A Simple and Sensitive Procedure for Measuring Isotope Fluxes through Ion-specific Channels in Heterogenous Populations of Membrane Vesicles*

In this paper, we describe a simple and highly sensitive manual assay for isotope fluxes through ion-conducting pathways, particularly cation-specific channels, in heterogeneous populations of small membrane vesicles. We measure uptake of tracer of the ion of interest, against a large chemical gradient of the same ion. As a result of the imposed chemical gradient, a transient electrical diffusion potential is set up across the membranes of those vesicles which are highly permeable to the ion of interest. The isotope tends to equilibrate with the diffusion potential and is therefore concentrated selectively and transiently into those vesicle containing the channels. Furthermore, when performed in this way, the time course of tracer equilibration occurs over several minutes, rather than the sub-second range expected for tracer equilibration into channel-containing vesicles in the absence of an opposing chemical gradient of the permeant ion.

The use of the procedure is demonstrated for three Na-conducting channels: gramicidin D incorporated into phospholipid vesicles, amiloride-blockable Na channels in toad bladder microsomes, and veratridine-activated tetrodotoxin-blockable Na channels in rat brain synaptic membranes. For all three cases, it proved simple to measure a specific \^22Na uptake, in a minute time range, using very low concentrations of the channel-containing vesicles. By comparison with isotope flux measurements performed without an opposing Na gradient, the power of the present assay derives from both the very large gain in sensitivity and the convenient time course.

Functional properties of ion-conducting channels can be studied by electrophysiological techniques on native cells or phospholipid bilayers. For isolation of channel proteins and their assay, one invariably wishes to reconstitute the protein into artificial phospholipid vesicles. In a number of cases where channel proteins have been isolated, binding of a specific ligand such as tetrodotoxin (7) or saxitoxin (8, 9) has been used as the criterion for preservation of function during purification, but not all channels show high affinity ligand binding. A transport assay would of course be preferable. However, the small diameter of phospholipid vesicles in the various reconstitution procedures (300–1000 Å) and the requirement for an excess of lipid compared to protein should lead to a very small fraction of the vesicle population containing channels and makes the problems of measurement even more severe, even in cell membrane vesicles. Assay of isotope uptake or release from reconstituted vesicles has proved to be possible only in favorable cases in which the channel protein is available in large quantities, e.g. acetylcholine receptors (10) and the tetrodotoxin receptor (11, 12). As expected, the tracer equilibration time with these systems is so fast as to preclude continuous monitoring of the time course with simple manual methods.

In this paper, we describe a simple, sensitive, and convenient flux assay for selective ionic channels in membrane vesicles and demonstrate its use to measure \^42Na fluxes through gramicidin incorporated into phospholipid vesicles, amiloride-sensitive Na channels in membranes isolated from toad bladder, and tetrodotoxin-sensitive Na channels in brain synaptic membranes. This assay was conceived partly on the basis of an interpretation offered by Glynn and Warner (13) for the transient accumulation in human red cells of \^42K flowing through Ca-activated K channels: the Gardos effect.

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* This research was supported in part by National Institutes of Health Grant AM 31089-82 ("Structural Analysis of Na/K ATPase") to I. S. Edelman. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ Supported by National Institutes of Health Grant GM 26976.

** Held a Ludwig-Sheaffer Visiting Associate Professorship in Biochemistry at Columbia University for 1981–1982.

(Received for publication, April 26, 1983)
**EXPERIMENTAL PROCEDURES**

**Methods**

**Vesicle Preparations**

Liposomes—80 mg of crude soybean lecithin were suspended in 2 ml of media containing 25 mM imidazole (pH 7), 5 mM EDTA, and 150 mM NaCl. The lipid suspension was vortexed for 5 min and then sonicated in a Branson 12 bath sonicator until it became translucent (~30 min).

