bral ventricle enlargement[2]. In the present study, the embryonic development of the SCO in affected rats could not be investigated, since the incidence of congenital hydrocephalus in CWS/Idr rats is very low, and cranial enlargement in affected rats is not clearly recognizable in prenatal life. However, dysplastic SCO was always observed in hydrocephalic CWS/Idr rats during postnatal day 1 and day 20, indicating that the hydrocephalic state in CWS/Idr rats may not induce degeneration of pre-existing dysplastic SCO. Thus, dysplasia of SCO in congenital hydrocephalic CWS/Idr rats may be a primary defect and closely related to the cause of congenital hydrocephalus.

Numerous reports have been published in the literature on the functional significance of the SCO and its secretory product, Reissner's fiber, but none have been confirmed by direct evidence[3]. The present results and also our previous work showing SCO dysplasia in congenital hydrocephalus[2,3] suggest that the SCO and Reissner's fiber may be involved in the regulation of the intraventricular flow of cerebrospinal fluid. The precise mechanism awaits further elucidation.

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Selective effects of PHA on rat brush border hydrolases along the crypt-villus axis

J.-M. Rouanet, J. Lafont*, J. L. Zambonino-Infante and P. Besançon

Laboratoire de Physiologie de la Nutrition, Centre de Génie et Technologie Alimentaires, F-34060 Montpellier (France)
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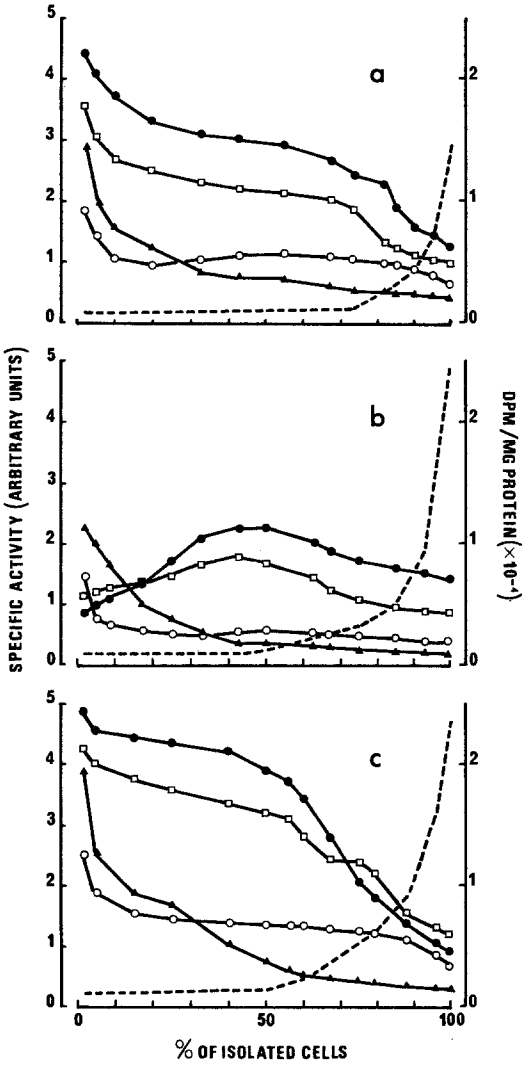
Summary. The mechanism of the toxicity of lectin from *Phaseolus vulgaris* seeds has been investigated on rat enterocytes. Cell isolation procedures showed a selectivity in the loss of brush border hydrolases; this indicated that the microvilli blebbing was not the only mechanism of action of lectins on rat enterocytes.
Key words. PHA; lectins; *Phaseolus vulgaris;* intestinal hydrolases; enterocytes; brush border membrane.

The toxicity of orally ingested lectins is primed by damage to the small intestine. Many studies have been devoted to the analysis of this cellular toxicity[1-4]. They concluded essentially that there is a microvillar fragility induced by lectins; this appeared as blebbing of brush border membranes leading to a decrease in hydrolase activities. The objective of the present study was to investigate this mechanism as a function of the age of enterocytes, i.e. along the crypt-villus axis.

