

## POLAR DISTRIBUTION OF INTRAMITOCHONDRIAL GRANULES OF MUCOSAL EPITHELIAL CELLS

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### INTRODUCTION

Evidence obtained from both biochemical and morphological studies indicates that mitochondria play major roles in maintaining intracellular ionic stability. In the case of whole cells, as well as for isolated mitochondria, an increase in size and/or number of intramitochondrial granules is associated with the stabilizing process during changes in the ionic environment. The increase in large granules within the mitochondrial matrix has been shown to be an indication of divalent cation accumulation (Peachey, 1964).

In our own studies of mucosal tissue of the small intestine of the rat, we have noted that during high rates of oxygen consumption by jejunal slices incubated in a mixture of amino acids (Bronk and Parsons, 1966) the mitochondria of the epithelial cells frequently show an apparent increase in the size and number of intramitochondrial granules. Although most mitochondria in any sample of tissue incubated under these conditions are highly condensed, those which have an orthodox configuration also contain larger or more numerous mitochondrial matrix granules. In the instances being described, mitochondria are widely distributed throughout the cytoplasmic regions, but those organelles exhibiting this phenomenon are primarily localized in the basal region of the cell. This paper describes this apparent structural polarity in brief form.

### MATERIALS AND METHODS

Details of methods have been described previously (Bronk and Parsons, 1965; Jasper and Bronk, 1968). Jejunal slices from male albino rats (200–300 g) were incubated 3–5 min at 38°C in 1 ml of a Krebs bicarbonate-Ringer medium containing 1 mg/ml of a mixture of amino acids. The pH of this medium was maintained at 7.5. Respiration rates were moni-

tored polarographically and sample slices were selected for fixation for electron microscopy. Fixatives routinely used were cacodylate-buffered glutaraldehyde followed by phosphate-buffered osmium tetroxide. After alcohol dehydration tissue samples were embedded in Epon. Sections were made on either a Huxley Cambridge ultramicrotome (Cambridge Scientific Instruments, Ltd., Cambridge, England) or a Porter-Blum MT-2 with glass or diamond knives. After mounting on bare copper grids, these sections were stained with uranium and lead salts. Electron microscope observations were made with the AEI EM6B (AEI Scientific Apparatus Inc., Elmsford, N. Y.), the Zeiss EM 9S, and the Siemens Elmiskop 1A.

### RESULTS

Mucosal epithelial cells of jejunal slices incubated in a mixture of amino acids alone respire rapidly and frequently contain 90% or more of their mitochondria in the condensed configuration (Jasper and Bronk, 1968; see also Fig. 1). As has been pointed out previously, the essential structural features of such mitochondria are a concentration of the matrix and a refolding of the inner membrane system. Recently, we have noted that another interesting feature of many condensed mitochondria observed under these conditions is their tendency to show an increase in the concentration of intramitochondrial granules (Figs. 1, 3, and 5). As shown in the figures, the granular deposits vary in size (averaging 50–100 nm) and density. They consist of numerous smaller units (~5 nm or less in diameter) which sometimes appear aggregated around a central lighter core, but which may also appear as dense, highly concentrated particles within the mitochondrial matrix. Differences in concentration or in the

