REDMOND CLAY: A HIGH AFFINITY SORBENT OF AFLATOXIN B_1 AND CHOLERA TOXIN

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ABSTRACT

Studies of the pathophysiology involved in the human gastrointestinal tract show that two types of diarrhea exist: osmotic and secretory. Osmotic diarrhea is caused by the presence of poorly absorbed luminol osmols in the lumen of the gut. Secretory diarrhea is usually caused by bacterial toxins or viral agents, which promote the secretion of an excess of water and electrolytes. These infections become dangerous due to dehydration, which can often lead to death if left untreated. Clays, such as bentonite, may reverse the dehydration caused by secretory diarrhea by replenishing lost electrolytes and adsorbing bacterial toxins; thereby promoting water reabsorption.

Enzyme-linked immunosorbent assays (ELISA) were conducted to measure the efficacy of hydrated sodium bentonite in binding aflatoxin B_1 (AFB_1) and cholera toxin (CT) from solution. Using 5ml of a 4μg/ml aqueous solution of AFB_1 and 1-2g of sodium bentonite, adsorption abilities ranged from 99.9990% to 99.9995%. Values of 20,000ppb were brought within concentrations safe for human consumption (<20ppb) (14, 23). Using a 0.1mM diluted aqueous solution of CT and 1-2g of sodium bentonite, adsorption values were similar to those of bentonite with AFB_1. These results implicate a possible use of hydrated sodium bentonite in the preventative management of bacterial enterotoxin induced secretory diarrhea; however, its efficacy in protecting against these should be verified further by *in vivo* testing.

I. INTRODUCTION

Severe dehydration due to acute diarrhea causes more than 5 million deaths per year worldwide (12, 34). Children and the elderly are the most susceptible to this dehydration because of underdeveloped, or decreased immune function. The World Health Organization (WHO) defines acute diarrhea as three or more liquid stools within 24 hours and chronic, or persistent, diarrhea as acute diarrhea that lasts at least fourteen days (12). "The WHO estimates that 3-20% of acute diarrheal episodes in children under 5 years of age in developing countries become persistent." (12). This statistic is alarming, considering every child under five years of age in these developing countries, on average, has 2-3 episodes of diarrhea per year (2, 12). Chronic diarrhea can lead to other serious problems besides mortality, such as malnutrition, dehydration, and morbidity. In Columbia, a scientific paper reported that chronic diarrhea can cause a negative effect on a child's growth in the first three years of life. This study estimated that a child's growth is stunted between 2.5 and 10 cm, resulting from a loss of nutrients essential to promote growth hormones (12).

The Dystrophic Center, in Iasi, Romania, starkly portrays the problems that diarrhea can cause in children. This orphanage contains about one hundred children ranging from a few months to three years of age. Due to cramped quarters, and the lack of sanitation, all of these children suffer from chronic diarrhea. The causes of diarrhea vary from malabsorbtion to intestinal infection. Because of these diarrheal problems, three year old children in the orphanage only have the body size and development of an average healthy Romanian one year old. (Figures 1 and 2 provide a graphical representation of the serious health detriments of chronic diarrhea.)

Apart from the problems suffered by the children and elderly in developing countries, 20-50% of adult travelers that visit these developing countries often suffer from this same diarrhea, commonly known as "travellers' diarrhea" or "Montezuma's revenge." (7). The effects of this ailment include discomfort, lost travel time, and impaired health. Because of the seriousness of chronic diarrhea, a search for more efficient antidiarrheals has increased greatly over the past twenty years. Currently, the only generally accepted treatment for acute diarrhea is not an antidiarrheal, but an oral rehydration solution. The purpose behind the

rehydration solution is to replenish the fluids and electrolytes lost during an episode of secretory diarrhea. Oral rehydration solutions do not decrease the duration or severity of diarrhea, but only prevent the severe dehydration that can lead to death.

The search for an antidiarrheal to accompany oral rehydration therapy has spanned a variety of drugs that target different aspects of diarrhea, such as antimicrobial and antimotility agents. Besides the high cost, most of these drugs have dangerous side effects, and therefore are not generally accepted. Antimotility agents, such as opiates and loperamide target slowing the passage of fluid through the intestinal tract, allowing time for the colon to reabsorb water. However, opiates cause drowsiness, respiratory depression, hypotension, nausea, and addiction; furthermore, Loperamide causes abdominal distension. There are many antimicrobial agents, all of which have some side-effects. These side-effects range from intestinal irritation and hemorrhages to accumulation of salicylate in the blood stream. Apart from dangerous side-effects, there is also a concern for the cost and availability of these drugs. In 1996, the WHO reviewed a large majority of antimotility drugs, antimicrobial drugs, and adsorbents; and concluded that they should not be implemented as treatments for diarrhea (13). The WHO determined that Oral Rehydration Therapy (ORT) was sufficient; and only in cases of dysentary, and severe cholera should an antimicrobial be used until scientific information supports a safe and effective antidiarrheal (13). This standpoint was taken because all but the adsorbents demonstrated dangerous side-effects and would be expensive to incorporate. Adsorbents

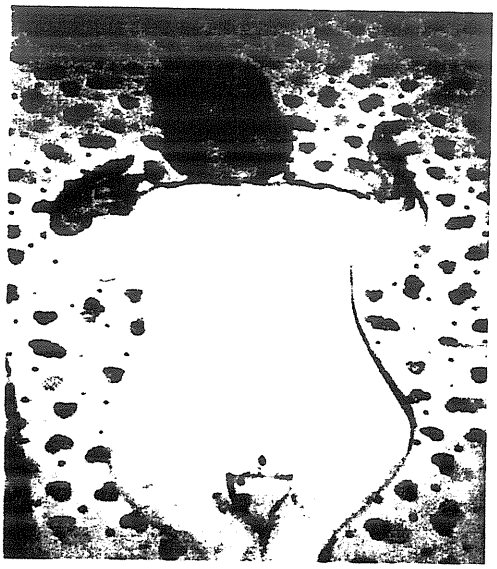

Figure 1: A two and a half year old Romanian baby with chronic diarrhea

Figure 2: A one year old healthy Romanian baby

were most likely disapproved because of the lack of consistent scientific evidence *in vivo* to support them.

