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Morpho-cytochemical and biochemical evidence for insulin absorption by the rat ileal epithelium

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Summary. In order to investigate the mechanism through which insulin is absorbed by the intestinal epithelium and transferred to the circulation where it exercises its biological activity of lowering blood glucose levels, a combined biochemical morpho-cytochemical study was undertaken on rat ileal tissue, in vivo. Insulin was introduced into the lumen of the ileum in combination with sodium cholate and aprotinin and allowed to be absorbed for various periods of time. Analysis of blood samples from the inferior vena cava, at different time points has demonstrated an increase in plasma insulin followed by a decrease in blood glucose levels. The ileal tissues were studied at different time points after the introduction of the insulin, by applying the protein A-gold immunocytochemical technique. Insulin antigenic sites were detected with high resolution, at various levels of the enterocytes but were absent from goblet cells. At 2 to 5 min, the labelling was mainly associated with the microvilli and endocytotic vesicles in the apical portion of the epithelial cells. Some gold particles were in contact with the lateral membranes. At 10 min, the labelling was found at the level of the trans-side of the Golgi apparatus and mainly along the baso-lateral membranes of the epithelial cells. Labelling was also detected in the interstitial space. The control experiments have demonstrated the specificity of the labelling and confirmed the nature of the insulin molecules detected. Furthermore, the morphological study has confirmed that exposure of the tissue to the insulin-cholate-aprotinin solution does not affect the integrity of the epithelium while promoting insulin absorption. Thus, insulin introduced in the lumen of the rat ileum in conjunction with sodium cholate and aprotinin, appears to be rapidly absorbed by the epithelial cells and transferred to the circulation through a transcytotic pathway.

Key words: Insulin absorption, ileum, immunocytochemistry, diabetes.

Since the discovery of insulin and its successful use for treatment of diabetes mellitus, numerous attempts have been made in search of alternative methods to injection [1]. In recent years several studies have reported successful results demonstrating that nasal [2–4], rectal [5–9] and oral-enteral [10–14] administration of insulin is feasible provided that particular conditions are provided. When considering the oral administration of insulin, two major difficulties are encountered, the intensive proteolytic activity of the gastrointestinal tract and the relative impermeability of the intestinal epithelium to the transport of peptide and proteins. These two limitations seem to be overcome by the use of proteinase inhibitors to protect the peptide from major proteolysis and the addition of surfactants to promote the absorption of peptides and proteins by the intestinal mucosa [7, 13]. Bile acids were found to be active for this latter function [7, 13]. It has been recently demonstrated that efficient insulin transport by the intestinal epithelium differs depending on the proteinase inhibitor and bile acid used. Amounts of insulin able to induce significant lowering of blood glucose levels are transported from the rat intestinal lumen to the blood when it is administered in combination with sodium cholate and aprotinin [7, 13].

While conditions for effective oral administration of insulin are still being defined, little is yet known about the mechanism by which insulin is absorbed by the intestinal epithelium and is subsequently transferred to the circulation. In an attempt to study this mechanism, we have investigated the pathway of insulin transport by the ileal epithelium. The use of a morpho-cytochemical approach has revealed the location of insulin molecules in the ileal epithelial cells. Indeed, application of the protein A-gold immunocytochemical technique [15, 16], allows for the detection of antigenic sites with high resolution, at the electron microscope level. Insulin was introduced in the lumen of the rat ileum in combination with aprotinin and sodium cholate and allowed to be absorbed for various

periods of time. The biochemical analysis of blood samples demonstrated the absorption of insulin by the ileal epithelium. The adsorbed insulin was able to reduce significantly the glycaemic levels of the animals. The subsequent ultrastructural localization of insulin antigenic sites on thin sections of the ileal tissues has enabled the tracing of the path taken by insulin during its internalization by the epithelial cells.

Materials and methods

Male rats of the Hebrew University strain weighing 250–350 g, were kept on a standard diet with pelleted chow. The animals were fasted for 16 h before the beginning of the experiment. Under sodium barbital (60 mg/kg) anaesthesia, a surgical operation was performed in order to inject an insulin solution into the ileum. A longitudinal incision of the abdomen was made to expose the small intestine. A loop of the ileum of about 7 cm, located 5 cm away from the caecum, was chosen and clamped at both ends. A 0.5 ml solution of 0.15 mol/l NaCl containing the different reagents was injected into the lumen of the ileum. The animals received 150 U of human insulin (Novo, Copenhagen, Denmark), mixed with 2000 Kallikrein inhibitor units (KIU) of aprotinin (Trasylol, Bayer, Leverkusen, FRG) and 5 mg of sodium cholate (22 mmol/l) (Sigma, St Louis, Mo, USA) in 0.5 ml of 0.15 mol/l NaCl. The addition of aprotinin was required in order to prevent insulin degradation; rates of degradation with and without aprotinin were reported previously [13]. In control experiments, the animals were injected with the same volume of saline containing only the aprotinin (2000 KIU) and the sodium cholate (5 mg) without insulin. The solutions were kept in the lumen of the ileum for periods of time, from 2 to 60 min. Six animals were selected for the morpho-cytochemical study of their ileal tissue, while three were kept for the biochemical analysis of their blood.

For the morphological study, at the end of each time point (2, 5, 10 and 30 min) after insulin administration, the loop of the ileum having been exposed to the solution was sampled, cut open and fixed by immersion with a 1% solution of glutaraldehyde in 0.1 mol/l phosphate buffer (NaH_2PO_4/Na_2HPO_4) for 2 h at room temperature. The tissue fragments were then processed for electron microscopy.

