Progress report

Membrane digestion

By the end of the 1950s the extensive investigations which laid the foundations for certain basic current concepts were complete, namely, the digestive function of the intestinal cells; the integration of the processes of digestion proper and of transport during digestion; and the pathology of enterocytes and other cells. And they may be summarized as follows: (1) the free surface of the enterocyte represents an aggregate of microvilli¹⁻¹⁴; (2) hydrolysis of oligomers, ie, the final stages of digestion, is being accomplished during absorption by enzymes bound to the enterocyte structures¹⁵⁻²⁰; (3) numerous enzymes, in particular alkaline phosphatase and the aminopeptidases, are concentrated within the brush border zone²¹⁻²⁷. The final link in the understanding of these processes is that oligomers as such penetrate inside the intestinal cells where the final stages of hydrolysis and further transport of monomers take place.

The Digestive Functions of Enterocytes

Simultaneously, at the end of the 1950s and the beginning of the 1960s, two different hypotheses to explain these facts were presented, namely, (1) the concept of apical intracellular digestion²⁸⁻³² and (2) that of membrane digestion³³⁻⁴⁶. Evidence is increasing to suggest that the first hypothesis is untenable.

Membrane digestion, in addition to the two early known types—distant extracellular and intracellular—is considered to be a system of the digestive processes occurring under the influence of the enzymes situated on the external surface of the membrane. We had the good fortune to be able to demonstrate that the external surface of the brush border is a morphological substrate of a new, previously unknown type of digestion (membrane) which appeared to be associated with the adsorption of pancreatic enzymes to the surface of the intestinal mucosa^{33-36,40,47}. (Due to the large size of amylase molecules (molecular weight is about 45 000)^{48,49} their penetration into the cells was excluded.) Evidence of the rapid and complete loss of enzyme from fluid as it adsorbs onto membrane (desorption) supported our arguments even more forcibly^{33-36,40,47}. Adsorbed amylase was shown to hydrolyze intensively the soluble (not colloidal) starch⁴⁰. In practice, it was found at the same time that enteric enzymes, in particular disaccharidases and dipeptidases, produce their effect not intracellularly but at the external surface of the cell membranes^{37,40,41,46}. In man and mammals, membrane digestion is accomplished at the surface of the microvilli of the enterocyte. This idea was first reported in 1959 and discussed in a number of reports^{36,40,50,51-53}.

Those enzymes which ensure membrane digestion (Fig. 1)* may have two

*The Editor and publishers of *Gut* are grateful to the Ciba Foundation and Associated Scientific Publishers, Amsterdam, for permission to reproduce the illustrations which originally appeared in the proceedings of a Ciba symposium on 'Peptide Transport in Bacteria and Mammalian Gut'.

Fig. 1 The enzymatic apparatus for membrane digestion. Adsorption of pancreatic enzymes from chyme (1), synthesis (3), and migration (2) of enteric enzymes.

origins: (1) enteric enzymes synthesized by enterocytes and structurally associated with the external surface of their membrane, and (2) enzymes adsorbed from the intestinal cavity.

Indeed, I was able to write in the *Physiological Review*⁵⁰: 'Cavital digestion is produced by enzymes secreted into the cavity of the gastrointestinal tract. Membrane digestion is due to enzymes adsorbed from chyme and enzymes structurally associated with the membrane of the intestinal cells. Consequently, study of enzymatic functions of digestive glands and properties of digestive juices cannot answer all questions of the physiology of digestion'.

It should be stressed that the nature of the bonds of the enteric enzymes with the membrane structures of the microvilli remains unknown. The terms employed in the literature 'structurally associated enzymes' or 'enzymes, which are the integral part of the membrane structure,' lack physical or chemical sense as they do not reflect the forces ensuring preservation of the enzyme-membrane complexes. It is known only that the majority of intestinal peptidases are easily solubilized *in vitro* while disaccharidases, alkaline phosphatase, and aminopeptidase can be solubilized by treating the enzyme-membrane complexes with proteases (papain, trypsin) and other detergents (Triton X-100)^{18, 54-62}.

Fig. 2 The detailed scheme of the interrelationship between cavital and membrane digestion with no food substances present (I) and in their presence (II). 1 Enzymes in the intestinal cavity; 2 microvilli; 3 enzymes (adsorbed and enteric) on the microvillous surface; 4 the brush border pores; 5 bacteria; 6, 7 food substances at the different stages of hydrolysis.

The general scheme of membrane digestion as a process completing the hydrolysis and associated with absorption was proposed in 1959-60 and is illustrated in Figure 2. Later it was developed and defined concretely in line with the success achieved in the study of the role of membranes in the body and with the progress in our knowledge of their digestive and transport functions^{51,52}.

During the 1960s about 20 independent experimental criteria were used to prove the fact that oligosaccharide and peptide hydrolysis occurs exclusively or mainly at the external membrane surface^{50,52,63,64}. Of particular significance are the electron-microscope studies by Oda and Seki, Johnston, and others⁶⁵⁻⁶⁹, who have demonstrated the presence of globules of 50 to 60 Å in diameter at the external membrane surface using the negative contrast of the brush border. These globules were solubilized and turned out to be at least the bearers of disaccharidase activity.

Four different viewpoints on the mechanism of the final stages of hydrolysis existed before 1966⁷⁰. Since that time investigators have accepted the membrane hypothesis. Judging from the later publications of Crane and his group, they themselves have joined this majority^{63,71-75,76}.

Following the first research refuting the significance of enzyme adsorption for membrane digestion came the first support for this mechanism^{77,81}. At present, adsorption of such pancreatic enzymes as amylase^{38,40,43,52,77-81}, lipase⁸²⁻⁸⁵, and protease^{37,47} is shown. It is very likely that other enzymes, such as pancreatic DNA-ase and RNA-ase, may be adsorbed and participate in membrane digestion.

