SODIUM AND POTASSIUM BINDING BY RAT LIVER CELL MICROSOMES*, ‡

By HISASHI SANUI AND NELLO PACE

(From the Department of Physiology, University of California, Berkeley)

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ABSTRACT

The effects of ion concentration, pH, and presence of competing ions on the sodium and potassium binding properties of rat liver cell microsomes were studied. Typical adsorption isotherms were obtained in the concentration dependence studies, with saturation being reached when 1.2 to 1.4 m.eq. cations were retained per gm. of microsome Kjeldahl nitrogen. The retention was shown to be due to a binding to specific sites rather than to a trapping of the cations. The binding showed a sharp pH dependence in the range 6.0 to 7.5. The presence of one cation depressed the binding of the other, indicating that Na^+ and K^+ as well as H^+ ions compete for the same sites. Potassium was bound slightly more strongly than sodium, while hydrogen was bound about 10^6 times more strongly than either. Calculations show that the binding follows the simple mass law.

Similarities between adsorption by microsomes and adsorption by synthetic cation exchange resins are discussed and compared to some of the characteristics of electrolyte behavior in living systems. A possible ion exchange elution, active cation transport mechanism is suggested, involving the preferential elution of Na^+ out of the cell by H^+ ions produced by metabolism.

INTRODUCTION

In the study of electrolyte transport by living cells the existence of cellular binding agents, either as carrier substances responsible for the transport of specific ions across cellular membranes or as fixed sites responsible for binding specific ions within cells, has long been postulated. Of the ions present in living material sodium and potassium are of particular interest because these ions show sharp differences in distribution despite the fact that they are very similar in their chemical behavior. The nature of the combination of these alkali metal cations with intracellular constituents of living matter is therefore of particular importance in the study of cation transport by living cells.

* A preliminary report of this investigation was presented at the meeting of the Federation of American Societies for Experimental Biology in April, 1957 (27).

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Various investigators have studied the binding of sodium and potassium ions by proteins (4, 18), lipides (8), nucleic acids (29), other organic acids (14), cell membranes of intact plant cells (26) and, more recently, by cell fractions such as nuclei (13) and mitochondria (9). However, a constituent with the necessary chemical and physical characteristics to fill the role of the cellular binding agent has yet to be demonstrated satisfactorily in biological material.

Because the cell membrane is probably intimately involved in cellular ion transport and the microsomal fraction is believed to consist mainly of fragments of cellular membrane systems, possibly including the cell membrane (24), the sodium and potassium binding properties of the microsomal material as isolated from tissue homogenates by the method of differential centrifugation deserve attention.

Evidence that the alkali and alkaline earth metal cations may be bound in some manner by the microsomal fraction as well as by other cell fractions has been presented by Griswold and Pace (12) and by Berger (1), among others. Griswold and Pace determined the distribution of sodium, potassium, calcium, and magnesium in the various fractions of rat liver cell homogenates and found that two additional washings of each fraction had only a minor effect upon the concentrations of any of the measured constituents. They concluded that these ions must be present in the fractions in some non-diffusible form.

In addition to the apparent adsorption of these cations, recent studies have shown that the microsomal fraction also possesses other striking adsorptive properties (20, 23). It therefore appeared of importance to investigate further the sodium and potassium binding properties of this fraction.

Methods

Preparation of Microsomes.—Male rats of the Long-Evans strain weighing between 300 and 450 gm. were fasted for about 15 hours and anesthetized with sodium pentobarbital. The abdominal and thoracic cavities were opened and the livers perfused *in situ* with 500 ml. of cold (0–5°C.) 0.25 M sucrose solution. The perfusion was done in retrograde fashion through the thoracic portion of the inferior vena cava, as described by Griswold and Pace (12). The infrahepatic vena cava was clamped off and the portal vein severed to permit a free flow of perfusion fluid through the liver. After perfusion the liver was snipped out and poorly perfused portions as well as hilar tissue were trimmed off. The remainder was blotted on filter paper, weighed, and pressed through a stainless steel screen. The resulting mash was weighed and the volume measured. The mashes from two rat livers were combined and suspended in 0.25 M sucrose solution before homogenization, using 35 strokes in 8 minutes in a water cooled Potter-Elvehjem type homogenizer. The homogenate was made up to 100 ml. volume with cold 0.25 M sucrose solution.

The microsomes were isolated by a modification of the method described by Schneider (28), with the entire procedure carried out in a cold room (0–5°C.) or in a refrigerated centrifuge except as otherwise indicated. All centrifugations were carried

out in a refrigerated Spinco model E ultracentrifuge, using lusteroid tubes of about 11 ml. capacity and the Spinco A-31 preparative rotor precooled to 0–5°C.

The whole homogenate (*H*) was centrifuged for 10 minutes at an average force of 9,214 × *g* (12,590 R.P.M.) to sediment the nuclear and mitochondrial fractions together. The pellets were washed once by resuspension in 0.25 M sucrose solution and centrifuged again for 10 minutes at 9,214 × *g*. The supernatants and wash fluids were combined and centrifuged for 2 hours at 132,050 × *g* (47,660 R.P.M.) to sediment the microsomal fractions as used in the present studies. The higher speed and longer centrifugation were used in order to include most of the macromolecular ribonucleoprotein in the microsome fraction. The pellets were washed once by resuspension in 0.25 M sucrose solution and recentrifuged at 132,050 × *g* for 2 hours. The final pellets were again resuspended in 0.25 M sucrose solution and the suspensions from the different tubes were combined and made to a total volume of 100 ml. to give the microsomal preparation (*Ms*) to be used in the cation binding studies.

Sodium and Potassium Saturation Studies.—Ten ml. of the microsome suspension (*Ms*) were pipetted into each of nine clean lusteroid tubes, capped, and centrifuged for 2 hours at 132,050 × *g*. The supernatants were decanted and the aliquot microsome pellets were resuspended in 10 ml. of the appropriate buffered medium containing sodium or potassium as a mixture of the chloride and phosphate at concentrations ranging from 10 to 400 m.eq./liter and at a pH of 6.8. The tubes were capped and left on a shaker in the cold room (0–5°C.) and shaken gently for 12 hours to insure complete equilibration. After equilibration the suspensions were centrifuged for 2 hours at 132,050 × *g* and the pellets were washed twice by resuspension in 0.25 M sucrose solution and centrifugation for 2 hours at 132,050 × *g* each time. The final pellet volumes were estimated by carefully comparing the pellets with measured volumes of water in tubes identical to those containing the pellets. The packed microsomes were resuspended in 0.25 M sucrose and made to 10 ml. volume to give the microsome fractions designated Ms_1 through Ms_9.

In a separate experiment microsome suspensions were equilibrated for different lengths of time before centrifugation and washing in order to determine the rate of uptake of sodium and potassium. It was found that uptake was about 95 per cent complete within 2 hours and essentially complete in about 12 hours. Pellets were also washed several times in order to study the effect of repeated washings on their cation content. Results showed that after the second wash the cation content remained nearly constant, with only a small decrease after each successive wash.

Effect of pH on Sodium and Potassium Binding.—The same procedure used in the saturation experiments was followed in the pH studies except that the microsomes were equilibrated in 10 ml. of media containing a constant concentration (about 40 to 50 m.eq./liter) of either sodium or potassium at various pH's ranging from 3.0 to 10.1.

