

ULTRASTRUCTURAL TRANSFORMATION IN MITOCHONDRIA ISOLATED FROM KIDNEYS OF NORMAL AND LEAD-INTOXICATED RATS

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ABSTRACT

Mitochondria isolated from kidneys of lead-intoxicated rats have been shown to have decreased oxidative and phosphorylative abilities. The purpose of this study was to determine whether these abnormal mitochondria would undergo ultrastructural transformation during controlled respiration in the absence of phosphate acceptor (State IV), as previously demonstrated for normal liver mitochondria. It was first shown that normal rat kidney mitochondria transform from a condensed ultrastructural conformation to an orthodox conformation after 5 min of State IV respiration with pyruvate-malate substrate. Reversal to a condensed conformation follows stimulation of respiration with adenosine diphosphate (ADP). A large portion of kidney mitochondria from lead-poisoned rats do not change from condensed to orthodox conformation during State IV respiration. Other mitochondria do transform to the orthodox form but they rapidly degenerate. State IV respiration decreases as these few orthodox mitochondria disintegrate. The conclusion is that those mitochondria that do not undergo change in ultrastructure have impairment of electron transport, and that those that do become orthodox have increased membrane lability and undergo degeneration.

INTRODUCTION

Mitochondria in proximal tubular lining cells in the kidneys of lead-intoxicated rats have been shown to be structurally and functionally abnormal (1). The basilar mitochondria of these cells are swollen and have marginal cristae and few matrical granules, and there is an associated decreased reabsorption of amino acids. After isolation these mitochondria show a decreased respiratory control ratio and partial uncoupling of oxidative phosphorylation in the presence of pyruvate-malate substrate (2). Osmotic swelling experiments and electron microscopy of the mitochondria suggest that the mitochondrial membranes have increased lability. Phosphoryla-

tive ability is only partially improved by treatment *in vivo* with EDTA¹. We have concluded, therefore, that kidney mitochondria from lead-poisoned rats have a deficiency in oxidative and phosphorylative abilities, as well as a defect in membrane integrity.

The present study was undertaken to determine whether the abnormal mitochondria isolated from

¹Abbreviations used in this paper are: EDTA, ethylenediaminetetraacetic acid; ADP, adenosine diphosphate; ADP:O, adenosine diphosphate to oxygen ratio; TMPD, tetramethyl-*p*-phenylenediamine; JEM, trade name for microscope produced by Japan Electrical and Optical Co., Ltd.

the kidneys of lead-poisoned rats would undergo ultrastructural transformation during controlled respiration in the absence of phosphate acceptor (State IV), as demonstrated for normal liver mitochondria by Hackenbrock (3). Transformation from a condensed to an orthodox conformation occurring during State IV is reversible by ADP stimulation of respiration (State III). This ultrastructural change appears to be dependent on an intact electron-transport mechanism and is believed to be related to the energy-conserving function of mitochondria. It is thought not to be an osmotically induced ultrastructural change (4). Since we are unaware of previous studies of ultrastructural transformation in kidney mitochondria comparable to reported studies of this transformation in liver mitochondria, our studies of pathological mitochondria were dependent upon our being able to demonstrate this phenomena in normal renal mitochondria.

MATERIALS AND METHODS

The oxidative and phosphorylative studies shown in Table I were performed on mitochondria isolated from the kidneys of four 200-g Sprague-Dawley rats fed a diet of pulverized laboratory chow that contained 1% lead acetate. Respiratory and ultrastructural studies were repeated on mitochondria from the kidneys of three other lead-fed rats. An equal number of similar rats fed the same chow without lead acetate served as controls. Rats were sacrificed by decapitation and the kidneys were quickly excised; the capsule was peeled free and the medullary zone was separated from the cortex. The cortical portion was then homogenized in 0.40 M sucrose containing 0.1 mM EDTA and centrifuged twice at 600 g for 10 min. The supernatant solutions were combined and

centrifuged at 12,000 g for 10 min. The crude mitochondrial pellet was resuspended in 30 ml of 0.25 M sucrose at pH 7.4 and centrifuged again at 12,000 g for 10 min. The washed pellet was resuspended in 1.5 ml of 0.25 M sucrose and stored in an ice bath. An aliquot was removed for protein determination by a biuret method.

