BBA 73009

Water, Na^+, and K^+ in rat-kidney microsomes

PALADE AND SIEKEVITZ[1] have observed that the size of rat-liver microsomes prepared for electron microscopy varies inversely with the concentration of sucrose added to the fixative. This "osmometer-like" behavior suggests that microsomal vesicles are surrounded by intact semipermeable membranes. In agreement with this interpretation TEDESCHI, JAMES AND ANTHONY[2] obtained photometric evidence that liver microsomes swell and shrink in response to changes in the osmolarity (mannitol and raffinose concentration) of the suspending medium. It is difficult to reconcile these observations with the findings of SHARE AND HANSROTE[3] that the sucrose space of liver microsomal pellets comprises up to 90% of the total water, and that it does not increase relative to the sucrose-inaccessible space as the osmolarity of the medium rises.

The present study demonstrates that microsomes prepared from another tissue, rat kidney, do show osmotic behavior as evidenced by changes in the water content of a sucrose-inaccessible space in response to alterations in the osmolarity of the suspending fluid.

Kidneys, freshly removed from pentobarbital-anesthetized rats, were placed in 20 ml 0.88 M sucrose, minced with scissors, and homogenized for 2 min in a motor-driven glass and teflon homogenizer of the Potter–Elvehjem type. The homogenate was centrifuged for 30 min at 15 500 $\times$ g. The supernatant was decanted, diluted with an equal volume of water, and centrifuged at 69 000 $\times$ g for 30 min to bring down the heavy microsomal fraction. The microsomal pellet was drained and resuspended in an electrolyte solution (NaCl, 40 mM; KCl, 40 mM; $MgSO_4$, 5 mM; Tris (pH 7.4), 10 mM). Aliquots of this suspension were transferred to centrifuge tubes, and sucrose solutions identical in electrolyte composition to the above, containing uniformly ^{14}C-labeled sucrose, were added. Final sucrose concentrations were 200, 150, 100, 50, 25 and 1 mM.

After a 10-min equilibration period the microsomes were resedimented at 68 000 $\times$ g for 20 min. Pellets were drained, solidly frozen by plunging the tubes into a solid CO_2–propanol bath, and transferred to tared Vycore test tubes. After obtaining the wet weight, pellets were dried for 24 h at 95° and then extracted for 48 h in 0.1 M HNO_3. Na^+ and K^+ concentrations in the extracted fluid were determined by flame photometry and [^{14}C]sucrose by liquid-scintillation counting. Water content of the sucrose space is calculated from data for the density of aqueous sucrose solutions[4]. Water contained in the sucrose-inaccessible space is the total water (difference between wet and dry weights) *minus* the sucrose space water. Water and electrolyte contents are given in units of mg or μmole per mg microsomal dry weight. Dry weights are corrected for pellet sucrose content.

If microsomes are indeed surrounded by semipermeable membranes which are not penetrated by sucrose, it should be possible to demonstrate in the microsomal pellet a sucrose-inaccessible space whose water content changes inversely with the sucrose concentration of the suspending medium. Assuming (1) that microsomes are permeable to all external solutes except sucrose, (2) that the amount and osmotic coefficient of solute contained within the microsomal vesicles remains constant, and (3) that no hydrostatic pressure gradients develop, we may write

$$W_m = MS_m + W_b \quad (1)$$

where W_m is the intramicrosomal (sucrose-inaccessible) water, W_b any portion of this water which is bound or otherwise unable to participate in osmotic shifts, and S_m the impermeant intramicrosomal solute. M is the water:sucrose molar ratio of the suspending medium.

Fig. 1 shows that a significant fraction (16–27%) of the microsomal pellet water is inaccessible to sucrose. In Fig. 2 this quantity, W_m, is plotted against M for sucrose concentrations between 25 and 200 mM. In accord with Eqn. 1 there is a linear relationship between these quantities ($r = 0.977$, $P<0.01$). The intercept of the least

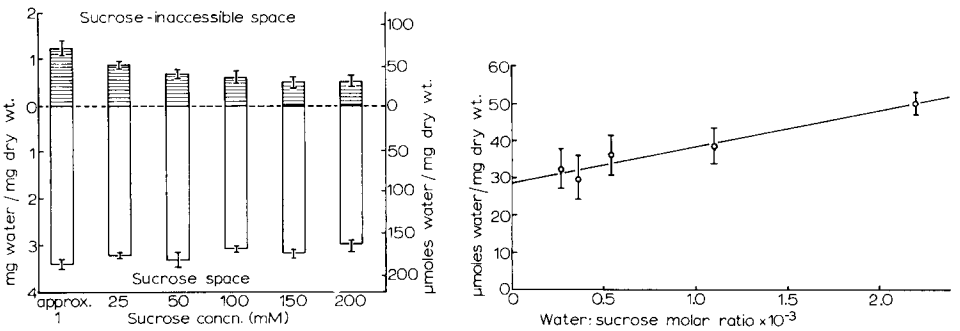

Fig. 1. Water contents of the sucrose, and sucrose-inaccessible spaces of kidney microsomal pellets sedimented from suspending media of various sucrose concentrations. The vertical lines extend ± 1 S.E. from the mean.

