Methanol production from the degradation of pectin by human colonic bacteria[1-3]

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ABSTRACT When ingested, pectin can lower serum cholesterol levels in humans. Pectin is degraded by fecal bacteria in the colon. We examined the release of methanol (MeOH) by this degradation. A 0.2% glucose (2 g/L) mixture was used as the control medium. A pure culture of pectinolytic *Erwinia carotovora* was the control bacterium. The chief substrates were, in set 1, 0.2% pectin (2 g/L) and, in set 2, 0.1% glucose (1 g/L) and 0.1% pectin (1 g/L). Cultures of fecal bacteria and *E carotovora* grew for 72 h in each of the solutions. By 72 h the fecal flora culture in set 1 cleaved 30% of the possible methoxyl groups on pectin. The fecal flora in set 2 cleaved 90.7% of all possible methoxyl groups. Balance studies suggest that all of the free MeOH comes from methoxyl groups on pectin. This study demonstrates that fecal bacteria are capable of degrading pectin to release MeOH. *Am J Clin Nutr* 1988; 47:848–51.

KEY WORDS Pectin, methanol, colon, bacteria

Introduction

Pectins are a group of heterogeneous polysaccharides with a high molecular weight (1). They can be found in the intercellular regions and cell walls of most fruits and vegetables, with the greatest abundance in limes, lemons, grapefruit, and oranges (2). Pectin is composed of D-anhydrogalacturonic acid units linked through α (1 → 4) glycosidic bonds, forming a polygalacturonic acid with some of the carboxyl groups esterified with methanol (MeOH) (2). Variations in methoxyl content exist among different plant pectins. Over 50% of the carboxyl groups of high-methoxyl or high-ester pectins are esterified, which corresponds to a methoxyl content of 7–10 g/100 g pectin (1). This degree of methoxylation is commonly present in citrus pectin.

When pectin is used as a dietary supplement it lowers serum cholesterol levels in humans as well as in several other animal species (3–7). However, if the diet is supplemented with high concentrations of pectin, it is possible that bacteria from human feces will degrade pectin, releasing the methoxyl groups as free MeOH into the lumen of the large intestine. It has already been demonstrated that human fecal bacteria are capable of degrading dietary fiber such as cellulose (8–9). This study examines the ability of normal colonic bacteria to degrade pectin and release free MeOH.

Materials and methods

Microbial cultures and medium

A sample of feces from the descending colon was obtained for culture from a patient undergoing routine diagnostic colonoscopy. The patient's diagnosis was irritable colon syndrome and no underlying pathology was demonstrated; the subject was not on antibiotics. The composition of the medium that was prepared for use as the basic substrate was as follows per liter of medium: 3 g H_2PO_4, 6 g Na_2HPO_4, 2 g NH_4Cl, 3 g NaCl, 2 mL 1 mol/L $MgSO_4$, 0.5 g $FeSO_4$, and 1 mL 0.5 mol/L $CaCl_2$. To prepare the inoculum, 5 mL of the medium was placed in each of two sterilized test tubes and to each was added 0.01 mL of liquid fecal aspirate. The cultures were incubated at 37 °C for 36 h to allow for adequate growth. The samples were then centrifuged at 7900 × *g* for 20 min (Surval RC-5, Dupont Co, Wilmington, DE). The supernatant was decanted and the pellet was washed with 5 mL of 0.9% sterilized saline (9 g NaCl/L). This procedure was repeated twice to remove any lytic enzymes that might have been present in the initial sample. After the last washing, 5 mL of 0.9% sterilized saline (9 g NaCl/L) was added to each culture tube.

A pure culture of *E carotovora* was used as the source of pectinolytic bacteria. It was grown in the medium with 0.1% glucose (1 g/L) at 37 °C for 36 h, centrifuged, and washed as stated above.

