Ultrastructural alterations of the rat intestinal epithelium fed with polymeric, oligopeptidic or elementary full diet, following starvation

D. Botsios[1], L. Economou[2], A. Manthos[2], M. Tsolaki[3], A. Sioga[2], S. Agelopoulos[1],
I. Dadoukis[1] and Ch. Foroglou[2]

[1]Department of Surgery, [2]Laboratory of Histology-Embryology and [3]Department of Neurology,
Faculty of Medicine, University of Thessaloniki, Thessaloniki, Greece

Summary. In this study the ultrastructure of rat jejunal epithelial cells was examined, following a starvation period of 72 hours and an enteral refeeding period of 12 days, with either Nutrison, Pepti 2000, or Nutri 2000. Most changes occurred in the animals examined immediately after the 72-hour starvation period; these mainly included a significant decrease in microvilli population, occasional cell membrane disintegration, and a usual microvesicular appearance and degranulation of the rough endoplasmic reticulum. No alterations were found in the normally-fed animals (control group). This was also practically the same for the Pepti 2000 group. In the Nutrison group, a small amount of changes were found, while in the Nutri 2000 group many alterations were detected, which nevertheless were fewer than in the starved animals. The results demonstrate that the micromorphological alterations of the intestinal epithelium caused by starvation improve faster when an oligopeptidic formula is provided, which consequently results in faster and better absorption of the nutrients.

Key words: Enteric nutrition, Ultrastructure, Jejunum

Introduction

Experimental and clinical studies have shown that prolonged starvation leads to serious metabolic disturbances as well as serious morphological and functional alterations of the intestinal tract (Dugue et al., 1975). However, many of these alterations could be attributed to coexisting illnesses, as well as to changes of the enteric flora. Therefore, the exact adverse effect caused by the lack of nutritional substances on the morphological and functional integrity of the gastrointestinal tract, cannot be accurately determined

(Viteri and Scheider, 1974; Baker, 1977).

On the other hand, the unfavourable consequence on the integrity of the intestinal mucosa following a short period of starvation, is well documented (Adibi and Allen, 1970; Clarke, 1975). It has been shown that these alterations are progressively restored following refeeding (Clarke, 1975; Gorostiza et al., 1985). In order to avoid the severe effects of malnutrition, various solutions of artificial enteral feeding for patients with functional or non functional integrity of the intestinal tract have been used. All these solutions were full diets, which provided the essential nutritional components, vitamins, salts and trace elements, in quantities sufficient to cover the mean daily needs of the patients. These various diets differed in the molecular type of their nutritional components, mainly of the proteins (Koretz and Meyer, 1980). Many studies have been performed in order to evaluate the degree of absorption and the nutritional value of the various types of solutions, which had been considered suitable for artificial enteral feeding (Smith et al., 1982; Meguid et al., 1984; Keohane et al., 1985; Poullain et al., 1989). Very often, the contradictory conclusions drawn from these studies, were mainly due to differences in the experimental models. The quantity and form of the contained protein, in addition to the method of its hydrolysation, as well as the quantity and form of carbohydrates and fats, are likely to influence the absorbability and nutritional value of the solution (Silk et al., 1980; Keohane et al., 1985; Poullain et al., 1989), and furthermore the morphology of the intestinal mucosa (Young, 1988).

The aim of this study was to examine the ultrastructural alterations of jejunal mucosa in animals refed with three different types of artificial enteral nourishing solutions, containing differents forms of proteins after a starvation period. An attempt was made to draw direct and indirect conclusions concerning the absorptional facility, and thus the better utilization of these nutritional

solutions.

Materials and methods

Three types of formulae were used: (1) a polymeric formula (Nutrison) with whole purified proteins of milk serum; (2) an oligopeptidic formula (Pepti 2000) containing a mixture of milk serum proteins hydrolysed by chymothrypsin and thrypsin; and (3) a formula of elementary diet (Nutri 2000) with a mixture of equal quantities of free aminoacids. These three formulae (1, 2, 3) contained the same basic nutritional components, mineral salts and vitamins, had the same caloric value, and presented small differences in osmolarity; they basically differed in the molecular forms of the contained proteins (Table 1).

Thirty male Wistar rats weighing 330-350 gr were used. Six of them were fed normally with the usual commercial food during the experimental period (pellets of mixture 510, EL.BI.Z., Table 2) (Andoniadis et al., 1987) (first control group). They were kept under the same conditions as the other animals and were sacrificed at the end of the experimental period using the same methodology, the same preparation methods and the same solutions as for the rest of the animals.