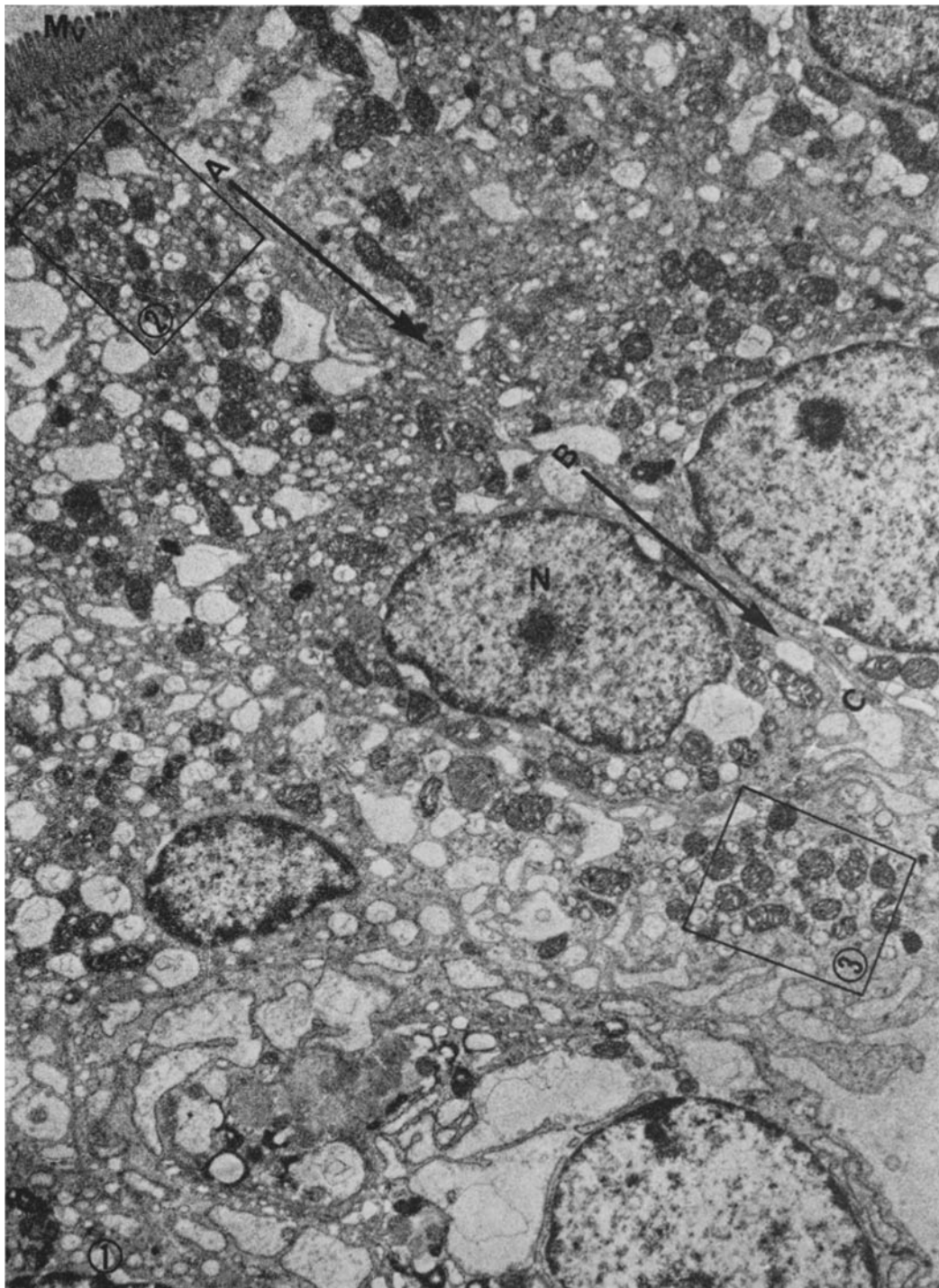


FIGURE 1 Electron micrograph depicting epithelial mucosal cells of a jejunal strip in longitudinal profile. The sample was incubated in a mixture of amino acids (1 mg/ml). Nearly all mitochondria are highly condensed. An apparent polar localization of those organelles with enlarged granules exists (see Figs. 3 and 5). Intramitochondrial granules are mostly concentrated in the basal two-thirds of the cells (supranuclear and nuclear, i.e. from tip of arrow *A* to tip of arrow *B*; infranuclear, area *C*). Few, if any, mitochondria in the apical third (to tip of arrow *A*) contain the enlarged granules (cf. Figs. 2 and 4). This regional distribution of intramitochondrial granules may indicate changes in ionic concentrations within the cellular cytoplasm as well as the mitochondria. Outlined areas are regions for Figs. 2 and 3. *Mv*, microvilli; *N*, nucleus.  $\times 6400$ .

degree of aggregation of the smaller units appear to be related to the density variations of the granules

A thin section through the long axis of the jejunal mucosal cell (as shown in Fig. 1) illustrates the regional localization of the intramitochondrial granules most favorably. It is clear that a polar distribution of the mitochondria containing the granules exists. Those organelles in the apical regions of the cells contain fewer or are essentially devoid of the enlarged dense deposits (Fig. 2), while those mitochondria in the basal cellular areas contain them in varying numbers (Fig. 3). Table I illustrates the percentage distribution of the intramitochondrial granules in the different regions of the cell. The apical third of the cell contains less than 10% of the observable granules whereas over 90% of the enlarged intramitochondrial granules are found in those organelles situated in the basal two-thirds of the cells.

