# Intracellular Redistribution of Sodium and Calcium During Stimulation of Sodium Transport in Epithelial Cells

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The localization and distribution of cations in the epithelial cells of tissues which actively transport sodium, such as the kidney tubular epithelium or the frog skin, is not well known. In all types of calculations involving compartmentalization of the tissue electrolytes, it has been assumed that they are evenly distributed inside the cells. However, the cell nucleus contains a substantial amount of sodium (1, 2), more than the cytoplasm as a whole, while mitochondria are known to contain little sodium, high potassium, and, in some conditions, great amounts of calcium precipitates (3). In some epithelia, sodium is apparently bound or trapped inside some cellular component (4).

The distribution of sodium in epithelial cells of frog skin and rat kidney was studied by means of the localization of the precipitates of sodium pyroantimonate in electron microscopy (5–7) during stimulation of sodium transport by vasopressin.

The changes found permit the conclusion that sodium is mobilized from the nucleus during stimulation of active transport and that there is an increase in the penetration of calcium into the mitochondria of the tissues. These findings in fixed tissues were confirmed in fresh isolated nuclei and mitochondria. The results gave an opportunity to construct a working hypothesis linking the increase in permeability produced by vasopressin to the intracellular redistribution of sodium and calcium.

## METHODS

Frog skins were mounted as a membrane in lucite chambers, and the potential difference and short circuit current were measured by the method of Ussing and Zerahn (8). One-half of the skin was treated with 0.05 U/ml of lysine-vasopressin (Sigma Chemical Co., St. Louis, Mo.), and the other half was used as control. When the effect of the hormone on the sodium transport was observed, as evidenced by the increase in the short circuit current, the treated and control skins were rapidly fixed for electron microscopy, as described below. Male white rats weighing approximately 150 g were injected with 5 U of ADH. (Pitressin from Parke, Davis & Co., Detroit, Mich.) subcutaneously. Controls were injected with saline. 1 hr after injection, the