Twenty-four animals had free access to water, but no food was provided to them for 72 hours. Immediately after that, six were sacrificed (second control group). The remaining 18 were separated into 3 groups of 6 animals each, and were refed with 80 ml (90 Kcal) of full diet solution daily, for 12 days. The quantity of nutritional components used was sufficient for the daily needs of the experimental animals (Committee on Animal Nutrition, 1978). The solution (1) (Nutrison) was given to the first group; the solution (2) (Pepti 2000)
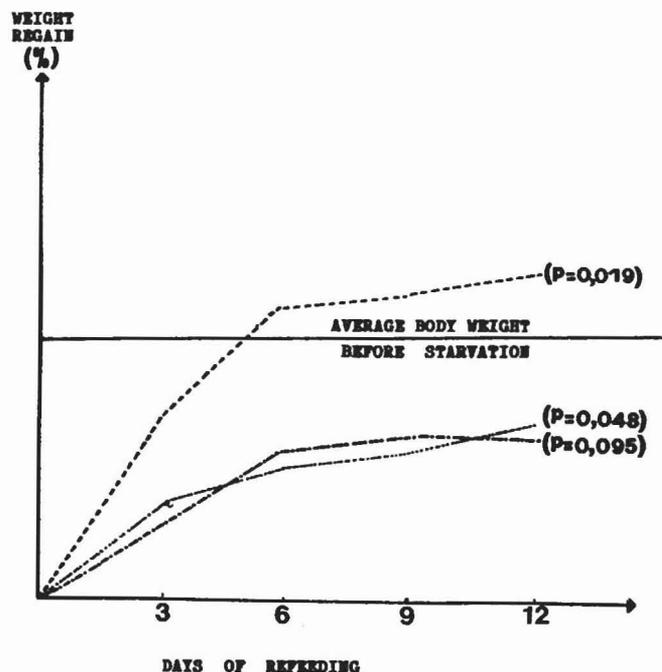
was given to the second group; the solution (3) (Nutri 2000) was used for the third group (Table 1). At the end of the twelve-day period of refeeding the 18 rats were sacrificed. All animals were weighed at the beginning of the experiment, on the third day of starvation, and then daily, until the last day of the refeeding period (12th day). To determine fluctuations of body weight, the Mann-Whietney test was used.

Tissue pieces of jejunal mucosa (approximately 1 mm^3) from the area along the mesenterium, and at distances of 1 cm to each other, were taken for TEM examination, from all the animals, following light chloroform anaesthesia. Fixation was accomplished by immersion in 3% glutaraldehyde solution (pH 7.3, Temp 0 °C) and then in 1% OsO_4 solution (pH 7.3, Room temp.). Following tissue staining with 1% aqueous solution of uranyl acetate, the tissue pieces were dehydrated using gradually denser alcohol solutions, and finally 100% alcohol. The procedure was concluded with embedding in EPON resins. Ultrathin sections (approx.

Table 2. Content in 100 gr of normal food (mixture 510 EL.BI.Z. company).

Proteins (gr)	22
Fats (Linoleic acid) (gr)	5
Carbohydrates (gr)	61.3
Cellulose (gr)	4
Ash (gr)	3
Moisture (%)	16
Metallic salts (gr)	0.65
Vitamins (gr)	0.95

Table 3. Increase in percentage of body weight following the three-day starvation. ····· = group A; − − − =group B; −·− = group C.

Table 1. Content in 100 ml solution of the three formulae.

NUTRIENTS	NUTRISON (gr)	PEPTI 2000 (gr)	NUTRI 2000(gr)
Total protein	4	-	-
Hydrolised protein	-	4	-
Free aminoacids	-	-	3.92
Fats	4	3.93	3.95
Corn oil	4	1.96	1.97
MCT	-	1.96	1.97
(Linoleic acid)	(1.2)	(0.92)	(0.96)
Carbohydrates	12	13.06	12.4
Lactose	0.2	0.25	0.25
Maltodextrine	11.6	11.9	11.8
Rest of carbohydrates	0.4	0.3	0.25
Moisture	0.87	0.87	0.87
Metallic salts	0.57	0.56	0.55
Vitamins	0.075	0.08	0.075
Caloric value (Kcal)	100	100	100
Osmolarity (mOsm/L)	260	410	520
Renal charge (mOsm/L)	340	240	196
pH	5.2	4.8	4.6

900 nm) were made using a Reichert ultratome OmV$_2$ and after treating them with lead citrate, they were examined in a JEOL 100 CX TEM, at 80 kv.

Results

The first control group, that is the animals fed normally during the experimental procedure, presented no significant differences in their weight (34 ± 7.55 gr). Their jejunal mucosa was normal, and no alterations or deviations from the normal ultrastructure were found (Fig. 1).

The 72-hour starvation period caused a mean body weight loss of approximately 12% in all starved animals. More specifically, the rats of the second control group, which were sacrificed immediately following starvation, lost 32.8 ± 2.4 gr; those of the first group lost 34.4 ± 4.27 gr, of the second 31.8 ± 2.85 gr, and of the third 36.2 ± 1.31 gr.

During refeeding all of the experimental animals gained weight; however they did not reach the initial weight on the 12th poststarvation day, except for the Pepti 2000 group. The rats of this group gained 14.3 ± 2.9%, which was 2.3% above their initial weight. The animals of the first group (Nutrison) increased their weight by 9.2 ± 0.6%, and the animals of the third group (Nutri 2000) by 7.3 ± 0.7%; that is correspondingly 3.1% and 3.8% less than the initial weight (Table 3).

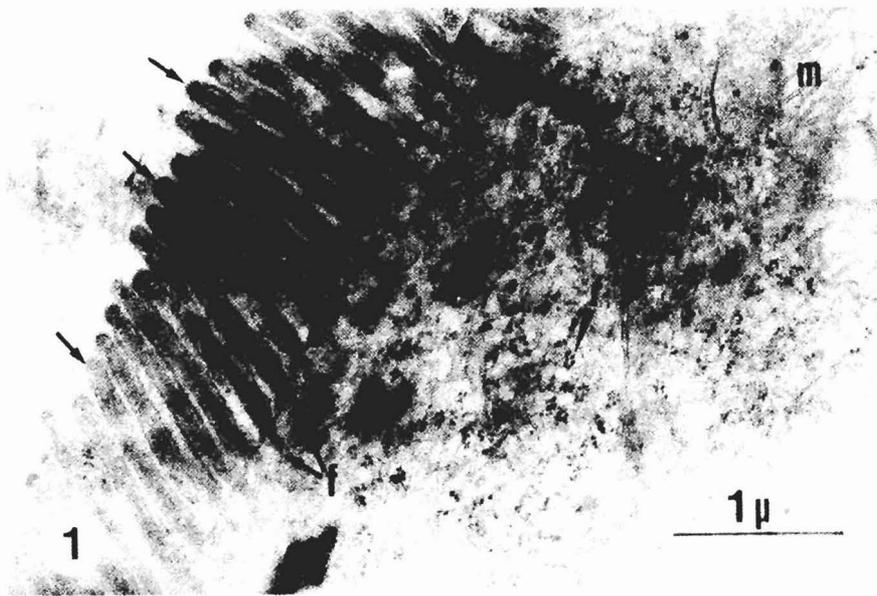In the second control group, the experimental animals which were examined immediately following