Substrates and culture conditions

For each test tube the basal medium remained the same while the substrate in the medium was varied. The substrates

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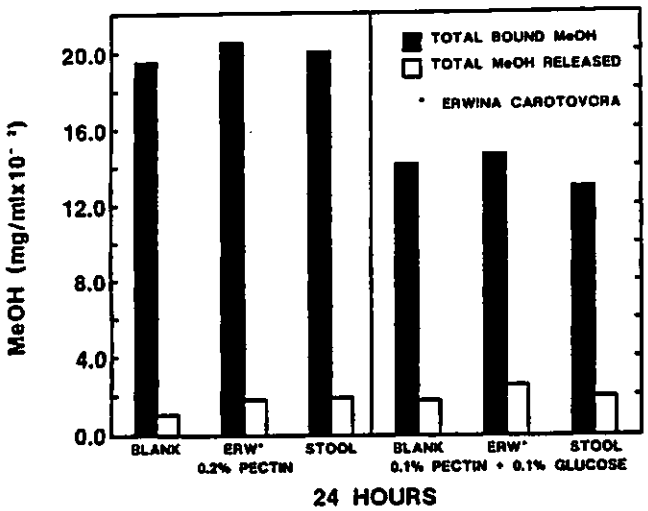

FIG 1. Total free MeOH and total bound MeOH at 24 h.

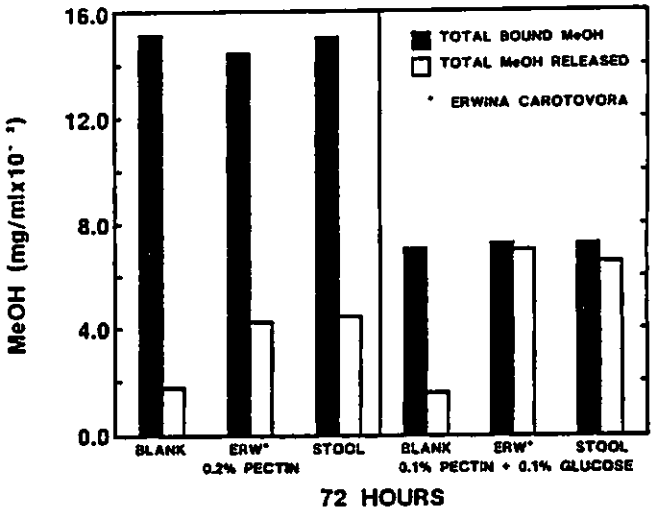

FIG 2. Total free MeOH and total bound MeOH at 72 h.

were 2 g/L glucose, 2 g/L pectin, and 1 g/L each of glucose and pectin. (The pectin was rapid-set orange, 11% esterified lot #A8142 (Sunkist Growers, Inc, Ontario, CA). The medium containing glucose as the sole energy substrate was the control medium for *E cartovora* and the fecal culture. There were six test tubes per substrate with 5.0 mL of substrate and medium in each test tube. For each group two test tubes contained no organisms (blanks), two contained 0.01 mL from the fecal flora inoculum; and two contained 0.01 mL from the *E carotovora* inoculum. One tube from each pair was incubated for 24 h and the other for 72 h at 37 °C. At the end of each set's growth time the vials were centrifuged at 7900 × *g* for 20 min, and two 1.0-mL aliquots of supernatant were removed and separated from each sample. To the first aliquot was added 0.025 mL of 2 mol/L HCl to fix all free MeOH in the solution. To the second aliquot was added 0.05 mL of 10 mol/L NaOH to free all bound MeOH into solution. It was therefore possible to quantify the amount of MeOH per sample. A gas-liquid chromatograph (GLC) (5830A, Hewlett-Packard Co, Palo Alto, CA) was used to determine the amount of MeOH in each sample (10).

Results

Figures 1 and 2 compare the amount of enzymatically hydrolyzed (free) MeOH with chemically bound methoxyl groups in each sample medium. The amount of free MeOH recorded for each blank represents the MeOH released by simple degradation over time. In the control medium (0.2% glucose [2 g/L]) no amount of MeOH was detected in either culture at any time.

Figure 3 represents the amount of MeOH released by the organisms in each sample at 24 and 72 h. This was calculated by subtracting the total MeOH released by each blank from the total MeOH released by each sample (ie, *E carotovora* and stool). The percentage of free MeOH in each sample is summarized in Table 1.