Oxygen consumption was determined polarographically (5), and rate of oxygen uptake, ADP:O ratio, and respiratory control ratio were calculated from oxygen electrode tracings (6). The reaction mixture contained 2.2 mM pyruvate and malate, 4.4 mM phosphate, and 0.16 M sucrose. For stimulation of State III respiration, 1 μ mole of ADP was added 2 min after addition of mitochondria. All reagents were made with ion-free water and adjusted to pH 7.4 with Tris base. 0.15 ml of mitochondrial stock solution (containing approximately 3 mg of protein) was added to the reaction mixture to make a final volume of 3.0 ml.

For ultrastructural studies State IV respiration and State III respiration were measured as above. The rates of oxygen consumption for the mitochondria from which the electron micrographs were made are shown in Figs. 1 and 5. Each tracing of oxygen uptake represents a separate aliquot of stock mitochondria. At the time indicated by the arrow at the end of each tracing, 0.12 ml of 25% glutaraldehyde was added to the reaction mixture. This treatment stopped respiration instantly. The mixture with fixative was allowed to remain in suspension in the cold (4°C) for a minimum of 1 hr. The reaction mixture was then centrifuged at 35,000 g for 5 min to form a pellet of mitochondria.

A narrow strip was then cut from the center of the pellet and further fixed with 1% osmium tetroxide in acetate-Veronal buffer at pH 7.4, dehydrated, embedded in Epon, sectioned with a Porter-Blum ultramicrotome, and examined with a JEM T-7 electron microscope.

TABLE I
*Oxidative and Phosphorylative Abilities of Kidney Mitochondria from Control and Lead-Fed Rats with Pyruvate-Malate Substrate**

Mitochondria	No. ‡	Rate of oxygen uptake μ atoms O/min/per g protein				Respiratory control ratio
		State IV	State III	ADP/O		
Control	8	43.2 \pm 1.9	130.3 \pm 13.9	2.7 \pm 0.2	3.3 \pm 0.5	
Lead-Fed	7	46.5 \pm 6.9	101.8 \pm 14.0	2.1 \pm 0.2	2.2 \pm 0.3	
		p < 0.15	p < 0.05	p < 0.05	p < 0.05	

* Reaction mixture as described in text (Methods).

‡ Number of measurements. Stock mitochondria are from four control and three lead-fed rats.

RESULTS

Mitochondrial Respiration

Table 1 compares the respiratory rates of kidney mitochondria isolated from normal and lead-intoxicated rats. Oxygen uptake of mitochondria from the lead-fed rats in the presence of pyruvate-malate substrate (State IV) is slightly increased, whereas State III respiration during phosphorylation is decreased. Likewise, ADP:O ratios and respiratory control ratios of kidney mitochondria from the lead-intoxicated rats are less than the comparable values obtained with control mitochondria.

Ultrastructural Transformation

The respiratory rates of mitochondria obtained from kidneys of a control rat are shown in Fig. 1. The time points at which mitochondria were fixed for Figs. 2, 3, and 4 are indicated.

The mitochondria shown in Fig. 2 respired in State IV with pyruvate-malate substrate for only 1 min. Nearly all of the mitochondria have a dense, contracted inner compartment and an expanded outer compartment. The conformation of these mitochondria resembles the condensed ultrastructural conformation demonstrated in liver mitochondria by Hackenbrock (3).