Fig. 1. Jejunal mucosa epithelium cell, in the normally-fed control animals. Normal appearance of the microvilli and of the other cellular elements. d: desmosome, m: mitochondrion, r: ribosomes, f: microfilaments.
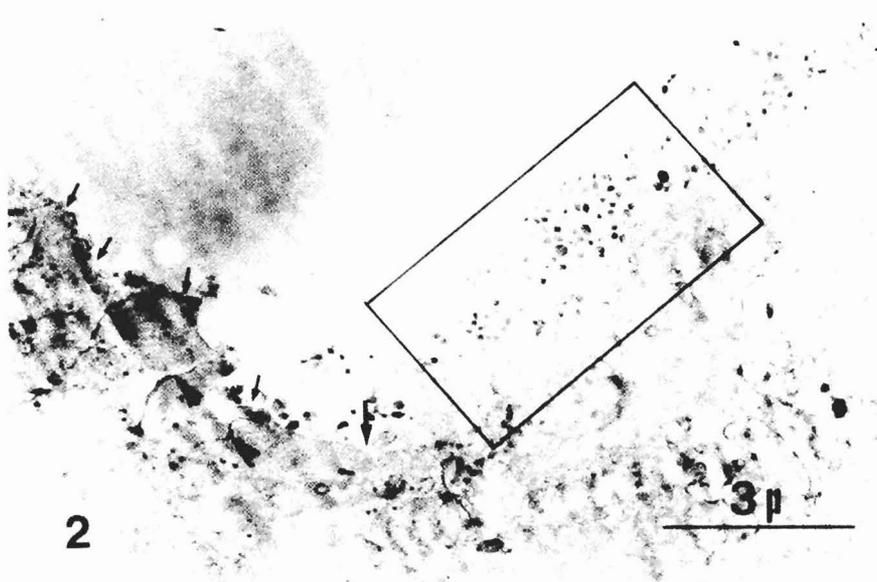

Fig. 2. Second control group of starved animals. Areas devoid of microvilli (arrows). Arrow heads show cellular boundaries. The area of the rectangle is presented enlarged in the next figure.

the 72-hour starvation period, many alterations of the ultrastructure of the small intestine epithelium were found. There were focal lesions along the jejunum, involving 30-35% of the whole mass of the mucosa, and in particular of the proximal part. Among areas with almost normal appearance, there were foci in which the cells presented intense microvesicular appearance, cystoid dilation and degranulation of granular endoplasmic reticulum. In some sites microvilli had completely disappeared. These areas were surrounded by cells with shorter and scantier microvilli (Figs. 2, 3). The cell surfaces lacking microvilli displayed intact cell membranes and normal junctions at their periphery. Rarely there were ruptures of the cell membrane at the luminal surface, while the other surfaces were intact (Fig. 4).

In the first group of animals, refed with a formula of polymeric diet (Nutrison), a great part of the cell surfaces retained microvilli which, however, in places were scantier and unequal in size. In certain sites there was a complete absence of microvilli, but there was no disintegration of the cell membrane. More characteristic findings were many cytoplasmic rarefactions, and areas of disorganization which were found within the cells, usually in the areas where there was loss of microvilli. In many places lytic areas of the cytoplasm presented an osmiophilic fine granular material (Figs. 5, 6).

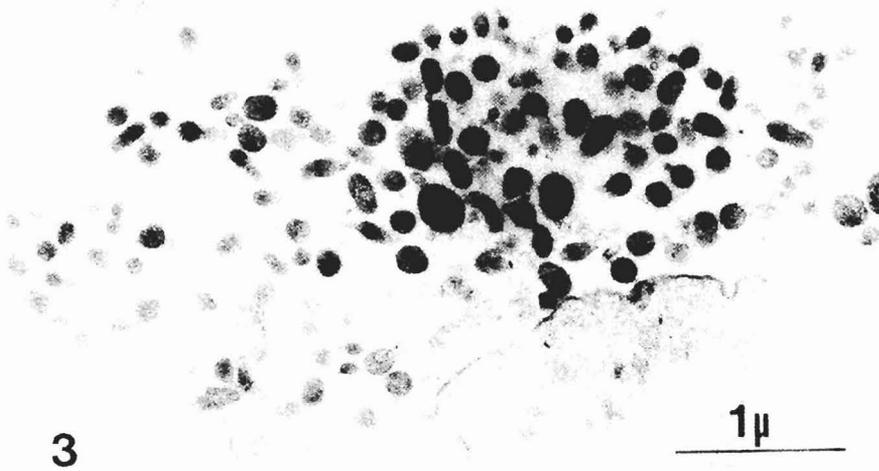In the second group of rats, fed with a formula of

