to presence of Mn^{2+} and Ba^{2+}. The previous conclusions that i_f contributes to pacemaker activity in the SA node, and that ACh can exert its negative chronotropic effect through inhibition of i_f remain unaltered.

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Effect of pancreatic secretions upon ileal disaccharidase activities of neonatal miniature pigs

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Summary. Mechanisms by which pancreatic secretions influence disaccharidase activities in the distal small intestine have been investigated in 1-week-old miniature pigs. Using a combination of biochemical, cytochemical and morphological techniques it has been found that the decrease in lactase specific activity is due solely to a reduction in villus surface area. By contrast, the increased sucrase-isomaltase activities, which occur despite the reduction in villus surface area, are due entirely to increased enzyme expression during enterocyte differentiation.
Key words. Disaccharidases; pancreatic proteases; cellular and structural adaptation; small intestine.

Expression of intestinal disaccharidases [1] and the secretion of pancreatic enzymes [2] in the pig are age dependent. The latter have been shown to affect disaccharidase activities of the adult small intestine through proteolysis of the enzymes concerned [3]. There is, on the other hand, evidence to suggest that pancreatic secretions also exert trophic effects in the small intestine [4] and this could increase the villus capacity to hydrolyze sugars. Changes in intestinal activities of disaccharidases along the length of the tract have recently been partitioned into components dependent upon enterocyte development and villus structure but it is not known whether these changes are due to local effects produced by pancreatic secretions [5]. The purpose of the present study was to estimate the relative importance of these two separate influences on ileal disaccharidase activities in the neonatal intestine after the elimination of pancreatic secretions from the lumen by ligation of the pancreatic duct.

Methods

Seven pairs of 1-week-old Hanford miniature pigs (Charles River Breeding Laboratories, Wilmington, MA) underwent either sham (S) or pancreatic duct ligation (PDL) operations. This procedure does not interfere with the bile duct since these ducts are discrete entities in the pig. All pigs received a prophylactic dose of 50 mg cefotaxime/kg b. wt prior to surgery. Pigs were anesthetized by i.m. injections of 0.03 mg fentanyl/kg followed 15 min later by 8 mg ketamine/kg b. wt. The abdomen was opened and the pancreatic duct ligated using 3-0 suture. Sham-operated animals underwent identical surgery except that ligation of the duct was omitted. The abdomen was then irrigated with 10 ml of sterile normal saline and closed. After the pigs had recovered they were returned to the same cage and maintained on a standard swine weaning formula (Soweena, Merrick's, Union Center, WI). All pigs received formula prepared from the same batch and were allowed free access to food which was provided fresh three times daily. Throughout the study, it was noted that all animals were feeding. Percentage composition of dry formula was 25% protein, 10% fat, 50% lactose and the remainder being inorganic solid and vitamins. At 14 days of age the animals were killed by a lethal i.m. injection of 0.05 mg fentanyl and 11.0 mg ketamine/kg b. wt, and samples of the distal small intestine were taken. Disaccharidase activities were determined both biochemically and cytochemically as described previously [1]. Villus surface area was determined

from micro-dissected Carnoy's fixed tissue. Data give the means ± SEM, significance of differences between means was determined by a paired Student's t-test.