Adsorbents such as kaolin and smectite have been tested in double-blind placebo studies as treatments for diarrhea. The results, however, have been deemed inconclusive by the WHO. The WHO currently does not support sorbents as an effective treatment for diarrhea on these grounds. We suggest that inconclusive data in the double-blind studies may be due to a lack of consideration made as to the source of diarrhea among the subjects in the studies. We hypothesize that sorbents such as bentonite would be more effective in treating a toxin-induced secretory diarrhea and not so effective with a case of osmotic diarrhea caused by malabsorption. Most studies that test sorbents as a treatment for diarrhea have targeted decreasing the motility of the lumen. We propose that the emphasis should be on the sorbents' ability to bind and remove toxins produced by enterotoxigenic bacteria. In a case of malabsorption, sorbents would only increase the osmotic load of the lumen and may result in a greater loss of fluids.

According to scientists Berschneider and Powell, "'The need for a safe, effective antidiarrhoeal agent is clear', and the search for such an agent continues. It should have the following characteristics: 'a high degree of activity, oral effectiveness, target its action on the intestine without systemic absorption or systemic effect, have no effect on a normally functioning gut and have a mechanism which is well understood." (12). Our study was to determine through *in vitro* tests, whether the adsorbent Redmond Clay fits this description.

Redmond Clay (activated sodium bentonite) has been shown to be one of the best swelling clays (adsorbents) (38). Swelling clays contain a large array of electrolytes that can function as cation and anion exchangers. We propose that this quality allows bentonite to bind harmful toxins and prevent them from causing damage. A wide variety of toxins exist in nature and cause illnesses ranging from severe diarrhea to cancer. We chose to test Redmond Clay against Aflatoxin B_1 (AFB_1) and cholera toxin, which are two of the most dangerous toxins in nature. Aflatoxins are toxic, carcinogenic, mutagenic, and teratogenic in animals including humans (8, 15). We chose to test the clay with aflatoxin first because of the availability of a simple ELISA to test for the presence of AFB_1 in solution. (Additional information on aflatoxin may be of interest and is provided in Appendix A.)

Cholera toxin is produced by the bacterial strain *Vibrio cholerae*. Cholera toxin, as well as many other enterotoxins, causes severe secretory diarrhea. *Vibrio cholerae* binds to the mucosal lining of the small intestine. Once bound, the toxin is synthesized and released into the lumen of the gut. The toxin is made up of two subunits. The B subunit's main function is to bind to the GM_1 receptor on an epithelial cell of the intestinal lining (12, 34). The A subunit enters the epithelial cell and activates the adenylate cyclase on the basolateral membrane. The adenylate cyclase in turn creates cAMP, which stimulates the chloride channels of the crypt cells, leading to the secretion of Cl-, electrolytes and water (12, 32, 34). In addition, The NaCl absorption transporter is completely blocked by the B subunit. (Fig. 3) (Background information on the physiology of the gastrointestinal tract and the pathophysiology of diarrhea can be found in Appendix B.)

We hypothesize that activated bentonite, in the lumen of the small intestine, can prevent cholera toxin and other enterotoxins from binding to their receptors. The inability to bind to their receptor would neutralize the damaging effects of the toxins. We propose that if our study shows Redmond Clay can remove a significant amount of cholera toxin from solution *in vitro*, it may function as an effective antidiarrheal treatment *in viv*

