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Pronounced Mitochondrial Response to Carbon Dioxide in the Epithelial Cells lining the Esophagus-Foregut Connection of the Pig *Ascaris* (*Ascaris suum*)

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Introduction

In *Ascaris suum* var. *lumbricoides* residing in the small intestine where the oxygen tension is low, a specific type of anaerobic energy metabolism was reported to occur (Rew and Saz, 1974), as in other parasitic helminths (Scheibel and Saz, 1966 ; Saz, 1972), in molluscs (Hammen, 1969 ; Hochachka and Mustafa, 1972), in turtle liver (Penney, 1974) and in anoxic mammalian heart (Cascarano *et al.* 1968 ; Wilson and Cascarano, 1970). In the *Ascaris* tissues mitochondria are known to contain fumarase and NAD-linked "malic" enzyme required for the anaerobic energygenerating system (Rew and Saz, 1974), in addition to enzymes for aerobic and related systems.

In the course of our current studies on the cytology of the epithelial cells lining the esophagus and gut of the pig *Ascaris* (*Ascaris suum*), we have found a pronounced mitochondrial response to carbon dioxide in particular epithelial cells lining the esophagus-foregut connection. The present investigation examines, therefore, the aspects of the ultrastructural response of these epithelial cells to carbon dioxide and discusses the possible cytophysiological functions of the epithelial cells.

Materials and Methods

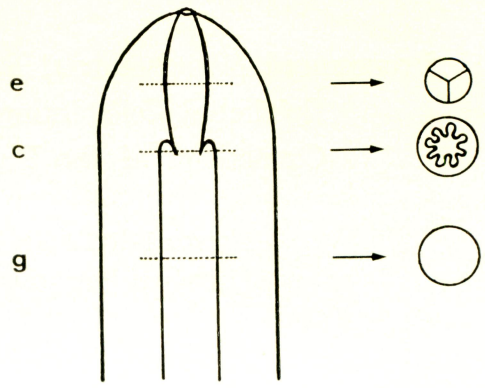
As an experimental group, five *Ascaris* worms (*Ascaris suum*) were obtained from pigs and placed in the Ringer solution saturated with carbon dioxide for 1 to 2 hours at room temperature, whereas other five worms obtained from the donors were used as controls and immersed in the Ringer solution under the same conditions of duration and temperature as those employed for the experimental worms. Immersion of the worms longer than 10 hours in the Ringer solution saturated with carbon dioxide was found to lead to the death of the animals and the specimens from such worms did not deserve the object of the present investigation. After being placed in the Ringer solutions with or without administered carbon dioxide, the worms were subjected to autopsy. Epithelial tissues lining the esophagus-foregut connection and those lining the rest parts of foregut, mid- and hindguts were dissected out from the worms of both the experimental and control groups. For light microscopy, the epithelial tissues were fixed in Bouin solution, embedded in paraffin and cut at 6 μ in thickness, and the sections were stained with hematoxylin and eosin for general observation. For electron microscopy, the epithelial tissues were fixed in chilled (4 C) phosphate or cacodylate buffered (pH 7.2) 2.5% glutaraldehyde for 1 to 2 hours, rinsed in chilled (4 C) buffer solutions (pH 7.2) and postfixed in chilled (4 C) cacodylate buffered (pH 7.2) 2.0% osmium tetroxide for 1 to 2 hours. The fixed tissue specimens were then dehydrated in an ethanol series of ascending concentrations and embedded in Epon 812, as prescribed by Luft (1961). Ultrathin

sections were cut from these tissue blocks, stained doubly with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in a Hitachi HS 8 electron microscope.

Results

As is illustrated in the figure (Fig. 1), in the pig *Ascaris* the esophagus-foregut connection is invaginated into the lumen of the foregut, and the epithelial cells lining the connection are tall columnar in shape and contain an oval nucleus situated basally (Fig. 2). Such structural features of the epithelial cells are nearly similar to those of the epithelial cells lining the rest parts of the guts.

