BBA 66719

RELEASE OF ENTEROPEPTIDASE AND OTHER BRUSH-BORDER ENZYMES FROM THE SMALL INTESTINAL WALL IN THE RAT

CHRISTER NORDSTRÖM

Research Department 1, E-blocket, Hospital of Lund and Division of Physiological Chemistry, Chemical Centre, University of Lund, Lund (Sweden)

(Received June 1st, 1972)

SUMMARY

1. The release of the enteropeptidase (EC 3.4.4.8; formerly known as enterokinase), sucrase (EC 3.2.1.26), trehalase (EC 3.2.1.28), maltase (EC 3.2.1.20) and alkaline phosphatase (EC 3.1.3.1) activities from the small intestinal wall was studied *in vivo* in gut segments in the rat for the purpose of clarifying the release mechanism and magnitude of the enterokinase activity in the lumen. First, the release following combined cholecystokinin and secretin stimulation was determined. Then, the release effect of different factors (bile, bile salts, pancreatic juice, pancreatic proteases and different gastrointestinal hormones) was tested in separate experiments.

2. The enterokinase activity was analyzed in presence of activating bile salts, which greatly improved the sensitivity and reliability of the method. Endogenous trypsin was removed from the samples by gel filtration prior to analysis.

3. About 15% of the total enterokinase and alkaline phosphatase activities were found in the lumen after stimulation with cholecystokinin and secretin. The corresponding value for sucrase was only about 7.5%. Other enzymes had intermediate release values. Only minor amounts of these enzymes were present in the lumen at zero time. Both bile salts and trypsin and chymotrypsin were able to release enterokinase, but they also released other brush-border enzymes.

4. The results do not support the concept that enterokinase is secreted or specifically released *in vivo*. Enterokinase behaved as other brush-border enzymes in all types of release experiments. The rise in luminal enterokinase activity after cholecystokinin and secretin stimulation seems to be partly an effect of a direct action of bile salts and proteases on the intestinal wall. At least part of the action is probably on cell fragments trapped in the surface mucus coating and on cells in a pre-desquamating condition in the tips of the villi. Cholecystokinin and secretin also contribute to the release of enzyme in another way, probably through the strongly increased motility and the fluid secretion.

INTRODUCTION

It has been a general concept since the time of Pavlov[1] that enteropeptidase

(EC 3.4.4.8; formerly known as enterokinase) is secreted from the intestinal wall and that the activation of trypsinogen takes place in the intestinal lumen. Recent investigations in man have also demonstrated the presence of significant enterokinase activity in the duodenal juice[2], in an amount proportionally larger (compared with the activity of the mucosa) than the amount of disaccharidases present[3]. However, we have recently found that enterokinase is located in the villi and bound to the superficial brush-border membrane of the villous epithelial cells like, *e.g.* the disaccharidases and alkaline phosphatase (EC 3.1.3.1)[4–6]. These other enzymes all exert their function in the membrane of the intact cells of the villi, and the activity which reaches the lumen is due to cell shedding in the tips of the villi. Whether that is also the case for the enterokinase activity, or if there is a specific release mechanism for this enzyme *in vivo*, is not known. Bile salts can solubilize enterokinase activity of isolated rat brush borders[7]. It has also been demonstrated that trypsin (EC 3.4.4.4) and chymotrypsin (EC 3.4.4.5) cause a specific release of enterokinase activity from isolated rat duodenal brush borders[8].

The present paper reports *in vivo* experiments in the rat, carried out to clarify the release mechanism and magnitude of the enterokinase activity in the lumen. First, the release of enterokinase and other brush-border enzymic activities from the intestinal wall, after strong, simultaneous cholecystokinin (pancreozymin) and secretin stimulation, was determined. Then, the release effect of different factors (bile, bile salts, pancreatic juice, pancreatic proteases and different gastrointestinal hormones) was tested in separate experiments.

