LOCALIZATION OF THE ACTION OF THE DIGESTIVE ENZYMES IN THE INTESTINE

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One of the more important aspects of intestinal activity is the enzymic breakdown of the food substances. The intestine contains many enzymes, some of which manifest their activity almost entirely extracellularly, in the cavity of the intestine (enterokinase, for example), while others do so mainly inside the cells of the intestinal epithelium (for example, some types of dipeptidases with narrow specificity); there is also an intermediate group of enzymes, exhibiting great activity both inside and outside the cells (for example, alkaline phosphatase, saccharase, and so on) [14].

Because of this great variety of enzyme processes taking place in the intestine, it is difficult to study them simultaneously and to compare their importance in the organism.

In their investigations of the secretion of the intestinal enzymes and of its regulation, I. P. Pavlov, B. P. Babkin, Nasset, and other authors [1,2,4,24] paid considerable attention to processes taking place within the lumen of the intestine. Dahlqvist and co-workers [15,17], who used a technique of intestinal incubation, studied digestion and absorption of a special food mixture and the content of enzymes in human chyme. They found that the digestion of starch is completed very quickly in the intestinal lumen, whereas disaccharides are broken down partly in the lumen of the intestine and partly intracellularly. In their study of the localization of enzymes in cells of the intestinal epithelium, Miller and co-workers [20-22] and some earlier investigators [16,19,27] classified the whole action of the intestinal enzymes as intracellular. These workers did not take into account the special features of the secretory process in the intestine where, in contrast to the other digestive glands, individual granules are not secreted but whole cells of the intestinal epithelium are detached. The enzymes contained in them, acting intracellularly, enter the lumen of the intestine, where they also take part in the breakdown of food substances [5,8,12,26].

Some investigations have shown that not only the intestinal, but also the pancreatic enzymes produce breakdown of food substances mainly after their adsorption on the surface of the intestinal mucous membrane. This mechanism assumes particular importance in the early postnatal period of development [3,6].

Several of the investigations cited above were carried out on isolated organs and tissues or in experiments on animals, but in conditions selected for the study of special and often very limited problems in digestion. These methods cannot be used to judge the course of the enzyme processes in the intestine as a whole.

It appeared interesting to study the intensity of the enzymic breakdown of the food in the lumen of the intestine and actually in the structural elements of the intestinal wall of man and certain animals in conditions as close to natural as possible. For this purpose, investigations were conducted on material obtained from human cadavers and experiments were carried out on dogs and rats.

EXPERIMENTAL METHOD AND RESULTS

Investigations of cadaver material. The cadavers of seven adults dying from accidental causes were autopsied 3-6 h after death, and the cadavers of four normally developed newborn full-term infants, dying from birth injury either at birth or during the first 24 h after birth, the cadaver of one premature infant (30 weeks) and one fetus aged 23-24 weeks were autopsied during the first 24 h after death. Taking into account the fact that at the height of the digestive process, the small intestine is filled comparatively uniformly with food chyme, and the areas not filled with chyme at this time play no direct part in the processing of the food masses, it was concluded that it was not necessary to investigate all the intestine, but only individual segments at 3 or 4 different levels. In the areas of the intestine excised between two ligatures, the volume of the contents and the weight of the epithelium scraped from

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TABLE 1. Quantity of Enzymes in the Adult Human Intestine*

Material	Weight (in g)	Entero-kinase	Phospha-tase	Saccha-rase	Entero-kinase	Phospha-tase	Saccha-rase
		Conventional units/g			Conventional units in the whole segment of intestine		
Duodenum:							
Contents	0.92	10,000	2000	615	9200	1840	565
Scraping of epithelium	1.54	610	150	114	940	232	176
Middle of small intestine:							
Contents	1.65	1,000	900	550	1650	1480	905
Scraping of epithelium	0.80	Less than 60	300	210	Less than 48	240	168

*The investigation was conducted on the cadaver of a woman aged 35 years dying suddenly from acute cardiac failure. Autopsy was performed 3 h after death. The stomach contained no food masses.

