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LABELLING OF INTESTINAL BRUSH BORDER MEMBRANE PROTEINS IN VIVO USING DIAZOTISED [^{125}I]IODOSULFANILIC ACID

GRAEME P. YOUNG * and DAVID H. ALPERS **

Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110 (U.S.A.)

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Summary

Membrane proteins of the intestinal brush border were labelled in vivo by intraluminal injection of diazotised [^{125}I]iodosulfanilic acid, a highly polar molecule. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of brush border membranes labelled in this manner showed 20 protein bands, 11 of which contained significant radioactivity. The most heavily labelled proteins had molecular weights greater than 150 000, indicating that they were the most exposed to the intestinal lumen. Little radioactivity was detected in proteins with molecular weights of less than 94 000. The majority of these smaller proteins were likely to have been brush border core proteins. The evidence that diazotised [^{125}I]iodosulfanilic acid bound primarily to brush border membrane proteins when administered in this way, was: (a) the specific activity of brush border proteins was up to 3-fold greater than that of total cell particulate proteins (pelleted at 27 000 × *g* from mucosal homogenates); (b) principal peaks in the gel radioactivity profile of total cell particulate proteins corresponded to the most heavily labelled proteins of the isolated brush border membrane; and (c) brush border core proteins showed minimal radioactivity in vivo, but considerably higher radioactivity when brush border membranes were labelled in vitro. A small amount of label was absorbed across the intestinal mucosa. However, secondary labelling of brush border proteins by this absorbed label was minimal, since the specific activity of brush border proteins in jejunum adjacent to the labelled loop was only 0.22%

* Present address: University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Victoria 3050, Australia.
** To whom reprint requests should be addressed.

of the level for those proteins in the labelled segment. Since this technique did not affect the cellular morphology, enzyme activity or biochemical integrity of the membrane, it should prove useful as a means of accurately studying in vivo turnover rates of brush border membrane proteins.

Introduction

The intestinal brush border is a complex organelle which carries out important digestive and absorptive functions. The proteins of this structure, which include hydrolases, receptors and transport systems [1], are subject to attack by proteases from both sides of the membrane. Both lysosomal [2] and pancreatic proteases [3,4] have been implicated in the rapid turnover of brush border proteins. Half-lives of degradation range from 4 to 18 h in the rat and mouse [5—10], the larger proteins having the shortest half-lives (4.4 h for sucrase [10]). Measurement of actual turnover rates of these membrane proteins has been complicated by the short life-span of the villous cell, estimated at between 3 and 6 days [11]. Such findings indicate that turnover of brush border proteins is not merely a function of cell loss from villi.

Unfortunately, all the methods used to date to study brush border protein turnover have been based on measurement of the rate of decrease in radioactivity which occurs after radioactive amino acids have been maximally incorporated into brush border proteins. Such methods have thus been affected by the synthetic rate of nascent proteins and the processing time needed to place them in the brush border membrane. The results are also likely to have been influenced by re-utilization of radioactive amino acids after breakdown of intracellular protein. To measure accurately degradation rates, the ideal in vivo labelling technique should be independent of the synthetic rate. It should label proteins at one point in time after they have already been incorporated into the membrane, without affecting biological function or biochemical characteristics of the proteins. Cellular morphology should remain unchanged. In addition, the radioisotope should be a gamma emitter for easy and sensitive detection and the label should be sufficiently polar so as not to penetrate membranes by simple diffusion.

Diazotised [^{125}I]iodosulfanilic acid appears to possess most of these characteristics. When used to label erythrocyte and platelet membrane proteins in vivo [12—14], neither morphology nor subsequent in vivo behavior of these cells was affected. The principal platelet membrane components which were labelled were glycoproteins which reflects their prominence on the exterior of the membrane. Diazonium compounds principally label tyrosine, histidine and lysine residues [15].

This report describes the successful use of [^{125}I]iodosulfanilic aicd to label intestinal brush border membrane proteins in vivo by intraluminal injection. Such labelling was achieved without damage to cellular morphology or enzymic activity. It should prove to be a useful and accurate means of studying turnover rates of membrane proteins in various physiological and pathophysiological states.