Fig. 2. Plot of non-sucrose space water, W_m, against the (water:sucrose) molar ratio of the suspending medium, M.

squares linear regression line shown, 28±1.4 (S.E.) μmoles (= 0.52 mg) water per mg microsomal dry weight, is W_b.

It is clear from these results that kidney microsomes do show osmotic behavior. The osmotic space is small, however, comprising only 0.3–4.2% of the total water, a finding which suggests that the microsomal pellets contained a preponderance of damaged or incomplete vesicles. A large fraction of the sucrose-inaccessible water does not participate in osmotic shifts. The amount of this "bound" water appears to be of the same order of magnitude as that in the human erythrocyte where 20% of the cell water, or about 0.37 mg water per mg dry matter, is osmotically inactive[5].

The Na^+ and K^+ contents of the microsomal pellets always exceed that calculated on the assumption that the total pellet water is available to these ions at a concentration equal to that in the suspending medium. This finding suggests that Na^+ and K^+ are either bound to the microsomal matrix or are present in the intramicrosomal water at concentrations greater than that in the external fluid. The former possibility appears to apply. In Fig. 3 microsomal Na^+ (computed as total pellet Na^+ *minus* that in the sucrose space) is plotted against the volume of microsomal (sucrose-inaccessible) water. The least squares linear regression line fitted to these points has a slope of 0.043±0.003 μmole/μl, not significantly higher than the Na^+ concentration of the outside fluid, 0.040 M. Na^+ binding is shown explicitly by the fact

that this regression line crosses the ordinate above the origin. The actual extent of binding depends upon the proportion of the microsomal water which is not accessible to Na^+. Making the assumption that this ion is totally excluded from the bound water, one obtains a mean figure for Na^+ binding of 0.058 ± 0.004 μmole/mg dry weight. Na^+ binding is overestimated in this calculation if some Na^+ does indeed penetrate the bound water, and a minimal figure is given by the y-intercept, 0.037 ± 0.002

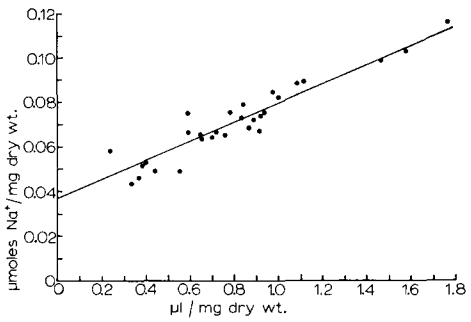

Fig. 3. Na^+ content plotted as a function of volume for the sucrose-inaccessible space.

μmole/mg. The data for K^+ are almost identical with those shown in Fig. 3. For this ion the least squares regression line has a slope of 0.042 ± 0.003 and its y-intercept is 0.039 ± 0.002.

These results are in agreement with those of SANUI AND PACE[6], whose findings of saturation in Na^+ and K^+ accumulation by liver microsomes suggested that a binding mechanism was operative. The magnitude of binding appears to be roughly comparable in both tissues; in neither is there selectivity between the two ions.

Department of Physiology,
New York Medical College,
New York, N.Y. (U.S.A.)

DAVID L. MAUDE

1 G. E. PALADE AND P. SIEKEVITZ, *J. Biophys. Biochem. Cytol.*, 2 (1956) 171.
2 H. TEDESCHI, J. M. JAMES AND W. ANTHONY, *J. Cell Biol.*, 18 (1963) 503.
3 L. SHARE AND R. W. HANSROTE, *J. Biophys. Biochem. Cytol.*, 7 (1960) 239.
4 C. D. HODGMAN, *Handbook of Chemistry and Physics*, 39th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1957, p. 1929.
5 D. SAVITZ, V. W. SIDEL AND A. K. SOLOMON, *J. Gen. Physiol.*, 48 (1964) 79.
6 H. SANUI AND N. PACE, *J. Gen. Physiol.*, 42 (1959) 1325.

Received September 12th, 1966