For the biochemical determinations of plasma glucose and insulin levels, blood samples of 0.3 ml were taken from the inferior vena cava at 0, 2, 5, 10, 30, and 60 min after insulin administration. Glucose was determined on plasma samples by the glucose oxidase method (Boehringer, Mannheim, FRG). On the other hand, insulin was determined by radioimmunoassay (Serono Diagnostic, Coinsins, Switzerland) using human insulin standards on plasma samples diluted 1:10 to 1:50. The assay lower and upper limits were 5 and 400 μU/ml respectively with intra- and inter-assay precisions of 5 to 10%.

Cytochemistry

For electron microscopy immunocytochemistry, the fragments of ileum fixed with glutaraldehyde were rinsed with the phosphate buffer and post-fixed with 1% osmium tetroxide for 1 h at 4°C. After dehydration in ethanol and propylene oxide, they were infiltrated with Epon and polymerized. The tissue blocks were embedded in flat molds for good orientation of the epithelium. Semi-thin sections were cut and examined at the light microscope level. Blocks demonstrating a good orientation of the ileal epithelium with cells cut along their major axis, were then chosen for electron microscopy. Thin sections were cut, mounted on Parlodion and carbon-coated nickel grids and processed for immunocytochemistry.

The grids with their mounted sections were incubated for 1 h on a saturated solution of sodium metaperiodate [17] at room temperature and then rinsed with distilled water. The grids were incubated on one drop of 0.01 mol/l phosphate buffered saline (NaH_2PO_4/Na_2HPO_4, NaCl 0.15 mol/l) pH 7.2 (PBS) containing 1% ovalbumin for 5 min and then transferred to one drop of the anti-insulin antibody (Miles Chemicals, Ontario, Canada) diluted 1/200. The incubation with the antibody was carried out at room temperature for 2 h. The grids were rinsed with PBS, and incubated on one drop of the protein A-gold complex for 1 h at room temperature. After a thorough wash with PBS and distilled water, the sections were dried, stained with uranyl acetate and lead citrate and examined in the electron microscope. The protein A-gold complex was prepared with 15 nm gold particles as described previously [15, 16].

Several control experiments were performed in order to assess the specificity of the labelling: (1) the anti-insulin antibody was adsorbed with its specific antigen for 24 h at 4°C before its use for the labelling protocol; (2) the tissue sections were incubated with the protein A-gold complex without a previous incubation with the specific antibody; (3) sections of ileum exposed to the aprotinin and sodium cholate solution but not to insulin, were incubated with the anti-insulin antibody and the protein A-gold complex.

Results

Biochemical determinations have indicated that absorption of biologically active insulin from the ileal lumen and its transfer to the blood circulation did occur, as demonstrated by the elevation of plasma insulin concentrations and the decrease in blood glucose levels. Table 1 shows that a rise in plasma insulin occurs as early as 2 min after the introduction of insulin into the ileal lumen. Insulin levels peaked between 5 and 30 min after the beginning of the experiment. From the data obtained, considering the

Table 1. Plasma glucose and insulin levels in rats at different time points after the injection of 150 U of insulin, 2000 KIU of aprotinin and 10 mg/ml of sodium cholate in the ileum

	Plasma glucose levels (mmol/l)				Plasma insulin levels (mU/l)			
Time	Rat 1	Rat 2	Rat 3	Relative change in % starting value	Rat 1	Rat 2	Rat 3	Average
0 min	4.3	5.8	5.9	100	35	62	22	40 ± 14
2	6.2	5.9	6.6	119 ± 15	476	1058	69	534 ± 353
5	6.2	7.4	4.3	114 ± 26	5556	3623	2228	3802 ± 1185
10	3.9	5.6	4.0	85 ± 9	6152	3685	2997	4278 ± 1177
30	2.4	2.8	2.1	47 ± 8	7733	7151	7679	7521 ± 228[a]
60	1.8	2.5	1.2	35 ± 9	4766	1708	3749	3408 ± 1104

[a] In spite of these high levels of circulating insulin, all the animals remained alive for more than two months after the experiment.
KIU = kallikrein inhibitor units

Table 2. Plasma glucose and insulin levels in rats at different time points after the injection of 2000 KIU of aprotinin and 10 mg/ml of sodium cholate without insulin, in the ileum

	Plasma glucose levels (mmol/l)				Plasma insulin levels (mU/l)			
Time	Rat 1	Rat 2	Rat 3	Relative change in % starting value	Rat 1	Rat 2	Rat 3	Average
0 min	5.9	4.0	5.6	100	57	27	35	40 ± 11
2	6.5	4.2	6.8	112 ± 6	77	27	49	51 ± 18
5	7.1	4.3	6.5	114 ± 5	86	21	42	50 ± 19
10	6.5	5.0	6.8	119 ± 5	74	23	52	50 ± 18
30	6.4	5.2	6.3	118 ± 8	65	23	51	46 ± 15
60	5.4	4.9	6.4	110 ± 11	58	30	49	46 ± 10