MATERIALS AND METHODS

Experimental procedure

Male and female rats (300–350 g) of Wistar strain were fasted for 16–20 h before the experiment. Anaesthesia was induced by ether and maintained with cyclopropane–oxygen through a tracheotomy tube[9]. Cyclopropane gave an anaesthesia that was easy to control and the tracheotomy facilitated bronchial suction. Body temperature was kept at 37 °C by aid of a rectal tele-thermometer and a heating lamp. The abdomen was opened by a midline incision and a thin, short polythene tube was inserted through a small incision in the duodenum at the level of the common bile and pancreatic duct. The cannula was tied in place with a ligature immediately proximal or distal to the duct opening, depending on if pancreatic and bile secretion into the segment was required or not during the subsequent experiment. The distal end of the experimental segment was made by tying off the intestine at about the ligament of Treitz. The length of the segment was 3–4 cm (unstretched tissue). No washing of the segment was carried out prior to the experiment.

Luminal factors to be tested for their influence on the release of enterokinase and other brush-border enzymes (bile, bile salts, pancreatic juice or pure trypsin and chymotrypsin) were slowly injected through the cannula into the intestinal segment in an amount that gave a moderate filling of the segment as judged from visual inspection. The segments used in these experiments were without the common bile and pancreatic duct. The abdomen was closed with clips. 35 min after injection the animal was killed by transsection of the carotid arteries. The segment was immediately dissected free, the distal end was opened and the intestinal content was washed out

with 2.6 ml 67 mM sodium phosphate–potassium phosphate buffer, pH 7.7 (made isotonic with NaCl) and collected. This volume was sufficient, as judged from control experiments with larger volumes. The intestinal segment was cut open and homogenized *in toto* in 10 vol. of water (Ultra-Turrax homogenizer). Enzyme analyses were carried out on the homogenate and on the fluid washed out.

When the release effect of gastrointestinal hormones was investigated, the procedure was the same as described above and the experimental segments lacked the common bile and pancreatic duct. However, the effect of simultaneous cholecystokinin and secretin stimulation was studied also with segments including the duct. The hormones were injected intravenously through the femoral vein.

When perfusions with bile salts were carried out, the segments were made about 5 cm long (unstretched tissue) and they were cannulated at both ends. The proximal end was made immediately distal to the duct opening. Constant flow rate was produced by a motor-driven syringe (Braun Perfusor). The perfusion solution was pre-warmed to 37 °C by a heat-jacket of circulating water around the inflow tube.

The collecting tube was tied in place in the bile duct as far up towards the liver as possible, when rat bile was wanted for experimental purposes, or when pancreatic juice was collected through a distal cannula for the same reason. When the effect of hormones was tested in bile fistula animals, the cannula was tied in place in the bile duct more distally towards the duct opening into the intestine. The purpose was to reduce the amount of bile present in the duct that could come into the lumen during the subsequent experiment.

Chemicals

Cholecystokinin (pancreozymin) and secretin from either Boots, Nottingham, England, or Gastro-Intestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden, were used. Glucagon was purchased from Lilly, Ind., U.S.A., and pentagastrin from ICI, Macclesfield ,England.

Sodium taurocholate was obtained from Fluka, Buchs, Switzerland. Sodium taurodeoxycholate was kindly supplied by Dr L. Krabisch, Chemical Centre, Lund, Sweden.

α-Chymotrypsin (thrice crystallized; lyophilized) was obtained from NBC, Cleveland, Ohio, U.S.A., and crystallized trypsin from bovine pancreas from Ferrosan, Malmö, Sweden.

Bovine trypsinogen (once crystallized) was purchased from Seravac, Maidenhead, England.

Enzymic activity assays

Enterokinase activity was determined by a method previously described in detail[6]. Bovine trypsinogen was used as the substrate. The amount of trypsin formed was estimated in a subsequent step with the chromogenic method of Erlanger *et al.*[10], using benzoyl DL-arginine *p*-nitroanilide·HCl as the substrate. The enterokinase activity is strongly depressed by a rise in the ionic strength of the activation mixture, thus the ionic strength was kept constant and rather low to allow a correct comparison between different samples. One modification was introduced (for several reasons,

see Result section): the activation mixture was made 3.6 mM with respect to bile salts (taurocholate–taurodeoxycholate; 2:1, w/w).

Many samples contained large amounts of trypsin which had to be removed from the enterokinase activity prior to analysis of this enzyme. This was accomplished by gel filtration on Sephadex G-100[2]. The eluant buffer was 34 mM sodium phosphate–potassium phosphate, pH 7.7, containing 27 mM NaCl.

Disaccharidase activities were measured with the two-step Tris–glucose oxidase method of Dahlqvist[11].

