Fecal Bacterial β-Glucuronidase: Control by Diet

Abstract. *The effect of a mixed Western, high meat diet or a nonmeat diet on the intestinal bacterial β-glucuronidase activity was studied in human volunteers. This enzyme was significantly higher in stools of subjects on a high meat diet as compared to the nonmeat regimen. Thus, intestinal flora of subjects on a high meat diet was more able to hydrolyze glucuronide conjugates than that of individuals on a nonmeat diet. This, in turn, may raise the amount of substances, such as carcinogens, within the colonic lumen.*

Epidemiologic studies indicate that colon cancer in man is associated with dietary habits (*1*). Regression analysis demonstrated that the consumption of animal protein and combined fat was a most important causal factor leading to colon cancer (*2*). Thus, such data reveal that populations on a high meat, high fat diet are more likely to develop colon cancer than individuals on vegetarian or similar low meat diets. There is now support for the concept that the suspected effect of diet on the formation of colon cancer may be mediated through changes in the intraluminal compounds secreted into the gut, and also in the composition of intestinal microflora (*3, 4*). Several ways by which the intestinal microflora could play a significant role are that (i) it could convert dietary components into cocarcinogens or carcinogens (or both); (ii) it could metabolize endogenous secretions, themselves controlled by diet, into such compounds; and (iii) it could produce carcinogenic toxins from suitable precursors (*5*).

To study these elements, we have investigated the effects of a mixed Western high meat diet and a balanced nonmeat diet on the fecal, and hence intestinal, microbial activity. Bacterial β-glucuronidase activity was used as an indicator because this activity is associated with many components of the anaerobic and aerobic microflora present in the feces (*4, 6*). This enzyme is necessary to release active carcinogen metabolites from their glucuronide conjugates in the lower gut (*5*).

Five males and one female (aged 30 to 52 years; five whites and one black) were selected for this study from the staff of the American Health Foundation; they consumed a mixed Western high meat, high fat diet.

All subjects were healthy and none of them received any medication for at least 3 weeks prior to or during the experimental period. The mixed Western high meat diet consisted of beef, pork, or chicken (454 g per day); vegetables (two servings); potato or rice (as needed); bread, cereal (four servings); fruit (one citrus, two others); milk (two cups, skim); and butter (as needed). Each subject was consuming at least 454 g of meat per day regularly before participating in this study. Individual 24-hour fresh stool samples were collected daily on consecutive days for 4 days from each volunteer. Then the test subjects were transferred to a nonmeat diet which contained the recommended dietary essential nutrients and the caloric value needed to maintain body weight. The composition of the nonmeat diet was as follows: eggs, one; skim milk, two cups; citrus fruit, one serving; other fruits, two servings; dried beans, peas, or nuts, one cup; vegetables, three to four servings; bread and cereals, eight servings; potatoes, rice, or pasta, one serving; peanut butter, two to four tablespoons, margarine, and vegetable oil. Both diets provided approximately 23 percent protein, 45 percent fat, and 32 percent carbohydrate. All the foods consumed were prepared by the volunteers or their families at home. From the prescribed 7-day menu plan (which was repeated for 4 weeks) food choices were altered when possible to meet the preferences of the study subject provided that the composition of the diet was not altered. Subjects consumed the exact portions called for in the diets. All subjects maintained their normal activity during the experimental period. Participants were maintained on the nonmeat diet for 4 weeks, and individual 24-hour stool samples were collected daily during the last 4 days of the dietary period. The fecal samples from subjects during two periods were voided during the morning. Each subject gave one sample per day. There was neither constipation nor diarrhea on changing the diet.

All subjects voided their stool specimens in sterile plastic bags attached to toilets in our laboratory. As soon as the stool was passed into a bag, it was gassed with oxygen-free CO_2, mixed well without exposure to air, and analyzed individually.

The preparative procedures were carried out anaerobically. All determinations were performed in triplicate from each sample. A portion of the sample was diluted with reduced anaerobic media (Robbins, Fiskeville, R.I.), flushed with oxygen-free CO_2, and centrifuged at 100*g* at 4°C for 30 minutes to remove undigested food particles and other coarse materials. The supernatant, which contained bacteria, was centrifuged for 30 minutes at 15,000*g* at 4°C. The pellet was washed with three portions (10 ml each) of sterile normal saline by resuspension and centrifugation at 15,000*g*. The supernatants from all washings were pooled. The final sediment containing bacteria was disrupted by sonication at 4°C for 15 minutes. β-Glucuronidase activity in the bacterial pellet and in the supernatant fraction was assayed as described (*4, 7*).

The β-glucuronidase activity in the feces and in the pellet was significantly higher while the subjects were consuming the high meat diet as compared to the nonmeat diet (Table 1). Thus, the intestinal microflora of individuals on a high meat diet has higher capability to hydrolyze glucuronic acid conjugates than that of individuals on a meat-free diet.

Glucuronide formation is a major detoxification mechanism in mammals. Many endogenous and exogenous com-

Table 1. Effect of a mixed Western high meat diet or a nonmeat diet on fecal β-glucuronidase activity in man. The β-glucuronidase activity in the bacterial pellet and the supernatant fraction was assayed as described (*7*). The reaction mixture, containing 0.1 ml of 0.01*M* phenolphthalein glucuronide (*p*H 5.5), 0.8 ml of 0.1*M* acetate buffer (*p*H 5.8), and 0.1 ml of enzyme (bacterial pellet fraction and supernatant fraction) was incubated for 4 hours at 37°C in a water bath. The reaction was terminated by adding 2.5 ml of 0.1*M* glycine and 0.1 ml of 5 percent trichloroacetic acid. The phenolphthalein liberated was measured at 540 nm in a spectrophotometer (Beckman DB-GT). The β-glucuronidase activity is expressed as micrograms of substrate hydrolyzed per hour at 37°C. The results are expressed as the mean value ± standard error of mean for six volunteers. Each determination was performed in triplicate.

