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Biosynthesis of intestinal microvillar proteins

Characterization of intestinal explants in organ culture and evidence for the existence of pro-forms of the microvillar enzymes

E. Michael DANIELSEN,* Hans SJÖSTRÖM,* Ove NORÉN,* Birgit BRO† and Erik DABELSTEEN‡
**Department of Biochemistry C and †Department of Biochemistry A, The Panum Institute, University of Copenhagen, Copenhagen, Denmark, and ‡Department of Oral Pathology, The Royal Dental College, Copenhagen, Denmark*

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Explants of pig small intestine were maintained at 37°C in organ culture for periods up to 24 h in a system using Trowell T-8 medium supplemented with 10% foetal-calf serum. The mucosal morphology was well preserved during culture, as judged by light and electron microscopy. The explant contents of protein and two brush-border enzymes, microvillus aminopeptidase (EC 3.4.11.2) and dipeptidyl peptidase IV (EC 3.4.14.5), were not significantly modified during culture compared with controls, but a moderate, continuous release of both protein and enzyme activities into the medium was observed. Continuous labelling with [^{35}S]methionine resulted in an even incorporation of radioactivity in the protein components, and the rate of labelling only moderately decreased over the 24 h period. The polypeptide compositions of sucrase (EC 3.2.1.48)–isomaltase (EC 3.2.1.10), maltase–glucoamylase (EC 3.2.1.20) lactase (EC 3.2.1.23)–phlorizin hydrolase (EC 3.2.1.62), microvillus aminopeptidase and aspartate aminopeptidase (EC 3.4.11.7) synthesized during culture were studied, and some were found to be similar to those of the pro-forms of the enzymes isolated from animals that had had their pancreatic duct disconnected 3 days before being killed. These results confirmed earlier findings of the existence of pro-forms of some of the microvillar enzymes and thus indicate a low activity of pancreatic proteinases in the culture system.

Since the pioneering work of Browning & Trier (1969), organ culture of intestinal explants has become an established method in studies on various aspects of metabolism of the small intestine. Contrary to experiments with open intestinal segments, everted rings or sacs of intestine (Parsons, 1968), which can only be performed for relatively short intervals (1–2 h), explants have been kept viable in organ culture for periods up to 24 or even 48 h. Organ culture has been carried out with intestinal explants from a number of different species; thus explants of human (Browning & Trier, 1969; Hauri *et al.*, 1975, 1977; Kedinger *et al.*, 1979), rabbit (Kagnoff *et al.*, 1972), guinea pig (Kedinger *et al.*, 1974*a*,*b*), rat (Shields *et al.*, 1979) and mouse (Ferland & Hugon, 1979*a*,*b*; Berteloot *et al.*, 1979) intestine have all successfully been cultured. In these previous studies the system has been used to provide information on the turnover of brush-border proteins as well as secretory activity of the enterocytes.

In order to establish a model system suitable for studying the biogenesis of individual intestinal microvillar proteins, the organ-culture technique has been applied in the present work to pig small intestine. The paper presents a microscopic and biochemical characterization of cultured intestinal explants, with particular emphasis on the synthesis and turnover of the microvillar proteins. Some of these, sucrase (EC 3.2.1.48)–isomaltase (EC 3.2.1.10), maltase–glucoamylase (EC 3.2.1.20), lactase (EC 3.2.1.23)–phlorizin hydrolase (EC 3.2.1.62), microvillus aminopeptidase (EC 3.4.11.2) and aspartate aminopeptidase (EC 3.4.11.7), were isolated from the cultured explants and their polypeptide compositions studied.

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Materials and methods

Materials

Trowell's T-8 medium, Hanks' buffered salt solution, foetal-calf serum, penicillin (10000 units/ml) and streptomycin (10mg/ml) were products of Gibco Europe, Glasgow, Scotland, U.K. Plastic dishes for organ culture supplemented with stainless-steel grids were purchased from Falcon, Los Angeles, CA, U.S.A. [^{35}S]Methionine (1390Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Aqualuma Plus (for liquid-scintillation counting) was from Lumac Systems, Basel, Switzerland. 2,5-Diphenyloxazole and dimethyl sulphoxide were from Merck, Darmstadt, Germany. The sources of other chemicals were as previously described (Sjöström *et al.*, 1978).

Pig small intestines were kindly given by the Department of Experimental Pathology, Rigshospitalet, Copenhagen, Denmark.