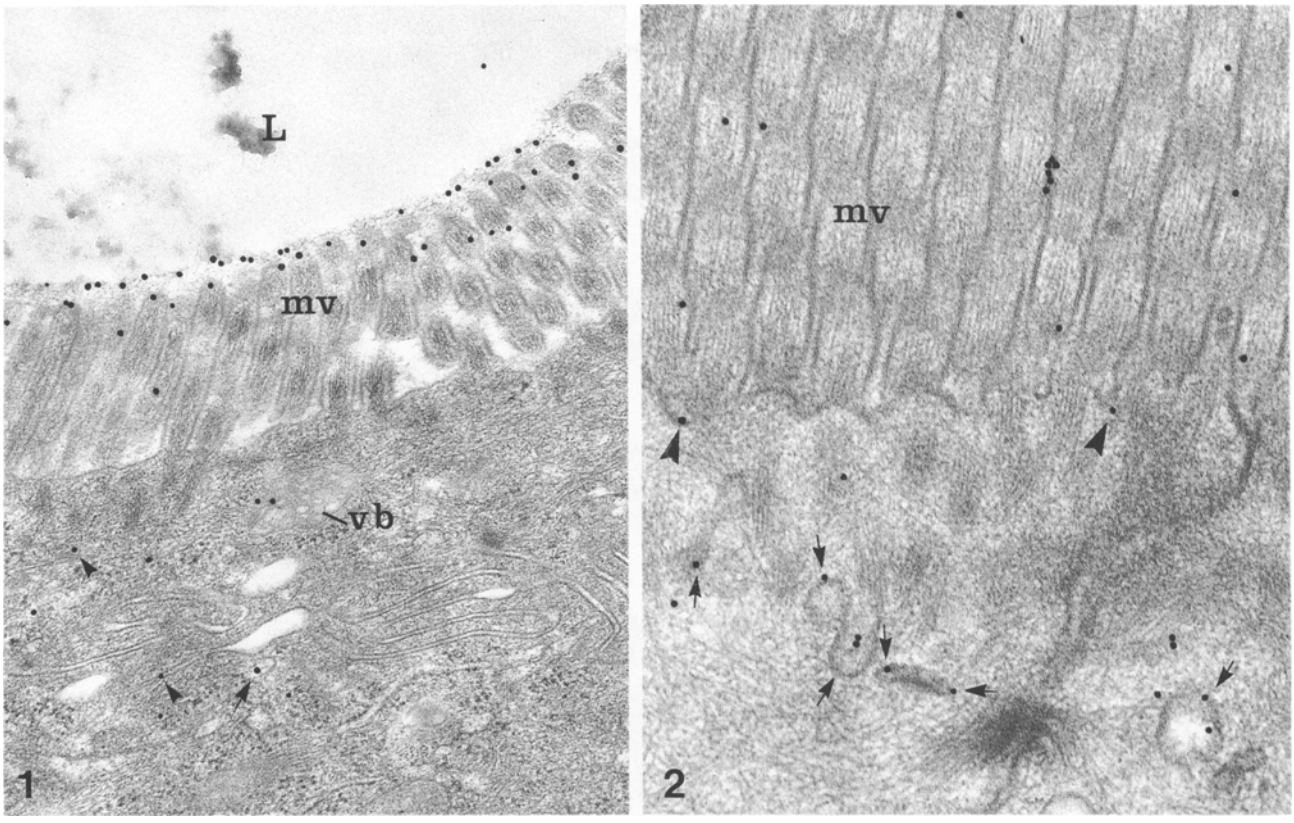


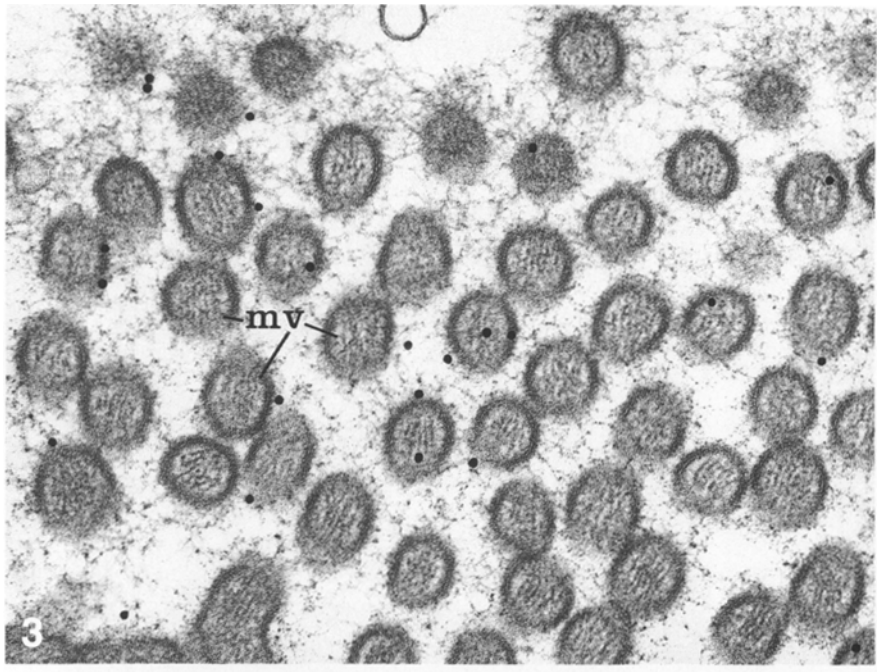
Fig. 1. Rat ileal tissue exposed to insulin for 2 min. Immunocytochemical detection of insulin antigenic sites by the use of an anti-insulin antibody in combination with the protein A-gold complex. The labelling with gold particles revealing insulin antigenic sites is present in the luminal space (L) either in contact or in close association with the microvilli (mv). Some particles (arrow) are associated with endocytotic vesicles in the apical part of the epithelial cell while few (arrowheads) seem to be in contact with the lateral membrane in interdigitations located in the apical portion of the cell. Multivesicular bodies (vb) are also labelled. × 55,000

Fig. 2. Rat ileal tissue exposed to insulin for 5 min. The labelling for insulin is associated with microvilli (mv), plasma membrane invaginations (arrowheads) and apical vesicles (arrows). The junctional complex between the cells is devoid of labelling. × 55,000

half-life of insulin and the blood volume, we can estimate that 0.5 U of insulin were absorbed during the first 30 min. Blood glucose on the other hand, started to decrease 10 min after insulin administration and reached its lower levels, 50% and 35% of its initial value, at 30 and 60 min, respectively after the beginning of the experiment. The results demonstrated very good correlations between the increase in circulating insulin and the decrease of glycaemic values. Furthermore, the time lapse between these changes showed that the decrease in blood glucose followed the increase in insulin levels. No significant changes in plasma insulin and glucose concentrations were detected in the control experiment in which sodium cholate and aprotinin were administered without insulin (Table 2).

