In bovine cardiac sarcolemmal vesicles, an outward H+ gradient stimulated the initial rate of amiloride-sensitive uptake of 22Na+, 42K+, or 86Rb+. Release of H+ from the vesicles was stimulated by extravesicular Na+, K+, Rb+, or Li+ but not by choline or N-methylglucamine. Uptakes of Na+ and Rb+ were half-saturated at 3 mM Na+ and 3 mM Rb+, but the maximal velocity of Na+ uptake was 1.5 times that of Rb+ uptake. Na+ uptake was inhibited by extravesicular K+, Rb+, or Li+, and Rb+ uptake was inhibited by extravesicular Na+ or Li+. Amiloride-sensitive uptake of Na+ or Rb+ increased with increase in extravesicular pH and decrease in intravesicular pH. In the absence of pH gradient, there were stimulations of Na+ uptake by intravesicular Na+ and K+ and of Rb+ uptake by intravesicular Rb+ and Na+. Similarly, there were trans stimulations of Na+ and Rb+ efflux by extravesicular alkali cations. The data suggest the existence of a nonselective antiporter catalyzing either alkali cation/H+ exchange or alkali cation/alkali cation exchange. Since increasing Na+ caused complete inhibition of Rb+/H+ exchange, but saturating K+ caused partial inhibitions of Na+/H+ exchange and Na+/Na+ exchange, the presence of a Na+-selective antiporter is also indicated. Although both antiporters may be involved in pH homeostasis, a role of the nonselective antiporter may be in the control of Na+/K+ exchange across the cardiac sarcolemma.

The existence of alkali cation/H+ antiporters in energy-transducing membranes was postulated by Mitchell (1), and an Na+/H+ antiport activity was first demonstrated by Mitchell and Moyle (2) in the mitochondrial inner membrane. Subsequently, it has been established that the plasma membranes of many cells, including the cardiac sarcolemma, also contain Na+/H+ antiporters that are involved in the regulation of cytosolic pH and a variety of other cellular functions (3-5). Although the role of a plasma membrane Na+/H+ antiporter in cellular pH homeostasis must clearly depend on its ability to discriminate among the various cellular cations, the ion selectivity of the antiporter has not been examined thoroughly in all membranes in which it has been detected. Here, we present data that suggest the existence of two distinct amiloride-sensitive antiporters in the purified cardiac sarcolemmal vesicles. One is the Na+-selective Na+/H+ antiporter that transports Na+ but not K+ or Rb+. The other is a nonselective alkali cation/H+ antiporter that transports all alkali cations including Na+, K+, and Rb+.

**EXPERIMENTAL PROCEDURES**

**Membrane Preparations**—Cardiac sarcolemmal vesicles were prepared from fresh beef heart by the method of Jones (6) as described before (7). The vesicles were suspended in 0.25 mM sucrose, 10 mM histidine (pH 7.5) and stored at -50°C. The purity of the vesicles was determined as described before (6, 8); contamination by mitochondria was in the range of 5-10% and by sarcoplasmic reticulum, less than 2%. Procedures described previously were used to prepare beef heart mitochondria (9) and submitochondrial particles (10).

**Trans Membrane Ion Influx and Efflux Measurements**—Uptakes of "Na", "Rb", and "K" by the vesicles were measured by modification of the procedures of Seiler et al. (9) as indicated below. Freshly thawed vesicles were centrifuged at 100,000 × g for 15 min in a Beckman Airfuge. The sedimented vesicles were washed once by suspension and centrifugation with a medium of specified pH, suspended in the same medium, and incubated on ice overnight. Unless indicated otherwise, this loading medium was either 0.1-0.3 mM mannitol, 40 mM MES, 20 mM MGA, and 1 mM EGTA (pH 6.0) or 0.1-0.3 mM mannitol, 40 mM glycylglycine, 20 mM MGA, and 1 mM EGTA (pH 8.0). The following day, the vesicles (30-50 μg) were diluted 10-20 times in an assay medium (one of the loading media to which "Na" or "Rb" and "K" had been added) for uptake studies at 22°C. (Changing the mannitol concentrations of the above solutions in the range of 0.1-0.3 mM had little effect on the results as long as the osmolarities of the loading and the assay media were the same.) After the indicated periods, uptake was terminated by the addition of a 15-fold excess of an ice-cold stop solution containing 100 mM mannitol, 100 mM MgCl2, 8 mM HEPES, and 4 mM Tris (pH 7.3). The diluted mixture was filtered and washed as indicated (9). When not specified, duration of the uptake experiment was 20 s. Experiments such as those of Fig. 1 showed that 20 s was at the upper limit of the period during which the H+ gradient-dependent uptake was a linear function of time. Hence, the uptake rates presented under "Results" may be somewhat underestimated. In some experiments (see Figs. 5, 6, and 10) the pH values of the above loading and assay media were changed by the replacement of the indicated buffers and MGA with 40 mM ACES and 20 mM MGA (pH 6.4-6.5); 40 mM MOPS and 20 mM MGA (pH 6.7); 40 mM HEPES and 20 mM MGA (pH 7.1); 40 mM HEPPS and 20 mM MGA (pH 7.4-7.5); 20 mM CHES, 27 mM glycylglycine, and 20 mM MGA (pH 8.3); 30 mM CHES and 30 mM MGA (pH 9.0); 20 mM CHES and 40 mM MGA (pH 9.4). The final pH of each medium was determined prior to use.

