

MITOCHONDRIAL CHANGES INDUCED BY POTASSIUM AND
SODIUM IN THE DUODENAL ABSORPTIVE CELL AS STUDIED
WITH THE ELECTRON MICROSCOPE

By JULES M. WEISS,* M.D.

WITH TECHNICAL ASSISTANCE OF DORIS FLEISCH

(From the Department of Anatomy, Washington University School of Medicine, St. Louis)

PLATES 91 AND 92

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Mitochondria are important in ion transport, as has been shown in recent biochemical studies. For example, Stanbury and Mudge have shown that isolated mitochondria are capable of maintaining high concentrations of potassium when suspended in potassium poor solutions (7), and Bartley and Davies have shown that isolated mitochondria can take up cations from the surrounding solution against a concentration gradient (1).

Mitochondria concentrate the cationic dye Janus green, as is well known from light microscopic observations. That the cationic dye neutral red is also concentrated by mitochondria has been shown by electron microscopic studies (10). This paper reports electron microscopic observations on the effect of sodium and potassium ions upon a cell normally engaged in their transport. The duodenal absorptive cell of the Swiss albino mouse was studied under varying salt and water loads. Mitochondria were found to develop internal granules when these salts were administered in large amounts.

Materials and Methods

The duodenal absorptive cells of Swiss albino mice were prepared and studied by the techniques described in the preceding paper. The adult mice were males, 8 to 10 weeks old.

In order to assess the relative number of mitochondrial granules, at least ten high resolution micrographs were obtained for each animal. In questionable cases at least another fifteen micrographs were taken from another part of the duodenum. A total of 69 animals was studied.

OBSERVATIONS

Mitochondrial Structure.—Mitochondria from duodenal absorptive cells are similar to those found elsewhere (3,4,6) (Figs. 1 and 2). They are bounded by a double membrane, the inner one of which folds inward to form the double membraned internal folds. Between the two 90 Å thick osmiophilic membranes bounding the mitochondrion and forming the internal folds is an electron-

* American Cancer Society Scholar.

lucent space about 90 A thick. Under our conditions of fixation the mitochondrial matrix is always homogeneous and moderately dense. Irregular, dense granules, about 500 A in diameter, may be present within the mitochondrial matrix (Fig. 2). The number of mitochondrial granules was found to vary with the salt intake of the animal.

Normal adult mice were given Purina lab chow and tap water *ad lib.* Our laboratory mice sleep from dawn to dusk, during which time they take only traces of food and water.

TABLE I

Treatment		No. of granules per ten mitochondrial profiles*		
Type	Duration	1 or less, No. of mice	5 or more, No. of mice	
Water <i>ad lib.</i> †		5	0	
Thirsted and replenished with:‡	Water	2 min.-¼ hr.	4	0
		¼-½ "	5	0
		½-1 "	2	0
		1½ hrs.	3	0
		2½ "	3	0
		3½ "	5	0
	0.15 M KCl	1½ hrs.	5	3
		2½ "	2	2
		3½ "	5	8
		5-6 "	3	1
	0.15 M NaCl	1½ hrs.	3	0
		3½ "	2	4
Synthetic diet§	2 days	0	1	
	4 "	0	1	
	7 "	0	2	

* At least 400 mitochondrial profiles were counted for each animal.

† Received lab chow *ad lib.* throughout the experiment.

§ Sugar, water, polyvisol, and Ringer's solution (9).

They become active at about 6 p.m. and eat and drink intermittently until about 6 a.m. Mice were killed at intervals throughout this cycle. Mitochondrial granules were infrequent in all animals, not exceeding one granule per 10 mitochondrial profiles (Table I). No significant diurnal variation in mitochondrial granules was detected.