Fig. 3. Enlarged area of the previous picture. The surface of the epithelial cells does not have microvilli. Above the cellular membrane there is a more or less transverse cut of adjacent microvilli, presenting differences in thickness.
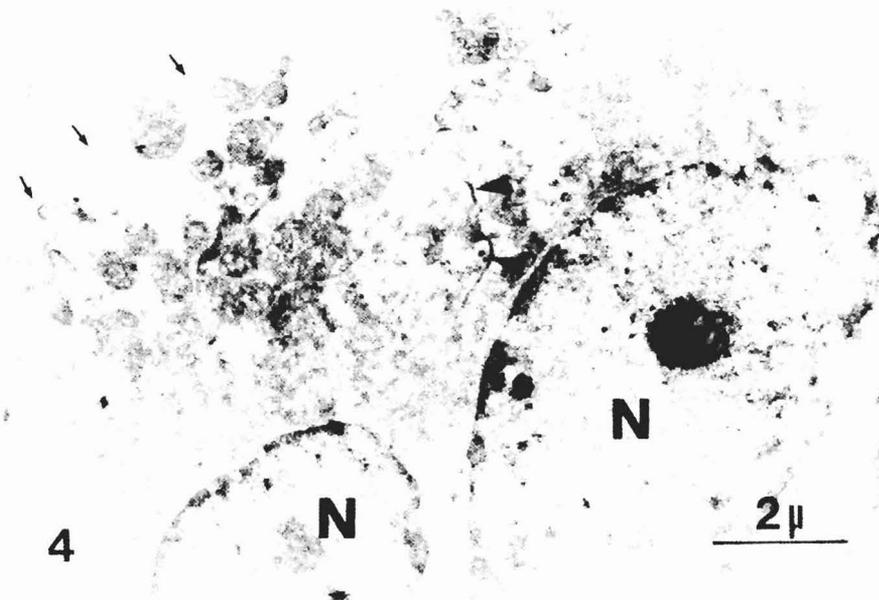

Fig. 4. Second control group of starved animals. Area with membrane disintegration (arrows). Arrow heads show cellular boundaries. N: nuclei.

oligopeptidic diet (Pepti 2000), the majority of the enteric epithelium had a normal appearance, and only very few alterations were found. The greater part of the microvilli were normal in size, shape and density. Rarely there was a decrease in the microvilli population, but these still had a normal appearance. Within the cytoplasm, in places, there was a limited number of small-sized vacuolated areas. However, in the majority of cells, the cytoplasm and the organelles had a normal appearance, except from some sparse sites with slightly oedematous mitochondria. The rough endoplasmic reticulum was normal and there were places with free ribosomes as well as polyribosomes (Figs. 7-9).

In the third group of experimental animals, fed with a mixture of free aminoacids (Nutri 2000), microvilli were preserved in the greater part of the surface, but in places they had vanished, and rarely in these sites there were ruptures of the luminal cell membrane. Oedema of the mitochondria was frequent and sometimes there was complete disorganization of their interior structure. The granular endoplasmic reticulum was generally normal (Figs. 10-12).

Discussion

The epithelium of the intestinal tract is a dynamic and constantly regenerating tissue; when affected by periods of long starvation the consequent results are serious nutritional problems. In these cases, lack of essential nutritive components (phylic acid, vitamin B_{12}, necessary fatty acids, etc) exerts an additional adverse effect on the enteric mucosa (Earnest, 1988).
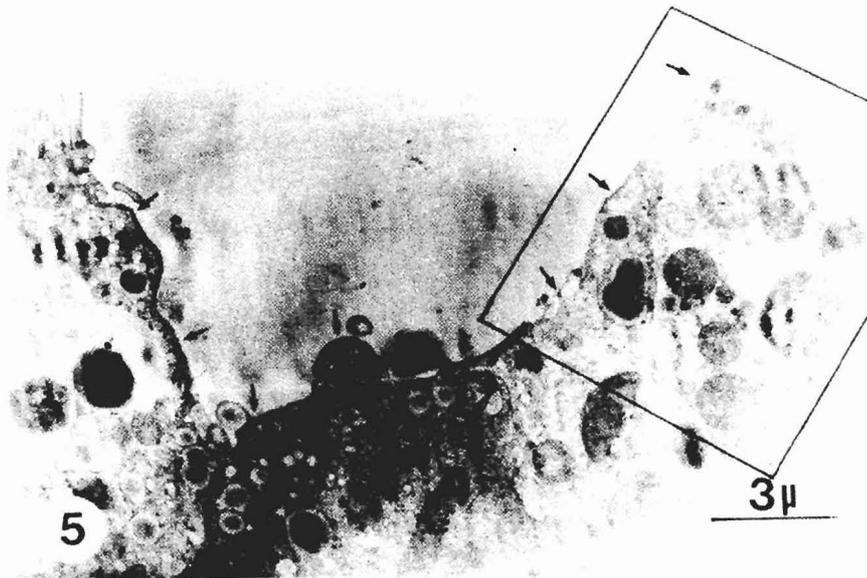