Sodium and Potassium Competition Studies.—Aliquot microsome pellets were resuspended in 10 ml. of media containing 40 to 50 m.eq./liter each of both sodium and potassium and with the pH ranging from 3.0 to 10.1. The samples were centrifuged and washed in the same manner as in the preceding studies.

Aliquots of the fractions in the various studies were taken for cation and nitrogen determinations. Equilibrium sodium and potassium concentrations and equilibrium

pH values were determined in all the studies, as were the sodium and potassium concentrations in the last wash fluids.

Nitrogen.—Nitrogen was determined in the various fractions by the Kjeldahl method, with steam distillation of the ammonia into a boric acid receiver carried out in a one piece Kirk type micro Kjeldahl distilling apparatus. Titration with standard 0.01 N HCl was carried to a methyl red color matching end point (22).

Ashing of Tissue Samples.—Aliquots of the microsome fractions were wet ashed in 30 ml. Vycor Kjeldahl flasks, first with concentrated nitric acid (sp. gr. 1.42) and then with concentrated perchloric acid (72 per cent). The samples were digested with nitric acid by slowly raising the oven temperature to 100°C. and drying until a pale brown or yellow residue remained. Perchloric acid was then added to the cooled samples and the temperature was gradually raised to 150°C., or slightly higher if necessary, until a white residue remained. Reagent blanks were also wet ashed in the same manner by using the appropriate volume of 0.25 M sucrose solution. In order to determine the small amounts of sodium found in most of the samples it was necessary to use vycor glassware because Pyrex vessels gave relatively high and variable blanks for sodium.

Sodium and Potassium.—The white residues from the wet ashing were dissolved in 1 ml. of 1 N HCl. Five ml. of a lithium nitrate solution containing 0.1 per cent lithium were then added as an internal standard for flame photometry. To minimize contamination, especially with sodium, the samples were made to 50 ml. volume in the vycor ashing vessels without transferring and were measured subsequently on a Perkin-Elmer model 52-C flame photometer. The duplicate samples were each read twice, a close standard being determined before and after each reading. The zero was checked after each set of sample and standard readings was completed. The wet ashed blanks generally contained 0.1 to 0.2 μ.eq. Na^+ per sample, with duplicate blanks in each experiment usually agreeing to within ± 0.01 μ.eq. per sample. The microsome samples contained from 0.2 to 10.0 μ.eq. Na^+ or K^+ per sample, with most of the samples containing between 2.0 and 5.0 μ.eq. Na^+ or K^+ per sample.

RESULTS

Sodium and Potassium Saturation Studies.—Figs. 1 and 2 summarize the data for the saturation studies. Typical adsorption isotherms were obtained for both sodium and potassium binding, with saturation apparently being nearly reached when the bathing medium ion concentration was about 150 to 200 m.eq./liter. The curves for sodium and potassium coincided fairly closely and appeared to level off at the same concentration of bound ions. In Fig. 1 the sodium and potassium ions bound to the microsomes are expressed in terms of microsome pellet volume. Near saturation about 16 to 18 m.eq. ions were bound per liter of microsome pellet volume. Also included in Fig. 1 are curves representing the sodium and potassium ion concentrations in the last wash fluids. It may be seen that the saturation level of bound sodium or potassium represents a concentration 25 to 35 times higher than that of the ions in the last wash fluid.

Fig. 2 shows the concentration dependence data for the binding of sodium and potassium ions by microsomes, expressed as milliequivalents of ions bound per gram of microsome Kjeldahl nitrogen. At saturation about 1.2 to 1.4 m.eq. sodium or potassium were bound per gram of microsome nitrogen. (This

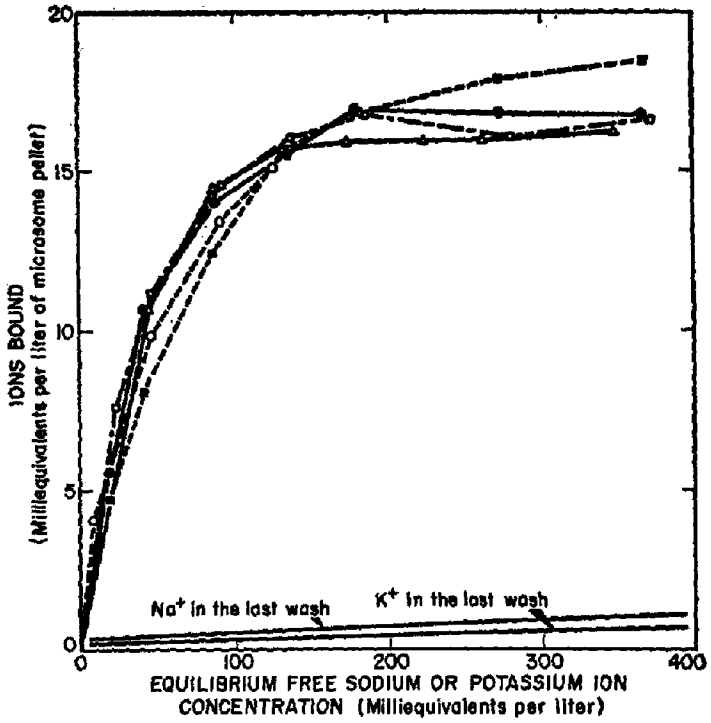

FIG. 1. Concentration dependence of sodium and potassium binding by rat liver cell microsomes, expressed in terms of microsome pellet volume. The microsomes were equilibrated for 12 hours at 0–5°C. in media containing a particular concentration of sodium or potassium ions, then were washed twice with 0.25 M sucrose solution before analysis. The open points are for sodium and the solid points are for potassium.

amounts to approximately 7.6 m.eq./kg. liver mash wet weight or 18 m.eq./kg. microsome wet weight.) This apparent maximum binding is much higher than the amount of sodium or potassium actually bound by microsomes as originally isolated from whole rat liver homogenate. On the average, the original isolated microsomes contained about 0.2 m.eq. Na^+/gm. N and 0.3 m.eq. K^+/gm. N.

pH Dependence of Sodium and Potassium Binding.—Fig. 3 summarizes the results of the pH dependence experiments. The sodium or potassium retained by the microsomal material was found to be close to zero at the lower pH values