Alkaline phosphatase activity was measured by the method of Kelly and Hamilton[12], except that the reaction mixture was made 1 mM with respect to Mg^{2+}.

Trypsin activity was estimated as in the method for the enterokinase activity. Crystallized bovine trypsin was used for preparation of the standard curve.

Chymotrypsin activity was measured with the method of Erlanger *et al.*[13], using *N*-glutaryl-L-phenylalanine *p*-nitroanilide as the substrate. Crystallized bovine α-chymotrypsin was used for preparation of the standard curve.

All readings were performed in a microcuvette with a Vitatron filterphotometer.

Calculation of enzyme release

The amount of each enzymic activity released, *i.e.* the amount of enzymic activity washed out from the lumen of the segment at the end of the experiment, is expressed as the percentage of the total intestinal activity (the sum of the activity in the lumen and that remaining in the wall). One exception is the perfusion experiment with bile salts, where it is more relevant to express the release as the percentage of activity present in the wall at the end of the perfusion only.

This comparative mode of expressing the release is important when the possible physiological significance of the release is evaluated. It also allows direct quantitative comparison between the release of different enzymes.

RESULTS

Determination of enterokinase activity

A considerable increase in the sensitivity of the assay was achieved by making the activation mixture 3.6 mM with respect to bile salts (taurocholate and taurodeoxycholate; 2:1, w/w). This increase is due to the ability of bile salts to activate the enterokinase activity[8] and is not an effect of the solubilization of membrane-bound enterokinase activity as stated by Hadorn *et al.*[7], who first demonstrated the increase in enterokinase activity in the presence of bile salts. The activation by bile salts was influenced by the ionic strength of the activation mixture, in that a stronger activation occurred at higher ionic strength. The degree of activation was more than ten times the original at the ionic strength usually present in the activation mixture. Higher concentrations of bile salts than 3.6 mM did not further appreciably increase the degree of activation. No attempts were made to find the exact optimum proportions of bile salts.

The reliability of the assay also increased in presence of bile salts: the assay system became insensitive to the addition of small, varying amounts of bile salts, as may be present in the samples, and less inert sample protein had to be introduced into

the activation mixture due to the increased sensitivity of the assay. Bile salts also had a stabilizing effect on enterokinase and trypsin during the analysis.

Samples containing large amounts of trypsin were filtered on Sephadex G-100 to remove the trypsin prior to analysis of the enterokinase activity. Enterokinase was eluted in the void volume, whereas trypsin elution was considerably delayed. The chromatography did not change the enterokinase activity, as judged from control experiments with samples containing sufficiently low trypsin activity to allow direct analysis, *e.g.* samples from release experiments with bile.

Enzyme release following cholecystokinin and secretin stimulation (bile and pancreatic secretions intact)

Visual inspection during the course of the experiment revealed that the intestinal segment became filled with fluid and the motility increased greatly. The colour of the fluid was strongly yellow from bile. Pancreatic secretion was also greatly stimulated, as judged from the high activities of trypsin and chymotrypsin found in the intestinal fluid (0.5–1.8 mg trypsin/ml and 4–10 mg chymotrypsin/ml).

The released amounts of enterokinase and some other brush-border enzymic activities found in five separate experiments are given in Table I. The enterokinase and alkaline phosphatase activities were always released to the greatest extent (mean about 15%), whereas the smallest release was always found for the sucrase activity (mean 7.5%). The maltase and trehalase activities showed intermediate release figures.

TABLE I

ENZYME RELEASE FOLLOWING CHOLECYSTOKININ AND SECRETIN STIMULATION (BILE AND PANCREATIC SECRETIONS INTACT)

A combined dose of cholecystokinin and secretin (4–6 units/kg of each hormone) was given intravenously. The experiment was finished 35 min later. The released amount of each enzymic activity is expressed as percent of the total intestinal activity, *i.e.* the sum of the activity in the lumen and the activity remaining in the wall. This way of expressing the release is always used if not otherwise stated.