Methods

Animal and tissue preparation. Male Wistar rats weighing approx. 325 g were obtained from National Laboratory Animal Co., O'Fallon, MO. In none of the experiments were rats fasted. The method of Schmitz et al. [16] was used with slight variations to isolate brush border fragments. Briefly, the mucosa was collected by scraping the gut with a glass slide. All following steps were performed at 4°C. Scrapings were then suspended in 100-times their volume of 2 mM Tris-HCl (pH 7.4) containing 50 mM mannitol. Homogenization was carried out using seven full strokes of a Potter-Elvehjem homogenizer with Teflon pestle rotating at full speed. Each homogenate was then adjusted to 10 mM $CaCl_2$ and stirred for 15 min. After centrifugation at 2000 × *g* for 10 min, the pellet was discarded. The brush border fragments * were finally collected by centrifugation at 27 000 × *g* for 15 min. Further purification to separate remaining core proteins from the brush border membrane was not carried out.

Enzyme and protein assay. Alkaline phosphatase was assayed by using the method of Forstner et al. [17] at pH 9.2, using *p*-nitrophenyl phosphate as substrate. Sucrase activity was determined according to the method of Dahlqvist [18]. Protein was assayed according to the method of Lowry et al. [19] using bovine serum albumin as standard. All chemicals were obtained from Sigma Chemical Co., St. Louis, MO. To determine the specific activity of ^{125}I-labelled proteins, proteins were first precipitated with 10% trichloroacetic acid. Radioactivity was determined in washed pellets using a Nuclear Chicago gamma-counter, with a counting efficiency of 74%. Samples were counted for at least 10 min or until counting error was below 2%. Protein pellets were then dissolved in 0.2 M NaOH and assayed for protein. Background radioactivity measured 14 cpm and was subtracted before specific activities were calculated. All of the preceding determinations were carried out in duplicate at least.

Diazotisation of [^{125}I]iodosulfanilic acid. [^{125}I]Iodosulfanilic acid (specific activity greater than 1000 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA), stored at 4°C and used within 4 weeks of production. Diazotisation was carried out immediately prior to use, following exactly the instructions provided by the manufacturer.

In vitro labelling with diazotised [^{125}I]iodosulfanilic acid. Brush border fragments containing 2 mg of protein were added to 0.3 ml of 50 mM potassium phosphate buffer at the following pH values: 7.4, 6.6 and 5.8. 5 μCi of diazotised [^{125}I]iodosulfanilic acid were added and the reaction allowed to proceed during gentle shaking at 37°C for 30 min. Membrane proteins were then collected by centrifugation at 27 000 × *g* for 15 min and washed three times in 1% bovine serum albumin in 10 mM Tris-HCl (pH 7.4) to remove loosely bound label.

In vivo labelling with diazotised [^{125}I]iodosulfanilic acid. Each rat was anesthesized with pentobarbital (35 mg/kg) given intraperitoneally. After

* The term brush border or brush border fragments refers to this preparation. It should not be confused with the term total cell particulate fraction which is used later to refer to those proteins pelleted at 27 000 × *g* from a mucosal homogenate which had not been pretreated with $CaCl_2$.

opening the peritoneal cavity, a 7 cm segment of proximal jejunum was cannulated at both ends with polyethylene tubing (2.1 mm external diameter). Each cannula was inserted through a small, longitudinal incision in the antimesenteric border and tied securely in place. The bowel lumen above and below the cannulated segment was tied off to prevent contents leaking into the peritoneum. The segment of bowel was then rinsed with 50 ml of 10 mM potassium phosphate-buffered isotonic saline (pH 7.4) warmed to 37°C. After draining the segment, the distal cannula was clamped. Freshly diazotised [^{125}I]iodosulfanilic acid in 1 ml phosphate-buffered saline was injected via the proximal cannula. Further non-radioactive phosphate-buffered saline was then added to distend the loop (in order to remove any folds), and the contents mixed by gentle squeezing. The proximal cannula was clamped, the loop of bowel returned to the abdominal cavity and the wound closed with a continuous suture. At the end of the labelling period, the wound was opened and the segment of jejunum removed with cannulae intact. The lumen was rinsed with 100 ml phosphate-buffered saline warmed to 37°C to remove as much non-mucosal-bound radioactivity as possible. Brush border fragments were prepared from the jejunal segment as described above. To achieve reproducible results from animal to animal, it was important to match carefully lengths of bowel labelled and to use rats of similar weights. Increasing the length of gut but not the dose of radioactive label resulted in a reduced specific activity of the recovered brush border proteins.