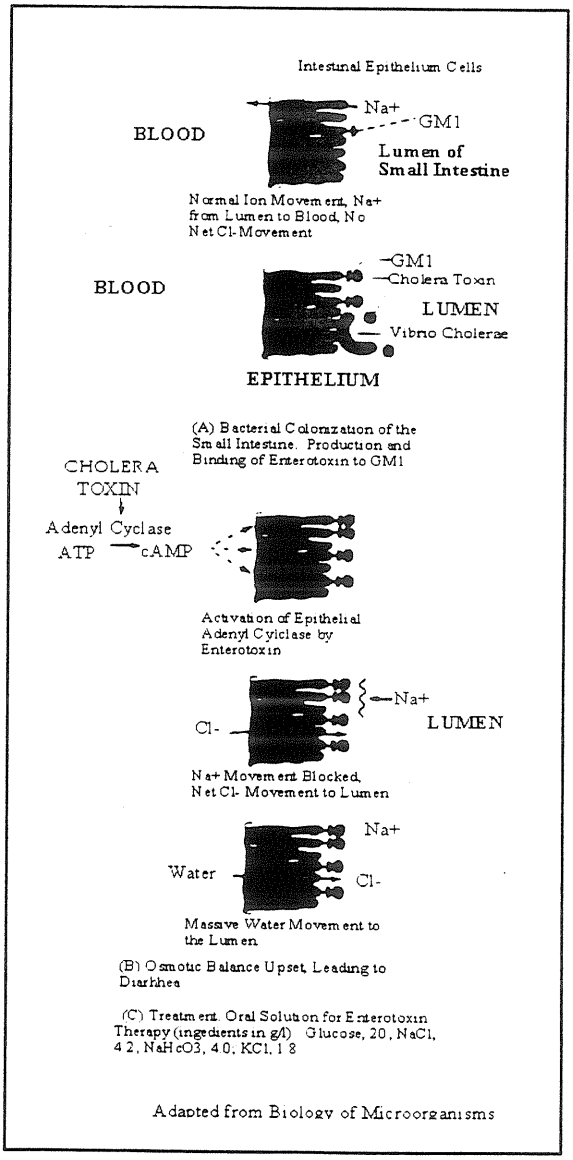

Figure 3: Action of Cholera Toxin

II. AFFINITY OF REDMOND CLAY

MATERIALS AND METHODS

Materials

The sodium bentonite, Redmond Clay, was provided by Redmond Minerals Inc. (Redmond, UT). Aflatoxin B_1 purity >99%, Cholera toxin, Anti-rabbit IgG peroxidase conjugate antibody, and Anti-cholera toxin antibody were all purchased from Sigma Chemicals (St. Louis, MO). An AFB_1 specific ELISA kit, Veratox Quantitative Aflatoxin Single Test (AST), was obtained from NEOGEN Corporation (Lansing, MI).

Sorption of AFB_1

A 70% methanol stock solution containing 1mg unlabeled AFB_1 was prepared to give a working solution of 4µg AFB_1/ml (20,000ppb). For each ELISA, 5ml of stock solution was aliquoted into four 15ml tubes. Two of these tubes were labeled and set apart as controls for the ELISA. The other two were labeled "w/bentonite." Total incubation time for the samples was 1 hour at 25°C . All tubes were vortexed at the beginning of incubation and at 15min. intervals throughout the incubation period. The sorption was terminated after 1hr. by centrifugation for 10min. at 1,500 rpm. 100µl of the supernatants were decanted and added to the proper microwells of the ELISA.

The Veratox AST was conducted according to NEOGEN guidelines. The test is a competitive ELISA between AFB_1 and a conjugate to the anti-aflatoxin B_1 antibody. One control consisted of an internal standard of 20ppb of AFB_1 solution provided by NEOGEN. A negative control was also used. The test is quantitative for concentrations of 5-350ppb of AFB_1. The optical densities are measured with a 650nm filter in a microwell plate reader. The results are then compared to a set calibration curve determined by NEOGEN. (Table 1) The ELISA

Std. ppb	Calc. O.D.
0	2.102
5	1.833
10	1.554
20	1.150
50	0.693
100	0.427
300	0.200
500	0.170

Table 1: Calibration Curve for Veratox

was repeated twice using 2mg of bentonite. A third ELISA was run using 1mg of bentonite.

Sorption of Cholera Toxin

A 50% PBS diluted stock solution was prepared with cholera toxin. 12ml of a .1mM dilution was added to two 15ml test tubes. Two negative controls were also run with 12ml of PBS. 1g of sodium bentonite was added to one of test solution and to one of the controls. The other test solution and control were left untreated. The tubes were left to incubate at 22-25°C for 1 hr. and were vortexed at the beginning of the incubation period and at 15min. intervals. The samples were then centrifuged at 1,500rpm for 10min. 100µl of supernatant from each tube was aliquoted into their respective wells. The microwell was left to incubate for 24hrs.

After 24hrs., the wells were emptied and washed twice with 300µl of a PBS solution with 0.5% tween20. The wells were then flushed a third time with PBS alone. 300µl of block solution (PBS with 3% FBS) was then aliquoted to each of the wells and left to incubate for another 24hrs. Afterwards, the same washing procedures as above were performed.

A 100µl of 1:10,000 PBS diluted solution of anti-cholera toxin antibody was added to each well and incubated for 2hrs. The same washing procedures were followed and 100µl of a 1:7,000 PBS diluted solution of anti-rabbit IgG labeled with horseradish peroxidase was added to each well. The microwell plate was left to incubate for 2hrs. and then washed. 100µl of 3,3′,5,5′- Tetramethylbenzidine (TMB) was then added to each well and left to react for 30min. The optical density (OD) was then measured with a 450nm filter. Results were compared with controls.

RESULTS

The AFB_1 ELISA results showed that the bentonite removed approximately 20,000ppb of aflatoxin, bringing the levels within ranges set as safe for human consumption (20ppb: All food except milk). The optical densities showed that the 4μg AFB_1 solution had levels well beyond the ranges measured by the ELISA. The samples with bentonite had levels of aflatoxin lower than the 20ppb control. (Fig. 4) The graph shows that the sample of aflatoxin treated with bentonite had approximately the same O.D. as the control sample with only 20ppb of AFB_1. This means that the antibody conjugate was in large concentration in proportion to the. The O.D. value for the sample not treated with bentonite was very low. This means that there was a very large concentration of AFB_1 in proportion to the antibody conjugate. Therefore the two samples began with the same concentration of AFB_1, but the sample treated with bentonite had almost all of the AFB_1 removed. In fact, the bentonite brought the level of AFB_1 down to below the standard set as safe for human consumption.