Enzymic activity	*Release (%) in experiment*					*Mean*
	1	*2*	*3*	*4*	*5*	
Enterokinase	17.1	15.6	11.6	15.8	12.9	14.6
Sucrase	6.6	9.5	6.0	9.7	5.7	7.5
Trehalase	9.6	13.8	7.5	8.7	9.5	9.8
Maltase	9.4	14.7	9.3	12.6	7.7	10.7
Alkaline phosphatase	15.8	21.6	9.4	16.1	14.6	15.5

In Table II the release of the various enzymes has been expressed in a relative way. The released sucrase activity has been arbitrarily set at 1.0 in each experiment and the released amounts of the other enzymic activities have been expressed as the number of times that they exceed the release of the sucrase activity. There is a rather good agreement between the ratios in the different experiments. The enterokinase activity in the intestinal fluid after hormone stimulation is apparently about twice the quantity of sucrase activity present.

Control experiments revealed that minor amounts of enzymic activity were

TABLE II

RATIOS FOR THE RELEASED AMOUNTS OF ENZYMES OF TABLE I WHEN THE SUCRASE ACTIVITY IS TAKEN AS UNIT

Enzymic activity	*Expt*					*Mean*
	1	*2*	*3*	*4*	*5*	
Enterokinase	2.6	1.6	1.9	1.6	2.3	2.0
Sucrase	1.0	1.0	1.0	1.0	1.0	1.0
Trehalase	1.5	1.5	1.3	0.9	1.7	1.4
Maltase	1.4	1.5	1.6	1.3	1.4	1.4
Alkaline phosphatase	2.4	2.3	1.6	1.7	2.6	2.1

already present in the lumen prior to hormone stimulation (the percent figures given below represent mean values for several experiments, the numbers of which are given in parentheses): Enterokinase 5.1% (4), sucrase (EC 3.2.1.26) 1.6% (6), trehalase (EC 3.2.1.28) 2.7% (3), maltase (EC 3.2.1.20) 2.1% (5) and alkaline phosphatase 3.9% (5). These minor amounts are included in the release values given in Table I and the following tables, since no washing of the segments was made prior to the start of the experiments. Only slightly higher values were found when isotonic buffer was allowed to moderately distend the segment for 35 min before the wash out (the segments did not include the common bile and pancreatic duct).

Effect of bile

Rat bile was introduced into the experimental segment and left there for 35 min. An increased motility in the segment was observed. The amounts of the various brush-border enzymic activities present in the lumen at the end of the experiment are given in Table III. The results resemble the results obtained after stimulation with cholecystokinin and secretin (Table I), both regarding the ratios and the absolute amounts of released enzymic activities. Bile from different animals was used in the different experiments.

Control analyses revealed no or negligible activities of enterokinase, alkaline phosphatase or disaccharidases in the bile itself.

TABLE III

ENZYME RELEASE BY BILE

Enzymic activity	*Release (%) in experiment*			*Mean*
	1	*2*	*3*	
Enterokinase	17.4	13.0	13.9	14.8
Sucrase	9.2	7.5	9.6	8.8
Trehalase	11.2	9.8	13.6	11.5
Maltase	10.8	8.7	12.1	10.5
Alkaline phosphatase	16.4	11.9	20.4	16.2

Effect of bile salts

A solution containing taurocholate and taurodeoxycholate was perfused at a constant rate through an intestinal segment that was cannulated at both ends. The

perfusate was collected in fractions and analyzed for enzymic activities. The results from experiments with two different rats are given in Fig. 1. The presence of bile salts increased the output of all enzymic activities studied by an amount several times larger than that in the control fractions (Fractions 1 and 2). Trehalase and alkaline phosphatase activities were always released to the greatest extent. A lower release was found for the enterokinase activity, which, however, was higher than that for the maltase and, especially, the sucrase activity. The ratios for the three latter enzymic activities were about the same as the corresponding ratios found in the experiments illustrated in Tables I (Table II) and III, whereas the ratios for the alkaline phosphatase and, especially, the trehalase activity were higher in this experiment. The absolute amounts of enzymic activity released in this experiment cannot be directly compared with the release values of the preceding experiments, since the experimental conditions, and also the way of expressing the release, are different.