The introduction of the insulin solution into the ileal lumen, did not alter the structural integrity of the ileal epithelium. Morphological examination performed at all time points has demonstrated that the enterocytes remained intact with well-developed microvilli. The cells were joined by tightly closed junctional complexes. A thin layer of cell coat was observed over and between the microvilli. The ultrastructural preservation of the epithelial cells was adequate, with no sign of cell degeneration, membrane hydrolysis or lysosomal proliferation.

Application of the anti-insulin antibody in conjunction with the protein A-gold complex on thin tissue sections, resulted in a labelling by gold particles over the epithelial cells. Fixation of the tissue with glutaraldehyde and osmium tetroxide allowed for very good ultrastructural preservation which in turn, yielded labellings of very high resolution allowing for an accurate identification of the labelled structures. The pattern of labelling differed among samples depending on the length of time the ileal epithelium was exposed to the insulin solution. Indeed, those tissues exposed to the insulin solution for 2 min, displayed a labelling concentrated in the apical region of the cells (Fig. 1). Most of the gold particles were seen associated with the cell coat and in contact with the membranes of the microvilli. Some particles were found in the bottom of the microvilli and in deep invaginations of the apical plasma membrane. These invaginations correspond to endocytotic figures leading to endosomal vesicles. Multivesicular structures located in the apical region of the cells were also labelled (Fig. 1). Some particles were also present in contact with the lateral membrane associated with the interdigitations located close to the junctional complexes in the apical portion of the epithelium. After 5 min (Fig. 2), most of the particles were detected over vesicular structures



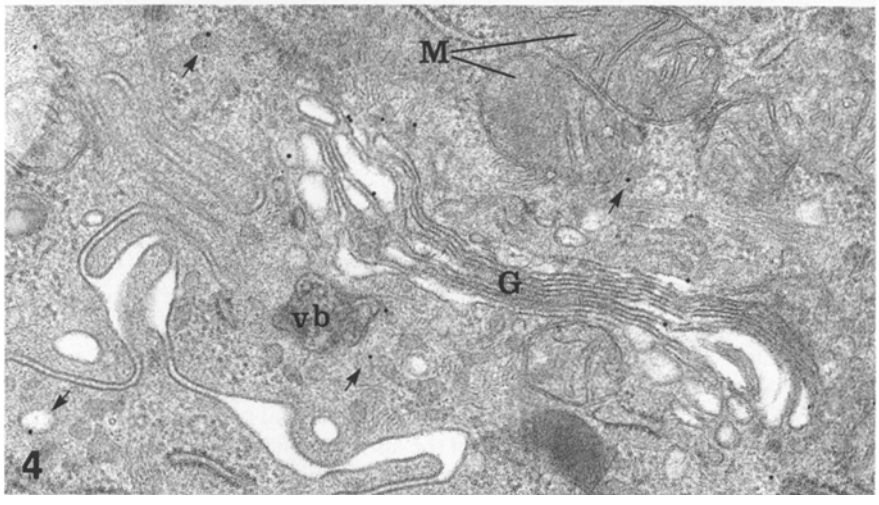


Fig. 3. Rat ileal tissue exposed to insulin for 5 min. The association of the labelling for insulin with the microvilli (mv) is conspicuous when these are cut transversely. × 80,000

Fig. 4. Rat ileal tissue exposed to insulin for 10 min. The labelling is associated with the Golgi apparatus (G) of the enterocytes, mainly with the cisternae in the trans side. Other particles (arrows) are associated with vesicular structures. Very few gold particles are seen over mitochondria (M). × 45,000

