

The Effects of Antibiotics on Nutrient Digestion

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Summary

Oral antibiotics are essential for the treatment of bacterial infections. A disadvantage of antibiotic therapy is gastrointestinal side effects caused in part by interfering with normal bacterial colonization of the gastrointestinal tract. However, other mechanisms could also be involved. We hypothesized that antibiotics might interfere with nutrient digestion. To test this hypothesis, we employed four tests: the biuret test (for protein digestion), the Lugol's iodine test (for polysaccharide digestion), the Benedict's test (for disaccharide digestion), and the litmus test (for lipid digestion). The *in vitro* effects of three different antibiotics (penicillin, tetracycline, and erythromycin) were assessed semi-quantitatively using these tests. We found that the antibiotics inhibited protein, polysaccharide, and disaccharide digestion, but not lipid digestion. Of the three antibiotics, erythromycin had the highest inhibitory effect. Interference with nutrient digestion could underlie, at least in part, the gastrointestinal side effects seen with antibiotics.

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Introduction

Antibiotics, also called antibacterials, are a mainstay for the treatment of bacterial infections. The most common form of antibiotic administration is oral administration, followed by intravenous and intramuscular routes. The mechanism of action of the antibacterial drugs can involve disruption of the bacterial cell wall, disruption of the cell membrane, or interference with bacterial enzymes [1].

Besides the targeted effects on the pathogens, antibiotics can also have unwanted effects. The most common side effects seen with antibiotic usage are gastrointestinal (GI) effects, including nausea, vomiting, abdominal pain, and diarrhea. The GI side effects occur more commonly with oral antibiotic administration and less often with intravenous or intramuscular administration [2]. One of the leading mechanisms underlying the development of GI side effects during antibiotic treatment is interference with normal bacterial colonization of the GI tract [3].

The main function of the GI tract is to digest nutrient macromolecules into small molecules that can

be absorbed across the intestinal wall into systemic circulation. The GI tract is equipped with a myriad of enzymes that break down the food macromolecules (proteins, polysaccharides, disaccharides, lipids) into small molecules (amino acids, monosaccharides, fatty acids) [4]. Several illnesses caused by the inability to digest certain food products (such as lactose intolerance and celiac disease) manifest with abdominal pain and diarrhea [5].

We hypothesized that the orally administered antibiotics could interfere with the digestion of the main food nutrients (proteins, polysaccharides, disaccharides, lipids). It is possible that some antibiotics might have a bigger impact than others on nutrient digestion, and the nutrients might not be equally affected by a certain antibiotic. In this study, we explored the effects of penicillin, tetracycline, and erythromycin on protein (albumin) digestion, polysaccharide (starch) digestion, disaccharide (sucrose) digestion, and lipid (vegetable oil) digestion.

Results

Protein digestion. To assess protein digestion, we used the biuret test (**Table 1**). The reagent used in the biuret test is a solution of copper sulfate (CuSO_4) and sodium hydroxide (NaOH); the latter component is used to raise the pH of the solution to alkaline levels, and the crucial component is the copper II ion (Cu^{2+}) from the CuSO_4 . Albumin was the protein substrate used to analyze protein digestion. Pepsin (a digestive enzyme) and hydrochloric acid were added for albumin digestion into peptide chains. When peptide bonds are present in an alkaline solution, the Cu^{2+} will form ionic bonds with four nitrogen atoms from the peptide bonds. The resulting complex of Cu^{2+} ions and nitrogen atoms makes

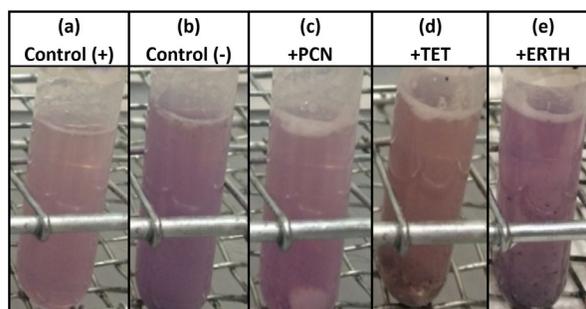


Figure 1: Representative results for the protein digestion experiments. All tubes contain albumin, pepsin, HCl, and Biuret reagent. Negative control tube (b) additionally contains Alka-Seltzer, and tubes c–e contain the indicated antibiotic (PCN, penicillin; TET, tetracycline; ERTH, erythromycin). Note differences in solution color in the tubes containing antibiotics (c–e) relative to the positive (a) and negative (b) control tubes.