SDS-polyacrylamide gel electrophoresis. Gel electrophoresis was carried out in 2-mm thick slab gels containing 0.1% sodium dodecyl sulfate (SDS) [20]. Separating gels consisted of 11.1% total acrylamide; 110 : 1 acrylamide to bisacrylamide (Polysciences, Warrington, PA). Samples of membrane proteins were processed immediately after isolation by boiling in 2.5% SDS for 3 min. Each sample, containing 100 μg of protein, was subjected to electrophoresis in duplicate. One of each sample pair was stained with Coomassie Brilliant Blue [21]. After destaining, gels were scanned at 555 nm in a modified Beckman DU-2 spectrophotometer. The second gel of each pair was immediately sectioned into 1 mm slices with a multiple razor blade holder and the radioactivity in each slice measured as described above for protein pellets. The following molecular weight marker proteins were used: β-galactosidase, phosphorylase B, bovine serum albumin and ovalbumin (all from Sigma Chemical Co., St. Louis, MO).

Morphological studies. The effect of iodosulfanilic acid labelling on intestinal morphology was studied in two rats. In each rat, three adjacent 7-cm segments of jejunum were cannulated and rinsed exactly as described above for the radioactive label. Phosphate-buffered saline was instilled into the lumen of each segment and the cannulae clamped. The buffer in the middle and distal segments also contained 0.2 and 2 nmol, respectively, of non-radioactive diazotised iodosulfanilic acid. The smaller amount was approximately equal to labelling in vivo with 200 μCi. After incubation for 1 h, each segment was removed and rinsed exactly as described for the in vivo experiments using radioactive label. Three full-thickness pieces (3 × 3 mm) of intestinal wall were removed from each loop and fixed in 10% formalin. Photomicrographs were prepared from all pieces and examined closely for damage to the villous cells. No evidence of damage was seen in any of the segments.

Results

In vitro labelling

Using 5 μCi of diazotised [^{125}I]iodosulfanilic acid, as described in Methods, $204.2 \cdot 10^3 \pm 1.4 \cdot 10^3$ cpm ($\bar{x} \pm$ S.E.) bound to 1 mg brush border protein at pH 7.4. Lower pH caused a reduction in labelling: $181.1 \cdot 10^3 \pm 2.3 \cdot 10^3$ cpm at pH 6.4 and $153.1 \cdot 10^3 \pm 9.2 \cdot 10^3$ cpm at pH 5.8. Labelling of membranes at pH 7.4 did not adversely affect enzymic activity when compared with control, unlabelled membranes otherwise treated in the same manner. For alkaline phosphatase, mean total activity in control membranes was 1.52 units and in labelled membranes 1.51 units. For sucrase, corresponding means were 0.074 units and 0.076 units, respectively. These enzymes were not solubilized by the labelling procedure.

In vivo labelling

The method used for isolation of brush border fragments yielded 0.3—0.4 mg of protein from the labelled segment of jejunum. Purification factors, when comparing enzymic specific activities of brush border preparations with mucosal homogenates, were 12.5 for alkaline phosphatase and 10.1 for sucrase. When specific activities in brush borders were compared with those of 27 000 × *g* pelleted membranes and intact organelles (the 'particulate fraction'), the purification factors were 5.5 for sucrase and 6.1 for alkaline phosphatase.