Organ culture

Immediately after the animal was killed, a 5cm segment of the small intestine, taken 2–3m from the pylorus, was everted and placed in 40ml of ice-cold Hanks' buffered salt solution, containing 25units of penicillin/ml and 25μg of streptomycin/ml. Within 15min, explants (approx. 1.5–2mg wet wt.) containing largely mucosa and submucosa were excised and placed with the villus side upwards on a triangular stainless-steel grid (10 explants/grid) in sterile plastic organ-culture dishes as described by Browning & Trier (1969). To the central well of the dish was carefully added 0.75ml of Trowell's T-8 medium, containing 100 units of penicillin/ml, 0.1mg of streptomycin/ml and 10% (v/v) foetal-calf serum. In protein-synthesis experiments, the medium also contained 25μCi of [^{35}S]methionine/ml. After addition of 3ml of water to the outer well, the dishes were covered and maintained at 37°C in a constant-temperature incubator for periods up to 24h. After 0, 5 and 10h of culture, the dishes were gassed for 3min with O_2/CO_2 (19:1).

Microscopy

Explants cultured for 0 (control explants), 5, 10 and 24h were taken for microscopy examinations. For light microscopy, the tissue was formalin fixed and paraffin embedded. Sections (5μm thick) were cut and stained with haematoxylin and eosin or with a periodic acid/Schiff/Alcian Blue stain.

For electron microscopy, the tissue was placed in a cold fixative containing 3% (v/v) glutaraldehyde in 0.1M-sodium cacodylate buffer, pH7.2, and then placed in 2% (w/v) OsO_4 in the same buffer for 2h. *En bloc* staining was performed overnight in 0.5% uranyl acetate (Weinstein *et al.*, 1963). After dehydration in increasing concentrations of ethanol, the tissue was embedded in Epon. Thin sections for electron microscopy were cut with a diamond knife by using a LKB Ultratome IV (LKB Produkter, Stockholm, Sweden), and sections of golden to silver colour were examined in an electron microscope (Philips 200 C, Eindhoven, The Netherlands) after staining with lead citrate (Reynolds, 1963).

Biochemical determinations

Unless otherwise stated, all procedures were performed at 4°C.

After culture, the explants, still on the grid, were briefly washed with about 3ml of ice-cold 0.9% NaCl, by using a Pasteur pipette. For further processing, explants from one culture dish were pooled. They were transferred by forceps to a pre-weighed 1.5ml plastic tube and, after weighing, 1.0ml of ice-cold 0.9% NaCl was added. The suspended explants were then transferred to a 5ml Potter–Elvehjem homogenizer and homogenized by approx. 20 strokes of a motor-driven pestle (1000rev./min). Samples for determination of protein concentration and enzyme activities were taken directly from the homogenates. Samples (0.7ml) of the homogenates were centrifuged at 48000*g* for 1h. Both the pellet (the total membrane fraction) and the supernatant (the total soluble-protein fraction) were collected. Ice-cold 0.8M-$HClO_4$ (1ml) was added to the supernatant. Precipitated protein was collected by centrifugation for 3min in a Beckman Microfuge (Beckman Instruments, Palo Alto, CA, U.S.A.). The pellet was resuspended in 1.0ml of 0.4M-$HClO_4$ and centrifuged again as above. The final pellet was dissolved in 100μl of 10% (w/v) sodium dodecyl sulphate and stored at −20°C until use.

