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Prevention by Mannan and other Sugars of *in Vitro* Damage of Rat Fetal Small Intestine Induced by Cereal Prolamin Peptides Toxic for Human Celiac Intestine[1]

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ABSTRACT. Peptic-tryptic-cotazym and peptic-tryptic digests were obtained, simulating *in vivo* protein digestion, from pure "bread" wheat gliadins and from rye, barley, and oats prolamine and tested on small intestine cultures from fetal rats. When tested at a concentration of 0.1 mg of peptides/ml of culture medium the peptic-tryptic-cotazym and peptic-tryptic digests of gliadin and prolamines were very active in slowing *in vitro* development of fetal rat intestine and in increasing the occurrence and severity of degenerative changes. The ability of some sugars to interfere with inhibition of fetal intestinal morphogenesis induced by these peptides was also tested. Mannan at a concentration of 0.1 mM was effective in allowing intestinal morphogenesis to take place in the presence of prolamine peptic-tryptic-cotazym and prolamine peptic-tryptic digests of the four toxic cereals. Some oligomers of N-acetyl-glucosamine were also effective in blocking the inhibitory effect of "bread" wheat gliadin peptides. These data are compatible with the hypothesis that some sugars may exert a protective effect on the toxic activity of cereal prolamin peptides on the human celiac intestine. (*Pediatr Res* 22: 703–707, 1987)

Abbreviations

PTC, peptic-tryptic-cotazym
PT, peptic-tryptic

Celiac disease is a well-known enteropathy whose symptoms are triggered by the presence in the diet of wheat, barley, rye, or oats. Rice and maize are well tolerated by celiac patients. Falchuk *et al.* (1) have proposed the organ culture of human small intestinal biopsies as an *in vitro* model of celiac disease. Flat intestinal mucosa of celiac patients with active disease showed morphological and biochemical improvement when cultured in a gluten-free medium. However, no improvement occurred when the tissue was cultured in the presence of gliadin fractions or peptides (2–5). In this system, mannan exhibited a protective effect and allowed morphological improvement of intestinal mucosa specimens of patients with active celiac disease also in the presence of wheat gliadin peptides (6).

Prolamin peptides from all the toxic cereals in celiac disease as well as some peptides prepared from A-gliadin (7), which are toxic *in vitro* for human atrophic small intestinal mucosa (8), are all able to agglutinate undifferentiated K 562 (S) cells (9, 10). However, prolamin peptides from rice and maize (10) and the peptides of A-gliadin which are not able to damage the cultured celiac mucosa (7), do not agglutinate these cells. Mannan and oligomers of N-acetyl-glucosamine, N, N′-diacetylchitobiose and N, N′, N″-triacetylchitoriose were very effective in inhibiting the agglutinating activity of prolamin peptides obtained from bread wheat, barley, rye, and oats as well as that of the active A-gliadin peptides.

Gliadin peptides are also able to reversibly inhibit the development and morphogenesis of the immature small intestine from 17-day-old rat fetus, whereas they have no effect on the *in vitro* cultured, differentiated jejunum from 21-day-old rat fetus (11, 12). Moreover, prolamin peptides from rye, oats, barley, and sorghum show in this *in vitro* system an activity similar to bread wheat gliadin peptides, whereas prolamin peptides from rice and maize are inactive (13).

Herein we describe the results of further investigations undertaken to confirm the hypothesis that the *in vitro* toxic effects induced by bread wheat, barley, rye, and oats on rat fetus intestinal mucosa may be prevented by mannan and other sugars.

MATERIALS AND METHODS

Pure bread wheat (*Triticum aestivum*, var. S. Pastore), rize (*Oryza sativa* var. Roma), maize (*Zea mays* var. B 73), rye (*Secale cereale* var. 500 2Gi), barley (*Hordeum vulgare* var. Arma), sorghum (*Sorghum vulgare* var. NK 120), and oats (*Avena sativa* var. Astra) were kindly supplied by the Istituto Sperimentale per la Cerealicoltura, Rome, Italy. Prolamin fractions were extracted from the above mentioned cereals with an experimental procedure identical to that described by Auricchio *et al.* (13). PTC digests of the prolamin fractions were prepared as described by Auricchio *et al.* (13). PT digests of the purified prolamin fractions were prepared following a two-step procedure reported by de Ritis *et al.* (12); in order to inactivate the proteolytic enzymes, PT digests were submitted to heating at 100° C for 30 min.