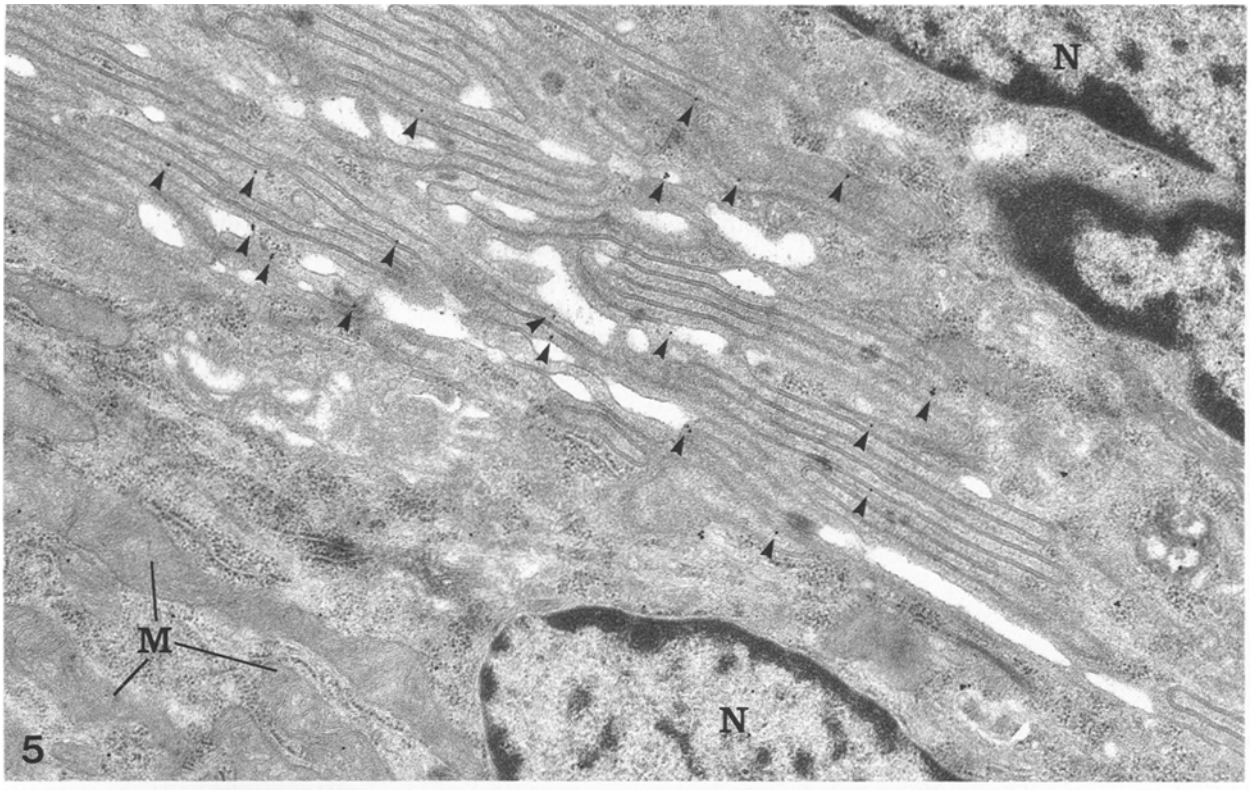
particularly those present in the apical region of the cell. Multivesicular bodies were also labelled. Some particles were found deeper in the cell while others were associated with the lateral membrane in areas of interdigitations. The intercellular junctional complexes remained tight and free of any label. The association of the gold label with the cell coat was prominent at higher magnification or when the microvilli were examined on cross section (Fig. 3). At 10 min (Figs. 4–6), gold particles were detected deeper inside the cells. They were particularly associated with vesicular structures, with the Golgi apparatus and at the baso-lateral membrane. In the Golgi area (Fig. 4), particles were mainly present in the trans cisternae and associated with Golgi related vesicles. Few were seen in cisternae on the cis-side of the Golgi. At 10 and 30 min a large number of gold particles were present aligned along the baso-lateral membrane, particularly at the level of the interdigitations (Fig. 5). Some particles were also found in the extracellular space, associated with the basal laminae

Fig. 5. Rat ileal tissue exposed to insulin for 10 min. This figure illustrates the lateral membranes of the epithelial cells with extensive interdigitations. The gold particles (arrowheads), revealing insulin antigenic sites, are seen aligned along the membranes. Very few gold particles are seen over the nuclei (N). × 24,000

Fig. 6. Rat ileal tissue exposed to insulin for 10 min. This figure illustrates the basal region of the epithelial cells. The gold particles are seen associated with vesicular structures (arrows) or with the plasma membrane. Few gold particles are also present over the extracellular space at the level of the basal laminae (BL). × 45,000

Fig. 7. Rat ileal tissue exposed to insulin for 10 min. The labelling by gold particles is present over the enterocyte (EC) but absent over the goblet cell (GC). × 32,000

