SOME EFFECTS OF EDTA AND TETRAPHENYLBORON ON THE ULTRASTRUCTURE OF MITOCHONDRIA IN MOUSE LIVER CELLS

CURTIS C. HARRIS and CHARLES A. LEONE. From the Department of Zoology, University of Kansas, Lawrence

Investigators who utilize tissue culture cells in viral, metabolic, and cytogenetic studies have generally overlooked the possible influences of cell-dispersing agents on their results. Our investigations, showing that EDTA and tetraphenylboron (TPB) cause extensive in vivo changes in the ultrastructure of several organelles of cells, will be presented fully in a later report. Reported herein are some of the in vivo effects of these dispersing agents on the mitochondria of mouse liver cells.

MATERIALS AND METHODS

EDTA is a widely used chelating agent for Ca^{++} and Mg^{++}. TPB chelates K^+ (1) and has recently been used as a cell-dispersing agent (2). Our dispersing media contained EDTA and/or TPB in variously modified Tyrode's solutions (Table I).

C_3H/Heston mice weighing 12–14 g were sacrificed by cervical dislocation and their livers removed. The livers were gently minced, using a scissoring action with scalpels on dental wax, and placed in approximately 10 ml of prewarmed EDTA- or TPB-dispersing medium per gram of tissue. 5 min later the dispersing medium was replaced with fresh, prewarmed dispersing medium and incubated for 1, 4, or 6 hr at 37°C. The cell-laden fluid was filtered through gauze and centrifuged at 500 RPM (75 × *g*) for 10 min.

The cells in the pellet were fixed by dispersing them in cold Veronal-buffered, 1% osmium tetroxide with sucrose (3). The cells were embedded in Epon 812, and sections were cut on a Porter-Blum microtome. The sections were picked up on uncoated grids and stained with Reynold's lead citrate (4) and/or 4% methanolic uranyl acetate (5). The sections were examined in an RCA EMU-3F electron microscope.

RESULTS

Some of the effects of deficiencies of Ca^{++}, Mg^{++}, and K^+ on the structure of mitochondria, summarized in Table I, are in close agreement with observations made by others on isolated mitochondria (6–9). The mitochondria in EDTA-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 1 hr) dispersed mouse liver cells exhibited: (*a*) condensation of the mitochondrial matrix; (*b*) ballooning of the outer membrane; and (*c*) distention of the intracristal spaces (Fig. 1 *d*). Adding K^+ to the EDTA-dispersing medium deferred or prevented the condensation of the mitochondrial matrix and the distention of the intracristal spaces (Fig. 1 *a*). After incubation for 4 or 6 hr in the K^+-supplemented EDTA (Ca^{++}- and Mg^{++}-free Tyrode's solution), the mitochondria were swollen and their matrices were irregularly distributed (Fig. 1 *b*). These swollen mitochondria appeared similar to substrate-swollen mitochondria (6); however, in our studies the swelling is probably a degenerative change from the normal organelle (Fig. 1 *c*).

A deficiency in K^+ resulted in coagulation of the cytoplasm. Incubation for 4 hr in EDTA (Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution) caused cytoplasmic coagulation (Fig. 1 *e*), as did incubation for 1 hr in TPB(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution) (Fig. 1 *g*). The importance of K^+ in cytoplasmic protein synthesis has been reported (10), and the observed coagula-

TABLE I

Effects of Dispersing Agents on the Ultrastructure of Mitochondria within Mouse Liver Cells

Conditions	I. T.*	Appearance	Fig.‡
0.1% EDTA in Ca^{++}- and Mg^{++}-free Tyrode's, pH 7.6	1.0	Irregular-shaped mitochondria, ballooning of outer membrane, cristae not evident	1 *a*
	4.0	Swelling, disappearance of matrix, lysis	1 *b*
	6.0	Similar to 4.0 hr	
0.1% EDTA in Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's, pH 7.6	1.0	Condensation of matrix, ballooning of outer membrane, distention of intracristal space	1 *d*
	4.0	Coagulation of cytoplasm, mitochondria not evident	1 *e*
	6.0	Dissociation of cytoplasm	1 *f*
0.05–0.5% trypsin in complete Tyrode's, pH 7.6	1.0	Normal mitochondria	1 *c*
3×10^{-3} M TPB in Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's, pH 8.0	1.0	(*a*) Swelling, disintegration of cristae and matrix	1 *h*
		(*b*) Coagulation of cytoplasm, mitochondria not evident	1 *g*
3×10^{-6} M – 3×10^{-3} M TPB in K^+-free Tyrode's, pH 7.6	1.0	Irregularly shaped mitochondria and cristae, ballooning of outer membrane, matrix of near normal density	1 *i*

* I.T. refers to incubation time in hours.