After respiring in State IV for 5 min, the mitochondria from the control animals changed in ultrastructure from a condensed to an orthodox conformation (Fig. 3), which resembles the appearance of kidney mitochondria usually observed in tissue sections. The inner compartment is expanded, the inner membrane forms thin cristae,

and the outer compartment is very narrow. 90% of the mitochondria examined were of the conformation shown in Fig. 3. Most exceptions were damaged mitochondria, single examples of which appear in both Figs. 2 and 3.

Fig. 4 was obtained from mitochondria 30 sec after State III respiration was stimulated by the addition of ADP. These mitochondria were allowed to respire in State IV for 4 min before addition of ADP. Approximately 80% of kidney mitochondria assume an orthodox conformation after 4 min of State IV respiration. If the mitochondria are allowed to respire a full 5 min before the respiration is stimulated with ADP, little or no respiratory control is obtained and many of the mitochondria appear damaged. However, if ADP is added after only 4 min of State IV respiration, oxygen uptake is stimulated and reversal to a condensed ultrastructural conformation occurs (Fig. 4).

Similar experiments were repeated with kidney mitochondria from lead-intoxicated rats. The respiratory rates and the time points at which mitochondria were fixed for electron microscopy are shown in Fig. 5. Initial State IV respiratory rates were similar to those observed for the control mitochondria, but after approximately 3 or 4 min the rate of oxygen uptake decreased. This change in State IV respiration was a consistent finding in experiments with mitochondria from lead-intoxicated rats. ADP only weakly stimulated respiration after 2 min of State IV respiration and produced no increase in oxygen uptake if added after 3 or more min of State IV respiration.

After 1 min of State IV respiration, the majority of the mitochondria from the lead-poisoned rats

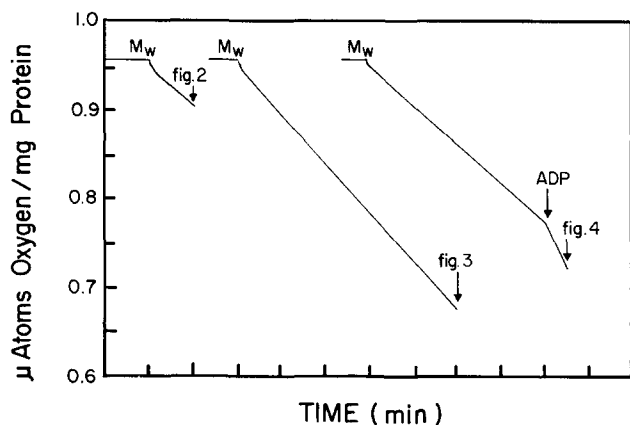


FIGURE 1 Oxygen consumption of control kidney mitochondria. Mitochondria for Figs. 2 and 3 were fixed after 1 min and 5 min of State IV respiration, respectively. Mitochondria for Fig. 4 were fixed after 4 min of State IV respiration, addition of ADP ($1 \mu\text{mole}/3.4 \text{ mg protein}$), and $\frac{1}{2}$ min of State III respiration. The reaction mixture was as described in text (Methods) except that ADP was added only as indicated.