The Effect of Water Deprivation and Replenishment.—

Mice were fed regular Purina lab chow *ad lib.* at all times. Water bottles were removed at 6 a.m. Mice were killed 24 and 48 hours later. Other mice were given either tap water or tap water containing 0.15 M NaCl or KCl to drink after thirsting 24 hours. The initial drink-

ing time and the amount of fluid taken were noted. Animals were killed at frequent intervals after first drinking.

Deprivation of water for 24 or 48 hours produced no detectable change in the number of mitochondrial granules. Furthermore giving thirsted animals tap water to drink produced no change (Table I) (Fig. 1). On the other hand, sodium or potassium chloride solution fed to thirsted animals produced striking increases in the number of mitochondrial granules, especially in the mitochondria located in the bases of the cells, in about half the animals (Table I). The intercellular spaces in thirsted animals contained no electron-lucent dilatations; whereas after feeding either tap water, or tap water containing salt, electron-lucent dilatations appeared.

The Effect of a Sugar, Water, and Salt Diet.—Because of the osmiophilia of the mitochondrial granules, the possibility of fat, protein, or nucleic acid absorption playing a role in their formation had to be evaluated.

Four animals were placed on a diet of sugar, water, mammalian Ringer's solution, and polyvisol (9). One mouse was killed after 2 days on this diet, one after 4 days, and two after 7 days.

In every case the number of mitochondrial granules exceeded five per ten mitochondrial profiles. In addition to these mitochondrial changes, it was noted that the Golgi complex was reduced in extent having the same appearance as in absorptive cells from animals starved and thirsted for 24 hours (11).

DISCUSSION

Electron microscopy has revealed that mitochondrial granules are prominent in cells across which large amounts of water and cation are transported. Proximal renal tubule mitochondria are especially rich in granules (4); distal tubule mitochondria contain a moderate number (12). Liver cell mitochondria are also rich in granules (12). We have found that pancreatic acinar cell mitochondria vary considerably in granule number from animal to animal, but we have not had an opportunity to correlate this change with the secretory phase of the cell (12).

Cells which do not transport large amounts of cation and water do not usually have many mitochondrial granules. We have found this to be true of cardiac and smooth muscle cells, resting thyroid cells, and anterior pituitary cells (12). The data reported here show that duodenal cells engaged in transporting water, small amounts of cation, and the digestion products of fat, protein, and carbohydrate, contain few mitochondrial granules, whereas an excess of potassium or sodium added to the same transport load significantly increases the number of mitochondrial granules. Adult animals on a fat-free and protein-free diet may have large numbers of mitochondrial granules in their duodenal

absorptive cells, indicating that these mitochondrial granules are independent of fat and protein absorption.

These electron microscopic data, taken with the biochemical data demonstrating cation concentration by mitochondria (1, 7), suggest that the mitochondrial granules may represent a segregation form for cations physiologically transported by cells. As mentioned before, Janus green and neutral red are two toxic cations which are concentrated in vacuoles within mitochondria. The mitochondrial vacuole represents a segregation mechanism for certain toxic cations; possibly this mechanism is a perversion of that which normally occurs.

The electron density of the mitochondrial granules strongly suggests the presence of an osmiophilic, solid phase material, perhaps analagous to an ion-exchange resin and capable of transforming osmotically active, soluble cation, into osmotically inactive cation.

There is an extensive and well corroborated literature describing the presence within cells of excessive amounts of potassium, amounts which would cause appreciable swelling of cells were the potassium osmotically active (2); see the review of Robinson and McCance (5). Some workers in the field have postulated a mechanism for segregating much of the intracellular potassium in an osmotically inactive form (2). Others have suggested that water is pumped out of the cell as fast as it is pulled in by the osmotic pressure of the potassium (5). Either mechanism would require energy, explaining the well known swelling of cells following any interference with their energy metabolism (5, 8). That the mitochondrion may represent an intracellular segregator of osmotically active potassium is suggested by the biochemical work on cation concentration by mitochondria.