Mannan, a mannose homopolysaccharide, was obtained from *Saccharomyces cerevisiae* (Sigma Chemical Company, St. Louis,

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MO). N, N′-diacetylchitobiose and N, N′, N″-triacetylchitotriose were prepared from Sigma Chemical Company.

For the *in vitro* culture of fetal jejunum, pregnant Wistar rats were anesthetized with ether and 17-day-old fetuses were removed at laparotomy. Fetal jejunum segments were isolated and cultured *in vitro* for 48 h in a serum-free medium according to the method described by de Ritis *et al.* (14). Jejunal segments from the same fetus were cultured in the absence and in the presence of the above mentioned digests. All the peptide mixtures were sterilized before addition to the incubation medium by filtration through 0.22 mμ Millipore filters. Differentiation of fetal rat jejunum was followed morphologically by light microscopy as reported by de Ritis *et al.* (14) without knowledge of the culture conditions. A morphologic assessment of histologic changes during organ culture of 17-day-old fetus intestine was performed on the more external part of the tissue where the epithelium showed more clearly developmental changes in the absence of gliadin peptides. In this part of the tissue specimen we constantly observed well-formed villi that do not develop in the more internal part of the intestinal segment probably as the consequence of the more difficult penetration of the tissue by the culture medium. The first five sections were cut off and the following 10–20 sections were evaluated. No appreciable variations in the developmental pattern of intestinal mucosa were observed among these histologic sections.

RESULTS

Before culture, jejunal mucosa from forty five 17-day-old fetuses did not show any villus, and only undifferentiated cuboidal stratified epithelia lining the lumen were present (Figs. 1*A* and 2*A*); no goblet cells could be detected in any uncultured fetuses (Tables 1 and 2). After 48 h of *in vitro* culture in the absence of peptides (Tables 1 and 2), formed villi were present in 33 fetal jejunum segments (Figs. 1*B* and 2*B*) and rudimentary villi were observed in jejunal segments from 10 other fetuses; villi did not develop in only two cultured fetuses. In all 45 cultured fetuses the epithelial lining consisted exclusively of simple columnar epithelium and goblet cells were present in 21 fetuses. Slight patchy degenerative changes were observed only in a few cultured jejunal segments from one fetus. These findings confirm previous results by de Ritis *et al.* (12) and Auricchio *et al.* (11, 13) showing that differentiation and maturation of small intestinal mucosa from rat fetuses take place *in vitro* in a way comparable to *in vivo* conditions.

When tested at a concentration of 0.1 mg of peptides/ml of culture medium the prolamine-PTC and prolamine-PT digest from bread wheat, rye, oats, and barley were very active in slowing down *in vitro* development of fetal rat intestine and in increasing occurrence and severity of degenerative changes (Tables 1 and 2). After 48 h culture in the presence of PTC or PT of prolamines digests from the four toxic cereals, well-developed villi were absent in most cultures (Figs. 1*C* and 2*C*) and rudimentary villi were present only in a few cultures. Furthermore goblet cells were present only in one culture with prolamin peptides from oats (Tables 1 and 2). Available findings do not indicate any significant difference between activities of PT- or PTC-digests of prolamines from each cereal in slowing down *in vitro* development of fetal rat intestine.