Fig. 8. Rat ileal tissue exposed to the sodium cholate and aprotinin solution but not to insulin for 5 min. Very few gold particles are present over the cells when the anti-insulin antibody is used in combination with the protein A-gold technique. × 32,000. mv = microvilli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M
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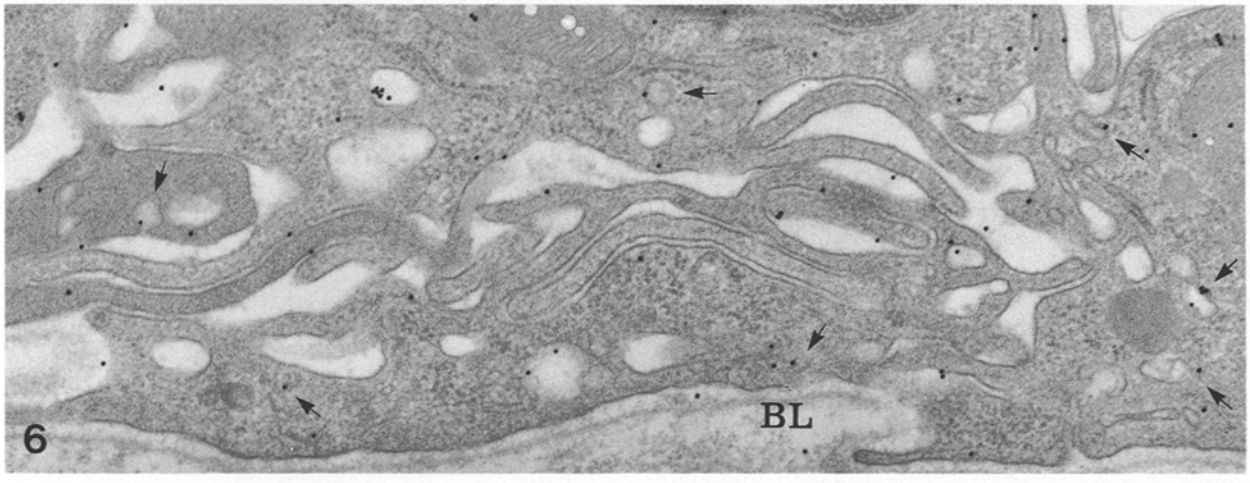
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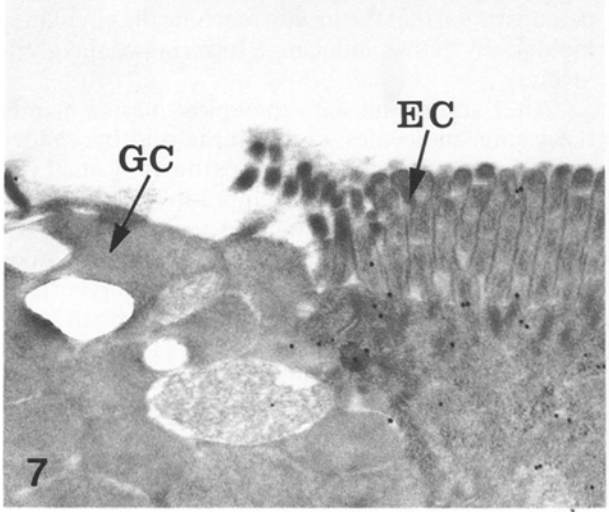
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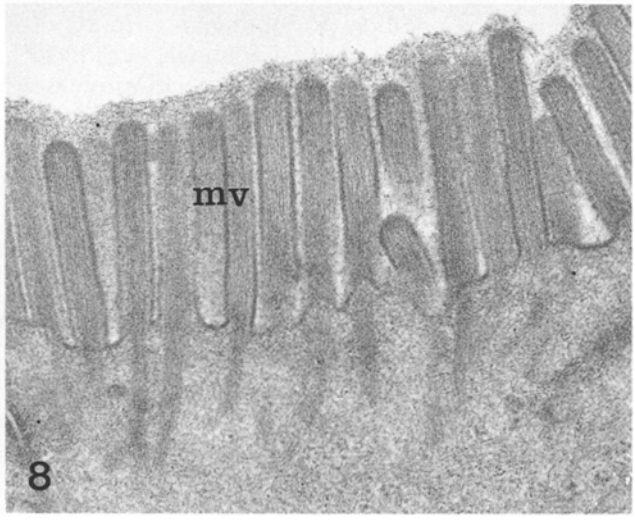
mv
8

(Fig. 6). In all instances, very few particles were detected over nuclei and mitochondria. However, some particles were seen apparently free in the cytoplasm. These could be associated with vesicular or other structures which, because of the cutting procedure, were located outside the section.

Control experiments did allow for assessing the specificity of the labelling. In all instances, when the ileal tissue was exposed to insulin, its antigenic sites as revealed by the immunogold were detected in absorptive cells and not in goblet cells (Fig. 7). This result speaks in favour of the internalization of insulin through the endocytotic activity of the absorptive epithelium. On the other hand, when insulin was absent from the solution injected into the lumen of the ileum, the immunogold approach was unable to yield any specific labelling in the epithelial cells, very few gold particles were more or less randomly distributed throughout the tissue (Fig. 8). Applying an anti-insulin antibody pre-adsorbed with its specific antigen, on ileal tissue having been exposed to the insulin, resulted in an absence of labelling (not illustrated). A similar result was obtained when the protein A-gold complex was used alone without the anti-insulin antibody. These control experiments, thus, confirm that the labelling obtained over the cellular structures in absorptive ileal epithelial cells, corresponds to the presence of insulin antigenic sites. The results also demonstrate that these insulin molecules originate from the injection protocol.

Discussion

Introduction of insulin into the rat ileum in combination with sodium cholate and aprotinin has enabled the internalization of the insulin molecules to such an extent that it induced lowering of the blood glucose levels. Indeed, analysis of blood samples collected in the inferior vena cava demonstrated an increase in insulin immunoreactivity followed by a decrease in blood glucose levels. These results together with those reported previously [7, 13], strongly indicate that under appropriate conditions, insulin molecules are transferred from the ileal epithelium to the circulation. Conditions for optimal absorption of peptides and proteins by intestinal epithelial cells have been found to require the presence of a specific protease inhibitor and an adjuvant [13]. Previous studies have demonstrated the effectiveness of the aprotinin and sodium cholate for such a purpose in rat tissue [7, 12, 13]. On the other hand, the question of the path for internalization remains to be elucidated. The morphological-cytochemical approach utilising specific immunoprobes enables the detection of insulin antigenic sites on tissue sections with high resolution [15, 18]. The use of the protein A-gold technique [15, 16] is an appropriate choice for this purpose because of its application on tissue-sections and its high resolution at the electron microscope level. Indeed, application of this approach on thin sections of ileal tissue previously exposed to the insulin solution did result in a labelling over various cellular structures. The control experiments performed in order to assess the validity of the approach demonstrated that the labelling obtained is specific and that the insulin antigenic sites revealed by the technique, correspond to insulin molecules internalized by the cells. Our results have indicated that the absorption of insulin from the ileal lumen occurred via the enterocytes, as the goblet cells were free of label. The internalization occurred via a pathway, which was revealed by the time schedule of the experimental protocol. Indeed, after very short periods of exposure, the insulin antigenic sites were detected mainly at the level of the microvilli. Intracellularly, the insulin antigenic sites were associated with endocytotic invaginations of the apical plasma membrane or with endosomal vesicles as well as with the lateral membrane in the apical region of the cells. After longer periods of exposure, the insulin was found in the Golgi area and mainly in great amounts in association with the baso-lateral plasma membrane. A significant amount of labelling was eventually found in the extracellular space demonstrating the transfer of insulin molecules from the lumen to the interstitial space. The fact that the junctional complexes present in the upper part of the epithelium remained tight and unlabelled, indicates that no significant passage of insulin seems to occur via this path.