Toad Bladder Microsomes—Toads (*Bufo marinus*, Dominican Republic—public origin) were obtained from National Reagents (Bridgeport, CT) and kept partially submerged in tap water. The animals were double pithed and perfused through the ventricle with about 500 ml of Ringer’s solution. The bladders were then excised and immersed sonicated in a Branson 12 bath sonicator until it became translucent double pithed and perfused through the ventricle with about 500 ml of sucrose, 12.5 mM imidazole (pH 7), 2.5 mM EDTA, and 0.1 mM amiloride. All the subsequent operations were carried out at 0 °C. The preparation of microsomes is based on a procedure developed by Palmer and Edelman. The epithelial cells were scraped from the supporting tissue with a glass microscope slide and washed twice in the above homogenization buffer by centrifugation at 800 × g for 10 min. Washed cells obtained from four to six hemibladders were suspended in 2 ml of homogenization buffer and disrupted by a single 5-s burst with a Polytron tissue grinder (Brinkmann Instruments) at setting 6. The homogenate was centrifuged at 800 × g for 5 min, yielding a cloudy supernatant, a loose white-yellow pellet, and a hard brown pellet. The loose layer was separated from the tight pellet by gentle shaking and was combined with the supernatant. The combined fraction was centrifuged for 60 min at 27,000 × g, yielding a clear supernatant and pellet. The pellet, “the microsomal fraction,” was suspended in the homogenization buffer to a final concentration of 9.5–1.5 mg of protein/ml and used for transport measurements within 4 h.

**Rat Brain Synaptic Membrane**—Brain membranes were obtained from synaptosomal lysates. Synaptosomes were prepared from rat brain homogenates by a procedure similar to that described by Krueger et al. (14). All operations were carried out at 0–4 °C. The synaptosome pellet was homogenized with four strokes at 600 rpm in a glass Teflon homogenizer (0.15-mm clearance) in 5 mM Tris HCl (pH 8.0) (about 40 ml/brain). Solid ultrapure sucrose was then added to the suspension to bring it to 15% w/v. Twenty ml of this suspension were layered on a 3-ml cushion of 32.5% sucrose and centrifuged at 120,000 × g for 90 min in an SW 28 Beckman rotor. The interface between the 15% and the 32.5% sucrose was collected, diluted in 2 volumes of the Na buffer described in the legend to Fig. 4, and centrifuged at 100,000 × g for 30 min. The pellets contain the membranes used in the experiments. All solutions also contained 0.1 mM phenylmethylsulfonyl fluoride, 1 mM phenacetamide, and 1 μM pepstatin A. For flux measurements, the membranes were sonicated for 20 s in a bath sonicator prior to being used.

**Transport Assay**

Two different procedures were used to remove Na ions from the external media of vesicle suspensions prior to the transport assay. For experiments with liposomes, a volume of 200 μl was centrifuged through a Sephadex G-50-40 column pre-equilibrated with 150 mM Tris-Cl, 25 mM imidazole (pH 7), and 5 mM EDTA, as described previously (15). This step exchanged the external NaCl by an equal amount of Tris-Cl without changing the total volume. The eluted vesicle suspension was mixed with 800 μl of the above solution to which gramicidin had been added. The transport assay was initiated 30 s later by adding 10 μl of 22NaCl (2 Ci) to the suspension.

For experiments with toad bladder microsomes and brain synaptic membranes, the external Na was removed by passing the vesicles through a cation-exchange column (Dowex 50-X8 Tris form). Volumes of 100–200 μl of the vesicle suspensions were applied to small Dowex columns (see below) and eluted with 1–1.5 ml of 157 mM sucrose. This step exchanged the external cations by Tris and diluted the suspension 5–15-fold. Various reagents (amiloride, veratridine, and tetrodotoxin) were added, as required, to the vesicle suspension. The assay was initiated 90 s later by adding 10–15 μl of 22NaCl (2–3 Ci).

The vesicle suspensions were incubated with the isotope in the different conditions and for the times indicated in the legends to the Figs. 1–4. In order to separate the vesicles from the medium, 100-μl aliquots of the vesicle suspension were applied to 5–6-cm columns of Dowex 50-X8 (Tris form) poured in Pasteur pipettes, and the vesicles were eluted into counting vials by addition of 1.5 ml of ice-cold sucrose suspension (175 mM brown pellet). The supematant was washed with 1–2 ml of 175 mM sucrose containing 25 mg/ml of bovine serum albumin and stored at 0 °C. The amount of 22Na trapped in the vesicles (eluted on the Dowex columns) was estimated by scintillation counting. The 22Na content is expressed everywhere as a fraction of the initial total radioactivity in the reaction medium. Transport assays were performed at 0 or 30 °C for experiments using cell membrane vesicles or liposomes, respectively.