Studies on the effect of dose of [^{125}I]iodosulfanilic acid and duration of labelling on the radioactive specific activity of brush border proteins were carried out to give an indication of suitable labelling conditions and to minimize the amount of label used. Table I shows that increasing the dose and period of labelling will increase the specific activity of brush border proteins. Satisfactory labelling was obtained with 200 μCi for 60 min, resulting in a labelling efficiency of 0.3%. Further increase in the duration of labelling was

TABLE I

RADIOACTIVITY OF MEMBRANE PROTEINS FOLLOWING IN VIVO LABELLING WITH DIAZOTISED [^{125}I]IODOSULFANILIC ACID

To determine suitable labelling conditions, rats were anesthetized with 35 mg/kg of pentobarbital and a 7 cm segment of jejunum was labelled by intraluminal injection of freshly diazotised [^{125}I]iodosulfanilic acid, as described in Methods. Following incubation and thorough rinsing of the jejunal segment, brush border fragments were isolated from homogenates according to the method of Schmitz et al. [16]. The particulate fraction was collected by centrifugation of a small aliquot of mucosal homogenate (not treated with $CaCl_2$) at 27 000 × *g*. Specific activity was measured in trichloroacetic acid-precipitable proteins as outlined in Methods. Stated specific activities are the means of individual experiments on two rats for each set of conditions. Results are expressed as the mean ± S.E.

Dose (μCi)	Duration (min)	Specific activity	
		Brush border membranes (cpm/mg protein) ($\times 10^{-3}$)	Particulate fraction (cpm/mg protein) ($\times 10^{-3}$)
100	30	57.25 ± 5.42	20.94 ± 2.71
100	30	82.86 ± 8.42	32.32 ± 4.32
200	60	258.24 ± 18.17	110.67 ± 15.89

TABLE II

RADIOACTIVITY OF NON-INTESTINAL TISSUES FOLLOWING IN VIVO LABELLING OF DIAZOTISED [^{125}I]IODOSULFANILIC ACID

The experimental procedure was the same as in Table I. Organs and blood were collected immediately after the labelled segment of jejunum was removed. Radioactivity in the total mass of each solid organ was determined in triplicate from aliquots of a 10% homogenate prepared in 50 mM mannitol/10 mM Tris-HCl (pH 7.4). Values in parentheses indicate the percentage of counts which were not protein-bound, as determined by trichloroacetic acid precipitation. Results are expressed as the mean ± S.E.

Dose (μCi)	Duration (min)	Spleen (cpm) ($\times 10^{-3}$)	Liver (cpm) ($\times 10^{-3}$)	Kidneys (cpm) ($\times 10^{-3}$)	Blood (cpm) ($\times 10^{-3}$/ml)
100	30	10.1 ± 1.1	1742 ± 80 (28)	323 ± 25 (33)	116 ± 12
100	60	10.5 ± 1.3	1972 ± 220 (25)	833 ± 60 (69)	149 ± 13

of little value. The radioactivity of particulate proteins pelleted at 27 000 × *g* from mucosal homogenates was about one-third that of brush border membranes. This was somewhat higher than expected, considering that sucrase specific activity (i.e., units enzyme activity/mg protein) in this particulate fraction was only one-fifth of sucrase specific activity in the isolated brush border membranes.

A number of factors were important in achieving maximal labelling at a given dose. Thorough rinsing of the gut lumen prior to instilling label was most important, presumably by rinsing out many of the soluble, more accessible proteins. Following instillation of label, it was found important to distend the gut lumen with buffer to minimize overlapping of mucosal folds. The use of [^{125}I]iodosulfanilic acid immediately after diazotisation also gave better results.

In Table II the radioactivity of non-intestinal tissues is detailed and suggests that diazotised [^{125}I]iodosulfanilic acid was absorbed across the jejunal mucosa. After labelling for 1 h, the proportion of counts in the liver represented 4% of the total dose. Not all of this was protein bound, however, since 25.2% was not precipitated by trichloroacetic acid. Nonetheless, only minute amounts of label appeared to be redistributed to intestinal mucosal tissues. The specific activity of brush border proteins in the 15 cm of jejunum distal to the labelled segment was 568 cpm/mg protein (in the rat labelled with 200 μCi for 60 min). This is 0.22% of the specific activity for brush border proteins in the labelled segment of that rat.

