STUDIES ON THE PHYSIOLOGICAL AND STRUCTURAL CHARACTERISTICS OF RAT INTESTINAL MUCOSA

Mitochondrial Structural Changes During Amino Acid Absorption

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ABSTRACT

Sections from mucosal strips and rings of rat jejunum were studied with the light microscope and the electron microscope before and after incubation in a modified Krebs bicarbonate Ringer. Various additions were made to the incubation medium, and their effects on both the structure and the respiratory activity of the mucosal tissue were noted. In those cases in which an amino acid mixture was added, there was a pronounced increase in the rate of respiration. When strips of intestine were used, the presence of the amino acid mixture more than doubled the rate of oxygen consumption. Along with the increased levels of respiration there was a sharp rise in the percentage of mitochondria assuming a condensed ultrastructural conformation. The amino acid mixture did not cause the condensation of jejunal mitochondria if glucose was included in the incubation medium or if 2,4-dinitrophenol was present. The evidence suggests that a high proportion of the jejunal mitochondria assumes a condensed conformation in response to an increased energy demand. Apparently glucose can prevent the amino acid mixture from increasing the energy drain on the oxidative processes in these cells. Although a high rate of respiration was obtained in the presence of dinitrophenol, the studies indicated that mitochondrial condensation was only associated with a high rate of coupled oxidative phosphorylation.

INTRODUCTION

We were prompted to study the relationship between cellular respiration and mitochondrial structural conformation by two rather unrelated investigations. First, Bronk and Parsons (1966 *a*) discovered that in the presence of an amino acid mixture the oxygen consumption of mucosal strips of rat intestine rose to almost three times the control value. This change from a reasonably normal rate of respiration to a high rate is reminiscent of the phenomenon of respiratory control in which the rate of mitochondrial respiration increases dramatically with the addition of a phosphate acceptor.

Second, Hackenbrock (1966), in a study of mitochondria in the different respiratory states (Chance and Williams, 1955), was able to show that isolated liver mitochondria had orthodox ultrastructure only when they were taken from incubations with no phosphate acceptor. On the other hand, tightly coupled mitochondria from

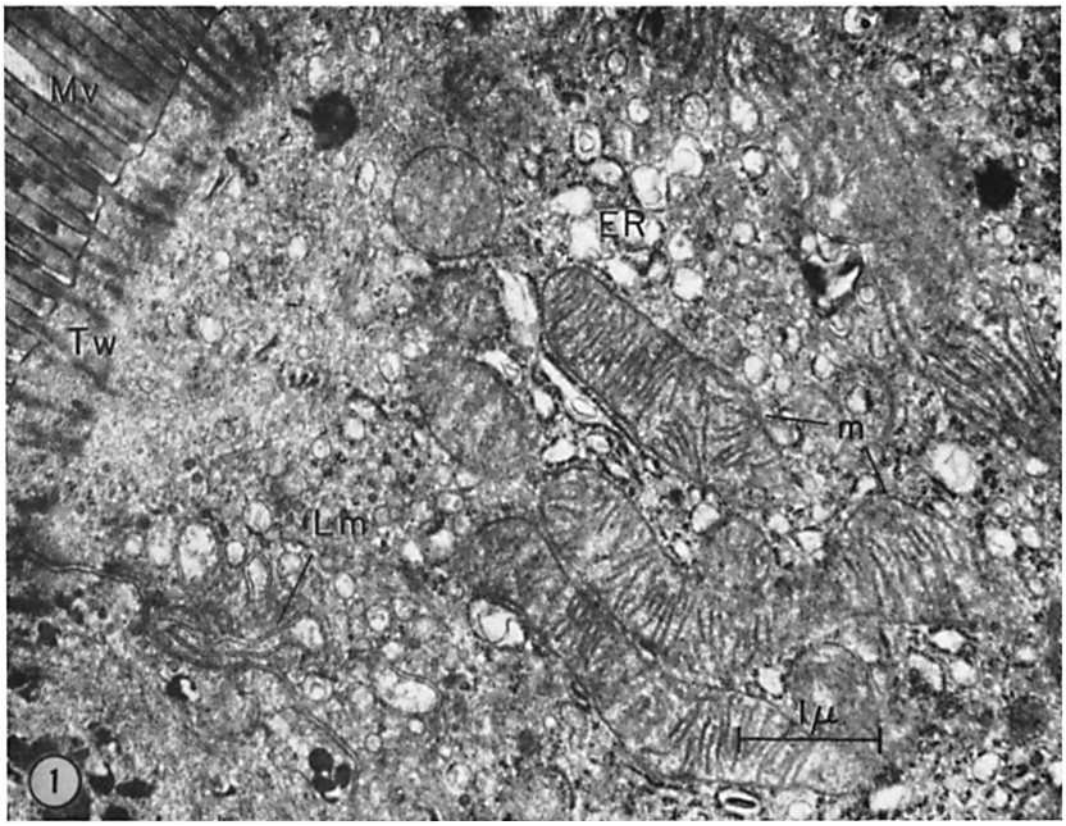


FIGURE 1 Electron micrograph of a section of a jejunal ring from unincubated tissue. The view shown is the apical region of three adjacent mucosal epithelial cells from a villus cut longitudinally. Mitochondria (*m*) are characteristically orthodox in appearance. Smooth- and rough-surfaced elements of the endoplasmic reticulum (*ER*) are in moderate abundance as vesicular or cisternal profiles. Other typical features of this cell type are recognizable: *Mv*, microvilli; *Tw*, terminal web; *Lm*, lateral cell membrane. × 20,000.

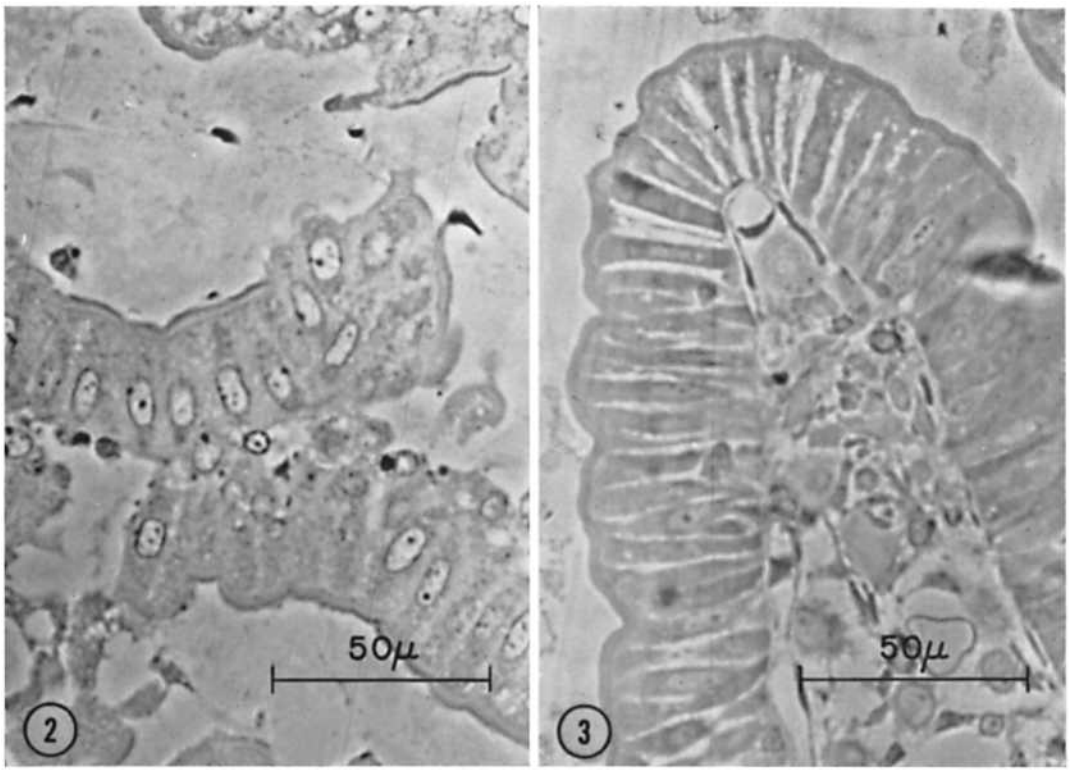
incubations in the presence of a phosphate acceptor, inorganic phosphate and substrate showed a highly condensed conformation. We decided to investigate the possibility that a change in mitochondrial ultrastructure might accompany the increase in respiration of intact rat intestinal mucosal cells incubated with amino acids. Preliminary reports of some of these findings have been made (Bronk and Jasper, 1967 and 1968).