**Materials**

Bovine albumin, (fraction V), soybean lecithin, granicidin D, Dowex 50X-8 (50–100 mesh), tetrodotoxin, phenylmethylsulfonyl fluoride, pepstatin A, iodoacetamide, and Sephadex G-50-40 were obtained from Sigma. Veratridine (99% pure) was obtained from Aldrich. Amiloride was a gift from Merck Sharp and Dohme. 22NaCl (200 μCi/ml, carrier-free) was purchased from Amersham Radiochemicals. All conventional chemicals were of analytical grade.

**RESULTS AND DISCUSSION**

The Principle of the Measurement—The principle of the assay is as follows. The vesicles are prepared to contain a relatively high concentration of NaCl. Shortly before the assay, the external Na is replaced by a relatively impermeant ion such as Tris. As a consequence of the Na gradient, an electrical diffusion potential will be set up, the magnitude of which will be determined by the relative permeabilities of Na, Cl, and Tris through the membrane. Only in those vesicles containing the Na channel is the Na permeability likely to be much greater than the Cl and Tris permeabilities, and hence a diffusion potential of maximal size and interior negative will be formed. If an isotope that permeates through the channel (in our case 22Na) is added to the exterior solution, it will tend to equilibrate with the membrane potential without itself significantly affecting the potential. It will therefore accumulate selectively into that fraction of the vesicle population containing the channels. In time, the Na gradient will dissipate, as will the interior negative membrane potential, and so 22Na will leave the vesicles. It will be shown that by arranging the flux assay in this way, the measurement of 22Na uptake is highly sensitive due to its accumulation, the time course of the selective 22Na uptake is convenient (in the minute range), and one can distinguish permeability properties of the channels of interest from nonselective Na permeabilities.

**Gramicidin Channels in Phospholipid Vesicles**—The experiments in this section demonstrate the use of the transport assay for the case of a well characterized channel-forming ionophore, gramicidin (17). Fig. 1 shows time course of 22Na uptake into soybean phospholipid vesicles prepared to contain 150 mM NaCl, in the presence and absence of gramicidin and a large Na gradient. In the presence of a Na gradient and the ionophore, a large amount of 22Na was taken up, and after reaching a maximal level of about 1% of the total added radioactivity, after about 8 min, the 22Na content of the vesicles declined slowly. If we assume that the average molecular weight of the phospholipids is 1000 and there are about 3000 phospholipid molecules/sonicated vesicle, then the molar concentration of vesicles is about 2–3 × 10^{-8} M. Assuming optimally that the gramicidin molecules are all incorporated into the vesicles and one requires two gramicidin molecules to produce an active channel (19), then at the concentration of ionophore used, 5 × 10^{-8} M, one could expect that not more than about one in every 100 vesicles contains the channel. The ratio of internal to external volume in our...
It is of interest that the transient accumulation of tracer in the gramicidin-containing vesicles is reminiscent of the classical "counter transport" phenomenon observed for carrier kineticochemical mechanisms. Although the phenomenon of isotope accumulation against a chemical gradient has not been taken as diagnostic of a "carrier" mechanism (21), the result with gramicidin, a well characterized channel-forming ionophore, shows that in the experimental conditions chosen, the distinction between carrier and "channel" kinetic mechanisms is not possible.

Amiloride-sensitive Na Channels—A crude preparation of membranes was prepared from epithelial cells scraped from toad bladders. The apical surface of the epithelial cells contains the amiloride-sensitive Na-specific channel (3, 22). The membrane preparation used consists of a mixture of vesicles derived from apical and basolateral membranes of the cells and is also heavily contaminated with fragmented mitochondria and probably other internal organelles. In the experiment of Fig. 2, the cells were homogenized and the membranes prepared in a medium containing 55 mM NaCl and 100 μM amiloride. Shortly before the assay, the external Na was replaced with Tris as described under "Methods." Amiloride was a weak base (pK = 8.7), and it is essentially all in the protonated form at the experimental pH of 7.4. External amiloride will therefore be removed on the Dowex column. Then 22Na uptake was monitored in the conditions described in the legend to Fig. 2. As seen in Fig. 2A, when unlabeled NaCl was added to the medium and the Na gradient was thus abolished, a small, slow, and monotonic uptake of 22Na was observed. Inhibition by externally added amiloride was not detectable. Conversely, when only 22Na was added to the medium and the transmembrane Na gradient was maximal, a very large uptake was found and it declined slowly from the maximum. Amiloride added to the external medium inhibited both the initial rate and the maximal extent of 22Na uptake by about 65%. In five different preparations, the average inhibition by amiloride was 69 ± 5% (mean ± S.E.). Since amiloride acts from the outside of epithelial cells, it is likely that the amiloride-sensitive flux is occurring in vesicles oriented right side out with respect to the cellular orientation. The purpose of adding amiloride to the membrane preparation media was that it might, when incorporated inside, block Na

