Scanning Microscopy

Volume 2 | Number 1 Article 24

10-19-1987

Ion and Water Retention by Permeabilized Cells

Carlton F. Hazlewood
Baylor College of Medicine

Miklos Kellermayer
University Medical School

Follow this and additional works at: https://digitalcommons.usu.edu/microscopy

Part of the Biology Commons

Recommended Citation

Hazlewood, Carlton F. and Kellermayer, Miklos (1987) "Ion and Water Retention by Permeabilized Cells," *Scanning Microscopy*: Vol. 2 : No. 1 , Article 24.
Available at: https://digitalcommons.usu.edu/microscopy/vol2/iss1/24

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

UtahStateUniversity
MERRILL-CAZIER LIBRARY

Scanning Microscopy, Vol. 2, No. 1, 1988 (Pages 267-273) 0891-7035/88$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

ION AND WATER RETENTION BY PERMEABILIZED CELLS

Carlton F. Hazlewood*, Miklos Kellermayer[1]

*Department of Physiology and Molecular Biophysics
Baylor College of Medicine, Houston, TX
[1]Department of Clinical Chemistry, University Medical School
Pecs, 7624 Hungary

(Received for publication May 18, 1987, and in revised form October 19, 1987)

Abstract

Nonionic detergents, Triton X-100 and Brij 58, removed, within 2-5 minutes, lipid membrane of suspended thymus lymphocytes and monolayer H-50 cells grown in culture. Studies of hydration, ionic asymmetry, and ionic and protein release kinetics were conducted on these membraneless cellular preparations. The hydration of nuclei isolated by Triton X-100 procedures appears to be influenced strongly by the monovalent ionic concentration of the buffer bathing the organelles. The putative monovalent ionic concentration of the cellular aqueous phase (i.e., 150 meq/L) caused nuclei to swell and coalesce. Monovalent ionic concentrations of 30 meq/L or less caused minimal changes in volume and in morphology. Triton X-100 treatment led to rapid mobilization and solubilization of membrane and cytoplasmic lipids and proteins, and the cellular potassium was reduced to very low levels. Brij 58 treatment of the lymphocytes for 5 minutes led to loss of membrane structure. Potassium, however, was retained at significant levels for over 10 minutes. Potassium and protein release kinetic studies in the H-50 monolayer cells following Brij treatment revealed that potassium and the detergent mobilized proteins may be co-compartmentalized and that 10 minutes or more are required before their release is completed. These results support the view that most of the potassium and "diffusible" proteins are not fully dissolved in the cellular water. Furthermore, the integrity of the membranes does not appear to be essential for the retention of the ions and the proteins.

Key Words:

Water, ions, adsorption, detergents, membraneless preparations.

***Address For Correspondence:**
C. F. Hazlewood, Baylor College of Medicine,
1200 Moursund - Physiol., Houston, TX 77030

phone number: (713) 799-5773

Introduction

Central to the more popular views of the living cell is the postulate that the membrane is the primary, if not sole controller of the asymmetric distribution of solutes [1,3,6,8,12,20-22,37]. Furthermore, the membrane mechanisms that bring about the cellular "gradients" of charged and uncharged solutes are purported to be regulated by membrane-situated energy-requiring pumps [1,3,6,8,12,20-22,37]. (The quotation marks have been placed around the word gradients for the following reason. From dilute solution theory, a gradient refers to differences in <u>concentrations of solutions</u> separated by a membrane. If significant adsorption of ions or non-charged solutes is involved in the development of the asymmetric distribution between cells and extracellular environment, then the "gradient" does not necessarily represent a difference in the concentration between the aqueous phase of the cell and the external solution bathing it.) Although the idea of adsorption of ligands to membrane cytoplasmic structures has become commonplace in the membrane pump views, the conceptual origins of the theory are traceable to dilute solution theory [9-11,28,38].

Theories that view the cytoplasm as playing the dominant role in the control of the cellular interior have existed for more than a century [28 (pp 81-111)]. A central theme of these theories, often referred to as the bulk phase theories, is that the cytoplasmic macromolecules adsorb significant amounts of solutes and water. Since the 1960's a large body of evidence has been published that provides support for the general tenets of the bulk phase theories [4,5,7,11,17,19,23-31,39].

It is obvious that these two theories or groups of theories (the membrane pump view and the bulk phase view) are based on different fundamental assumptions. It is important to remember this point, even though the membrane pump view has evolved to a position that is inconsistent with its historical physical-chemical origins (dilute solution theory). Discussions of the membrane-pump view, without the awareness of these deviations from its origins, often lead incorrectly to the viewpoint that the differences of interpretation are merely semantic. Nevertheless, of major significance for our report is the fact that the importance of the membrane structure is viewed differently by the proponents of each of the views. In the case of the proponents of the membrane-pump view, the integrity of the membrane structure is all important in the control of the internal milieu of the cell (see for example reference 35). For the bulk phase theories in general and the Association-Induction Hypothesis [28] in particular, the membrane, although important, is not considered the primary controller

of the cellular milieu. To address this difference in viewpoint we utilized techniques that remove the membrane structure of the cell and permit the observation of the subsequent redistribution of ions and proteins. Our objective was to provide decisive results consistent with one or the other of these theories.