‡ Fig. refers to the appropriate figure in the text.

tion likely reveals a breakdown of this function. After 6 hr in the EDTA-dispersing media, the coagulated cytoplasm begins to dissociate (Fig. 1 *f*).

While these results imply that the effects of the absence of the ions, Ca^{++}, Mg^{++}, and K^+, are additive, deficiences of individual ions display characteristic effects. The active removal of K^+

FIGURE 1 Mouse liver cells containing mitochondria (*M*). Nucleus (*N*).

(*a*) Irregularly shaped mitochondria; EDTA-(Ca^{++}- and Mg^{++}-free Tyrode's solution, 1 hr) dispersed cell. × 34,500.

(*b*) Disintegration of mitochondrial matrix and cristae; EDTA-(Ca^{++}- and Mg^{++}-free Tyrode's solution, 6 hr) dispersed cell. × 20,500.

(*c*) Normal mitochondria; Trypsin-(Complete Tyrode's solution, 1 hr) dispersed cell. × 34,500.

(*d*) Condensation of mitochondrial matrix, ballooning of outer membrane (arrows); EDTA-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 1 hr) dispersed cell, × 48,500.

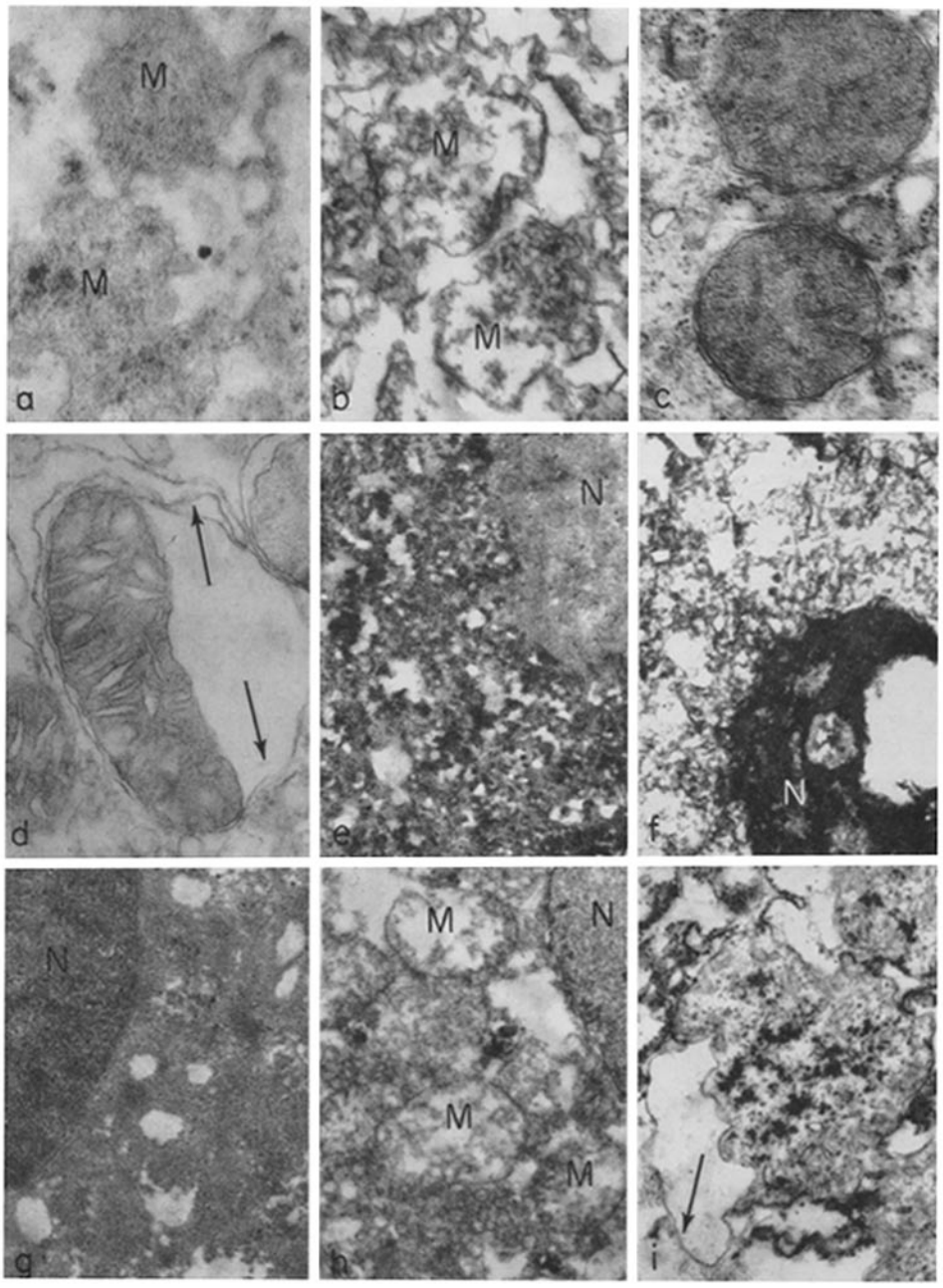
(*e*) Coagulation of cytoplasm and nucleus related to K^+ deficiency; EDTA-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 4 hr) dispersed cell. × 15,000.