Fig. 5. First group of experimental animals fed with polymeric diet. Complete absence of microvilli (arrows). Round to ovoid rarefied areas of cytoplasm partly filled with fine granular material. The area of the rectangle is presented enlarged in the next picture.
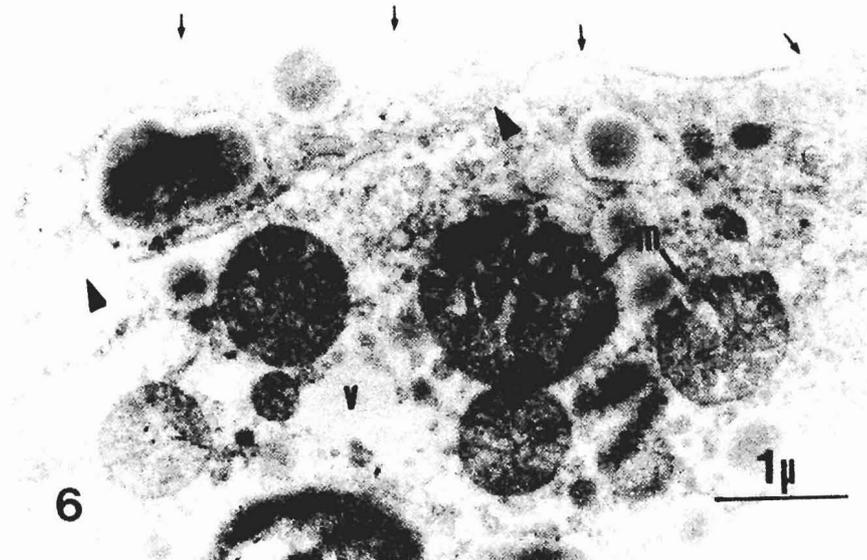

Fig 6. Enlarged area of the previous picture. The arrows show the cell surface and the arrow heads the cellular boundaries. v: vacuolized areas, m: mitochondria.

Many researchers have studied the effects of chronic malnutrition upon the morphology and function of the intestinal mucosa (Dugue et al., 1975; Earnest, 1988). However, since in these patients other disorders often coexisted, such as parasites, disturbances of the intestinal flora, etc, it was usually difficult to determine alterations of the intestinal mucosa that could be attributed exclusively to chronic food deprivation. The morphological and functional effects of short-term food deprivation upon the mucosa of the small intestine started to be stressed two decades ago.

Adibi and Allen (1970) established that a short period of per os food deprivation, resulted in a prominent decrease in the activity of microvilli disaccharidase in the epithelial cells, and in a reduced absorbability of peptides and aminoacids. Other researchers observed

that rats, which were fed parenterically for a period of 7 days, showed a significant decrease in intestinal weight, decrease of the weight of intestinal mucosa, lowering of protein and DNA content of the epithelial cells, reduction of microvilli height, significant reduction of disaccharidase activity, and reduction of the rate of multiplication and immigration of the crypt cells. These findings were considered to be an indication of epithelial cell hypertrophy, aiming at preserving the absorptive capability (Altaman, 1972; Levine et al., 1974). These observations not only support the theory that deprivation of essential nutritive components has certain effects upon the intestinal tract, but also underline the significance of food presence intraluminally, for the preservation of functional and morphological integrity of intestinal mucosa (Young, 1988).
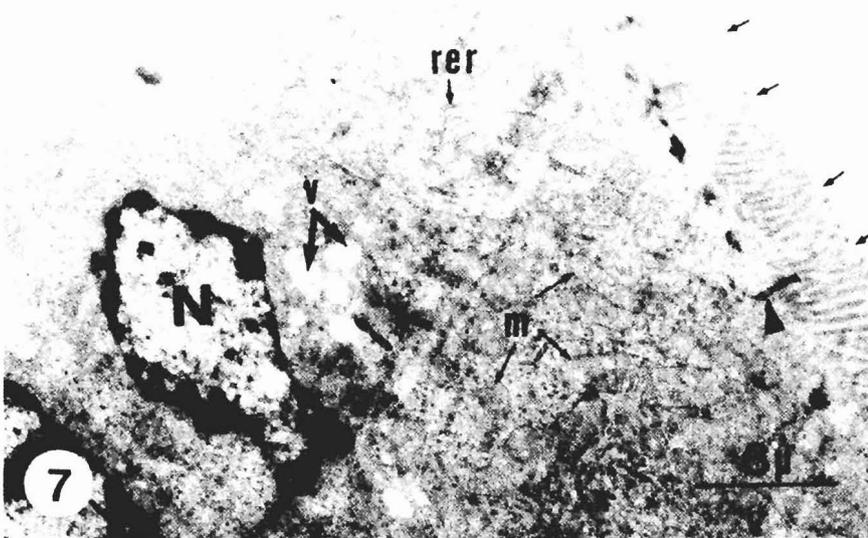

Fig. 7. Second group of experimental animals fed with oligopeptidic diet. Practically normal appearance of the epithelial cells. Arrows show the luminal surface presenting normal microvilli; arrow head, the cellular boundaries; m: mitochondria; rer: rough endoplasmic reticulum; v: small vacuoles; N: nucleus.
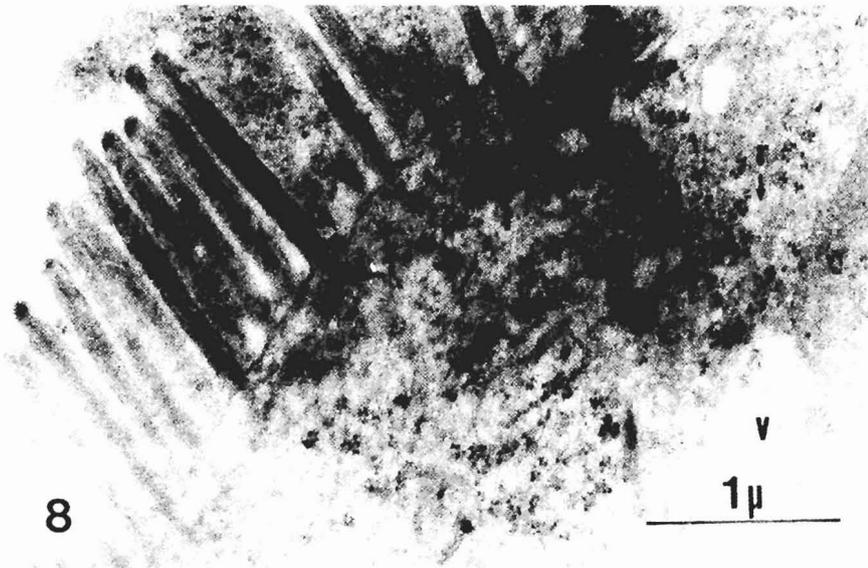

Fig. 8. Second group of experimental animals fed with oligopeptidic diet. Area with not heavily populated microvilli (arrows). f: microfilaments, r: ribosomes, v: vacuolated areas.