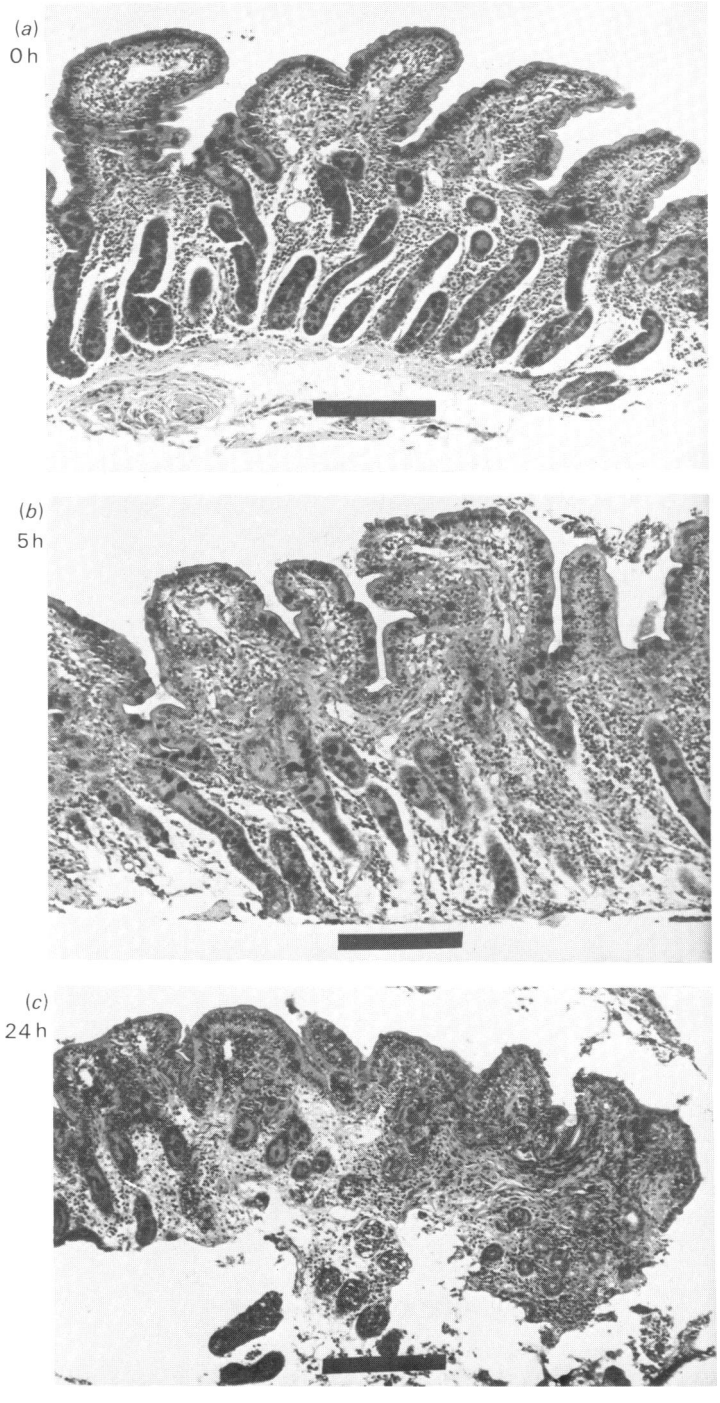
The total membrane fraction was resuspended in 100μl of 50mM-Tris/HCl, pH8.0, and solubilized by the addition of 50μl of 10% (w/v) Triton X-100. After frequent stirring for at least 1h, the suspension was centrifuged at 48000*g* for 1h. The supernatant (the solubilized total membrane fraction) was stored at −20°C until use.

After culture, the media were collected and samples were taken directly for enzyme-activity determinations. Samples (0.5ml) of the media were precipitated with $HClO_4$ as described above and the pellets washed twice with 0.4M-$HClO_4$ before being dissolved in 100μl of 10% sodium dodecyl sulphate.

Radioactivity in the explant and medium fractions was determined by liquid-scintillation counting of 5μl samples, dissolved in 3ml of Aqualuma Plus scintillator.

Immunoelectrophoresis

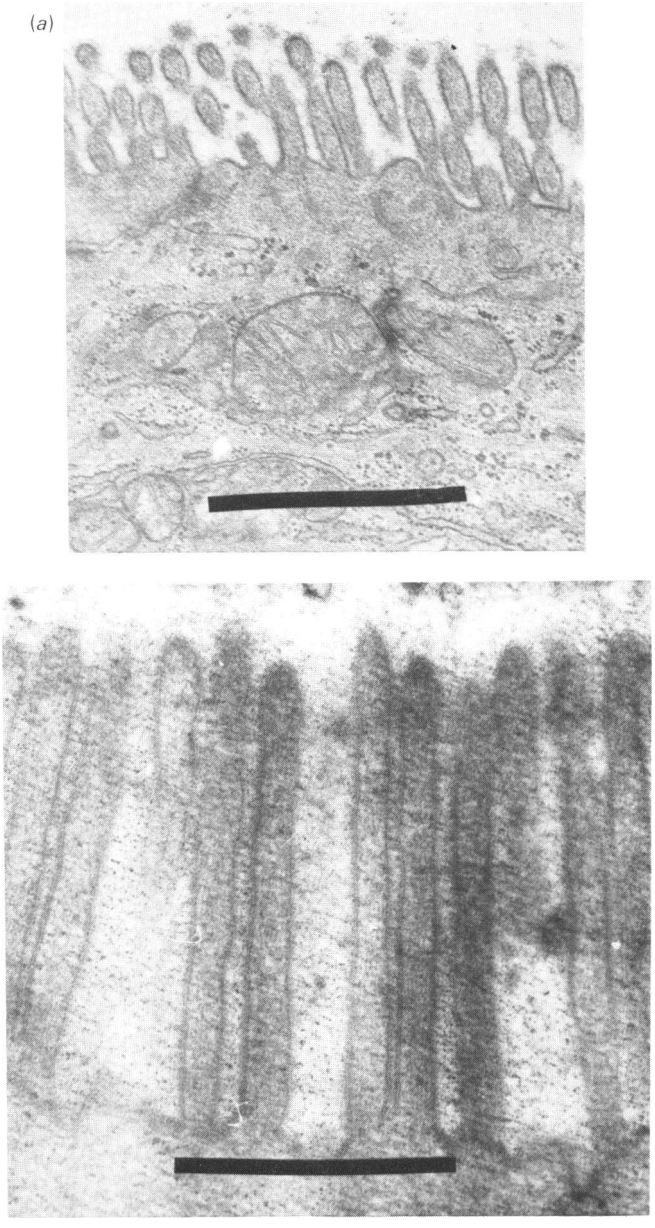
Crossed immunoelectrophoresis (Axelsen *et al.*, 1973) against antibodies directed towards microvillar-membrane proteins was performed as described by Danielsen *et al.* (1977). Immunospecific



