14}C]DMO) (18). Once we established, using [^{14}C]inulin, that the trapped extracellular water volume is a constant fraction of the total volume of the pellet water, the measurements of intracellular pH using [^{14}C]DMO and ^3H_2O could be done in the same samples as the measurements of total intracellular concentrations of Cl^-, Na^+, Ca^{2+}, and Mg^{2+}. All results are reported as mean $\pm$ SD.

RESULTS AND DISCUSSION

The total ion concentrations in the RPMI 1640 media used for cell incubation were (in mM) 137 Na^+, 113 Cl^-, 5.4 K^+, 0.57 Ca^{2+}, and 0.15 Mg^{2+}. The pH and Pco_2 averaged 7.23 and 46.5 mmHg.

Table 1 shows cell volume, the total intracellular ion concentrations, and intracellular pH measured in the pre-B lymphocyte cell line, 70Z/3. All measurements were done in the same lysate of 70Z/3 pellet. Total $[Cl^-]_i$ averaged 54.1 mmol/l cell H_2O. To validate this value, we performed a group of experiments to compare the measurements of total $[Cl^-]_i$ in 70Z/3 cells using the conventional coulometric titration (Chloridometer, Buchler-Cotlove, Saddle Brook, NJ) (9) and the new microelectrometric titration method. With the chloridometer, the minimum number of cells required was 10 $\times$ 10^6 per measurement. Through the use of 300 μl of the 500 μl of cell lysate, the $[Cl^-]_i$ averaged 60.2 $\pm$ 13.9 mM compared with 59.7 $\pm$ 10.3 mM ($n = 6$), using 0.4 μl samples from the same lysate and measured by the microelectrometric titration *method 1*. In the same group of experiments, $[Cl^-]_i$ averaged 56.5 $\pm$ 13.7 ($n = 6$) when determined with *method 1* from lysates obtained from 1 $\times$ 10^6 cells. In another group of measurements, $[Cl^-]_i$ was 69.5 $\pm$ 10 mM determined by the chloridometer compared to 71.9 $\pm$ 8.0 mM ($n = 4$) using the microelectrometric titration *method 2*. Thus these results further validate our method for measurement of total $[Cl^-]_i$.

With the use of the same method, $[Cl^-]_i$ averaged 65.5 $\pm$ 14.4 mmol/l cell H_2O ($n = 6$) in human B lymphocytes (Table 1); 46.6 $\pm$ 10.2 mmol/l cell H_2O ($n = 4$) in human neutrophils; 53.6 $\pm$ 8.3 mmol/l cell H_2O ($n = 2$) in mouse thymocytes. These values compare favorably with a value of 43 mmol/l cell H_2O [Cl^-] obtained in rat thymocytes (12) and 58 mmol/l cell H_2O in Ehrlich ascites tumor cells (14), both studies used coulometric titration and 49.9 mmol/l cell H_2O in smooth muscle cells of guinea pig vas deferens measured by chemical titration (2). Our measurements are higher than those reported using ^{36}Cl uptake: 30 mmol/l cell H_2O in lymphocytes from pig mesenteric lymph nodes (10) and 38 mmol/l cell H_2O

TABLE 1. *Total intracellular ion concentration and cell water volume in 70Z/3 and human B lymphocytes*

	Cell H_2O Volume, 10^{-12} liter	pH	P_{CO_2}, mmHg	Intracellular Ion Concentration, mmol/l H_2O			
				Cl^-	Na^+	Ca^{2+}	Mg^{2+}
Media		7.23	46.5	113	137	0.57	0.15
70Z/3	0.561±0.042	7.06±0.07		54.1±13.3	28.6±8.1	2.29±0.77	11.3±1.4
B cell	0.139±0.040			65.5±14.4	51.7±8.9	1.65±0.50	13.3±1.7

Values are means ± SD. Before measurements, cells were incubated in RPMI media at 37°C in an incubator gassed with CO_2-air mixture. All measurements were done in same lysate of 1×10^6 70Z/3 cells or 4×10^6 human B lymphocytes. Mean values for 70Z/3 cells were obtained from 13 different cultures. Mean values for human B lymphocytes were obtained from tonsils of 6 different individuals. Intracellular pH in B lymphocytes was not measured.

reported in human lymphocytes (11). Our values, however, are lower than 104 mmol/l cell H_2O reported in human lymphocytes when measured with Cl^--sensitive electrode (16). In rat peritoneal macrophages, we obtained [Cl^-] that averaged 35.5 ± 6.4 mmol/l cell H_2O (n = 2), which is lower than 64 mmol/l cell H_2O reported in rat alveolar macrophages using coulometric titration (6). The differences in Cl^- measurements among the various studies can not be easily explained because they do not appear to depend on the cell type or method used.