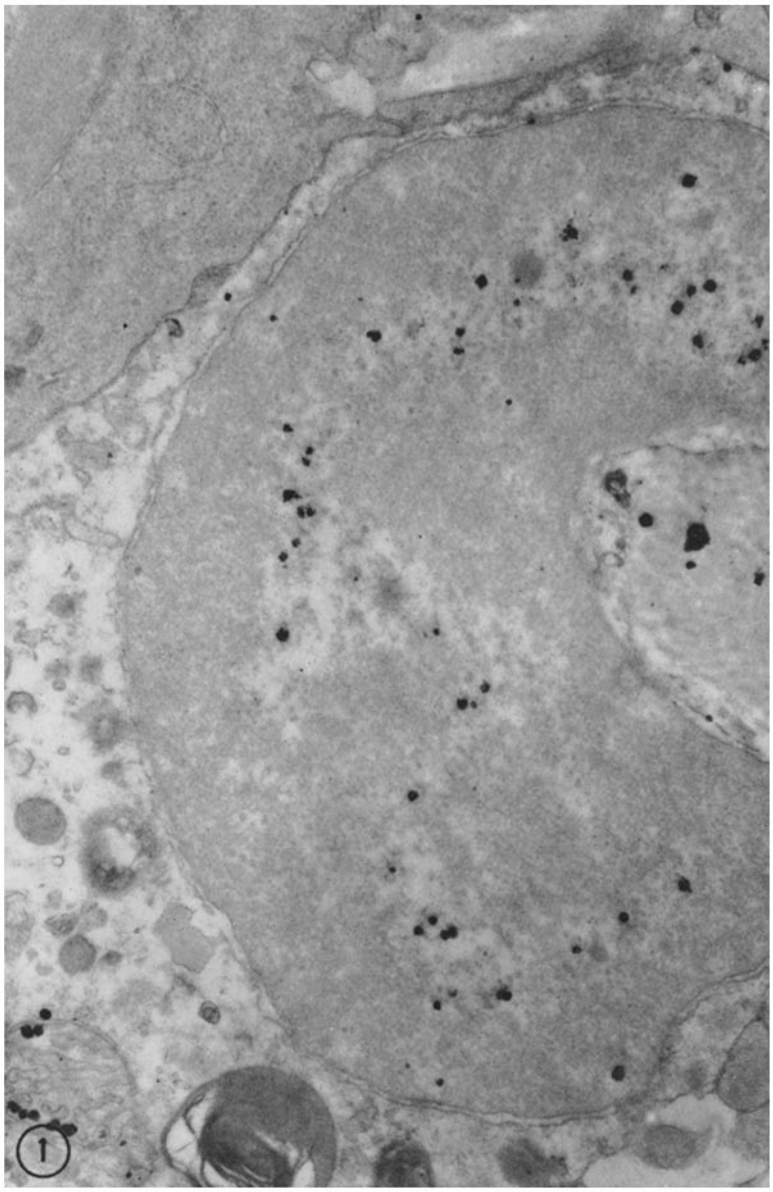

FIGURE 1. Nucleus of an epithelial cell of frog skin shows the dark dots of the sodium pyroantimonate precipitates. Intense precipitation in the nucleus of the epithelial cells was found. × 20,000.

rats were sacrificed, and the kidneys were dissected out and fixed for electron microscopy, as indicated below.

For electron microscopic examination the skins were sectioned into fine strips about 2 mm wide and 10 mm long, and slices about 2 mm thick were made from the rat kidneys. Two pro-

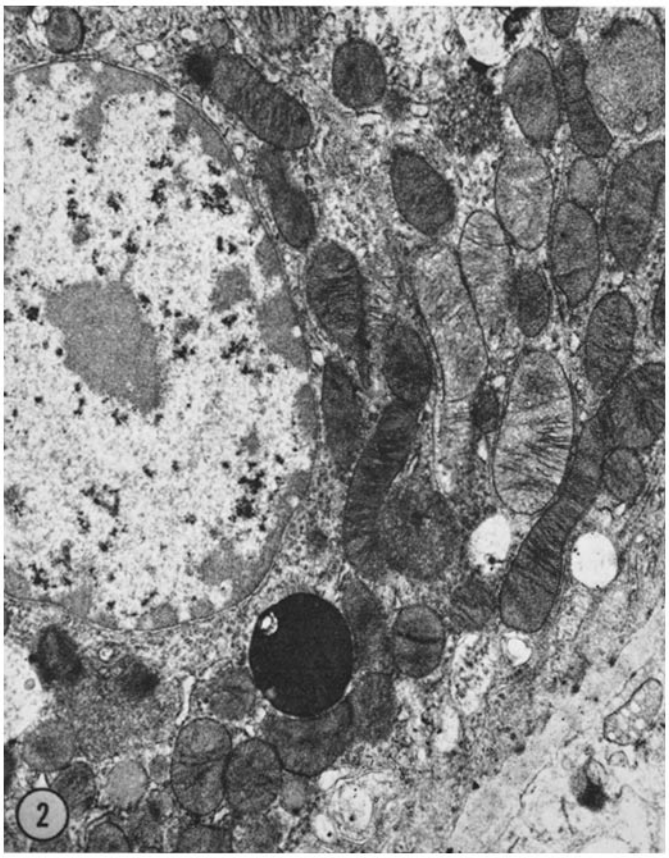


FIGURE 2. A view of an epithelial cell of the rat kidney, at the level of the proximal tubule. Observe also the dark precipitates inside the cell nucleus at left and the presence of a darkly stained microbody very close to the nuclear envelope. × 12,000.

cedures were used for the histochemical localization of sodium with the potassium pyroantimonate salt. One followed the same method described previously (7) for skeletal muscle. The other method consisted of exposing the pieces of tissue for 1 hr to a choline–Ringer solution containing no sodium and 2% potassium pyroantimonate. After this pretreatment at room temperature, the pieces were fixed in 3% glutaraldehyde, dissolved in 0.1 M potassium phosphate buffer (pH 7.4) containing no other salt, rinsed, and postfixed as described previously for skeletal muscle. In all the cases the sections were poststained with lead citrate (9).

Incubations were done with 0.2 ml of nuclei suspension (10) plus 0.1 ml of $^{22}NaCl$ with a final specific activity of 0.05 $\mu c/\mu M$ in a total volume of 0.8 ml of media. Mitochondria from

rat liver or kidney were prepared according to Dallam and Stoler (11) and treated with the same amount and activity of $^{22}NaCl$. $^{45}CaCl$ was used at a specific activity of 0.03 $\mu c/\mu M$. Lysine-vasopressin was used at a final concentration of 0.2 U/ml. Total activity in nuclear or mito-