For efflux measurements, the sarcolemmal vesicles were washed and loaded as indicated above with 0.1 mM mannitol, 40 mM glycylglycine, 20 mM MGA, 1 mM EGTA (pH 8.0). The vesicles were suspended in the same medium containing 0.3 mM NaCl or 0.5 mM RBCl and incubated at 22°C for 2 min. The suspension was then cooled and
centrifuged in the Airfuge. The supernatant solution was discarded, and the vesicles were diluted 10-fold into the above pH 8 buffer solution, with the indicated additions, to initiate the efflux measurements at 22 °C. After specified periods, cold stop solution was added, and the vesicles were filtered and washed as indicated above.

3Na+, 86Rb+, and 42K+ were counted by conventional procedures. Unless indicated otherwise, each experimental point shown is the average of at least three determinations, with the standard error of the mean not larger than the size of the symbol.

Measurement of H+ Transport—This was done with the use of the fluorescent dye acridine orange (9, 11, 12). When vesicles are added to a solution of this weak base whose pH is higher than that of intravesicular pH, the dye is accumulated inside the vesicles, resulting in the quenching of fluorescence. Appropriately loaded sarcolemmal vesicles (380–420 μg) were added to 3 ml of the indicated solutions containing 10 μM acridine orange. The fluorescence of the dye was measured at 22 °C as a function of time using a SLR8000C spectrofluorometer at an excitation wavelength of 491 nm and an emission wavelength of 527 nm.

(Na+ + K+)-ATPase Assay—Orientation of the sarcolemmal vesicles was determined as described under "Results" through the assay of digitonin-sensitive (Na+ + K+)-ATPase. Vesicles were added to reaction mixtures containing 100 mM NaCl, 25 mM KCl, 3 mM MgCl2, 1 mM EDTA, 1 mM NaN3, 25 mM MOPS, 25 mM Tris (pH 7.4), and other additions as indicated. Oubain (1 mM) and 0.1 mM digitoxigenin were used as inhibitors. Alamethicin (1 mg/mg of protein) was used to unmask the activity of all sealed vesicles (13). Valinomycin (10 μM) and 1 μM carbonylcyanide m-chlorophenylhydrazone were used to unmask the activity of the inside-out vesicles (14). The initial rate of release of P, was determined colorimetrically.

Materials—22Na+, 86Rb+, and 48K+ were purchased from Du Pont-New England Nuclear. "Vanadate-free" ATP, alamethicin, amiloride, valinomycin, acridine orange, ouabain, digitoxigenin, and carbonylcyanide m-chlorophenylhydrazone, and all buffering agents were obtained from Sigma.

RESULTS

Sarcolemmal Na+/H+, K+/H+, and Rb+/H+ Exchange Activities—Cardiac sarcolemmal vesicles were preequilibrated in acid (pH 6.0) or alkaline (pH 8.0) media and then diluted into media of varying pH, all of which contained 1 mM 22Na+. When the uptake of 22Na+ by these vesicles was monitored as a function of time, the results (Fig. 1a) confirmed the finding of others (9) by showing that the initial rate of 22Na+ uptake in the presence of an outward H+ gradient was severalfold greater than the rate of uptake either in the absence of a gradient or in the presence of an inward H+ gradient. To test the ion specificity of the system, similar experiments were done in which 22Na+ was replaced with either 86Rb+ or 48K+. The results (Fig. 1b) were nearly identical for 86Rb+ and 48K+ and qualitatively the same as those obtained with 22Na+.