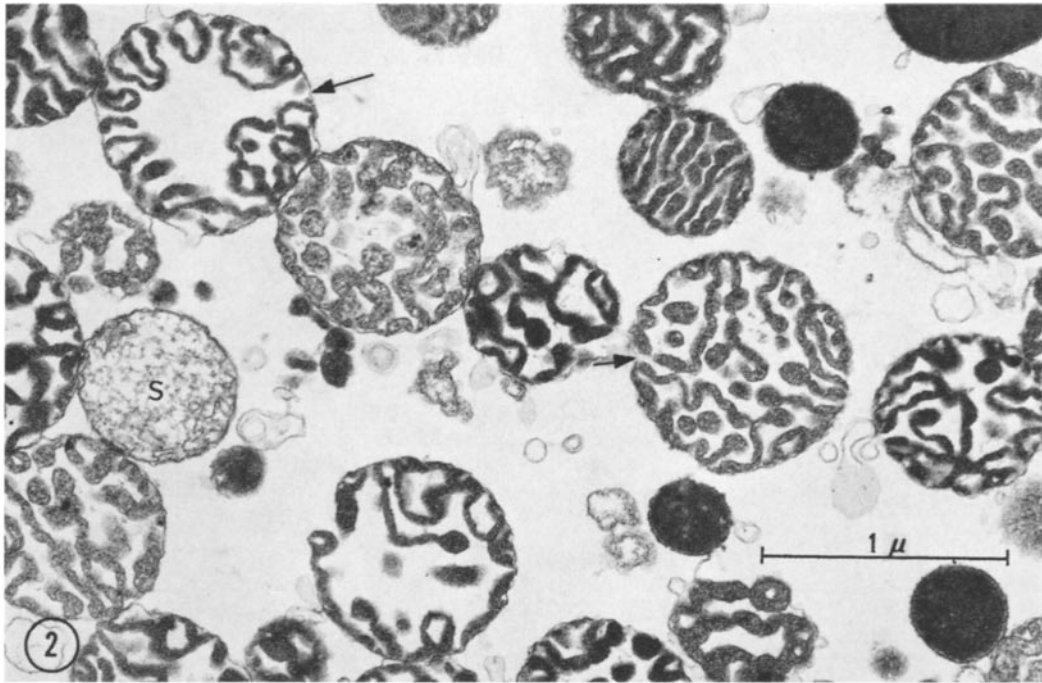


FIGURE 2 Mitochondria fixed after 1 min of State IV respiration as shown in Fig. 1. Mitochondria have a condensed conformation indicated by the increased density and contraction of the inner matrix. The density or degree of condensation varies in different mitochondria. The inner membranes appear to be adhered to the outer membranes at multiple points. The expanded clear areas within the mitochondria are continuous with the outer compartment as indicated by arrows. A swollen damaged mitochondrion (s) is present. $\times 32,000$.

(Fig. 6) are in the condensed conformation, which resembles the conformation of the control mitochondria in Fig. 2. However, after only 3 min of State IV respiration, about half of the mitochondria have become orthodox in conformation; the remaining mitochondria remain condensed (Fig. 7). Some of the mitochondria that have undergone ultrastructural transformation to an orthodox configuration appear swollen and damaged. Continuation of State IV respiration seems to worsen the ultrastructural appearance of the mitochondria, resulting in a higher proportion with ruptured membranes (Fig. 8). Nevertheless, many more mitochondria remained in the condensed conformation than were observed in preparations of control mitochondria after 5 min of State IV respiration. Similar results were obtained on mitochondria isolated from three different lead-intoxicated rats.

DISCUSSION

The experiment demonstrates that mitochondria isolated from rat kidney cortex undergo an ultrastructural transformation from a condensed to an orthodox form during State IV respiration. This transformation resembles the change in ultrastructure described by Hackenbrock for liver mitochondria under similar circumstances (3). The present experiment differs from Hackenbrock's study in only minor aspects. We initially fixed the mitochondria in the reaction mixture with glutaraldehyde before later fixation with osmium tetroxide. Pyruvate-malate rather than succinate, was used for substrate and the temperature of the reaction mixture was 25°C rather than 30°C. A pyruvate-malate substrate has been consistently used in previous studies of the phosphorylating defect of mitochondria of lead-intoxicated animals in this laboratory. Recent studies suggested that mitochondria from lead-