The limitations of the technical approach used, correspond to its sensitivity and to the nature of the antigenic sites that are detected. Indeed, due to the sensitivity of the approach, large amounts of insulin had to be introduced in the lumen of the ileum in order for the immunocytochemical probes to detect a signal inside the cells. Previous studies [7] have revealed that only a small proportion, 3 to 5%, of the total insulin is absorbed during the first 15 to 20 min. Thus, for its detection on thin tissue sections, relatively large amounts had to be introduced into the lumen of the ileum. The specificity of the anti-insulin antibody used together with the various control experiments performed, confirmed the nature of the antigenic sites detected. However, insulin antigenicity may be exhibited by intact molecules as well as by peptidic fragments of insulin resulting from its breakdown. No control or experimental protocol could allow for discrimination between both types of antigenic sites. However, the biochemical determinations of insulin immunoreactivity in the plasma, together with the variations in blood glucose levels have demonstrated that the insulin reaching the circulation was biologically active, inducing a lowering of the glycaemic levels.

After adsorption onto the apical plasma membrane, the insulin molecules were internalized by endocytosis and reached, via an endosomal pathway of small vesicles, the baso-lateral plasma membrane where they were secreted into the interstitial space as are several other secretory products of this cell. The pathway taken by insulin molecules through the epithelial cells corresponds to a transcytotic route similar to that described for IgG in these same cells in the neonatal rat [19]. The participation of the Golgi complex differs, however, from the previous study on IgG in which the Golgi cisternae were only labelled when cationic ferritin was used. Under our study conditions however, no tracers such as peroxidase or cationic ferritin were used, since the cytochemical labelling of insulin was performed on a post-embedding

approach. The fact that labelling of the baso-lateral membrane was observed as soon as 2 to 5 min after the introduction of insulin into the lumen of the ileum and the appearance of insulin molecules in the plasma, speak in favour of a very fast transfer of insulin from the lumen to the interstitial space, most probably bypassing the Golgi apparatus. Thus, labelling of the trans-cisternae of the Golgi may correspond to membrane recycling pathway as described in many cells [20–22] and/or to the transcytosis of insulin. What remains to be elucidated, is the presence of insulin receptors at the level of the apical plasma membrane. This question is in fact, relevant for all pancreatic hormones since they are normally present in the pancreatic juice and reach the intestinal lumen [23]. Insulin receptors were described in enterocytes [24–27]. However, while some studies have demonstrated the presence of insulin receptors in the luminal plasma membrane, at the level of the microvilli [25], others seem to indicate that insulin receptors are mainly in the baso-lateral membrane of the enterocytes [24, 26]; differences in the experimental protocols used can account for these discrepancies. Thus, whether insulin internalization by the luminal surface of ileal epithelial cells takes place through the classical receptor-mediated endocytosis, remains to be elucidated.

The introduction of sodium cholate and aprotinin into the lumen of the intestine could produce cellular alterations at the level of the epithelium. However, morphological examination of the tissue has demonstrated that the conditions used did not induce any changes in the integrity of the epithelium or in the junctional complexes. Intracellularly, the epithelial cells did not seem to be affected, since no structural modification of the cells such as lysosomal proliferation and membrane degeneration were detected. These results indicate that the solution introduced into the lumen of the ileum had little negative effect on the tissue. On the other hand, these conditions were found to be efficient in protecting the peptide from being hydrolyzed to an extent which would prevent any biological activity and further promoted its internalization. The fact that sodium cholate by itself did not induce any cellular or tissue alteration should have been expected since cholate derivatives are normally present in the ileal lumen as part of the bile juice. Less well known is the fact that insulin as well as other pancreatic peptides such as glucagon and somatostatin do reach the lumen of the intestine through the pancreatic juice [28–31]. Beside their secretion into the pancreatic interstitial space for reaching the circulation, the pancreatic hormones are also present in the pancreatic juice as a result of their exocrine secretion by particular endocrine cells located in the wall of the pancreatic ducts [23]. The role of these peptides in the pancreatic juice has only been the subject of speculation. The present study demonstrates that in fact peptides can be absorbed by intestinal epithelial cells in order for them to act directly on the metabolism of these cells [32, 33] or to be transported into the intestinal interstitial space to reach the circulation.

Various studies have been performed demonstrating that the administration of insulin at various levels of the digestive tract may be adequate alternative routes for insulin treatment. Our present study was focussed on the evaluation of the passage of insulin through the ileal epithelium, revealing the pathway of this internalization. No attempt was made to compare this site of insulin administration to others previously used, such as the rectal or nasal sites.