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Fig. 1. 22Na uptake through gramicidin channels in liposomes. Sonicated phospholipid vesicles (40 mg/ml) were prepared in 150 mM NaCl solution, and the external Na was substituted by Tris as described under "Methods." A, the eluted vesicles (200 μl) were diluted 5-fold in one of the following solutions: 150 mM Tris-Cl, 25 mM imidazole (pH 7), 5 mM EDTA, 5 × 10^-8 M gramicidin (○), same solution but without gramicidin (△), same solution using a solution that contains NaCl instead of Tris-Cl (□). About 30 s after the dilution, 10 μl of 22NaCl (2 μCi) were added to each suspension. The traces were shown at 1°C was measured as described under "Methods." B, the initial stages of this assay were as in part A, using a diluting solution containing 150 mM Tris-Cl and gramicidin (○). At t = 4.5 min (indicated by the arrow), the suspension was divided into two portions of 600 and 100 μl, respectively. The larger volume of suspension was mixed with 80 μl of a solution containing 150 mM KCl, 25 mM imidazole (pH 7), and 5 mM EDTA. 110-μl aliquots of this suspension were removed to the Dowex columns at the times indicated (O). The smaller portion (100 μl) served as a control. This portion was mixed with 10 μl of Tris-imidazole buffer and was applied to a Dowex column at t = 10 min (△).

Fig. 2. Amiloride-blockable 22Na uptake in toad bladder microsomes. Membrane vesicles from epithelial cells were isolated as described under "Methods." Volumes of 100 μl (1 mg of protein/ml) were applied to Dowex 50-X8 columns and eluted with 1.0 ml of sucrose (175 mM). A, the eluted vesicles were diluted with an equal volume of four different reaction mixtures consisting of 175 mM sucrose (○), 175 mM sucrose, 0.2 mM amiloride (□), 110 mM NaCl (△), and 110 mM NaCl, 0.2 mM amiloride (○). All reaction mixtures contained 10 μCi of 22Na (50 μl). Samples of 100 μl were removed at the indicated times and applied to Dowex columns. B, The experiment was initiated as in A, and the symbols have the same meaning. At the time indicated by arrows, the vesicles were diluted 1:1 with solution of 110 mM NaCl.

2 Portions of this paper (including Figs. 5–7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9630 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-1146, cite the authors, and include a check or money order for $2.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
fluxes through channels in any membranes oriented inside out with respect to the cellular orientation. The uptake of \(^{22}\text{Na}\) which persists in the presence of amiloride added to the assay medium indicates the presence of other Na- or cation-specific permeation pathways, possibly in a different population of vesicles. The fact that most of the tracer is retained even 120 min after imposition of the NaCl gradient implies the vesicles both in the absence and presence of amiloride to C1 or cannot contract to a great extent (see "Analysis of blockable flux measured in the vesicles is passing through the membrane-enriched fraction isolated from toad bladder cells. The necessity of using the rapid flow equipment makes such measurements less convenient than the manual assay described in this paper. Labelle and Valentine (26) have reported a much slower \(^{22}\text{Na}\) flux in toad bladder microsomes. This flux was inhibited by amiloride but only at concentrations higher than 0.6 mM. The slow time course and relative insensitivity to amiloride make it doubtful that the apical Na channels were being observed.

**Tetrodotoxin-sensitive Na Channels**—The third system that we have utilized to demonstrate the efficacy of the assay is the veratridine-activated, tetrodotoxin-inhibitable Na channel in rat brain synaptic membranes (Fig. 4). For this experiment, sonicated synapticosomal membrane fragments, equilibrated with 150 mM NaCl, were prepared as described under "Methods." Fig. 4 shows the time course of \(^{22}\text{Na}\) uptake into synaptic membranes and the effects of veratridine, tetrodotoxin, and a transmembrane Na gradient.