Experiments similar to those of Fig. 1 were done in which the sarcolemmal vesicles were replaced with beef heart mitochondria or submitochondrial particles. Based on the results (not shown) and considering the extent of contamination of the sarcolemmal vesicle preparation by mitochondria (see "Experimental Procedures"), it was established that no more than 3% of the H+ gradient-dependent uptakes of alkali cations noted in Fig. 1 could be due to mitochondrial contamination.

When experiments similar to those of Fig. 1a were done with vesicles that were exposed to 1 mM ouabain during the overnight loading period, the results (not shown) were nearly identical to those of Fig. 1a, suggesting that the observed Na+ uptake was not mediated by the sodium pump.

To determine if the H+ gradient-dependent uptake of an alkali cation was accompanied by the release of H+ from the vesicles, experiments of Fig. 2 were done. Vesicles that were preloaded with the acid medium (pH 6.0) were added to the alkaline (pH 8.0) medium containing acridine orange. There was a rapid quenching of acridine orange fluorescence, which is known to be due to the outward H+ gradient in such vesicles (9, 11, 12), followed by a slow increase in fluorescence, which is due to the spontaneous dissipation of the H+ gradient (9).

Addition of Na+, K+, Rb+, or Li+ at 20 mM concentrations increased the rate of H+ gradient dissipation, but the same concentrations of choline and MGA were without effect (Fig. 2). The combined data of Figs. 1 and 2 suggest the existence of both Na+/H+ and K+/H+ (or Rb+/H+) exchange activities in sarcolemmal vesicles.

In experiments of Fig. 3 the effect of extravesicular Na+ concentration on the rate of Na+/H+ exchange and the effect of extravesicular Rb+ concentration on the rate of Rb+/H+ exchange were determined in the same vesicle preparation. The double-reciprocal plots of the data of Fig. 3 showed the same Kmax value of 2–3 mM for Na+ and Rb+ and maximal velocities of 52 nmol/mg/min for Na+ and 32 nmol/mg/min for Rb+. Similar experiments in two other preparations revealed similar Kmax values and the same ratio (about 1.5) of Na+ maximal velocity to Rb+ maximal velocity.

Amiloride had similar inhibitory effects on Na+/H+ and Rb+/H+ exchange. When these activities were assayed at 1

Fig. 2. Stimulation of H+ release from sarcolemmal vesicles by extravesicular alkali cations. Vesicles were loaded overnight with acid (pH 6.0) or alkaline (pH 8.0) buffer. At the first bold arrow on the left, the suspensions containing the acid-loaded vesicles were added small volumes of NaCl, KCl, RbCl (not shown), LiCl, choline chloride, and MGA hydrochloride to obtain a 20 mM final concentration of each. At the third bold arrow, 5 mM (NH4)2SO4 was added to obtain the dissipation of the pH gradient due to the rapid passage of NH4+ across the membrane. Additions of 20 mM gluconate salts of Na+ or K+ had the same effects as those shown for NaCl and KCl. Quenching of the fluorescence that is observed after the addition of control vesicles (pH1 = pH2 = 8) is most likely due to the binding of the dye to the vesicles (9, 11). For other details, see "Experimental Procedures."
Ion Specificity of Na+/H+ Antiporter

FIG. 3. Effects of extravesicular Na⁺ and Rb⁺ concentrations on the H⁺ gradient-dependent uptakes of Na⁺ and Rb⁺. Vesicles were loaded with the acid (pH 6.0) medium and then diluted into alkaline (pH 8.0) and acid (pH 6.0) media containing the indicated concentrations of NaCl or RbCl. Uptake rates were measured as indicated under "Experimental Procedures." The indicated value at each Na⁺ or Rb⁺ concentration is the difference between uptake at pH 8 and uptake at pH 6.

FIG. 4. Inhibition of Na⁺/H⁺ and Rb⁺/H⁺ exchange by amiloride. a, vesicles were loaded with the pH 6 buffer and diluted into the pH 8 buffer solution containing 1 mM Na⁺ or 1 mM Rb⁺ and the indicated amiloride concentrations. Uptake rates were then measured as described under "Experimental Procedures." b, Na⁺ uptake rates were determined as indicated above in media containing the indicated Na⁺ concentrations in the absence of amiloride and in the presence of indicated concentrations of amiloride.