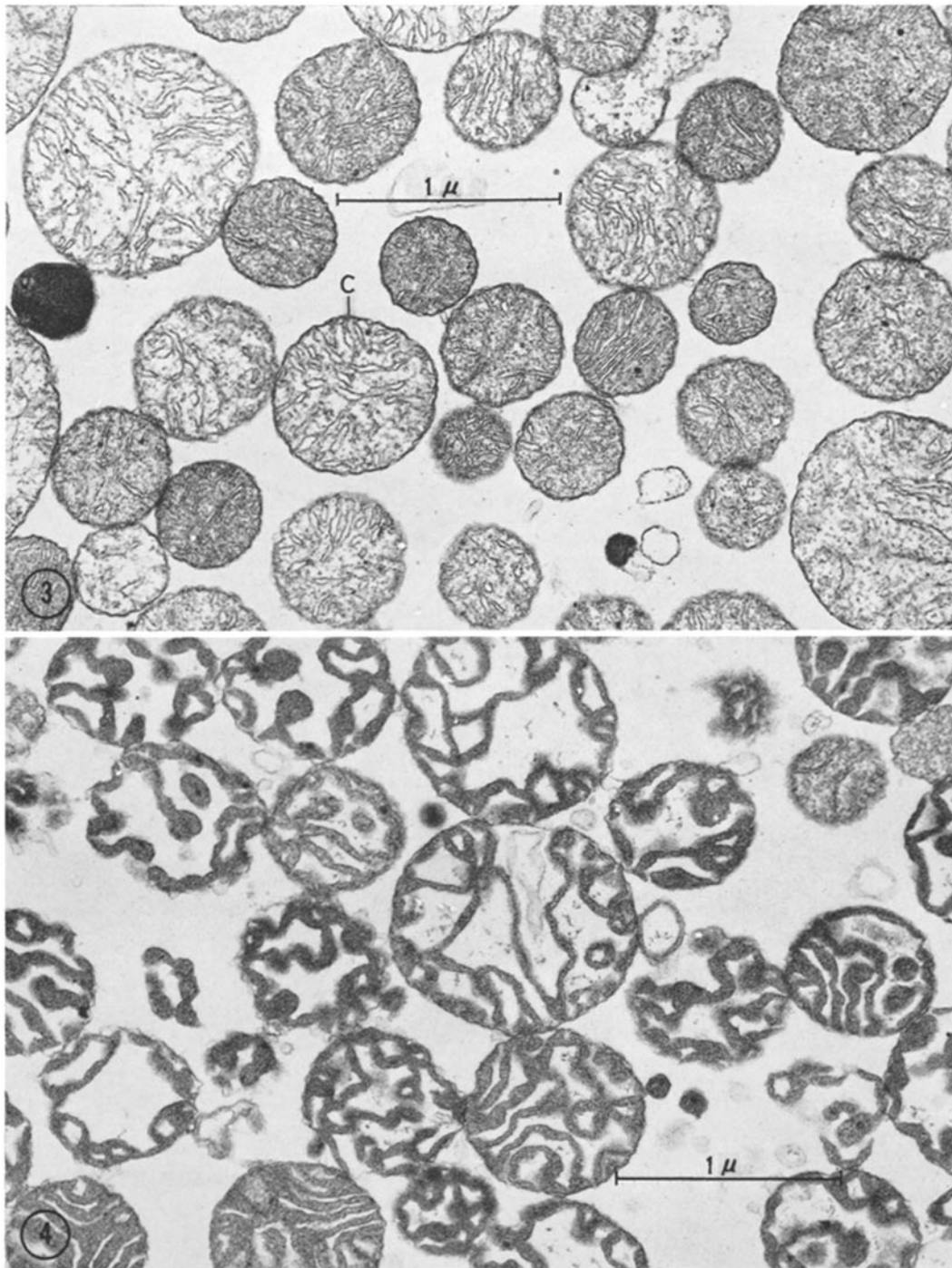


FIGURE 3 Mitochondria fixed after 5 min of State IV respiration as shown in Fig. 1. The mitochondria have undergone ultrastructural transformation from condensed to orthodox conformation. The inner compartment is finely granular and homogeneous. The cristae and outer compartment (c) are thin. The variation in size is characteristic of mitochondria isolated from kidney. $\times 32,000$.