It is expected that in the presence of veratridine these channels will be open, while tetrodotoxin should block them. The criterion of a successful measurement will therefore be inhibition by tetrodotoxin of a flux observed in the presence of veratridine. As seen in Fig. 4, when such measurements are made in synaptic membranes without a Na gradient, the isotope uptake is small and inhibition by tetrodotoxin is only just detectable. However, with a transmembrane Na gradient, \(^{22}\text{Na}\) uptake into the veratridine-activated synaptic membranes was large and showed the by now expected biphasic kinetics. Here, tetrodotoxin inhibited the \(^{22}\text{Na}\) uptake by about 60–70%, to the same level as that observed in synaptic membranes which were not preincubated with veratridine.

Stimulation of the Na Flux by veratridine could be observed at concentrations as low as \(10^{-6}\) M. The concentration dependence of the flux followed a simple hyperbolic saturation curve with half-maximal activation at \(1 \times 10^{-5}\) M veratridine. Tetrodotoxin inhibited the stimulation of the flux produced by \(1 \times 10^{-4}\) M veratridine. The concentration of tetrodotoxin giving a half-maximal inhibition of the initial rate, i.e. \(I_{50}\), was about \(3 \times 10^{-3}\) M. These affinities are similar to those observed in excitable cells (27). The \(^{22}\text{Na}\) uptake in the absence of both veratridine and tetrodotoxin presumably reflects the presence in the vesicle preparation of a Na conductance which is neither activated by veratridine nor inhibited by tetrodotoxin.

The resolution of the tetrodotoxin-sensitive \(^{22}\text{Na}\) flux shown in Fig. 4 is far better than that obtained in previous

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**Fig. 4. Tetrodotoxin-blockable \(^{22}\text{Na}\) uptake in brain synaptic membranes.** Rat brain synaptic membranes were obtained from synaptosomes as described under "Methods." The membranes in 100 mM NaCl, 0.4 mM MgCl\(_2\), 5 mM Tris-Hepes, and 1 mM iodoacetamide were sonicated for 20 s in a bath sonicator prior to use. The membranes were then incubated at room temperature for 15 min in 100 \(\mu\)M veratridine (triangles) or 100 \(\mu\)M veratridine + 1 \(\mu\)M tetrodotoxin (TTX) (squares) or with no extra additions (circles). Volumes of 100 \(\mu\)l (2 mg of protein/ml) were applied to Dowex 50-X8 columns and eluted with 1 ml of sucrose (175 mM). The eluted vesicles were diluted with an equal volume of five different reaction mixtures consisting of 100 mM Tris-Cl, 100 \(\mu\)M veratridine (A), 100 mM Tris-Cl (B), 100 \(\mu\)M veratridine, 1 \(\mu\)M tetrodotoxin (C), 100 mM NaCl, 100 \(\mu\)M veratridine (D), and 100 mM NaCl, 100 \(\mu\)M veratridine, 1 \(\mu\)M tetrodotoxin (E). To 1 ml of each mixture were added 10 \(\mu\)Ci of \(^{22}\text{Na}\) (50 \(\mu\)l), and samples of 100 \(\mu\)l were removed at the indicated times and applied to Dowex columns.

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**Fig. 3. Sensitivity of \(^{22}\text{Na}\) uptake to amiloride.** \(^{22}\text{Na}\) uptake was measured in toad bladder microsomes as described in the legend to Fig. 2 and under "Methods." Increasing concentrations of amiloride (0–16 \(\mu\)M) were used.

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\(^{3}\) The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
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studies using synaptic vesicles without an opposing Na gradient (11, 12, 28). Measurements of $^{22}$Na uptake into phospholipid vesicles reconstituted with the partially purified Na channel protein have failed to show any stimulation by veratridine, although in two reports evidence has been produced that active Na channels were incorporated into the vesicles (29, 30). By contrast, it has been shown that in vesicles reconstituted with partially purified Na channel protein, veratridine produces a 400-500% increase in $^{22}$Na flux when assayed in the presence of an opposing Na gradient. This flux is sensitive to tetrodotoxin and occurs in a time scale of minutes.

Significance and Potential Uses of the Assay—Comparison of the experiments described in this paper with previous work on membrane vesicles from both toad bladder and rat brain synaptic membranes emphasizes the convenient time course and tremendous gain in sensitivity of the flux measurements when performed according to the present procedure.