MATERIALS AND METHODS

Physiological

Mucosal slices and rings were cut from the jejunum of male rats (225–300 g) of local stock according to the methods of Bronk and Parsons (1965). The tissue was incubated for 3–5 min in 2 ml of a modified Krebs bicarbonate Ringer with the following composition: NaCl, 118 mM; $NaHCO_3$, 25 mM; KCl, 4.7 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2 \cdot 6H_2O$, 1.3 mM. The pH of the incubation medium was maintained at 7.5 by equilibration with 95% air mixed with 5% CO_2. All incubations were carried out at 38°C. Other substances were added to the incubation medium where indicated in the tables and figures. These additions were made to give the following final concentrations: D-glucose, 28 mM; amino acid mixture (see Bronk and Parsons, 1966 *a*) 1 mg/ml; oligomycin-B, 10 μg/ml; 2,4-dinitrophenol (DNP), 5×10^{-4} M.

Respiration rates were followed polarographically by recording changes in oxygen tension during the incubations (Bronk and Parsons, 1965). Oxygen consumption was expressed as Q_{O_2} (microliters of O_2 consumed per milligram tissue dry weight, per hour). The dry weights were 3–6 mg for rings and 1–3 mg for strips.



FIGURES 2 and 3 Light micrographs of intestinal tissue after incubations in Ringer medium. Fig. 2 shows parts of a mucosal strip after incubation in the Ringer medium with 28 mM D-glucose added. The area shown is a longitudinal cut through the cells. Intact lamina propria regions are absent. Fig. 3 depicts an apparently active region of a villus from a jejunal ring incubated with added glucose and the amino acid mixture (1 mg/ml). The areas suggesting greater activity are at the tip and on the left. In contrast with these areas, the right villus region does not show intra- and intercellular distensions. Expansion of the lamina propria also is in evidence. × 600.

Structural

For morphological studies, portions of unincubated control tissue were placed immediately into fixatives after removal from the animals. Other tissue samples were placed directly into fixatives at the end of each incubation.

Fixations of 1–2 hr were routinely done at 0°–5°C in a 2.5% solution of glutaraldehyde (Sabatini et al., 1963) buffered with 0.2 M sodium cacodylate plus 2 mM calcium chloride at pH 7.4–7.5. This first fixation was followed by two-½ hr rinses in chilled (0°–5°C) 10% sucrose plus calcium chloride (2 mM) and sodium cacodylate buffer (0.2 M). The tissue was further fixed for about 1 hr in 1% osmium tetroxide buffered with 0.15 M sodium phosphate at pH 7.0–7.2 (0°–5°C). Dehydration was done in ethanol-water mixtures at room temperature (approximately 18°C).

Epikote (Epon) 812 was the standard embedding medium (Luft, 1961). For proper orientation, tissue was selectively oriented during embeddings or remounted after the resin hardened. Thick (1–2 μ) and thin (≃300–800 A) sections were cut on a Huxley Cambridge Ultramicrotome with glass or diamond knives. Thick sections were mounted on glass slides in oil (N_D 1.46), and studied with the phase-contrast microscope. The thin sections were mounted on bare 200-mesh copper grids and stained first with a saturated solution of uranyl acetate; subsequently they were stained with lead citrate (Reynolds, 1963). These sections were viewed with an AEI EM6B.

RESULTS

Mitochondrial Structure after Control Incubations

Our first step was to define the structural characteristics of epithelial mucosal mitochondria

TABLE I

Respiration of Strips of Rat Intestinal Mucosa in Bicarbonate Ringer

Substrate added	No. incubations	Q_{O_2}*
		(μl O_2/mg tissue dry wt/hr)
None	17	19.2 ± 2.6
D-glucose, *28 mM*	15	23.8 ± 2.2
Amino acid mixture, *1 mg/ml*	14	49.2 ± 7.6
Amino acid mixture + glucose	6	23.7 ± 3.1
Amino acid mixture + oligomycin, *10 μg/ml*	10	32.3 ± 2.8
Amino acid mixture + 2,4-dinitrophenol, 5×10^{-4} *M*	9	41.8 ± 3.8

* Mean ± standard error of mean

TABLE II

Respiration of Rings of Rat Intestinal Mucosa Incubated in Bicarbonate Ringer

Substrate added	No. Incubations	Q_{O_2}*
		(μl O_2/mg tissue dry wt/hr)
None	25	6.8 ± 0.3
D-glucose, *28 mM*	10	6.0 ± 0.3
Amino acid mixture, *1 mg/ml*	5	9.8 ± 0.5
Amino acid mixture + glucose	6	7.5 ± 0.5
Amino acid mixture + oligomycin, *10 μg/ml*	4	7.5 ± 0.7

* Mean ± standard error of mean

under various control conditions. Although a small proportion of the mitochondria of unincubated tissue were condensed, most mitochondrial profiles were similar to those commonly found in cells of other mammalian tissues and thus are characterized as orthodox. Such mitochondria appear as either rod-shaped or spherical units which average about 0.5–0.7 μ in diameter and have lengths of 3 μ or more. These organelles contain cristae surrounded by scattered matrix in the manner usually seen in mitochondria of the intact cell. An example is shown in Fig. 1.

To assess structural integrity and morphological relationships of incubated jejunal tissue, we studied all preparations consistently with the light microscope as well as with the electron microscope. Figs. 2 and 3 are phase-contrast micrographs of portions of jejunal slices and rings; they illustrate that the mucosal epithelium remains essentially intact after incubation. Fig. 2 shows a sample section of an intestinal slice incubated in 28 mM D-glucose. Although the basal lamina propria region is lacking, the epithelium is remarkably intact. Fig. 3 shows part of an active region of a villus from a ring incubated in the presence of an amino acid mixture with added glucose. Intra- and intercellular distensions are observable at the tip and on the left of the villus but are absent on its right. An enlarged lamina propria region also is evident.