FIGURE 4 Mitochondria fixed after State IV respiration has continued for 4 min followed by ADP-stimulated respiration (State III) for 30 sec as shown in Fig. 1. State IV to State III transition is accompanied by transformation of ultrastructure from orthodox to condensed conformation in nearly all of the mitochondria. $\times 32,000$.

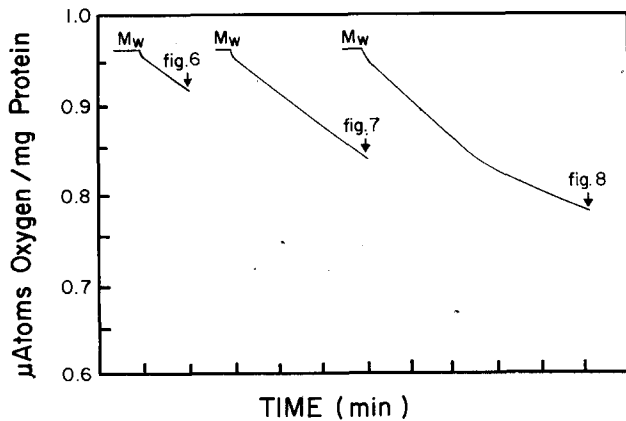


FIGURE 5 Oxygen consumption of mitochondria from kidneys of lead-intoxicated rats. Mitochondria for Figs. 6, 7, and 8 were obtained after 1, 3, and 5 min of State IV respiration, respectively. The reaction mixture was as described in text (Methods).

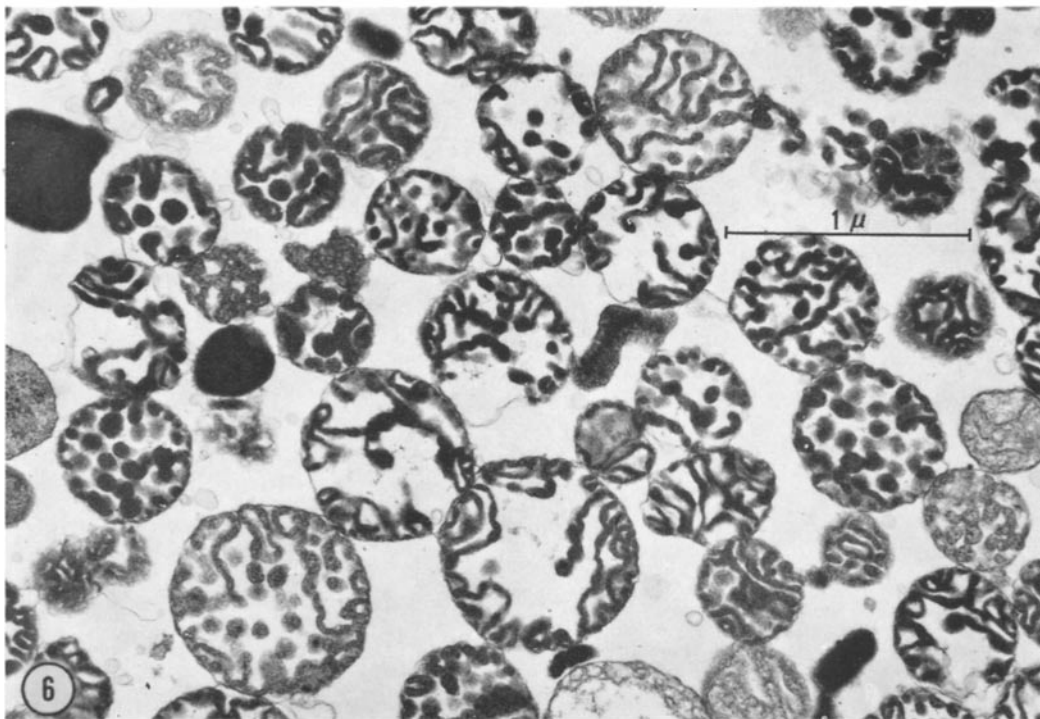


FIGURE 6 Mitochondria from kidneys of a lead-intoxicated rat fixed after 1 min of State IV respiration (Fig. 5). The majority of the mitochondria are in the condensed conformation and do not differ from control mitochondria after 1 min of State IV respiration (Fig. 2). $\times 32,000$.

intoxicated rats have normal phosphorylative abilities in the presence of succinate.