In the control worms maintained in the Ringer solution without administered carbon dioxide, the cytoplasm of the epithelial cells lining the esophagus-foregut connection contain usual cell organelles such as mitochondria, rough and smooth varieties of endoplasmic reticulum, free ribosomes and Golgi apparatus (Fig. 3). In addition, varying amounts of inclusions such as glycogen particles and lipid droplets are detected in the cytoplasm (Fig. 3). The mitochondria within the cytoplasm of the control epithelial cells range in size from 200 to 600 mμ, are spherical or rob-like in shape and provided with rather sparsely arranged cristae, but the organelles are devoid of any dense intramitochondrial granules (Figs. 3 and 5). In the cytoplasm of the epithelial cells from experimental worms immersed in the Ringer solution saturated with carbon dioxide, mitochondria are found to undergo pronounced changes in size, shape and structure; they are significantly larger than those in the control epithelial cells, ranging from 0.7 to 5.5 μ in size, mostly thick thread-like in appearance and provided with an abundance of cristae (Figs. 4 and 6). In the cytoplasm of the experimental epithelial cells these mitochondrial changes are accompanied by a series of changes in other cell organelles and inclusions; the extension of the rough



e : esophagus

c : connection

g : gut

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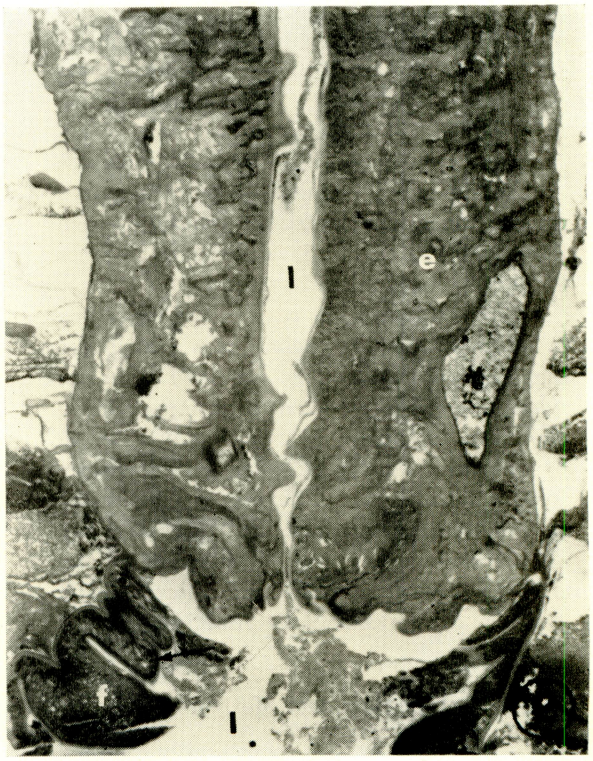