We used conventional detergent treatments to remove the lipid components from the cytoplasmic and surface membrane structure of cells [16,17,19]. These procedures also resulted in the time-dependent release of proteins [17,19,36]. The employment of these procedures allowed us the opportunity to determine the time-dependent dissipation of the electrolyte "gradient" of the cell.

Materials and Methods

We have used two general methods in these studies. In the first method, lymphocytes were isolated from the thymus glands of young calves. The isolated lymphocytes were then placed in various buffers, some containing monovalent (Na^+ and K^+) ions and one without monovalent ions. Both types of buffer contained 0.01 M Tris-HCl, 3mM $CaCl_2$, and 3mM $MgCl_2$ [16,17]. (The buffer without monovalent ions is referred to as TSCM, whereas the buffer with monovalent ions is referred to as TNKCM.) To these buffers we added either Triton X-100 or Brij 58 to a final concentration of 0.2%. The lymphocytes were incubated in the various buffers for 5, 10, 20, or 90 minutes and centrifuged at various g forces. The pellets were then analyzed for water and/or electrolyte concentration. The specific procedures are described in detail elsewhere [16,17].

The second general method involved the use of simian virus 40-transformed H-50 cells in monolayer culture. Two different analyses were performed on the Triton X-100 and Brij 58 detergent treated cells: ^{42}K-release and release of ^{35}S-methionine labeled proteins. The details of these studies may be found in reference 19.

In both methodologies electron micrographs were obtained to assess the degree of membrane structure removal [16,17,19].

The Hydration of Isolated Membraneless Lymphocyte Nuclei

In this study three buffers were used: (1) TSCM (no monovalent electrolytes); (2) TNKCM (monovalent ion concentration equal to 150 mM/liter-75 mM/liter Na^+ and 75 mM K^+); and (3) 1/5 TNKCM (a buffer containing 15 mM/liter Na^+ and 15 mM/liter K^+ with sucrose making up most of the difference in osmotic pressure). Each of these buffers contained Triton X-100 (0.2%). Batches of the isolated calf thymus lymphocytes were incubated in each of the Triton-containing buffers for 90 minutes. Aliquots of the cells were then centrifuged for 30 minutes at 500, 1000, 5000, 10,000, 20,000, 40,000, 80,000, 100,000, or 150,000g. Electron micrographs of the Triton-treated cells were obtained from the samples centrifuged at 500 g. Figure 1 contains representative electron micrographs of the calf thymus lymphocytes treated with Triton in each of the buffers. Figure 2 displays the changes in sample hydration (i.e., percentage of water) as a function of centrifugal force. The electron micrographs, as well as the centrifugation studies (Figure 2), show that the exposure of the membraneless nuclei to the buffer containing the full complement of monovalent ions (TNKCM-150 mM/liter Na^+ plus K^+) results in a significant increase in hydration.

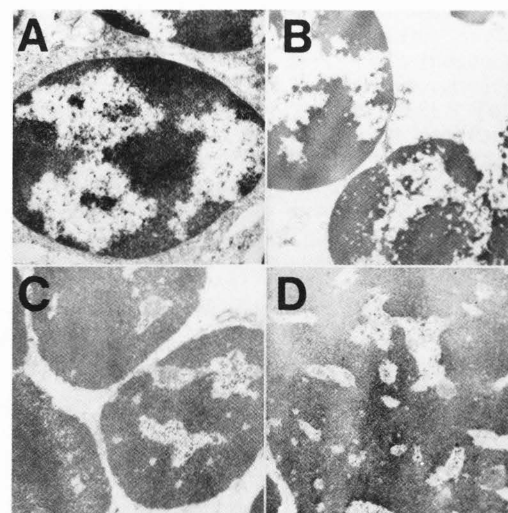

FIGURE 1. Electron micrographs of: (A) intact calf thymus lymphocytes; (B) Triton X-100 treated thymus lymphocytes isolated in TSCM buffer; (C) Triton X-100 treated thymus lymphocytes isolated in 1/5 TNKCM buffer; and (D) Triton X-100 treated thymus lymphocytes isolated in TNKCM buffer. (Photo width ≈ 6 micrometers)

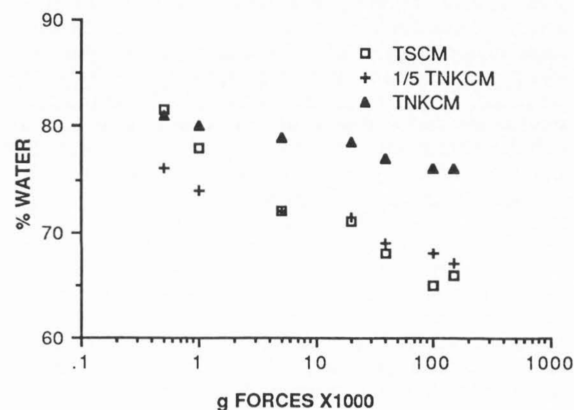

FIGURE 2. The percentage of water remaining in the nuclear pellets as a function of g forces. The specific g forces were 500, 1,000, 5,000, 20,000, 40,000, 100,000, and 150,000. Electron microscopy (EM) and light microscopy were conducted on pellets centrifuged at 500 g. The Ca^{++} and Mg^{++} concentrations are 3 mM in each of the solutions with the monovalent Na^+ / K^+ concentrations varying from 0/0 in TSCM, to 15/15 mMol/l in 1/5 TNKCM, and 75/75 mMol/l in TNKCM solution. Each point represents the average of 5-15 samples and the standard error of the mean is <2% for these data. (For more detail see the text and reference 16.)