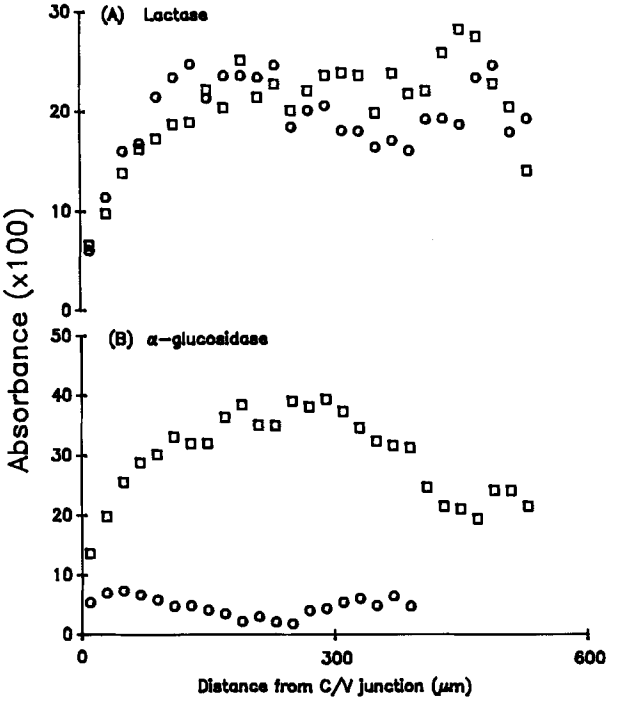
Results

PDL caused a significant 42% decrease in ileal villus surface area (0.144 ± 0.18 vs 0.084 ± 0.008 mm^2; $p < 0.05$). Data for the specific activities of intestinal disaccharidases are summarized in the table. Ligation of the pancreatic duct produced a 58% decrease in the specific activity of lactase, and a 6.3-fold increase in the combined sucrase and isomaltase specific activity. Moreover, the ratio of sucrase to isomaltase activity was close to unity after the removal of proteolytic enzymes. This suggests a greater susceptibility of the sucrase over the isomaltase moiety to proteolysis and is in agreement with Goda and Koldovsky[6].

Specific activities of intestinal disaccharidases determined in mucosal scrapings of distal small intestine

Enzyme	Sham (μmole/g protein/min)	PDL
Lactase	67.5 ± 5.6	27.7 ± 6.2[a]
Sucrase (S)	5.8 ± 3.3	61.6 ± 4.2[a]
Isomaltase (I)	12.0 ± 3.7	57.2 ± 3.7[a]
S/I ratio	0.48	1.08

[a] $p < 0.001$.



Development profile for enterocyte expression of (A) lactase and (B) α-glucosidase, combined assay for sucrase, isomaltase and maltase (absorbance (× 100), along the crypt villus (c/v) axis for ileal villi from either sham (○) or PDL (□) pigs.

Cytochemical analysis for lactase and the α-glucosidases sucrase, isomaltase and maltase are shown in the figure. No difference between groups with respect to the average maximal activity determined between 100 and 300 μm from the crypt/villus (C/V) junction (22.2 ± 3.7 vs 21.8 ± 4.4, Ab (× 100); S vs PDL) and the profile of expression along the crypt/villus axis for lactase were observed. By contrast, PDL caused a significant ($p < 0.01$) 10.5-fold increase from 3.4 ± 0.84 to 35.8 ± 6.1 (Ab(× 100); S vs PDL) in the average maximal α-glucosidases activity between 100 and 300 μm from the C/V junction. This increase was also associated with a changed development profile along the crypt/villus axis.

Discussion

Hauer-Jensen et al.[7] demonstrated that occluding the pancreatic duct in adult rats produced increased specific activities of sucrase and isomaltase in jejunal and ileal segments. However, lactase specific activity either remained constant or was increased by this procedure. This latter observation has also been observed in humans with pancreatic insufficiency[8]. The present results on the effects of PDL decreasing neonatal pig intestinal lactase specific activity is thus an effect not seen in the studies of Hauer-Jansen et al.[7] and Seetharam et al.[8]. However, in these earlier studies either no changes in mucosal mass and thickness were noted or no assessment of structural parameters was made. From the lactase cytochemistry it is clear that the cellular expression of this enzyme in the neonatal pig ileum is not influenced by pancreatic secretions. The reduction in lactase specific activity is accounted for solely by a reduced villus surface area. Removal of a trophic agent present in the pancreatic juice may account for this effect directly[4].