A detailed analysis of adsorption mechanisms revealed, first, that both the physical and chemical aspects of adsorption are of importance; secondly, adsorption is possible on both lipoprotein and mucopolysaccharide structures^{40,43,51,52,70,86-93}.

The analysis of the structure and functions of the brush border glycocalix made first by Ito⁹⁴ and later by other authors⁹⁵⁻¹⁰¹ appeared of particular significance for the development of the membrane digestion concept. Indeed Ito was the first to pay attention to the importance of this structure in the processes of membrane digestion.

The Characteristics of Membrane Digestion

The basic functional and structural characteristics of membrane digestion are illustrated in Fig. 2 and are set out as follows:

Membrane digestion is carried out at the external surface of the membrane of microvilli by the enteric and adsorbed enzymes. Therefore, the final stages of digestion are integrated and the initial stages of transport are begun. It occurs in the zone inaccessible to bacteria, thus ensuring sterility of the final stages of hydrolysis. Membrane digestion, as any other catalysis on the surface, depends on the rate at which substances pass from liquid medium onto the surface and under normal conditions that depends upon intestinal motility.

The size of the pores of the brush border and of the glycocalix allows the segregation of larger structures from smaller ones thus providing for the consequent treatment of food, first in the cavity then at the surface. Thanks to the continuous elimination of the intermediate products of hydrolysis into the membrane digestion zone, digestion in the cavity is intensified.

Many characteristics of the solubilized enzymes and those bound to membrane differ from each other, in particular K_m , V_{max} , and thermostability.

Fig. 3 Enteric and adsorbed enzymes during membrane digestion (the scheme of the fragment of the microvillus luminal surface). A Distribution of enzymes; B interrelationship of enzymes, carriers and substrates. I cavity; II glycocalix; III three-layered membrane; IV glycocalix luminal surface; V three-layered membrane luminal surface. 1 Intestinal enzymes proper; 2 adsorbed enzymes; 3 carriers; 4 substrates.

Owing to fixation of enzymes at the external surface of the membrane, the structural organization of membrane hydrolysis and transport processes is possible. Physicochemical conditions in the membrane digestion zone vary from those of the digestive cavities.

Figure 3 shows a fragment of membrane with a glycocalix, the approximate correlation of enzymes and structures being observed. As can be seen, the normal course of membrane digestion requires the presence of both enteric enzymes proper and adsorbed enzymes. In fact, the enteric and adsorbed enzymes functionally supplement each other and simultaneously seem to enhance the reliability of the system as a whole. It is assumed that enteric enzymes are structurally connected with the external surface of a three-layered membrane which ensures the effective conveyance of the final products (glucose, amino acids) from enzyme onto the carrier. Adsorbed enzymes can be situated on all the structures of the external surface of the microvillus.

It is obvious that substrates included in chyme do not directly contact the three-layered membrane; they must pass through the glycocalix space. Their penetration into the glycocalix is facilitated by enzymes, adsorbed to the free surface of the latter, and further on passing enzymes inside the glycocalix space. Thus, the movement across the glycocalix space is accompanied by an intense depolymerization of food substances. Some supplementary hydrolysis by means of adsorbed enzymes is possible at the external surface of the lipoprotein 'membrane', but the chief enzymes are enteric enzymes represented mainly by dimer and oligomerhydrolases.

Such localization of disaccharidases, di-amino-, and oligopeptidases is likely to ensure the highly effective conveyance from the final enzyme onto the entrance into the transport system, in particular, onto the labile carrier (Fig. 4).

Within the limits of the main arguments concerning membrane digestion (hydrolysis is completed at the external surface of the membrane and its

Fig. 4

Fig. 4 Hypothetical distribution of enzymes and carriers on the external membrane surface. E Enzyme; C carrier; M membrane.

Fig. 5

Fig. 5 The enzyme-carrier pool. S Substrate; P hydrolysis products; E enzyme; C_1 , C_2 , C_3 carriers forming a transport conveyor.

products are transported across the membrane) a number of hypotheses have been suggested to explain the extreme efficiency of the digestive-transport conveyor^{40,44,52,53,76,58-60,64,70,103-112}. They all, finally, agree that enzyme and carrier form the spatial and functional integration; one such scheme is represented in Figure 5. Thus the membrane digestion apparatus represents a highly complex structure with elements of a different nature and origin, which is in agreement with the current idea of 'thick' membranes^{113,114}.

The membranous surface of the microvilli seems to exercise not only digestive-transport functions but those of a protective barrier as well. In fact, the glycocalix, together with its enzymes, forms a specialized filter which allows adequate amounts of substrates to pass but traps the other molecules. The glycocalix is also an ion exchanger⁹⁸ and a molecular sieve¹⁰⁰.

Thus, membrane digestion is one of the three basic stages in the activity of the alimentary tract: it accepts the products of intermediate hydrolysis from the cavity (by which all the cavital processes are intensified); it accomplishes the hydrolysis of 70 to 80% of the primary molecular bindings in food biopolymers; and it couples the digestive processes proper and those of transport^{40,50,52,53}.

Membrane Digestion as a Clinical Problem

Membrane digestion should be taken into account for the better under-

Fig. 6 Amylase activity in the different fractions obtained during successive desorption and reflecting cavital and membrane digestion. A Normal; B in patients with defective pancreatic function; C in patients with chronic enteritis. Abscissa: separate fractions and subfractions; ordinate: amylolytic activity in $\mu\text{g}/\text{mg}$ of hydrolyzed substrate. C Activity of pancreatic α -amylase not adsorbed to the surface of small intestine. D₁ Easily desorbed amylase; D₂ more slowly desorbed amylase; D₃ slowly desorbed amylase; H homogenate amylolytic activity.

standing of the aetiology of a number of diseases and for promoting progress in diagnostic and therapeutic research. We have considered this problem in detail earlier and it will be the subject of a special review. Here we shall dwell only on certain consequences following upon the idea of membrane digestion.

