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TURNOVER OF RAT BRUSH BORDER PROTEINS AFTER MASSIVE INTESTINAL RESECTION

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SUMMARY : Turnover of rat intestinal brush border proteins has been evaluated by measuring their relative degradation rates after a 75 % proximal resection. The ^{3}H : ^{14}C ratios determinated after an intraluminal double labeling technique show a significant decrease of the high molecular weight protein turnover rates after ileal regeneration when compared to equivalent size proteins isolated from sham-operated animals. This is interpreted as the reflect of the villus epithelial cells immaturity in the remaining ileum after a small bowel resection.

Morphological and physiological modifications following a massive resection of the small bowel are well documented (for a review see ref. 1). They lie essentially in a compensatory cellular hyperplasia leading to a more or less complete reconstitution of the intestinal mucosa. This adaptative hyperplasia after excising various lengths of the small intestine is characterized by changes in the epithelial cell kinetics (2,3,4,5). At the cell membrane level modifications of the enzymatic activities of enterocyte brush borders have been described but the results were often conflicting, depending on the enzyme studied or the type of resection performed.

In order to determine if the turnover of brush border membrane proteins is affected by a massive intestinal resection we have compared the relative degradation rates of brush border proteins isolated from rats after a 75 % proximal small bowel resection or after sham surgery.

MATERIAL AND METHODS :

Operative techniques : Male Wistar rats weighing between 250 and 300 g were starved for 24h. before surgery, but were allowed free access to water. Under ether anesthesia 75 % of the small intestine was removed starting from a point 3 cm distal to the ligament of Treitz.

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Sham-operated animals underwent only a transection at a point on the middle of the ileum followed by an anastomosis end to end without tissue excision. After surgery rats were fasted for 24 h. and then fed a standard rat chow diet (UAR. France) ad libitum.

Isotope administration : The relative degradation rates of brush border membrane proteins were determinated 4 weeks after the intestinal resection by the intraluminal double labeling technique adapted by Alpers to rat intestine (6). All animals were fasted 5 h. before the first injection. At 12 noon 25 µCi of ^{14}C leu (270 mCi/mmole) in 2 ml of NaCl 0.154 M were injected at the anastomosis point towards the coecum. Ten hours after the first injection (10 P.M.) 50 µCi of ^{3}H leu (40 to 60 Ci/mmole) in 2 ml NaCl 0.154 M were injected in the same conditions. Both isotopes were obtained from the Radiochemical Center, Amersham (U.K.). Ten hours after the 2nd. injection animals were sacrified (8 A.M.).

Brush border purification : Brush borders were purified by the method of Schmitz et al (7) with some minor modifications. After an extensive washing of the ileal segment between the anastomosis and the ileocecal valve the mucosa was gently scraped in the 2 groups of animals. For each rat 200 mg of mucosa was homogeneized with an Ultra-Turrax apparatus (JANKE & KUNKEL. Germany) at slow speed in 20 ml of a Mannitol 50 mM, Tris 2 mM pH 7,1 buffer. $CaCl_2$ at 10 mM final concentration was added and after 10 min. the precipitate was centrifuged for 10 min. at 3000 g. The supernatant was centrifuged again 20 min. at 27 000 g. Sucrase and aminopeptidase specific activities were increased 15 to 20 fold in the pellet with a recovery of 25 %.

Protein fractionation : The brush border pellet was resuspended in 100 ml water, homogeneized by sonication and finally solubilized by 2 % sodium dodecyl sulfate. Membrane proteins were then analysed by polyacrylamide gel electrophoresis in presence of sodium dodecyl sulfate (8). Proteins (150 to 200 µg) containing 600 - 1100 cpm of ^{14}C and 2500 - 5000 cpm of ^{3}H were layered on each gel ; after electrophoresis gels were immediately frozen and cut in 2 mm slices. Each slice was solubilized in a scintillation vial by an overnight incubation with 200 µl of hydrogen peroxyde at 37° C. Ten ml of Instagel scintillator (PACKARD), 200 µl of acetic acid were added before counting radioactivity in each vial in a PACKARD TRICARB spectrometer.

RESULTS AND DISCUSSION :

Four weeks after a 75 % proximal resection a thickening of the mucosa were observed in the remaining ileum of operated rats whereas no anatomic modifications were noted in the sham-operated animals. This increase of the mucosa thickness is illustrated by the mean villus height found for the 6 resected animals (518 ± 91 µm) when compared to the mean villus height of the sham-operated animals (225 ± 52 µm). This hypertrophy is consistent with the process of intestinal regeneration as it was described by others (1). The double labeling technique by intraluminal injections for estimating the relative degradation rates of brush border proteins has been validated in the rat (6) and confirmed in the mouse (9). Fig 1A shows that in control experiments high molecular weight proteins (MW > 70000) turnover at a faster rate than smaller ones. This difference

of membrane proteins turnover rates has already been described in rat liver (10) and intestine (6,11).