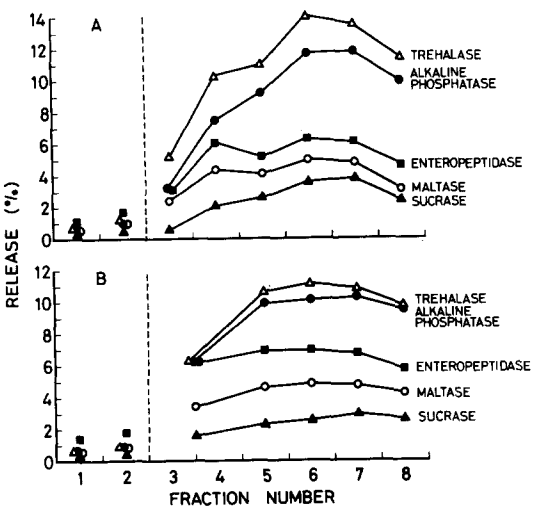

Fig. 1. Enzyme release by bile salts in two separate perfusion experiments (A and B). A 10 mM mixture of sodium taurocholate and taurodeoxycholate (2:1) in 0.78% NaCl was perfused through the intestinal segment (fractions to the right of the broken line). The first two fractions (to the left of the broken line) correspond to perfusion with only NaCl. Constant flow rate: 3.3 ml/h. Collection time: 25 min per fraction, except for a single fraction of 50 min in Expt B as indicated in the figure. The release in the fractions is expressed as percent of the activity present in the wall at the end of the experiment. The segment was washed through with 8 ml of saline at a rate of 32 ml/h before the collection of Fraction 1.

The first two fractions in each experiment show that there is more enterokinase activity than any other enzymic activity in the outflow when the segment is perfused with only NaCl. However, the absolute amounts of activity are very small.

Attempts to determine the content of DNA (the diphenylamine and indole methods) in the fractions were not successful due to insufficient sensitivity and interference by the bile salts present. However, protein determination revealed that fractions with high enzyme release values also had a high protein content.

Experiments were also carried out in which bile salts were injected into the seg-

ment and left there for 35 min. They caused a release that was similar to the release by rat bile (Table III).

Enzyme release in rats with bile fistula following cholecystokinin and secretin stimulation

Visual inspection revealed that the motility greatly increased after stimulation (4–6 units/kg of both hormones) but the segment was not as rapidly filled with fluid as in rats with intact bile secretion. Therefore, the major part of the fluid normally present in the segment after cholecystokinin and secretin stimulation seems to be created by bile secretion, rather than by pancreatic or intestinal secretion. To compensate for the reduced intestinal fluid volume in the animals with bile fistula, the segments were moderately filled with 67 mM sodium phosphate–potassium phosphate buffer, pH 7.7 (made isotonic with NaCl), about 10 min after hormone stimulation (thus making the conditions of luminal fluid volume more normal). The experiment was finished 35 min after stimulation. There was always a significant release, in spite of the absence of bile secretion. The magnitude of the release was usually, but not always, comparable with the release observed in segments with intact bile secretion (Table I). The ratios for the various enzymic activities released were similar to the ratios found in preceding experiments.

The amounts of trypsin and chymotrypsin activity in the lumen at the end of the experiment were lower than those found in segments with intact bile secretion.

Some rats were found to have empty bile ducts and significant amounts of bile in the duodenum. Such rats were not used in this or other experiments, where the absence of bile in the lumen is a pre-requisite for a correct interpretation of the results.

Effect of pancreatic juice

Rat pancreatic juice (containing activated proteases) was introduced into the experimental segment and left there for 35 min. The amounts of the various brush-border enzymic activities present in the lumen at the end of this period are given in Table IV. They indicate that a significant release occurred. The ratios for the released enzymic activities were similar to the ratios found in other experiments, such as the one in Table II.

Control analyses revealed no, or negligible, activities of enterokinase, alkaline phosphatase or disaccharidases in the pancreatic juice itself.

TABLE IV

ENZYME RELEASE BY PANCREATIC JUICE

Enzymic activity	*Release (%) in experiment*			*Mean*
	1	*2*	*3*	
Enterokinase	13.9	9.7	9.7	11.1
Sucrase	6.6	5.9	7.2	6.6
Trehalase	—	7.8	6.8	7.3
Maltase	9.6	8.1	9.7	9.1
Alkaline phosphatase	18.7	14.8	11.0	14.8

Effect of pancreatic proteases

Bovine trypsin and chymotrypsin (1 and 2 mg/ml, respectively) in 67 mM sodium phosphate–potassium phosphate buffer, pH 7.7 (made isotonic with NaCl) were injected into the intestinal segment. The filling caused an increased motor activity. 35 min later, the content of the segment was washed out and the amount of luminal enzymic activities was determined (Table V). The results resemble the release results obtained after stimulation by cholecystokinin and secretin (Table I) both qualitatively and quantitatively.