Gel electrophoresis of brush border proteins

Fig. 1 demonstrates the results of gel electrophoresis after mucosal cells were labelled in vivo with diazotised [^{125}I]iodosulfanilic acid. The electrophoresis results shown are typical of consistently occurring patterns obtained in four experiments, each in a different rat. The lower portion of Fig. 1 gives the results of electrophoresis of proteins of membranes and intact organelles pelleted from a non-$CaCl_2$-precipitated homogenate at 27 000 × *g*. There were five regions which showed the most dense Coomassie blue staining. Two of these regions, designated A and B, corresponded to molecular weights of 200 000—300 000 and 130 000, respectively. Although radioactivity through-

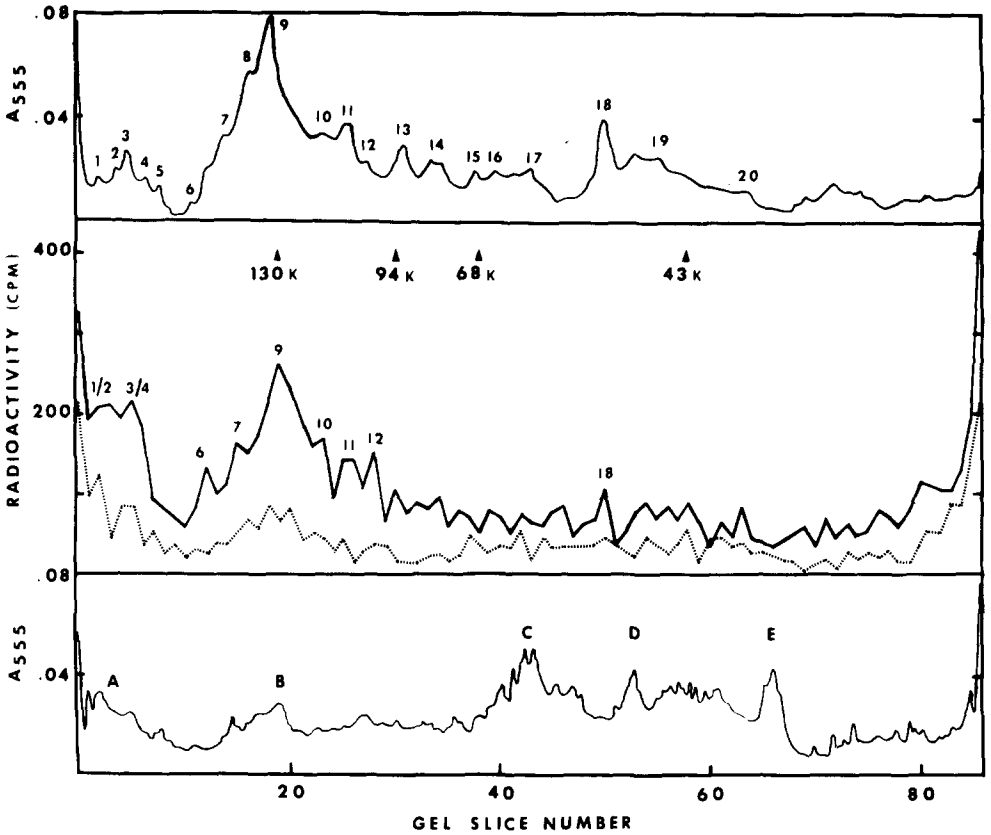

Fig. 1. Polyacrylamide gel electrophoresis of brush border fragments and total cell particulate proteins from jejunal mucosal cells labelled in vivo with diazotised [^{125}I]iodosulfanilic acid. Under general anesthesia, a 10 cm segment of jejunum was labelled by intraluminal injection of 200 μCi of label for a duration of 60 min. Details are given in Methods. The total cell particulate fraction was collected from the mucosal homogenate by centrifugation at 27 000 $\times g$. Brush border fragments were isolated following the method of Schmitz et al. [16] as outlined in Methods. Gel electrophoresis was carried out in 11.1% acrylamide gels containing 0.1% SDS, as detailed in Methods. The upper (brush border membrane) and lower (particulate fraction) panels show the results of scanning the Coomassie blue-stained gels at 555 nm. Consistently occurring protein bands in brush border membranes are indicated by numbers. The most prominent regions of protein staining in the profile of the particulate fraction are designated A—E. The radioactivity profiles of 1 mm gel slices (see Methods) are shown in the middle panel, the radioactivity peaks in brush border membranes being related to corresponding protein bands by the appropriate numbers; (brush border membrane, ———; particulate fraction, · · · · · ·). The positions of molecular weight markers are indicated by the arrows. Profiles shown are typical of four experiments on four rats.