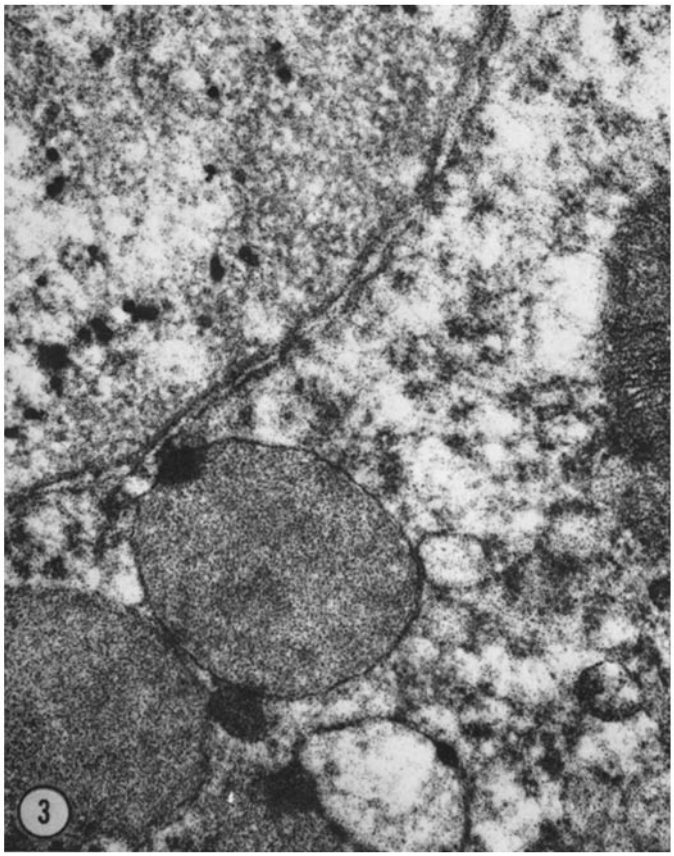


FIGURE 3. A high magnification of a rat kidney epithelial cell showing two microbodies containing fine sodium precipitates near the nuclear membranes. Fine precipitates are also seen in the cytoplasm and a coarser accumulation inside the nucleus. × 41,000.

chondrial pellets was counted in a well scintillation detector for $^{22}Na$, and a liquid scintillation counter for $^{45}Ca$. No correction was made for trapped fluid in the pellets. Identical volumes of suspension were used in controls and treated samples.

## RESULTS

*Normal Distribution of Sodium in the Epithelial Cells of Frog Skin and Renal Tubule* Sodium precipitate was always found in the nuclei of both frog skin and kidney tubular cells (Fig. 1). The accumulation of sodium in the nuclei was comparable to that found in areas of the dermis where the precipitate was associated with the col-