On the other hand, membraneless nuclei exposed to buffers containing a reduced complement of monovalent ions (0 or 30 mM/liter Na^+ plus K^+) have a greatly reduced hydration. An obvious conclusion from these studies is that nuclear hydration is extremely sensitive to the free monovalent ionic environment to which it is exposed. Furthermore, free ionic concentrations of the order purported to exist in living cells are not tolerated well by these nuclear preparations. For example, using figure 2, compare (at

150,000 g) the hydration of nuclei incubated in 1/5 TNKCM, 3.07 grams H_2O / gram of dry solids, with those incubated in TNKCM solutions, 4.22 grams H_2O / gram dry solids. From this and other studies we raise the question: Could it be that the free (aqueous) monovalent ionic concentration of living cells (during interphase) is of the order of 30 mM/liter or less? Certainly, the integrity of membrane structures do not appear to be necessary for the retention of water in these cells. Thus, it also appears unlikely that the osmotic theory of cellular volume regulation which requires a membrane separating two solutions, can offer an adequate explanation for these findings.

Electrolyte Composition of Isolated Thymus Lymphocytes Following Detergent Treatment

From our earlier findings and from findings of several others, it seems unlikely that DNA within the nuclei of living cells is exposed to free electrolyte concentrations near those calculated from the water and inorganic element contents [14,15]. In other words, observations made on cell nuclei strongly support the notion that the concentration of electrolytes is low in the aqueous cytoplasm of the living cell [28,33,18]. We have tested this idea on isolated calf thymus lymphocytes. The lymphocytes were first released from the thymus glands in calf serum. The cells were pelleted by centrifugation at 500 g for 10 minutes and the pellet resuspended in TSCM solution in 1:5 vol:vol ratio. This suspension was divided into three aliquots: (1) control with no detergent treatment: (2) 0.1% Brij 58 treatment; and (3) 0.1% Triton X-100 treatment. Thus the bathing solutions of each of these aliquots were very similar at the beginning of each treatment (see Tables 1 and 2 for the average values).

The separated cells were treated with Triton X-100 or Brij 58 for 5, 10, or 20 minutes and then centrifuged at 50,000 g for 10 minutes. The pellets were weighed, dried to a constant weight, and processed for flame photometric K^+, Na^+ and atomic absorption photometric Ca^{++}, Mg^{++} measurements. The electrolyte composition of the supernatants (i.e. bathing solutions) was determined in like manner. Parallel with measurements of the inorganic elements, a careful electron microscopic study was performed on the detergent opened cells. In good agreement with the findings of others, both detergents solubilized the lipid membranes within 5 minutes; only the so-called detergent resistant cellular elements remained [18,34,36].

If the specific inorganic element composition of living cells (the high intra-cellular K^+ content) is maintained only by the lipid membranes, immediate equilibrium would follow the removal of the membranes. The analyses of the pellets of Brij 58 treated cells showed that after 15 minutes (5-minute exposure in suspension and 10-minute centrifugation) a high K^+ content remained (Table 1). As the exposure time was extended, the K^+ content decreased in the Brij-treated cells. The Na^+ content did not change, but a significant increase in Ca^{++} and a slight increase in the Mg^{++} content were also found. The protein content of the supernatants (i.e., proteins released from the cells) increased in parallel with the equilibrium of inorganic elements between the intra- and extracellular spaces (Table 1).

At the beginning of our experiments with the different detergents, we assumed that, following the solubilization of the lipid membranes, the protein release followed passively. However, additional observations suggested that the detergent molecules actively participate in the release of the different intracellular proteins. Thus the lipolitic and protein-mobilizing effects of the detergents are events well-separated in time as documented by Schliwa et al [36].

The time course for changes in the concentration of K^+, Na^+, Ca^{++}, and Mg^{++} in the detergent-opened cells may also be evaluated with respect to changes in the external concentration of these ions [14]. The ratio of the internal to external ion concentrations (i.e., the ρ values) are presented in Table 2 as a function of the exposure time to the detergents. A ρ-value of 15 in the case of 15-minute Brij treatment indicates a considerable K^+ "gradient" in the "membrane free" lymphocytes (Table 2).

Table 1. K^+, Na^+, Ca^{++}, and Mg^{++} in lymphocytes treated with Triton X-100 and Brij 58 detergents.