When mucosal strips are incubated in the absence of added substrate, the mean oxygen consumption is 19.2 μl/mg tissue dry weight per hour (Table I). In the presence of 28 mM D-glucose there is no statistically significant rise in respiration. In contrast to the slices, intestinal rings incubated with or without added glucose show consistently lower respiratory activity (Table II). Similar findings were reported by Bronk and Parsons (1965).

When slice preparations of well-preserved rat jejunum incubated in the absence of added substrate or in the presence of glucose are viewed in the electron microscope, mitochondria appear in the form shown in Figs. 4 and 5. A major proportion of the mitochondria observed under these conditions contain a rather diffuse matrix, which is similar to that of mitochondria from unincubated rat small intestine. It should be made clear, however, that some compartmental or cristal reorganization occurs particularly in the absence of added glucose (Fig. 4). This change in conformation is referred to as an intermediate type of condensation and could be due to the changes in endogenous substrate levels during the incubation. The mitochondria of most ring preparations under similar experimental conditions show little structural change as the result of incubation.

Effects of Amino Acids on Respiration and on Mitochondrial Conformation

When strips of rat jejunum are incubated with an amino acid mixture, their respiration increases to a very high level (Bronk and Parsons, 1966 a). In the present study with mucosal strips, incubation with amino acids more than doubled the Q_{O_2} obtained without any addition or in the presence

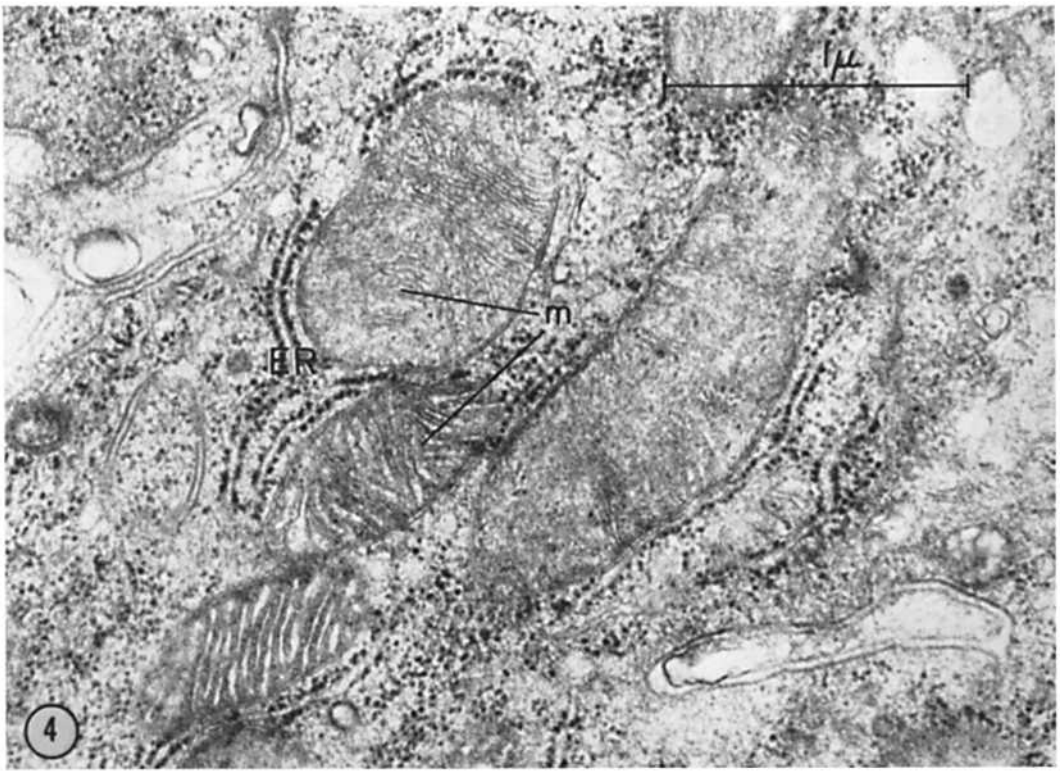


FIGURE 4 A portion of an epithelial cell from a jejunal slice incubated without added substrate. The electron micrograph depicts mitochondria (*m*) in the apical region of the cell. Although mitochondrial compartmental reorganization is evident in some cases, most mitochondria under these conditions do not show a highly "condensed" conformational change. The organelles with the somewhat denser or more concentrated matrix are referred to as an intermediate type. *ER*, endoplasmic reticulum. × 40,000.

of glucose (Table I). The changes in Q_{O_2} shown by the more intact intestinal rings are less pronounced (Table II). Nevertheless, the average Q_{O_2} of the rings is significantly higher during incubation with added amino acids.

Electron microscopic observations reveal that the large increase in metabolic activity is accompanied by definite structural changes in the tissue. In this paper only the changes in mitochondrial structure will be reported in detail; however, distension of the rough-surfaced endoplasmic reticulum (ER) also commonly accompanies amino acid uptake by the intestinal mucosa (Fig. 6).

A very large proportion of the mitochondria in intact cells of this highly respiring epithelial tissue have a dense matrix (Figs. 6, 7, and 12). In most of the mucosal strip preparations this condensation of the matrix is associated with a refolding and reorganization of the cristae and an increase in the intracristal spaces. The profiles of most condensed mitochondria are $\simeq$0.2–0.5 μ in diameter and are often only 1–2 μ long.

Mitochondrial condensation occurred during the incubation of both mucosal strips and jejunal rings, but the cells of the epithelial strips show mitochondria with the highest degree of condensation and the most extensive refolding of the internal membrane (Figs. 6 and 7). These mucosal strip preparations also have the highest rates of respiratory activity (Table I) and the greatest number of condensed mitochondria (Table III) when incubated with amino acids. Nevertheless, although the respiration rates of the rings generally seem low, a much greater proportion of condensed mitochondria is found in the incubations with amino acids (Table III). In such incubations the Q_{O_2} is also increased significantly above the normal value (Table II). Thus, within certain