EXPLANATION OF PLATE 1

Histology of cultured explants

After 0 h (*a*), 5 h (*b*) and 24 h (*c*) of culture, the explants were prepared for light microscopy as described in the Materials and methods section, by using the periodic acid/Schiff/Alcian Blue staining. The horizontal bars represent 300 μm.



EXPLANATION OF PLATE 2

Ultrastructure of cultured explants

After culture, the explants were prepared for electron microscopy as described in the Materials and methods section. (*a*) Electron micrograph of the apical part of enterocytes from explants cultured for 24h. The horizontal bar represents 1 μm. (*b*) Electron micrograph of the microvilli of enterocytes from explants cultured for 24h. The horizontal bar represents 0.5 μm.

precipitation of microvillus aminopeptidase, sucrase–isomaltase, maltase–glucoamylase, lactase–phlorizin hydrolase and aspartate aminopeptidase was performed by line-immunoelectrophoreses (Axelsen *et al.*, 1973) against monospecific antibodies directed towards these enzymes as follows. Separate squares (about 2 cm^2) of antibody- and antigen-containing gels were cast on the immunoplate, and electrophoresis at 3 V/cm was done for 20 h. After electrophoresis the gels were pressed, washed for 15 min in water and pressed again. The immunoprecipitates, visible without any staining, were then excised with a scalpel and transferred to 1.5 ml plastic tubes, where they were kept at −20°C until further processing.

Polyacrylamide-gel electrophoresis

Gel electrophoresis in 10% polyacrylamide gels in the presence of sodium dodecyl sulphate was performed as described by Laemmli (1970). Before electrophoresis, samples were denatured by boiling for 5 min in the presence of 1% sodium dodecyl sulphate and 2.5% 2-mercaptoethanol. The following molecular-weight indicators were used: prosucrase–isomaltase (M_r 260000) (Sjöström *et al.*, 1980), subunits A (M_r 162000) and B (M_r 123000) of microvillus aminopeptidase (Sjöström *et al.*, 1978), albumin (M_r 67000) and ovalbumin (M_r 45000).

Fluorography

Polyacrylamide gels were prepared for fluorography by the method of Bonner & Laskey (1974), and agarose gels as described by Norén & Sjöström (1979).

Other methods

Protein was determined by the method of Bradford (1976) (microassay procedure from Bio-Rad Laboratories, Munich, Germany) with bovine serum albumin as standard. Microvillus aminopeptidase and dipeptidyl peptidase IV activities were determined as described by Sjöström *et al.* (1978). One unit of enzyme activity is defined as the amount of enzyme hydrolysing 1 μmol of substrate/min.

Results

Histology and ultrastructure

Explants kept in organ culture for 5 h showed no changes in the histology compared with control explants (Plate 1). After 10 and 24 h of organ culture, minor changes in the histological picture occurred: degeneration of inflammatory cells could be seen, and in some enterocytes the position of the nuclei had changed from the normal, basal, location to the apical part of the cell. The ultrastructure of the enterocytes appeared well maintained in explants even after 24 h in organ culture (Plate 2). Microvillar architecture seemed intact and the individual microvilli had retained their fibrillar roots protruding into the cytoplasm. Mitochondria, with well-preserved inner granules, were evenly distributed in the cytoplasm of the cells and the rough endoplasmic reticulum showed no dilatations.