	Exposure time *	mMol/kg water				Proteins in the supernatant mg/ml
		K^+	Na^+	Ca^{++}	Mg^{++}	
Control (no detergent)		125.80 ±4.90	39.80 ±1.29	15.77 ±0.88	27.46 ±0.53	8.25 ±0.54
Brij 58	15 min.	73.90 ± 3.17	48.51 ±2.68	17.68 ±3.86	25.00 ±3.49	8.42 ±0.57
	20 min.	48.82 ± 3.43	49.28 ±3.62	21.99 ±2.83	30.51 ±5.70	8.63 ±0.51
	30 min.	36.13 ±1.43	47.17 ±4.39	27.87 ±0.78	28.94 ±3.98	10.04 ±0.53
Triton X-100	15 min.	18.25 ±0.28	42.74 ±1.58	27.14 ±0.35	33.09 ±2.80	10.85 ±0.35
	20 min.	18.07 ±0.25	39.99 ±3.91	24.92 ±2.19	29.26 ±2.5	10.80 ±0.28
	30 min.	19.12 ±0.54	43.01 ±4.51	27.31 ±4.62	28.29 ±6.73	11.30 ±0.28

*Exposure time = 5, 10, 20 minutes in suspension and 10 minutes needed for centrifugation and sample preparations. There are ten samples for each group; all data are presented as the mean ± the standard error of the mean. These data are from a separate study and the values for sodium and potassium may be compared with another similar study published in reference 25. (Control supernatant concentrations mMol/l:
K = 1.95±0.4; Na = 23.48±0.12; Ca = 4.71±0.2; Mg = 5.37±0.12).

Table 2. ρ - values*

	Exposure Time	K^+	Na^+	Ca^{++}	Mg^{++}
Control (no detergent)		64.51 ±2.13	1.7 ±0.06	6.63 ±0.2	10.23 ±0.25
Brij 58	15 min.	15.0 ±0.84	3.07 ±0.10	7.27 ±0.95	9.75 ±0.60
	20 min.	7.30 ±0.31	3.06 ±0.23	10.93 ±0.62	12.83 ±0.97
	30 min.	5.14 ±0.37	3.19 ±0.11	15.94 ±0.84	12.90 ±1.49
Triton X-100	15 min	2.28 ±0.06	2.13 ±0.06	14.07 ±0.12	14.02 ±0.64
	20 min.	2.29 ±0.07	2.36 ±0.21	13.77 ±0.78	12.41 ±0.72
	30 min.	2.40 ±0.06	2.59 ±0.29	14.90 ±1.14	12.19 ±1.34

*ρ - value = (conc. in the pellet) / (conc. in the supernatant). (Control supernatant concentrations mMol/l: K = 1.95±0.4; Na = 23.48±0.12; Ca = 4.71±0.2; Mg = 5.37±0.12).

The findings reported in Tables 1 and 2 were obtained under conditions in which "ion-gradients" were maintained long after removal of lipid membranes. Thus, a second major question arises: Is it possible that these "ion-gradients", and those of intact cells, are maintained by something other than the membranes and the membrane situated pumps?

The evidence from these studies also provides information that may be interpreted to implicate the detergent-mobilized proteins in the selective accumulation of K^+ over Na^+. We suspect that the proteins mobilized by the detergents are not in a solubilized form within the living cell. Rather we propose that they are dynamically associated not only to the fixed charged elements of the nucleus but also to the detergent resistant filaments of the cytoskeleton. We further propose that when these potentially mobile proteins are associated, they selectively accumulate K^+ over Na^+.

The Time Dependent Release of Potassium and Protein From Detergent Treated Cells

From the studies thus far reviewed it is clear that the non-ionic detergents Triton X-100 and Brij 58 are similar in their lipo- or membranolytic effects but quite dissimilar in their effects on protein mobilization. As confirmed by electron microscopic observations, both detergents remove the lipid membranes within 2-5 minutes; however, the release of K^+ was much faster in Triton X-100 treated cells than in Brij 58 treated cells. A study was then undertaken to take advantage of the differences in the detergents, and thereby test further the idea that protein and K^+ release are correlated.

A set of experiments was carried out on SV40 virus transformed H-50 monolayer culture cells. Three different analyses were performed on Triton X-100 and Brij 58 detergent-treated cells: (1) ^{42}K-release, (2) release of ^{35}S-Methionine "labelled" proteins, and (3) electron microscopic studies. The details of the methods are described elsewhere [19].

The release of ^{42}K from the detergent-treated cells showed several interesting features (Fig. 3). The spontaneous release of ^{42}K from the control (untreated) cells did not exceed 30% of the total ^{42}K, even at the longest or 20 minute time point (Fig. 3). In the case or the Triton X-100 treatment, the release of ^{42}K was very rapid; 97% of the total ^{42}K was released in the first 2 minutes (19). The release of ^{42}K from Brij 58 treated cells was slower. At the 2 minute exposure only 25% and at the 5 minute exposure only 37% of the total ^{42}K had leaked from the cells.

"Labelling" most of the proteins of the H-50 culture cells in situ (by exposing them to ^{35}S-Methionine during an entire generation cycle) gave us an excellent tool by which to study the detergent-induced protein release. In the case of the Brij 58 treatment, at the 2 minute exposure time only 0.5% and after 5 minutes still only 1.2% of the ^{35}S-Methionine "labelled" proteins were released from the cells (see Table I of reference 19 and Figure 4). By the end of the 20 minute exposure, the Brij 58 detergent had mobilized 45% of the proteins from the "membraneless" cells. The Triton X-100 detergent seemed to be more effective in the mobilization of the intracellular proteins. Within 2 minutes, Triton X-100 removed as many proteins as Brij 58 did in 20 minutes (Figure 3, reference 19).