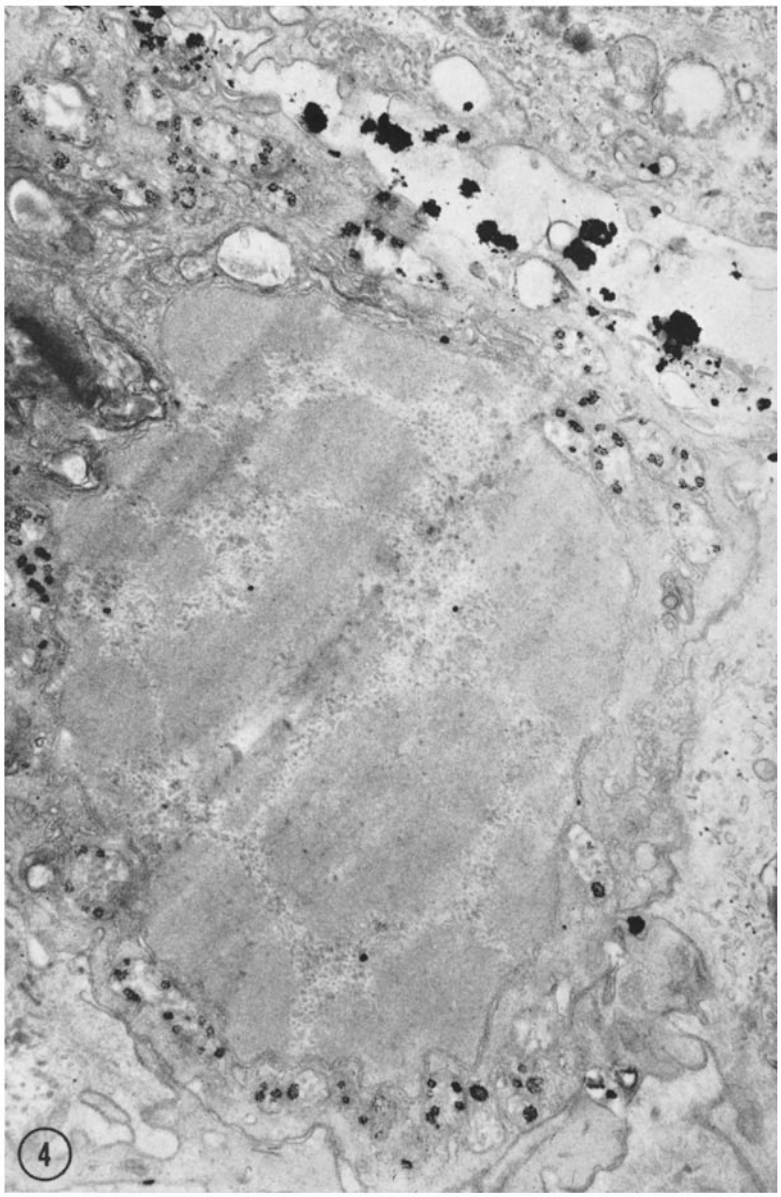


FIGURE 4. General view of an epithelial cell of frog skin after treatment with vaso pressin. Note the scarce amount of precipitates inside the nucleus, the dark precipitates in the mitochondria, and the intercellular spaces. × 10,000.

lagen fibers. Sodium precipitates were also found inside rounded bodies resembling microsomes, specifically in the vicinity of the nuclei (Fig. 2). These bodies sometimes contained great amounts of dense sodium precipitates (Fig. 3). The sacs of the endoplasmic reticulum showed sodium precipitate occasionally in a finely diffused manner. The interstitium of both tissues also contained sodium precipitates, specifically near the basement membranes and between the interdigitation at that level. Here the

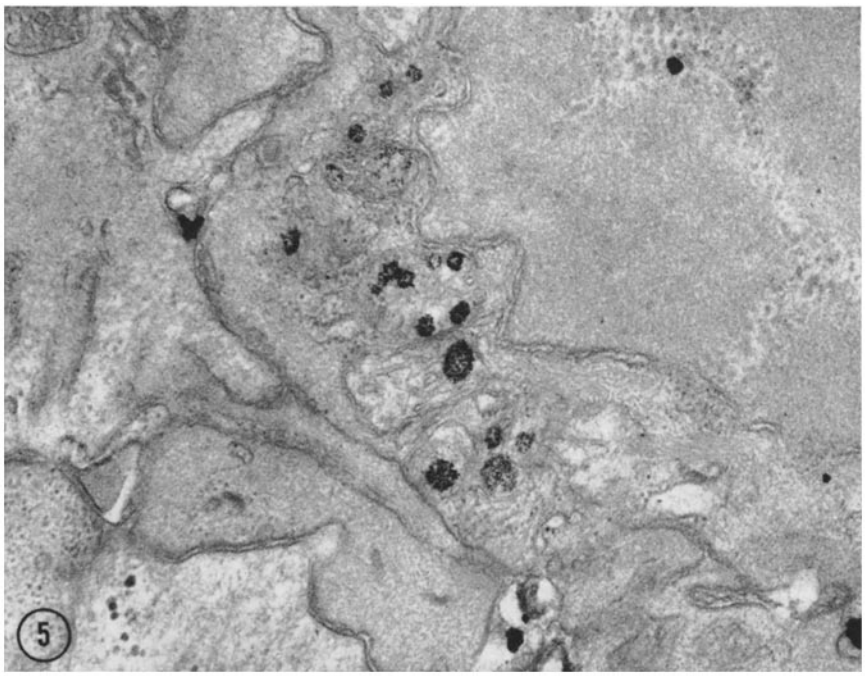


FIGURE 5. At higher magnification, the precipitates inside mitochondria after treatment with vasopressin are clearly seen in this epithelial cell from frog skin. The accumulation is identical with those produced after uptake of calcium phosphate by isolated mitochondria.

precipitates appeared as larger conglomerates than inside the cells. The normal granules of calcium precipitate were seen in the mitochondria of cells treated with pyroantimonate, which were no different in frequency and density to those found in tissues fixed without pyroantimonate.

*Action of Vasopressin* Important changes were observed during the stimulation with vasopressin. The sodium precipitate of the nuclei (Fig. 4) was less frequent and dense and the mitochondria showed increased amounts of granules (Figs. 5–7). The interstitium contained more sodium precipitates than the controls, and there was no apparent change in the frequency of microbodies containing precipitates.