Contents of protein and microvillar enzymes

Table 1 shows the effect of increased periods of organ culture on the contents of protein and microvillus aminopeptidase and dipeptidyl peptidase IV activities of the cultured explants. In relation to wet weight, de-proteinization as observed by Hauri *et al.* (1975) did not occur. The contents of activity of microvillus aminopeptidase and dipeptidyl peptidase IV in the explants also appeared fairly constant, both enzymes being released into the medium at a slow rate during the culture (Table 2). The total amount of these enzymes released after 24 h of culture (approx. 65% of the activity in the explants) was somewhat less than what others have found (90–120%) in organ culture of human (Hauri *et al.*, 1975, 1977), rat (Shields *et al.*, 1979) and mouse (Berteloot *et al.*, 1979) intestinal explants.

The presence in the culture medium of cycloheximide had a striking effect on all parameters determined (Tables 1 and 2). Cycloheximide caused a heavy deproteinization of the explants, and after 24 h of culture most of the activity of microvillar aminopeptidase and dipeptidyl peptidase IV was present in the medium.

To examine the state (i.e. soluble or membrane-bound) of the released activity of microvillus aminopeptidase, the distribution of the enzyme in the membrane-bound and soluble protein fractions of the media was determined. (Table 3; see experimental details in the Table legend). The vast majority of the released enzyme was found to be in the membrane-bound fraction, as was also observed to be the case for the glycosidases and alkaline phosphatase by Hauri *et al.* (1977). The finding suggests that the release of microvillar proteins occurs as a result of microvillar fragmentation and extrusion rather than by proteolysis of the individual components, as also observed by Misch *et al.* (1980).

In contrast, the release of radiolabelled protein was found to be more evenly distributed between the membrane-bound and soluble protein fractions (Table 3).

Incorporation of radioactivity

Fig. 1 shows the incorporation of radioactivity into explants and released protein during continuous labelling with [^{35}S]methionine. By 24 h of culture, about 20% of the [^{35}S]methionine added to the medium had become incorporated into synthesized

Table 1. *Protein content and enzyme activities of cultured explants*

After 0 (control explants), 5, 10 and 24 h of culture the protein contents and microvillus aminopeptidase and dipeptidyl peptidase IV activities were determined as described in the Materials and methods section. Each value represents the data for ten explants cultured in parallel in the same dish and is the mean of five experiments. Where applicable, S.D. values are given in parentheses. In one experiment, cycloheximide (1 mg/ml) was added to the culture medium.

Time of culture (h)	Protein (μg/mg of tissue)	Microvillus aminopeptidase (munits/mg of tissue)	Dipeptidyl peptidase IV (munits/mg of tissue)
0	52.8 (±2.4)	10.42 (±1.33)	2.63 (±0.27)
5	51.4 (±4.5)	9.80 (±2.87)	2.30 (±0.46)
5 (+cycloheximide)	42.2	3.09	1.00
10	52.5 (±6.8)	11.47 (±5.35)	2.84 (±0.95)
10 (+cycloheximide)	34.1	2.13	0.72
24	51.4 (±6.3)	8.58 (±0.83)	2.81 (±0.36)
24 (+cycloheximide)	18.9	1.70	0.57

Table 2. *Release from cultured explants into the medium of microvillus aminopeptidase and dipeptidyl peptidase IV*

After 5, 10 and 24 h of culture, enzyme activities in the media were determined as described in the Materials and methods section. Each value is the mean of five experiments and expresses the activity in the medium relative to that of the explants. Where applicable, S.D. values are given in parentheses. In one experiment, cycloheximide (1 mg/ml) was added to the culture medium.

Time of culture (h)	Microvillus aminopeptidase (% of explant activity)	Dipeptidyl peptidase IV (% of explant activity)
5	29.4 (±11.7)	26.8 (+1.6)
5 (+cycloheximide)	120	111
10	38.5 (±8.0)	41.7 (±5.3)
10 (+cycloheximide)	197	167
24	67.3 (±15.4)	61.7 (±13.6)
24 (+cycloheximide)	446	341

Table 3. *Partition of microvillus aminopeptidase and radioactivity in the soluble and membrane-bound fractions of protein released from the explants into the medium*

After 5, 10 and 24 h of culture, the media were collected and centrifuged at 48000 ***g*** for 1 h. Microvillus aminopeptidase activity and radioactivity were determined in the supernatant (soluble protein) and pellet (membrane-bound) fractions as described in the Materials and methods section. Each value given is the mean for two experiments, and expresses the relative amounts of enzyme activity and radioactivity in the two fractions.