After a small bowel resection our results clearly show (Fig 1B) a statistically significant decrease of the ^{3}H : ^{14}C ratios for high molecular weight brush border proteins when compared to proteins of similar size isolated from control animals.

During intestinal regeneration processes different modifications of epithelial cell kinetic parameters have been described (2,3,4,5). The migration rate of these cells along the villus is increased (1,2,3), which may lead to more or less important modifications of the cellular turnover rate, depending on the villus height in the regenerated intestine. An earlier desquamation of these cells from the villus should increase the ^{3}H : ^{14}C ratio measured in rats that underwent an intestinal resection. The ^{3}H : ^{14}C ratios found after resection in the present study are thus probably over-estimated and the differences between the relative degradation rates of the high molecular weight proteins in normal and experimental animals could be even greater that those measured.

Warren and Glick (12) found that in growing mice L cells the newly synthetized proteins are incorporated in the membrane whereas in nongrowing cells the proteins are synthetized and eliminated at comparable rates. In the same way, Alpers (11) recently showed that high molecular weight brush border proteins of proliferating cells in the crypts turnover at a slower rate than proteins of similar size of migrating cells along the villus. The relative degradation rates of brush border proteins we have observed after intestinal regeneration (Fig 2B) are very similar to those found by Alpers (11) in crypt cells. The slow turnover rates of epithelial cells in regenerating ileum could thus be the result of the cellular immaturity. This would be in close agreement with the hypothesis of the enterocyte functional immaturity after intestinal resection as proposed by Loran (3) on functional data and confirmed by Weser (13) on the basis of a larger number of cells per mg of protein on the remaining ileum. Moreover, an important decrease of ^{3}H : ^{14}C ratio of brush border high molecular weight proteins after double labeling turnover experiments has been found after a 95 % pancreatectomy in the rat (14).

It appears from Alpers' study (14) that intraluminal proteases play an important role in the control of membrane protein degradation. Modification of the intraluminal content resulting from the excision of 75 % of the small bowel could also be responsable for the brush border turnover variations observed during intestinal regeneration.

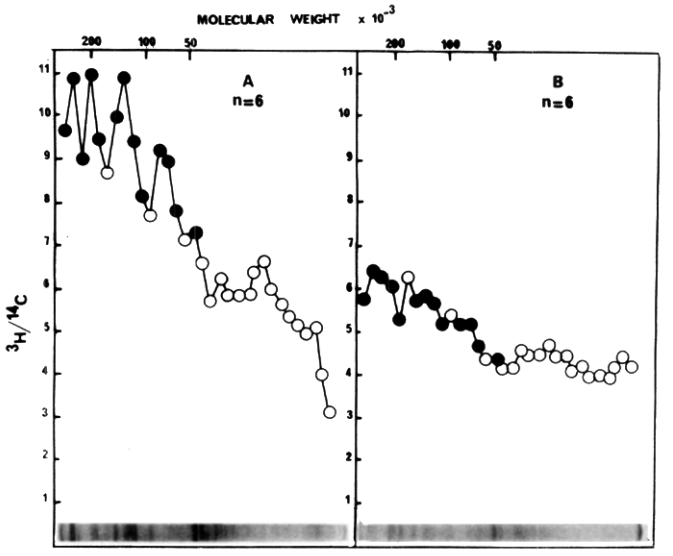

Figure 1. Effect of a 75 % proximal small bowel resection on relative degradation rates of brush border proteins.

Relative degradation rates were determinated by a double labeling technique (see material and methods) on brush border proteins isolated from 6 sham-operated rats (A) and 6 rats with a 75 % intestinal resection (B). Brush borders were prepared from each animal, solubilized in sodium dodecyl sulfate and proteins analysed by a polyacrylamide gel electrophoresis in which the bromophenol blue dye was allowed to migrate exactly 65 mm from the origin. The ^3H : ^{14}C ratios were determinated on 2 mm slices and compared with the ratios found on equivalent slices in all the gels of the control experiments. Means and standard deviations were calculated for each fraction and compared by a Student's test to corresponding mean values obtained with experimental animals.

● statistically significant differences ($p < 0,05$) between ^3H : ^{14}C ratio means of equivalent molecular weight proteins in the two series of experiments.

○ non significant differences ($p > 0,05$) between ^3H : ^{14}C ratio means of equivalent molecular weight proteins in the two series of experiments.

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