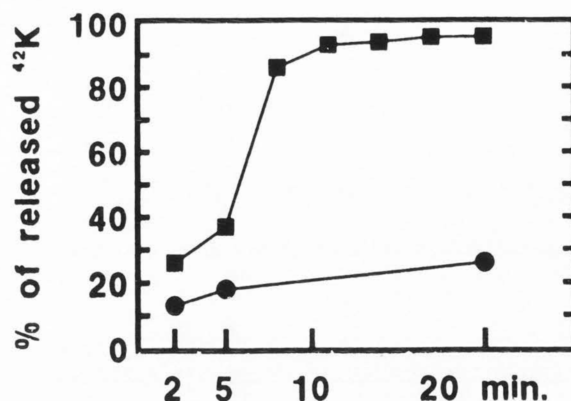

FIGURE 3. Release of ^{42}K from H-50 cells grown in culture and treated with Brij 58 detergent (filled squares). The spontaneous release of ^{42}K to the medium is presented in lower trace (filled circles). The cells were exposed to Brij 58 treated cells for 2, 5, 8, 11,14,17 and 20 minute intervals. The release of ^{42}K appears sigmoidal.

Working with the ^{35}S-Methionine "labelled" proteins provided an additional advantage: although the total amount of protein in the single samples (2-3 x 10^7 H-50 cells/sample) was low, an extended characterization of the individual proteins could be carried out with two-dimensional polyacrylamide gel electrophoresis technique and its concomitant fluorographic detection (Fig. 4). The electrophoretic pattern of proteins mobilized by the Triton X-100 treatment was practically identical at every exposure time (Figs. 4a and b). While at the beginning Brij 58 solubilized only a few proteins, characteristic proteins were mobilized by this detergent as early as 2 - 5 minutes (Fig. 4c). Overall, the pattern of proteins mobilized by each of the Triton X-100 and the 20 minute Brij 58 treatments were very similar (Figs. 4a and b compared to Fig. 4d).

Discussion

Schliwa et al [36] observed that, although Brij 58 removed the lipid membranes of BSC-1 cells in 5 minutes, the collapse and release of the microtrabecular lattice took place several minutes later. On the other hand, in the case of the Triton X-100 treatment, the removal of the microtrabecular lattice occurred practically parallel with the removal of the membranes. Our findings with Brij 58 and Triton X-100 are consistent with these studies.

A major finding resulting from the experiments reported herein is the restricted release of K^+ and proteins from the Brij 58 treated cells. Our electron microscopic observations are in good agreement with those of others [36]; they clearly indicate that the lipid membranes were removed within a few minutes by both detergents. The bulk of the K^+ and proteins, however, remained associated with the framework of the cytoskeleton for several minutes during the Brij 58 treatment. Considering the diffusion coefficients of the free solutes at 25° C [28,32] and the thickness of the monolayer cells [13], if both the proteins and K^+ were freely dissolved in the aqueous phase of the living cell, equilibration would have occurred in a time frame of a few seconds rather than the minutes observed in our studies [17,19].

The observation that it takes several minutes (rather than seconds) for K^+ to obtain equilibrium has been

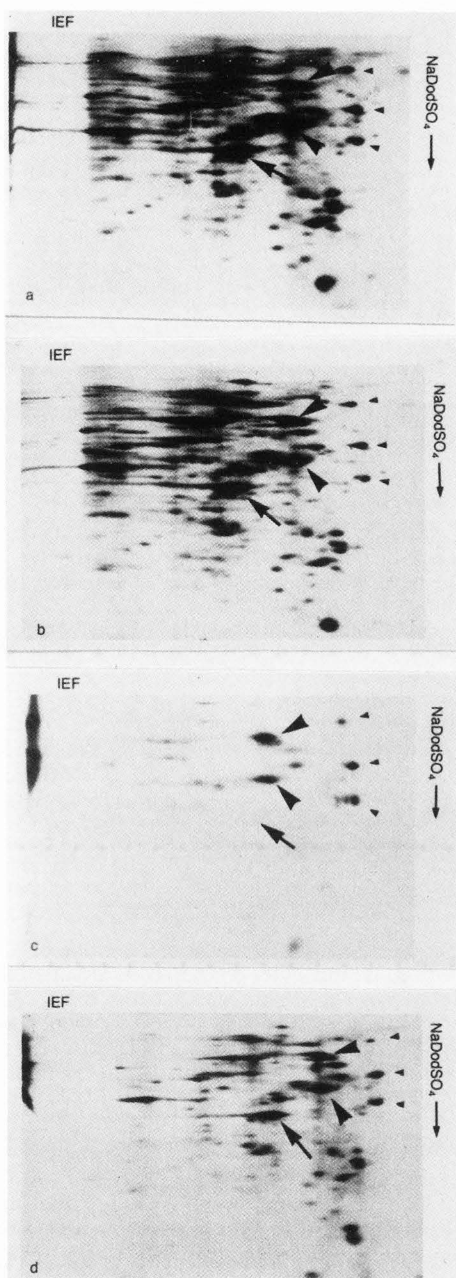

FIGURE 4. Two-dimensional polyacrylamide gel electrophoresis of proteins labeled with ^{35}S-methionine and released from H-50 cells grown in culture. In frames a and b the cells were treated with Triton X-100 (0.2% final concentration) for 5 and 20 minutes, respectively. The arrows with shaft point to actin; whereas the large and small arrows without shafts point to unidentified proteins easily mobilized even with 5 minutes exposure to Brij 58. IEF refers to isoelectric focusing.

observed by Bashford et al [2] in other permeabilized cells [2,35]. However, electron micrographs of their preparation were not presented and the extent of membrane damage is not known. Nevertheless, it appears that permeabilization of cells in general, and by Brij 58 treatments in particular, reveals a retention of the cation K^+ for some time.