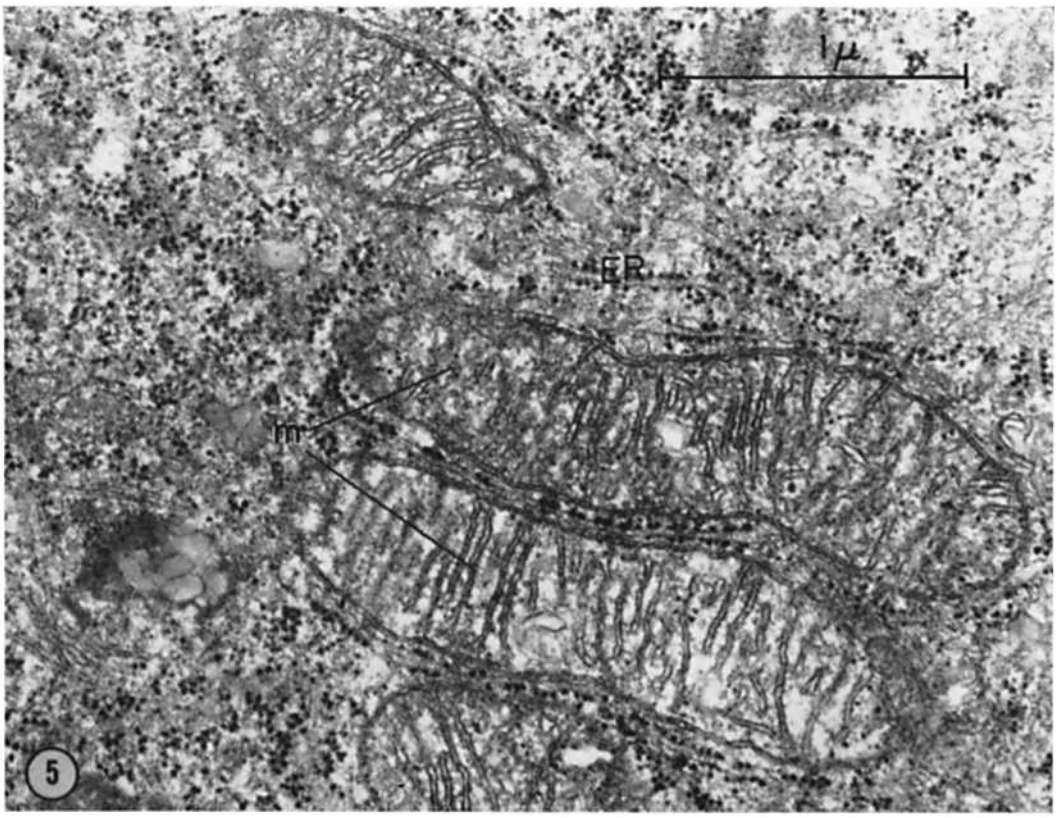


FIGURE 5 Mitochondria (*m*) in the apical region of a cell from a mucosal strip incubated with added glucose (28 mM). These mitochondria have little or nothing to distinguish them from typical orthodox organelles. *ER*, endoplasmic reticulum. × 40,000.

limits, changes in mitochondrial conformation and condensation are directly correlated with the rate of oxygen consumption.

Q_{O_2} *and Mitochondrial Structure in the Presence of Amino Acids Plus Glucose or Oligomycin*

Slices of mucosal tissue incubated in the presence of an amino acid mixture with added glucose show a much slower rate of respiration than that found in the presence of the amino acid mixture alone (Bronk and Parsons, 1966 *a*; and Table I). This effect of added glucose is also shown for rings (Table II). In spite of the presence of the same concentration of the amino acid mixture under these conditions, the respiratory rate was closer to that obtained with the addition of no substrate at all or with glucose alone.

It should be noted that neither the accumulation of the amino acid mixture nor the incorporation of amino acids into protein is diminished by the presence of glucose (Bronk and Parsons, 1966 *b*). On the other hand, it is significant that the reduced respiration rate in the presence of the amino acid mixture and glucose is directly correlated with a reduction in the number of mitochondria showing condensed ultrastructural conformation (Table III). Fig. 8 shows typical fields of mitochondria in a mucosal slice which was incubated with amino acids and glucose. Mitochondrial structures are clearly of the orthodox type.

As a further check on the possible relationship between the rate of tissue respiration in the presence of amino acids and the observed conformational shifts in mitochondria, we incubated mucosal slices in a medium including both the amino acid mixture and oligomycin, the inhibitor of ATP formation (Lardy et al., 1958). About one-half the rise in respiration caused by the amino acid mixture is prevented by the presence of 10 μg/ml oligomycin (Table I). In addition, few of the

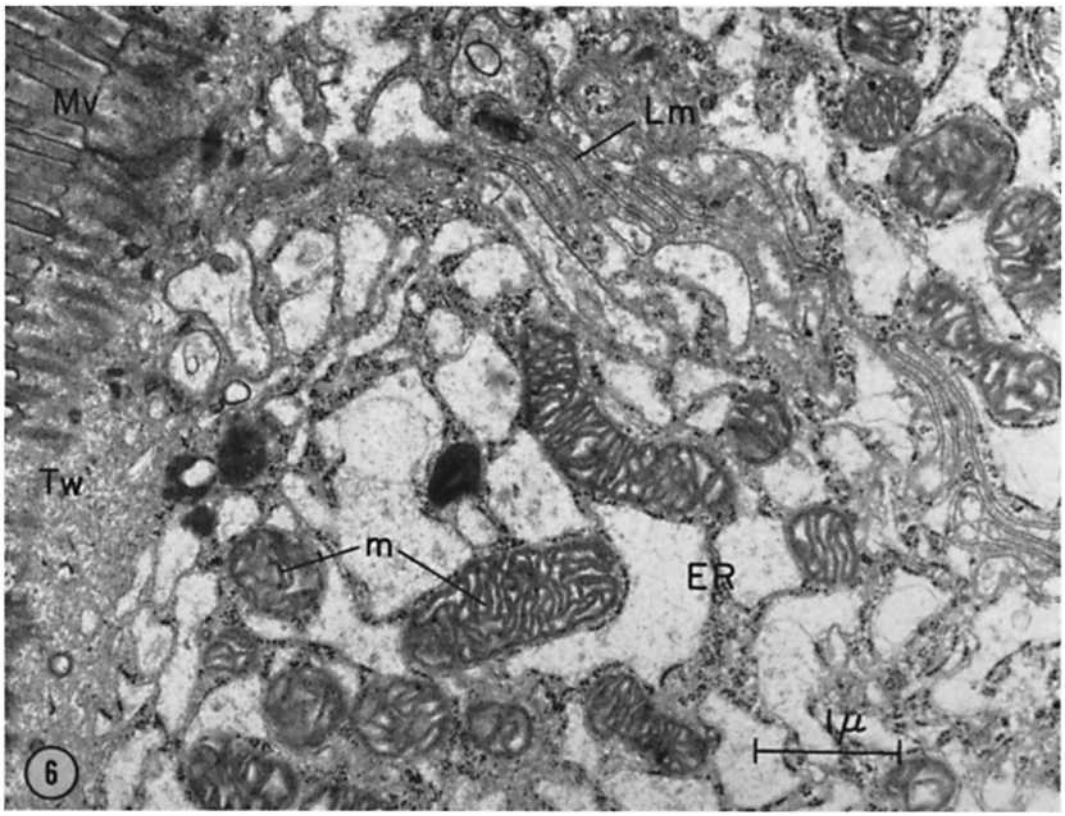


FIGURE 6 Mitochondria (*m*) of the apical portions of epithelial cells from mucosal strips incubated in the presence of 1 mg/ml amino acid mixture. These mitochondria show deep infoldings of inner membranes and marked reorganization of inner and outer compartments. Cristal profiles no longer exhibit their typical orderly parallel arrangement, as frequently encountered in orthodox mitochondria. A condensed and more tightly packed mitochondrial matrix and a widening of intracristal areas are both evident. These mitochondria are termed condensed. Note the widening of luminal areas of the endoplasmic reticulum (*ER*). *Mv*, microvilli, *Tw*, terminal web, *Lm*, lateral cell membrane. × 20,000.

mitochondria assumed condensed ultrastructural conformation (Table III). Fig. 9 shows a portion of a mucosal slice from an experiment of this type. From this field it can be seen that, while these mitochondria may not appear typical or orthodox, they certainly lack a condensed ultrastructural conformation.