FIG. 5. Effects of extravesicular pH on the H⁺ gradient-dependent uptake of Na⁺ or Rb⁺. Vesicles were loaded with the pH 6 buffer and then diluted into buffers of the indicated pH values containing either 1 mM Na⁺ or 1 mM Rb⁺. Uptake rates were determined according to "Experimental Procedures." Each point represents uptake in excess of that obtained in the absence of a pH gradient.

FIG. 6. Effects of intravesicular pH on the H⁺ gradient-dependent uptake of Na⁺ or Rb⁺. Vesicles were loaded with buffers with the indicated pHi values. For experiments with Na⁺, the vesicles were diluted into pH 8 buffer containing 1 mM Na⁺. For experiments with Rb⁺, the vesicles were diluted into pH 8.3 buffer containing 1 mM Rb⁺. Uptake rates were determined as described under "Experimental Procedures." Each point represents uptake in excess of that obtained in the absence of a pHi gradient.

FIG. 7. Dixon plots of the inhibition of Na⁺/H⁺ exchange by K⁺ (a) and the inhibition of Rb⁺/H⁺ exchange by Na⁺ (b). Vesicles were loaded with the pH 6 buffer and then diluted into buffers for uptake measurements containing either 1 mM Na⁺ or 1 mM Rb⁺ and the indicated concentrations of KCl or NaCl. Uptake rates were measured as described under "Experimental Procedures." For each indicated condition, uptake was measured at pH 8 and pH 6; the difference between the two was taken as the value of the exchange rate.

Effects of pHi and pHe on Na⁺/H⁺ and Rb⁺/H⁺ Exchange Activities—When pHi was kept constant at 6, and pHe was varied in the range of 6–9.4, both Na⁺ uptake and Rb⁺ uptake rates increased with increase in pHe, and seemed to level off at pHe values higher than 8.3 (Fig. 5). The value of pHe for half-maximal stimulation of Rb⁺ uptake seemed to be lower than the same value for the stimulation of Na⁺ uptake, but the data were not good enough to establish this point (Fig. 5). In experiments of Fig. 6, pHe was kept constant (at 8 in experiments with Na⁺ and at 8.3 in experiments with Rb⁺), and the effects of changes in pHe on Na⁺ and Rb⁺ uptake rates were studied. Both rates increased with decrease in pHe, (Fig. 6). Although there was no evidence that either uptake rate had reached a maximal value at the lowest tested pHe, both exchange systems clearly seemed to approach saturation with increase in intravesicular H⁺ concentration (inset to Fig. 6).

Relation of Na⁺/H⁺ Exchange to Rb⁺/H⁺ or K⁺/H⁺ Exchange Activities—Extravesicular K⁺ inhibited the H⁺ gradient-dependent uptake of 22Na⁺, and extravesicular Na⁺ inhibited the H⁺ gradient-dependent uptake of 36Rb⁺. Dixon plots of these inhibitory effects (Fig. 7) revealed K₀ values of 4 mM K⁺ (Fig. 7a) and 2.5 mM Na⁺ (Fig. 7b), in close agreement with the K₀ values obtained in Fig. 3. Taken together, these data suggest that Na⁺ and K⁺ (or Rb⁺) are alternate substrates.
concentrations were varied in the range of 100-300 mM to maintain concentrations of Na⁺ (0) and Li⁺ (0), and inhibition of Na⁺/H⁺ exchange was obtained with increasing Li⁺ concentrations (Fig. 8c). In similar experiments on the effects of K⁺ on ²²Na⁺ uptake, however, the maximal level of inhibition of Na⁺ uptake obtained at saturating K⁺ concentration was less than 100% (Fig. 8h; see also Fig. 11, below). When Rb⁺ was used instead of K⁺ in experiments similar to those of Fig. 8h, the results were the same (not shown). This partial inhibition of Na⁺/H⁺ exchange by K⁺ or Rb⁺ is not consistent with competition between Na⁺ and K⁺ or Na⁺ and Rb⁺ on a single carrier (see “Discussion”).

In experiments similar to the above, complete inhibition of either Na⁺/H⁺ or Rb⁺/H⁺ exchange activity was approached by increasing Li⁺ concentrations (Fig. 8, a and b); however, up to 50 mM concentrations of choline and tetraethylammonium ions did not inhibit either activity (data not shown).