By contrast, the effect of PDL upon neonatal pig intestine sucrase and isomaltase-specific activities agrees with earlier findings. Increased disaccharidase activity in the neonate is probably due to the removal of the proteolytic enzymes as suggested previously for adult animals[6], a conclusion reinforced by the finding that sucrase, an easily degraded enzyme is affected more than isomaltase by PDL. Cytochemical determination of α-glucosidases showed that increase in specific activity was mediated by a raised cellular expression. However, the increase of the sucrase-isomaltase specific activities in PDL pigs was only 6.3-fold compared with a 10.5-fold increase in the plateau activity for these enzymes determined by cytochemistry. This discrepancy is accounted for by the 42% reduction in villus surface area. It is concluded that ileal villus lactase and α-glucosidase activities are selectively modified by pancreatic secretions in the neonatal pig by either a structural component alone or in combination with altered enterocyte expression. These results emphasize the caution required when assessing mechanisms of intestinal adaptation by relating enzyme activities to homogenate protein alone.

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Age-related lipid peroxidation in the digestive gland of mussels: The role of the antioxidant defence systems

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Summary. The main cellular defence systems against free radical-mediated oxidative stress are significantly reduced in the digestive gland of aged (> 10 years old) compared to younger (2–4 years old) mussels (*Mytilus edulis* L.). Moreover, the concentration of lipid peroxidation products (malondialdehyde) is increased in the same age group with respect to younger animals. The obtained data indicate that an impairment of the antioxidant defence systems would render the older animals more susceptible to peroxidative stress, thus supporting the general significance of the free radical theory of aging.
Key words. Aging; mussels; free radicals; antioxidant systems; lipid peroxidation.

Among the various concepts of biological aging[1], the free radical theory[2] has aroused particular interest because of its general significance and applicability. According to Harman, the structural and metabolic changes which occur in aging cells are mainly related to free radical reactions and, among these, to the peroxidation of membrane lipids.

Free radicals generally arise from: i) exposure of cells to ionizing radiation; ii) non-enzymatic reactions of oxygen with metal cations and organic compounds; iii) enzymatic reactions involved in the electron transport of the respiratory chain, in phagocytosis and in the activity of the cyt-P450 system. These reactions, in which oxygen is the main source of free radicals, seem to be responsible for the lipid peroxidation as well as for aging processes.

A related concept is that the intracellular redox state becomes more pro-oxidizing, and that aging organisms are more susceptible to oxidative stress. Indeed, recent studies indicate that cellular aging may be related to a decreased capacity to inactivate free radicals, due to a reduced activity of the antioxidant systems involved in protection against peroxidative damage[3–6].

Even though this subject has been studied in different mammalian tissues, information on marine invertebrates, including mussels, is still lacking. These intertidal, sessile, filter-feeding organisms are adapted to, and therefore tolerant of, variations of a wide range of environmental parameters (such as oxygen, temperature, food availability, etc.)[7]. In particular, during tidal exposure they are periodically subjected to either hypoxia or anoxia followed by aerobic recovery. Such conditions are thought to give rise to an enhanced free oxygen radical production in tissues, due to the simultaneously enhanced flux of reducing equivalents and oxygen[8,9]. Therefore, because of the characteristics of their metabolism, mussels seem particularly suited for studies on the role of free radicals in age-related lipid peroxidation. Reduced coenzyme-dependent oxyradical production has been observed in digestive gland microsomes and cytosol[10] of mussels, and it is stimulated by redox cycling quinone[11] and nitroaromatic compounds[12].

Data are presented here on the antioxidant systems and lipid peroxidation in the digestive gland of mussels (*Mytilus edulis* L.) of three different age groups (I age = 2–4 years; II age = 6–8 years; III age = > 10 years). The average life span of this species is about 12 years[7].

In this work, the concentration of both aqueous-phase (GSH) and lipid-soluble (vitamin E, carotenoids) antioxidant compounds, as well as the activity of antioxidant enzymes such as superoxide dismutase (SOD – EC 1.15.1.1.), catalase (EC 1.11.1.6.) and GSH peroxidase (EC 1.11.1.9.) were evaluated. As is already known, naturally-occurring antioxidants can act as reductants or as free radical scavengers, while antioxidant enzymes play a fundamental role in scavenging superoxide anion radicals (superoxide dismutase) and in the metabolism of H_2O_2 and lipid hydroperoxides (catalase, GSH peroxidase)[13–15]. Finally, the determination of the tissue levels of malondialdehyde (MDA) was utilized as an indicator of the lipid peroxidation process.