Enzyme Adsorption Disorders

Hooft and others¹¹⁵ were the first to show that in certain forms of starch malabsorption α -amylase activity in the cavity remains normal but the capacity of the mucosa, obtained from biopsy in such patients, to adsorb amylase is greatly decreased.

Masevich, Zabelinskii, and Ugolev¹¹⁶ have developed the fraction technique of investigating enzyme adsorption. Figure 6 shows typical examples of the mucosal amylolytic spectral disorders. As can be seen, such a technique allows the differentiation of disorders in the amylolytic activity in the intestinal content, the adsorption properties of the surface, and α -amylase activity dependent upon proteolytic processes in the enterocyte itself.

Recently, changes in lipase adsorption have been seen in patients suffering from atherosclerosis when compared with healthy controls¹¹⁷.

Different enzyme maladsorptions were revealed also in such diseases as enteritis, enterocolitis, and diseases of the biliary ducts⁸¹. Adsorption defects could be both isolated and combined with damage to the villous structures and disturbances in the epithelial element in proteosynthesis.

It is of interest that disorders of membrane digestion are likely to appear earlier than can be seen by histological and histochemical changes.

Synthesis and Migration of Enteric Enzymes in Disease

Studies of genetic and acquired defects of the enzymatic properties of enterocytes shed light upon a great number of diseases of the digestive system. Information was obtained by means of loading studies and by investigation of the enzymatic properties of mucosa obtained by biopsy. In both cases homogenates of a specimen were mainly used.

In terms of membrane digestion, functioning of the enteric enzymes (disaccharidases, peptidases, etc) is determined by the two successive processes of intracellular synthesis and further progress to the external membrane surface.

There are two possibilities: enzymes are being included either in the composition of a waiting microvillous membrane structure or built into the subunits of the membrane intracellularly so that the enzyme-membrane blocks are included in the composition of the microvillus⁵².

It is obvious that investigation of homogenates provides only a notion of the state of enzyme synthesis but not of the transfer of enzymes and therefore not of their actual functioning. For this reason our laboratory has developed a technique based on the comparison of intact and homogenized mucosa^{52,118-120} as the investigation of homogenates led to a considerable loss of information. Many enzymatic defects remain beyond the understanding both of the laboratory investigator and the clinician.

It is important to remember that in the same disease certain enzymatic defects can depend on disorders of synthesis and others on disturbances of

Fig. 7 Effect of x-ray treatment on the hydrolysis of sucrose (A) and glycyl-L-leucine (B). Abscissa: time after x-ray treatment (in hours); ordinate: enzyme activity (in conventional units). Dotted line: enzymatic activity of homogenates; continuous line: enzymatic activity of the everted pieces of small intestine.

transfer⁵². For example, in severe x-ray injuries hydrolysis of both disaccharides and peptides is completely stopped. But the absence of invertase activity depends on the suppression of synthesis whereas peptidase is synthesized in amounts close to normal but it is not included in the membrane of the microvillus (Fig. 7)^{52,119}. Restoration of normal interactions between the synthesis and transfer of the enteric enzymes after irradiation injuries is essentially important for a better insight into the restoration processes¹²¹⁻¹²⁴.

It is interesting that under the influence of different stresses disorders of the enterocyte digestive function depend rather on disturbances of transfer than on enteric enzyme synthesis.

Other Defects of Membrane Digestion

It is clear that disorders of membrane digestion can take place not only as a result of disturbances in the enteric cell functions but in all those situations in which hydrolysis is decreased at the intestinal surface as a whole. When hydrolysis in the digestive cavity is slowed as a result of deficiency of the basic digestive glands membrane digestion is impaired due to an insufficient supply of substrates into the brush border zone^{53,125-127}. This is accompanied by the enzymatic and transport functions of distal portions of

small intestine being greatly enhanced, a situation which is defined by Booth as a 'reserved zone'¹²⁸. In severe cases food which has not been utilized can enter the large intestine unchanged.

In disturbances of motility of the small intestine the increased passage shortens the time of contact between food and digestive surface and, therefore, decreases the volume of membrane digestion. As soon as the entry of substances into the brush border is determined by convection and not by diffusion, the effectiveness of membrane hydrolysis is reduced so as to impair the action of chyme stirring the contents of the small intestine^{43,46}.

The total volume of membrane hydrolysis can diminish as a result of a reduction in the epithelial population in the small intestine, first, due to its shortening, as in surgical operations, and secondly to smaller numbers of enterocytes in the villi ('atrophy of villi').

Thus, disorders of the enterocyte digestive functions are all disorders of membrane digestion, but the pathology of membrane digestion is wider since it can be brought about by factors other than those of the enterocytes^{40,50,52}.

Until now we have been considering membrane digestion as a process which when disordered can be the cause of various diseases. It should be noted, however, that in many cases membrane digestion is a process compensating for the defects of cavital digestion. This was demonstrated, in particular, in connexion with the assimilation of polysaccharides when the external secretory function of the pancreas was excluded^{53,125,127}.

Conclusion

Membrane digestion is the third fundamental type of digestive processes, whose functional regulation in a number of aspects is highly specific. This mechanism is widespread and probably universal.

The actual functioning of the digestive apparatus in higher animals and man comprises three successive and interconnected stages: cavital digestion, membrane digestion, and absorption.

The study of membrane digestion is not only of theoretical interest but of practical importance as well.