Discussion

Before 1940 it was commonly believed that pectin was a digestible carbohydrate, hydrolyzed and utilized in the animal. Kertesz (11), who incidentally was the first investigator to study the fate of ingested pectin in animals, found no evidence to support that belief. Furthermore, he showed that saliva collected from human subjects, dogs, and cows and jejunal secretions collected from dogs had no effect on pectin after several weeks of incubation. In vivo attempts to digest pectin in the stomach and small intestine of dogs and humans showed no indication of the slightest breakdown of this fiber. However, when pectin was incubated with a sample of human feces, it was rapidly degraded. This suggested to Kertesz that when pectin is ingested, it passes through the upper gastrointestinal tract unaltered, but it is subsequently degraded by the abundant microorganisms in the large intestine.

The ileostomy experiments conducted by Werch and

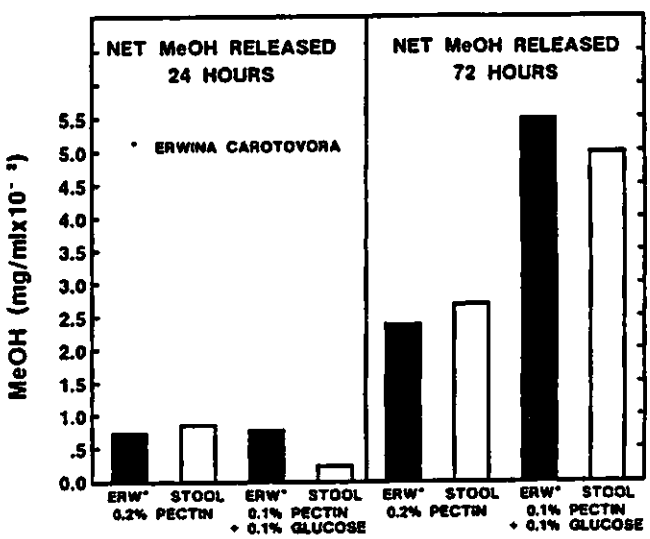

FIG 3. Amount of MeOH released by the organisms in each sample after 24 and 72 h.

TABLE I
Percentage of free methanol in each sample

Cultures	Growth time	Solution*	Free MeOH
	h		%
Blank	24	1	5.56
E carotovora	24	1	8.92
Stool	24	1	9.77
Blank	24	2	12.55
E carotovora	24	2	17.54
Stool	24	2	15.65
Blank	72	1	11.98
E carotovora	72	1	29.55
Stool	72	1	30.03
Blank	72	2	23.50
E carotovora	72	2	97.69
Stool	72	2	90.71

* Solution 1 = 0.2% pectin (2 g/L) solution; solution 2 = 0.1% glucose and 0.1% pectin (1 g/L each).

Ivy in 1941 (12) helped to confirm the findings of Kertesz. Similar quantities of pectin in a mixed diet and a diet of pectin alone were fed to human subjects and dogs with ileostomies. The recovery of completely intact pectin from the ileostomies ranged from 84 to 89% in dogs and from 94 to 97% in humans. By contrast, when the same diets of pectin were fed to normal dogs and normal human subjects, examination of the feces showed that 90% of the pectin had disappeared, demonstrating that the breakdown of pectin occurs chiefly in the colon and not in the upper gastrointestinal tract.

Werch and Ivy (12) also observed that when pectin was fed with a mixed diet for 7 d, 90% of the pectin was degraded by canine and human feces. When pectin alone was fed to both dogs and human subjects, an average of only 50% was degraded. Also, if dogs or humans on pectin alone defecated frequently (1 bowel movement/d), 75–96% of the pectin could be recovered in the feces. However, if the subjects defecated only once during the 7 d, only 2–30% of the pectin was recovered. The results showed that the degradation of pectin was greater on the mixed diet than when given alone and was also greater when pectin was retained for longer periods of time.

In 1942 Werch et al (13) isolated the fecal organisms that were responsible for the destruction of pectin. By collecting feces from dogs fed a diet solely of pectin, they were able to isolate several groups of pectinolytic bacteria. The more active organisms belong to the groups *Aerobacillus, Lactobacillus, Micrococcus,* and *Enterococcus.* In addition, these organisms were found to work synergistically on pectin.