We also tested the ability of some sugars to interfere with inhibition of fetal intestinal morphogenesis induced by peptides of bread wheat, barley, rye, and oat prolamines. The data summarized in Tables 1 and 2 show that mannan at a concentration of 0.1 mM was largely effective in allowing intestinal morphogenesis to take place in the presence of PTC- or PT-digests of prolamines from any of the four toxic cereals (Fig 1*D*). Mannan did not show any effect on the *in vitro* development of the fetal rat intestine in the absence of toxic prolamine peptides. N, N′-diacetylchitobiose and N, N′, N″-triacetylchitotriose at a concentration as low as 3 mM were both effective (Fig. 2*D*) and completely removed the inhibitory effect of bread wheat gliadin peptides. Mannose and N-acetylglucosamine were ineffective even when tested at concentration as high as 50 and 200 mM, respectively.

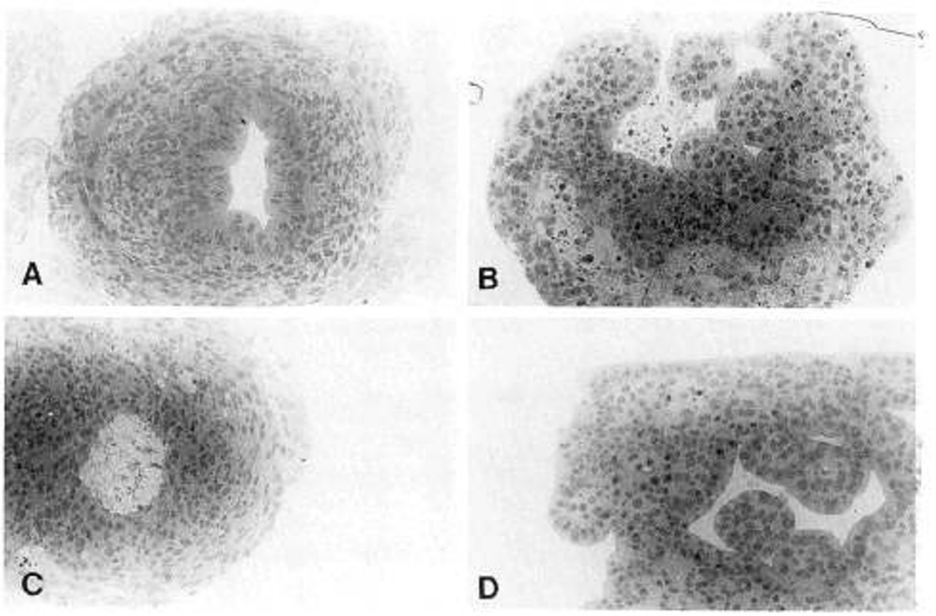

Fig. 1. Effect of PT digest of prolamines from barley and protective effect of mannan on *in vitro* development and morphogenesis of jejunum from 17-day-old rat fetus. *A*, jejunal mucosa before culture. The epithelium consists of stratified undifferentiated cells and no villi are present. *B*, jejunal mucosa after 48 h culture and *D*, jejunal mucosa after 48-h culture in the presence of the PT prolamin digest (0.1 mg/ml) from barley and mannan (0.1 mM): well-developed villi and an epithelium consisting of single layer of relatively differentiated cells are evident. *C*, jejunal mucosa after 48-h culture in the presence of the prolamin digest (0.1 mg/ml) from barley. No villi developed on the surface which is lined by cuboidal and columnar epithelial cells. Degenerative changes are evident.