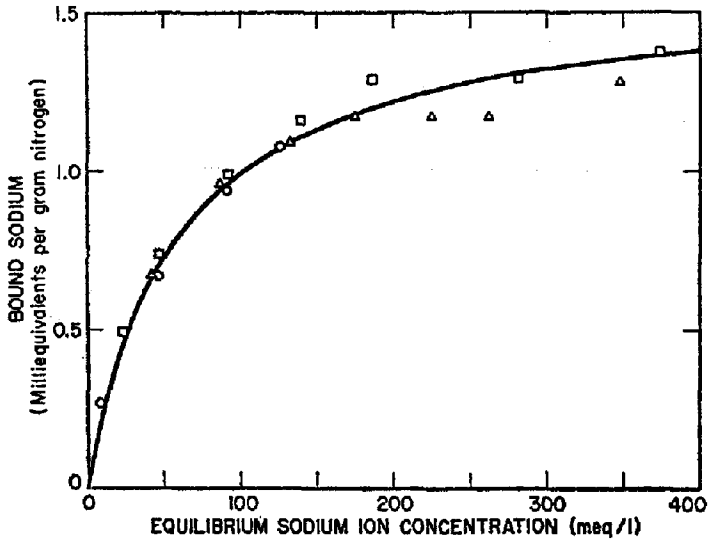

FIG. 2 *a*

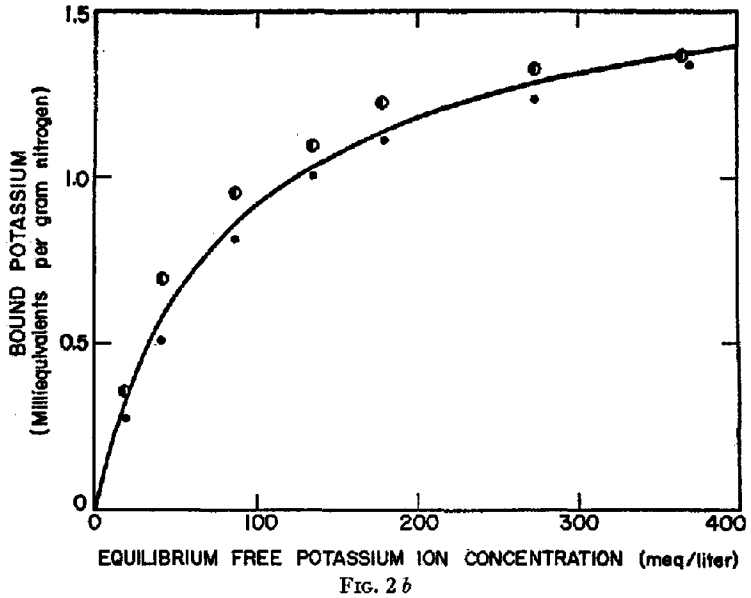

FIG. 2 *b*

FIG. 2. Concentration dependence of sodium binding (Fig. 2 *a*) and potassium binding (Fig. 2 *b*) by rat liver microsomes at 0–5°C. and a bathing medium pH of 6.8. The bound ions are expressed in terms of microsome Kjeldahl nitrogen. The points are from replicate saturation experiments. The solid curves were calculated from mass law Equation 4 of the text, using values of constants obtained from the experimental data.

and rose sharply in the pH range 5 to 7, reaching a maximum binding of about 1.2 m.eq./gm. nitrogen at pH values above 7. The curves for sodium and potassium were found nearly to coincide with calculations showing that sodium was displaced by hydrogen ions somewhat more readily than was potassium.

Sodium and Potassium Competition Studies.—Because sodium and potassium appeared to be bound to the same extent by the microsomes it was of interest

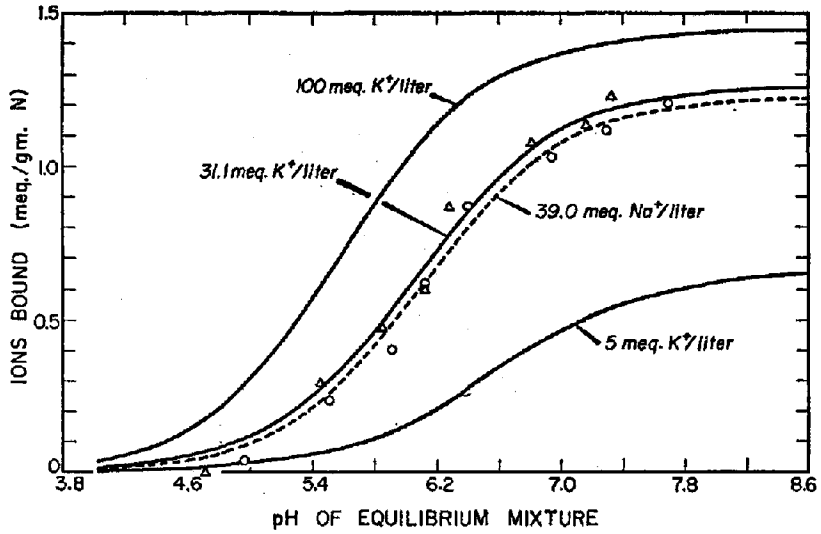

FIG. 3. The pH dependence of sodium (circles) and potassium (triangles) binding by rat liver microsomes at 0–5°C. Sodium binding was studied in media at various pH values, all containing 39.0 m.eq. Na^+/liter. Potassium binding was studied in media containing 31.1 m.eq. K^+/liter. The smooth curves for these two concentrations were calculated from mass law Equation 6 of the text, using values of constants obtained from the experimental data. Included also are the smooth curves calculated for expected binding in media containing 5 and 100 m.eq. K^+/liter to indicate the effect of cation concentration on the pH dependence curve for one ion species.

to determine whether or not these cations competed for the same binding sites. To investigate the effect of the presence of one ion on the binding of the other, aliquot microsome pellets were resuspended in media containing 48.6 m.eq. Na^+/liter and 40.0 m.eq. K^+/liter with the pH ranging from 3.0 to 10.1. The microsome suspensions were equilibrated for 12 hours in the cold room (0–5°C.) to insure complete equilibration. The equilibrium mixtures were then centrifuged for 2 hours at 132,050 × *g* and the pellets were washed twice by resuspension in 0.25 M sucrose solution and recentrifuged each time. Sodium and potassium were determined on duplicate aliquots of the wet ashed samples.

Fig. 4 summarizes the results obtained when microsomes were equilibrated

in solutions containing both sodium and potassium. It may be seen that the individual amount of sodium and potassium bound by the microsomes in the competition studies was considerably less than that bound when sodium or

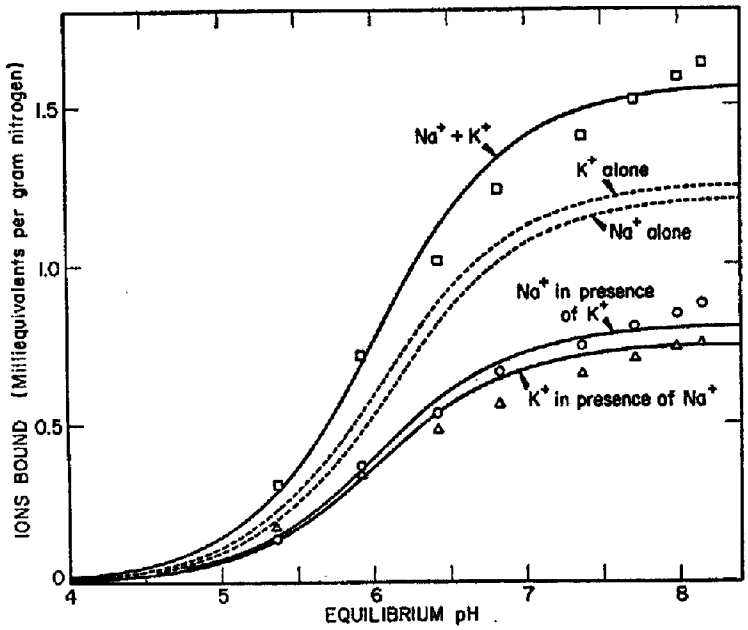

FIG. 4. Competition between sodium and potassium ions for microsome binding sites at 0–5°C. The microsomes were equilibrated for 12 hours in media at different pH values, all containing 48.6 m.eq. Na^+/liter and 40.0 m.eq. K^+/liter. After two washes with 0.25 M sucrose the microsome pellets were analyzed for bound Na^+ (circles), bound K^+ (triangles), and the sum of the two (squares). The solid curves were fitted using mass law Equations 8 and 10 of the text. When corrected for concentration difference the competition data reveal a preference for potassium over sodium binding. The dotted curves were calculated using Equation 6 for sodium alone at 48.6 m.eq./liter and for potassium alone at 40.0 m.eq./liter. It may be noted from Fig. 2 that saturation of available binding sites is not complete in this cation concentration range.

potassium alone was present in the suspending medium at approximately the same single ion concentration as for the competition studies. The two ion species apparently compete against one another for the same microsome binding sites. However, the sum of the bound ions was somewhat greater for the competition studies than for the single ions bound in the presence of only a single ion species in the bathing medium. This was due to the difference in total cation concentration, as may be seen in Fig. 3.