From the electron microscopic observations then, it appears that the nuclear sodium

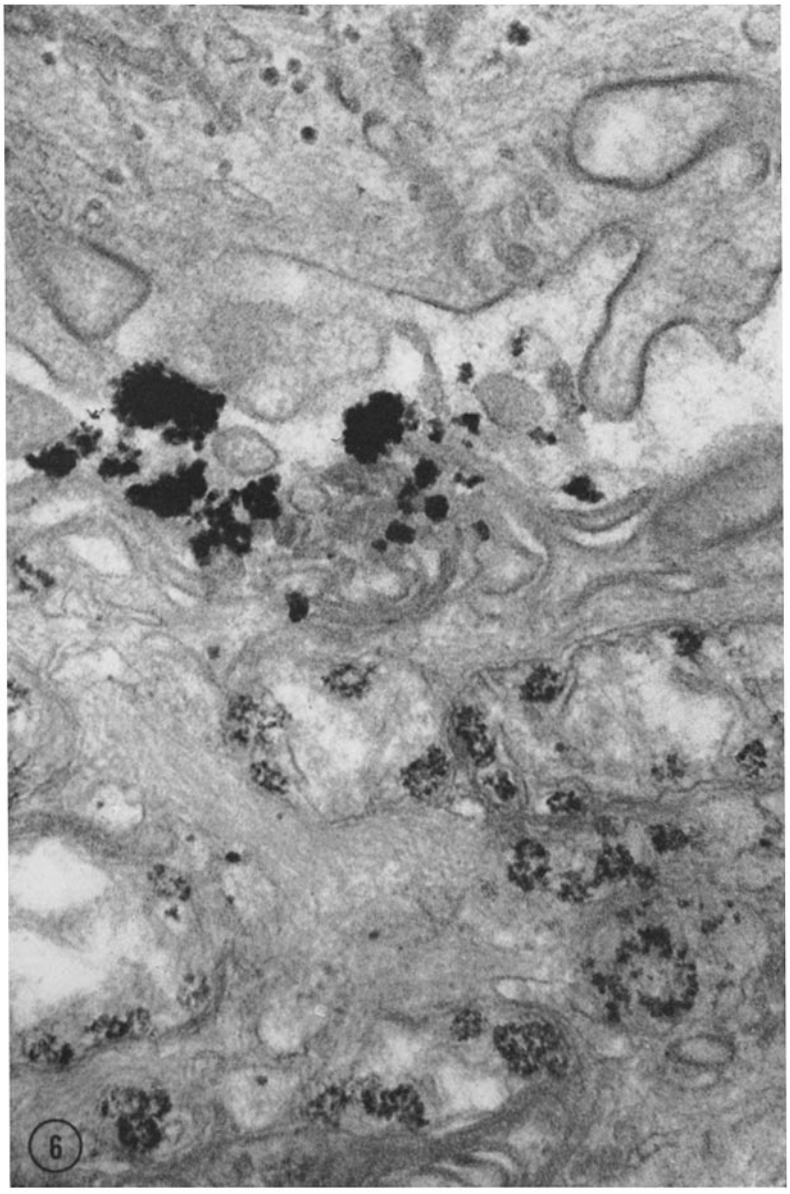


FIGURE 6. Another view of highly magnified mitochondria containing precipitates after vasopressin treatment. The intercellular spaces show the typical interdigitations of the cell close to the basement membrane and the dark precipitates of sodium pyroantimonate. × 60,000.