(*f*) Dissociation of cytoplasm, darkly staining nucleus; EDTA-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 6 hr) dispersed cell. × 15,000.

(*g*) Coagulation effect, in part, as the result of K^+ deficiency, similar to that in Fig. 1 *e*; TPB-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 1 hr) dispersed cell. × 15,000.

(*h*) Swelling and disintegration of mitochondria, slightly before coagulation of cell as seen in Fig. 1 *g*, cytoplasm and nucleus granulated; TPB-(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution, 1 hr) dispersed cell. × 11,100.

(*i*) Swollen mitochondria, ballooning of outer membrane (arrow), irregular cristae; TPB-(K^+-free Tyrode's solution, 1 hr) dispersed cell. × 20,500.



by TPB(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution) resulted in swelling of the mitochondria (Fig. 1 *h*). The active removal of Ca^{++} and Mg^{++} by EDTA(Ca^{++}-, Mg^{++}-, and K^+-free Tyrode's solution) resulted in condensation of the mitochondrial matrix (Fig. 1 *d*). Mitochondria in mouse liver cells dispersed by EDTA(Ca^{++}- and Mg^{++}-free Tyrode's solution, 1 hr) or TPB(K^+-free Tyrode's solution, 1 hr) were irregular in shape (Fig. 1 *a* and *i*).

While the influence of ion deficiences on isolated mitochondria has been actively investigated, the in vivo effects of these conditions have received little attention. Our observations show similarities to those made on isolated mitochondria; however, it is obvious that complex ionic interactions are occurring which need further study.

The effects on mitochondria that are presented may be somewhat exaggerated because of the OsO_4 fixations. However, in some of our other work on the McCoy cell strain in tissue culture, cells were fixed in buffered glutaraldehyde and postfixed in buffered OsO_4, or fixed in buffered OsO_4 alone. The ultrastructural appearances of such cells were similar.

The formulations for the various Tyrode's solutions were taken from Parker (11). Small differences in osmolarities do exist among them. Control experiments in which the molar concentrations of the dispersing media were varied indicated that the described effects could not be attributed to the small differences in molarity among the several Tyrode's solutions.

After 1 hr in the EDTA-dispersing media, over 90% of the mitochondria showed some ballooning. Longer exposures in the media caused changes primarily in the density of the mitochondrial matrix.

Swollen mitochondria were regularly found in an entanglement of the endoplasmic reticulum; others were free of the reticulum. They were so characteristic in appearance (Fig. 1 *d*) that their "swollen membranes" could not be mistaken for membranes of the closely adhering endoplasmic reticulum.

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