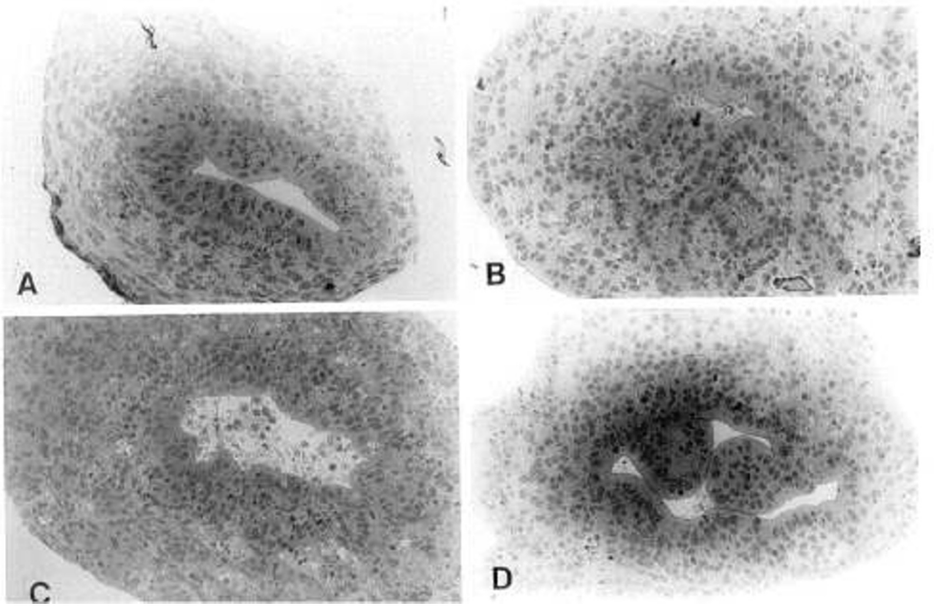

Fig. 2. Effect of PTC digest of gliadins from bread wheat (var. S. Pastore) and protective effect of N, N′, N″-triacetylchitotriose on *in vitro* development and morphogenesis of jejunum from 17-day-old rat fetus. *A*, jejunal mucosa before culture. The epithelium consists of stratified undifferentiated cells and no villi are present. *B*, jejunal mucosa after 48-h culture and *D*, jejunal mucosa after 48-h culture in the presence of PTC gliadin digest (0.1 mg/ml) of bread wheat (var. S. Pastore) and N, N′, N″-triacetylchitotriose (3 mM): well-developed villi are present which are lined by a single layer of differentiated columnar epithelial cells. *C*, jejunal mucosa after 48-h culture in the presence of PTC gliadin digest (0.1 mg/ml) of bread wheat (var. S. Pastore). Villi are absent from the mucosal surface which is lined primarily by columnar epithelial cells. Degenerative changes are evident.

Table 1. *Effect of mannan and Other Sugars On Jejunum from 17-day-old rat fetus cultured in vitro in presence of PTC and PT gliadin peptides from hexaploid wheat*

		Villi			Epithelium				Degenerative changes†	
						Monostratified				
Sample*		Absent	Rudimentary	Present	Stratified	Cuboidal	Columnar	Goblet cells	±	++
Uncultured 17-day-old fetuses	(32)	32			32					
After 48-h culture										
Without peptides[a]	(32)	2	8	22			32	15		
Gliadin peptides from hexaploid wheat 0.1 mg/ml‡§[b]	(36)	31	5			4	32		7	4
Gliadin peptides from hexaploid wheat 0.1 mg/ml + mannan 0.1 mM‖¶[c]	(37)	3	15	19		1	36	16	9	
Gliadin peptides from hexaploid wheat 0.1 mg/ml + diacetylchitobiose 3 mM**	(3)			3			3	1	1	
Gliadin peptides from hexaploid wheat 0.1 mg/ml + triacetylchitotriose 3 mM††	(3)			3			3	2	1	

* Number of fetuses examined or of fetal jejuna cultured is shown in parentheses.
† ±, slight degenerative changes; ++, large necrotic areas.
‡ Six cultures at 0.5 mg/ml.
§ Fifteen cultures with PT gliadin peptides.
‖ Eight cultures at 0.5 mg/ml.
¶ Twenty cultures with PT gliadin peptides.
** One culture at 1 mg/ml.
†† One culture at 2 mg/ml.
Frequency distribution analysis by χ^2. The two groups rudimentary and present are pooled for the analysis.
[a] Difference from the value before culture, $\chi^2 = 49.05$, $p < 0.001$.
[b] Difference between the values after culture with and without peptides, $\chi^2 = 40.11$, $p < 0.001$.
[c] Difference between the values after culture with gliadin peptides and gliadin peptides with mannan, $\chi^2 = 41.35$, $p < 0.001$.