TABLE 2. Quantity of Enzymes in the Intestine of the Newborn Infant*

Material	Weight (in g)	Enterokinase	Phosphatase	Saccharase	Peptidase	Lipase	Amylase	Enterokinase	Phosphatase	Saccharase	Peptidase	Lipase	Amylase
		Conventional units/g						Conventional units per whole segment of intestine					
Duodenum													
Contents	0.80	170	2,250	220	—	450	15	136	1,800	176	—	360	12
Scrapings of epithelium	0.85	30	90	58	—	200	Traces	26	76	49	—	170	—
Middle of small intestine:													
Contents	1.37	90	8,500	960	770	520	—	123	1,170	1330	1060	710	—
Scrapings of epithelium	0.92	—	225	103	334	420	—	—	207	95	308	390	—
Distal part of ileum:													
Contents	1.50	200	20,100	460	800	45	—	300	30,200	690	1200	810	—
Scrapings of epithelium	0.90	—	337	140	334	37	—	—	303	126	300	330	—

*The investigation was carried out on the cadaver of a newborn girl (weight 3000 g, length 50 cm), living 14 h; the child had received 5 ml of breast milk twice. Autopsy 18 h after death. A small quantity of acid contents was present in the stomach.

the resected portion of intestine were determined. In the material thus obtained, estimations were made of the content of the enzymes enterokinase, alkaline phosphatase, and lipase by methods developed in the author's laboratory [9,11,13]. Lipase was also determined by a titration method based on the hydrolysis of tributyrin, saccharase by the hydrolysis of sucrose by a polarimetric method, peptidase by the hydrolysis of peptone and subsequent titration with potassium hydroxide solution [8], and amylase by the micromethod of Smith and Roe [25] and of Wohlgemuth.

In every case the pattern of distribution of enzyme activity between the contents and the intestinal mucous membrane was of the same type. In adults (Table 1) and in children (Table 2), the activity of the intestinal and pancreatic enzymes investigated per unit weight of intestinal contents was much higher than in the scrapings of epithelium from the corresponding part of the intestine. The same ratio was observed when the activity of the enzyme was calculated for the whole segment of intestine. This distribution of the enzymes shows that the quantity of food substances which can be broken down in the intestinal lumen is much greater than the quantity broken down by the intestinal epithelium.

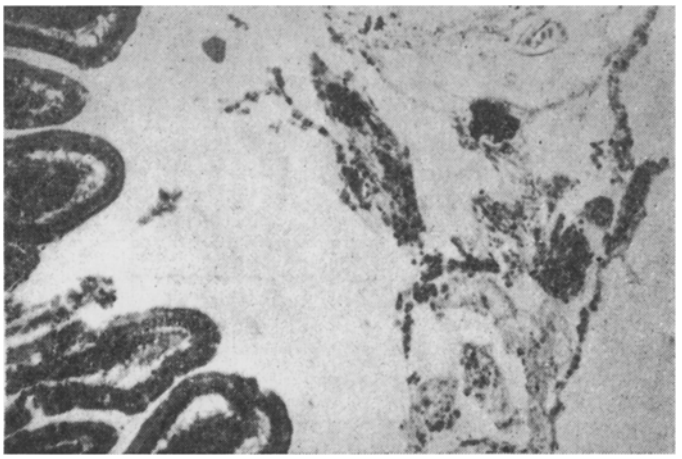
TABLE 3. Quantity of Enzyme in the Intestine of Dogs in the Period of Digestion

Material	Weight (in g)	Entero-kinase	Phospha-tase	Saccha-rase	Lipase	Amylase	Entero-kinase	Phospha-tase	Saccha-rase	Lipase	Amylase
		Conventional units/g					Conventional units in whole segments of intestine				
Duodenum (dog 3):											
Contents	8.50	2000	6,700	240	400	450	17,000	57,000	2040	3400	3800
Scrapings of epithelium	5.22	337	6,700	510	22	34	1760	35,000	2600	117	177
Intestinal wall	21.1										
Per g of wall		18	500	130	—	2	384	10,600	2700	—	35
Per g of epithelium		74	2,020	250	—	7					
Ileum (dog No. 4):											
Contents	6.98	3370	22,500	490	60	—	23,500	157,000	3410	418	—
Scrapings of epithelium	4.88	Less than 15	18,000	480	22	2	Less than 70	88,000	2350	98	—
Intestinal wall	14.67										
Per g of wall		Less than 2	100	115	Traces	Traces					
Per g of epithelium		Less than 6	300	345	"	"	Less than 30	1467	1690	5	—