While the mechanism of this retention is unclear, approximately 45% of the proteins are mobilized with the loss of K^+. The bulk of the K^+ and protein release occurs after 5 minutes exposure to Brij 58. We propose that the proteins which are mobilized by the detergents are anchored to the cytoskeletal framework [14,15,17-19]. We refer to these proteins as "potentially mobile". We propose further that when these proteins anchor or adsorb to the detergent-resistant cytoskeleton, they change from a globular form to a fibrillar form which selectively adsorbs K^+ over Na^+. The desorption of the proteins, which would occur with detergent treatment, results in a fibrillar to globular transition and the loss of K^+ selectivity.

Conclusions

The integrity of membrane structure does not appear to be the primary controller of K^+ retention. Most of the K^+ and "mobile" proteins are not freely dissolved in the cellular water, but are co-compartmentalized with the cellular cytoskeletal system.

References

1. Andreoli TE, Hoffman JF, Fanestil DD, Schultz SG. (1986). Physiology of Membrane Disorders, second edition. Plenum Medical Book Co., New York, NY. pp. 1066.
2. Bashford CL, Alder GM, Menstrina G, Kingsley JM, Murphy JJ, Pasternak CA. (1986). Membrane Damage by Hemolytic Viruses, Toxins, Compliment, and Other Cytotoxic Agents. J. Biol. Chem. 261, 9300-9308.
3. Bonting SL. (1970). Sodium-Potassium Activated Adenosinetriphosphatase and Cation Transport. In: Membranes and Ion Transport. Vol. 1. EE Bittar, editor. Wiley Interscience, New York. pp. 257-363.
4. Damadian R. (1969). Ion Exchange and Escherichia Coli: Potassium Binding Proteins. Science 165, 79-81.
5. Damadian R. (1971). Biological Ion Exchanger Resins 1. Quantitative Electrostatic Correspondence of Fixed Charge and Mobile Counter Ion. Biophys. J. 11, 739-760.
6. Dean RB. (1941). Theories of Electrolyte Equilibrium in Muscle. Biol. Symp. 3, 331-348.
7. Ernst E. (1975). Inorganic Materials in Striated Muscle. Acta Biochem. Biophys., Sci. Hung. 85, 173-181.
8. Glynn IM, Karlish SJD. (1975). The Sodium Pump. Ann. Rev. Physiol. 37, 13-55.
9. Hazlewood CF. (1972). Pumps or No Pumps. Science 177, 815-816.
10. Hazlewood CF. (1975). A Role for Water in the Exclusion of Cellular Sodium - is a Sodium Pump Needed? Cardiovasc. Dis. Bull. Tex. Heart Inst. 2, 83-104.
11. Hazlewood CF. (1979). A View of the Significance and Understanding of the Physical Properties of Cell-Associated Water. In: Cell Associated Water, W Drost-Hansen, JS Clegg, editors. Academic Press, pp. 165-259.