The problem may now be restated, do the mitochondria actively pump out water, or do they inactivate the osmotically active cation? We prefer this latter view, and would like to suggest that the mitochondrial granules represent the hypertrophy of a system occurring in all mitochondria, a system for complexing cation onto solid anion. The failure to observe granules in all mitochondria cannot be taken as evidence that the system is not present in all mitochondria, since extreme rates of ion transport may be required to accumulate visible quantities of complex. Without any supporting evidence, we would like to advance one further suggestion, and that is that the granule (or the vacuole as the case may be) containing inactivated cation may be excreted by being bodily discharged from the mitochondrion and then the cell. This may be too simple and mechanistic a device for transporting cations, and indirectly water. It would be a shame, however, to dismantle such a carefully prepared package of cation as the mitochondrial granule may be, and the mitochondrial vacuole is, and then have to devise a second mechanism for the cation's further transport.

SUMMARY

Duodenal absorptive cells from animals fed large amounts of sodium or potassium contain many mitochondria with internal granules. Many mitochondrial granules are also present in cells which transport large amounts of cation. The hypothesis is advanced that the mitochondrial granule represents cation segregated within the mitochondrion. A mechanism is suggested for the segregation of excessive intracellular potassium, and for the transport of cations across cells.

BIBLIOGRAPHY

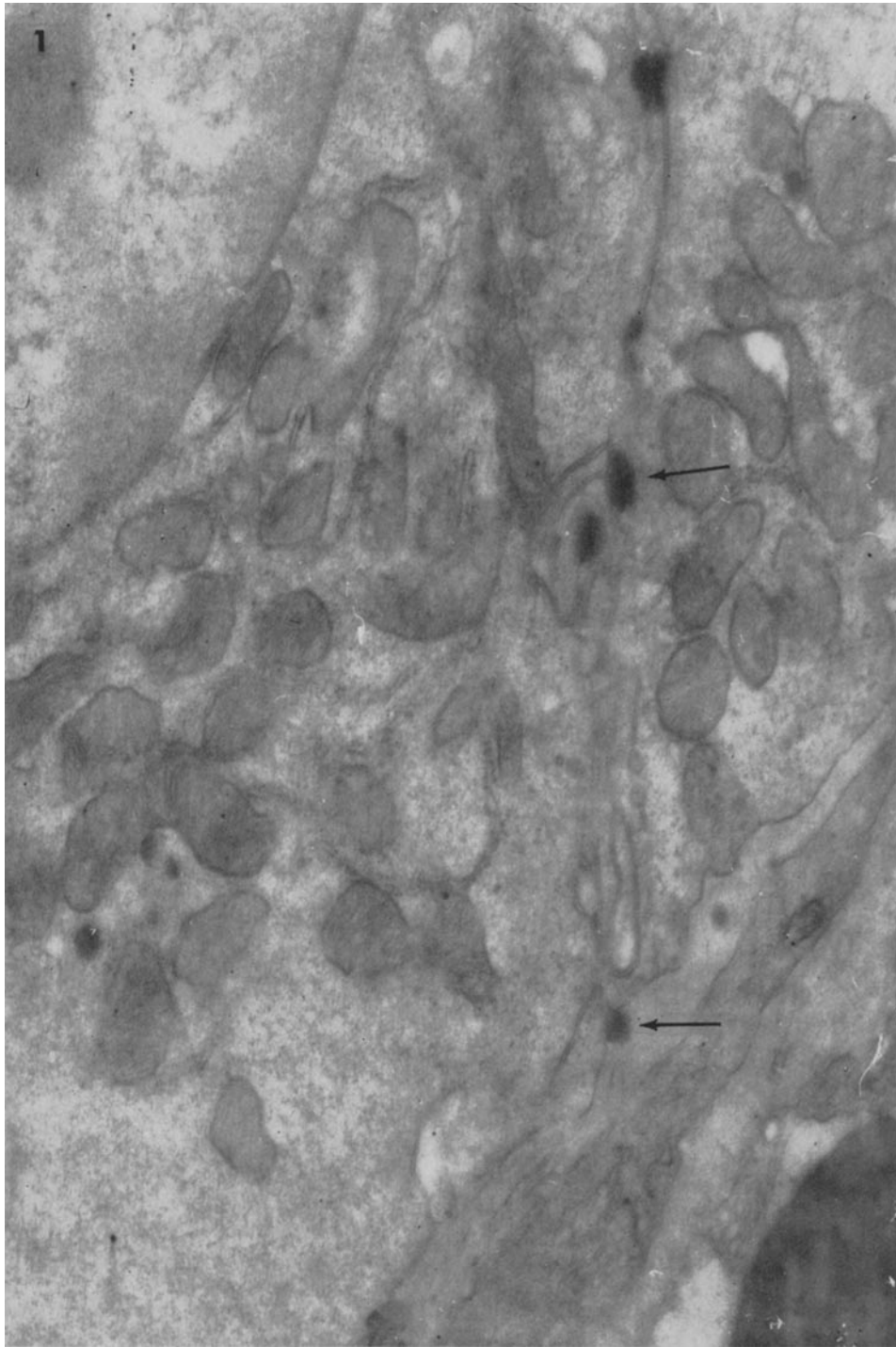
1. Bartley, W. O., and Davies, R. E., *Biochem. J.*, 1954, **57**, 37.
2. Danowski, T. S., *Am. J. Med.* 1951, **10**, 468.
3. Palade, G. E., *Anat. Rec.*, 1952, **114**, 427.
4. Rhodin, J. Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney, Karolinska Institutet, Stockholm, Aktiebolaget Godvil, 1954.
5. Robinson, J. R., and McCance, R. A., *Ann. Rev. Physiol.*, 1952, **4**, 115.
6. Sjostrand, F. S., and Rhodin, J., *Exp. Cell Research*, 1953, **4**, 426.
7. Stanbury, S. W., and Mudge, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1953, **82**, 675.
8. Stern, J. R., Eggleston, L. V., Hems, R., and Krebs, H. A., *Biochem. J.*, 1949, **44**, 410.
9. Weiss, J. M., *J. Exp. Med.*, 1953, **98**, 607.
10. Weiss, J. M., *J. Exp. Med.*, 1955, **101**, 213.
11. Weiss, J. M., *J. Exp. Med.*, 1955, **102**, 000.
12. Weiss, J. M., unpublished observations.