**Alkali Cation/Alkali Cation Exchange Activities**—To determine if the Na⁺/H⁺ and the Rb⁺/H⁺ exchangers could also carry out alkali cation/alkali cation exchange, experiments of Table I were done. The values of pHi and pH, were kept constant at 8. The rate of amiloride-sensitive uptake of ²²Na⁺ or ⁸⁶Rb⁺ by vesicles that did not contain alkali cations was compared with the uptake rate by vesicles that contained 20 mM concentrations of unlabeled Na⁺, K⁺, or Rb⁺ as indicated. (Note that for technical reasons when the intravesicular cation was different from the labeled extracellular cation, it was necessary that the extravesicular medium also contain some of the intravesicular cation.) The results showed that uptake of ²²Na⁺ was stimulated by intravesicular Na⁺ or K⁺ and that uptake of ⁸⁶Rb⁺ was stimulated by intravesicular Rb⁺ or Na⁺. The results also showed that the largest degree of trans stimulation was that of Na⁺ uptake by intravesicular Na⁺ (Table I).

Cardiac sarcolemmal vesicles develop a membrane potential if vesicles with a transmembrane K⁺ gradient are exposed to valinomycin, and this potential is not significantly affected by a transmembrane Na⁺ gradient (15, 16). When the experiments of Table I on the effects of intravesicular K⁺ on ²²Na⁺ uptake were repeated with the inclusion of 2 μM valinomycin during the course of the assay, the results were not different from those of Table I, suggesting, but not establishing, that the trans stimulatory effects of alkali cations observed in Table I are independent of the membrane potential.

Experiments of Fig. 9 showed that ²²Na⁺ efflux from the vesicles was stimulated by extravesicular Na⁺ or K⁺ (Fig. 9a) and that ⁸⁶Rb⁺ efflux was stimulated by extravesicular Na⁺ or Rb⁺ (Fig. 9b). Here, as in the case of influx experiments, the largest trans stimulation was that of Na⁺ efflux by extravesicular Na⁺ (Fig. 9). Taken together, the data of Table I and Fig. 9 establish the existence of alkali cation/alkali cation exchange activities and indicate that the rate of such exchange is dependent on the nature of the alkali cation on the two sides of the membrane.

The above experiments were done in the absence of an imposed pH gradient. To examine the effect of such gradient on Na⁺/Na⁺ exchange, the rate of exchange was determined at several pH, values in the range of 6-9 under two conditions: when pH, was equal to pHi, and when pH, was held constant at 6. The results (Fig. 10) showed that (a) Na⁺/Na⁺ exchange was reduced when there was a transmembrane H⁺ gradient; and (b) either with or without a pH gradient, Na⁺/Na⁺ exchange increased with increase in pH. These results are consistent with the assumption that Na⁺/H⁺ exchange and Na⁺/Na⁺ exchange occur on the same antiporter and that Na⁺ and H⁺ compete at both sides of the membrane.

In experiments of Fig. 11, the effects of extravesicular K⁺ on amiloride-sensitive Na⁺/Na⁺ exchange, measured through

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### Table I

**Alkali cation/alkali cation exchange in cardiac sarcolemmal vesicles**

Vesicles were loaded with the standard pH 8 medium to which was added either 20 mM Na⁺ gluconate, 20 mM K⁺ gluconate, or 20 mM RbCl. Control vesicles without internal alkali cations were prepared similarly but with the addition of either 20 mM MGA gluconate or 20 mM MGA hydrochloride. For uptake measurements, the vesicles were then diluted into the same medium containing the final concentrations of the indicated labeled and unlabeled alkali cation salts. For each condition, the rate of uptake was measured in the absence of amiloride and in the presence of 2.5 mM amiloride. The number of experiments is indicated in the parentheses. The indicated values are mean ± S.E. in all four sets, p < 0.05.