Analysis	Activity of β-glucuronidase	
	High meat	Nonmeat
	Bacterial pellet	
Protein (per mg)	285 ± 25*	127 ± 17
Dry feces (per mg)	20 ± 2.5*	5.6 ± 0.98
Daily total ($\times 10^{-2}$)	3440 ± 408*	1198 ± 229
	Supernatant	
Protein (per mg)	84 ± 3.8*	42 ± 3.8
Dry feces (per mg)	15 ± 1.8*	3.6 ± 0.41
Daily total ($\times 10^{-2}$)	2610 ± 295*	770 ± 99

* Significantly different from nonmeat group, $P < .01$.

pounds that are excreted in the bile as glucuronide conjugates are deconjugated by bacterial β-glucuronidase and further modified by intestinal bacteria in the large bowel (*5*, *8*). Since the intestinal microflora is changed by diet, these changes might alter the biological activity, toxicity, excretion, and resorption of many endogenous and exogenous compounds, such as carcinogen metabolites, acid and neutral sterols, ammonia and select amines, major products of urea and protein degradation, and tryptophan metabolites. Diet may also control the secretory and functional ability of the liver to yield potentially harmful metabolites that are subsequently split and released by gut microflora. Since the microflora is more active in populations on a mixed Western high meat diet, these reactions, including the release of carcinogens or other toxic components, would be more likely to occur in the gut of these populations on a mixed Western diet.

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Synaptic Transmission between Photoreceptors and Horizontal Cells in the Turtle Retina

Abstract. *Low calcium, high magnesium, and cobalt hyperpolarize the horizontal cell membrane and suppress the response to light, but only partially affect the response of receptor cells. These observations are consistent with the interpretation that a depolarizing transmitter is released by photoreceptors in darkness. The hyperpolarizing response to light of the horizontal cells would then result from a reduction in the amount of transmitter released.*

Vertebrate photoreceptors do not generate nerve impulses, but respond to light with a graded increase in transmembrane potential (hyperpolarization). The mechanism by which receptor hyperpolarization affects second order neurons and causes a hyperpolarization in the luminosity-type horizontal cell is not completely known. It has been suggested that the hyperpolarization of the receptor membrane by light reduces the release of a depolarizing transmitter which, according to this view, would flow continuously from the receptor pedicle (*1*). If this explanation is correct one would expect that reducing the release of transmitter by the action of blocking agents would result, not only in a decrease of the response to light, but also in a hyperpolarization of the horizontal cell membrane.

Divalent cations play a fundamental role in the process of transmitter release from presynaptic terminals both of the neuromuscular junction and of spinal cord neurons (*2*). Calcium must be present in the extracellular fluid for the transmitter to be released and its action is antagonized by an excess of magnesium or by the presence of other divalent cations. On the assumption that the principle for such an action is extensible to retinal synapses, we studied the effects of varying concentrations of calcium, magnesium, and cobalt on the intracellularly recorded responses of photoreceptors and horizontal cells in the perfused retina of turtle (*Pseudemys scripta elegans*). The effect of magnesium on horizontal cell activity in the skate retina has been reported by Dowling and Ripps (*3*).

The receptors of the turtle retina are predominantly cones (*4*) and their synaptic organization has been studied in detail by Lasansky (*5*).

The turtle eye was removed and cut along the medial lateral axis. After the vitreous chamber was drained, the eyecup was mounted in a chamber where an oxygenated and buffered Ringer solution continuously flowed over the vitreous side at 4 to 5 cm^3/min. The ionic composition of the Ringer solution used was similar to that of the cerebrospinal fluid (*6*). The *p*H was adjusted to 7.7 ± 0.2 with appropriate amounts of sodium bicarbonate. The room temperature was kept around 20°C. Test solutions containing magnesium and cobalt in different proportions were not compensated for changes in osmolarity when changes did not exceed 5 meq. Calcium-free solutions were prepared by omitting calcium chloride and adding 1 m*M* ethylenediaminetetraacetic acid (EDTA).

Intracellular recordings were made with glass micropipettes filled with 4*M* potassium acetate. With microelectrodes of low tip potential there was no need to correct the results for changes of

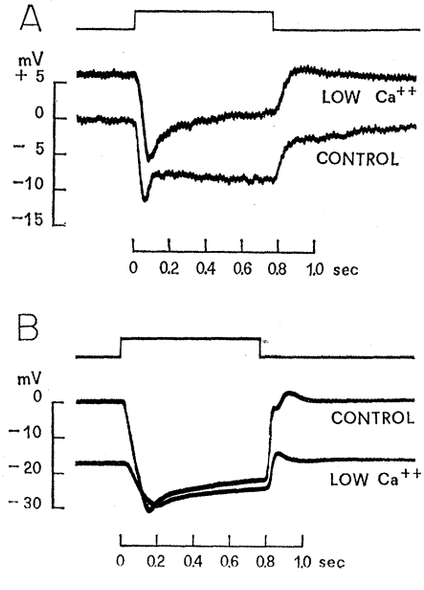

Fig. 1. Effects of low calcium on the activity recorded from (A) a cone and (B) a horizontal cell. Responses to light were obtained during perfusion with normal Ringer solution (control) and after the retina was perfused for 15 minutes with a calcium-free solution containing 1 m*M* EDTA (low Ca^{2+}). The light intensity used was attenuated 1.8 log units with respect to the maximum available energy; the area illuminated was 1500 μm in diameter. The raised bars at the top indicate durations of illumination. The zero level of membrane potential is arbitrary and indicates the level of membrane potential in darkness.