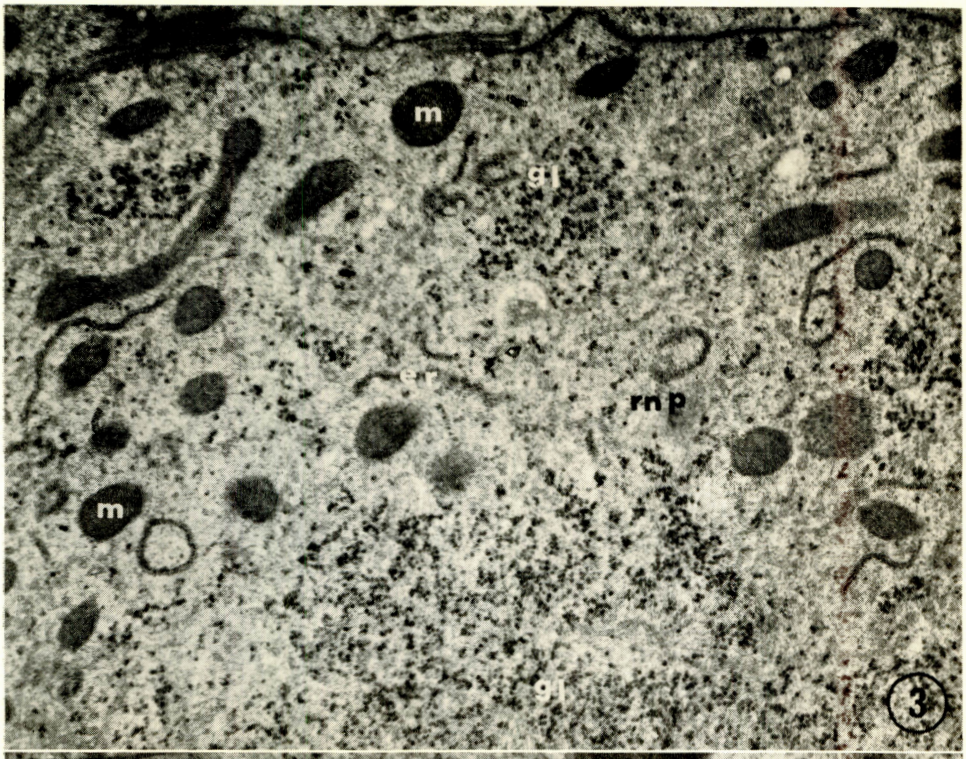
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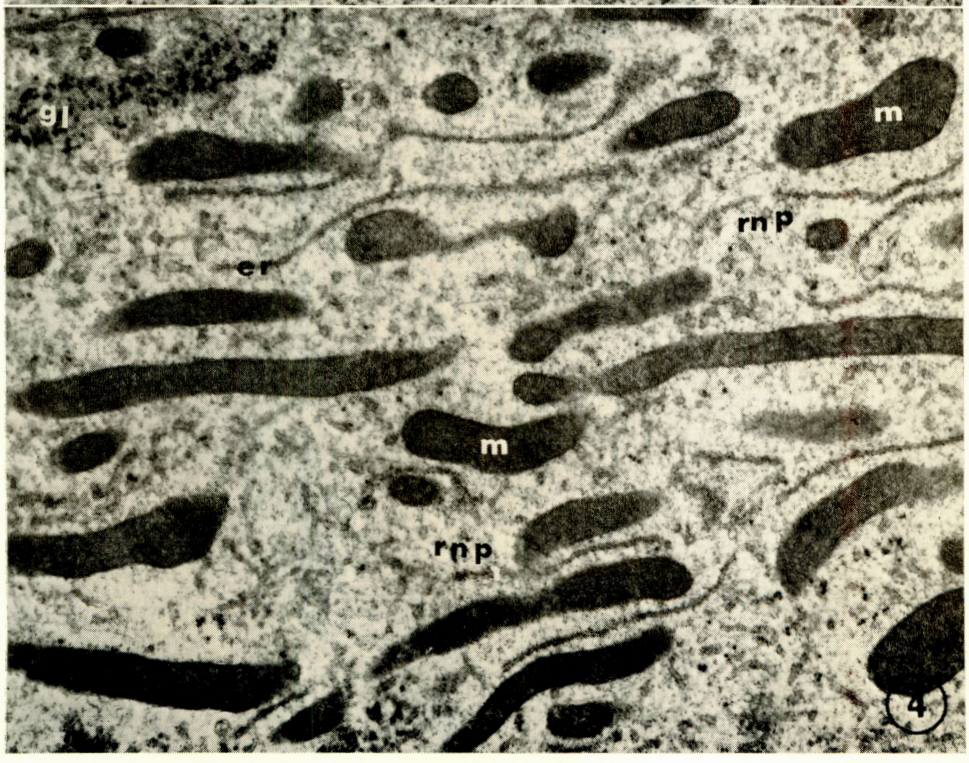
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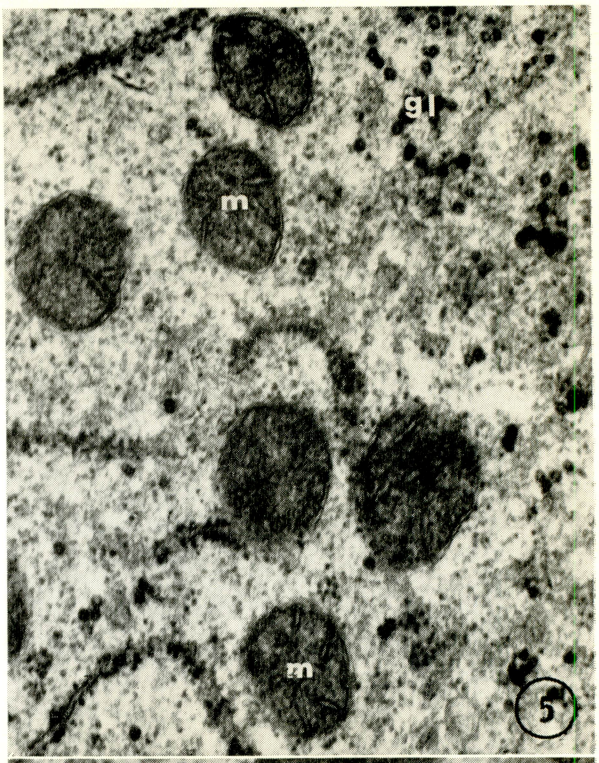
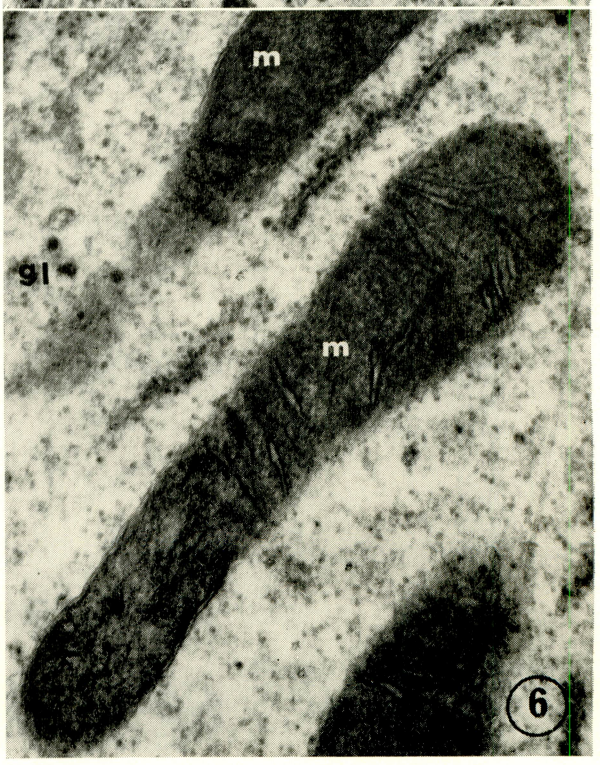
surfaced endoplasmic reticulum cisternae associated with mitochondria, an abundance of free ribosomes and a markedly decreased amount of glycogen particles (Fig. 4). In the epithelial cells from experimental worms, however, no apparent changes are noted in other structural elements such as the nucleus, Golgi apparatus, smooth varieties of endoplasmic reticulum and lipid droplets.