A. M. UGOLEV

*Laboratory of the Physiology of Nutrition
I. P. Pavlov Institute of Physiology
Academy of Sciences of the USSR
Leningrad, USSR*

References

- ¹Granger, B., and Baker, R. F. (1949). Electron microscope investigation of the striated border of intestinal epithelium. (Abstr.) *Anat. Rec.*, 103, 459.
- ²Granger, B., and Baker, R. F. (1950). Electron microscope investigation of the striated border of intestinal epithelium. *Anat. Rec.*, 107, 423-441.
- ³Dalton, A. J., Kahler, H., Striebig, M. J., and Lloyd, B. (1950). Finer structure of hepatic, intestinal and renal cells of the mouse as revealed by the electron microscope. *J. nat. Cancer Inst.*, 11, 439-461.
- ⁴Dalton, A. J., Kahler, H., and Lloyd, B. J. (1951). The structure of the free surface of a series of epithelial cell types in the mouse as revealed by the electron microscope. *Anat. Rec.*, 111, 67-77.
- ⁵Schiebler, T. (1955). Neuere morphologische Anschauungen über die Darmresorption. *Dtsch. med. Wschr.*, 80, 1885-1887.
- ⁶Sjöstrand, F. S. (1956). The ultrastructure of cells as revealed by the electron microscope. *Int. Rev. Cytol.*, 5, 455-533.
- ⁷Goldin, L. S. (1956). Electron microscopy of the mucosa of the small intestine. (Russian). *Dokl. Akad. Nauk SSSR*, 106, 721-724.

- ⁹Zetterqvist, H. (1956). *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejenum*. Aktiebolaget Godvil, Stockholm.
- ¹⁰Johnston, J. M., and Wiggans, D. S. (1958). The absorption in vitro of a lanyl-phenylalanine. *Biochim. biophys. Acta (Amst.)*, 27, 224.
- ¹¹Palay, S. L., and Karlin, L. J. (1959). An electron microscopic study of the intestinal villus. I. The fasting animal. *J. biophys. biochem. Cytol.*, 5, 363-372.
- ¹²Palay, S. L., and Karlin, L. J. (1959). An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. *J. biophys. biochem. Cytol.*, 5, 373-384.
- ¹³Shestopalova, N. M. (1959). The regeneration of mucosa of the small intestine of mammals and birds after experimental injury. (Russian). In *Problems of Regeneration and Cell Division: Proceedings of a Conference on Problems of Regeneration and Cell Multiplication*. pp. 154-158. Moscow.
- ¹⁴Shestopalova, N. M., Avakyan, A. A., Reingold, V. N., and Tkal, V. V. (1960). Electron microscopic study of plastic processes of intestinal epithelium of certain mammals. (Russian). *Ark. Anat. Gistol., Embriol.*, 38, (3), 34-44.
- ¹⁵Shestopalova, N. M., Avakyan, A. A., and Reingold, V. N. (1961). Electron microscopic comparative study of the structure of the epithelium of duodenum and of mammals and amphibious animals. (Russian). *Tsitologiya*, 3, 125-136.
- ¹⁶Cajori, F. A. (1933). The enzyme activity of dog's intestinal juice and its relation to intestinal digestion. *Amer. J. Physiol.*, 104, 659-668.
- ¹⁷Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J. (1957). Studies of intestinal digestion and absorption in the human. *J. clin. Invest.*, 36, 1521-1536.
- ¹⁸Borgström, B., and Dahlqvist, A. (1958). Cellular localisation, solubilization and separation of intestinal glycosidases. *Acta chem. scand.*, 12, 1997-2006.
- ¹⁹Dahlqvist, A. (1960). Hog intestinal α -glucosidases. Solubilization, separation and characterization. Thesis, Lund.
- ²⁰Smyth, D. H. (1961). Intestinal absorption. *Proc. roy. Soc. Med.*, 54, 769-773.
- ²¹Borgström, B., Dahlqvist, A., and Lundh, G. (1962). On the site of absorption of fat from the human small intestine. *Gut*, 3, 315-317.
- ²²Bourne, G. (1944). Distribution of alkaline phosphatase in various tissues. *Quart. J. exp. Physiol.*, 32, 1-19.
- ²³Deane, H. W., and Dempsey, E. W. (1945). The localization of phosphatases in the Golgi region of intestinal and other epithelial cells. *Anat. Rec.*, 93, 401-417.
- ²⁴Dempsey, E. W., and Deane, H. W. (1946). The cytological localization, substrate specificity, and pH optima of phosphatases in the duodenum of the mouse. *J. cell. comp. Physiol.*, 27, 159-179.
- ²⁵Emmel, V. M. (1946). The intracellular distribution of alkaline phosphatase activity following various methods of histologic fixation. *Anat. Rec.*, 95, 159-175.