12. Hodgkin AL (1951). The Ionic Basis of Electrical Activity in Nerve and Muscle. Biol. Rev. (Cambridge) 26, 339-409.
13. Kellermayer M, Somfai M, Jobst K. (1975). Determination of Saline Extractable Material of HeLa Cell Nuclei. Cytobiologie 11, 240-246.
14. Kellermayer M, Hazlewood CF. (1979). Dynamic Inorganic Ion-Protein Interactions in Structural Organization of DNA of Living Cell Nuclei. Cancer Biochem. Biophys. 3, 181-188.
15. Kellermayer M, (1981). Soluble and "loosely bound" Nuclear Proteins in Regulation of the Ionic Environment in Living Cell Nuclei. In: International Cell Biology. HG Schweiger, editor. Springer Verlag, Heidelberg. pp. 915-924.
16. Kellermayer M, Rouse D, Gyorkey F, and Hazlewood CF. (1983). Ionic Milieu and Volume Adjustments in Detergent-Extracted Thymic Nuclei. Physiol. Chem. Phys. Med. NMR 15, 345-354.
17. Kellermayer M, Rouse D, Gyorkey F, Hazlewood CF. (1984). Potassium Retention in Membraneless Thymus Lymphocyte Nuclei. Physiol. Chem. Phys. Med. NMR 16, 503-511.
18. Kellermayer M, Hazlewood CF. (1985). Dynamic Interaction of Water Molecules, Inorganic Ions and Chromatin Structures in Isolated Thymus Nuclei. In: Water and Ions in Biological Systems. A Pullman, V Vasilescu, L Packer, editors. Plenum Press, London. pp. 57-67.
19. Kellermayer M, Ludany A, Jobst K, Szucs G, Trombitas K, Hazlewood CF. (1986). Co-compartmentation of Proteins and K^+ Within the Living Cell. Proc. Natl. Acad. Sci. (USA) 83, 1011-1015.
20. Knight AB, Welt LG. (1974). Intracellular Potassium: A Determinant of the Sodium-Potassium Pump Rate. J. Gen. Physiol. 63, 351-373.
21. Krasne S. (1980). Ion Selectivity in Membrane Permeation. In: Membrane Physiology. TE Andreoli, JF Hoffman, DD Fanestil, editors. Plenum Medical Book Co., New York, NY. p. 217.
22. Krogh A. (1946). The Active and Passive Exchanges of Inorganic Ions Through the Surfaces of Living Cells and Through Living Membranes Generally. Proc. Roy. Soc. Ser. B. 133, 140-200.
23. Ling GN, Cope FW. (1969). Potassium Ion: Is the Bulk of Intracellular K^+ Adsorbed? Science 163, 1335-1336.
24. Ling GN, Ochsenfeld MM, Walton C, Bersinger TJ. (1980). Mechanism of Solute Exclusion from Cells: The Role of Protein-Water Interaction. Physiol. Chem. Phys. 12, 3-10.
25. Ling GN, Negendank W. (1980). Do Isolated Membranes and Purified Vesicles Pump Sodium? A Critical Review and Reinterpretations. Pers. Biol. and Med. 23, 215-239.
26. Ling GN. (1981). Water and the Living Cell as Seen from the Viewpoint of a New Paradigm. In: International Cell Biology 1980-1981. HG Schweiger, editor. Springer-Verlag. pp. 904-914.
27. Ling GN. (1981). Active Solute Transport Across Frog Skin and Epithelial Cell Systems According to the Association-Induction Hypothesis. Physiol. Chem. Phys. 13, 356-384.
28. Ling GN. (1984). In Search of the Physical Basis of Life. Plenum Press, New York. pp. 791.
29. Ling GN, Ochsenfeld MM. (1986). Membrane Lipid Layers vs Polarized Water Dominated by Fixed Ions: A Comparative Study of the Effects of Three Macrocyclic Ionophores on the K^+ Permeability of Frog Skeletal Muscle, Frog Ovarian Eggs and Human Erythrocytes. Physiol. Chem. Phys. Med. NMR 18, 109-124.
30. Ling GN. (1986). Cooperative Interaction Among Surface β- and γ-carboxyl Groups Mediating the Permeation of Ions into Frog Muscle Cells. Physiol. Chem. Phys. Med. NMR 18, 125-129.
31. Ling GN. (1986). A New Theory of Water Contents of Living Cells in Solutions Containing Different Concentrations of Permeant Solutes. Physiol. Chem. Phys. Med. NMR 18, 132.
32. Mastro AM, Keith AD. (1984). Diffusion in the Aqueous Compartment. J. Cell Biol. 99, 180-187.
33. Negendank W. (1982). Studies of Ions and Water in Human Lymphocytes. Biochem. Biophys. Acta 694, 123-161.
34. Osborn M. Weber K. (1977). The Detergent- Resistent Cytoskeleton of Tissue Culture Cells Includes the Nucleus and the Microfilament Bundles. Exp. Cell Res. 106, 339-349.
35. Pasternak CA. (1985). Cell Surfaces. Interdisciplinary Science Reviews 10, 42-55.
36. Schliwa M. van Blerkom J, Porter KR. (1981). Stablization of the Cytoplasmic Ground Substance in Detergent-Opened Cells and a Structural and Biochemical Analysis of its Composition. Proc. Nat. Acad. Sci. U.S.A. 78, 4329-4333.
37. Semenza G, Kinne P. (1985). Membrane Transport Driven by Ion Gradients. Ann. New York Acad. Sci. 456, 1-462.
38. Smith H. (1960). "A Knowledge of the Laws of Solution...". Circulations 21, 808-817.
39. Trantham EC, Rorschach HE, Clegg JS, Hazlewood CF, Nicklow RM, Wakabayashi N. (1984). Diffusive Properties of Water in Artemia Cysts as Determined from Quasi-elastic Neutron Scattering Spectra. Biophys. J. 45, 927-938.

Discussion with Reviewers

C.A. Pasternak: The authors' results on retention of K^+ by detergent-treated lymphocytes or H-50 cells are interesting, but not too surprising. First, lymphocytes are known to contain very little cytoplasm (unless they are stimulated or transformed), the nucleus occupying an exceptionally large proportion of total cell volume. Second, it has been shown by others (e.g. Paine et al. (1981) Nature 291, 258; Tluczek et al. (1984) Dev. Biol. 104, 97) that certain cells (in this instance amphibian oocytes) contain surprisingly large amounts of bound K^+; (that work did, however, show that for other cells, K^+ content is approximately equal to K^+ activity, i.e. little bound K^+). In any case, authors' own results (Table 1) show very extensive loss of K^+ (125 to < 20 mMol/kg water) with only slight protein leakage (3mg/ml over controls) in Triton-treated cells.

Authors: We agree that our results demonstrating that the release of K^+ does not follow promptly the removal of the cell membrane are not surprising for those who have long been aware that K^+ is not freely dissolved inside the living cell. These findings may be new, however, to those who only know about the conventional view of the living cell or to those who continue to use equations derived from dilute solution theory to explain cellular potentials and ion distribution. Indeed the textbooks continue to present the cell as a membranous bag surrounding what may be taken as

a dilute solution. Also our results should be surprising to the majority of the cell physiologists who consider the cell membrane to be filled with hypothetical pumps and channel proteins that control all that comes and goes from the cell.