Rates of Respiration and Mitochondrial Conformation in the Presence of Amino Acids Plus 2,4-dinitrophenol

2,4-dinitrophenol prevents the formation of ATP in mitochondrial systems by uncoupling oxidative phosphorylation. In mucosal tissue slices incubated in the presence of amino acids, dinitrophenol does not increase the mean respiration rates (Table I), but the percentage of condensed mitochondria is significantly reduced in its presence (Table III). Mitochondrial conformation in experiments of this type shows considerable variation, especially in cristal and compartmental organization. Fig. 10 illustrates examples of the two most common variations. In some cases the mitochondrial structure is hardly altered by dinitrophenol (Fig. 10 *a*), while in others there is obvious mitochondrial disorganization (Fig. 10 *b*).

The Percentage of Condensed Mitchondria per Cell Profile

Because of the wide variations in the percentage of condensed mitochondria, it seemed worthwhile

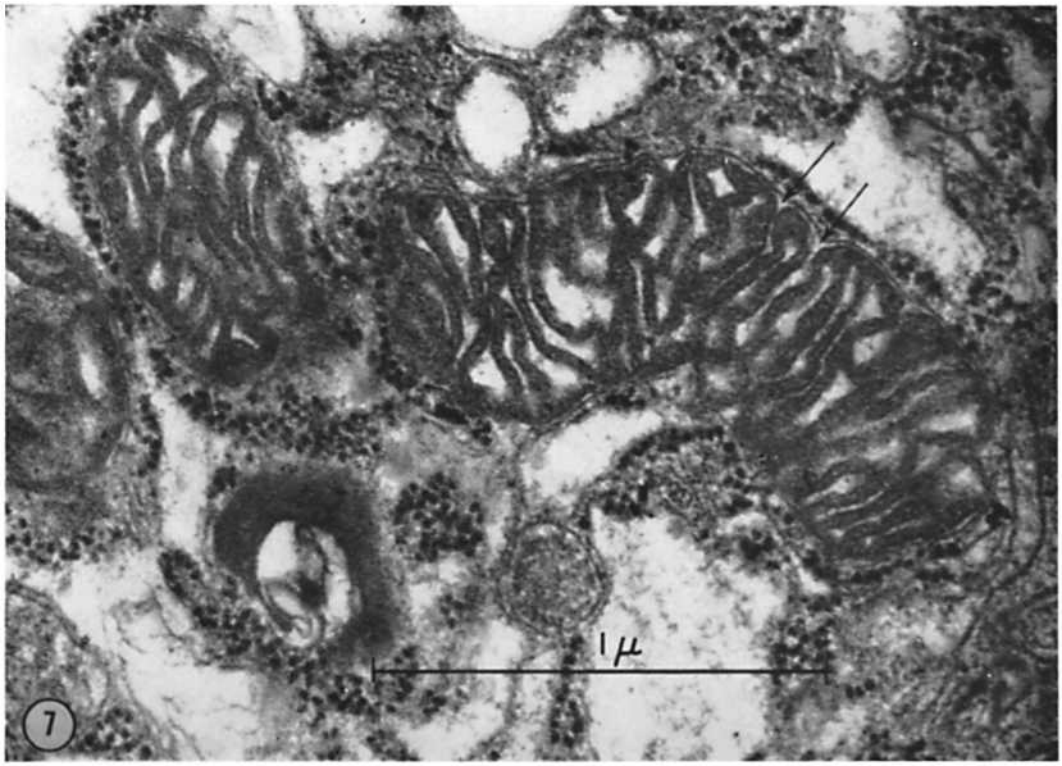


FIGURE 7 The deep infoldings of a condensed mitochondrion's inner membrane is shown at a higher magnification. These infoldings enclose the enlarged intracristal spaces which run more or less perpendicular to the mitochondrion's long axis and are seen to be continuous with the outer compartment (arrows). Matrical packing and condensation and the comparative fineness of mitochondrial membranes also are shown. Section from intestinal slices incubated in the amino acid mixture. × 60,000.

to note the distribution of mitochondrial types per cell in these intestinal strips. Fig. 11 shows the proportion of orthodox and condensed mitochondria in tissue incubated under three sets of conditions. Depending on the plane of section, mitochondrial profiles per cell range from approximately 5 to 50 or more. Average sections show about 20 mitochondrial profiles per cell. In the presence of amino acids, there is an increase in the percentage of cells having a predominance of condensed mitochondria and a corresponding decrease in cells with orthodox mitochondria or a mixture of mitochondrial types. In the presence of amino acids plus glucose, on the other hand, a high proportion of cells maintain predominately orthodox mitochondria. These results suggest that most of the mitochondria in a cell will respond to the strong stimulation of respiration caused by the amino acid mixture. However, it is also true that conditions which ensure a more normal Q_{O_2} also lead to a larger proportion of cells with a mixture of condensed and orthodox mitochondria.

This general relationship between respiration rate and the proportion of cells with condensed mitochondria is most marked in mucosal slices, but it is also clearly evident in rings of intestine. In rings, however, less complete ultrastructural changes are frequently encountered in comparable mitochondrial populations. Fig. 12 depicts an area from a jejunal ring in which a mixed population of mitochondria with variable conformational changes is found. Our results indicate that, in incubations in which the presence of amino acid mixture increased the rate of respiration, mitochondria with condensed ultrastructure are a consistent and characteristic feature. The only exception to this is found in the presence of the uncoupler, 2,4-dinitrophenol.

TABLE III

Proportion of Mitochondria Showing Condensed Ultrastructural Conformation in Rat Intestinal Mucosa under Various Conditions

Substrate added	Mitochondrial profiles counted	Condensed mitochondria
		%
Incubated Strips		
None	234	13
Glucose, *28 mM*	220	20
Amino acid mixture, *1 mg/ml*	214	73
Amino acid mixture + glucose	182	24
Amino acid mixture + oligomycin, *10 μg/ml*	335	22
Amino acid mixture + 2,4-dinitrophenol, 5×10^{-4} *M*	322	12
Unincubated Strips		
None	250	14
Incubated Rings		
Amino acid mixture	118	56
Amino acid mixture + glucose	180	7

DISCUSSION

The results of the present study indicate an interrelationship between the respiration of the intact jejunal cell and the ultrastructure of mitochondria. This relationship is most striking in mucosal strips which exhibit a 2.5-fold increase in respiration rate in the presence of an amino acid mixture. In such experiments, over two-thirds of the mitochondria may show a condensed ultrastructural conformation. It is also clear that under these conditions there are many cells in which virtually all of the mitochondria are condensed.