- ²⁶Martin, B. F., and Jacoby, F. (1949). Diffusion phenomenon complicating the histochemical reaction for alkaline phosphatase. *J. Anat. (Lond.)*, 83, 351-363.
- ²⁷Barnett, R. J. (1959). The demonstration with the electron microscope of the end-products of histochemical reactions in relation to the fine structure of cells. *Exp. Cell Res.*, Suppl. 7, 65-89.
- ²⁸Burstone, M. S., and Weisburger, E. K. (1961). Histochemical demonstration of aminopeptidase by a new oxidation technique. (Letter). *J. Histochem. Cytochem.*, 9, 712-713.
- ²⁹Holt, J. H., and Miller, D. (1961). The intestinal brush border as a digestive surface. Localization of aminopeptidase and phosphatase in the mucosal epithelial cell. (Abstr.) *J. Lab. clin. Med.*, 58, 827.
- ³⁰Miller, D., and Crane, R. K. (1961). The digestive function of the epithelium of the small intestine. I. An intracellular locus of disaccharide and sugar phosphate ester hydrolysis. *Biochim. biophys. Acta (Amst.)*, 52, 281-293.
- ³¹Miller, D., and Crane, R. K. (1961). The digestive function of the epithelium of the small intestine. II. Localization of disaccharide hydrolysis in the isolated brush border portion of the intestinal epithelial cells. *Biochim. biophys. Acta (Amst.)*, 52, 293-298.
- ³²Holt, J. H., and Miller, D. (1962). The localization of phosphomonoesterase and aminopeptidase in brush borders isolated from intestinal epithelial cells. *Biochim. biophys. Acta (Amst.)*, 58, 239-243.
- ³³Miller, D., and Crane, R. K. (1963). The digestion of carbohydrates in the small intestine. *Amer. J. clin. Nutr.*, 12, 220-227.
- ³⁴Ugolev, A. M. (1960). On the existence of membrane (contact) digestion. (Russian). *Byull. eksp. Biol. Med.*, 49, (1), 12-17.
- ³⁵Ugolev, A. M. (1960). Certain general patterns in cavital and membrane digestion. (Russian). In *Proceedings of Scientific Conference on the Problems of Physiology and Pathology of Digestion, Dedicated to the Memory of Academician K. M. Bykov*, pp. 829-834. Ivanovo.
- ³⁶Ugolev, A. M. (1961). *Digestion and its Adaptation Evolution*. (Russian). 'Vysshaya Shkola', Moscow.
- ³⁷Ugolev, A. M. (1961). Data on membrane digestion. III. Comparison of enzymatic hydrolysis of starch in the intestine and in vitro. (Russian). *Byull. eksp. Biol. Med.*, 52, (8), 8-12.
- ³⁸Ugolev, A. M., Iezuitova, N. N., Kashina, N. A., Sabsai, B. I., Spiridenkova, T. I., Timofeeva, N. M., Shchelovanova, I. N., Shalygina, T. V., and Sherstobitov, O. E. (1961). Newer data on the physiology of membrane digestion. In *Scientific Conference on the Problems of Physiology and Pathology of Digestion and Absorption, Abstracts of Reports*, pp. 240-243. Odessa.
- ³⁹Iezuitova, N. N. (1963). On hydrolysis of certain poly- and oligosaccharides in the mammalian small intestine. (Russian). In *First All-Union Biochemical Conference. Abstracts*, vol. 2, Supplementary (Sectional) Conference 1-9, p. 43. Akad. Nauk SSSR, Moscow-Leningrad.
- ⁴⁰Timofeeva, N. M. (1963). Localization of the hydrolysis of certain dipeptides in the small intestine of white rats. (Russian). In *First All-Union Biochemical Conference. Abstracts*, vol. 2, Supplementary (Sectional) Conference 1-9, p. 50. Akad. Nauk SSSR, Moscow-Leningrad.
- ⁴¹Ugolev, A. M. (1963). *Membrane (Contact) Digestion*. (Russian). Izd. AN SSSR, Moscow.
- ⁴²Iezuitova, N. N., Ugolev, A. M., and Fediushina, I. N. (1963). The effect of the rate of perfusion on cavital and membranal hydrolysis of starch and sucrose in the intestine. (Russian). *Dokl. Akad. Nauk SSSR*, 149, 746-749.
- ⁴³Iezuitova, N. N., De Laey, P., and Ugolev, A. M. (1964). An analysis of invertase localization in small intestine cells by a comparison of the concentration of hydrolysis products in intra- and extracellular fluids. (Russian). *Dokl. Akad. Nauk SSSR*, 159, 1191-1193.
- ⁴⁴Iezuitova, N. N., De Laey, P., and Ugolev, A. M. (1964). Digestion of starch *in vivo* and *in vitro* in a rat intestine. *Biochim. biophys. Acta (Amst.)*, 86, 205-210.