TABLE V

ENZYME RELEASE BY PANCREATIC PROTEASES

Enzymic activity	*Release (%) in experiment*			*Mean*
	1	*2*	*3*	
Enterokinase	10.6	14.0	12.9	12.5
Sucrase	5.5	5.4	7.3	6.1
Trehalase	7.0	7.2	8.3	7.5
Maltase	7.4	7.9	10.7	8.7
Alkaline phosphatase	15.9	14.2	14.0	14.7

Effect of gastrointestinal hormones in absence of bile and pancreatic secretions

The following hormones were tested: cholecystokinin (4–8 units/kg) and secretin (4–8 units/kg), separately and together, pentagastrin (8 μg/kg) or glucagon (0.2 mg/kg). The doses were made high to give maximal stimulation. 35 min after hormone injection the experiment was finished and the luminal content of the segment was washed out and analysed for enzymic activities. The segments used were without the bile and pancreatic duct.

The luminal content of enterokinase and other brush-border enzymic activities clearly increased after cholecystokinin and/or secretin stimulation, in spite of the absence of bile and pancreatic secretion. Possibly, there was also some small release after gastrin and glucagon stimulation. However, the release values obtained after cholecystokinin and/or secretin stimulation were always considerably lower than those obtained after combined cholecystokinin and secretin stimulation in animals with intact bile and pancreatic secretion. The ratios for the released amounts of enzymes were always similar to the ratios found in all preceding experiments.

DISCUSSION

The results do not support the concept that enterokinase is secreted or specifically released *in vivo*, at least not in the rat. Enterokinase behaved as other brush-border enzymes in all types of release experiments. The released amounts of the enzymes were always a minor fraction of the total intestinal activity. The rise in luminal enterokinase activity after cholecystokinin and secretin stimulation in the intact rat (Table I) seems to be mainly an effect of a direct action of bile salts and proteases on the intestinal wall. The strongly increased motility in the gut under influence of cholecystokinin probably contributes to the release. It is likely that at least

part of the action of bile salts and proteases is on cell fragments trapped in the surface-mucus coating and on cells in a pre-desquamating condition in the tips of the villi. The quantitative differences in the release of different enzymes, which were found to be about the same in all types of release experiments, may well be explained by the differences in the distribution profiles of the enzymes along the villi. The alkaline phosphatase, enterokinase and trehalase activities, which have shown the highest release values, are located more distinctly toward the tips of the duodenal villi than the sucrase and maltase activities[6].

Stimulation with cholecystokinin and secretin also caused an enzyme release in the absence of bile and pancreatic juice, but it was smaller than the release found with intact bile and pancreatic secretions. In view of all the present findings, this release is probably not caused by a direct hormone action on the surface membrane of the intact cells of the villi. It is more likely that it results from the influence of increased motility and fluid secretion on desquamated cell material and on cells in a pre-desquamating condition at the tips of the villi.

Recently, it was reported that bile salts may be responsible for a physiological release of enterokinase from the intact cells of the villi, as judged from experiments with isolated rat brush borders[7]. The present study shows that bile salts are able to release *in vivo*, but this effect is unspecific since other brush-border enzymes are released in equivalent amounts. Moreover, it is doubtful whether the bile salts in fact do act by releasing enzymes from the intact cells on the villi.

That pancreatic proteases cause a release of enterokinase activity from the intestinal wall is in agreement with previous observations by Pavlov[1] and Lepkovsky *et al.*[14]. However, the present study has revealed that also other brush-border enzymes are released in equivalent amounts. Moreover, the pancreatic proteases are not the only release factor and they are probably less important than bile salts during physiological conditions. The release by trypsin and chymotrypsin cannot be due to an enzyme-solubilizing action on the membrane of the intact cells of the villi, since studies with isolated duodenal brush borders have shown that these proteases can not solubilize any brush-border enzymes except enterokinase[8].

After cholecystokinin and secretin stimulation, the concentrations of trypsin and chymotrypsin found in the lumen were lower in rats with bile fistula than in animals with intact bile secretion. This indicates that the activation of the enterokinase activity caused by the bile salts present in the secreted bile is of physiological importance. In this context, the observation that bile salts seem to counteract the depressive effect of high ionic strength on the enterokinase activity should be kept in mind.