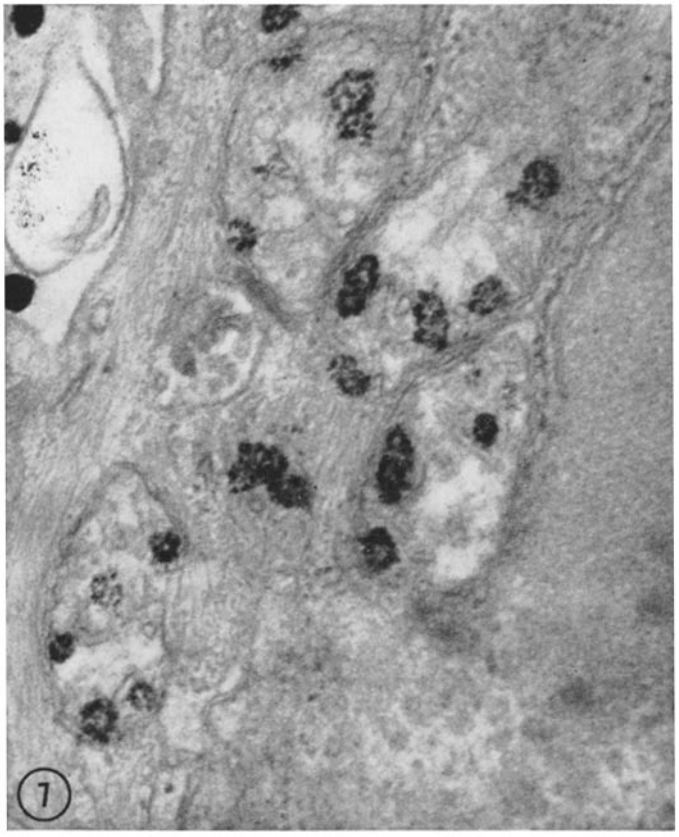


FIGURE 7. This figure shows again mitochondria of an epithelial cell of frog skin after vasopressin treatment containing the typical granules, most probably of calcium phosphates. × 62,000.

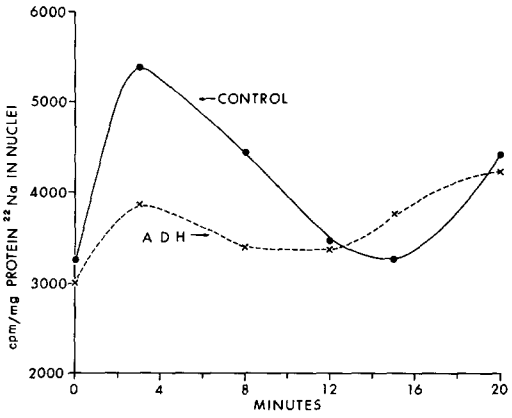


FIGURE 8. Action of vasopressin on the uptake of $^{22}$Na by isolated thymus nuclei. A rapid effect of the hormone on the sodium content of the nuclei is observed. The dots and crosses are means of four experiments.

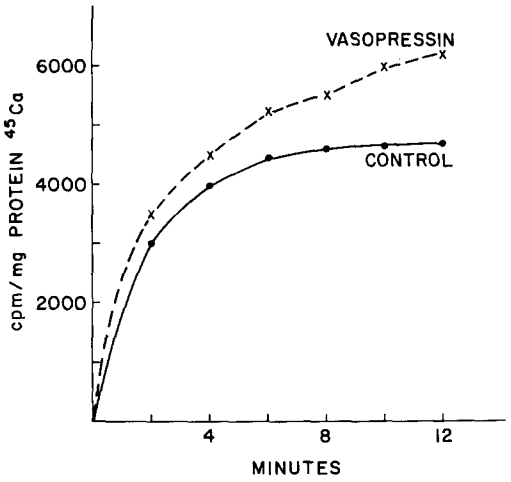


FIGURE 9. Action of vasopressin on $^{45}Ca$ accumulation by isolated kidney mitochondria. Calcium penetration into the mitochondrial pellet is stimulated by vasopressin. The points and crosses are means for four experiments.

is reduced under the effect of vasopressin and that the granules of the mitochondria increase appreciably in number and size.

*Isolated Nuclei and Mitochondria* In order to establish whether these changes would occur in isolated nuclei and mitochondria under the action of vasopressin, we performed the experiments shown in Figs. 8–11. In Fig. 8 a rapid effect of vasopressin on the sodium content of isolated nuclei can be observed. The fact that thymus nuclei were used in these experiments indicates that the effect is probably a general one, and that the nuclei of the target organs for this hormone might show even a greater effect.

Vasopressin increased the calcium uptake of isolated kidney mitochondria as shown in Fig. 9. The release of labeled calcium from previously loaded mitochondria was also increased by vasopressin as indicated in Fig. 10. With respect to sodium movements, it can be seen in Fig. 11 that there is no effect of vasopressin on $^{22}Na$ uptake by isolated kidney mitochondria.