- ⁴⁴Ugolev, A. M. (1964). Interrelationship of digestive and absorption processes in the intestinal cell. (Russian). In *Symposium on Physiology and Pathology of Absorption in the Gastrointestinal Tract*, pp. 7-20. Odessa.
- ⁴⁵Ugolev, A. M., Iesuitova, N. N., and De Laey, P. (1964). Localization of invertase activity in small intestinal cells. *Nature (Lond.)*, 203, 879-880.
- ⁴⁶Ugolev, A. M., Iezuitova, N. N., Timofeeva, N. M., and Fediushina, I. N. (1964). Location of hydrolysis of certain disaccharides and peptides in the small intestine. *Nature (Lond.)*, 202, 807-809.
- ⁴⁷Ugolev, A. M. (1966). Influence of the surface of the small intestine on enzymatic hydrolysis of starch by enzymes. *Nature (Lond.)*, 188, 588-589.
- ⁴⁸Dixon, M., and Webb, E. C. (1964). *Enzymes*, 2nd ed., Longmans, Green, London.
- ⁴⁹Barman, T. E. (1969). *Enzyme Handbook*, vol. 1. Springer, Berlin, Heidelberg, New York.
- ⁵⁰Ugolev, A. M. (1965). Membrane (contact) digestion. *Physiol. Rev.*, 45, 555-595.
- ⁵¹Ugolev, A. M. (1966). Die Membran-(Kontakt)-Verdauung in der Physiologie und Pathologie der Magen-Darm-Traktes. *Die Nahrung*, 10, 483-498.
- ⁵²Ugolev, A. M. (1968). *Physiology and Pathology of Membrane Digestion*, translated by J. A. Stekol. Plenum Press, New York.
- ⁵³Ugolev, A. M. (1970). Organization and regulation of membrane digestion and transport processes. (Russian). *Fiziol. Zh. (Mosk.)*, 56, 651-662.
- ⁵⁴Semenza, G., and Auricchio, S. (1962). Chromatographic separation of human intestinal disaccharidases. *Biochim. biophys. Acta (Amst.)*, 65, 172-175.
- ⁵⁵Auricchio, S., Dahlqvist, A., and Semenza, G. (1963). Solubilization of the human intestinal disaccharidases. *Biochim. biophys. Acta (Amst.)*, 73, 582-587.
- ⁵⁶Semenza, G., Auricchio, S., and Rubino, A. (1965). Multiplicity of human intestinal disaccharidases. I. Chromatographic separation of maltases and of two lactases. *Biochim. biophys. Acta (Amst.)*, 96, 487-497.
- ⁵⁷Eggermont, E. (1968). The biochemical defects of sucrose intolerance and in glucose-galactose malabsorption. Thesis, Université Catholique de Louvain, Belgium.
- ⁵⁸Semenza, G. (1968). Intestinal oligosaccharidases and disaccharidases. In *Handbook of Physiology, Sect. 6, Alimentary Canal*, edited by C. F. Code, vol. 5, pp. 2543-2566. American Physiological Society, Washington, D.C.
- ⁵⁹Semenza, G. (1968). Pancreatic amylase. In *Handbook of Physiology, Sect. 6, Alimentary Canal*, edited by C. F. Code, vol. 5, pp. 2637-2645. American Physiological Society, Washington, D.C.
- ⁶⁰Semenza, G., and Kolinská, J. (1968). An 'enzyme-substrate chromatography': intestinal sucrose-isomaltase on sephadex G-200. *Prot. biol. Fluids*, 15, 581-583.
- ⁶¹Dahlqvist, A., and Telenius, U. (1969). Column chromatography of human small-intestinal maltase, isomaltase and invertase activities. *Biochem. J.*, 111, 139-146.
- ⁶²Grudkov, A. A., Egorova, V. V., Iezuitova, N. N., Timofeeva, N. M., Tulyaganova, E. H., Chernyakhovskaya, M. Y., and Ugolev, A. M. (1970). Distribution of some enzymatic activities along the small intestine in white rats in fraction ultracentrifuging. In *Physiology and Pathology of the Small Intestine*, pp. 53-56. (Proceedings of the All-Union Conference of Gastroenterologists, Riga).
- ⁶³Greenberger, N. J. (1969). The intestinal brush border as a digestive and absorptive surface. *Amer. J. med. Sci.*, 258, 144-149.
- ⁶⁴Newey, H., and Smyth, D. H. (1970). Basic concepts in intestinal absorption. In *The Alimentary Tract*, part 3, p. 347.
- ⁶⁵Oda, T., Seki, S., Takesue, J., and Sato, R. (1964). *Kōso Kagaku Shinposhūmu* 17, 389.
- ⁶⁶Oda, T., and Seki, S. (1965). Molecular structure and biochemical function of the microvilli membrane of intestinal epithelial cells with special emphasis on the elementary particles. *J. electron. Microsc.*, 14, 210-217.
- ⁶⁷Oda, T., and Seki, S. (1966). Molecular basis of structure and function of the plasma membrane of the microvilli of intestinal epithelial cells. In *Electron Microscopy, 6th International Congress for Electron Microscopy*, vol. II. Biology, pp. 387-388. Maruzen, Tokyo.
- ⁶⁸Johnson, C. F. (1967). Disaccharidase: localization in hamster intestine brush borders. *Science*, 155, 1670-1672.
- ⁶⁹Nishi, Y., Yoshida, T. O., and Takesue, Y. (1968). Electron microscope studies on the structure of rabbit intestinal sucrose. *J. molec. Biol.*, 37, 441-444.
- ⁷⁰Crane, R. K. (1966). Structural and functional organization of an epithelial cell brush border. In *Intracellular Transport*. (Symposia of the International Society for Cell Biology, vol. V), pp. 71-103. Academic Press, New York and London.
- ⁷¹Eichholz, A., and Crane, R. K. (1965). Studies on the organization of the brush border on intestinal epithelial cells. I. Tris disruption of isolated hamster brush borders and density gradient separation of fractions. *J. Cell Biol.*, 26, 687-691.
- ⁷²Overton, J., Eichholz, A., and Crane, R. K. (1965). Studies on the organization of the brush border in intestinal epithelial cells. II. Fine structure of fractions of Tris-disrupted hamster brush borders. *J. Cell Biol.*, 26, 693-706.
- ⁷³Eichholz, A. (1967). Structural and functional organization of the brush border of intestinal epithelial cells. III. Enzymic activities and chemical composition of various fractions of tris-disrupted brush borders. *Biochim. biophys. Acta (Amst.)*, 135, 475-482.
- ⁷⁴Eichholz, A. (1968). Studies on the organization of the brush border in intestinal epithelial cells. V. Subfractionation of enzymatic activities of microvillous membrane. *Biochim. biophys. Acta (Amst.)*, 163, 101-107.
- ⁷⁵Crane, R. K. (1969). Introductory remarks, to Intersociety Symposium of Gastroenterology: structure and function of a digestive-absorptive surface. *Fed. Proc.*, 28, 5.
- ⁷⁶Crane, R. K. (1968). Absorption of sugars. In *Handbook of Physiology, Sect. 6, Alimentary Canal*, edited by C. F. Code, vol. 3, pp. 1323-1351. American Physiological Society, Washington, D.C.
- ⁷⁷De Laey, P., and Iesuitova, N. N. (1962). On the mechanism of enzymatic cleavage of starch in the intestine. (Russian). *Dokl. Akad. Nauk SSSR*, 146, 731-733.
- ⁷⁸Iezuitova, N. N. (1965). On the role of membrane digestion in the hydrolysis of poly- and oligosaccharides. (Russian). In *Data of Scientific Conference on the Problems of 'Physiology and Pathology of Cortico-Visceral Interrelationships and Functional Systems of the Organism'*, pp. 414-417. Ivanovo.
- ⁷⁹Iezuitova, N. N., Timofeeva, N. M., Chernyakhovskaya, M. Yu., Zabelinskii, E. K., and Ugolev, A. M. (1967). Distribution of some enzymes (amylase, invertase, dipeptidase, lipase and monoglyceride lipase)