Digestion Experiment	Test	Reactants			Colorimetric assessment (estimated % of digestion)					
		Positive control	Negative control	Independent variable	100%	99-75%	74-50%	49-25%	24-1%	0%
Protein digestion	Biuret test	Albumin, Pepsin, HCl, Biuret reagent	Albumin, Pepsin, HCl, Alka-Seltzer, Biuret reagent	Antibiotic with Albumin, Pepsin, HCl, Biuret reagent	Light Pink 	Medium Pink 	Dark Pink 	Light Purple 	Dark Purple 	Purple 
Polysaccharide digestion	Lugol test	Starch, Pancreatin, Lugol reagent	Starch, Lugol reagent	Antibiotic with Starch, Pancreatin, Lugol reagent	Light Yellow-Brown 	Medium Yellow-Brown 	Dark Yellow-Brown 	Brown 	Dark Brown 	Black 
Disaccharide digestion	Benedict test	Sucrose, Invertase, Benedict reagent	Sucrose, Distilled water, Benedict reagent	Antibiotic with Sucrose, Invertase, Benedict reagent	Light Green 	Medium Green 	Dark Green 	Med. Ash-Green 	Dark Ash-Green 	Blue 
Lipid digestion	Litmus test	Vegetable oil, Bile Salt, Pancreatin, Litmus milk	Vegetable oil, Bile Salt, Litmus milk	Antibiotic with Vegetable oil, Bile Salt, Pancreatin, Litmus milk	Dark Magenta-Red 	Medium Magenta-Red 	Light Magenta-Red 	Magenta-Violet 	Dark Magenta-Purple 	Light Magenta-Purple 

Table 1: Summary of digestion experiments and colorimetric estimates of nutrient digestion

the color of the CuSO_4 solution change from blue to pink, violet, or purple depending on the number of peptide bonds in the solution. **Figure 1** is an illustrative example of the colorimetric changes noted in one set of protein digestion experiments. Albumin digestion was affected by all three antibiotics, of which erythromycin exerted the highest inhibition with an average of 20% protein digestion, followed by penicillin with an average of 67% protein digestion, and tetracycline with an average of 89% protein digestion (**Figure 5**).

Polysaccharide digestion. The Lugol test was used to assess polysaccharide digestion (**Table 1**). In this test, the reagent is potassium triiodide (I₃K, also called Lugol's solution), which is yellow-brown in color. Starch was the polysaccharide substrate used for these experiments; pancreatin (a digestive enzyme) was added to break down the macromolecules of starch into small-chain carbohydrates. Starch is a glucose polymer connected through glycosidic linkages. The reaction between I₃K and the glycosidic bonds in starch leads to a black-colored product. Because small-chain carbohydrates lack glycosidic bonds, starch digestion is expected to lead to a product of various tones of brown nearing the brown-yellow of the iodine reagent. In our experiments, all three antibiotics inhibited starch digestion because all test tubes that contained antibiotics had various shades of brown; the darker the brown tone, the less starch digestion occurred. **Figure 2** is an illustrative example of the colorimetric changes seen in one set of starch digestion experiments. Erythromycin had the most negative effect with an average of 31% starch digestion,

followed by penicillin with an average of 50% starch digestion, and tetracycline with an average of 80% starch digestion (**Figure 5**).