out the gel was low, the most radioactive regions corresponded to regions A and B. The other three prominent regions of Coomassie blue staining, designated C, D and E, do not have corresponding peaks of radioactivity.

The upper half of Fig. 1 shows the results of gel electrophoresis of brush border proteins labelled in vivo. 20 consistently occurring bands were found after Coomassie blue staining. Comparison of this protein profile with the radioactivity profile revealed corresponding peaks of radioactivity for bands 1/2, 3/4, 6, 7, 9, 10, 11, 12 and 18. It is apparent that the intensity of labelling was disproportionate to the amount of protein present as assessed from the

density of the stain. For example, radioactivity in peaks 1/2 and 3/4 was almost as high as in peak 9, even though there was considerably more staining of band 9. Similarly, the relationship of radioactivity in peaks 10 and 12 to peak 11 was the reverse of the density of Coomassie blue staining. In addition, band 18 stained prominently with Coomassie blue but was barely distinguishable as a peak on the radioactivity profile. Radioactivity in bands 13—17 was low.

A comparison of the two radioactivity profiles in Fig. 1 demonstrates the 3-fold increase in radioactive specific activity of proteins of the isolated brush border membrane over particulate proteins of the 27 000 × *g* pellet. The prominently labelled regions A and B of particulate proteins corresponded to the areas of highest radioactivity in isolated brush borders, namely bands 1—4 and 7—9, respectively.

The results of gel electrophoresis of in vitro-labelled brush border proteins are shown in Fig. 2. The brush border membranes used were isolated from the 15 cm segment of gut adjacent (caudally) to the in vivo-labelled loop of the same animal depicted in Fig. 1. The results shown are typical of experiments