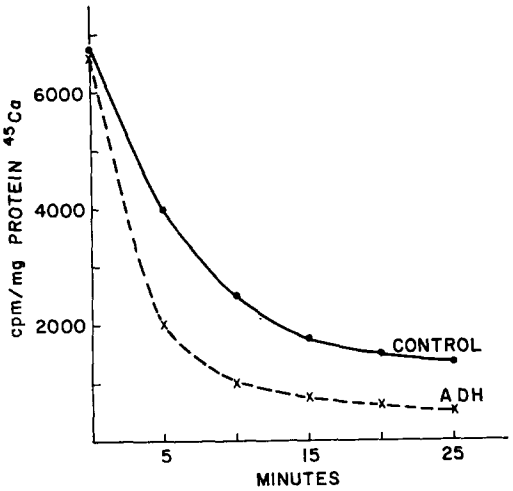


FIGURE 10. $^{45}Ca$ release from previously loaded, isolated kidney mitochondria. The addition of vasopressin to the incubation media produced a faster release of Ca in the treated than in the control mitochondria. The curves represent the mean of three experiments.

## DISCUSSION

The two salient features of both the electron microscopic observations and the experiments with isolated nuclei and mitochondria are that sodium is rapidly lost from the nucleus, probably towards the cytoplasm, and that calcium is gained by the mitochondria during stimulation of sodium transport. Accumulation of calcium in mitochondria has been shown to occur when they are suspended in media containing increasing amounts of calcium and phosphate (3). This accumulation is known to be oxygen dependent and to consist of the deposition of calcium in the form of crystals that are easily observed in electron microscopic preparations of mitochondria (3, 12). The possibility that sodium had penetrated into the mitochondria of the preparations

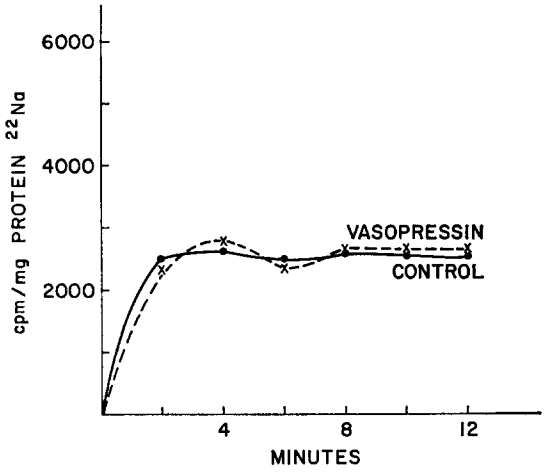


FIGURE 11. The uptake of $^{22}$Na by isolated kidney mitochondria is not affected by vasopressin.

of frog skin and kidney under vasopressin treatment seems to be ruled out by the fact that sodium is not accumulated by isolated mitochondria under the action of vasopressin. The accumulation of calcium as typical crystals, identical with the ones observed by Peachey and Leningher, is substantiated by the increased incorporation of calcium during action of vasopressin in isolated mitochondria.

These changes on sodium and calcium distribution during increased transport of sodium are probably linked. The actions of vasopressin on transporting epithelia can be reduced to (*a*) an increase in the permeability of the outside facing border of the cells with (*b*) a concomitant increase in sodium and water transport transcellularly.

It is known that calcium controls permeability of epithelial cells as well as that of excitable tissues (13, 14). Calcium apparently controls the permeability of the cell membrane and also is a constituent of the cement at the level of the junctional complexes (15).

It is probable then, that under the action of vasopressin the calcium associated with the cell membrane would be released from the membrane structure and subsequently diffuse into the cytoplasm, thereby increasing the permeability of the cell membrane to sodium. This free calcium in the cytoplasm then would be accumulated by mitochondria, which would act as reservoirs of calcium and indirectly control permeability.