Monosaccharide digestion. The Benedict test was used to assess monosaccharide digestion (**Table 1**). In this test, the Benedict solution is a clear blue solution that contains CuSO_4 , sodium carbonate (Na_2CO_3), and sodium citrate ($\text{C}_6\text{H}_7\text{NaO}_7$). Sucrose was the disaccharide substrate used in these experiments, which is glucose linked with fructose molecules. In the presence of invertase (a digestive enzyme), sucrose is

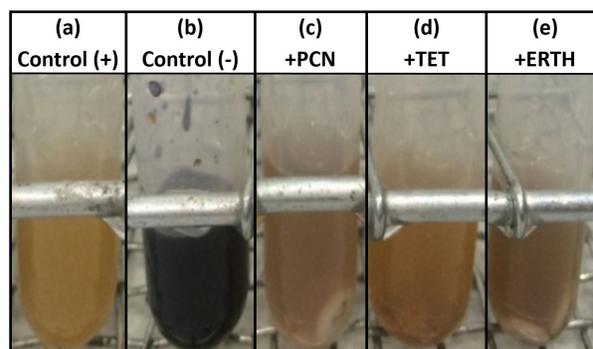


Figure 2: Representative results for the polysaccharide digestion experiments. All tubes contain starch and Lugol Reagent. All tubes except the negative control (b) additionally contain pancreatin. Tubes c–e contain the indicated antibiotic (PCN, penicillin; TET, tetracycline; ERTH, erythromycin). Note differences in solution color in the tubes containing antibiotics (c–e) relative to the positive (a) and negative (b) control tubes.

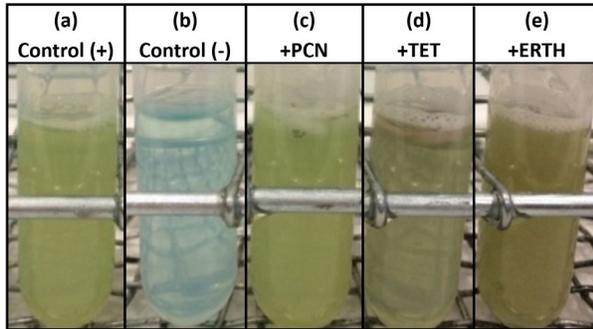


Figure 3: Representative results for the disaccharide digestion. All tubes contain sucrose and Benedict reagent. All tubes contain invertase except the negative control (b), which instead contains distilled water. Tubes c–e contain the indicated antibiotic (PCN, penicillin; TET, tetracycline; ERT, erythromycin). Note differences in solution color in the tubes containing antibiotics (c–e) relative to the positive (a) and negative (b) control tubes.

broken down into glucose. In the presence of glucose, which is a simple sugar, the blue Benedict solution changes color to shades of green based on the amount of sugar. Colorimetric reactions obtained in one set of five Benedict tests are shown in **Figure 3**. Of the three antibiotics, erythromycin exerted the highest inhibition on sucrose digestion with an average of 69% sucrose digestion seen in the presence of this antibiotic, followed by 80% digestion in the presence of tetracycline, while penicillin had a minimal effect on sucrose digestion (**Figure 5**).

Lipid digestion. To assess lipid digestion, the litmus test was used (**Table 1**). Vegetable oil was the lipid substrate used in these experiments. Bile salts and pancreatin were added for lipid digestion; the end products of lipid digestion are fatty acids, which lower the

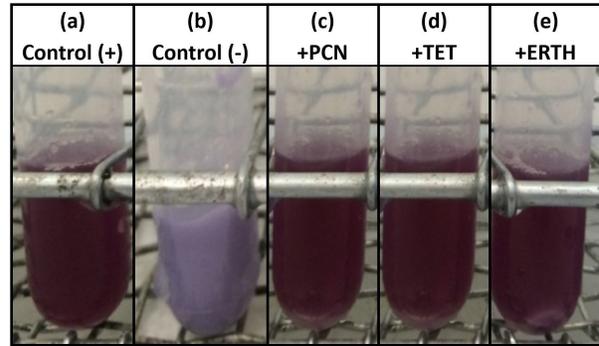


Figure 4: Representative results for the lipid digestion experiments. All tubes contain vegetable oil, bile salt, and litmus milk. The positive control tube (a) additionally contains pancreatin, and tubes c–e contain the indicated antibiotic (PCN, penicillin; TET, tetracycline; ERT, erythromycin). Note lack of solution color differences in the tubes containing antibiotics (c–e) relative to the positive control tube (a).

solution's pH. In the presence of litmus, the solution turns red; under basic conditions, the solution is purple-blue. A representative example of the effects of antibiotics on vegetable oil digestion is shown in **Figure 4**. All test tubes containing antibiotics were dark magenta-red, very similar to the positive control test tube. All test tubes were checked for results of lipid digestion at identical time points. Across all lipid digestion experiments, none of the antibiotics interfered with lipid digestion (**Figure 5**).