The protein loss which occurs together with the loss of K^+ is not small. In fact the protein loss is quite substantial if we consider the large volume of the extracelluar liquid into which the protein is released. From other studies we know that the amount of proteins released by detergents can exceed 50% of the total cellular proteins.

C.A. Pasternak: Authors refer extensively (references 4,5,7,11,17,19,23-31,39) to results from laboratories that challenge the conventional view of a permeable membrane, containing ion pumps, that regulates cation content in cells. Apart from some general reviews (1,3,6,8,12,20-22,37) to the latter, experiments showing extensive leakage of K^+ out of, and Na^+ into, cells permeabilized by a whole host of different agents, are scarcely mentioned. This reviewer's work (Bashford et al. (1983) Biosci. Rep. 3, 631), showing an equilibration between intracellular and extracellular K^+ in permeabilized cells, is only one of hundreds of such reports; note that in most of these, where detergents are used to permeabilize cells, the concentrations are often less than those used here. Equally, there are many experiments in which K^+ activity is measured by intracellular electrodes and is found to be close to K^+ concentration (total K^+ divided by water content). In short, the authors' results with lymphocytes (the H50 cells do show K^+ leakage when permeabilized with Triton; hence the Brij results may reflect merely the lesser potency of Brij as a solubilizing agent) are the exception, not the rule.

Authors: We also are well aware of the hundreds of papers - in fact we have chosen references for the conventional membrane-pump view which would lead the reader to a good sampling of that general view point. Likewise, we have chosen only a few papers which offer alternative interpretations. It is correct that Bashford, et al. (2) have demonstrated, using various permeabilizing agents, an equilibration between intracellular and extracellular K. These findings are comparable to our studies with Brij 58; the equilibration, however, occurs over several minutes. If K^+ activity were close to K^+ concentrations as purported for the intracellular K^+ electrode studies, then the equilibration would occur over a period of a second or less with the loss of membrane integrity. Problems inherent in the K^+ selective electrode studies are well addressed in reference 28 (pp. 250-257). The evidence now available is quite substantial favoring the adsorption of K^+ within cells (reference 33 and pp. 227-269 of reference 28).

It is quite evident that Brij 58 has lesser potency than Triton X-100; however, the potency of Brij 58 to disrupt membrane integrity rapidly (often less than one minute) and to completely dissolve membrane structure within 5 minutes is well documented.

C.A. Pasternak: Why should not the detergent, having solubilized the plasma membrane and released its proteins, proceed to solubilize intracellular membranes and release additional membrane proteins, as well as cytoplasmic proteins?

Authors: It has been well proved that detergents are directly involved in both solubilization of lipids and solubilization of protein from the cell surface and also from the interior of the cell. Electron micrographs clearly document the disruption of membrane integrity, even the complete loss of membrane structure in specific studies.

I.L. Cameron: What is the basis for your proposal that fibrillar proteins in the intact cell selectively absorb K^+ over Na^+ and that these proteins selectively desorb K^+ upon transformation back to the globular shape with detergent treatment?

Authors: Fibrillar proteins like F-actin do not accumulate K^+ selectively in the test tube. A globular protein like G-actin also does not accumulate K^+ selectively in vitro. We are hypothesizing that both the cytoskeletal proteins and proteins associated to the skeleton form a three dimensional protein system where the individual polypeptides must be in their extended configuration, and this state is what we call high energy state with selective K^+ accumulating capability.

I.L. Cameron: What is the rationale for use of the symbol ρ instead of a descriptive title such as Ratio of concentration in the pellet over concentration in the supernatant?

Authors: The symbol ρ is a shorthand notation for the ratio of the "concentration" of the pellet over the concentration in the supernatant. It should be realized that the "concentration" in the pellet includes both adsorbed and aqueous phase solutes.

I.L. Cameron: In Table 2 the concentration of Na^+ in the supernatant is listed as 23.48 mMol/l while the concentration of Na+ in the lymphocyte "control" is listed as 39.80 mMol/l in Table 1. How is the relatively high concentration of Na^+ in the lymphocyte compared to the extracellular environment explained?

Authors: The value for the lymphocyte control (39.80 mMol/l) in Table 1 is comparable to the values for human lymphocytes (see reference 33). Unfortunately, we do not know the fractional distribution of intracellular sodium between the nucleus and the cytoplasm. The low extracellular (supernatant) sodium concentration results from the fact that the cells have been washed with TSCM solution (sodium free); therefore, most of the supernatant sodium comes from that which was trapped between cells and the small amount which may have leaked from the cells during the procedure.

G.H. Pollack: Contrary to common expectation, the authors find that potassium may diffuse rather slowly from cells that are functionally membrane-free. If they are right - and they seem to have carried out appropriate controls - the implications are no less than staggering; for if monovalent cations are largely bound to the cell's proteins, most of what is assumed about the status of the sarcoplasm and cell water may be all wet (or perhaps dry). It will be of great interest to see what comes of follow-up studies. The implications are fundamental.

Authors: Thank you.