The morphologic appearance of condensed kidney mitochondria differs from that of liver mitochondria only in that the dense inner compartment appears adhered or fixed to the outer membrane at more points than were suggested for

liver mitochondria. Also, the time required for State IV condensed to orthodox mitochondria to occur is less for kidney mitochondria, 5 min rather than 15 min. The orthodox ultrastructural conformation of State IV kidney mitochondria was rapidly reversible to a condensed conformation during State III, as occurs in liver mitochondria.

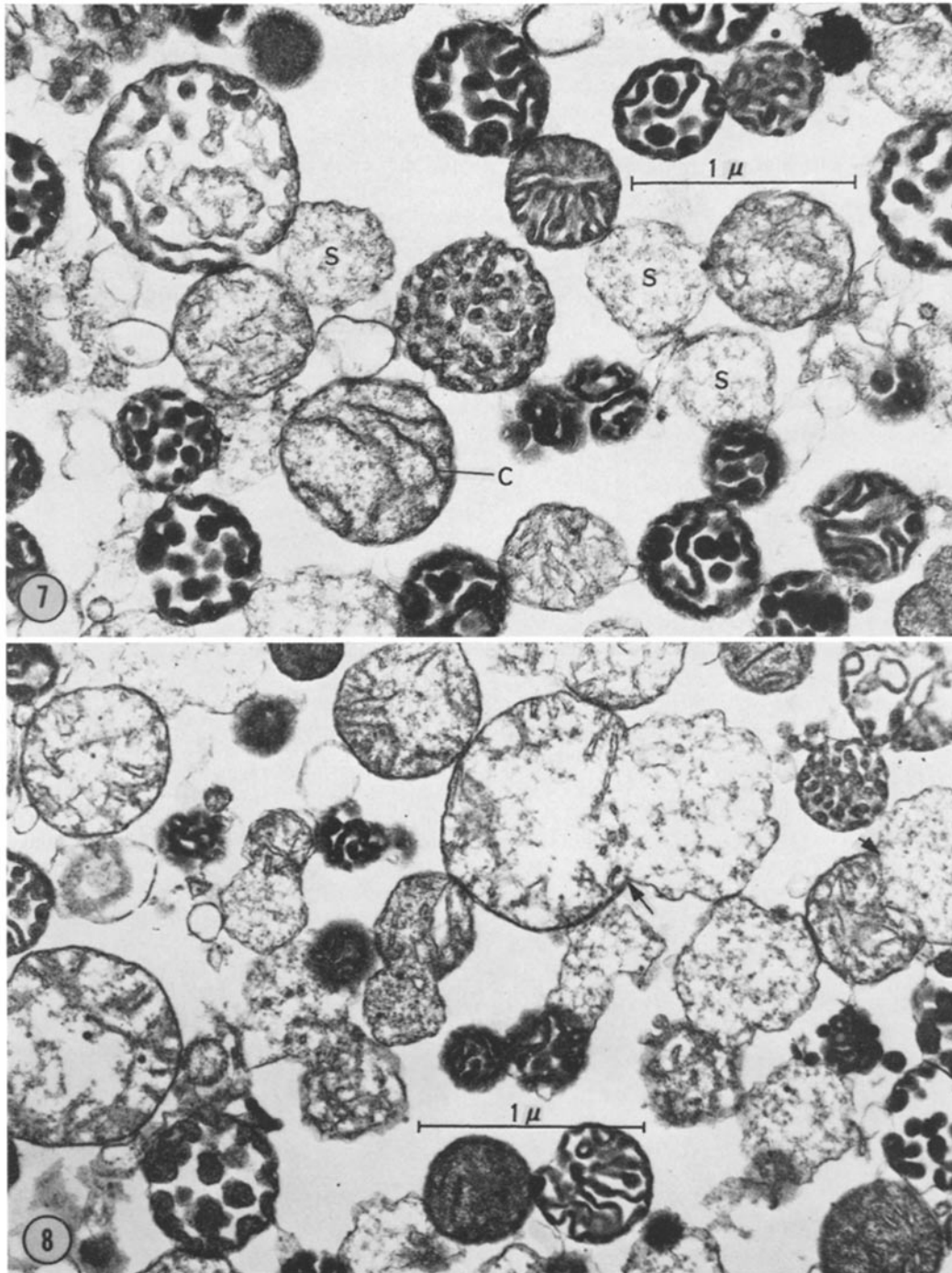


FIGURE 7 Mitochondria from kidneys of a lead-intoxicated rat fixed after 3 min of State IV respiration (Fig. 5). About 50% of the mitochondria are transformed to orthodox conformation. These mitochondria differ from control mitochondria with orthodox conformation (Fig. 3) in that cristae are sparse and irregular (*c*). Many of the mitochondria are swollen (*s*). $\times 32,000$.

FIGURE 8 Mitochondria from kidneys of a lead-intoxicated rat after 5 min of State IV respiration (Fig. 5). The decrease in oxygen consumption occurring after 3 min of State IV respiration is accompanied by swelling and rupture of membranes (arrows) of many of the mitochondria that have undergone transformation to the orthodox conformation. Many mitochondria persist in the condensed conformation. $\times 32,000$.

The significance of the ability of mitochondria to undergo ultrastructural transformation is not completely understood. Hackenbrock has demonstrated that this morphologic change may be prevented by agents which impair electron transport, such as cyanide or antimycin (4). The latter compound may be bypassed with TMPD, which restores electron flow and ultrastructural transformation. It has been suggested that these ultrastructural changes are electron-transport energized mechanochemical transformations, rather than ion-induced osmotic ultrastructural transformations energized by electron transport (4).