We have found that the respiration rates of mucosal strips consistently exceed those of intestinal rings. In an earlier paper (Bronk and Parsons, 1965) it was shown that this difference could not be due to the presence of nonmucosal tissue. Two possible reasons for these disparate levels of oxygen consumption were considered: tissue damage and the effects of exposing the internal face of the mucosal cells to the medium. While the present morphological studies have confirmed that the structural integrity of jejunal tissue is better preserved in the rings than in the mucosal strips, the extent of the damage found in the strips seems unlikely to account for all of the differences or for the greatly increased respiration rate. For example, despite the high variability of the tissue respiration in the presence of the amino acids, the mean Q_{O_2} of strips incubated with this mixture is increased to a level near 50. This level represents a significant rise in tissue respiration compared with that observed in the absence of additions or in the presence of the amino acid mixture plus glucose. Furthermore, this rise in metabolic activity is correlated with an increase in the number of condensed mitochondria. Since the Q_{O_2} of the rings approximates only 20% of that obtained with slices, it seems quite likely that much of the mucosal tissue of the rings is not stimulated to give a high rate of respiration in the presence of the amino acid mixture. This lack of response may be partly due to a failure of the amino acids to reach all of the cells in the rings. Indeed, our observations with the light microscope suggest that the comparatively high levels of respiration characteristic of jejunal slices are not due primarily to tissue damage, but rather to a greater exposure of the epithelial cellular surfaces to the incubation medium. Other factors, such as an apparent asynchrony in the metabolic activity of mucosal epithelial cells, obviously contribute to some of the variability in the response of both slices and rings to the different conditions.

The shifts in ultrastructural conformation which we have observed in our experiments are attended by increases in intracristal areas and matrical condensation. These changes are most similar to the reversible *ultrastructural* changes observed by Hackenbrock (1966) in isolated liver mitochondria during state III oxidative phosphorylation. However, the observations made by Packer (1960) on isolated mitochondria which demonstrated that the state III phosphorylation was accompanied by nonspecific light-scattering changes may also be relevant, if the reduction in the profile areas of the condensed mitochondria can be shown to reflect a volume decrease.

We feel that the condensed conformational changes and cristal reorganization observed in mitochondria during high rates of respiratory activity are mainly indicative of a high rate of coupled oxidative phosphorylation within these mitochondria. This view is substantiated by the absence of the condensed configuration in the presence of the uncoupler, 2,4-dinitrophenol. It should also be pointed out that the condensed

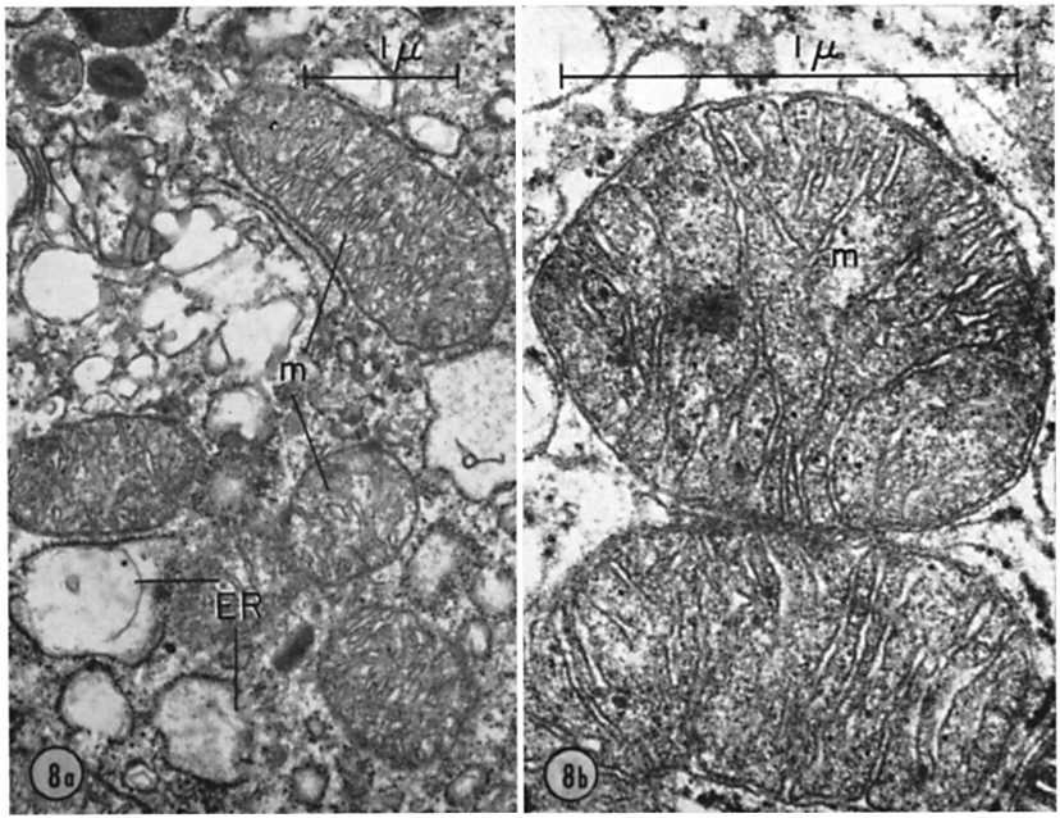


FIGURE 8 Mitochondria (*m*) from intestinal strips incubated in Ringer medium with the amino acid mixture and added glucose. Conformation of these profiles is termed orthodox. *ER*, endoplasmic reticulum. Fig. 8 *a*, × 20,000; Fig. 8 *b*, × 60,000.

ultrastructural conformation is rarely, if ever, observed in the mitochondria of noticeably damaged cells and never observed in obviously disrupted cells or isolated fragments. This suggests that essentially intact cells are required for a continual supply of phosphate acceptor in the presence of the amino acid mixture.

The high rate of respiration which is obtained in the presence of the amino acid mixture must result either from an increase in the energy demand in the mucosal cells or from an uncoupling of oxidative phosphorylation; otherwise the high Q_{O_2} would result in an overproduction of ATP. In the presence of the amino acid mixture alone, there is a high rate of respiration, a high proportion of condensed mitochondria, and rapid incorporation of the absorbed amino acids into protein (Bronk and Parsons, 1966 *b*). When 2,4-dinitrophenol is added along with the amino acids, the high rate of respiration remains, but few mitochondria are condensed and, as reported in the earlier paper, the incorporation of amino acids into protein is reduced to less than 10%. In our view, these observations indicate that the condensed mitochondria in the actively respiring mucosal strips remain tightly coupled. The fact that dinitrophenol does not increase the respiration rate also suggests that the high rate of respiration in the presence of the amino acid mixture alone is close to the maximum rate. As defined by Chance and Williams (1955), the maximum rate of coupled oxidative phosphorylation occurs in state III, that is, when the mitochondria have an excess of substrate and phosphate acceptor. Thus, from our results we conclude that mitochondria in intact mucosal cells adopt a condensed structural conformation when they are in state III. This conclusion concerning mitochondria in the intact cell