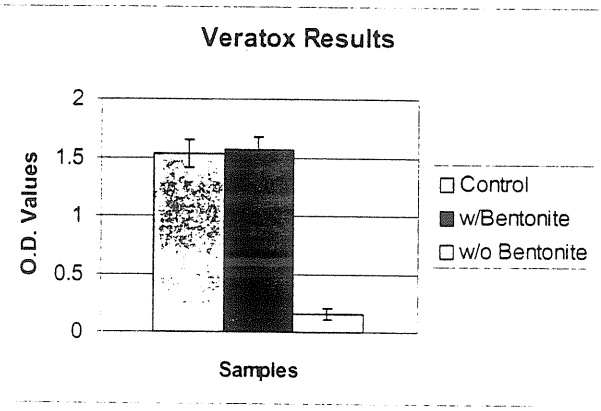

Figure 4: O.D. Values of Veratox ELISA

The cholera toxin ELISA worked a bit differently than the Veratox AST ELISA. Instead of being a competitive ELISA, it simply measured for the presence of cholera toxin. The larger the concentration of cholera toxin present resulted in a larger O.D. This is the reverse of what occured with the Veratox AST ELISA. The cholera toxin results showed that the O.D. of the negative controls and the cholera toxin sample with bentonite were of about equal value. They both had a reasonably low O.D. Even though the control did not have any cholera toxin present, it had a very small O.D. which is common to occur. It is customary to set the O.D. of the control as the negative point (no cholera toxin). Therefore the sample treated with bentonite tested negative for the presence of cholera toxin. The bentonite effectively bound the toxin and removed it from solution during centrifugation. The positive control of cholera toxin not treated

with bentonite had a significantly higher O.D. which means that it tested positive for the presence of cholera toxin. (Fig. 5) We did not determine a concentration value of how much cholera toxin was removed from the sample treated with bentonite, but we can assume that almost all of the toxin was removed from solution. These results are exciting because we know that the toxins in both tests were bound and not just inactivated by the bentonite. We know this because we used polyclonal antibodies in both of the ELISA's. Polyclonal antibodies recognize several epitopes on the toxin. Therefore if the toxin was mutated at one epitope by a mutation, it's other epitopes would still be recognized by the antibody and counted as present in solution. Our results, however, showed that the toxins were completely removed from solution. It is therefore determined by these tests that Redmond Clay is an effective binder of AFB_1 and cholera toxin *in vitro*, and may be an effective anti-toxin with all toxins.

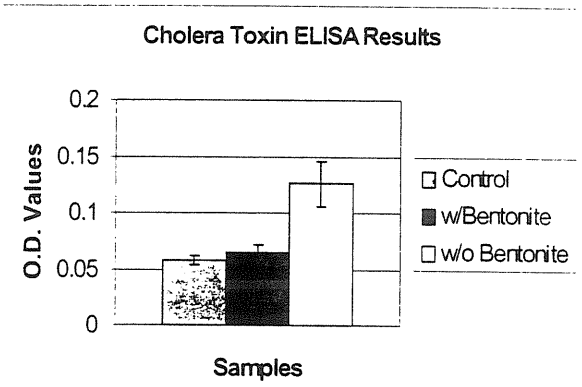

Figure 5: O.D. Values of Cholera Toxin ELISA

III. DISCUSSION

Redmond Minerals Inc. approached our lab on the premise that they had a clay (Redmond Clay) that they and many personal testimonies claimed to cure diarrhea. Our lab agreed to look at the clay's attributes and test it for a possible mechanism by which it might cure diarrhea. We first tested the Redmond Clay for any antimicrobial qualities. We ran tests with several bacterial strains, but the clay showed no inhibition on their growth. Our second approach on the matter was to test the clay for any immune enhancement attributes. We tested the clay with macrophage response and immune function. None of our tests, however, were statistically significant to conclude that it did enhance immune response.

After thorough research in the literature on the topic, we hypothesized that the clay may not be affecting the bacteria directly; but rather, it may be affecting the toxins that the enterotoxigenic bacterial strains produce. Many studies have shown that sorbents such as Redmond Clay have the ability to adsorb dangerous toxins such as carcinogenic aflatoxin (29, 30, 31), killer yeast toxins in wines (26), T-2 toxin (6), staphylococcal enterotoxin A (20), and *E-coli* enterotoxins (5, 6, 19). Successful *in vivo* studies have been conducted with cholestyramine (4, 28) and even one with smectite, conducted on egyptian children (18, 27). This last study found that 48hrs after treatment, "19 patients (42%) of the group receiving smectite were free of diarrhea, while this was true of only 6 patients (13%) receiving placebo."(27).

In our study we first tested Redmond Claywith AFB_1 to see if this theory is plausible. Our results show that Redmond Clay did effectively bind AFB_1 and removed it from solution. These positive results encouraged us to devise an ELISA for cholera toxin. We could use this ELISA to test Redmond Clay against an actual enterotoxin. After determining the proper concentrations of antibodies for the ELISA, the results gave proof that Redmond Clay does in fact bind cholera toxin in solution and may do likewise with other enterotoxins.

In addition the positive results of our study, we also analyzed the biochemical make-up of sodium bentonite and found an interesting possibility. A biochemical assay of sodium bentonite shows that it contains the proper electrolytes at the same concentrations of the average oral rehydration solution. (Table 2

) Bentonite lacks only a glucose-glutamine additive that would make it function properly as a rehydration treatment for children suffering from acute diarrhea. (The biochemical assay of sodium bentonite and background on oral rehydration therapy can be consulted in Appendix C.)