The success of this assay depends on the existence of a large differential membrane permeability between the ion of interest and the other ions present. It is therefore applicable to all transport systems involving net conductance of the ion of interest, but especially to the case of channel mechanisms.

Reconstitution of functionally active channel protein into artificial phospholipid vesicles will also make use of the advantages of the assay. The experiment of Fig. 1 clearly demonstrates that a successful reconstitution can be detected even if the channel of interest is incorporated into a small fraction of the lipid vesicles (less than 1%), and the assay provides also a sensitive measure of the concentration of the channels. By assaying channel fluxes in different vesicle fractions isolated from structurally complex tissues such as muscle, it should also be possible to use the procedure to localize channels to particular membranes.

Acknowledgments—We (H. G. and S. J. D. K.) would like to express our appreciation for the hospitality and encouragement of Prof. I. S. Edelman of The Biochemistry Department, Columbia University, where part of this work was performed. The technical assistance of Jeff Bennett is greatly appreciated.

REFERENCES

1. Montal, M., Darszon, A., and Schindler, H. (1981) Q. Rev. Biophys. 14, 1–79

2. Lindemann, B., and Van Driessche, W. (1977) Science (Wash. D. C.) 195, 292–294

3. Palmer, L. G., Li, J. H.-Y., Lindemann, B., and Edelman, I. S. (1982) J. Memb. Biol. 64, 91–102

4. Van Driessche, W., and Zeiske, W. (1980) J. Physiol. (Lond.) 299, 101–116

5. Sigworth, F. J., and Neher, E. (1980) Nature (Lond.) 287, 447–449

6. Neher, E. (1981) in Techniques in Cellular Physiology (Baker, P. E., ed.) pp. P121–P121/6, Elsevier/North-Holland Biomedical Press, Amsterdam

7. Alegre, W. S., Levinson, S. R., Brahson, J. S., and Raftery, M. A. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2606–2610

8. Barchi, R. L., Cohen, S. A., and Murphy, L. E. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1306–1310

9. Hartshorne, R. F., and Catterall, W. A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4820–4824

10. Epstein, M., and Racker, E. (1978) J. Biol. Chem. 253, 6660–6662

11. Weigle, J. B., and Barchi, R. L. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3651–3655

12. Talvenheimo, J. A., Tamkun, M. M., and Catterall, W. A. (1982) J. Biol. Chem. 257, 11888–11871

13. Gynnn, K. M., and Warner, A. E. (1972) Br. J. Pharmacol. 44, 271–278

14. Krueger, B. K., Ratzlaff, R. W., Strichartz, G. R., and Blaustein, M. P. (1979) J. Memb. Biol. 50, 287–310

15. Peneefsky, H. S. (1977) J. Biol. Chem. 252, 2891–2899

16. Gasko, O. D., Knowles, A. F., Shertorz, H. G., Soulinia, E. M., and Racker, E. (1976) Anal. Biochem. 72, 57–65

17. Hladky, S. B., and Haydon, D. A. (1972) Biochim. Biophys. Acta 274, 294–312

18. Watts, A., Marsh, D., and Knowles, P. F. (1978) Biochemistry 17, 1792–1801

19. Urry, D. W. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 672–676

20. Clement, N. R., and Gould, J. M. (1981) Biochemistry 20, 1544–1548

21. Lieb, W. R. (1982) in Red Cell Membranes. A Methodological Approach (Ellory, J. C., and Young, J. D., eds) pp. 135–164, Academic Press Inc., Ltd., London

22. Macknight, A. D. C., Dibona, D. R., and Leaf, A. (1980) Physiol. Rev. 60, 615–715

23. Bentley, P. J. (1969) J. Physiol. (Lond.) 205, 372–380

24. Gathy, J. T. (1971) J. Pharmacol. Exp. Ther. 178, 580–594

25. Chase, H., and Al-Awqati, Q. (1983) J. Gen. Physiol. 81, 643–666

26. Lavelle, E. F., and Valentine, M. E. (1980) Biochim. Biophys. Acta 601, 195–205

27. Catterall, W. A. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 15–43

28. Mathews, J. C. Albuquerque, E. X., and Eldefrawi, M. E. (1980) Life Sci. 25, 1651–1659

29. Goldin, D. M., Rhoden, V., and Hess, E. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6884–6886

30. Tamkum, M. M., and Catterall, W. A. (1981) J. Biol. Chem. 256, 11457–11463

31. Rodriguez, H. J., and Edelman, I. S. (1979) J. Memb. Biol. 45, 215–232

32. Hodgkin, A. L., and Keynes, R. D. (1955) J. Physiol. (Lond.) 128, 61–88

33. Hille, B. (1973) in Membranes A Series of Advances (Eisenman, G., ed) Vol. 3, pp. 255–323, Marcel Dekker, Inc., New York