	Soluble protein		Membrane-bound protein	
Time of culture (h)	Microvillus aminopeptidase (%)	Radioactivity (%)	Microvillus aminopeptidase (%)	Radioactivity (%)
5	15.6	58.6	84.5	41.6
10	8.6	61.7	91.4	38.2
24	10.0	60.2	90.0	39.9

protein. The total rate of [^{35}S]methionine incorporation decreased only moderately during the 24 h period of culture. The rate of release of radiolabelled protein into the medium was a relatively low constant proportion (8–10%) of the rate of labelling of explant protein. This contrasts with the findings of Kagnoff *et al.* (1972) and Hauri *et al.* (1977), who observed an increasingly progressive release of radiolabelled protein into the medium during culture, so that about 60% of the radioactive explant protein after 24 h of culture was released (Hauri *et al.*, 1977).

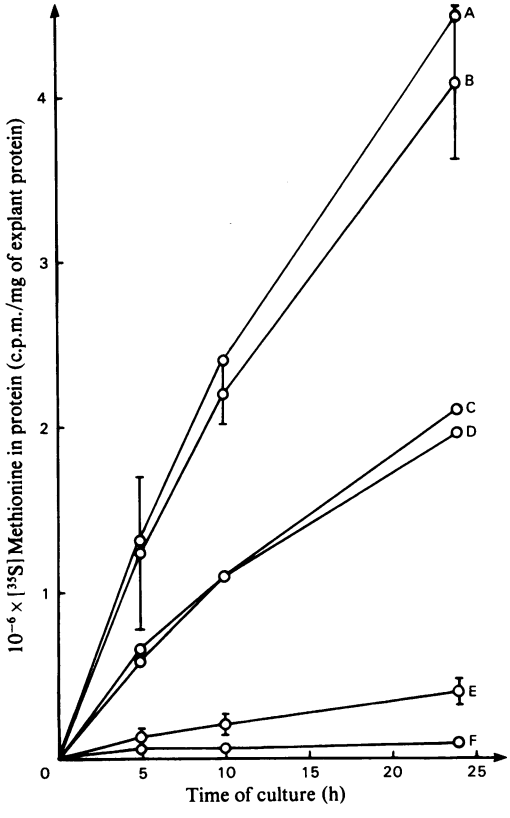


Fig. 1. *Incorporation of [^{35}S]methionine into explant protein and protein released into the medium*

After 5, 10 and 24h of culture, incorporation of radioactivity into soluble and membrane-bound explant protein and protein released into the medium was determined as described in the Materials and methods section. Each point represents the data for ten explants cultured in the same dish and is the mean for five such experiments. Bars indicate ±S.D. In one experiment, cycloheximide (1mg/ml) was added to the culture medium. A, Soluble + membrane-bound explant protein + protein released into the medium. B, Soluble + membrane-bound explant protein. C, Membrane-bound explant protein. D, Soluble explant protein. E, Protein released into the medium. F, Soluble + membrane-bound explant protein in the presence of cycloheximide.

The presence of cycloheximide in the medium inhibited the incorporation of radioactivity by 95–98% during the entire period of culture.

Labelling of microvillar enzymes

The use of a high-specific-radioactivity label (>1000Ci/mmol) ensured a high degree of incorporation of radioactivity into the explant protein, making it possible to study the labelling of individual components.

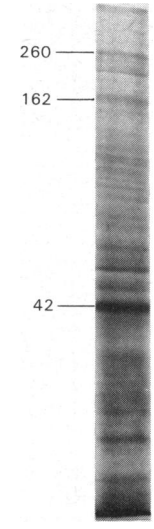


Fig. 2. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of total explant protein after 24h of culture*

After culture, explants were homogenized as described in the Materials and methods section; 1vol. of 0.8M-$HClO_4$ was then added to the homogenates to precipitate total explant protein. After washing in 1ml of 0.4M-$HClO_4$, precipitated protein was dissolved in 100μl of 10% sodium dodecyl sulphate and denatured by boiling for 5min in the presence of 2.5% 2-mercaptoethanol. After electrophoresis, the gel was prepared for fluorography (about 200000 c.p.m. was applied to the gel). Exposure time was 3days. Apparent M_r values ($\times 10^{-3}$) are shown.