| Extravesicular alkali cation | Amiloride-sensitive uptake of ²²Na⁺ or ⁸⁶Rb⁺ by vesicles containing | Stimulation of uptake by the trans alkali cation |
|-----------------------------|---------------------------------------------------------------|-----------------------------------------------|
|                             | No alkali cation | 20 mM Na⁺ | 20 mM K⁺ | 20 mM Rb⁺ |                          |
|                             | n mole/mg/min    | (n = 11)  | (n = 11) | (n = 15) | (n = 8) | (n = 5) | %     |
| 3 mM ²²Na⁺                  | 22.93 ± 1.53     | 41.61 ± 4.05 |          |          | 40.80 ± 2.55 |          | 81    |
|                             | (n = 11)         | (n = 11)  | (n = 15) | (n = 8)  | (n = 5) |       |
| 3 mM ²²Na⁺ + 2 mM K⁺        | 8.25 ± 0.60      | 11.40 ± 0.72 |          |          | 17.58 ± 2.31 |          | 38    |
|                             | (n = 15)         | (n = 15)  | (n = 8)  | (n = 5)  |       |
| 3 mM ⁸⁶Rb⁺                  | 32.19 ± 2.49     |          | 17.58 ± 2.31 |          |          |       |
|                             | (n = 8)          |            | (n = 5)  |          |       |
| 3 mM ⁸⁶Rb⁺ + 2 mM Na⁺       | 11.37 ± 1.23     |          |          |          |          |       |
|                             | (n = 5)          |            |          |          |       |

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**Experimental Procedures**
to keep intra- and extravascular osmolarities the same (see "Experimental Procedures").

The extravesicular medium contained the following additions: 20 mM KCl (O), 20 mM NaCl (●), 20 mM MGA gluconate (△), 20 mM choline chloride (▲).

**FIG. 9.** Stimulation of \(^{22}\)Na\(^{+}\) efflux and \(^{86}\)Rb\(^{+}\) efflux by extravascular alkali cations. Vesicles were loaded with the appropriate isotope, and efflux was measured as described under "Experimental Procedures." The extravesicular medium contained the following additions: 20 mM KCl (O), 20 mM NaCl (●), 20 mM MGA hydrochloride (△), 20 mM choline chloride (▲).

**FIG. 10.** Effect of H\(^{+}\) gradient on Na\(^{+}/Na^{+}\) exchange. Vesicles were loaded with the media with the indicated pH\(_{\text{in}}\) values. At each pH\(_{\text{in}}\), value the loading media contained either 20 mM Na\(^{+}\) gluconate or 20 mM MGA gluconate. The vesicles were then diluted into uptake media with the indicated pH\(_{\text{ext}}\) values, all of which contained a final concentration of 1 mM \(^{22}\)Na\(^{+}\). Uptake rates were measured as described under “Experimental Procedures.” The indicated exchange rate for each condition is the difference between \(^{22}\)Na\(^{+}\) uptake into vesicles containing 20 mM Na\(^{+}\) and uptake into vesicles with no intravesicular Na\(^{+}\).

**FIG. 11.** Inhibition of Na\(^{+}/Na^{+}\) exchange by extravascular K\(^{+}\). Amiloride-sensitive Na\(^{+}/Na^{+}\) exchange was measured as described in the legend to Table I in the absence of extravascular K\(^{+}\) and in the presence of the indicated concentrations of extravascular K\(^{+}\). Mannitol concentrations were varied in the range of 100–300 mM to keep intra- and extravascular osmolarities the same (see “Experimental Procedures”).

the uptake of \(^{22}\)Na\(^{+}\) by Na\(^{+}\)-loaded vesicles, were studied. K\(^{+}\) was a partial inhibitor of Na\(^{+}/Na^{+}\) exchange (Fig. 11) as it was a partial inhibitor of Na\(^{+}/H^{+}\) exchange (Fig. 8b).

**Orientation of the Sarcolemmal Vesicles**—Although there is agreement that purified cardiac sarcolemmal vesicle preparations are mixtures of leaky vesicles, sealed inside-out vesicles, and sealed right-side-out vesicles, there is considerable disagreement on the relative proportions of these vesicles in the various preparations (e.g. 6, 17–19). Since for the interpretation of the present data it was desirable to know the orientation of the sealed vesicles, we used the assay of (Na\(^{+} + K^{+}\))\(-ATPase\) (see “Experimental Procedures”) to determine the sidedness of the vesicles as others have done (6, 17, 18).