Since the cadavers were autopsied several hours after death (3-20 h), the possibility was not ruled out that during this period postmortem changes could have taken place in the intestine, reflected in the distribution of enzyme between the mucous membrane and the intestinal contents. In addition, it was not always possible to answer one question which is very important for this particular investigation: in what stage was the person's digestion at the moment of death. As an additional control, it was decided to carry out experiments on animals in order to obtain material for investigation in stricter and more nearly physiological conditions.

Experiments on dogs. Healthy adult dogs were given an ordinary mixed diet or bread with lard. The animals were sacrificed 3 h after eating, and parts of the intestine 12-15 cm long were immediately removed for investigation between two ligatures from the region of the duodenum, the distal portion of the ileum, and sometimes from the middle of the small intestine. The weight of the contents and of the whole wall of the resected portion of intestine was determined. The intestine was then divided longitudinally into two approximately equal parts. From one part scrapings were made from the epithelium, while the second was used for investigating the enzymic activity of the whole intestinal wall. The content of several intestinal and pancreatic enzymes was determined in the intestinal contents, the intestinal wall, and the scrapings of epithelium. The enzymic activity was calculated per gram of tested material and per weight of segment of intestine.

Investigation of all parts of the dogs' intestine revealed consistent results, generally speaking similar to those obtained in the cadavers, although differing in details from them. In the adult human intestine, the content of enzymes (especially phosphatase) as a rule was lower than in the corresponding segment of the dog's intestine. But the main difference was evidently associated mainly with the stage of digestion. At the height of digestion, corresponding to the period when the dogs were sacrificed, a considerable accumulation of enzyme takes place in the mucous membrane by comparison with the amount of enzyme in the mucous membrane of the fasting animal, and conversely the concentration of phosphatase and saccharase in the chyme falls slightly [10]. It is clear from Table 3 that the concentration of enterokinase and of pancreatic enzymes in the intestinal contents of the dogs in the stage of digestion of the food was many times (frequently several tens of times) greater than in the scrapings of the mucous membrane from the same parts of the intestine. Equally marked differences were observed in the total content of these enzymes in the whole segment of the intestine. The concentration of phosphatase and saccharase in the intestinal contents differed to a lesser degree from the concentration of these enzymes in the scrapings of the mucous membrane. Sometimes these concentrations were equal but sometimes, in animals in the stage of digestion, the amount of saccharase per gram of epithelium was actually rather less than the quantity per gram of contents of the same segment of the intestine. It is interesting that the concentration of the enzyme in the chyme of the distal portion of the small intestine was appreciably higher on account of the absorption of water and of a number of other components of the chyme. The enzymes, however, were not absorbed, so that their content in the distal portion was often



Section of intestine of a rat after 30 ml of starch solution had passed through it for 10 min. Bands of mucous, intestinal epithelial cells, and residues of food masses (the black structures) may be seen in its lumen. Magnification 100×.

much higher than in the proximal (Tables 2 and 3). This is seen particularly clearly in the case of enterokinase, which is secreted only in the most proximal portion of the intestine and which reaches the ileum only in the composition of the chyme.

The fact was noted that the enzyme activity of the intestinal epithelium when forming part of the whole structure of the intestinal wall, per unit weight and in relation to the weight of the whole investigated segment of the intestine was much lower for nearly all the enzymes than in the scrapings of epithelium, i.e., after disturbance of the structure of the mucous membrane and of the integrity of the epithelial cells the digestive power of the enzymes in the intestinal wall increased. Only the saccharase activity in some cases remained the same in scrapings of the epithelium as in the whole intestinal wall, but in other cases it also showed a slight increase. Evidently, distribution of the structure of the mucous membrane and of the integrity of the epithelial cell did not prevent, but on the contrary, facilitated interaction between the enzyme and the substrate. The same pattern was observed when the activity of the peptidases in the intestine of rats was investigated by other authors [23].