Fig. 2 shows the gel-electrophoretic pattern of the total explant protein after 24h of culture, shown by fluorography. A uniform radioactive labelling of the protein components over the entire molecular-weight range is seen.

Fig. 3 shows crossed immunoelectrophoresis of the solubilized total membrane fraction of the explants against antibodies directed towards microvillar-membrane proteins. At least seven precipitates can be detected, of which the four major ones have been shown to correspond to microvillus aminopeptidase, sucrase–isomaltase, maltase–glucoamylase and lactase–phlorizin hydrolase (Danielsen *et al.*, 1977). All microvillar precipitates were radioactively labelled during the 24h period of culture.

The immunospecifically purified explant microvillus aminopeptidase and sucrase–isomaltase both exhibited a different polypeptide composition in gel electrophoresis compared with the corresponding

enzymes usually obtained from the microvillar membrane (Fig. 4, gels *a* and *b*) (Sjöström *et al.*, 1978, 1980). Sucrase–isomaltase, which normally is composed of its constituent enzymic polypeptides, appeared largely in the form of the single chain pro-sucrase–isomaltase of M_r 260000. Only one polypeptide was seen in the position usually occupied by the two polypeptides of the separate sucrase and isomaltase of M_r 140000 and 150000 respectively. The microvillus aminopeptidase was found almost exclusively in the form of the A-subunit, of M_r 162000. This contrasts with the normal pattern, which is dominated by subunits B and C, of M_r 123000 and 62000 respectively. A faint band of about the same molecular weight as two polypeptides of M_r 142000 and 137000 suggested to represent precursor forms of the enzyme (Danielsen *et al.*, 1981) was also detectable. Subunit compositions of microvillus aminopeptidase and sucrase–isomaltase comparable with those obtained in the present work are otherwise only seen with animals which 3 days before being killed had had their pancreatic duct disconnected. Thus the organ-culture technique appears to simulate conditions *in vivo* with limited access of pancreatic enzymes in the intestinal lumen.

Maltase–glucoamylase, purified from the microvillar membrane, has been shown to consist of polypeptides of M_r 245000, 135000 and 125000 (S. Hedeager-Sørensen, O. Norén, H. Sjöström & E. M. Danielsen, unpublished work).

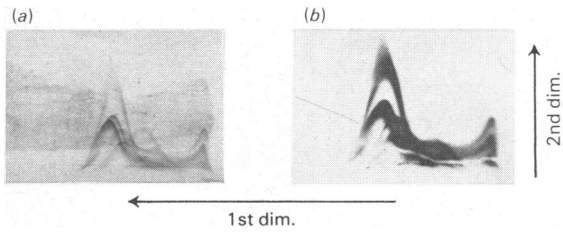


Fig. 3. *Crossed immunoelectrophoresis of solubilized total membrane protein fraction after 24 h of culture against antibodies to brush-border membrane proteins*
The first dimension was run at 10 V/cm for 1 h and the second dimension at 2 V/cm for 20 h. The immunoglobulin-G content of the gel was 150 μg/ml, and approx. 200 μg of protein (470000 c.p.m.) was applied to the gel. (*a*) After electrophoresis, immunoprecipitates were stained with Coomassie Brilliant Blue R 250. (*b*) After electrophoresis, the gel was prepared for fluorography (exposure time 3 days).