Administration of carbon dioxide to the worms fails to give rise to any notable effects upon the ultrastructures of the epithelial cells lining the rest parts of foregut, mid-and hindguts.

Discussion

According to the results of a previous biochemical study on the mitochondrial fractions of *Ascaris lumbricoides* (Rew and Saz, 1974), fumarase and NAD-linked "malic" enzyme required for the anaerobic energy-generating system were shown to be localized in the intermembrane space of mitochondria. In view of these results, the pronounced response of mitochondria with an increased number of cristae to carbon dioxide observed in the particular epithelial cells of the present *Ascaris* worms is to be correlated with the possibly high activity of such enzymes as fumarase and NAD-linked "malic,, enzyme. In the present study, however, the mitochondria in the particular epithelial cells from experimental worms are found to exhibit a general increase in size, and it is, therefore, conceivable that other enzymes than those for anaerobic energy-generation such as succinate and pyruvate dehydrogenases, NADH oxidase, Mg^{++}- dependent ATP-ase, adenylate kinase, citrate synthase and cytochrome C reductases are more or less activated as well. In the *Ascaris* worms, all these enzymes were reported to exhibit a distribution pattern comparable to that of the enzymes in mammalian mitochondria (Rew and Saz, 1974). In the particular epithelial cells from experimental worms the pronounced mitochondrial response is accompanied by