Implementing bentonite as an oral rehydration solution would be somewhat unnecessary unless it offered some other aspect in the fight against secretory diarrhea. Sodium bentonite's ability to sequester toxins in solution may be that other aspect. If sodium bentonite can perform this function within the lumen of the small intestine, it is possible that it may perform the roles of an oral rehydration solution and an antidiarrheal.

Solution	Sodum	Potassium	Chloride	Glucose *
	mmol/L	mmol/L	mmol/L	mmol/L
WHO solution	90	20	80	111 (20)
Rehydralyte	75	20	65	139 (25)
Pedialyte	45	20	35	139 (25)
Resol	50	20	50	111 (20)
Ricelyte	50	25	45	(30)
Gatorade	23.5	<1	17	(40)
Bentonite**	90	25	25	------

* Figures in parenthesis represent grams of carbohydrate

Table 2: Composition of Oral Replacement Solutions for the Treatment of Diarrhea

IV. CONCLUSION

Before a new antidiarrheal can be permitted into the health institutions of the world it should be endorsed by the World Health Organization. History shows that the WHO will not accept any new treatment for secretory diarrhea unless it is cost effective and safe. Redmond Clay does not have to be engineered but is rather a natural resource. It could be modified for glucose content and taste for a minimal amount. As for its safety, the FDA has labeled activated bentonite as GRAS, meaning Generally Regarded As Safe.

The antidiarrheal must also be proven scientifically first through *in vitro* studies and then through *in vivo* studies. Berschneider and Powell set forth basic qualifications of such an antidiarrheal: it must have "a high degree of activity, oral effectiveness, target its action on the intestine without systemic absorption or systemic effect, have no effect on a normally functioning gut and have a mechanism which is well understood." We believe that after further studies Redmond Clay may be proven to fit each of these categories.

Redmond Clay has demonstrated a potential for a high degree of activity through its high affinity to cholera toxin. Should the activated bentonite bind enterotoxins just as successfully *in vivo*, it would be very effective. The central concern that must be answered through *in vivo* trials is whether activated bentonite can sequester enterotoxin after they have bound to a receptor. Should the bentonite have a higher affinity for the toxin than the receptor, it may be concluded that it definitely has a high degree of activity.

Activated bentonite is oral effective because it is easily swallowed and kept down. Although Redmond Clay does not have a completely unpleasant taste, some may show reserve in taking it orally. It may be possible to modify the taste or take the solution with food without hindering its action. Because Redmond Clay is taken orally, its action is targeted to the lumen of intestine. As previously addressed, the solution of activated bentonite is constituted of mainly electrolytes and trace metals which are necessary for the diet and essential as a part of oral rehydration therapy during acute diarrhea. Many scientists have voiced a concern that clay based sorbents may cause granuloma in the intestine. Granuloma is the build up

of macrophages which can be detrimental to self cells. Only further trials will show if this will be a side-effect of the treatment.

As for the final qualification set forth by Berschneider and Powell, the mechanism by which sodium bentonite may functions as an antidiarrheal has been shown with the presented research. Activated bentonite should not be used to treat osmotic diarrhea because its mechanism is through binding the enterotoxins that cause secretory diarrhea. A sorbent should be used to target the source of the problem and not used to slow motility. The effect of allowing more time for water to be reabsorbed will be offset by the increase in luminal osmolarity. Our study only endorses the possible use of Redmond Clay as a treatment for enterotoxigenically induced secretory diarrhea.

We conclude that our study on Redmond Clay's action against the mycotoxin, AFB_1, and the enterotoxin, cholera toxin, was successful. In solution, Redmond Clay does effectively bind these toxins. These toxins can then be removed from the solution through centrifugation. We propose that this action may make Redmond Clay an effective antidiarrheal against secretory diarrhea. Its biochemical make up lends it to be an effective oral rehydration solution as well. Should Redmond Clay function as both an anti-toxin and oral rehydration solution *in vivo*, not only would it be a cost effective replacement of the current ORS, but it also has an excellent shelf life. We have not found or know of any other functions that Redmond Clay might have as a treatment for diarrhea. We hope that our research may prove useful to others that may continue this work.

APPENDIX A

Aflatoxin is considered a Group I carcinogen in humans by the International Agency for Research on Cancer (IARC). In the human liver, aflatoxin is activated by cytochrome p450 into an 8,9-epoxide that readily forms DNA adducts (8). Binding of this reactive intermediate to DNA results in disruption of transcription, leading to abnormal cell proliferation and the formation of tumors. In certain parts of the world, levels of aflatoxins in food produced for human consumption are monitored because aflatoxin is a product of grain mold, *Aspergillus flavus*, commonly found in animal feed. It is estimated that one quarter of the world's food crop may be affected with mycotoxins (16).

Detoxification of aflatoxin-contaminated foods and feed is a current problem in agriculture. The ingestion of aflatoxins can lead to an illness known as aflatoxicosis in animals. The symptoms of aflatoxicosis are: decreased carbohydrate, lipid, nucleic acid, and protein metabolism, reduced growth rate of young animals, and impaired immunological responsiveness (8, 16, 23). These problems leave the animal susceptible to additional infections which may lead to death.