Mitochondria uncoupled with small amounts of dinitrophenol are more rapidly transformed from condensed to orthodox forms during State IV and are not reversed by stimulating respiration with ADP (State III). Other workers have shown that that mitochondria from experimentally-induced hepatomas and fetal liver have an orthodox conformation when isolated in State IV but will not assume a condensed conformation in State III. Also, respiration of these mitochondria is not stimulated by ADP, which suggests an inability to perform phosphorylation (7).

It has been demonstrated that transformation from orthodox to condensed conformation may occur when phosphorylation is stimulated *in situ* (8). Addition of amino acids and amino acids plus glucose to "rings" of mouse jejunum results in stimulation of respiration and a condensed appearance of mitochondria in tissue sections, again demonstrating that condensed mitochondrial

conformation is associated with a high rate of coupled phosphorylation.

The present study shows that ultrastructural transformation of mitochondria from kidneys of lead-intoxicated rats differs from that of control mitochondria. A portion of the mitochondria from kidneys of lead-intoxicated rats does not change from condensed to orthodox configuration during State IV respiration. Other mitochondria do transform to the orthodox form but they rapidly degenerate. State IV respiration decreases as the mitochondria of orthodox configuration degenerate. Interpretation of these changes can, of course, only be predicated on the incomplete understanding of ultrastructural transformation available at the present time. However, it is suggested that those mitochondria that do not undergo condensed to orthodox transformation during State IV respiration have impairment of electron transport. Those mitochondria that do transform to orthodox conformation have some impairment of phosphorylative ability manifested morphologically by increased membrane lability and eventual degeneration.

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