- between the small intestinal content and mucose during digestion in dog and rat. (Russian). *Dokl. Akad. Nauk SSSR*, 173, 475-478.
- ⁸⁰Ugolev, A. M., and Ustinkova, E. M. (1967). On the problem of the chemosorption role in fixation of amylase at the surface of small intestine. (Russian). In *Physiology of Digestion* (IX Conference, Dedicated to the 50th Anniversary of the Great October Socialist Revolution, Abstracts of Reports, part II), pp. 122-123. Odessa.
- ⁸¹*Physiology and Pathology of the Small Intestine* (Proceedings of the All-Union Conference of Gastroenterologists, Riga, 1970).
- ⁸²Ugolev, A. M., and Chernyakhovskaya, M. Y. (1966). Analysis of enzymes role in the hydrolysis of some glycerides in the small intestine. (Russian). In *Data of the XVI Scientific Session of the Institute of Nutrition of the Academy of Medical Sciences of the USSR*, pp. 146-147. Moscow.
- ⁸³Chernyakhovskaya, M. Y. (1967). On the mechanism of the initial and final stages of the hydrolysis of triglycerides with short chains. (Comparative-physiological date.) (Russian). In *Biochemical, Pharmacological and Toxicological Aspects of Adaptation Investigations*, pp. 112-115. (Proceedings of 3rd All-Union Conference on Ecological Physiology, Biochemistry and Morphology, Novosibirsk).
- ⁸⁴Chernyakhovskaya, M. Y. (1968). Characterization of the initial and final stages of triglycerides hydrolysis. In *Physiology and Biochemistry of the Organism Functional Systems* (Data of the Conference, part II), pp. 180-182. Kiev.
- ⁸⁵Chernyakhovskaya, M. Y., and Ugolev, A. M. (1969). Localization of final stages of hydrolysis of tributyrine in epithelial cells of the small intestine. (Russian). *Dokl. Akad. Nauk SSSR*, 187, 701-704.
- ⁸⁶De Laey, P. (1965). Development of the intestinal digestion mechanism of starch as a function of age in rats. *Nature (Lond.)*, 212, 78-79.
- ⁸⁷De Laey, P. (1966). Die Membranverdauung der Stärke. I. Mitt. Der Einfluss von Seiten der Perfusionsgeschwindigkeit und der amylolytischen Aktivität des Pankreassaftes auf die 'in vivo'-Verdauung der Stärke. *Nahrung*, 10, 641-648.
- ⁸⁸De Laey, P. (1966). Die Membranverdauung der Stärke. II. Der Einfluss von Mucinen auf die Membranverdauung der Stärke. *Nahrung*, 10, 649-653.
- ⁸⁹De Laey, P. (1966). Die Membranverdauung der Stärke. III. Der Einfluss von alimentären Komponenten des Chymus auf die Membranverdauung der Stärke. *Nahrung*, 10, 655-663.
- ⁹⁰De Laey, P. (1967). Die Membranverdauung der Stärke. IV. Der Einfluss der Grösse der intestinalen Schleimhaut auf die Membranverdauung. *Nahrung*, 11, 1-7.
- ⁹¹De Laey, P. (1967). Die Membranverdauung der Stärke. V. Zur Zweigestaltigkeit der Membranverdauung der Stärke. *Nahrung*, 11, 9-15.
- ⁹²De Laey, P. (1967). Die Membranverdauung der Stärke. VI. Die Bindung der Amylase auf der Intestinal-Mucosa. *Nahrung*, 11, 17-30.
- ⁹³De Laey, P. (1968). Adsorption of pancreatic enzymes on the intestinal membrane. In *Prot. biol. Fluids*, 15, 159-165.
- ⁹⁴Ito, S. (1964). The surface coating of enteric microvilli. (Abstr.) *Anat. Rec.*, 148, 294.
- ⁹⁵Ito, S., and Revel, J. P. (1964). Incorporation of radioactive sulfate and glucose on the surface coat of enteric microvilli. (Abstr.) *J. Cell Biol.*, 23, 44A-45A.
- ⁹⁶Ito, S. (1965). The enteric surface coat on cat intestinal microvilli. *J. Cell Biol.*, 27, 475-491.
- ⁹⁷Ito, S. (1967). The surface components of cells. In *The Specificity of Cell Surfaces*, edited by B. D. Davis and L. Warren, p. 211. Prentice Hall, Englewood Cliffs, N.J.
- ⁹⁸Deren, J. J. (1968). Development of intestinal structure and function. In *Handbook of Physiology, Sect. 6, Alimentary Canal*, edited by C. F. Code, vol. 3, pp. 1099-1123. American Physiological Society, Washington, D.C.
- ⁹⁹Ito, S. (1969). Structure and function of the glycocalyx. *Fed. Proc.*, 28, 12-25.
- ¹⁰⁰Komissarchik, Y. Y., and Ugolev, A. M. (1970). The ultrastructure and probable functional role of the glycocalyx of microfibrillae intestinal cells. (Russian). *Dokl. Akad. Nauk SSSR*, 194, 731-733.
- ¹⁰¹Sjöstrand, F. S. (1968). Molecular structure and function of cellular membranes. *Prot. biol. Fluids*, 15, 15-36.
- ¹⁰²Crane, R. K. (1960). Intestinal absorption of sugars. *Physiol. Rev.*, 40, 789-825.
- ¹⁰³Crane, R. K. (1966). Enzymes and malabsorption: a concept of brush border membrane disease. *Gastroenterology*, 50, 254-262.
- ¹⁰⁴Semenza, G. (1967). Selected topics in carbohydrate biochemistry: digestion and absorption of sugars in the intestinal tract. *Carles Res.*, 1, 187-207.
- ¹⁰⁵Kolinská, J., and Semenza, G. (1967). Studies on intestinal sucrase and on intestinal sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. *Biochim. biophys. Acta (Amst.)*, 146, 181-195.
- ¹⁰⁶Crane, R. K. (1968). Digestive-absorptive surface of the small bowel mucosa. *Ann. Rev. Med.*, 19, 57-68.
- ¹⁰⁷Semenza, G. (1968). Digestion and absorption of sugars. *Mod. Probl. Pediat.*, 11, 32.
- ¹⁰⁸Semenza, G. (1968). Sucrase and sugar transport in the small intestine. *Prot. biol. Fluids*, 15, 210-208.
- ¹⁰⁹Ugolev, A. M. (1968). Some properties and functions of hydrolytic enzymes of the surface of intestinal cells. *Prot. biol. Fluids*, 15, 149-158.
- ¹¹⁰Crane, R. K. (1969). A perspective of digestive-absorptive function. *Amer. J. clin. Nutr.*, 22, 242-249.
- ¹¹¹Parsons, D. S. (1969). Black box models of intestinal mucosal function. In *International Symposium on Intestinal Transfer of Electrolytes, Amino Acids and Sugars*, edited by W. Armstrong. Thomas, Springfield, Illinois.
- ¹¹²Semenza, G. (1969). In search of molecular mechanisms in intestinal sugar transport. *Enzym. biol. clin.*, 10, 323-324.
- ¹¹³Sjöstrand, F. S. (1967). The structure of cellular membranes. *Protoplasma (wien)*, 63, 248-261.
- ¹¹⁴Sjöstrand, F. S. (1968). Molecular structure and function of cellular membranes. *Prot. biol. Fluids*, 15, 15-36.
- ¹¹⁵Hoof, C., van Hauwaert, J., De Laey, P., and Adriaenssens, K. (1963). Malabsorption after total gastrectomy in childhood. *Helv. pediat. Acta*, 18, 502-517.
- ¹¹⁶Masevich, T. G., Ugolev, A. M., and Zabelinskii, E. K. (1967). Methods of membrane digestion investigation in clinical conditions. (Russian). *Ter. Arkh.*, 39, 53-57.
- ¹¹⁷Churina, S. K., and Chernyakhovskaya, M. Y. (1971). The activity of small intestine lipolytic enzymes (membrane digestion of lipids, in patients with ischemic heart disease. (Russian). *Kardiologiya*, 11, (7), 38-41.
- ¹¹⁸Timofeeva, N. M., Iezuitova, N. N., Nadirova, T. Y., and Ugolev, A. M. (1965). Characterization of diges-