In this work, the ultrastructural study of the jejunal epithelium of the experimental animals, examined immediately after food deprivation, supports the conclusion of the aforementioned authors. The cell membrane disintegration, the microvesicular and cystoid appearance, as well as the degranulation of the rough endoplasmic reticulum were frequent findings. The large areas of jejunal mucosa, presenting sparseness and shortening or disappearance of the microvilli, was also a characteristic finding, which is in opposition to the findings of Levine et al. (1974), who found thinness and elongation of the microvilli in rats fed parenterically. This is probably due to the short period of food deprivation of the animals in our group, which did not allow the epithelial cells to develop hypertrophy.

Many researchers have shown that alterations of the intestinal epithelium caused by food deprivation are restored, following refeeding (Betzhold and Howard, 1984; Gorostiza et al., 1985; Pitchumoni et al., 1986; Earnest, 1988). Earnest (1988) reports that the functional and morphological reconstitution of the small intestine epithelium occurs on the third to sixth day following refeeding. At that time, new cells from the crypts colonise the surface of the enteric villi. In the experimental animals of this study, 12 days after refeeding, part or almost all of the alterations caused by the three-day food deprivation, were restored. The rate and extent of restoration was related to the composition
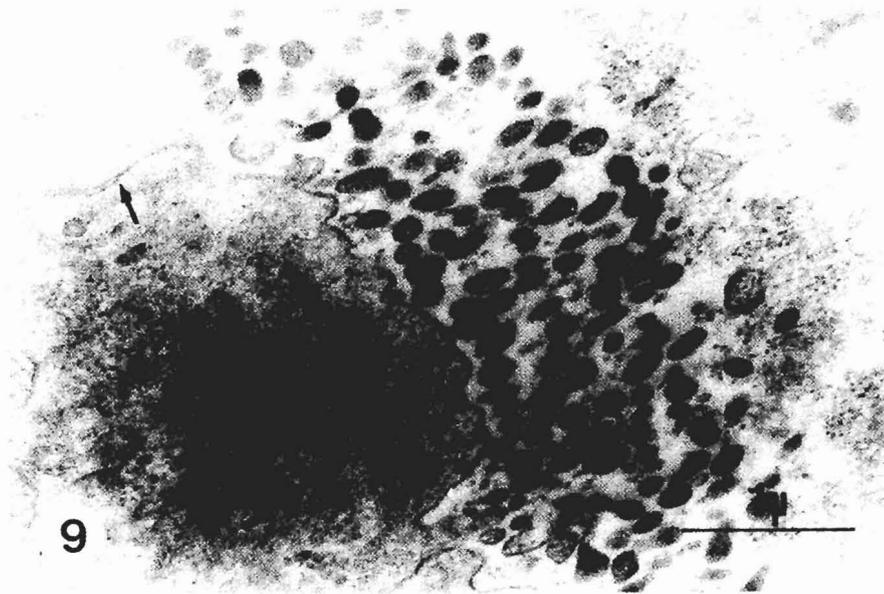

Fig. 9. Second group of experimental animals fed with oligopeptidic diet. Area devoid of microvilli. Adjacent microvilli are cut transversely and present more or less equal thickness. Arrow shows cellular boundaries.
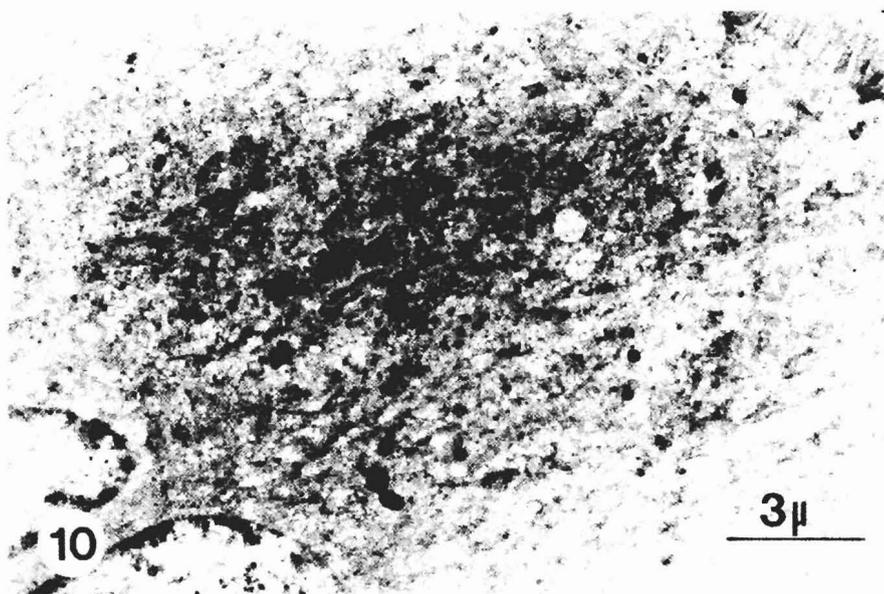