That same year Werch et al (13) isolated some of the end products of pectin digested by the bacteria mentioned above. The chief products found in the degradation of pectin were formic and acetic acid but no galacturonic acid was recovered. Werch assumed that if pectin was degraded to formic and acetic acids it might also be degraded to substances such as acetone and MeOH. However, until now no one has been able to isolate MeOH as an end product of pectin digestion by human fecal bacteria.

Our experiment demonstrates that human fecal bacteria over a 3-d period are capable of releasing MeOH from pectin. The amount of MeOH produced in each vial is related to two factors: the amount of time necessary for the bacteria to replicate and utilize the pectin, and the number of organisms in each solution.

Figure 1 shows a small increase in MeOH concentration in the inoculated samples compared with the blank samples. The stool culture in the pectin medium showed a 79.8% increase in MeOH. This means that 9.77% of available methoxyl groups were cleaved from the pectin. At 72 h (Fig 2) a much larger degree of MeOH production was observed. Stool culture in the pectin solution showed a 148% increase in MeOH over the blank. The total portion of methoxyl groups released during this time period for this solution was 30% (Table 1). There are several possible explanations for why we may see a significantly larger degree of MeOH production at 72 h. To begin with, 72 h may be required before enough organisms can grow to cleave a substantial number of methoxyl groups. It is possible that 72 h is needed before enzyme induction is fully activated. Also, the presence of glucose may help contribute to enzyme induction as well as to the growth of the bacteria.

To increase the growth rate of organisms per vial, a more effective nutrient source, glucose, was added to the pectin. Solutions of 0.1% glucose–0.1% pectin (1 g/L each) were used for 24 and 72 h. The fecal bacteria at 24 h in this solution showed for only a 13.3% increase in MeOH production over the blank. However, at 72 h there was a rapid and almost complete release of all bound MeOH by fecal flora in the glucose-pectin medium. This sample showed a 292% increase in MeOH over the blank. The total portion of methoxyl groups released from the pectin corresponds to 90.7% (Table 1). The release of MeOH by fecal bacteria was much greater in the glucose-pectin solution (90.7%) than in the pectin solution alone (30%). We conclude that the differences between these solutions were due to a greater number of organisms in the glucose-pectin mixture than in the pectin solution. These results suggest that during the first 24 h the microorganisms utilized glucose as the chief nutrient source, sparing the majority of pectin. At 72 h, when most of the glucose had been digested, the chief nutrient source then became pectin. Concomitantly, by the time more organisms were present in the solution, more of the available pectin had been degraded.

The sum of bound and released MeOH for the pectin and glucose-pectin solutions at 24 and 72 h are not proportional to the amount of pectin. It is possible that MeOH acts as a substrate to form another substance such as methane. This substance has been found in the human colon (14). Also, it is possible that some bacteria use MeOH as a carbon source.

Because MeOH is produced as an end product of pec-

tin digestion by fecal flora, is it safe for humans to consume therapeutic quantities of pectin for lowering serum cholesterol. Ingestion of 55–79 g of MeOH is usually fatal (15). However, there have been cases where as little as 4 g of MeOH have caused blindness and where as much as 425 g did not result in any toxic manifestations (15–17). In our study it took 72 h for the fecal bacteria to release 90.7% of the bound methoxyl groups in a 0.1% glucose-0.1% pectin (1 g/L each) solution. This resulted in a total amount of 66 mg of free MeOH/g of pectin. This low concentration of MeOH is far below toxic levels. In addition, previous studies involving human subjects on large doses of pectin (15–50 g/d) showed no reportable toxic side effects (3, 12).

To summarize, we believe that fecal bacteria were capable of releasing MeOH from pectin in our in vitro experiment. This has not yet been studied in vivo. However, if this does occur in the human bowel, we believe that the concentration of MeOH released would be so small that even if totally absorbed it would not be sufficient to cause toxic complications. Studies (eg, 18) involving human subjects on large quantities of pectin (50 g/d) for extended periods of time have not resulted in any MeOH poisoning. Finally, the fate of MeOH produced in the colon from pectin needs further investigation.

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