In addition to the sodium and potassium binding studies, preliminary acid-base titrations of the microsomes were carried out in order to learn something about the acid- and base-binding capacities of the microsomal material. Isolated microsomes were titrated with approximately 1 N HCl and 1 N NaOH. Results show that at saturation 5.5 to 7.5 m.eq. H^+/gm. microsome nitrogen were bound and that there may be more than one species of microsome binding

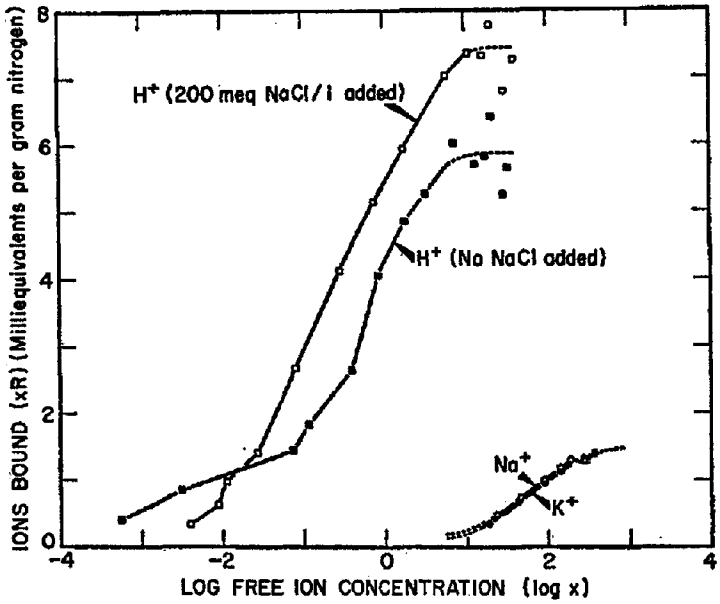

FIG. 5. Semilogarithmic plot of the concentration dependence of sodium, potassium, and hydrogen ion binding by rat liver microsomes. The points for sodium and potassium binding are from the concentration dependence experiments and those for hydrogen binding are from the acid titration experiments. Note that at higher hydrogen ion concentrations sodium seems to enhance the binding of hydrogen ions, but at lower concentrations sodium ions depress the binding of hydrogen ions and apparently compete with them for binding sites.

sites for H^+ ions, so that the maximum binding capacity for H^+ is much greater than for Na^+ or K^+. A semilogarithmic plot of the cation binding curves (Fig. 5) shows evidence of the existence of sites binding H^+ which may correspond to those which bind Na^+ and K^+ ions. It will be noted in the H^+ binding curves with and without added NaCl that at higher H^+ ion concentrations the microsomes appear to bind more H^+ ions in the presence of added Na^+ than in its absence, suggesting that Na^+ improves H^+ binding, rather than implying a competition. However, at the lower H^+ concentrations where the binding is presumably by sites which also bind Na^+ and K^+, it is seen that Na^+ actually

depresses H^+ binding, suggesting in this case a competition between Na^+ and H^+ for the same sites. Although many problems are encountered in titration studies of this kind, so that calculations of bound H^+ are only approximate and involve considerations which need not be covered here, the data in Fig. 5 do show the great difference in the strength with which the microsomes bind H^+ as compared to Na^+ or K^+ at the same concentration.

Base titration of the microsomes indicates that they possess an anion as well as a cation binding capacity. However, much work is necessary to characterize more completely the microsome acid- and base-binding properties.

DISCUSSION

The results of the sodium and potassium binding experiments demonstrate that significant amounts of these cations are retained by the microsomal material from rat liver homogenates after two washes with cold 0.25 M solution. Inasmuch as it has been postulated that the microsomes may represent vesicular fragments (24), the retained sodium and potassium could presumably represent either *bound* or *trapped* cations. The present study shows that the retention is typical of adsorption rather than of bathing medium entrapment.

The concentration dependence data in Fig. 1 demonstrate that ion retention by the microsomes, expressed in terms of pellet volume, exhibits a saturation type relationship which is characteristic of adsorption to specific sites, and not the straight line relationship which would be expected if the retained ions represented material trapped in tiny "osmometers."

In another series of experiments the uptake of the sodium or potassium retained after two washes was found to be rapid, being approximately 95 per cent complete within 2 hours, whereas the slow component of the washing data (Fig. 6) showed that in the second and succeeding washes the microsome cations were lost very slowly, indicating that they must be bound in some manner and not merely trapped in membranous sacs highly permeable to sodium and potassium.

In addition, preliminary studies show that microsomal material which has been solubilized with alkali and reprecipitated with dilute acid still retains its cation binding properties. The reprecipitated material, which can no longer be in the form of tiny "osmometers," has a maximum binding capacity for sodium which is very close to that found for freshly prepared, unsolubilized microsomes. Thus there appears to be no doubt that the ions retained in the present studies are in fact *bound* and not merely trapped. The term "bound" is used here to mean that the ions involved are attracted by the microsomal material strongly enough that they will migrate against a concentration gradient along with particles which are sedimented through an aqueous medium.

The close correspondence of the binding curves for sodium and potassium separately and the results of the mixed ion and pH dependence experiments indicate strongly that these cations as well as hydrogen ions compete for the

same sites. The manner in which the three cations apparently compete suggests that the binding involved is similar in some respects to that found in synthetic cation exchange resins (2).

The occurrence and importance of ion exchange binding of sodium and potassium as well as other cations by biological materials have been suggested