Dairy cows often suffer from aflatoxicosis when levels of aflatoxins in their feed is high. Besides the previously mentioned symptoms, aflatoxicosis results in high counts of aflatoxins in their milk. Dairy cows convert from .25% to 4.8% of the aflatoxins in the feed to afltatoxin M_1 in the milk (15). This poses a problem for dairy farmers when their milk productions does not meet FDA standards for aflatoxin levels in the milk. Treatment with antibiotics may cure the aflatoxicosis but it will also be toxic to the normal flora of the lumen which will contribute to high cell counts in the milk. The most recent form of treatment, to keep supplementary feed under 5μg/kg of aflatoxin, is the addition of sorbents in contaminated feeds to bind the aflatoxins. This will prevent aflatoxicosis while keeping the somatic cell counts of the milk low (15, 34). *In vitro* studies have shown that activated carbons (10) and hydrated sodium calcium aluminosilicates (22) are capable of binding AFB_1 in aqueous solution. *In vivo* tests on weanling piglets (16, 30, 31) and broiler chickens (29) show that treatment with bentonite may prevent aflatoxicosis.

APPENDIX B

GENERAL INTESTINAL PHYSIOLOGY

All of the vitamins, carbohydrates, fat, proteins, electrolytes, and trace minerals essential to the human body's cellular and organ functions are obtained from ingested foods. "Knowledge of the normal physiology of the transport of water and electrolytes across the gastrointestinal tract is an essential prerequisite for understanding of the pathophysiology of electrolyte disturbances resulting from diarrheal disorders." (11). In this section, a basic description of electrolytes and fluid transport involved in the digestion process will be given to provide a background understanding as to why diarrhea occurs and how it might be prevented or cured by bentonite.

As food enters the mouth, it is broken down into smaller pieces by enzymes. The food travels down the esophagus until it reaches the stomach, where enzymes and acids are secreted from its walls. Once in the stomach, all of the ingested food is liquified. This liquification is necessary for absorption through the walls of the intestines. Upon leaving the stomach, this liquid food first travels to the duodenum, the first part of the small intestine. "9-10 liters of fluid enter the duodenum daily...2 liters are dietary...7-8 liters are from secretions." (21). Absorption of the dietary nutrients begins in the duodenum and continues as this fluid travels to the large intestine, called the colon. (Fig. 6)

Most of the electrolytes (sodium and potassium) are absorbed into the lining of the small intestine. To the naked eye the lining of the intestine looks like a fleshy shag carpet. Upon microscope examination of a single section of that "carpet" it would appear to be packed with small bumps called villi.

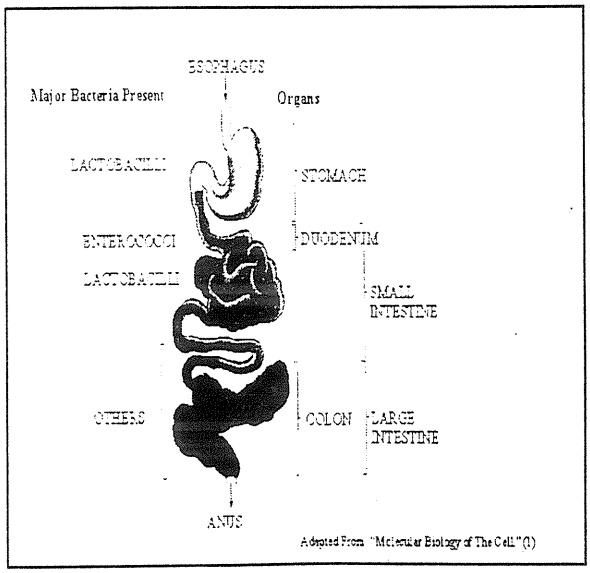

Figure 6: The Human Gastrointestinal Tract

Examination of one individual villi would reveal trillions of cells with more finger-like bumps called microvilli. The sum of the surface area created by all of these bumps is equal to about 200 square meters, an area larger than the size of a football field. This extensive surface area becomes important in allowing maximum absorption of essential body nutrients.

At the tips of the villi are the cells (tip cells) involved in absorption, while the cells at the base of the villi (crypt cells) primarily function as secretory cells (11). Sodium and potassium (Na+ and K+ respectively), can enter the bloodstream either through "tight" junctions between these cells or through tip the cells themselves. It is important to note that whenever Na+ and K+ are transported across these cell, water is brought with them, into the circulatory system. The reverse of this statement is true as well. Whenever Na+ and K+ are secreted out of the crypt cells into the lumen, water is brought with them. It is necessary that more water is absorbed than secreted, in order to prevent dehydration. Many scientists have conceded that when glucose, a basic sugar, is present, the tip cells will allow the junction to open up even larger, thus increasing permeability(3, 7, 12, 32). (Fig. 7)

To enter the bloodstream and from there to be taken to the various parts of the body, Na+ and K+ must pass through the basolateral membrane of the cells, which requires energy. This action is important because Na+ and K+ are used for nerve impulses in the body. Three pathways exist in the body for sodium to obtain the energy necessary to pass through the basolateral membrane and into the bloodstream. One, uncoupled absorption of Na+; two, co-transport of Na+ and Cl-; and three, Na+ coupled to absorption of organic solutes (sugars and amino acids). The first pathway does not play a large role in diarrhea, so it will not be detailed. It is a simple exchange between K+ and Na+ in and out of the lumen. The second pathway is the