We considered (a) the ouabain-sensitive activity in the presence of alamethicin as the total activity of all vesicles; (b) the ouabain-sensitive activity in the absence of any ionophore as that of the leaky vesicles; and (c) the digitoxigenin-sensitive activity in the presence of valinomycin and carbonylcyanide m chlorophenylhydrazone as the sum of the activities of the leaky and the inside-out vesicles. When three preparations selected at random were assayed by this procedure, we found 38.7 ± 2.6% of the vesicles to be leaky. Of the remaining sealed vesicles, 94.0 ± 5.7% were right side out and the remainder inside out. In agreement with Jones (6) we conclude that the sealed vesicles are predominantly right side out. Evidently, variations among the preparative procedures lead to significant differences in the ratio of the two types of sealed vesicles.

**Experiments with Other Sarcolemmal Vesicles**—Limited experiments showed that the Na\(^{+}/H^{+}\), Rb\(^{+}/H^{+}\), and alkali cation/alkali cation exchange activities described above also existed in the sarcolemmal vesicles of the canine heart obtained by the same procedure used to prepare the beef heart vesicles.

**DISCUSSION**

The Two Antiporters of the Cardiac Sarcolemma—Seiler et al. (9), who were the first to demonstrate an Na\(^{+}/H^{+}\) exchange activity in cardiac sarcolemmal vesicles, also noted (Footnote 2 of Ref. 9) the existence of a "small amount" of K\(^{+}/H^{+}\) exchange in these vesicles but did not pursue their study of this activity. The data presented here characterize this K\(^{+}/H^{+}\) (or Rb\(^{+}/H^{+}\)) exchange and its relation to the Na\(^{+}/H^{+}\) exchange activity. Previous studies with cardiac sarcolemmal vesicles demonstrated that an outward H\(^{+}\) gradient stimulates Na\(^{+}\) uptake by the vesicles (9, 20) and that this amiloride-sensitive Na\(^{+}\) uptake represents the operation of an electroneutral Na\(^{+}/H^{+}\) antiporter (9). We have shown that (a) Na\(^{+}\) uptake on this antiporter is inhibited by K\(^{+}\) or Li\(^{+}\) on the cis side (Figs. 7 and 8); (b) there are also amiloride-sensitive uptakes of K\(^{+}\) and Rb\(^{+}\) that are stimulated by H\(^{+}\) on the trans side (Figs. 1 and 4) and inhibited by Na\(^{+}\) or Li\(^{+}\) on the cis side (Figs. 7 and 8); (c) release of intravesicular H\(^{+}\) is stimulated by Na\(^{+}\), K\(^{+}\), Rb\(^{+}\), or Li\(^{+}\) on the trans side (Fig. 2); (d) there are sodium stimulations of Na\(^{+}\) uptake by Na\(^{+}\) or K\(^{+}\) and of Rb\(^{+}\) uptake by Rb\(^{+}\) or Na\(^{+}\) (Table I); and (e) release of intravesicular Na\(^{+}\) is stimulated by Na\(^{+}\) or K\(^{+}\) on the trans side, and release of intravesicular Rb\(^{+}\) is stimulated by Rb\(^{+}\) or Na\(^{+}\) on the trans side (Fig. 9). Taken together, these findings clearly indicate the existence of a nonselective antiporter that is capable of catalyzing either alkali cation/H\(^{+}\) exchange or alkali cation/alkali cation exchange.

The same data that establish the nonselectivity of the antiporter, however, also show significant quantitative differences in the exchange activities involving Na\(^{+}\), K\(^{+}\), and Rb\(^{+}\). Some of these differences may be explained simply by assum-
ing that alkali cations share a common binding site on the same antiporter but that within the antiport reaction cycle there are differences between one or more kinetic constants depending on the nature of the bound alkali cation (21). Two sets of our experiments, however, are difficult to reconcile with such a single antiporter: the data showing that K$^+$ and Rb$^+$ are partial inhibitors of Na$^+$/H$^+$ exchange and Na$^+$/Na$^+$ exchange (Figs. 8 and 11, and "Results"), and the results indicating that Na$^+$ causes complete rather than partial inhibition of Rb$^+$/H$^+$ exchange (Fig. 8). These findings can be explained if we assume the existence of two distinct antiporters in the sarcolemmal vesicles: the nonselective antiporter that transports Na$^+$, K$^+$, and Rb$^+$ and on which the alkali cations compete; and a selective antiporter that carries Na$^+$ but not K$^+$ or Rb$^+$. These antiporters can also account (a) for the higher maximal velocity of Na$^+$/H$^+$ exchange than that of Rb$^+$/H$^+$ exchange (Fig. 3); and (b) for the observation that in alkali cation/alkali cation exchange experiments (Table I and Fig. 9), the largest trans stimulation is that of Na$^+$ flux by Na$^+$. We should also note that our limited data on Li$^+$ (Figs. 2 and 8) suggest that it is a substrate for both antiporters.