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References

1. Banga AK, Chien YW (1988) Systemic delivery of therapeutic peptides and proteins. Int J Pharmacol 48: 15–50
2. Hirai S, Ikenaga T, Matasuzawa T (1978) Nasal absorption of insulin in dogs. Diabetes 27: 296–299
3. Salzman R, Manson JE, Griffing GT, Kimmerle R, Ruderman N, McCall A, Stoltz EI, Mullin C, Small D, Armstrong J, Melby JC (1985) Intranasal aerosolized insulin mixed meal studies and long-term use in type I diabetes. N Engl J Med 312: 1078–1084
4. Gordon GS, Moses AC, Silver RD, Flier JS, Carey MC (1985) Nasal absorption of insulin: enhancement by hydrophobic bile salts. Proc Natl Acad Sci USA 82: 7419–7423
5. Ichikawa K, Ohata I, Mitomi M, Kawamura S, Maeno H, Kawata H (1980) Rectal absorption of insulin suppositories in rabbits. J Pharm Parmacol 32: 314–318
6. Ziv E, Kidron M, Berry EM, Bar-On H (1981) Bile salts promote the absorption of insulin from the rat colon. Life Sci 29: 803–809
7. Ziv E, Kleinman Y, Bar-On H, Kidron M (1984) Treatment of diabetic rats with enteral insulin. In: Shafrir E, Renold AE (eds) Lessons from animal diabetes. John Libbey, London, pp 642–647
8. Ritschel WA, Ritschel GE (1984) Rectal administration of insulin. Methods Find Exp Clin Pharmacol 6: 513–529
9. Raz I, Bar-On H, Kidron M, Ziv E (1984) Rectal administration of insulin. Isr J Med Sci 20: 173–175
10. Nishihata T, Rytting JH, Kamada A, Higuchi T (1981) Enhanced intestinal absorption of insulin in rats in the presence of sodium 5-methoxy-salicylate. Diabetes 30: 1065–1067
11. Bar-On H, Berry EM, Eldor A, Kidron M, Lichtenberg D, Ziv E (1981) Enteral administration insulin in the rat. Br J Parmacol 73: 21–24
12. Kidron M, Bar-On H, Berry EM, Ziv E (1982) The absorption of insulin from various regions of the rat intestine. Life Sci 31: 2837–2841
13. Ziv E, Lior O, Kidron M (1987) Absorption of protein via the intestinal wall a quantitative model. Biochem Pharma 36: 1035–1039
14. Damge C, Michel C, Aprahamian M, Couvreur P (1988) New approach for oral administration of insulin with polyalkylcyahoacrylate manocapsules as drug carrier. Diabetes 37: 246–251
15. Bendayan M (1984) Protein A-gold electron microscopic immuno-cytochemistry: Methods, applications and limitations. J Electron Microsc Tech 1: 243–270
16. Bendayan M (1989) Protein A-gold and protein G-gold postembedding immunoelectron microscopy. In: Hayat MA (ed) Colloidal gold cytochemistry techniques and applications. Academic Press, New York, pp 33–94
17. Bendayan M, Zollinger M (1983) Ultrastructural localization of antigenic sites on osmium-fixed tissues applying the protein A-gold technique. J Histochem Cytochem 31: 101–109
18. Bendayan M (1989) Ultrastructural localization of insulin and C-peptide antigenic sites in rat pancreatic B cell applying the quantitative high resolution protein A-gold approach. Am J Anat 185: 205–222

19. Rodewald R, Abrahamson DR (1982) Receptor-mediated transport of IgG across the intestinal epithelium of the neonatal rat. Ciba Foundation Symp 92: 209–232
20. Herzog V, Farquhar MG (1977) Luminal membrane retrieval after exocytosis reaches most Golgi cisternae in secretory cells. Proc Natl Acad Sci USA 74: 5073–5077
21. Farquhar MG (1982) Membrane recycling in secretory cells: pathway to the Golgi complex. Ciba Foundation Symp 92: 157–183
22. Steinman RM, Mellman IS, Muller WA, Cohn ZA (1983) Endocytosis and the recycling of plasma membrane. J Cell Biol 96: 1–27
23. Bendayan M (1987) Presence of endocrine cells in pancreatic ducts. Pancreas 2: 393–397
24. Bergeron JJM, Rachubinski R, Searle N, Borts D, Sikstrom R, Posner BI (1980) Polypeptide hormone receptors, in vivo: Demonstration of insulin binding to adrenal gland and gastro intestinal epithelium by quantitative radioautography. J Histochem Cytochem 28: 824–835
25. Pillion DF, Ganapathy V, Leibach FH (1985) Identification of insulin receptors on the mucosal surface of colon epithelial cells. J Biol Chem 260: 5244–5247
26. Gingerich RL, Gilbert WR, Comens PG, Gavin III JR (1987) Identification and characterization of insulin receptors in basolateral membranes of dog intestinal mucosa. Diabetes 36: 1124–1129
27. Fernandez-Moreno MD, Serrano-Rios M, Prieto JC (1987) Identification of insulin receptors in epithelial cells from duodenum, jejunum, ileum, caecum, colon and rectum in the rat. Diabete Metabolism 13: 135–139
28. Lawrence AM, Prinz RA, Paloyan E, Kokal WA (1979) Glucagon and insulin in pancreatic exocrine secretions. Lancet II: 1354–1355
29. Colon JM, Rouiller D, Boden G, Unger RH (1979) Characterization of immunoreactive components of insulin and somatostatin in canine pancreatic juice. FEBS Lett 105: 23–26
30. Carr-Locke DL, Track NS (1979) Human pancreatic polypeptide in pancreatic juice. Lancet I: 151–152
31. Sarfati PD, Green GM, Brazeau P, Morisset J (1986) Presence of somatostatin-like immunoreactivity in rat pancreatic juice: a physiological phenomenon. Can J Physiol Pharmacol 64: 539–544
32. Ménard D, Malo C, Calvert R (1981) Insulin accelerates the development of intestinal brush border hydrolytic activities of suckling mice. Dev Biol 85: 150–155
33. Kellett GL, Jamal A, Robertson JP, Wollen N (1984) The acute regulation of glucose absorption, transport and metabolism in rat small intestine by insulin in vivo. Biochem J 219: 1027–1035

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