The various luminal factors and gastrointestinal hormones were tested in high concentrations in order to obtain the maximal effect in each case. Therefore, it is probable that some release values would have been even lower with a physiological concentration of the factor under investigation.

Further studies are required to determine whether the minor amount of the total activity that is present in the lumen after stimulation is completely or mainly responsible for the physiological activation of trypsinogen, or if an important part of this activation takes place on the membrane of the intact cells of the villi.

It has recently been suggested that enterokinase may be produced and secreted by the Brunner glands which are located in the submucosal tissue of duodenum[15].

Furthermore, it has been demonstrated that the output of enterokinase activity in the lumen of dog duodenum increases after stimulation of the Brunner gland secretion by glucagon[16]. On the other hand, other scientists have recently found enterokinase to be localized in the goblet cells[17]. However, our previous findings on the localization of enterokinase[4–6] strongly speak against the possibility that enterokinase is produced in any other place than in the villous epithelial cells and the present study on the release of the enzyme has not revealed anything that alters the concept of enterokinase as a true brush-border enzyme.

Concerning the localization of enterokinase to the goblet cells[17], immunochemical technique was used and a commercial enterokinase preparation from NBC was used directly as the antigen. The present author has investigated this enterokinase preparation (even the same control number) and has found it not to be pure. It has low specific enterokinase activity and also contains, for instance, other enzymic activities such as maltase and alkaline phosphatase. Therefore, no conclusions about the localization of enterokinase can be drawn from the results.

ACKNOWLEDGEMENTS

Thanks are due to Dr Arne Dahlqvist for valuable discussions and criticism. The investigation was supported by grants from the Medical Faculty of the University of Lund, the Swedish Nutrition Foundation and the Swedish Medical Research Council (grant to A.D. project No. 13X-157 and 13P-2262). Miss Ulla Iwarson is thanked for skilful technical assistance.

REFERENCES

1 I. P. Pavlov, in *Les Prix Nobel en 1904*, P. A. Norstedt and Söner for the Nobel Foundation, Stockholm, 1907.
2 B. Hadorn, M. J. Tarlow, J. K. Lloyd and O. H. Wolff, *Lancet*, (i) (1969) 812.
3 C. Nordström and A. Dahlqvist, *Abstr. Annual Meeting of European Society for Pediatric Gastroenterology, Lund, 1970, Acta Paediat. Scand.*, 60 (1971) 369.
4 C. Norström and A. Dahlqvist, *Biochim. Biophys. Acta*, 198 (1970) 621.
5 C. Nordström and A. Dahlqvist, *Symp. on Intestinal Mucosal Enzymes, 4th World Congress of Gastroenterology, Copenhagen, 1970*, Abstr. No. 130.
6 C. Nordström and A. Dahlqvist, *Biochim. Biophys. Acta*, 242 (1971) 209.
7 B. Hadorn, N. Steiner, C. Sumida and T. J. Peters, *Lancet*, (i) (1971) 165.
8 C. Nordström, *Biochim. Biophys. Acta*, 268 (1972) 711.
9 A. M. Dawson and H. B. McMichael, *J. Physiol.*, 196 (1968) 32 P.
10 B. Erlanger, N. Kokowsky and W. Cohen, *Arch. Biochem. Biophys.*, 95 (1961) 271.
11 A. Dahlqvist, *Anal. Biochem.*, 22 (1968) 99.
12 M. H. Kelly and J. R. Hamilton, *Clin. Biochem.*, 3 (1970) 33.
13 B. Erlanger, F. Edel, A. G. Cooper, *Arch. Biochem. Biophys.*, 115 (1966) 206.
14 S. Lepkovsky, F. Furuta, M. K. Dimick and I. Yamashina, *Poultry Sci.*, 49 (1970) 421.
15 E. Eggermont, A. M. Molla, L. Rutgeerts and G. Tytgat, *Lancet*, (ii) (1971) 369.
16 R. Goldman, Y. Kim, R. Jones and M. Sleisenger, *Proc. Soc. Exp. Biol. Med.*, 138 (1971) 562.
17 K. Takano, T. Suzuki and K. Yasuda, *Okajimas Folia Anat. Jap.*, 48 (1971) 15.