*. Rudy, unpublished experiments.
Assay of Isotope Fluxes through Channels in Vesicles

Supplemental material to:
A SIMPLE AND SENSITIVE PROCEDURE FOR MEASURING ISOTOPE fluxES THROUGH SPECIFIC CHANNELS IN VESICULAR MEMBRANES

(Shattuck, Bershadsky, and Smith J. H.)

Assay of Isotope Fluxes through Channels in Vesicles

In this section we attempt to analyze in a semi-quantitative manner the time-course of \( \text{Na}^{+} \) uptake into the vesicles. The overall kinetic behavior is determined by the permeability and concentration of all the ions present, as well as by the dimensions of the vesicles (A/v and V/Na). In this case we have calculated the turnover rate of \( \text{Na}^{+} \) uptake into vesicles (V/V/Na) from the following equation:

\[
\text{V} = \frac{\text{d}[\text{Na}]}{\text{d}t} = \frac{\text{P}_{\text{Na}} \cdot \text{EF} \cdot \text{RT}}{1 + \text{exp} \left( \frac{\text{EF} \cdot \text{RT}}{12} \right)}
\]

where \( \text{P}_{\text{Na}} \) is the net flux of \( \text{Na}^{+} \) into the vesicles, \( \text{EF} \) is the electric field, and \( \text{RT} \) is the rate constant of the reaction.

The change in concentration of \( \text{Na}^{+} \) in each ion is calculated for a small increment in time, \( \Delta t \), as:

\[
\Delta [\text{Na}^{+}] = \frac{\text{P}_{\text{Na}} \cdot \text{EF} \cdot \text{RT}}{1 + \text{exp} \left( \frac{\text{EF} \cdot \text{RT}}{12} \right)} \Delta t
\]

The change in total vesicle volume is calculated for the time increment \( \Delta t \), assuming that the osmotic equilibrium holds.

\[
\Delta [\text{Na}^{+}] = \frac{\text{P}_{\text{Na}} \cdot \text{EF} \cdot \text{RT}}{1 + \text{exp} \left( \frac{\text{EF} \cdot \text{RT}}{12} \right)} \Delta t
\]

and then new values of \( \text{P}_{\text{Na}} \) and \( \frac{\text{d}[\text{Na}]}{\text{d}t} \) are calculated for the next time increment. We then reconcile the proposed flux equations with the total volume of the vesicle as calculated for a further increment, \( \Delta t \), and the accumulation of the reaction to calculate the final time course of \( \text{Na}^{+} \) uptake into vesicles under various conditions. For simplicity, we consider two restrictive situations. In the first, we assume that the only ion with appreciable permeability is \( \text{Na}^{+} \) and \( \text{Na}^{+} \). The membrane potential created by the diffusion of \( \text{Na}^{+} \) into the vesicles will be equal to the time course of \( \text{Na}^{+} \) uptake into vesicles under varying conditions. In the second scenario, we assume that the only ions with appreciable permeability are \( \text{Na}^{+} \) and \( \text{Cl}^{-} \). The membrane potential created by the diffusion of both ions into the vesicles will be equal to the time course of \( \text{Na}^{+} \) uptake into vesicles under varying conditions.

In the figure, the higher the \( \text{Na}^{+} \) uptake into vesicles, the steeper the time course of \( \text{Na}^{+} \) uptake into vesicles. The lower the \( \text{Na}^{+} \) uptake into vesicles, the flatter the time course of \( \text{Na}^{+} \) uptake into vesicles. The opposite is true for the time course of \( \text{Cl}^{-} \) uptake into vesicles.
A simple and sensitive procedure for measuring isotope fluxes through ion-specific channels in heterogenous populations of membrane vesicles.

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J. Biol. Chem. 1983, 258:13094-13099.

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