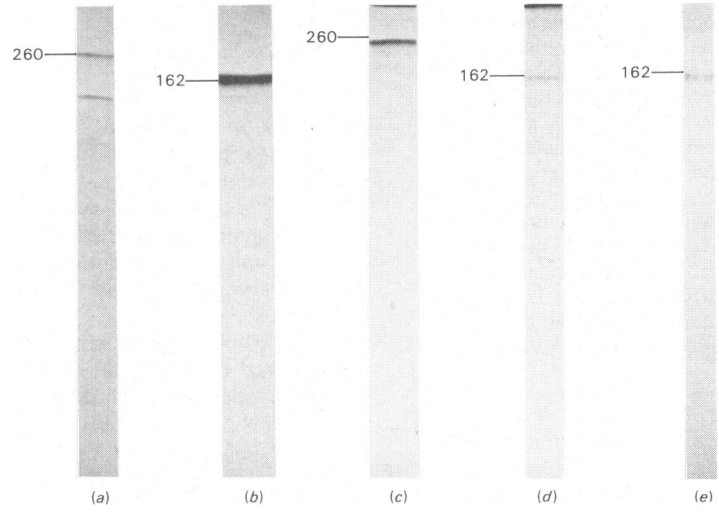


Fig. 4. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified explant microvillar enzymes after 24 h of culture*
The enzymes were immunoprecipitated from the solubilized total membrane protein fraction of ten explants by line immunoelectrophoresis against antibodies to the respective enzymes as described in the Materials and methods section. After electrophoresis, the gels were prepared for fluorography (exposure time 3–10 days). Gels: *a*, sucrase–isomaltase; *b*, microvillus aminopeptidase; *c*, maltase–glucoamylase; *d*, lactase–phlorizin hydrolase; *e*, aspartate aminopeptidase. Apparent M_r values ($\times 10^{-3}$) are shown.

The enzyme isolated from the cultured explants was found only to contain the polypeptide of M_r 245000 (Fig. 4, gel *c*). This result may indicate that maltase–glucoamylase, like sucrase–isomaltase, is synthesized as a large single polypeptide, which is then split post-translationally by pancreatic proteinases. In contrast, the polypeptide compositions of explant lactase–phlorizin hydrolase and aspartate aminopeptidase were comparable with those usually obtained from microvillar lactase–phlorizin hydrolase (Skovbjerg *et al.*, 1981; H. Skovbjerg, O. Norén, H. Sjöström, E. M. Danielsen & B. S. Enevoldsen, unpublished work) and aspartate aminopeptidase (O. Norén, H. Sjöström & E. M. Danielsen, unpublished work; Benajiba & Maroux, 1980), respectively (Fig. 4, gels *d* and *e*).

Discussion

The present work demonstrates that the organ-culture technique which has previously been used to culture explants from various other species also can be applied to pig small intestine. The morphology of intestinal explants cultured for periods up to 24h suggests a fairly well-preserved tissue architecture. On the molecular level, it is difficult to state the exact biochemical criteria by which to measure the viability of explants in organ culture, but determination of protein synthesis and contents of microvillar enzyme activities has been widely used to evaluate the system. If viable, cultured explants should be expected to maintain a constant rate of protein synthesis and enzyme activities. In the present investigation, these parameters remained fairly constant during the entire period of culture. In contrast, the strikingly different data obtained in the experiment where cycloheximide was included in the culture medium are probably typical for degenerating tissue.

Previous papers invariably report the observation of release of protein and microvillar enzyme activities into the medium, albeit at different rates. The phenomenon is usually referred to as secretion, suggesting the occurrence of a specific secretory mechanism. However, we consider it unlikely that a secretion of this kind is performed by the enterocytes, but believe that the appearance in the medium of explant protein and microvillar enzyme activities reflects the degradative part of the metabolism of explant protein. The moderate release of enzyme activities into the medium observed in the present work therefore does not indicate an excessive decay of the cultured explants, since the culture period is comparable in length with the half-life of enterocytes under normal conditions *in vivo*.

By 24h of culture, the microvillar enzymes detectable by crossed immunoelectrophoresis were all intensively radioactively labelled, indicating that their biogenesis still occurred at an appreciable rate during culture. Nothing is yet known about the time required for synthesis and transport to the microvillar membrane of these enzymes. In cell-culture systems, it has been shown that secretory proteins such as albumin and transferrin take about 20–40min (Strous & Lodish, 1980) to complete their synthesis and transport to their final extracellular destination. Despite the differences in the culture systems and the relatively high molecular weight of the microvillar enzymes, the time required for their biogenesis, including polypeptide synthesis, glycosylation, membrane insertion and cellular transport, will be unlikely to exceed 1–2h. With regard to time, the organ-culture technique therefore easily allows a study of the biosynthetic mechanisms and transport routes involved in the making of microvillar enzymes. Furthermore, in contrast with systems *in vivo*, it is possible with the organ-culture systems to perform pulse-labelling experiments with short pulse intervals, making it feasible to perform kinetic analyses of the protein synthesis with high resolution. Individual microvillar enzymes can thus be studied during their biosynthesis, because the system allows labelling to a high specific radioactivity to be performed.

The finding that the molecular forms of microvillus aminopeptidase and sucrase–isomaltase were similar to those obtained from animals that had had their pancreatic duct disconnected 3 days before being killed supports our earlier proposals on the role of pancreatic proteinases in the post-translational modification of these enzymes (Sjöström *et al.*, 1978, 1980). Organ culture therefore might be a suitable system for studying the effect of pancreatic enzymes on the molecular form of microvillar enzymes.

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