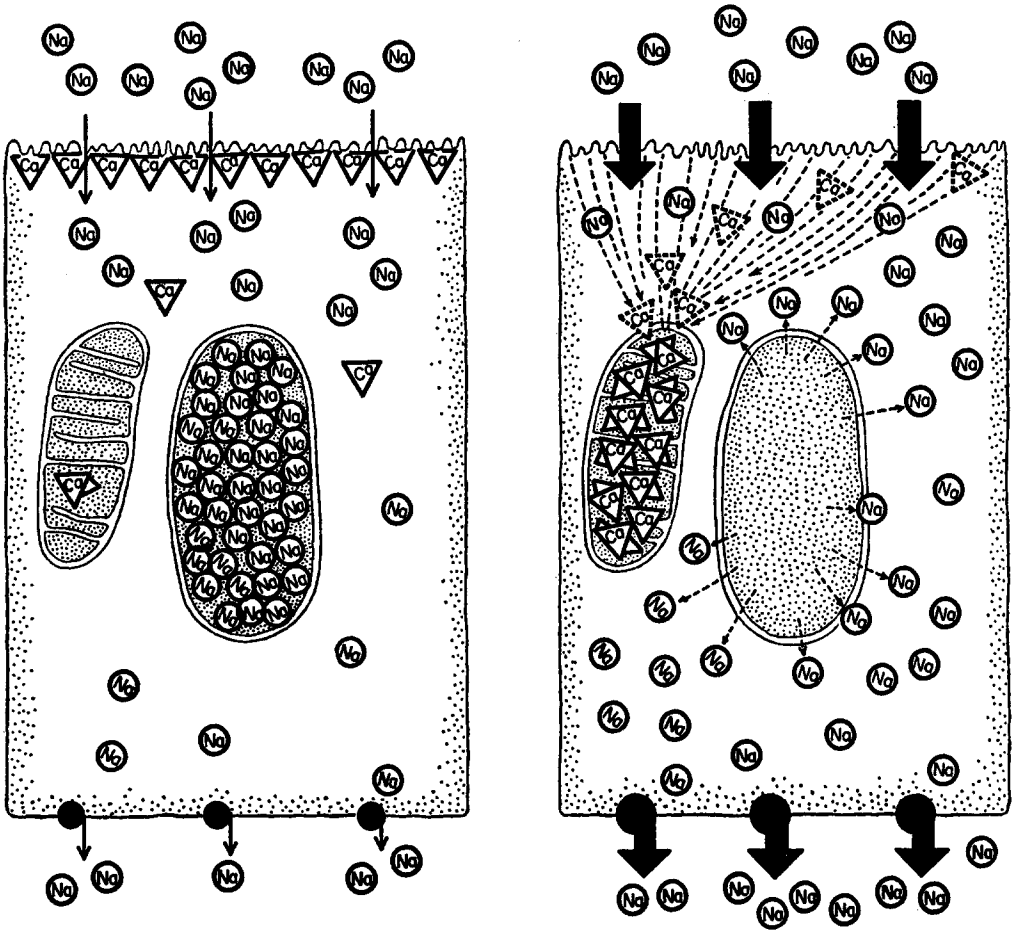

FIGURE 12. Highly simplified diagram showing the interpretation of the findings. In the sequence at left the epithelial sodium-transporting cell is shown at rest, with the high concentration of sodium in the nucleus and the calcium structurally bound to the cell membranes. In the diagram of the right, the effect of vasopressin is seen as an increase in both the penetration of sodium and the active transport shown by the arrows as well as the migration of calcium from the membranes to the mitochondria and the release of sodium from the nucleus. In this working hypothesis it is assumed that vasopressin has an effect on the intracellular distribution of both calcium and sodium ions with a resulting increase in permeability and transcellular rate of sodium transport.

Also, as proposed by Leaf (16), the increased concentration of sodium in the cytoplasm, which occurs after the accelerated penetration through the cell membrane, would induce a greater rate of sodium transport by the sodium pump located at the cell membranes. The release of sodium from the nucleus could be the first event which would increase sodium concentration in the cytoplasm to activate the sodium carrier, that is to make more sodium substrate available for translocation across the membrane.

This working hypothesis is shown in the diagram of Fig. 12. The main aspects would consist then in a steady state flux of calcium from cell membranes to mitochondria, which will control both sodium permeability and sodium transport. The action of the hormone apparently agrees with the model and reminds of the mechanism of calcium activation in skeletal muscle where it has been proven that calcium movements from and into a reservoir of calcium ions are the link between excitation and contraction (17). In the epithelial cells, therefore, we would have a reservoir of calcium, the mitochondria, and a reservoir of sodium; the nucleus, which works in a correlated manner, could control sodium permeability as well as sodium transport.

In summary, the sodium is distributed into the nucleus, the sacs of the sarcoplasmic reticulum, and microbodies in epithelial cells. Under stimulation of sodium transport by vasopressin, the sodium of the nucleus is rapidly mobilized. The calcium content of mitochondria increases during the stimulation. These observations were made in both electron micrographs of tissues treated with vasopressin and in isolated nuclei and mitochondria. On the basis of the findings, the hypothesis is advanced that during stimulation of transport, calcium ions are removed from the cell membrane which becomes more permeable to sodium. The calcium released is accumulated in mitochondria, and the increased sodium in the cytoplasm, both by accelerated entrance and release from the nucleus, activates the sodium-pumping mechanism.

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