EXPLANATION OF PLATES

PLATE 91

FIG. 1. Electron micrograph of part of the basal portions of two duodenal absorptive cells from an animal which had been thirsted for 24 hours, then given water, and killed $3\frac{1}{2}$ hours later. A portion of the nucleus is in the upper left-hand corner. In the lower right-hand corner is part of a red blood cell in a capillary. Between the capillary endothelium and the basal plasma membrane of the absorptive cells are, in order, extracellular connective tissue space, part of a fibroblast, and basement membrane of the absorptive cells. In the space between the two absorptive cells, and in the basement membrane region are several fat droplets (arrows).

The mitochondria within the absorptive cells contain no granules. A few ergastoplasmic sacs, and many free ergastoplasmic granules are present. Approximate magnification, 32,000.

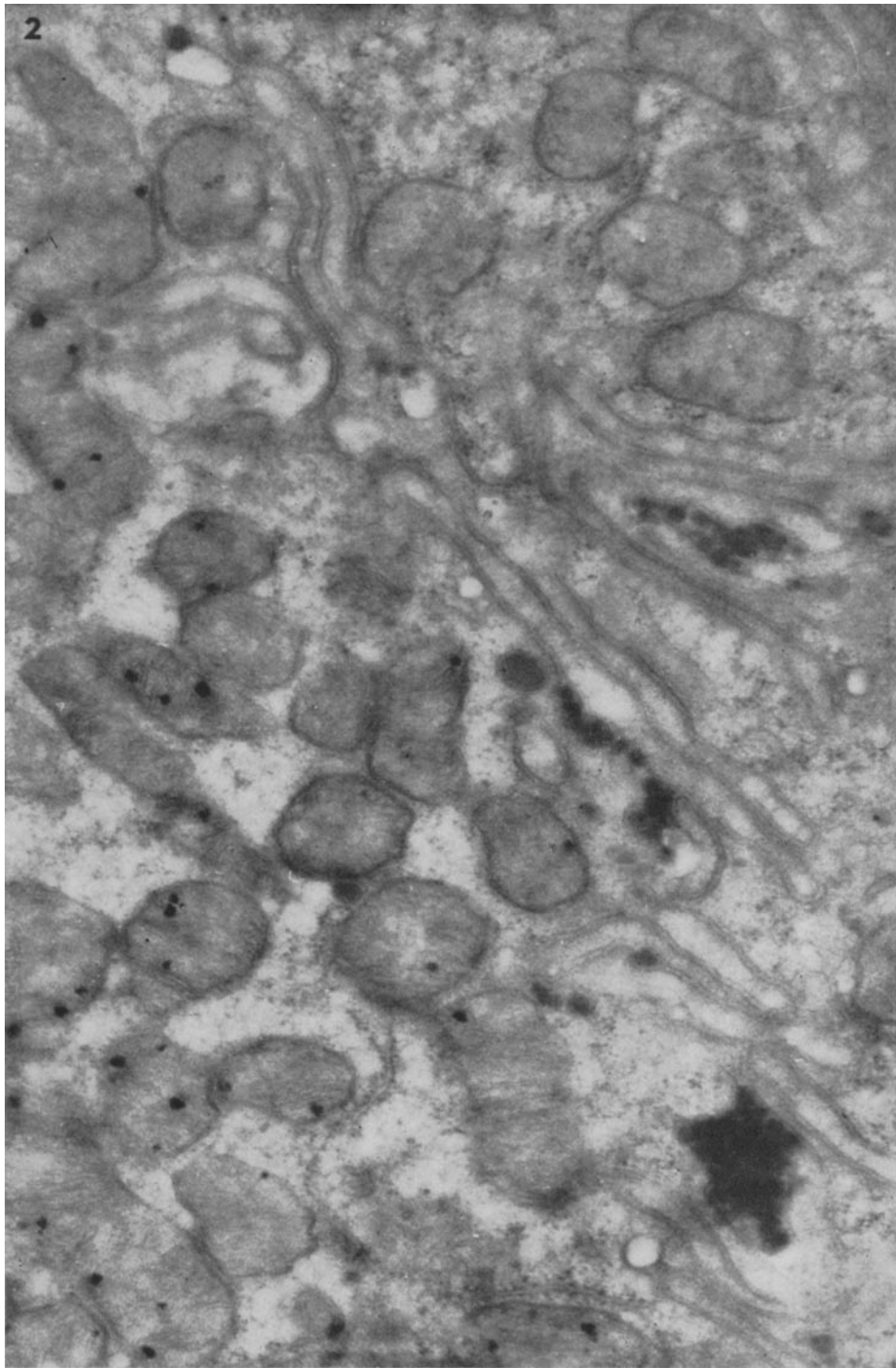


(Weiss: Mitochondrial changes in duodenal absorptive cell)

PLATE 92

FIG. 2. Electron micrograph of part of the basal portions of two duodenal absorptive cells from an animal which had been thirsted for 24 hours, then given 0.15 M KCl *ad lib.*, and killed 3½ hours later. Fat droplets are present in the intercellular space.

The mitochondria contain many granules. A few ergastoplasmic sacs, and many free ergastoplasmic granules are present throughout the cytoplasm. Approximate magnification, 50,000.



(Weiss: Mitochondrial changes in duodenal absorptive cell)