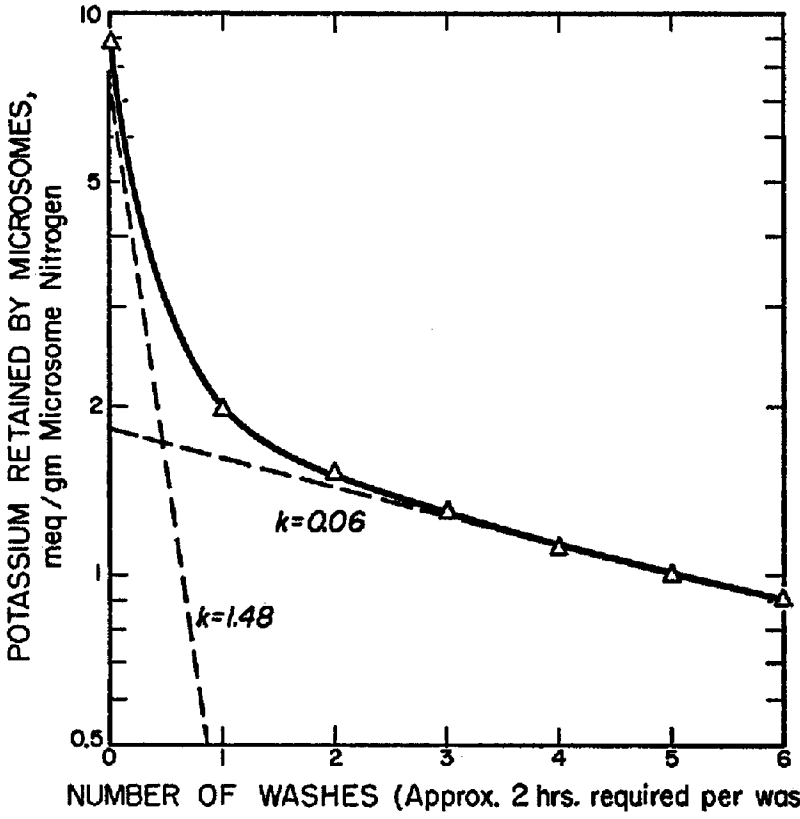

FIG. 6. Semilogarithmic plot of data showing the effect of repeated washings on the potassium retained by rat liver microsomes.

or indicated by many investigators (10, 11, 21), and for plant cells are well attested in numerous investigations on many tissues (7). To date, however, ion exchange binding of sodium and potassium in animal tissues has not been studied extensively. Among the more recent studies are those on myosin A and B by Lewis and Saroff (18).[1]

[1] The book Ion Exchangers in Organic and Biochemistry, edited by Calmon and Kressman (3), contains several very interesting and illuminating chapters concerning the apparently widespread existence and importance of ion exchange materials in living systems.

If, as it appears, an ion exchange type system is involved in the binding of cations by the microsomal material, the equilibrium between sodium, potassium, or hydrogen ions and the binding sites may be formulated either on the basis of an adsorption mechanism or according to the law of mass action (16). These relationships have been shown to be formally equivalent (2).

If it is assumed that the cation binding is analogous to an ordinary metathetic reaction and that at equilibrium the mass law applies to the heterogeneous system: microsomes + aqueous solution, then the equilibrium between the various cations and the binding sites may be represented as:

$$NaR \rightleftharpoons Na^+ + R^- \quad (1\,a)$$

$$KR \rightleftharpoons K^+ + R^- \quad (1\,b)$$

$$HR \rightleftharpoons H^+ + R^- \quad (1\,c)$$

The apparent maximum binding capacity for sodium and potassium may be represented as:

$$R_t^- = HR + KR + NaR + R^- \quad (2)$$

The general expression for the dissociation constants for the equilibria represented by Equations 1 *a*, 1 *b*, and 1 *c* is:

$$k_D = \frac{f_m(M^+)f_r(R^-)}{f_{mr}(MR)} \quad (3)$$

in which (M^+) represents the free Na^+, K^+, or H^+ ion concentration in the equilibrium mixture; (R^-) represents the concentration of the free microsome binding sites; (MR) represents the concentration of the cations bound by the microsomes; k_D is the intrinsic dissociation constant; and f_m, f_r, and f_{mr} are the appropriate activity coefficients. The value of f_m can be estimated from the ionic strength of the mixture, but the activity coefficients f_r and f_{mr} for the solid phases are not known. If it can be assumed that the ratio of the activity coefficients f_r/f_{mr} is constant (26), the following expression for the amount of bound cation (MR) can be derived from Equation 3:

$$(MR) = \frac{f_m(M^+)(R_t^-)}{a + f_m(M^+)} \quad (4)$$

in which a is the apparent dissociation constant. It is assumed that each microsome binding site behaves independently of all other sites; *i.e.*, a bound ion exerts no electrostatic influence on succeeding bindings.

By rearrangement, Equation 4 can be put in a form similar to one form of Langmuir's adsorption isotherm:

$$\frac{1}{(MR)} = \frac{a}{f_m(M^+)(R_t^-)} + \frac{1}{(R_t^-)} \quad (5)$$

Equation 5 is of a form similar to that used by Klotz, Walker, and Pivan (17)

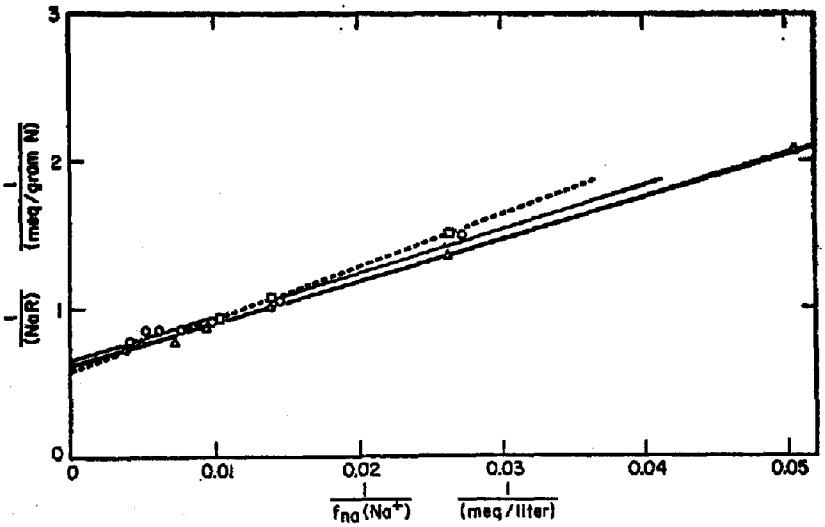

FIG. 7 *a*

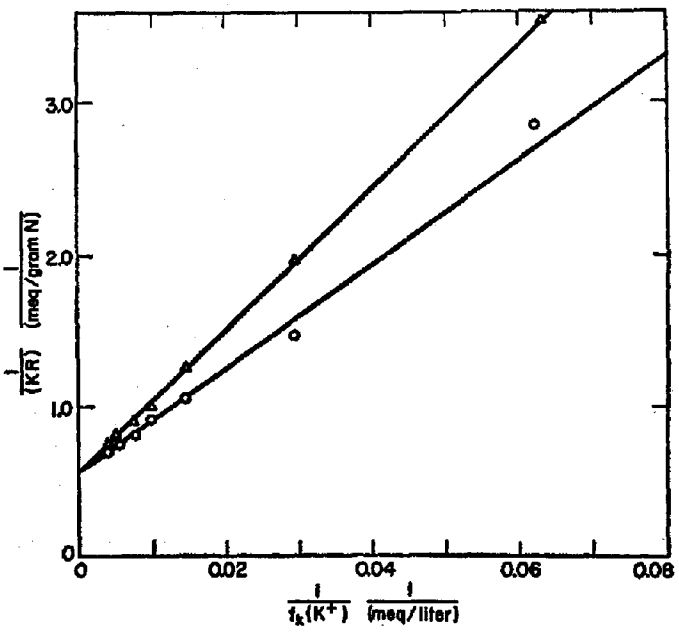

FIG. 7 *b*

FIG. 7. Mass law plots for the concentration dependence of sodium binding (Fig. 7 *a*) and potassium binding (Fig. 7 *b*) by rat liver microsomes. Experimental data are plotted in the form of mass law Equation 5 of the text.

in their study of the binding of ions by proteins and, when applicable, is useful in determining the maximum binding capacity and the dissociation constant.