Discussion

In this study, the effects of three antibiotics on *in vitro* nutrient digestion were evaluated. We hypothesized that the antibiotics could inhibit digestion, although not all antibiotics might reduce digestion to a similar degree, and

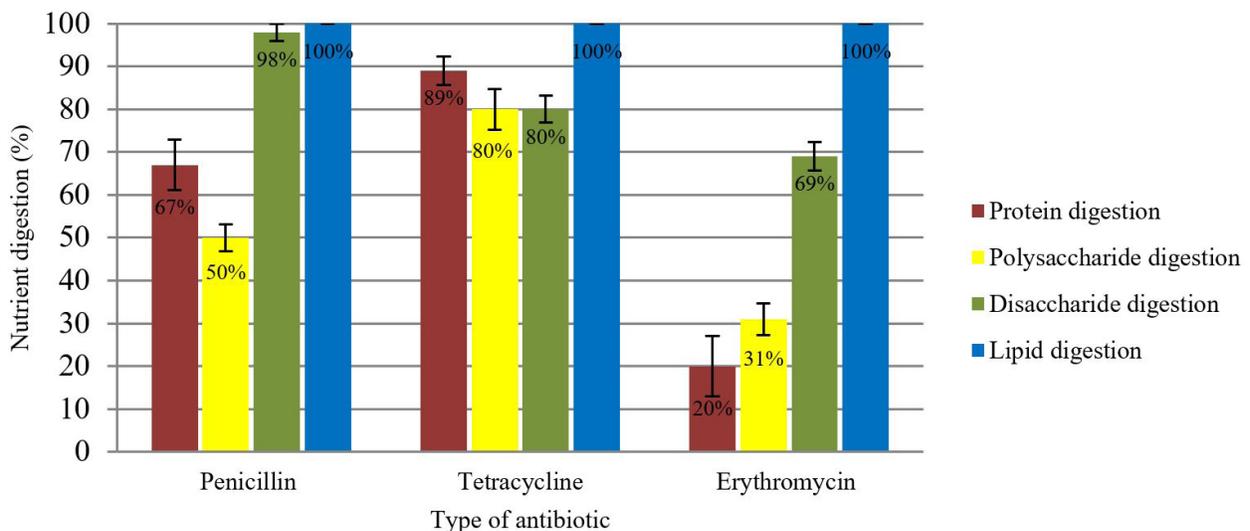


Figure 5: The efficiency of nutrient digestion in the presence of antibiotics. Mean (standard error of mean displayed as error bars) nutrient digestion across the five experiments performed for each antibiotic to assess protein digestion (red bars), polysaccharide digestion (yellow bars), disaccharide digestion (green bars), and lipid digestion (blue bars).

not all of the main nutrients would be similarly affected. The results obtained in these experiments support our hypotheses because nutrient digestion overall decreased in the presence of antibiotics; polysaccharide digestion was impaired the most, followed by protein digestion and disaccharide digestion, while lipid digestion was not impaired. Of the three antibiotics we tested, erythromycin had a greater impact on nutrient digestion than penicillin and tetracycline.

The results observed in these experiments suggest that antibiotics could interfere with the activity of the digestive enzymes on the large chemical molecules composing the nutrients. The positive control experiments showed that the tests were valid given that the expected colorimetric changes with nutrient digestion were seen in these controls. Negative control experiments demonstrate how the results would look in the absence of nutrient digestion. The results obtained in the presence of antibiotics could be due to the binding of the antibiotics to the nutrient substrate or to the enzymes. This binding could hinder the direct interaction between the nutrient substrate and the enzyme and explain the negative effects on digestion in the presence of antibiotics seen in these experiments.