the extension of the rough surfaced endoplasmic reticulum cisternae associated with mitochondria, an abundance of free ribosomes and a markedly decreased amount of glycogen particles. It is well established that ribosomes are the central sites of protein synthesis and the elements of rough surfaced endoplasmic reticulum play an important role in the segregation and transport of proteins synthesized in ribosomes (Porter, 1962; Fawcett, 1966; DeRobertis *et al.* 1975). Thus, the extension of the rough surfaced endoplasmic reticulum cisternae closely associated with mitochondria and the abundance of free ribosomes observed in the present study are taken to indicate the elevated synthesis and transport of proteins such as the mitochondrial enzymes involved in the anaerobic energy generating and other pathways. In mitochondria of *Ascaris lumbricoides*, cytoplasmic glucose is changed through oxalacetate into malate which is transferred to mitochondria and is then subjected to a specific type of anaerobic energy metabolism, in which fumarase and NAD-linked "malic" enzyme take an essential part (Rew and Saz, 1974). Therefore, the decreased amount of glycogen particles observed in the present study is consistently interpreted as reflecting the elevated consumption of glucose for the activated anaerobic energy-generating processes of carbohydrate dissimilation in mitochondria.

Taken altogether, the present ultrastructural evidences have indicated that in the pig *Ascaris* the epithelial cells lining the esophagus-foregut connection are specifically differentiated for carbon dioxide sensitive metabolic activity for anaerobic energy-generation. These particular epithelial cells may, accordingly, be called specified cells of anaerobic respiration, and the results obtained in the present study are to be regarded as a discovery of specifically differentiated respiratory cells in the nematode.

Summary

The ultrastructure of the epithelial cells lining the esophagus-foregut connection was examined in the pig *Ascaris* (*Ascaris suum*) following administration of carbon dioxide. The administration of carbon dioxide was found to induce specifically a pronounced increase in size of mitochondria in the cytoplasm of the particular epithelial cells. These epithelial cells are conceived to be specifically differentiated for carbon dioxide sensitive metabolic activity for anaerobic energy-generation. Thus, they are to be called specified cells of anaerobic respiration in the *Ascaris*.

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Descriptive Legends for Figures

Fig. 1 A schematic illustration of the structure of the upper parts of the pig *Ascaris* (*Ascaris suum*). Longitudinal and cross sections.

Fig. 2 Low power view of the esophagus-foregut connection in the pig *Ascaris*. The connection is invaginated into the lumen of the foregut (arrow) and the epithelial cells lining the connection are tall columnar in shape and contain an oval nucleus. Esophagus (e), foregut (f), lumen (l). Hematoxylin and eosin stained. × 70

Fig. 3 Distal parts of the cytoplasm of epithelial cells lining the esophagus-foregut connection in a control *Ascaris*. Usual cell organelles and inclusions are shown. Mitochondria (m), rough surfaced endoplasmic reticulum cisternae (er), free ribosomes (rnp), glycogen particles (gl). Uranyl acetate and lead citrate stained. × 11,200

Fig. 4 Distal parts of the cytoplasm of epithelial cells lining the esophagus-foregut connection in an experimental *Ascaris*. Note large thick thread-like mitochondria (m) with well developed cristae, extended cisternae of rough surfaced endoplasmic reticulum (er), abundant free ribosomes (rnp) and only small amounts of glycogen particles (gl). Uranyl acetate and lead citrate stained. × 11,200

Fig. 5 Mitochondria (m) in the cytoplasm of an epithelial cell lining the esophagus-foregut connection in a control *Ascaris*. The cell organelles are spherical or rod-like in shape and provided with rather sparsely arranged cristae. Glycogen particles (gl). Uranyl acetate and lead citrate stained. × 38,200

Fig. 6 Mitochondria (m) in the cytoplasm of an epithelial cell lining the esophagus-foregut connection in an experimental *Ascaris*. The organelles are larger than those in Fig. 5 and provided with an abundance of cristae. Glycogen particles (gl). Uranyl acetate and lead citrate stained. × 38,200

ブタ回虫食道前腸移行部上皮細胞糸粒体の二酸化炭素に対する著明な感応

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二酸化炭素飽和 Ringer 液中で生活させたブタ回虫の食道前腸移行部上皮細胞の超微構造を検索した．二酸化炭素の投与は特異的に同部上皮細胞質内糸粒体の顕著な増大ならびに糸粒体櫛の増数を来す．糸粒体のこれらの変化に伴つて，粗面小胞体槽の延長拡大，リボゾームの増加，グリコーゲン粒子の著明な減少などが認められる．ブタ回虫の食道前腸移行部上皮細胞は嫌気的エネルギー産生に必要な二酸化炭素感受性代謝活動のために特異的に分化した細胞と考えられ嫌気性呼吸のために特殊化した細胞というべきものである．