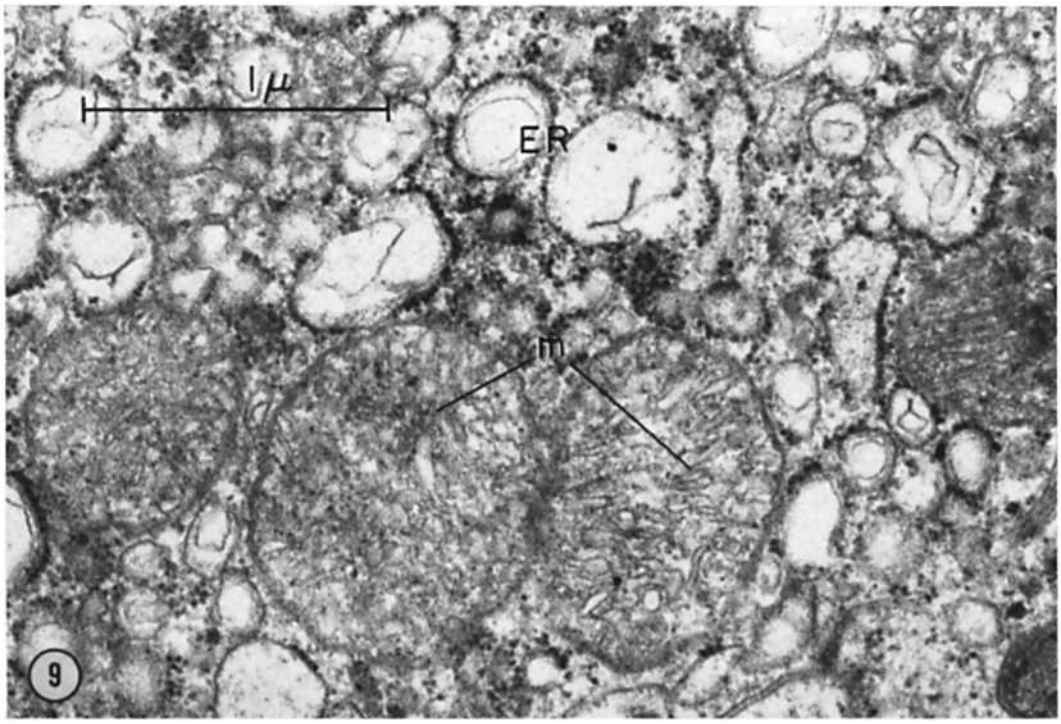


FIGURE 9 Mitochondria (*m*) from a mucosal preparation of strips incubated in the amino acid mixture with 10 μg/ml oligomycin added. In general, these mitochondria lack distinct cristal organization and cannot be termed typically orthodox, but they are not condensed. Note vesicular appearance of the endoplasmic reticulum (*ER*) studded with ribosomes. × 40,000.

is strongly supported by the results obtained by Hackenbrock (1966) with isolated mitochondrial preparations. The present results also suggest that, in mucosal cells under reasonably normal conditions, most mitochondria are not in the highly active state III and that therefore the levels of available phosphate acceptor must be low. The preponderance of cells which have all their mitochondria in the orthodox form in control incubations supports this contention.

It is interesting to note that the presence of glucose appears to be sufficient to prevent the rise in phosphate acceptor levels since it stops the consequent increases in respiration and in the percentage of condensed mitochondria. One interpretation of such findings is that glycolysis may serve to support amino acid uptake in the presence of added glucose, but it is also possible that the glucose can act in some other way to prevent the process of amino acid absorption from causing a drain on the supply of energy by oxidative systems.

Although one may question the effectiveness of oligomycin in intact mucosal tissue, it is known that in isolated mitochondria oligomycin prevents the formation of ATP (Lardy et al., 1958). This means that it will also inhibit respiration in a tightly coupled system, even though it has no direct effect on the electron transport chain. Any respiration which was not associated with ATP formation would be unaffected by the addition of oligomycin.

When oligomycin is added, the rise in cellular respiration caused by the amino acid mixture is less dramatic and over 50% of the increased respiration shown by mucosal strips in the presence of the amino acids is prevented. This observation is in agreement with the decreased number of condensed mitochondria as well as with the lower Q_{O_2} value (Tables I and III). Again, it should be noted that the uptake and incorporation of the amino acids cannot be considered directly responsible for the increased Q_{O_2}, since Bronk and

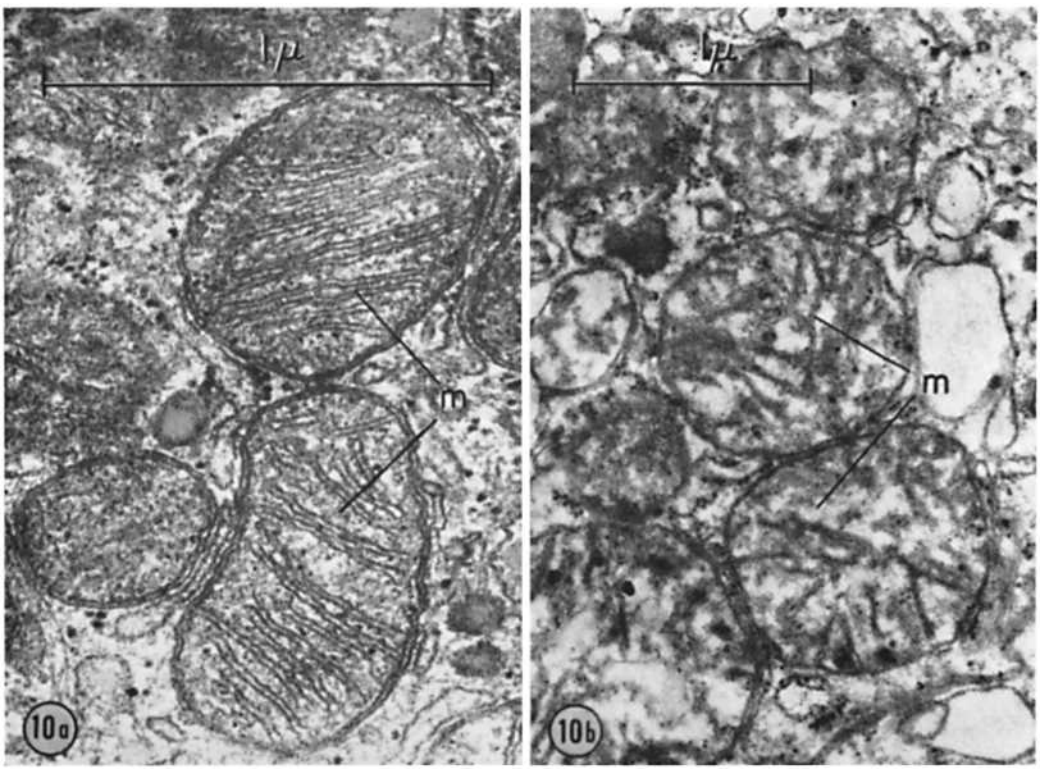


FIGURE 10 Examples of mitochondrial profiles (*m*) commonly encountered in intestinal slices after incubation with the amino acids plus 5×10^{-4} M 2,4-dinitrophenol. Under these conditions the profiles exhibit considerable structural variation. Fig. 10 *a* shows mitochondrial structures with little or no alteration from an orthodox conformation. Fig. 10 *b* depicts samples of mitochondria with highly disorganized internal regions. Fig. 10 *a*, × 60,000; Fig. 10 *b*, × 30,000.