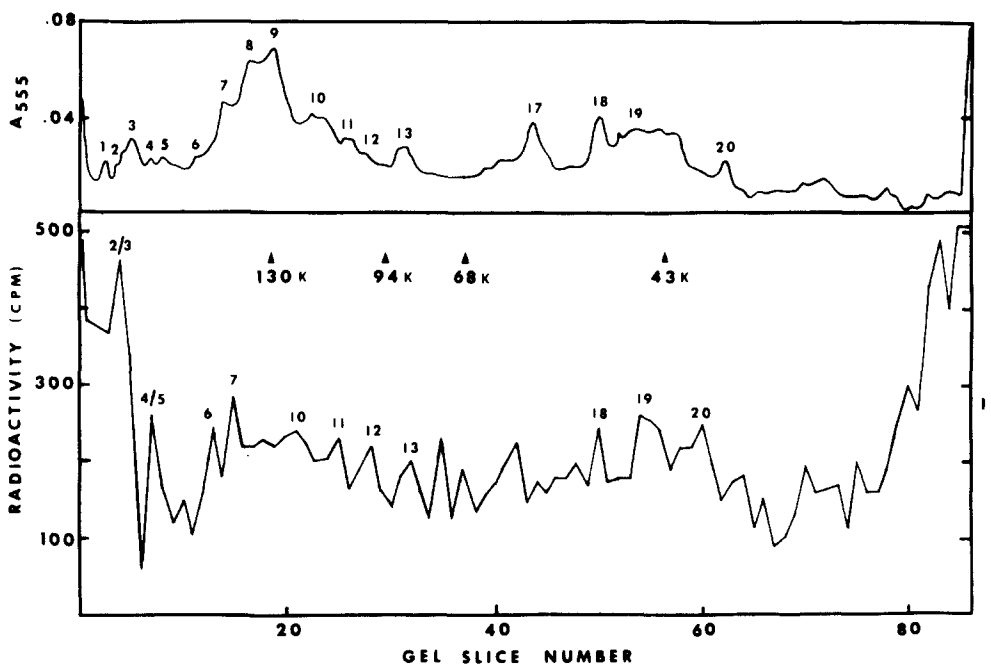

Fig. 2. Gel electrophoresis of intestinal brush border proteins labelled in vitro with diazotised [^{125}I]-iodosulfanilic acid. Brush border membranes were isolated as described in Methods from the jejunum just distal to the labelled segment of the rat, of which the findings are also depicted in Fig. 1. The entire amount of brush border protein recovered, about 0.8 mg, was suspended in 0.25 ml of 10 mM potassium phosphate-buffered isotonic saline (pH 7.4) containing 50 μCi of diazotised [^{125}I]iodosulfanilic acid. The mixture was allowed to react at 4°C for 30 min and then washed in exactly the same way as in vivo-labelled material. Gel electrophoresis was carried out in 11.1% acrylamide gels containing 0.1% SDS as detailed in Methods. The upper panel shows the results of scanning the Coomassie blue-stained gel at 555 nm. The lower panel depicts the radioactivity of 1 mm gel slices (see Methods). The position of molecular weight markers is the same as in Fig. 1.

for a total of four animals. The protein profile was similar to that of in vivo-labelled material (Fig. 1), although bands 9, 11 and 14—16 were less prominent and band 17 was more prominent in the more distal brush border material. These minor changes were likely to be related to the known differences of brush border proteins along the length of the intestine [22]. The radioactivity profile showed much more important differences, however. Fig. 2 shows that protein band 9 was much less radioactive than when labelled in vivo. The pattern of labelling of protein bands 1—5 was considerably altered. Although it is conceivable that gel-slicing geometry might cause minor differences in this pattern, it is clearly evident that in vitro labelling of proteins 2/3 relative to protein 9 was much heavier than in in vivo-labelled material. This was a reproducible result.

Most importantly, the radioactivity profiles for proteins with molecular weights of less than 94 000 are quite different when comparing in vitro- and in vivo-labelled tissues. In vivo-labelled membranes (Fig. 1) show little labelling in this region. In contrast, these smaller proteins are more intensely labelled in the in vitro system (Fig. 2).

Discussion

These results show that brush border proteins can be labelled in vivo with [^{125}I]iodosulfanilic acid, after these proteins have been incorporated into the membrane. The labelling agent did not modify activity of the enzymes, sucrase or alkaline phosphatase, and cell morphology was unaffected under optimal labelling conditions. Three findings indicated that when the radioactive label was administered by the intraluminal route, it bound primarily to brush border membrane proteins. Firstly, the specific activity of proteins of the isolated brush border membrane was 3-times higher than that of particulate proteins obtained by centrifuging mucosal homogenates at 27 000 $\times g$. Secondly, the regions of maximum radioactivity in this particulate fraction (regions A and B in Fig 1) corresponded to the most heavily labelled proteins of the isolated brush border preparations (bands 1—4 and 7—9 in Fig. 1). Since the particulate fraction contained brush border membranes, the radioactivity in the particulate fraction probably came from the brush border component. Thirdly, brush border core proteins showed minimal labelling in vivo. In Figs. 1 and 2, these would be the proteins with molecular weights of less than 68 000 [7,23]. Core proteins were more susceptible to labelling in vitro probably because they were no longer physically separated from diazotised [^{125}I]-iodosulfanilic acid by the cell membrane. This result supports the conclusion that brush border membrane proteins are the major proteins labelled in vivo.