If the concentration dependence data shown in Fig. 2 for sodium and potassium binding by the microsomes are plotted according to Equation 5 it is found that straight lines can be fitted to the points (Fig. 7), indicating that the mass law is applicable to the system even though the activity coefficients for the solid phases are not known, and that apparently only one species of microsome binding sites is involved. The slope of the line is equal to $a/(R_t^-)$ and the y-intercept is equal to $1/(R_t^-)$, so that a and (R_t^-) can be obtained. The average values obtained for these parameters for sodium and potassium are summarized in Table I.

Variations in slope (and therefore in a) occurred, which were due largely to the slight differences in pH of the equilibrium mixture from one experiment to the next. Therefore, an accurate estimate of the relative strength of sodium and potassium binding could not be made from these data. In general, however, it could be concluded that there was no great difference between sodium and potassium in their strength of binding by the microsomes.

By using Equation 4 and the experimentally determined values of a and (R_t^-), calculated concentration dependence curves could be constructed. In Fig. 2 the solid curves were calculated from Equation 4, and the points were those obtained experimentally. The calculated curves are seen to represent a satisfactory fit to the data.

Assuming direct competition between sodium or potassium ions and hydrogen ions for the same microsome binding sites, the following expression relating the sodium or potassium binding to the hydrogen ion concentration of the equilibrium mixture can be derived from Equation 1 *c* together with 1 *a* or 1 *b*:

$$(AR) = \frac{f_a(A^+)(R_t^-)}{b^A + f_a(A^+) + (b^A/b^B)f_h(H^+)} \tag{6}$$

in which (A^+) represents the equilibrium free Na^+ or K^+ ion concentration; (AR) represents the concentration of Na^+ or K^+ bound by the microsomes; f_h and f_a are the appropriate activity coefficients; and b^A and b^B are the respective apparent dissociation constants under the conditions of the pH dependence experiments.

By rearrangement, Equation 6 can be put in the form of an equation for a straight line:

$$\frac{f_a(A^+)/f_h(H^+)}{(AR)} = \frac{1}{(R_t^-)}\left[1 + \frac{b^A}{f_a(A^+)}\right]\frac{f_a(A^+)}{f_h(H^+)} + \frac{b^A}{b^B(R_t^-)} \tag{7}$$

Fig. 8 shows the data from the sodium and potassium pH dependence studies plotted according to Equation 7. It is seen that a straight line can be readily fitted to the experimental points in each case, suggesting again that

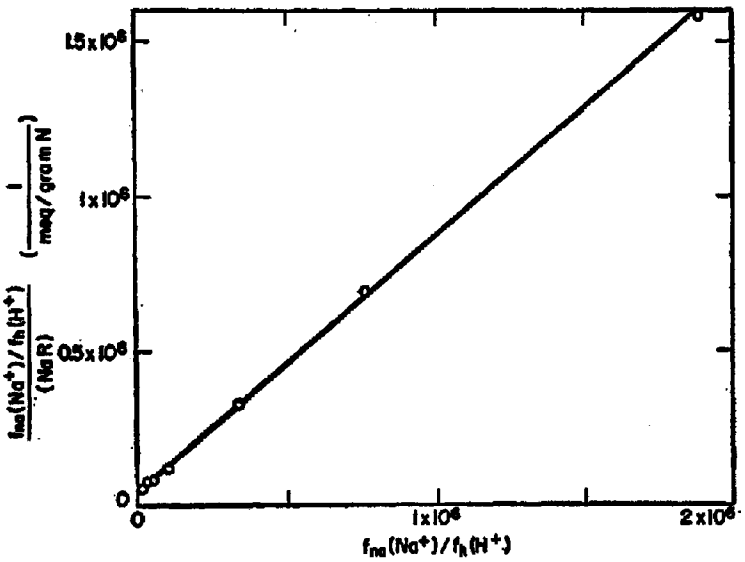

FIG. 8 *a*

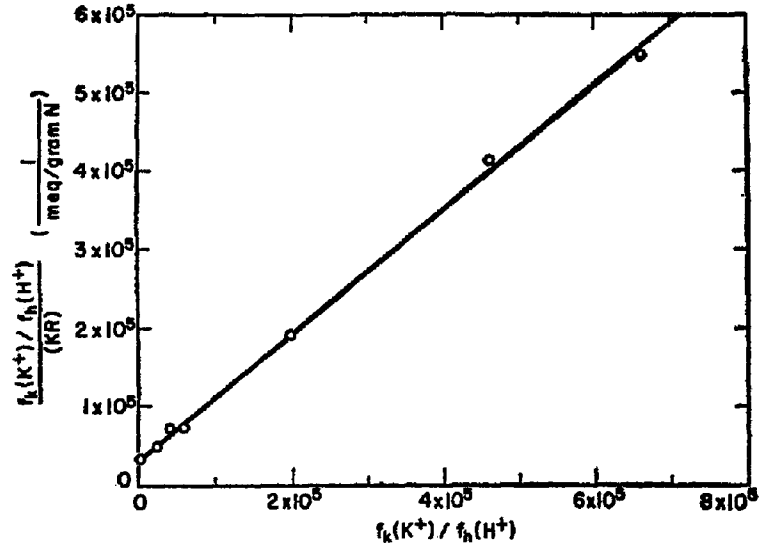

FIG. 8 *b*

FIG. 8. Mass law plots for the pH dependence of sodium binding (Fig. 8 *a*) and potassium binding (Fig. 8 *b*) by rat liver microsomes. Experimental data are plotted in the form of mass law Equation 7 of the text.

only one species of binding sites is involved in the competition with hydrogen ions. The slopes and intercepts yield values for b^A, b^B, and (R_t^-) for the systems involved. The values obtained are summarized in Table I.

By substituting values determined from the pH dependence experiments for

the constants in Equation 6, curves showing the hydrogen ion dependence of the binding of sodium and potassium by the microsomes can be calculated from mass law relationships (Fig. 3). Again the calculated curves are found to represent a satisfactory fit to the data. The curves for 5 and 100 m.eq. K^+/liter were also calculated using Equation 6, and indicate the shapes of the pH dependence curves at different total cation concentrations.

In studying the competition between sodium and potassium ions for microsome binding sites several approaches exist. If the competition is studied over a wide pH range, as was done in the present case, the following treatment would appear to be suitable. In general, the results can be represented by solving simultaneously the dissociation equations for sodium, potassium, and hydrogen ion binding. By making the same assumptions as in the preceding studies and using Equation 2, an expression for the amount of bound sodium can be derived:

$$(\mathrm{Na}R) = \frac{c^{\mathrm{H}}c^{\mathrm{K}}f_{na}(\mathrm{Na}^+)(R_t^-)}{c^{\mathrm{H}}c^{\mathrm{Na}}f_h(\mathrm{K}^+) + c^{\mathrm{H}}c^{\mathrm{K}}f_{na}(\mathrm{Na}^+) + c^{\mathrm{K}}c^{\mathrm{Na}}f_h(\mathrm{H}^+) + c^{\mathrm{H}}c^{\mathrm{K}}c^{\mathrm{Na}}} \tag{8}$$