Fig. 10. Third group of experimental animals fed with mixture of free aminoacids. Area with more or less normal appearance.

of the nutritional solution. The molecular type of nutritional substances, primarily of the proteins, seems to play an important role in the reconstitution of the morphological and functional integrity of the intestinal mucosa. Young et al. (1980) showed that a solution of a mixture of free aminoacids, given to rats following a 5-day food deprivation, did not successfully restore the body weight, the weight of the mucosa, and the DNA content of the epithelial cells, while these parameters were restored by an equicaloric quantity of the usual laboratory diet. Vázquez et al. (1985) also noticed that the absorption of the nutrients was faster and better when a solution of hydrolysed proteins was used, compared to an equivalent solution of full proteins or a

diet of free aminoacids. Poullain et al. (1989) drew the same conclusions by comparing these three types of solutions for enteric nutrition. They found that animals fed with oligopeptidic diet attained an improved nitrogen balance, at higher level of blood protein, and a faster rate of recuperation of their body weight, while there was no difference in the levels of blood urea and fat absorption. Finally, Brinson et al. (1989) observed that patients who received solutions of oligopeptidic diet had better absorption and utilization of nitrogen, higher levels of aminoacids and increased insulin secretion, compared to patients fed with diets of full proteins or with a mixture of free aminoacids.

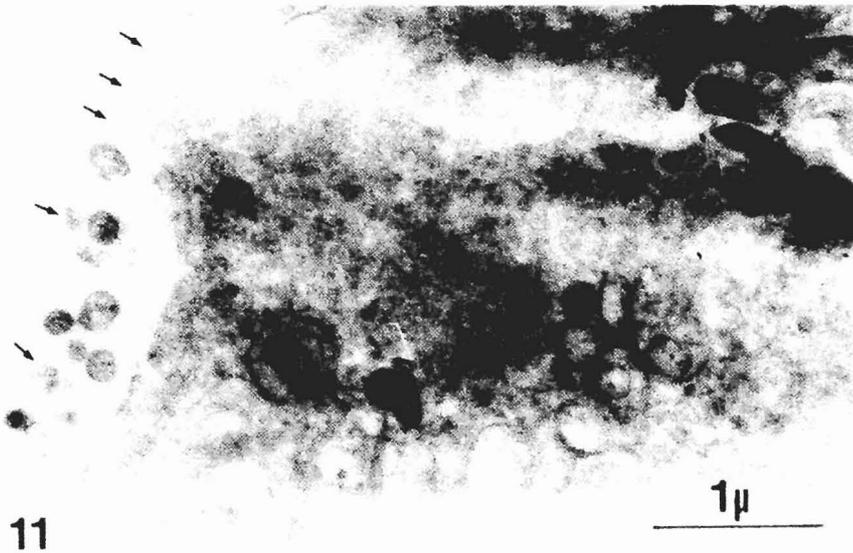In this work, the ultrastructural findings of the jejunal

Fig. 11. Third group of experimental animals fed with mixture of free aminoacids. Area devoid of microvilli and presenting cellular disintegration.
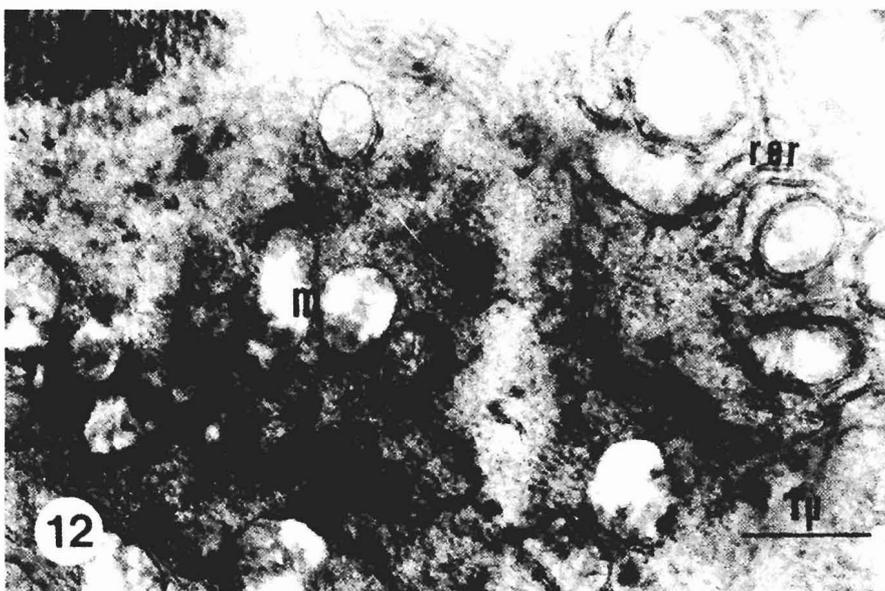

Fig. 12. Third group of experimental animals fed with mixture of free aminoacids. Mitochondrial oedema.

mucosa depended upon the molecular type of the solution used for artificial enteric nutrition. In the animals of the first group (macromolecular solution of total milk serum proteins) microvilli were found in a large area of the surface of the epithelial cells, but they were scant and unequal in size. At the sites that lacked microvilli, the cell membrane was intact. At the epithelium there were also foci which showed intracellular disorganization and alterations.

Scantier lesions were found in the experimental animals of the third group (solution of a mixture of free aminoacids). Microvilli existed in most of the surface, but there were places devoid of them and presenting also ruptures of the cell membrane. A characteristic finding in this group was the intense oedema of mitochondria, which sometimes caused complete mitochondrial disorganisation.