Expressed another way, the average number of granules per mitochondrion ranged from 0.20 in the apical region to 1.76 in the basal region. Structural details of representative mitochondria from these cellular regions are depicted in Figs. 4 and 5.

At this point it should be made clear that the high concentrations of the intramitochondrial granules and the polar localization of the mitochondria containing them as enlarged precipitates have not been consistent observations in all of our mucosal preparations. At the moment, such findings have not been made in cells of non-incubated mucosal tissue or in those samples incubated in the absence of added substrate.

Nor have we been able to observe high polar concentrations of intramitochondrial granules in cells of tissue samples incubated with glucose in the presence or absence of added amino acids. Aside from the necessity of obtaining sample sections which consistently show the mitochondrial profiles most favorably, there are several additional, possible reasons for these variations. We shall briefly discuss one.

#### DISCUSSION

Although at present we have no information on the specific nature of the intramitochondrial granules noted in this report, it seems rather reasonable to attribute their apparent polar distribution to localized changes in ionic concentrations. Studies on other cell types have indicated that intramitochondrial granules form as a result of divalent cation accumulation within mitochondria (Peachey, 1964; Rasmussen, 1966; Zadunaisky et al., 1968). We believe that the evident increase in concentration of intramitochondrial granules reported here is an indication of divalent cation accumulation in these organelles.

Thus, it may be of interest to point out that information from experiments on isolated liver mitochondria (Gear and Lehninger, 1968) shows that endogenous  $K^+$  is lost to the incubation medium in the absence of permeability-inducing agents, and this may provide a basis for an increase in mitochondrial  $Ca^{++}$  concentration levels during  $Na^+$  binding. In addition, it is well known that mucosal cells, like those of other tissues, maintain high intracellular gradients of potassium ions (Parsons, 1967).

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FIGURE 2 This electron micrograph depicts at higher magnification condensed mitochondria in the apical third of a cell shown in Fig. 1 (see outlined area). No indication of enlarged intramitochondrial granules appears.  $\times 32,000$ .

FIGURE 3 Mitochondria in the basal cellular region outlined in Fig. 1. Note the enlarged granular precipitate in the mitochondrial matrix. Generally, mitochondrial profiles in this area contain two or more of these granules, which are interpreted as divalent cation accumulation.  $\times 32,000$ .

FIGURE 4 Higher magnification of a mitochondrion in the apical region of a cell treated as those for Fig. 1. Although no clear indication of enlarged matrical granules can be seen, numerous particles (black arrow) with characteristics of mitochondrial ribosomes are observable; these average  $\sim 10$ – $15$  nm in diameter.  $\times 99,000$ .

FIGURE 5 Similar particles as those shown in Fig. 4 are observable in basal region mitochondria (black arrow), but, in addition, four enlarged intramitochondrial granules can be seen at the broad arrow points. The granules appear to be composed of smaller, dense units bound to a lighter core.  $\times 99,000$ .