Our study has some limitations. First, the results were evaluated in a semi-quantitative manner. More studies are needed to better quantify the effects of antibiotics on nutrient digestion and means to prevent these untoward effects. For instance, a definite way to assess the antibiotic effects on digestion would be to measure the concentration of amino acids, monosaccharides, and fatty acids obtained in the absence and presence of antibiotics. Second, it is possible that the experimental approach for lipid digestion was not sensitive enough; more sensitive methods of assessing lipid digestion in the presence of antibiotics would need to be done to better ascertain whether antibiotics have any effect on lipid digestion. Another possible explanation for no differences noted in the lipid digestion experiments is that the antibiotics may stimulate lipid digestion. Our study design would have detected an inhibitory effect on digestion; if lipid digestion occurred faster in the presence of antibiotics, then our tests could have yielded false negative results. Future studies should take this possibility into account and test lipid digestion at different time points. Finally, we acknowledge that the *in vitro* experiments are limited by the inability to simulate many other factors present *in vivo* in the GI tract such as peristalsis, bacteria, and other enzymes. It is believed that the GI side effects seen with the oral antibiotics are due, at least in part, to the antibiotics annihilating the harmless bacteria that colonize the GI tract [6, 7]. However, it is conceivable that interference with macromolecular digestion, and by extension interference with nutrient absorption, might contribute to the GI side effects seen with the antibiotics. Erythromycin is associated with the highest incidence of GI side effects (nausea, vomiting, diarrhea) compared with other antibiotics. Studies report an incidence of GI side effects with erythromycin that ranges between 50 to 70% but some studies reported it up to 95% [8].

Erythromycin can increase intestinal motility, which is believed to contribute to the higher rate of GI side effects seen with this antibiotic [9]. The incidence of GI side effects has been reported to be between 15–20% with penicillin, and 5–10% with tetracycline [10, 11]. In our experiments, erythromycin had the highest inhibitory effect on nutrient digestion compared to penicillin and tetracycline.

Our study is the first to report effects of antibiotics on nutrient digestion. It would be warranted to have studies in the future that quantify the interference of the antibiotics with nutrient digestion. By finding the exact mechanism of interference with digestion, pharmaceutical companies and research scientists might then design antibacterial drugs in a way to prevent the specific interference with nutrient digestion.

In conclusion, in our experiments, penicillin, tetracycline, and erythromycin interfered with carbohydrate and protein digestion but did not interfere with lipid digestion. Of these antibiotics, erythromycin had the most negative impact on nutrient digestion. Further studies are needed to evaluate in detail the interaction between the antibiotics and GI enzymes in order to design better drugs.

Methods

Using three different antibiotics – penicillin (PCN), tetracycline (TET), erythromycin (ERTH) – we evaluated the digestion of the main nutrients: proteins, carbohydrates, and lipids [12]. Nutrient digestion in the absence of antibiotic represented the positive control experiments, which are depicted in the first test tube in each experiment. Negative controls were run in each experiment and depicted in the second test tube (Table 1).

Protein digestion

Protein digestion was assessed with the biuret test using 0.1g of albumin powder, 2.5 mL pepsin solution, 2.5 mL hydrochloric acid, and 0.2 mL of biuret reagent. The negative control experiment contained 0.1g of Alka-Seltzer. Following albumin digestion, the presence of peptide bonds in the reactant turns the blue biuret reagent into pink-violet: darker shades of pink-purple signify less albumin digestion, and lighter shades of pink-violet signify more albumin digestion [13].

Polysaccharide digestion

Polysaccharide digestion was assessed with the Lugol test using 2 mL of starch solution, 0.1g of pancreatin powder, and 0.2 mL of Lugol reagent. The negative control experiment did not contain pancreatin. The test tubes with starch digestion display various tones of reddish-brown color: darker shades of brown signify less starch digestion, meanwhile lighter shades of brown signify more starch digestion [13].

Disaccharide digestion

Disaccharide digestion was assessed with the Benedict test using 3 mL of sucrose solution (a

disaccharide combination of the monosaccharides glucose and fructose), 1 mL of invertase, and 0.2 mL of Benedict reagent. The tube with negative control experiment contained an equal amount of distilled water instead of invertase (1 mL distilled water). In the absence of sucrose digestion, the product in the test tube remains blue (the color of Benedict reagent). The tubes in which sucrose digestion took place exhibit shades of green, with darker green tones signifying less sucrose digestion, and lighter green tones signifying more sucrose digestion [13].