- tive functions of intestinal epithelium in severe irradiation. In *Physiology and Pathology of Digestion*, (Brief Account in Reports of the Scientific Conference, 24-28 September), pp. 272-274. Lvov.
- ¹¹⁹Ugolev A. M., Iezuitova N. N., Nadirova, T. Y., and Timofeeva, N. M. (1966). Digestive functions of intestinal epithelium in severe radiation injuries. (Russian). *Dokl. Akad. Nauk SSSR*, **166**, 472-475.
- ¹²⁰Ugolev, A. M., Timofeeva, N. M., Iezuitova, N. N., Dombrovskaya, M. P., and Rakhimov, K. R. (1969). The effect of certain stress agents on the digestive functions of intestinal epithelium. (Russian). *Dokl. Akad. Nauk SSSR*, **188**, 489-492.
- ¹²¹Gavrilov, R. I., and Domanskii, V. Y. (1966). Alterations in membrane digestion caused by X-ray action. (Russian). *Radiobiologiya*, **6**, 394-398.
- ¹²²Gavrilov, R. I. (1968). Membrane digestion in cases with the injurious effects upon intestine. (Russian). In *Membrane Processes in the Organs of the Digestive System*, pp. 12-18. (Proceedings of a Symposium). Lvov.
- ¹²³Gavrilov, R. I., and Ovdeichuk, R. A. (1970). The peculiarities of alterations in the enzymatic processes of small intestine in case of damaging effects in the early period of ontogenesis. (Russian). In *Physiology and Pathology of the Small Intestine* (Proceedings of the All-Union Conference of Gastroenterologists), pp. 338-341. Riga.
- ¹²⁴Gavrilov, R. I., and Remizova, I. V. (1970). Alkaline phosphatase activity in small intestine under X-ray effect upon organism. (Russian). In *Physiology and Pathology of the Small Intestine* (Proceedings of the All-Union Conference of Gastroenterologists), pp. 43-45. Riga.
- ¹²⁵Loginov, G. I. (1968). Relationships between the membrane and cavital carbohydrate hydrolysis in normal state and after exclusion of external secretion of pancreas. In *Proceedings of 3rd Scientific Conference of Young Scientists from Tashkent Medical College*, pp. 200-202. Tashkent.
- ¹²⁶Loginov, G. I. (1969). Physiological mechanisms of pancreatic deficiency compensation at the cost of contact (membrane) digestion. (Russian). In *Problems of Prophylaxis, Diagnostics and Treatment of the Digestive Diseases*, (Proceedings of the Republic Conference), pp. 124-125. Kiev.
- ¹²⁷Loginov, G. I., and Ugolev, A. M. (1970). Digestive-resorptive functions of the intestinal epithelium organization, regulation and compensation. (Russian). *Proceedings of the 4th Inter-college Scientific Conference of Physiologists and Morphologists of the Educational Colleges in Honour of I. P. Pavlov's 120th birthday*, pp. 231-233. Yaroslav.
- ¹²⁸Booth, C. C. (1968). Effect of location along the small intestine of absorption of nutrients. In *Handbook of Physiology, Sect. 6, Alimentary Canal*, edited by C. F. Code, vol. 3, pp. 1513-1527. American Physiological Society, Washington, D.C.