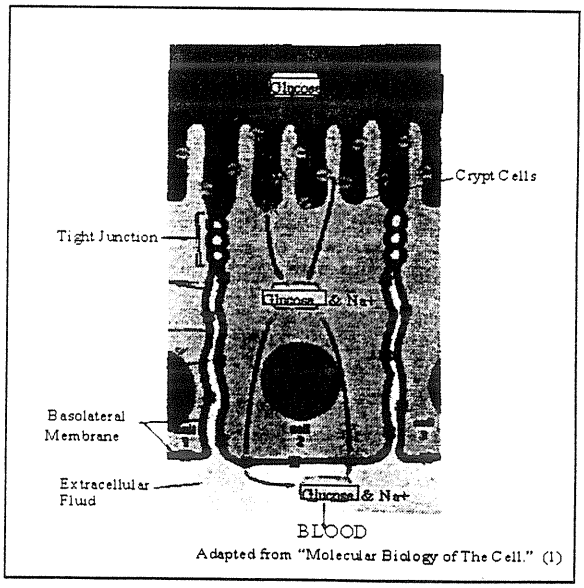

Figure 7: Epithelium Cell of the Small Intestine

common method of diffusion. Na+ and Cl- coupled together can create enough energy to pass through the basolateral membrane into the bloodstream. In the third pathway, energy is derived from sugars such as glucose (as previously mentioned) and amino acids. This pathway occurs through the "tight" junctions. This third pathway plays a very important role in Oral Rehydration Therapy because it is the only transport mechanism that continues to function during secretory diarrhea. More details on this subject is covered in the following section.

The electrolytes and water that are not absorbed in the small intestine are passed into the colon. Here, 80% of the water from the fluid passing into the colon is absorbed along with 90% of the remaining electrolytes (12). "Normally 1-1.5 liters [of fluid] enters the colon. The colon absorbs all but 200mL [of that]...[in cases of diarrhea,] the maximum colonic absorptive capacity [is] 4-6 liters." (21). This mass absorption of water turns the remaining waste back into solid form that is later excreted from the body as fecal matter. "Normal stool output is approximately 100 to 200 g/day." (2, 21). Most cases of diarrhea occur because the amount of fluid exceeds the maximum absorptive capacity of the colon. The fluid that is not absorbed is excreted as watery stool. Therefore, an antidiarrheal should target on preventing secretion of electrolytes, which will in turn prevent large volumes of water from being secreted and causing secretory diarrhea.

PATHOPHYSIOLOGY OF DIARRHEA

The symptoms involved in diarrhea include an increase in frequency and volume of watery stools. Diarrhea can be classified as osmotic or secretory. Osmotic diarrhea is the result of a decrease in water absorption in the colon. Secretory diarrhea, on the other hand, results from an increased secretion of fluid into the intestinal tract (21). These two forms of diarrhea differ widely in their causes, signs and symptoms. The following portion details the differences between these two classifications of diarrhea.

OSMOTIC DIARRHEA

Osmotic diarrhea is typically caused by non-infectious conditions. The major condition leading to osmotic diarrhea is malabsorption. Malabsorption is the inability of certain ingested carbohydrates to be absorbed by the microvilli (11, 12, 21, 32). When these water-soluble carbohydrates reach the colon they are fermented by colonic bacteria into luminol osmols, which not only can not be absorbed but also prevent reabsorption of water by the colon, resulting in watery stools (11, 12, 21).

Several types of molecules in food can lead to osmotic diarrhea. One example is glucose-galactose (dissacharide). This molecule will not be absorbed due to a defect in transport mechanisms or if the intestinal microvillus membrane is damaged (11). Another example of an unabsorbable carbohydrate is lactose. The body can not absorb lactose. Lactobacillus, a bacteria that is part of the normal intestinal flora, produces lactase, an enzyme capable of breaking down lactose into an absorbable form. Thus, if lactobacillus is absent, a person will be unable to absorb lactose which causes lactose intolerance. Thus, lactose intolerance causes osmotic diarrhea, abdominal distention, abdominal pain, and excessive gas (32).

Laxatives are another cause of osmotic diarrhea. When a person is suffering from constipation or is unable to defecate, a laxative is take to loosen up their stools. Laxatives are made up of nonabsorbable inorganic components that are fermented by bacteria in the colon. When these components are fermented, they become luminol osmols which means they prevent reabsorption of most of the water. The extra water in the lumen is what induces a looser stool or diarrhea, depending on the strength of the laxative. Because

water is only prevented from being absorbed and no water is secreted. osmotic diarrhea is always small in volume (<1 liter/day) (21). Children that suffer from malabsorption may experience severe weight loss and dehydration if their diets are not adjusted. The carbohydrates, essential to growth, that are not being absorbed must be substituted by a similar carbohydrate that can be absorbed. Fructose, a simple sugar often found in fruit, is the suggested substitute for glucose and sucrose for malabsorption caused by glucose-galactose. Lactose can be replaced by Soy milk, or lactase pills can be taken. The most common sign that diarrhea is osmotic is discontinuance of symptoms upon fasting (21, 32). Without ingesting carbohydrates that create an osmotic load, watery stool will not continue.

SECRETORY DIARRHEA

Infectious bacterial enterotoxins are the most common cause of secretory diarrhea (12). Bacterial enterotoxins cause an increased secretion of Chloride (Cl-) and Na+ in the crypt cells of the mucosal membrane (12). As previously mentioned, when Na+ is transported across the mucosal membrane into the intestinal lumen water also enters the intestinal lumen. This increase of fluid in the intestinal tract can become too large for the colon to reabsorb, resulting in large stool volume (>1 liter/day) (21). Other causes of secretory diarrhea include viral agents, tumor hormones and neurotransmitters.