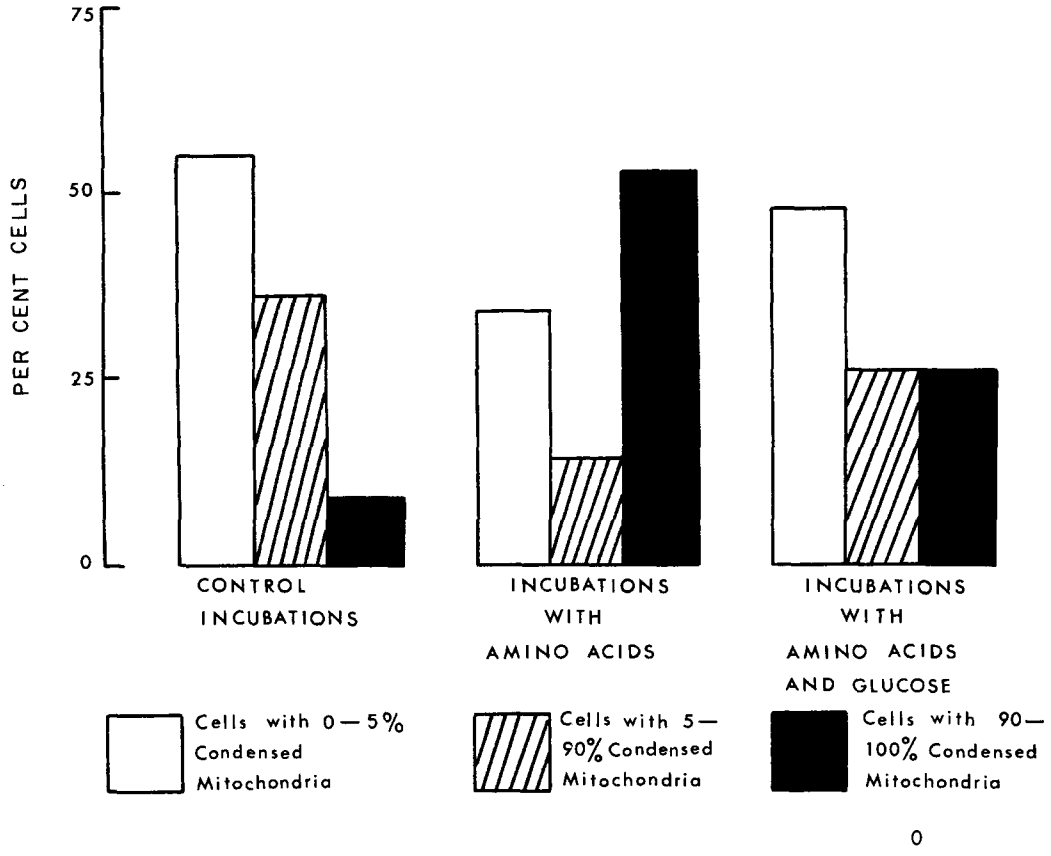


FIGURE 11 Per cent of mucosal cells showing mitochondria with condensed structural conformation. This graph shows the distribution of mitochondrial types in the profiles of 62 representative mucosal cells of epithelial strips. Under conditions in which the rates of respiration were nearer normal, e.g. incubation without added substrate or with glucose plus amino acids (Table I), more cells with orthodox or a mixture of mitochondrial types were encountered. Mitochondria were counted in 37 cells from incubations with the amino acid mixture as the only added substrate, and there was a high percentage of cells with a predominance of condensed mitochondria. Mitochondria were counted in 11 cells from incubations without added substrate, and in 14 cells from incubations with both amino acids and glucose.

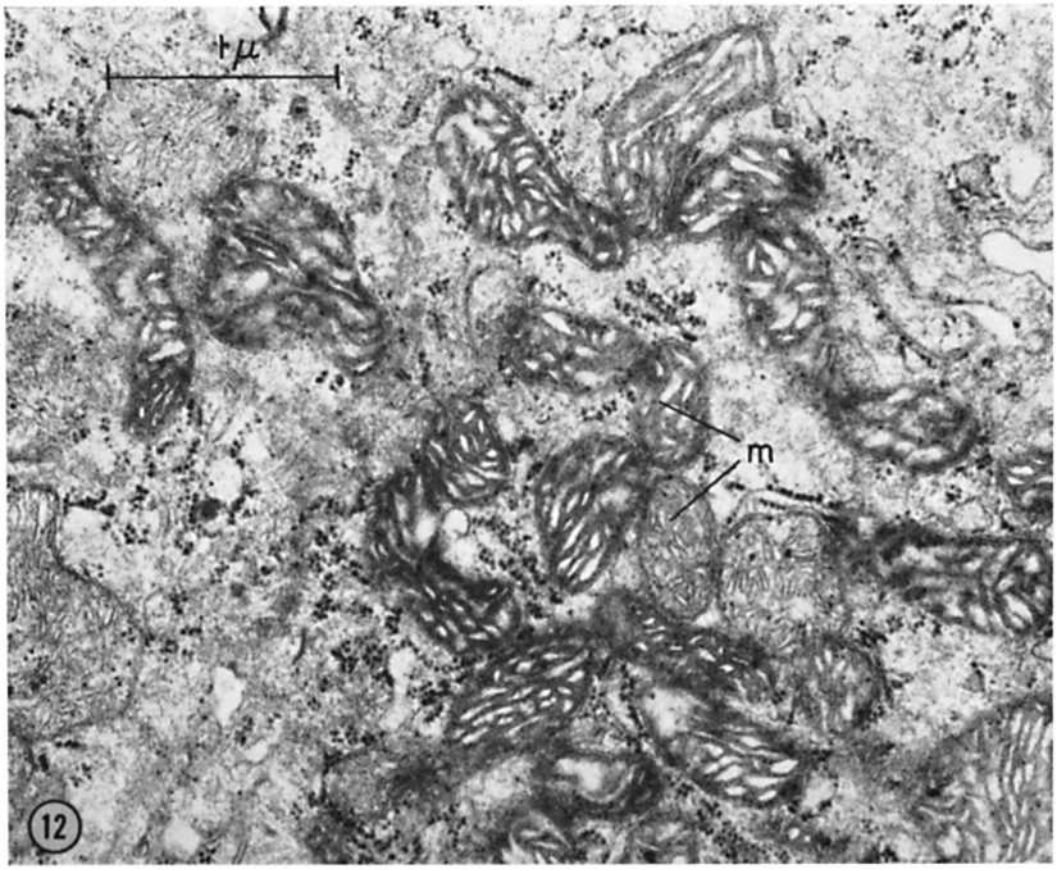


FIGURE 12 A somewhat oblique section showing mitochondria in mucosal epithelium from a jejunal ring incubated with the amino acids. The electron micrograph is representative of an apparently active region of a villus. A mixture of mitochondrial conformational types (*m*) is shown, but most profiles exhibit a remarkable degree of condensation. × 30,000.

Parsons (1966 *a* and *b*) have shown that these processes occur at an undiminished rate in the presence of either glucose or oligomycin. Nor, as the present results have shown, can these cellular activities readily account for the condensing of the mitochondria. Although oligomycin effectively prevents a considerable amount of the increased respiration by jejunal strips in the presence of amino acids, some mitochondria do show a condensed conformation in the presence of this inhibitor. One explanation for this phenomenon could be that the oligomycin, under these conditions, may not be a completely effective inhibitor of oxidative phosphorylation in all of the mitochondria in the intact mucosal tissue.

Additional studies are now underway to clarify the manner in which the amino acid mixture stimulates mucosal respiration.

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