The facts described above agree well with published data [7] showing that when the solid part of the intestinal juice is treated with solutions of proteinases and the structure of the cells of the intestinal epithelium is disturbed, the activity of the intestinal enzymes (alkaline phosphatase, saccharase, and particularly strongly, enterokinase) is appreciably increased.

The results described above disagree with those reported by A. M. Ugolev [6] regarding the special significance of structural integrity of the cells of the intestinal epithelium for the action of the enzymes. Ugolev broke up the intestinal epithelial cells by producing lysis by heating the intestinal secretion for 2 h at 38°. In the present investigation, the alkaline phosphatase activity in the liquid part of a dog's intestinal juice, completely free from cells, was studied before and after heating for 2 h at 38°. In these conditions, a decrease in activity of the enzyme was found. In one case, for example, the phosphatase content fell from 380 to 250 units/ml, and in the other case from 840 to 670 units/ml. This decrease can be explained only by inactivation of the enzyme as a result of the action of the proteinases of the intestinal juice. In Ugolev's experiments, evidently it was not the destruction of the cells of the intestinal epithelium which was important, but inactivation of the phosphatase for some other reason.

Next an attempt was made to discover the cause of the differences in the principle between the results obtained in this series of investigations on dogs and those obtained by other authors working with rats which were anesthetized while solutions of dipeptides and starch were passed through their intestine. The results of these experiments, according to their authors, show that a high rate of hydrolysis of the substrate is dependent on the action of enzymes adsorbed on the outer surface of the intestinal epithelium [6], or on intracellular breakdown of the substrate [23]. The experiments on digestion of starch solution were repeated, using the method described above. Some of the rats were fasted for 24 and 48 h before the experiment. A high degree of hydrolysis of starch was found, especially in the first minutes of the experiment. However, throughout the period of passage of the starch (10-15 min), traces of intestinal contents were found macroscopically in the solution flowing from the intestine, and in the lumen of the segment of intestine used for the experiment, not only bands of mucous and free-lying intestinal epithelial cells were found on morphological investigation, but also particles of food substances (see figure).

The difficulty of washing out the intestinal chyme is described by Dahlqvist and Thomson [18]. By carefully washing the tissues of the gastrointestinal tract of rats, they were able to wash out only 80% of the polyethyleneglycine (a substance which is not adsorbed and which is indifferent to the digestive secretion), administered to the animals with their food. These findings show that the breakdown of food substances during their passage through an intestinal loop takes place not only as a result of the enzyme of the intestinal wall, but also on account of the chyme which remains behind for a long time in the lumen of the intestine which contains intestinal and pancreatic enzymes.

In a series of experiments, the author attempted to discover the effect of pieces of surviving intestine, treated with trichloroacetic acid (by A. M. Ugolev's method [6]) on the breakdown of sucrose and starch by the corresponding enzyme. In no case could an activating action of the structural elements of the intestinal wall on these enzymes be observed.

The facts described above show that the enzymic activity of the chyme of both the proximal and the distal portion of the small intestine is several tens of times more active in pancreatic and certain intestinal enzymes (enterokinase) and several times more active in other intestinal enzymes (alkaline phosphatase, saccharase) than the same portion of the intestinal wall. Consequently, the hydrolytic action of the enzyme on the food substances is exerted mainly in the lumen of the intestine. Some intestinal enzymes, components of the cell bodies, also take part in the breakdown of food products and the products of their hydrolysis taking place in the cells of the intestinal epithelium, and thus performing a barrier function. When the cells of the intestinal epithelium are detached and destroyed, the enzymes which they contain pass into the chyme where they also take part in digesting the food masses. In this way, the fullest use is made of the enzyme capacity of the alimentary tract.

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