An insignificant quantity of lesions was found in the rats of the second group (solution of hydrolysed protein of milk serum), the jejunum of which displayed a more or less normal appearance. The reconstitution of mucosal changes, may have resulted in faster and better absorption of the nutritive components of the solution. This is highly indicated by the better statistically significant rate of recuperation of body weight noticed in this group, compared to the rate of the other groups. Moreover, the same conclusions have been reached by Poullain et al. (1989) and Brinson et al. (1989) following experimental and clinical observations.

In conclusion, it may be stated that a short period of food deprivation causes significant alterations in the jejunal mucosa. In the majority of cases these alterations may be reversed during refeeding. The composition of the dietic solution seems to play a significant role in the reconstitution of the morphological and functional integrity of the intestinal mucosa. Solutions of oligopeptidic diet restore the normal morphology of the jejunal mucosa faster, and this evidently results in better and faster absorption of the nutritional substances.

References

Adibi S.A. and Allen E.R. (1970). Impaired jejunal absorption rate of essential amino acids induced by either dietary caloric or protein deprivation in man. Gastroenterology 59, 404-413.

Altaman G.G. (1972). Influence of starvation and refeeding on mucosal and epithelial renewal in the rat small intestine. Am. J. Anat. 133, 391-398.

Andoniadis K., Bachtsevanos K., Kosta-Tsolaki M. and Gourtas K. (1987). Laboratory rat. Ann. Fasc. Med. Univ. Thessaloniki 104, 33-37.

Baker S.J. (1977). Malnutrition and intestinal disease. In: Progress in Gastroenterology. Jerzy-Glass G (ed). Vol. 3. Grune and Stratton Inc. New York. pp 563-594.

Betzhold J. and Howard L. (1984). Enteral nutrition and gastrointestinal disease. In: Enteral and tube feeding. Rombeau F.L. and Caldwell M.D. (eds). W.B. Saunders Comp. Philadelphia. pp 338-361.

Brinson R.R., Hanumantu K.S. and Pitts M.W. (1989). A reappraisal of the peptid-based enteral formulas: clinical applications. Nut. Clinic. Prac. 4, 211-217.

Clarke R.M. (1975). The time-course of changes in mucosal architecture and epithelial cell production and cell sheeding in the small intestine of the rat fed after fasting. J. Anat. 120, 321-327.

Committee on animal nutrition (1978). Nutrient requirements of laboratory rat. U.S. Nat Acad. Sci. 10, 7-28.

Dugue E., Bolanos O. and Loreto H. (1975). Enteropathy in adult protein malnutrition: Light microscopic findings. Am. J. Clin. Nutr. 28, 901-909.

Earnest L.D. (1988). Gastrointestinal mucosal changes from starvation and stress. In: Gastrointestinal response to injury. Starvation and enteral nutrition. Roche A.F. (ed). Ross Laboratories. Columbus. pp 11-16.

Gorostiza E., Poullain M.G. and Marche C. (1985). Effect of fasting and refeeding on small intestinal adaptation in the rat. Gastroenterol. Clin. Biol. 9, 790-796.

Keohane P.P., Grimple G.K. and Brown B. (1985). Influence of protein composition and hydrolysis method on intestinal absorption of protein in man. Gut 26, 907-913.

Koretz R.L. and Meyer J.H. (1980). Elemental diets, facts and fantasies. Gastroenterology 78, 393-410.

Levine G.M., Deren J.J. and Steiger E. (1974). Role of oral intake on maintenance of gut mass and disaccharidase activity. Gastroenterology 67, 975-982.

Meguid M.M., Landel A.M. and Terz J.J. (1984). Effect of elemental diet on albumin and urea synthesis. Comparison with partially hydrolyzed protein diet. J. Surg. Res. 37, 16-24.

Pitchumoni C.S., Schelle G. and Lee P.C. (1986). Effects of nutrition on the exocrine pancreas. In: Exocrine pancreas: Biology, Pathobiology and diseases. Go V.L.W. (ed). Raven Press. New York. pp 387-406.

Poullain M.G., Cezard J.P., Roger L. and Mendy F. (1989). Effects of whey proteins, their oligopeptide hydrolysates and free amino acid mixtures on growth and nitrogen retention in fed and starved rats. J. Pen. 13, 382-386.

Silk D.B.A., Fairchlough P.D. and Clark M.L. (1980). Uses of peptides rather than free amino acid nitrogen source in chemically defined «elemental diets». J. Pen. 4, 548-553.

Smith J.L., Arteaga C. and Heymsfiedl S.B. (1982). Increased urea genesis and impaired use during infusion of a synthetic aminoacid formula. New Engl. J. Med. 306, 1013-1018.

Vázquez J.A., Morse E.L. and Adibi S.A. (1985). Effect of starvation on aminoacid and peptide transport and peptide hydrolysis in humans. Am. J. Physiol. 249, 563-569.

Viteri F.E. and Scheider R.E. (1974). Gastrointestinal alterations in protein-caloric malnutrition. Med. Clin. North Am. 58, 1487-1492.

Young A.E. (1988). The effect of intraluminal nutrients on gastrointestinal mucosa. In: Gastrointestinal response to injury, starvation and enteral nutrition. Roche A.F. (ed.). Ross Laboratories. Columbus. pp 45-50.

Young A.E., Cioletti L.A., Winborn W.B., Traylor J.B. and Weser E. (1980). Comparative study of nutritional adaptation to defined formula diets in rats. Am. J. Clin. Nutr. 33, 2106-2128.