There are three ion secretion mechanisms: One, cyclic adenosine monophosphate (cAMP); two, Cyclic guanosine monophosphate (cGMP); and three, calcium (Ca++) (11, 12, 32). All three of these mechanisms function almost identically by phosphorylating mucosal membrane proteins via protein kinases. This phosphorylation results in a conformational change in the mucosal membrane proteins. This change in conformation prevents neutral NaCl absorption (the second sodium transport mechanism) and stimulates secretion of Cl-, which takes Na+ and water along with it. The Na+ absorption by means of the glucose carrier mechanism continues to function (12, 32). This provides a method of replenishing electrolyte and water losses by means of oral rehydration solutions.

APPENDIX C

ORAL REHYDRATION THERAPY

In the mid 1970's, Oral Rehydration Therapy (ORT) was the most revolutionary advance in the treatment of acute diarrhea and today it remains the cornerstone (7). The WHO has stated that ORT is "potentially the most important medical advance this century [20th century]." (12). It took an understanding of the physiologic concepts of water and electrolyte transport for researchers to find a solution to remedy the loss of fluids and electrolytes. The key is in the fact that although bacterial enterotoxins shut down the NaCl co-transport absorption pathway, the sodium and glucose paired pathway remains functional.

Supplementing the diet of a child suffering from secretory diarrhea with only electrolyte salts (NaCl, KCl and MgCl) would not do much for the child's increasing dehydration. It was found that adding electrolyte salts to a solution of water and glucose would make a solution that would function amazingly in replenishing the lost fluids and electrolytes in that child. The WHO has even gone as far to say that ORT is sufficient treatment for the majority of episodes of acute diarrhea (13). The WHO estimates that 90-95% of patients with acute diarrhea can be successfully treated with ORT alone, including those with travellers' diarrhea (7, 12, 25).

ORT, however, is not very beneficial for children with osmotic diarrhea caused by malabsorption. Because malabsorption diarrhea is caused by the inability to digest sugars such as glucose, a solution of glucose-based oral rehydration solution would only enhance the problem. If a child that suffers from malabsorption is diagnosed with secretory diarrhea, ORT may induce an osmotic diarrhea (11, 12). It is suggested that sucrose or amino acids can replace glucose, but the result is not nearly as effective. If the case is severe, intravenous therapy could be implemented without any problems. Intravenous therapy is when fluids and electrolytes are put directly into the blood system, without the patient having to orally take anything.

A concern that many scientists had about the WHO accepted ORT was its high concentration of sodium. Too much sodium in the human system can result in hypernatremia. This fear has subsided with

further studies that have shown the concentration to be harmless (11, 25). The cost of such prepared solutions is cheaper than medications but still can be burdensome for poor families with several children.

BIOCHEMICAL ASSAY OF SODIUM BENTONITE

Note: Aluminum and Silicon are also part of the elemental content Bentonite, but they are bonded in a compund called Aluminosilicate which is the basis of the clay. All trace minerals attach themselves to this compound which constitutes the clay.

Analyte	mg/L units in Bentonite
Antimony	0.84
Arsenic	0.05
Barium	98.6
Beryllium	0.13
Bismuth	0.57
Boron	2.3
Bromine	0.68
Cadmium	0.019
Calcium	1130
Carbon	3000
Cerium	6.55
Cesium	0.089
Chloride	990
Chromium	0.75
Cobalt	0.3
Copper	4.21
Dysprosium	0.39
Erbium	1.2
Europium	less than 0.1
Flouride	1.5
Gadolinium	0.62
Gallium	0.59
Germanium	7.3
Gold	less than 0.1
Hafnium	less than 0.33
Homium	less than 0.4
Indium	0.23
Iodine	4.1
Iridium	less than 0.5
Iron	550
Lanthanum	5.29
Lead	0.97
Lithium	26
Lutetium	less than 0.3
Magnesium	1080
Manganese	28.9
Mercury	less than 0.005

Molybdenum	less than 0.5
Neodymium	8.22
Nickel	0.52
Niobium	less than 0.3
Osmium	less than 0.2
Palladium	less than 0.3
Phosphorus	4.95
Platinum	less than 0.3
Potassium	990
Praseodynium	0.57
Rhenium	1.45
Rhodium	less than 0.5
Rubidium	12
Ruthenaim	less than 0.2
Samarium	0.26
Scandium	0.33
Selenium	0.11
Silver	0.26
Sodium	2070
Strontium	5062
Sulfur	275
Tantalum	less than 0.2
Tellurium	less than 0.2
Terbium	less than 0.2
Thallium	60.3
Thorium	1.57
Thulium	less than 0.3
Tin	0.25
Titanium	215
Tungsten	1.95
Vanadium	12.2
Ytterbium	0.19
Yttrium	2.1
Zinc	2.9
Zirconium	859

Adapted From: "The Contents of Bentonite Clay" (33)

Table 3: Biochemical Assay of Bentonite Clay

Concentration in mg/L can be converted to mmol/ml by the following equation:

$$\frac{X\ mg}{1L} \times \frac{1g}{100mg} \times \frac{1mol}{Y\ g} \times \frac{100mmol}{1mol} = \frac{mmol}{L}$$

X= The amount of mg/L

Y= Constant of g/mol

(**Y** constant can be found on the periodical table)

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