Lipid digestion

Lipid digestion was assessed with the litmus test using 1 mL of vegetable oil, 0.1g of bile salt, 0.1g of pancreatin powder, and 1 mL of litmus milk. Litmus is a water-soluble mixture of different dyes extracted from lichens and is used to test for acidity. The tube with the negative control experiment did not contain pancreatin powder. In the absence of vegetable oil digestion, the product in the test tube is alkaline, with a purple-blue color. The tubes in which vegetable oil digestion took place contain acidic products of various shades of red-purple color depending on the degree of lipid digestion [13].

Antibiotic experiments

For all the experiments, the last three tubes in each experiment contained antibiotic disks: one tube with 3 penicillin disks, one tube with 3 tetracycline disks, and one tube with 3 erythromycin disks. Each test tube was placed in a 40°C water bath for 60 minutes before the respective reagent was added. The color noted in the positive control tube represented 100% nutrient digestion, while the color noted in the negative control tube represented 0% nutrient digestion. Color tones obtained in the test tubes containing antibiotics were semi-quantitatively estimated as percentage of nutrient digestion relative to the positive and negative control experiments. **Table 1** summarizes the reactant in each type of experiment and the digestion percent estimate based on the color tones obtained in each experiment. All the experiments were performed five times. The average nutrient digestion across the five experiments was calculated to estimate the overall effect of each antibiotic. To determine the precision of the mean values, standard error of the mean (SEM) was calculated with Excel and displayed on bar graphs as error bars.

References

1. Jean Martin. *Core Chemistry*. Chapter 2. Keeping healthy. Cambridge: Cambridge University Press, 2011. Print.
2. Kim DH. "Gut Microbiota-Mediated Drug-Antibiotic Interactions." *Drug Metab Dispos*. 43.10 (2015): 1581-9.
3. Rafii F, Sutherland JB, and Cerniglia CE. "Effects of treatment with antimicrobial agents on the human colonic microflora." *Ther Clin Risk Manag*. 4.6 (2008): 1343–1358.

4. Paddy Gannon. *Framework Science*. Chapter 8. Food and digestion. Oxford: Oxford University Press, 2010. Print.
5. Farré R, Tack J. "Food and symptom generation in functional gastrointestinal disorders: physiological aspects." *Am J Gastroenterol*. 108.5 (2013): 698-706.
6. Brüssow H. "Growth promotion and gut microbiota: insights from antibiotic use." *Environ Microbiol*. 17.7 (2015): 2216-27.
7. Aguilera M, Cerdà-Cuellar M, Martínez V. "Antibiotic-induced dysbiosis alters host-bacterial interactions and leads to colonic sensory and motor changes in mice." *Gut Microbes*. 6.1 (2015): 10-23.
8. Anastasio GD, Little JM Jr, Robinson MD, Pettice YL, Leitch BB, Norton HJ. "Impact of compliance and side effects on the clinical outcome of patients treated with oral erythromycin." *Pharmacotherapy*. 14 (1994): 229-34.
9. Catnach SM, Fairclough PD. "Erythromycin and the gut." *Gut*. 33 (1992): 397-401.
10. Bhattacharya S. "The facts about penicillin allergy: A review." *J Adv Pharm Technol Res*. 1 (2010): 11–17.
11. Kuehn J, Ismael Z, Long PF, Barker C, Sharland M. "Reported Rates of Diarrhea Following Oral Penicillin Therapy in Pediatric Clinical Trials." *J Pediatr Pharmacol Ther*. 2 (2015): 90–104.
12. Charlotte W. Pratt. "A Biology Laboratory Exercise Using Macromolecule Assays to Distinguish Four Types of Milk." *Journal of Microbiology & Biology Education* (2011) 44-45.
13. Biology, IGCSE. "Food Digestion Tests." <http://biology-igcse.com/food-tests.html>