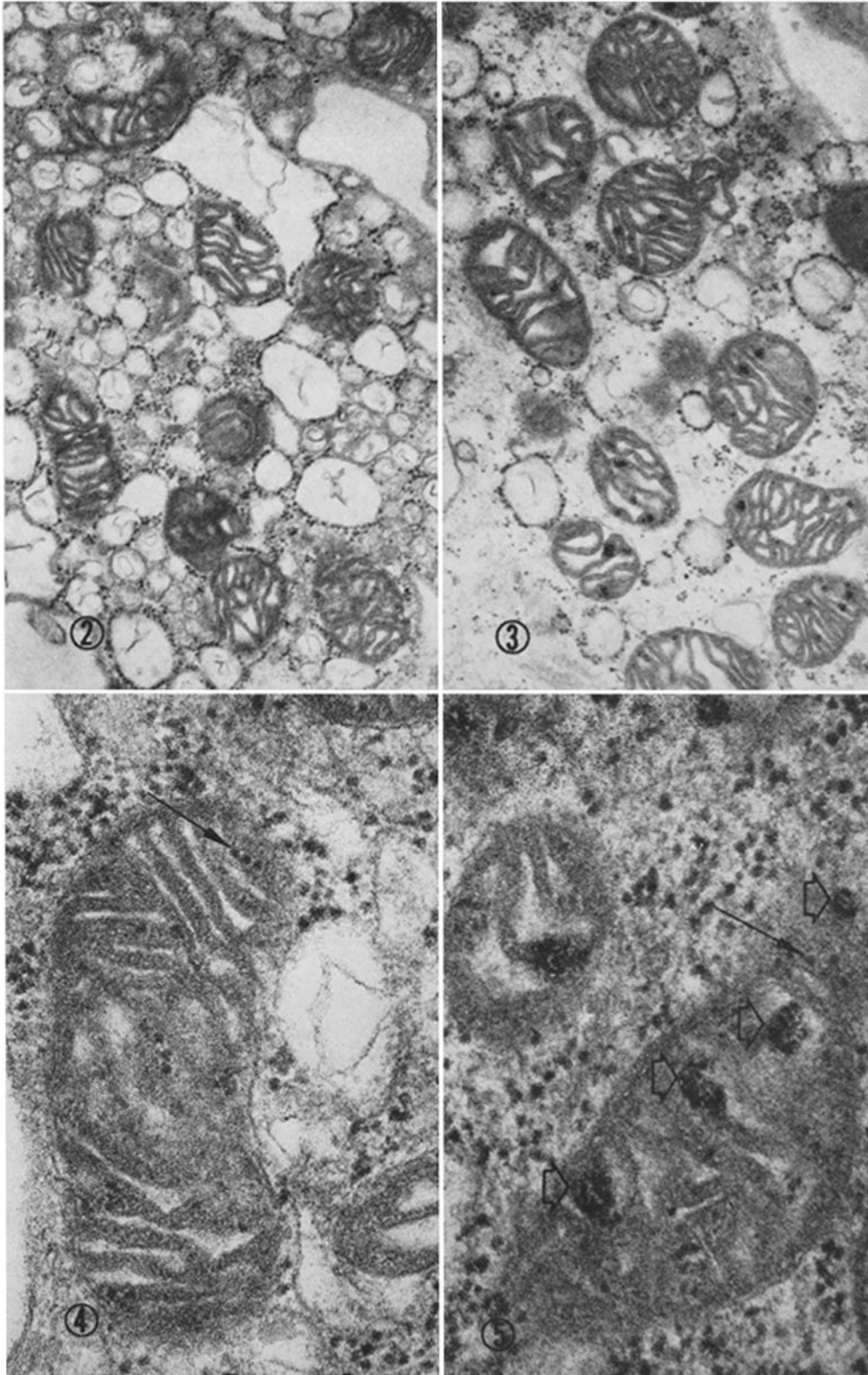


TABLE I  
Regional Distribution of Intramitochondrial Granules

Cellular region*	Mitochondrial profiles counted	Number of granules	% Granules (of total counted)	Average number of granules per mitochondrial profile
A (Apical)	109	22	6.0	0.20
B (Supranuclear, nuclear)	143	126	34.4	0.88
C (Infranuclear, basal)	124	218	59.6	1.76

\* See Fig. 1.

It was recently reported (Jasper and Bronk, 1970) that 50% of the  $K^+$  in mucosal slices was lost when the slices were incubated in an amino acid mixture for 5 min. In this connection, it may well be that  $K^+$  loss is indicative of ionic changes within mitochondria as well as within the cytoplasmic mass. It has been suggested (Parsons, 1967) that the maintenance of an intracellular  $K^+$  balance, at least in part, could be presumed to operate with an active accumulation of the ion at the epithelial mucosal pole and a passive outward leak at the basal pole. If to some extent the loss of  $K^+$  during amino acid accumulation by mucosal tissue is from within mitochondria, one

might see a redistribution of intracellular cations, notably  $Ca^{++}$ , since this ion tends to accumulate in mitochondria (Peachey, 1964; Rasmussen, 1966; Gear and Lehninger, 1968; Zadunaisky *et al.*, 1968). Additional studies on mucosal epithelium which include experiments to test this possibility are in process.

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