The protein profile obtained in the present study by gel electrophoresis is similar to that found by Seetharam et al. [24] who used similar methods for both brush border membrane isolation and gel electrophoresis. As we boiled samples before electrophoresis to prevent proteolytic digestion, we were unable to relate directly protein bands to specific enzymes. Comparison with the profile obtained by Seetharam et al. [24] revealed that the disaccharidases, maltase-glucoamylase, lactase and sucrase-isomaltase, were likely to be related to the region of bands 1—5. The molecular weight of sucrase-isomaltase is in

the region of 200 000, corresponding to the positions of bands 2/3 [25]. As rat intestinal alkaline phosphatase has an apparent molecular weight of 130 000 on gel electrophoresis (Yedlin, S.T., Young, G.P., Seetharam, B. and Alpers, D.H., unpublished data), this enzyme most likely migrates in the region of bands 8/9. However, enzyme activities cannot be ascribed with certainty to individual bands, since different methods of sample preparation can result in different electrophoretic characteristics, e.g., for maltase-glucoamylase [26].

George et al. [12,13] showed that the platelet membrane proteins most readily labelled by diazotised [^{125}I]iodosulfanilic acid were glycoproteins. These were the most exposed of the integral membrane proteins of the platelet. This phenomenon, i.e., that the most exposed proteins are the most heavily labelled, is likely to explain the lack of correlation between protein staining and radioactivity of in vivo-labelled membranes. This was especially evident for proteins with molecular weights greater than 94 000. In particular, bands 1—4 were more heavily labelled than bands 8—9 when the relation of protein staining to radioactivity is considered (Fig. 2). The molecular weights of bands 1—4, which include some of the disaccharidases, were greater than 150 000. The prominence of these glycoproteins on the brush border membrane has been demonstrated by the electron microscope (see Ref. 27 for review). The difference in the labelling pattern of the higher molecular weight proteins (greater than 94 000) between the in vitro and in vivo techniques may have been due to an alteration in the physical state of some membrane proteins during isolation, perhaps due to the production of some 'inside-out' vesicles.

Because of its highly polar nature, [^{125}I]iodosulfanilic acid does not cross the plasma membrane of platelets or erythrocytes [12—14]. Nonetheless, in this study the label clearly crossed the intestinal mucosa since one-quarter of the radioactivity in the liver was not protein-bound (Table II). This unbound [^{125}I]iodosulfanilic acid is likely to be able to label intracellular proteins, thus accounting for the higher than expected radioactivity of proteins within membranes and the still-intact intracellular organelles contained with the 27 000 × *g* pellet of the mucosal homogenate. The low labelling efficiency of brush border proteins is likely to have been caused in part by the physical barrier presented by mucus, the relative inaccessibility of brush borders on the sides of villi and the presence of more readily labelled soluble proteins in the gut lumen. Furthermore, it has been shown that pH 7.4 and a temperature of 4°C were optimal for labelling of erythrocyte membrane proteins [14], conditions which are not practicable in vivo.

Clearly, the labelling of membrane proteins by this technique is independent of protein synthetic rates. Re-utilization of labelled amino acids would seem quite unlikely to occur. Also, the very low level of labelling observed in brush border proteins of jejunum adjacent to the labelled segment indicated that any secondary labelling by the small amounts of [^{125}I]iodosulfanilic acid which were absorbed would be insignificant. This satisfies most of the criteria outlined earlier which are desirable for a labelling technique by which the turnover of individual or multiple brush border membrane proteins can be studied. Modification of the technique described, by restoring intestinal continuity at the end of the labelling period, should enable such studies to be made. The conditions chosen for labelling are mild and do not affect cell histology

and enzyme activity (sucrase and alkaline phosphatase), nor the biochemical integrity of the membrane (since sucrase and alkaline phosphatase were not solubilized). Hence, any effect of the technique itself on protein turnover is likely to be small.

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