**Relation to Other Alkali Cation/H$^+$ Antiporters**—The plasma membrane Na$^+$/H$^+$ antiporter that has been studied so extensively (3-5, 21) seems to have great selectivity for Na$^+$ versus K$^+$ in most tissues. K$^+$/H$^+$ exchangers, however, have been identified in the brush-border membrane of the ileum (12) and suggested in red cell and bacterial plasma membrane (22, 23). In mitochondria, in which the necessity of K$^+$/H$^+$ exchange was recognized long ago, a nonselective alkali cation/H$^+$ antiporter that accepts either Na$^+$ or K$^+$ but functions as a K$^+$/H$^+$ exchanger physiologically has been identified as an 82,000-kDa protein and reconstituted (24, 25). Interestingly, the mitochondria also contain a second selective Na$^+$/H$^+$ antiporter that does not carry K$^+$ (24, 25). Garlid (26) has discussed the possibility that these antiporters may be members of the same family.

**Physiological Implications**—In the literature dealing with the function of Na$^+$/H$^+$ exchange in cardiac myocytes, it is often stated that K$^+$ and Rb$^+$ can not substitute for Na$^+$ (e.g., 27-29). A closer examination shows that in some cases (28, 29) the stated selectivity of the cardiac antiporter for Na$^+$ versus K$^+$ is based on cited references on the selectivity of the antiporter in other tissues. In experiments in which the direct test of the specificity has been attempted in heart cells (Fig. 8 of Ref. 30), the data show the operation of a selective Na$^+$/H$^+$ exchange activity but do not rule out the existence of an additional nonselective exchange mechanism that can catalyze K$^+$/H$^+$ and K$^+$/K$^+$ exchanges. We consider it prudent to assume the existence of both antiporters in the reexamination of previous work and in the design of new experiments on intact cardiac myocytes.

Because the sealed sarcolemmal vesicles used here are about 90% right side out (see "Results"), the apparently equal K$^+$ and Na$^+$ values of Na$^+$, K$^+$, and Rb$^+$ (Figs. 3 and 7) must refer to cation affinities at the extracellular side of the membrane. Considering this and the high ratio of extracellular Na$^+$ to K$^+$, it is likely that under physiological conditions, the non-specific antiporter will also be carrying extracellular Na$^+$ in preference to K$^+$. The situation at the intracellular side will depend on the relative affinities of Na$^+$, K$^+$, and H$^+$ for the inward facing conformation of the nonselective antiporter. Although these affinities remain to be determined, our present experiments on alkali cation/alkali cation exchange (Table I and Fig. 9) clearly show that the antiporter is not oo asymmetrical that it would exclude K$^+$ at the intracellular side.

There is also the possibility that the antiporter properties in the purified sarcolemmal vesicles may be different from those in the intact cell. That the selective Na$^+$/H$^+$ antiporter of the plasma membrane is regulated in a variety of tissues other than the heart is known (4, 5), and the physiological regulation of the nonselective mitochondrial antiporter is well established (26). In intact cardiac myocytes, there is evidence to suggest that the pH, dependence of the Na$^+$/H$^+$ exchange can be regulated by protein kinase C (31). In this regard, we should note that our experiments on the dependence of Na$^+$/H$^+$ exchange and Rb$^+$/H$^+$ exchange on pH (Fig. 6) do not reveal an intracellular H$^+$ modifier site that has been suggested by experiments on some intact myocyte preparations (31-33) and in other tissues (5, 34). It remains to be seen if this reflects the absence or the regulated alteration of the H$^+$ modifier site in the nonselective antiporter that is predominant in our sarcolemmal preparation.

The above uncertainties notwithstanding, we believe it is reasonable to consider the possibility that without the imposition of an intracellular acid load, the nonselective antiporter may tend to catalyze Na$^+/K^+$ exchange and the coupled dissipation of the gradient that is maintained by (Na$^+$ + K$^+$)-ATPase. This may seem wasteful at first sight. Regulation of such a futile cycle, however, is a prevalent mechanism for the efficient control of many biological systems. It is appropriate to recall the long standing evidence for the coordinated control of the "pump/leak" system in the maintenance of cell volume (35).

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