This expression can be put in the form of an equation for a straight line:

$$\frac{1/f_h(\mathrm{H}^+)}{(\mathrm{Na}R)} = \frac{c^{\mathrm{Na}}f_h(\mathrm{K}^+) + c^{\mathrm{K}}f_{na}(\mathrm{Na}^+) + c^{\mathrm{K}}c^{\mathrm{Na}}}{c^{\mathrm{K}}f_{na}(\mathrm{Na}^+)(R_t^-)f_h(\mathrm{H}^+)} + \frac{c^{\mathrm{Na}}}{c^{\mathrm{H}}f_{na}(\mathrm{Na}^+)(R_t^-)} \tag{9}$$

Going through the same steps for potassium yields:

$$(\mathrm{K}R) = \frac{c^{\mathrm{H}}c^{\mathrm{Na}}f_h(\mathrm{K}^+)(R_t^-)}{c^{\mathrm{H}}c^{\mathrm{K}}f_{na}(\mathrm{Na}^+) + c^{\mathrm{H}}c^{\mathrm{Na}}f_h(\mathrm{K}^+) + c^{\mathrm{K}}c^{\mathrm{Na}}f_h(\mathrm{H}^+) + c^{\mathrm{H}}c^{\mathrm{K}}c^{\mathrm{Na}}} \tag{10}$$

Rearranging:

$$\frac{1/f_h(\mathrm{H}^+)}{(\mathrm{K}R)} = \frac{c^{\mathrm{K}}f_{na}(\mathrm{Na}^+) + c^{\mathrm{Na}}f_h(\mathrm{K}^+) + c^{\mathrm{K}}c^{\mathrm{Na}}}{c^{\mathrm{Na}}f_h(\mathrm{K}^+)(R_t^-)f_h(\mathrm{H}^+)} + \frac{c^{\mathrm{K}}}{c^{\mathrm{H}}f_h(\mathrm{K}^+)(R_t^-)} \tag{11}$$

in which c^{K}, c^{Na}, and c^{H} are the respective apparent dissociation constants for the conditions of the competition studies.

Under the conditions of the present competition experiments, plots of the data according to Equations 9 and 11 should yield straight lines if only one species of sites is involved. Such plots (Fig. 9) show that straight lines can be fitted to the experimental points. From the slopes and y-intercepts, values for the constants were determined and are summarized in Table I.

The constants obtained in the pH dependence and competition studies as well as the calculated selectivity coefficients (3) for the various samples over the pH range studied indicate that under the same conditions of pH, temperature, and ionic strength potassium ions are bound slightly more strongly than sodium ions. Hydrogen ions appear to be bound much more strongly than either alkali metal cation.

Substitution of the constants derived from the mixed ion experiment into Equations 8 and 10 yields the solid curves in Fig. 4. Although the fit to the

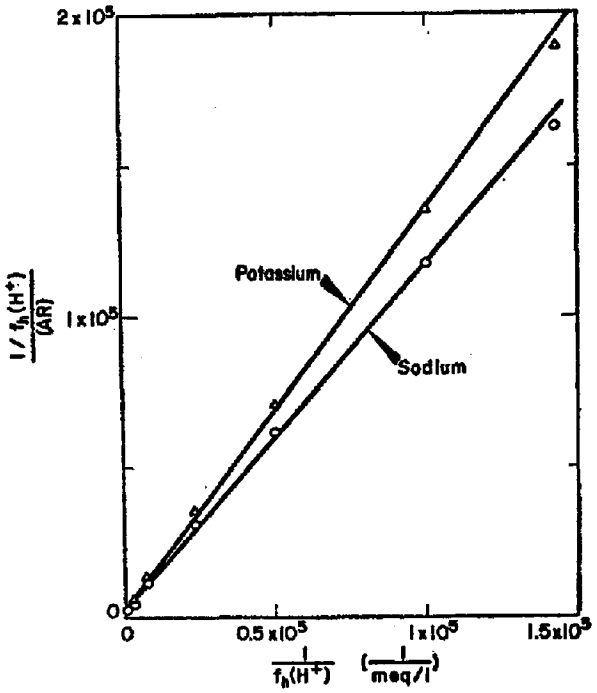

FIG. 9. Mass law plots for the competition between sodium and potassium for rat liver microsome binding sites. Experimental data are plotted in the form of mass law Equations 9 and 11 of the text.

TABLE I

Type of experiment	Apparent maximum binding capacity (R_1^1)	Apparent dissociation constants	
		Symbol	Values obtained
	m.eq./gm. N		*eq./liter*
Saturation	1.63	a^{Na}	50.3×10^{-3}
	1.75	a^{K}	71.3×10^{-3}
pH dependence	1.53	b^{Na}	10.2×10^{-3}
		b^{K}	6.9×10^{-3}
		b^{H}	1.6×10^{-7}
Competition	1.61	c^{Na}	3.1×10^{-3}
		c^{K}	2.7×10^{-3}
		c^{H}	0.4×10^{-7}

experimental points is not as good as in the other cases presented, it appears to be reasonably close, considering the conditions of the experiments and the number of assumptions underlying the calculations.

The apparent dissociation constants obtained in the various experiments are valid only for the particular conditions employed, and are related to the intrinsic constants by a number of factors. The conditions influencing the apparent constants have not been fully determined, but important factors must include pH, number and kind of competing ions present, and total ion concentration. The maximum binding capacities for the different studies were assumed to be the same and appeared to be approximately equal under the conditions employed.

Altogether, results from the present experiments indicate that the binding of sodium, potassium, and hydrogen ions by the microsomes generally resembles ion exchange type binding and follows the simple mass law. It is recognized that the analogy between microsomes and synthetic ion exchange resins may not be a complete one, each system possessing some characteristics differing from those of the other. It is believed, however, that differences which may exist are for the most part in the physical characteristics of the ion exchange particles and the effects of different conditions on the gross structure of the particles, rather than in the actual nature of the bonding involved.

One noteworthy difference between adsorption by the microsomes and by strong acid exchange resins is found in the binding of hydrogen ions. In strong acid cation exchange resins such as amberlite IR-1 sodium and potassium ions are bound slightly more strongly than hydrogen, whereas in the microsome system hydrogen ions are bound much more strongly than sodium or potassium, presumably owing to the presence of structurally bound weak acid groups (2). Some of the reports on ion binding by biological materials (*e.g.*, references 18, 26) appear to lend support to such an explanation. In the microsome system it would be expected that weak acid groups such as carboxyl or phosphoryl must be responsible for the adsorptive properties.

What role the ion exchange binding of sodium and potassium by the microsomal material may play in cellular ion transport is not yet clear. However, there are some striking similarities between the ion binding properties of the microsomes on the one hand and the observed ion adsorption properties of living material on the other. Some important similarities are as follows:—(*a*) The microsomal material is saturable; *i.e.*, there is a specific number of sites which can bind sodium or potassium ions. Likewise, the cellular ion transport mechanism appears to be saturable (6).

(*b*) Sodium, potassium, and hydrogen ions compete for microsome binding sites. In living cells sodium and potassium appear to be exchangeable under certain circumstances, and ions close to sodium and potassium in the lyotropic series for adsorption affinities can substitute for them in the cellular ion transport mechanism in a manner similar to the exchange found in synthetic cation exchange systems (*e.g.*, reference 25).