Table 2. *Effect of mannan on jejunum from 17-day-old fetus cultured in vitro in presence of PT prolamin peptides from different cereals*

Sample*		Villi			Epithelium			Goblet cells	Degenerative changes†	
						Monostratified				
		Absent	Rudimentary	Present	Stratified	Cuboidal	Columnar		±	++
Uncultured 17-day-old fetuses	(13)	13			13					
After 48-h culture										
Without peptides[a]	(13)		2	11			13	6	1	
Prolamin peptides from barley 0.1 mg/ml[b]	(11)	8	3			2	9	1	7	
Prolamin peptides from barley 0.1 mg/ml + mannan 0.1 mM‡[c]	(15)	2	2	11			15	7	5	2
Prolamin peptides from rye 0.1 mg/ml[b]	(12)	9	3			4	8		5	5
Prolamin peptides from rye 0.1 mg/ml + mannan 0.1 mM[c]	(13)		8	5			13	4	8	1
Prolamin peptides from oat 0.1 mg/ml[b]	(13)	9	3	1		1	12	1	7	2
Prolamin peptides from oat 0.1 mg/ml + mannan 0.1 mM[c]	(12)	2	6	4			12	4	5	2

* Number of fetuses examined or of fetal jejuna cultured is shown in parentheses.

† ±, slight degenerative changes; ++, large necrotic areas.

‡ One culture at 0.5 mg/ml.

Frequency distribution analysis by χ^2. The two groups rudimentary and present are pooled for the analysis.

[a] Difference from the value before culture, $\chi^2 = 15.38$, $p < 0.001$.

[b] Difference between the values after culture without peptides and with prolamin peptides $p < 0.01$: barley ($\chi^2 = 8.15$); rye ($\chi^2 = 9.14$); oat ($\chi^2 = 7.9$).

[c] Difference between the values after culture with prolamin peptides and prolamin peptides with mannan: barley ($\chi^2 = 8.1$, $p < 0.01$); rye ($\chi^2 = 9.14$, $p < 0.01$); oat ($\chi^2 = 5.02$, $p = 0.02$).

DISCUSSION

The mechanism of the toxic action of some cereal proteins on celiac intestine is still unknown. An abnormal immune response to these proteins has been postulated as the basis for the mucosal damage in celiac patients (15, 16). However, evidence for a direct toxic action of gliadin peptides on the small intestinal mucosa in some phases of its development and morphogenesis comes from our previous studies (11–13) and from the data presented herein, showing that the rat fetal intestine culture is an adequate model of screening and investigating cereal peptides which are toxic for celiac small intestinal mucosa. In particular, we have shown that this *in vitro* system responds not only to PTC digests of toxic prolamins fractions (13), but also to PT digests.

We have also demonstrated herein that mannan is able to protect fetal intestinal mucosa from *in vitro* effects of all the toxic prolamin peptides. These findings are likely related to the fact that mannan is also able to prevent K 562 (S) cell agglutination induced by gliadin and prolamin peptides from toxic cereals in celiac disease (9, 10) and is able to protect *in vitro* cultured atrophic celiac mucosa from the damaging effect of gliadin peptides (6).

Similar considerations apply to the results obtained with N-acetylglucosamine oligomers, *i.e.* N, N′-diacetylchitobiose and N, N′, N″-triacetylchitotriose. The results indicate the highly significant correlation existing, for all the sugars tested, between inhibition of agglutinating activity on K 562 (S) cells and protection of rat fetal intestine from *in vitro* damaging effect of toxic gliadin peptides.

Such protective effects would be compatible with the hypothesis that the peptides were acting as lectins in these *in vitro* systems. However, conflicting results have been reported on the lectin activity of gliadin peptides (17–20). Whatever the mechanism of sugars might be, the problem arises whether these *in vitro* studies apply to the *in vivo* situation; *i.e.* whether a similar protective effect might be observed *in vivo* on the celiac small intestine exposed to the toxic cereals after opportune administration of adequate amounts of sugars.

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