To the best of our knowledge the microelectrometric titration is the first method to measure Cl^- content in picomole range in samples containing micromolar Cl^- concentrations. The ability to reproducibly measure Cl^- down to 5 μM range in submicroliter samples is the most important feature of this new method. Because we can reliably measure Cl^- content down to 2 pmol (5 μM in 0.2–0.6 μl samples), theoretically the minimum cell number required for Cl_i^- content determination is <100. However, in practice a minimum of 5×10^5 cells is required to reproducibly measure the volume of the cell pellet water. The limiting factor here is the measurement of the trapped extracellular water in the cell pellet as determined by [^{14}C]inulin. In the cell pellet from 5×10^5 cells, total volume water (determined by ^3H_2O) in cell pellet was 0.365 ± 0.012 μl, the trapped extracellular water was 0.124 ± 0.014 μl, and the calculated intracellular water volume was 0.243 ± 0.015 μl (n = 4). With 10^5 cells per pellet, the measurement of the total water volume of the cell pellet remains reproducible, 0.0351 ± 0.001, but because the estimate of the trapped extracellular water volume is not sufficiently reproducible, 0.0126 ± 0.0029 μl, the calculated volume of the intracellular water of the pellet is not reliable, 0.0229 ± 0.038 (n = 4). In addition, [Cl^-] in the trapped extracellular water in the cell pellet is greater than the $[Cl^-]_i$ so that small variations in the measurements of the trapped water result in larger variations of the calculated $[Cl^-]_i$. For the experiments with 10^5 cells per aliquot, we used 0.7 ml Eppendorf tubes. With these smaller tubes we consistently found that up to half of these cells would not sediment through the oil compared with nearly complete sedimentation with the 1.5-ml tubes. This loss may be a result of increased surface tension in the more concave meniscus in the oil-overlay interface not allowing some cells to go through. This loss of cells accounts for the fact that in the above experiment reducing the cell number by a factor of five decreased volume of the pellet water by a factor of 10.

Because submicroliter samples were used to measure Cl^-, we could use the same lysate of a cell pellet to measure intracellular pH (DMO method) and total intracellular concentrations of Na^+, Ca^{2+}, and Mg^{2+} (Table 1). In 70Z/3 cells, total intracellular [Na^+] averaged 28.6 mM and in human B lymphocytes it averaged 51.7 mM. Magnesium averaged 11.3 mM in 70Z/3 and 13.3 mM in B cells. In human fibroblasts using electronmicroprobe others reported [Na^+] at 25.2 mM and Mg^{2+} at 10.1 mM (13). Total [Ca^{2+}] in 70Z/3 cells averaged 2.29 mM compared with 1.65 mM in B lymphocytes. The reported total intracellular [Ca^{2+}] ranged from 1.0 mM in human erythrocytes to 6.0 mM in human fibroblasts (13, 15). Our results show significant differences in total Na^+ and Ca^{2+} ion concentrations between 70Z/3 and human B lymphocytes. There is also a wide range of values reported for other cell types. These differences can not be readily accounted for but we do find significant differences in the values that we obtain from different 70Z/3 cultures or human tonsilar B lymphocytes from different donors. Using the flameless atomic absorption spectrometry, we initially reported total intracellular [Ca^{2+}] in the range of 1.0–1.5 mM in 70Z/3 cells (19), values that are lower than our recently obtained averages in this cell line. Although we do not have an explanation, we believe that these variations may represent real differences between cell cultures and cell types.

The ability to measure Cl^- together with the other ions in the same cell lysate is an advantage over the previously used methods. Cl^- transport can be coupled so that the microelectrometric titration may prove to be useful to study the relationship between Cl^- fluxes and transport of other ions measured in the same sample of a cell lysate. Because reproducible measurements of the volume of cell pellet water can be done with 5×10^5 cells, and because the microelectrometric titration method requires the equivalent of fewer than 10^3 cells, studies of changes in $[Cl^-]_i$ over a time course following various experimental maneuvers can be readily done. Also, the atomic absorption spectrophotometer measures picomole amounts of cations so that $[Cl^-]_i$ determinations over a time course can be done together with the other ion measurements in a small number of cells. These methods should be especially useful in studies that until now were difficult to carry out because of a limited number of cells available.

In summary, we developed a new method to measure

picomole amounts of Cl^- in solutions containing micromolar concentration of Cl^- and have used this method to measure total $[Cl^-]_i$ in leukocytes.

This work was supported by the National Science Foundation Grant PCM 8315 753, Grant-in-Aid from the American Heart Association with funds contributed in part by the American Heart Association, Washington Affiliate, 85 689, and by the Northwest Kidney Foundation.

Received 18 November 1986; accepted in final form 12 August 1987.

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