(*c*) Cation binding by the microsome system exhibits a very great hydrogen ion dependence. Numerous investigations on the effect of pH on cation distri-

bution in living systems give evidence of the great dependence of the cellular ion transport mechanism on hydrogen ion concentration (*e.g.*, reference 30). Although the manner in which hydrogen ions act is not always clear, it is evident nonetheless that this cation is vitally involved in some way with the mechanism of cation transport in living cells.

The foregoing discussion shows that there is good evidence of the presence in the cellular ion transport mechanism of a system exhibiting ion exchange properties. The findings reported here establish the existence of cellular membrane material capable of binding sodium and potassium ions in such a manner, the binding being highly dependent upon hydrogen ion concentration in the physiological pH range.

A clue to the possible role of this ion-binding system may be found in studies with synthetic resins. Many investigators (*e.g.*, reference 15) have demonstrated conclusively that sodium, potassium, and other alkali and alkaline earth metal cations can be separated by ion exchange chromatography, using dilute HCl as the eluting agent. Generally they have found that sodium is eluted before potassium and can be separated almost completely from it. Thus there is suggested a possible cellular active cation transport mechanism, based on the presence of ion exchange material near the cell boundary and involving the preferential elution of sodium ions out of the cell by hydrogen ions produced by cell metabolism. Such a system would be affected by many factors, including the presence of complexing agents such as citrate and oxalate, membrane "pore" size, rate of hydrogen ion production (and therefore rate of metabolic activity), presence of organic and inorganic competing ions, extracellular medium composition, and cellular water production.

Whether a mechanism of this nature could be operative in living cells is not yet known. On the other hand, it is known that thus far no cellular material has been shown to possess the great selectivity between sodium and potassium required by the cellular binding agents postulated in most of the cation transport mechanisms proposed by other investigators (*e.g.*, references 5, 19, 30). The results of the present experiments as well as of preliminary erythrocyte ghost cation-binding studies present strong evidence against the existence of a cellular membrane material which can bind large amounts of either sodium or potassium with a high degree of selectivity. In view of this fact, the elution mechanism suggested here, based on the ion exchange properties of the microsomal material, would appear to offer a possibly more satisfactory explanation of the means by which the cell is able to differentiate between these two chemically similar cations. Experimental work is in progress to attempt to test the feasibility of such a process.

REFERENCES

1. Berger, M., Studies on the distribution of potassium in the rat liver cell and the mechanism of potassium accumulation, *Biochem. et Biophysica Acta*, 1957, **23**, 504.

2. Boyd, G. E., Schubert, J., and Adamson, A. W., The exchange adsorption of ions from aqueous solutions by organic zeolites. I. Ion-exchange equilibria, *J. Am. Chem. Soc.*, 1947, **69**, 2818.
3. Calmon, C., and Kressman, T. R. E., editors, Ion Exchangers in Organic and Biochemistry, New York, Interscience Publishers, Inc., 1957.
4. Carr, C. W., Studies on the binding of small ions in protein solutions with the use of membrane electrodes. VI. The binding of sodium and potassium ions in solutions of various proteins, *Arch. Biochem. and Biophysics*, 1956, **62**, 476.
5. Conway, E. J., Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle, *Physiol. Rev.*, 1957, **37**, 84.
6. Epstein, E., Ion transport in plants; absorption by roots and passage through roots, *Plant Physiol.*, 1956, suppl. 31, vii.
7. Epstein, E., Mineral nutrition of plants; mechanisms of uptake and transport, *Ann. Rev. Plant Physiol.*, 1956, **7**, 1.
8. Folch, J., Biochemical problems related to psychiatry, *in* Psychiatric Research, (J. F. Enders, A. B. Hastings, J. H. Means, and S. B. Wolbach, editors), Cambridge, Harvard University Press, 1947, 17.
9. Gamble, J. L., Potassium binding and oxidative phosphorylation in mitochondria and mitochondrial fragments, *J. Biol. Chem.*, 1957, **228**, 955.
10. Graf, E., Base exchange in casein, *Kolloid-Beihefte*, 1937, **46**, 229.
11. Greene, C. H., and Power, M. H., The distribution of electrolytes between serum and *in vivo* dialysates, *J. Biol. Chem.*, 1931, **91**, 183.
12. Griswold, R. L., and Pace, N., The intracellular distribution of metal ions in rat liver, *Exp. Cell Research*, 1956, **11**, 362.
13. Itoh, S., and Schwartz, I. L., Sodium and potassium distribution in isolated thymus nuclei, *Am. J. Physiol.*, 1957, **188**, 490.
14. Jardetzky, O., and Wertz, J. E., The complexing of sodium ion with some common metabolites, *Arch. Biochem. and Biophysics*, 1956, **65**, 569.
15. Kayas, G., Séparation complète des métaux alcalins par échange ionique, *J. chim. phys.*, 1950, **47**, 408.
16. Klotz, I. M., The application of the law of mass action to binding by proteins. Interactions with calcium, *Arch. Biochem.*, 1946, **9**, 109.
17. Klotz, I. M., Walker, F. M., and Pivan, R. B., The binding of organic ions by proteins, *J. Am. Chem. Soc.*, 1946, **68**, 1481.
18. Lewis, M. S., and Saroff, H. A., The binding of ions to the muscle proteins. Measurements on the binding of potassium and sodium ions to myosin *A*, myosin *B* and actin, *J. Am. Chem. Soc.*, 1957, **79**, 2112.
19. Ling, G., Muscle electrolytes, *Am. J. Physic. Med.*, 1955, **34**, 89.
20. Lynn, W., Brown, R., and Mullins, J., Catalytic and adsorptive properties of testicular microsomes, *J. Biol. Chem.*, 1958, **232**, 995.
21. Mazia, D., The binding of Ca, Sr and Ba by *Elodea* protoplasm, *J. Cell. and Comp. Physiol.*, 1938, **11**, 193.
22. Meeker, E. W., and Wagner, E. C., Titration of ammonia in the presence of boric acid, *Ind. and Eng. Chem., Anal. Edition*, 1933, **5**, 396.
23. Paigen, K., Hemoglobin as the red pigment of microsomes, *Biochim et Biophysica Acta*, 1956, **19**, 297.

24. Palade, G. E., and Siekevitz, P., Liver microsomes. An integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.
25. Relman, A. S., Lambie, A. T., Burrows, B. A., and Roy, A. M., Cation accumulation by muscle tissue: The displacement of potassium by rubidium and cesium in the living animal, *J. Clin. Inv.*, 1957, **36**, 1249.
26. Rothstein, A., and Hayes, A. D., The relationship of the cell surface to metabolism. XIII. The cation binding properties of the yeast cell surface, *Arch. Biochem. and Biophysics*, 1956, **63**, 87.
27. Sanui, H., and Pace, N., Sodium and potassium binding by the microsome fraction from rat liver cell homogenates, *Fed. Proc.*, 1957, **16**, 111.
28. Schneider, W. C., Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions, *J. Biol. Chem.*, 1948, **176**, 259.
29. Shack, J., Jenkins, R. J., and Thompsett, J. M., The binding of sodium chloride and calf thymus desoxypentose nucleic acid, *J. Biol. Chem.*, 1952, **198**, 85.
30. Ussing, H. H., General principles and theories of membrane transport, *in* Metabolic Aspects of Transport Across Cell Membranes, (Q. R. Murphy, editor), Madison, University of Wisconsin Press, 1957, 39.