Material and methods. Phaseolus vulgaris bean agglutinin (PHA) was purified from seeds (var. Lingot blanc) by affinity chromatography on fetuin-Sepharose CL-4B[5]. Growing male Sprague-Dawley rats, weighing 82 ± 1 g, were divided into 3 equal groups of 6 animals, according to the average body weight. They were kept in individual metabolic cages at constant temperature and given water ad libitum. The control diet of which the detailed composition was previously reported[6] contained 67% carbohydrates, 10% protein and 4% fat. The experiment was conducted as published elsewhere[7]. Group 1 was fed the diet ad libitum; group 2 received the same diet plus 0.25% pure PHA; group 3 was pair-fed with group 2, but received the control diet. All diets were isonitrogenous. Methyl-^{3}H-thymidine was used as an indicator for cell proliferation. For this purpose 50 µCi/rat of methyl-^{3}H-thymidine (77 Ci/mmol, NEN, Boston) were injected i.p. 1 h before sacrifice. After 17 days, rats were killed by cervical fracture. The entire duodenum, extending to the ligament of Treitz, was removed, washed with ice-cold saline, and everted. Duodenal samples were pooled for each group. Enterocytes were isolated by a sequential cell release technique according to Weiser[8] and modified by Raul et al.[9]. Samples were incubated in series successively for 10 min at 37 °C in phosphate-buffered saline (no Ca^{++}, Mg^{++}), EDTA 1.5 mM, dithiothreitol 0.5 mM, under agitation in a water bath shaker. After each incubation, the medium containing the released enterocytes was spun at 900 × g for 10 min at 4 °C. The pelleted cells were resuspended in cold NaCl 0.15 M, homogenized in a Dounce apparatus and assayed for alkaline phosphatase according to Bessey et al.[10]. Disaccharidases were measured by the method of Dahlqvist et al.[11] and aminopeptidase according to Maroux et al.[12] using L-alanine-p-nitroanilide as substrate. Specific activities were calculated as µmol of substrate hydrolyzed per min at 37 °C; nevertheless, they were plotted as arbitrary units, in order to avoid a graph littered with many ordinates. Proteins were estimated according to Lowry et al.[13]. For radioactivity determination, one aliquot of each cell fraction was applied to a 2.5 cm glass-fiber filter (GF/C, Watman). The filters were extensively rinsed with 5% (v/v) trichloroacetic acid, then dried. The radioactivity of each filter was determined in a liquid scintillation spectrometer (Tri-Carb, Packard).

Results. The figure shows the gradient of specific activity of enzymes along the crypt-villus axis of the duodenum of rats fed either the control diet or the PHA diet. The 100% of cells isolated corresponds to the sum of the fractions expressed as protein.

Group 1, which was fed the control diet, displayed the usual gradient of enzyme activity. In the group fed the PHA diet (fig., b), a different pattern of activity for each enzyme, particularly for maltase and to a lesser extent for aminopeptidase, was observed at the top of the villus and in the midvillus area. On the other hand ^{3}H-thymidine incorporation increased in the crypt cell compartment when compared to the group 1 (multiplying factor: 1.6). A more complex phenomenon was observed for pair-fed animals (fig., c) where enzyme activities are clearly higher than those of the control



Enzymatic gradient in intestinal cells isolated along the crypt-villus axis in control rats (a), PHA-fed rats (b) and in pair-fed rats (c). The 100% of cells isolated corresponds to the sum of the fractions expressed as protein, with 0% representing the top-most part of the villus and 100% the bottom-most part of the crypt. The percentage of cells isolated in each successive fraction was determined by the proportion of cell protein isolated in a given fraction. Activities assayed were: aminopeptidase (□), lactase (○), maltase (●) and alkaline phosphatase (▲); the dotted line (– – –) represents Me-^{3}H-thymidine incorporation. Specific activities, first calculated as μmol/min/mg protein, are plotted as arbitrary units in order to avoid a graph littered with too many ordinates. Contribution of non-lactase activity (acid β-galactosidase) was elsewhere assayed according to Koldovsky et al.[14] and found to be minimal (on average, 11.5% of the whole β-galactosidase activity).

group. As seen in the group 2, figure c shows a similar increase (ratio: 1.6) for ^{3}H-thymidine incorporation when compared to the control group.

Discussion. These results corroborated those previously reported[7] on the whole intestinal mucosa. Similarly, we find that a decrease in specific activity is triggered off by dietary lectins (PHA), whereas it is increased among pair-fed animals. We also observed an increased ^{3}H-thymidine incorporation in these two groups compared to controls. It should be pointed out that specific activities and Me-^{3}H-thymidine incorporation patterns are qualitatively similar in control and pair-fed groups; on the contrary, it is noteworthy that the lectin-fed group patterns are quite different from the control ones. Furthermore, the mechanism of action of several lectins on enterocytes has already been reported[1–3]; PHA acts in the same way[4] which implies a loss of vesicular material from the brush border membrane. At a submicroscopic level, this membrane displayed a typical composition which is a function of the age of the enterocytes. Thus at a particular level of the villus, the loss of the membrane material should induce a similar relative decrease for any enzyme activity; this does not happen here, especially in the top villus area. In this zone, the activities of the hydrolases were decreased to different extents since the aminopeptidase, lactase and chiefly maltase activities decreased heavily. These results are inconsistent with a unique mode of membrane erosion by lectins, based only on the formation of small vesicles from microvilli. One may suppose two opposite mechanisms for the lectin effect:

– either an unknown mode of selective membrane erosion acting at the molecular level,

– or an indirect mechanism which could modulate a hypothetical synthesis of membrane hydrolases within the mature enterocyte.

It would now be of interest to determine which of these is present.

* Present address: Prabiotec S.A., 647 Route d'Alès, F-30380 Saint-Christol les Alès, France.

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